

Proceedings of  
2<sup>nd</sup> International Conference on Agriculture and  
Forestry  
ICOAF – 2015

10<sup>th</sup> - 12<sup>th</sup> June 2015

The International Institute of Knowledge Management (TIKM)

Colombo, Sri Lanka

Committee of the ICOAF- 2015

The International Institute of Knowledge Management (TIKM)

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Proceedings of the 2<sup>nd</sup> International Conference on Agriculture and Forestry, 2015

Edited by Prof. D.K.N.G. Pushpakumara and Others

ISSN: 2362 – 1036 online

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Published by The International Institute of Knowledge Management (TIIKM)

Tel: +94(0) 11 3132827

Fax: +94(0) 11 2835571

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**MESSAGE FROM PROFESSOR M.S. SWAMINATHAN**  
**CHIEF GUEST AND KEYNOTE SPEAKER**

Several parts of the world are undergoing unprecedented water shortage due to drought. California is an example of the serious water crisis. 97% of world's water is sea water. Therefore, the time has come for us to make use of the sea water in an effective way both for domestic use as well as for agriculture. For domestic purposes, the reverse osmosis method is being used. Another method used in desert areas is solar dehydration of saline water. There is still need for adequate water for farming. This is where the sea water farming technologies developed by the scientists of MSSRF assume relevance. Sea water farming involves, an integrated sylvi-aquaculture system or identifying suitable plants for such a farming system, MSSRF has established at Vedaranyam a Genetic Garden of Halophytes. Many halophytes like *Salicornia* and mangrove species are also economically valuable since they can provide food, feed, fodder, and fuel. The fish species could be chosen on consideration of both adaptation to sea water conditions and market demand. In addition to sylvi-aquaculture systems, coconut, casuarina and cashew nut can also be planted near the sea. Thus, there are possibilities for using sea water for food, jobs and income. I hope that the serious water shortage conditions prevailing in several parts of India as well as other parts of the world will stimulate more research and application in this field. Through the Dandi Salt March, Mahatma Gandhi emphasized in 1930 that sea water is an invaluable social resource. The use of sea water for coastal area farming is an idea whose time has come. Sea water farming will also help fisher families to have additional income and work opportunities when the sea is closed for fish regeneration.

**Professor M.S. Swaminathan**

Founder, Emeritus Chairman and Chief Mentor

MS Swaminathan Research Foundation

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Taramani, Chennai 600113

India

**MESSAGE FROM PROFESSOR BUDDHI MARAMBE**  
**KEYNOTE SPEAKER**

It gives me pride and pleasure to send this message to the 2<sup>nd</sup> Annual International Conference on Agriculture and Forestry (ICOAF-2015), which will be held during 10<sup>th</sup>-12<sup>th</sup> June, 2015 in Colombo, Sri Lanka. The theme of the conference "Sustainable Agriculture and Global Food Security", is of global significance and the conference is held on highly opportune time. Agriculture, forestry and fisheries can provide nutritious food for all and generate decent incomes, while supporting people-centered rural development and protecting the environment. According to the Food and Agriculture Organization (FAO) of the United Nations (UN), to be sustainable, agriculture must meet the needs of present and future generations for its products and services, while ensuring profitability, environmental health and social and economic equity. In such a situation, the global transition to sustainable food and agriculture will require major improvements in the efficiency of resource use, in environmental protection and in systems resilience. The presentations made at the ICOAF conference would no doubt strengthen the global efforts to tackle many issues in relation to sustainability in the agricultural systems and food security. I sincerely hope that the deliberations would assist in future policy directives, and planning and implementation of programs in relation to the theme of the conference.

**Professor Buddhi Marambe**  
Department of Crop Science  
Faculty of Agriculture  
University of Peradeniya  
Sri Lanka

## **MESSAGE FROM PROFESSOR GAMINI PUSHPAKUMARA CONFERENCE CHAIR**

It is a great pleasure and privilege to send this message as the Chair of the 2<sup>nd</sup> Annual International Conference on Agriculture and Forestry (ICOAF-2015) organized by the International Institute of Knowledge and Management (TIKM) which will be held from 10-12 June 2015, Colombo, Sri Lanka. The technical program is rich and varied with Prof. M.S. Swaminathan as the Chief Guest and 3 well known keynote speeches, 42 oral, 2 poster and 5 virtual presentations split between 4 parallel oral and 1 poster sessions. During the Conference, over 100 scientists from 12 countries will participate in sharing their findings in the theme of "Sustainable Agriculture and Global Food Security". This Conference is unique in the sense that it try to link traditional and modern thinking on Agriculture and Forestry together. Such integration is fundamental to reach sustainable development. The Conference, in addition to provide opportunity to present and share recent research findings of scientists, integrates and networks research scientists with well known figures in scientific arena in the world. I sincerely hope that the deliberations would assist in future policy directives, and planning and implementation of programs in relation to the theme of the conference.

As the conference Chair, I know that the success of the conference depends ultimately on the many people who have worked with us in planning and organizing both the technical program and supporting social arrangements. In particular, I thank the Chief Guest and Keynote speakers, all scientists, program and publication chairs and entire TIKM team for their hard work to stage ICOAF-2015, in the second successive time.

I welcome you all to the ICOAF-2015 and wish the Conference all success!

**Professor Gamini Pushpakumara**

Department of Crop Science

Faculty of Agriculture

University of Peradeniya

Sri Lanka

10<sup>th</sup> June 2015

Table of Contents	Page No
01. Survey on Commonly used Medicinal Plants-in Muthunagar Grama Niladhari Division, Trincomalee District, Sri Lanka <i>Dr. K. Vidya Dharshini</i>	01
02. Determination of Flower Biology of Underexploited Pollinator Attracting Vegetable, Luffa Cylindrica (L.) Growing in Home Gardens of Jaffna <i>Ms. Sathyakala Kodeeswaranathan</i>	13
03. Production, Quality Assessment and Shelf Life Evaluation of Protein - Rich Biscuits Made From Blends of Wheat and Defatted Coconut Flours <i>Ms. Sujirtha Nadarajah</i>	19
04. Biosurfactant Process Synthesis and Stabilization of Silver Nanoparticles for Modified Preservation Methods on Common Fermented Foods <i>Dr. Tholkappian Panchaksharam</i>	28
05. Is Feed and Fodder Shortage a Major Impediment to Accelerated Livestock Development in Bhutan? <i>Dr. Nar Bahadur Tamang</i>	36
06. Studies on Phenological Behavior of Two Cassia Species in Girnar Reserve Forest, Gujarat, India <i>Dr. Rupesh. N. Nakar</i>	43
07. Key Issues in Application of Community Use Zone (CUZ) to Crocker Range Park from the Perspective of Social Work <i>Dr. Peter Voo</i>	49
08. Assessment of the Variability of Yield of Maize in Lilongwe District (MALAWI) in Response to Climate Change Using DSSAT Model <i>Ms. Mphangera Kamanga</i>	54
09. Peasant Climate Knowledge, Application on Crops of Onion Bulb ( <i>Allium cepa</i> L.) <i>Dr. Pablo Pomboza-Tamaquiza</i>	68
10. Potato Production under Brakish Water and Compost Use <i>Prof. Samih Mohammad Abubaker</i>	77

11. Influence of Supplemental Lighting on Reducing Pre-Mature Fruit Drop and Increasing Fruit Yield of Greenhouse Gherkins	82
<i>Ms. Chathuri Lankani Karunarathne</i>	
12. Orientational Effect of Aqueous Leaf Extract of Citrus Aurantifolia (LIME) on Housefly, Musca Domestica (DIPTERA:MUSCIDAE)	90
<i>Mrs. Nithiyagowry Rajan</i>	
13. Evaluation of Growth of Listeria monocytogenes With Cinnamon Oil and Powder under Different Temperatures by Indirect Conductimetry	95
<i>W.B.C Wijamunige</i>	
14. A Potential Biosurfactant Mediated Control of Fusarium Wilt in Tomato Crop and a Novel Method for Cleaning Pesticide Residues in Tomatoes	102
<i>Dr. Parthasarathi Rengasamy</i>	
15. Interaction Effect of AM Fungi and Salt Stress on the Growth of Curcuma Longa L. Grown under Greenhouse Condition	113
<i>Dr. Sadhana Balasubramanian</i>	
16. Biochemical Characterization and Insecticidal Activity of Different Solvent Crude Extracts of Lantana Camara L. on Diamondback moth (DBM), Plutella Xylostella (Linn.)	117
<i>Mr. G. Thanavendan</i>	
17. Increasing the Yield of Onion through Improved Production Technology in Kalaburagi District of Karnataka	139
<i>Mr. Raju G. Teggelli</i>	
18. Inter-Relationship of Environmental and Managemental Parameters on Bull Semen Evaluation	146
<i>Dr. M. Pagthinathan</i>	
19. Effect of NaCl and Root-Media Selection on Yield Attributes, Oil Composition and Mineral Composition of Rose Geranium	152
<i>Mr. Zenzile Peter Khetsha</i>	
20. Use of Three Computer Models in Agriculture: A Review	167
<i>Mr. Pradeep Kumara</i>	

21. Gender and Productivity Differentials among Rice Farmers in Niger State, Nigeria 176  
*Dr. OJO, Michael Akindele*
22. Natural Rubber Prices Forecasting Using Simultaneous Supply-Demand and Price System Equation and Vecm Model: Between Theory and Reality 184  
*Dr. Aye Aye Khin*



# **SURVEY ON COMMONLY USING MEDICINAL PLANTS IN MUTHUNAGAR GRAMA NILADHARI DIVISION, TRINCOMALEE DISTRICT, SRI LANKA**

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## **Abstract**

Medicinal plants are the mainstay of the traditional medical system of Sri Lanka. The Muthunagar Grama Niladhari division is a rural area, including rich flora and fauna, spans an area of 8 km<sup>2</sup>, which belongs to Trincomalee district, Sri Lanka. The aim of the study is to identify the medicinal plants commonly used by traditional physicians. One year field survey was done and the plants were identified using herbariums, sample specimens, literatures, helps of traditional healers and senior yokels of the area. The survey was documented, 176 species of medicinal plants available belonging to 59 families. The floristic diversity was dominated by higher plants belonging to family Leguminosae (13.06%), ensuing by Cucurbitaceae and Labiatae (each 5.68%). Further, 2.27 % of rare medicinal plants were identified which are specially using by the Sri Lankan traditional medical practitioners. The study revealed that the Muthunagar area is one of the stockpile for medicinal plants. Currently, the reduction of availability of the herbs has reached a very critical phase with the growth of civilization and urbanization of this area. Unless evolve conservation of the medicinal plants, departing to be grave the flora and fauna including invaluable medicinal herbs in Muthunagar Grama Niladhari division in future.

Keywords: Medicinal plants, herbarium, field survey

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## **Introduction**

Traditional medical system is a prehistoric system of medicine recognized throughout the world as a trustworthy healthcare resource. It has been practiced since chronological times and draws its roots to ancient civilization. Nowadays, folk medicine is recognized throughout the world as a credible healthcare resource and about 80% of the world's population depends on traditional medicine for treatment of different ailments (Shinwari and Qaisar, 2011). The World Health Organization reveal the current estimative suggests, that many developed countries have a great proportion of the population making use of traditional practice of health, especially the use of medicinal plants (WHO, 1999). Although the access to the modern medicine is available in these countries, the use of medicinal herbs

has kept popularity for historical and cultural reasons. In the developing countries, 65 – 80 % of the population depends exclusively on the medicinal plants for basic cares of health (Maria de Fatima et al, 2008). Officially recognized that, the 2500 plant species have medicinal value while over 6000 plants are estimated to be explored in traditional, folk and herbal medicine (Huxley, 1984).

Medicinal plants are the mainstay of indigenous system of medicine in Sri Lanka including Siddha, Ayurveda, Unani, Homeopathy and traditional medicine. This herbal system of medicine thrives on naturally occurring floral diversity. Medicinal plants are involving with multi sectional systems including

pharmaceutical, cosmetic, agriculture and food industry nowadays (Siva Rama Krishna & Sujatha, 2012).

Trincomalee district has a rich floral diversity with 594 species of plants (Red list, 2012). Traditional physicians are commonly collecting medicinal plants in Muthunagar Grama Niladhari division for preparing and prescribing medicines. Currently, the reduction of availability of the jungle area reached a very critical phase with the civilization and urbanization of this area. The aim of the study is to identify the medicinal plants in this area, commonly using by the traditional physicians.

## Materials and Methods

### Study area

The study was conducted in Muthunagar Grama Niladhari division extends an area of 8 sq.km. The area situated in the Eastern province of Trincomalee district, Sri Lanka, integrated with Muthunagar and Vilankulam villages (Fig 1). The area is inhabit with small forests, marshy land and paddy field. The local people are mainly depending on farming in this area.

The study was focused on a survey of commonly used medicinal plants in traditional medicinal system in Sri Lanka. The Survey has been demeanor from January 2014 to January 2015 for all season in various habitats in this area as mentioned above. A comprehensive list of various medicinal plants has been prepared after proper photo documentation and identification of plant species were done with the help of herbariums, sample specimens, literatures, assistances of traditional healers and senior yokels of the area. Habit of plants and distribution of plants were recorded while taking the photo documentation. The species were further confirmed with abet of medicinal botany books and traditional medical text books.

## Result and Discussion

The present study reveals 176 species of medicinal plants available belongs to 59 families. The taxa are arranged in alphabetically according to family. In addition Tamil and English name tabulated in the table

(Table 1). The floristic diversity is dominated by higher plants belongs to family Leguminosae (13.06%), followed by Cucurbitaceae and Labiatae (each 5.68 %) and Euphorbiaceae and Malvaceae (each 5.11 %). 32 families were represented by single species each (Table 1, Chart 1). In addition, 2.27 % of rare medicinal plants were identified as, *Hugonia mystax* (Linaceae), *Salacia reticulate* (Hippocrateaceae), *Salvadora persica* (Salvadoraceae) and *Erythroxylum monogynum* (Erythroxylaceae) which are specially using by the Sri Lankan traditional medical practitioners for various disease patterns. Thus 5.68% of medicinal plants including *Nerium oleander*, *Cerbera thevetia*, *Calotropis gigantean*, *Excoercaria agallocha*, *Jatropha curcus*, *Jatropha glandulifera*, *Jatropha multifida*, *Ricinus communis*, *Gloriosa superba* and *Datura metel* were identified as poisonous plants, which are using by traditional physicians for various illness in proper dosage forms.

Among the 176 medicinal plants, 76.70% of medicinal plants were locally distributed and 18.75% of plants were cultivated and the remaining 4.54 % were both locally distributed and cultivated medicinal plants (Table 1, Figure 2). Further, demonstration of habit was evaluated, as 34.65 % of herbs, shrubs 21.59 %, trees 21.02 %, climbers 10.79 %, twiners 4.54 %, semi shrubs 2.84 %, palms and grasses each 1.70% and lianas and vines each 0.56% (Table 01).

These findings has demonstrated, the Muthunagar Grama Niladhari division engaged with categories of medicinal plants, further the diminution of jungle area may lead to big reduction of medicinal plants in this area, as our study indicating, 76.70 % of medicinal plants distributed locally.

## Conclusion

The study revealed that the Muthunagar Grama Niladhari division is one of the stockpile for medicinal plants. Currently, the reduction of availability of the herbs has reached a very critical phase with the growth of civilization and urbanization of this area. Unless evolve conservation of the medicinal plants, departing to be grave the flora and fauna including invaluable

medicinal herbs in Muthunagar Grama Niladhari division in future.

**Table 1:** List of identified medicinal plant species in Muthunagar Grama Niladhari division

Family name	Botanical name	Tamil name	Distribution	Habit
Acanthaceae	<i>Acanthus ilicifolius</i> Linn.	T. Kazhuthai mulli E. Sea holly	L	H
	<i>Adhatoda vasica</i> Nees.	T. Aadathodai E. Malabar nut	L	S
	<i>Hygrophila spinosa</i> T Ander	T. Neermulli E. Long leaved barleria	L	H
	<i>Justicia gendarusa</i> Burm.f.	T. Karunochchi /neernoichi E. Willow-leaved justicia	L	S
	<i>Barleria prionitis</i> Linn.	T. Semmulli E. Porcupine flower	L	S
	<i>Peristrophe bicalyculata</i> Nees.	T. Kattu nilavembu	L	S
	<i>Trianthema portulacastrum</i> Linn.	T. Saaranai E. Horse purslanes	L	H
Aizoaceae	<i>Mollugo pentaphylla</i> Linn.	T. Kattu Patpadagam E. Wild Indian chickweed	L	H
	<i>Achyranthes aspera</i> Linn.	T. Nayuruvi E. Prickly chaff flower	L	H
Amaranthaceae	<i>Aerva lanata</i> (Linn.) Juss	T. Sirupeelai E. Common way sideweed	L	H
	<i>Alternanthera sessilis</i> Linn.	T. Ponnankani E. Sessil joyweed	C	H
	<i>Amaranthus spinosus</i> Linn.	T. Mullukeerai E. Prickly Amaranth	L	H
	<i>Celosia argentea</i> Linn.	T. Panankeerai E. Silver cocks comb	L	H
	<i>Amaranthus viridis</i> Linn.	T. Mulaikeerai E. Green amaranth	L	H
	<i>Anacardium occidentale</i> Linn	T. Kottaimunthiri E. Cashew nut	C	Tr
	<i>Odina wodier</i> Roxb.	T. Othi E. Woider	L	Tr
Anacardiaceae	<i>Mangifera indica</i> Linn.	T. Maa E. Mango tree	C	Tr
	<i>Annona squamosa</i> Linn.	T. Annamunna E. Sugar apple	C	Tr
Apocynaceae	<i>Carissa carandas</i> Linn.	T. Kala	L	S

		E. Ceylon Damson		
	<i>Nerium oleander</i> Linn.	T. Alari	L	S
		E. Oleander		
	<i>Cerbera thevetia</i> Don.	T. Alari	L	Tr
		E. Yellow oleander		
	<i>Nerium divaricatum</i> Linn.	T. Nanthiyavattam	C	S
		E. Crape - jasmine		
	<i>Catharanthus roseus</i> (Linn.) G. Don	T. Patti poo	L	S
		E. Rosy periwinkle		
	<i>Pergularia daemia</i> (Forsk.) Chiov.	T. Uththamagani	L	Tw
		E. Dog's bane white low plant		H
Aristolochiaceae	<i>Aristolochia bracteolata</i> Lam.	T. Aadutheendapaalai	L	H
		E. Worm killer		
	<i>Aristolochia indica</i> Linn.	T. Peru marunthu	L	Tw
		E. Indian birthwort		H
Asclepiadaceae	<i>Calotropis gigantea</i> (Linn) Ait.f	T. Erukku	L	S
		E. Gigantic swallow wort		
	<i>Gymnema sylvestre</i> (Retz.) R.Br.exSchult.	T. Sirukurinja	C	C
		E. Small Indian Ipecacuanha		
	<i>Hemidesmus indicus</i> (L.) R.Br	T. Nannari	L	Tw
		E. Indian sarsaparilla		
	<i>Dregea volubilis</i> (Linn.f.) Hook.f.	T. Perunkurinja	L	Tw
		E. Sneezewort		S
Asteraceae	<i>Helianthus annuus</i> Linn.	T. Sooriyakanthi	C	H
		E. Sun flower		
Basellaceae	<i>Basella alba</i> Linn	T. Pasali	C	C
		E. Indian spinach		
Boraginaceae	<i>Heliotropium indicum</i> Linn.	T. Thelkudukku	L	H
		E. Heliotrope		
	<i>Cordia dichotoma</i> Forst.	T. Naruvili	L	Tr
		E. Sebesten plum		
Capparidaceae	<i>Polanisia icosandra</i> Linn.	T. Naaivelai	L	H
		E. Dog mustard		
	<i>Capparis zeylanica</i> Linn.	T. Kaatoti	L	S
		E. Ceylon caper		
	<i>Crataeva religiosa</i> hook.	T. Maavilangu	L	Tr
		E. Three leaved caper		
Caricaceae	<i>Carica papaya</i> Linn.	T. Pappali	C	Tr
		E. Papaw		
Combretaceae	<i>Terminalia arjuna</i> W & A	T. Maruthu	L	Tr
		E. White murdah		
Compositae	<i>Elephantopus scaber</i> Linn	T. Aanai suvadi	L	H

		E. Elephant's Foot		
	<i>Eclipta prostrata</i> Roxb.	T. Karisaalai	L	H
		E. False daisy		
	<i>Vernonia cinerea</i> Less.	T. Seetheviyar sengaluneer	L	H
		E. Ash coloured fleabane		
	<i>Vernonia zeylanica</i> Less.	T. Kuppilai	L	S
	<i>Sphaeranthus indicus</i> Linn.	T. Kottaikaranthai	L	H
		E. East Indian globe- thistle		
Convolvulaceae	<i>Evolvulus alsinoides</i> Linn.	T. Vishnu kranthi	L	H
	<i>Ipomoea obscura</i> Linn.	T. Siru thaali	L	Tw
	<i>Ipomoea pes tigridis</i> Linn.	T. Pulichuwadi	L	Tw
		E. Tiger's foot		
	<i>Ipomoea aquatica</i> Forsk.	T. Kankun	L	H
		E. Swamp cabbage		
Cucurbitaceae	<i>Coccinia grandis</i> Linn.	T. Kovai	L	C
		E. Ivy gourd		
	<i>Corallocarpus epigaeus</i> hook.	T. Akashagarudan	L	C
		E. Bryoms		
	<i>Luffa acutangula</i> (Linn) Roxb.	T. Peerku	C	C
		E. Ridged gourd		
	<i>Melothria heterophylla</i> Cogn.	T. Peyppudal	L	C
	<i>Melothria maderaspatana</i> Linn.	T. Musumusukkai	L	C
		E. Rough bryony		
	<i>Momordica charantia</i> Linn.	T. Pakal	C	C
		E. Bitter gourd		
	<i>Diplocyclos palmatus</i> Linn.	T. Ivirali	L	C
	<i>Cucurbita maxima</i> Duchesne.	T. Pushani	C	C
		E. Melon pumpkin		
	<i>Trichosanthes cucumerina</i> Linn.	T. Pudal	C	C
		E. Snake gourd		
	<i>Benincasa hispida</i> (Thunb.) Cogn.	T. Kalyana pushinikkay	C	C
		E. Ash pumpkin		
Cyperaceae	<i>Cyperus rotundus</i> Linn.	T. Korai	L	H
		E. Nut grass		
Chenopodiaceae	<i>Suaeda maritime</i> (L.) Dumort	T. Umari	L	H
		E. Marsh samphire		
Dioscoreaceae	<i>Dioscorea pentaphylla</i> Linn.	T. Allal kizhangu	L	C
		E. Five leaved yam		
Ebenaceae	<i>Diospyros malabarica</i> Pers.	T. Panichai	L	Tr
		E. Riber ebony		
Erythroxylaceae	<i>Erythroxylum monogynum</i> Roxb.	T. Semmanathi	L	Tr
		E. Bastard sandal		

Euphorbiaceae	<i>Acalypha indica</i> Linn.	T. Kuppaimeni E. Indian acalypha	L	H
	<i>Euphorbia hirta</i> Linn.	T. Ammanpatchaiyarissi E. Australian asthma weed	L	H
	<i>Euphorbia thymifolia</i> Linn.	T. Sinnanmanpatchaiy Arisi E. Asthma plant	L	H
	<i>Excoecaria agallocha</i> Linn.	T. Thillai E. Blinding Tree	L	Tr
	<i>Jatropha curcas</i> Linn.	T. Kadalamanaku E. Purging nut	L	S
	<i>Jatropha glandulifera</i> Hook.	T. Kattamanakku	L	SS
	<i>Jatropha multifida</i> Linn.	T. Eliyamanaku E. Physic nut	L	S
	<i>Ricinus communis</i> Linn.	T. Amanakku E. Castor oil plant	L	Tr
	<i>Tragia involucrata</i> Linn.	T. Sirukanchori E. Indian stinging-nettle	L	H
Gentianaceae	<i>Enicostema axillare</i> Lam.	T. Vellaruku E. Indian Gendian	L	H
Gramineae	<i>Cynodon dactylon</i> Linn.	T. Aruku E. Bermuda grass	L	G
	<i>Panicum crus galli</i> Linn.	T. Kuthiraivaalpul E. Cockspur Grass	L	G
	<i>Oryza sativa</i> Linn.	T. Nel E. Paddy, rice	C	G
	<i>Saccharum officinarum</i> Linn.	T. Karumbu E. Sugar cane	C	H
Hippocrateaceae	<i>Salacia reticulata</i> Wight.	T. Kadal raanji	L	C
Labiateae	<i>Leucas zeylanica</i> (Linn.) R.Br.	T. Mudithumpai E. Thumbe	L	H
	<i>Leucas aspera</i> Spreng.	T. Mudithumpai	L	H
	<i>Leonotis nepetifolia</i> (Linn.) R.Br.	T. Kaasithumpai	L	H
	<i>Ocimum americanum</i> Linn.	T. Kanjaakorai	L	H
	<i>Ocimum sanctum</i> Linn.	T. Ven thulasi E. Holy basil	L	H
	<i>Ocimum sanctum</i> Linn. (Black)	T. Karunthulasi	CL	H
	<i>Ocimum gratissimum</i> Linn.	T. Elumicham thulasi E. Lemon basil	L	H
	<i>Anisochilus carnosus</i> (Linn.) Wall.ex Benth	T. Katpooravalli E. Thick leaved lavender	C	H
	<i>Pogostemon heyneanus</i> Benth.	T. Pachchilai	L	S

Leguminosae	<i>Anisomeles indica</i> O.Ktze.	T. Pei miratti	L	H
	<i>Abrus precatorius</i> Linn.	T. Kundrimani	L	Tw
		E. Indian Liquorice		
	<i>Alysicarpus vaginalis</i> DC.	T. Pulladi	L	H
	<i>Arachis hypogaea</i> Linn.	T. Nilakadalai	C	H
		E. Ground nut		
	<i>Bauhinia tomentosa</i> Linn.	T. Thiruvathi	CL	S
		E. Wild champak		
	<i>Bauhinia purpurea</i> Linn.	T. Mantarai / Neelathiruvaththi	L	S
		E. Purple bauhinia		
	<i>Cassia alata</i> Linn.	T. Vandukolli	L	S
		E. Winged senna		
	<i>Cassia occidentalis</i> Linn.	T. Oosi thakarai	L	H
		E. Fetid cassia		
	<i>Cassia auriculata</i> Linn.	T. Avaarai	L	S
		E. Tanner's cassia		
	<i>Cassia sophora</i> Linn.	T. Ponnavaarai	L	S
		E. Negro coffee		
	<i>Cassia fistula</i> Linn.	T. Sarakkondrai	L	Tr
		E. Purging cassia		
	<i>Cassia tora</i> Linn.	T. Pandi thagarai	L	H
		E. Fetid cassia		
	<i>Clitoria ternatea</i> Linn.	T. Kaakatan / kakkanavan	CL	Tw
		E. Butterfly pea		
	<i>Indigofera tinctoria</i> Linn.	T. Avuri	L	S
		E. Indigo		
	<i>Indigofera enneaphylla</i> Linn.	T. Seppunerunjil	L	S
	<i>Mimosa pudica</i> Linn.	T. Thottatchurunki	L	H
		E. Sensitive plant		
	<i>Sesbania grandiflora</i> Pers.	T. Agathi	C	Tr
		E. Sesban		
	<i>Tephrosia purpurea</i> Linn.	T. Kaavila	L	H
		E. Wild indigo		
	<i>Desmodium triflorum</i> (Linn.)DC.	T. Sirupulladi	L	H
		E. Three flower ticktrefoil		
	<i>Tamarindus indica</i> Linn.	T. Puli	C	Tr
		E. Tamarind tree		
	<i>Piliostigma racemosum</i> (Lam.) Benth.	T. Aaththi	L	Tr
	<i>Crotalaria verrucosa</i> Linn.	T. Kilukilupai	L	H
		E. Rattle wort		

	<i>Dichrostachys cinerea</i> Wight & Arn.	T. Vidathal	L	Tr
	<i>Albizia lebbbeck</i>	T. Vaahai	L	Tr
		E. Lebbeck		
Liliaceae	<i>Asparagus racemosus</i> Willd.	T. Sathawari	L	S
		E. Wild asparagus		
	<i>Asperagus falcatus</i> Linn.	T. Sathawari	L	S
		E. Large forest asparagus		
	<i>Gloriosa superba</i> Linn.	T. Kalapai kizhangu	L	C
		E. Super lily		
	<i>Aloe vera</i> Linn.	T. Katralai	CL	H
		E. Aloe		
	<i>Sansevieria zeylanica</i> (Linn.) Willd.	T. Marul	L	H
		E. Bow string hemp		
Linaceae	<i>Hugonia mystax</i> Linn.	T. Mothirakanni	L	S
		E. Climbing flax		
Lythraceae	<i>Lawsonia inermis</i> Linn.	T. Maruthondri	C	S
		E. Henna plant		
Malvaceae	<i>Abutilon indicum</i> Sweet.	T. Thuththi	L	SS
	<i>Abutilon asiaticum</i> G. Don	T. Perunthuththi	L	S
		E. Country mallow		
	<i>Hibiscus rosa-sinensis</i> Linn.	T. Semparuthi	C	S
		E. Shoe flower		
	<i>Pavonia odorata</i> Willd.	T. Peramatti	L	H
	<i>Sida acuta</i> Burm.f.	T. Arivaalmanaipoondur	L	SS
	<i>Sida cordifolia</i> Linn.	T. Sitramatti	L	SS
		E. Yellow sticky mallon		
	<i>Thespesia populnea</i> Soland.	T. Poovarasu	L	Tr
		E. Portia- tree		
	<i>Sida veronicaefolia</i> Lamk.	T. Palampassi	L	H
	<i>Sida rhombifolia</i> Linn.	T. Kurunthoti	L	S
Meliaceae	<i>Azadirachta indica</i> A. Juss.	T. Vembu	L	Tr
		E. Neem tree		
Menispermaceae	<i>Tinospora cordifolia</i> Miers.	T. Seenthil	L	C
		E. Heart leaved moon seed		
Moraceae	<i>Artocarpus heterophyllus</i> Lam.	T. Pala	L	Tr
		E. Jak fruit tree		
	<i>Ficus benghalensis</i> Linn.	T. Aal	L	Tr
		E. Banyan tree		
	<i>Ficus racemosa</i> Linn.	T. Aththi	L	Tr
		E. Country fig tree		
	<i>Ficus infectoria</i> Roxb. F. tjakela.	T. Iththi	L	Tr
	<i>Ficus religiosa</i> Linn.	T. Arasu	L	Tr



		E. Bo tree		
Moringaceae	<i>Moringa oleifera</i> Lam.	T. Murunkai	C	Tr
		E. Drumstick tree		
Myrtaceae	<i>Psidium guajava</i> Linn.	T. Koiya	C	Tr
		E. Guava tree		
Nyctaginaceae	<i>Boerhaavia diffusa</i> Linn.	T. Mookarattai	L	H
		E. Hogweed		
Oleaceae	<i>Nyctanthes arbor-tristis</i> Linn.	T. Pavalamallikai	C	Tr
		E. Night-flowering jasmine		
Oxalidaceae	<i>Averrhoa bilimbi</i> Linn.	T. Vilimbi	C	Tr
		E. Bilimbi		
Palmae	<i>Areca catechu</i> Linn.	T. Kamuku	C	P
		E. Areca nut		
	<i>Borassus flabellifer</i> Linn.	T. Panai	L	P
		E. Palmyra palm		
	<i>Cocos nucifera</i> Linn.	T. Thennai	C	P
		E. Coconut tree		
Passifloraceae	<i>Passiflora edulis</i> Sims.	T. Kodithodai	C	L
		E. Passion fruit		
	<i>Passiflora foetida</i> Linn.	T. Sottup pazham	L	C
		E. Bush passion fruit		
Pedaliaceae	<i>Pedaliium murex</i>	T. Aanai nerunchil	L	H
<b><u>Phyllanthaceae</u></b>	<i>Phyllanthus niruri</i> Linn.	T. Kilkkaynelli	L	H
Piperaceae	<i>Piper betle</i> Linn.	T. Vetrilai	C	V
		E. Betel		
Punicaceae	<i>Punica granatum</i> Linn.	T. Maathulai	C	S
		E. Pomegranate		
Rhamnaceae	<i>Zizyphus jujuba</i> Mill & Lamk.	T. Ilanthai	L	S
		E. Common jujube		
	<i>Zizyphus oenoplia</i> Linn.	T. Soorai	L	S
		E. Jackal jujube		
Rubiaceae	<i>Borreria hispida</i> Linn.	T. Naththaichuri	L	H
		E. Shaggy button weed		
	<i>Oldenlandia umbellata</i> Linn.	T. Impooral	L	H
		E. Chaya root		
	<i>Canthium parviflorum</i> Lam.	T. Kaarai	L	S
		E. Carray cheddile		
	<i>Randia dumetorum</i> Lamk.	T. Marukkarai	L	S
		E. Common emetic nut		
	<i>Morinda tinctoria</i> Roxb.	T. Nuna	L	Tr
		E. Indian mulberry		
Rutaceae	<i>Aegle marmelos</i> Correa.	T. Vilvam	CL	Tr
		E. Bael afruit tree		

	<i>Feronia limonia</i> Linn.	T. Vila E. Wood apple	L	Tr
	<i>Murraya koenigii</i> Spreng.	T. Karivembu E. Curry leaf	C	Tr
	<i>Toddalia asiatica</i> Lamk.	T. Milakaranai E. Forest pepper	L	S
Salvadoraceae	<i>Azima tetracantha</i> Lam.	T. Iyangu	L	S
	<i>Salvadora persica</i> Linn.	T. Uga E. Tooth brush tree	L	Tr
Sapindaceae	<i>Cardiospermum microcarpum</i> H.B.K	T. Mudakkothan	L	C
Sapotaceae	<i>Madhuca longifolia</i> (Linn.) J.F. Macbr.	T. Illuppai E. Honey tree	L	Tr
Scrophulariaceae	<i>Bacopa monniera</i> (Linn.) Vatke.	T. Brammi E. Thyme leaved gratiola	L	H
Solanaceae	<i>Solanum nigrum</i> Linn.	T. Manathakkali E. Black nightshade	L	H
	<i>Solanum surattense</i> Burm.f.	T. Vattu kaththari	L	H
	<i>Solanum trilobatum</i> Linn.	T. Thoothuvalai E. Climbing brinjal	L	S
	<i>Solanum torvum</i> Swartz.	T. Sundam kaththari E. Unarmed night shade	L	SS
	<i>Datura metel</i> Linn.	T. Umaththai E. White datura	L	H
Sterculiaceae	<i>Pterospermum suberifolium</i> Lam.	T. Vinnangu	L	Tr
Umbelliferae	<i>Centella asiatica</i> Urb.	T. Vallarai E. Indian pennywort	C	H
Verbenaceae	<i>Clerodendrum inerme</i> (Linn.) Gaertn.	T. Sangan kuppi	L	S
	<i>Phyla nodiflora</i> (Linn.) Greene.	T. Poduthalai	L	H
	<i>Vitex negundo</i> Linn.	T. Notchi E. Indian privet	L	S
Vitaceae	<i>Cissus quadrangularis</i> Linn.	T. Pirandai E. Bone setter	L	C
Zygophyllaceae	<i>Tribulus terrestris</i> Linn.	T. Siru nerunjil E. Calthrops	L	H

L-Locally distributed medicinal plants, C- Cultivated medicinal plants and CL –cultivated and locally distributed medicinal plants

H-Herb, S-Shrub, Tr-Tree, Tw-Twiner, TwH-Twining herb, TwS-Twining shrub, SS-Semi shrub, C-Climber, G-Grass, P-Palm, L- Liana, V- Vine,

T-Tamil name, E-English name

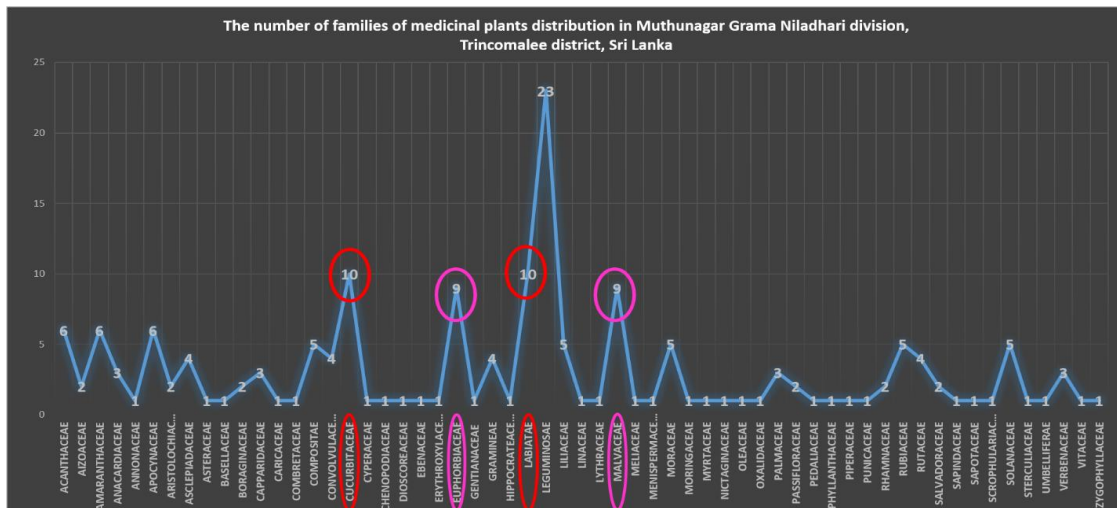


Chart 1. The families of medicinal plants distribution

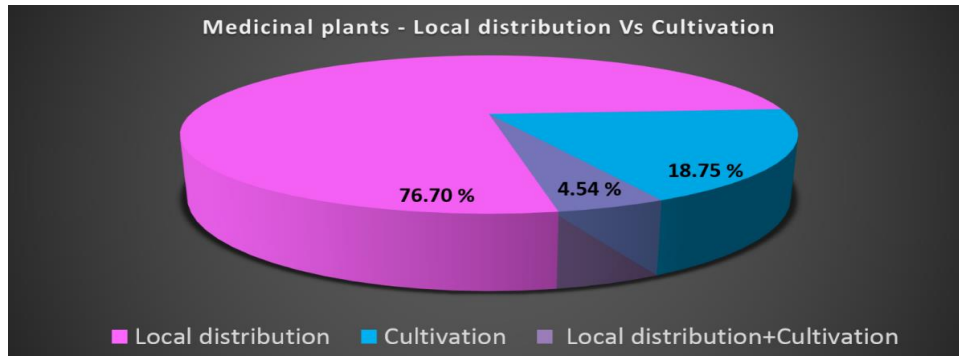


Chart 2. Showing the status of medicinal plants distribution

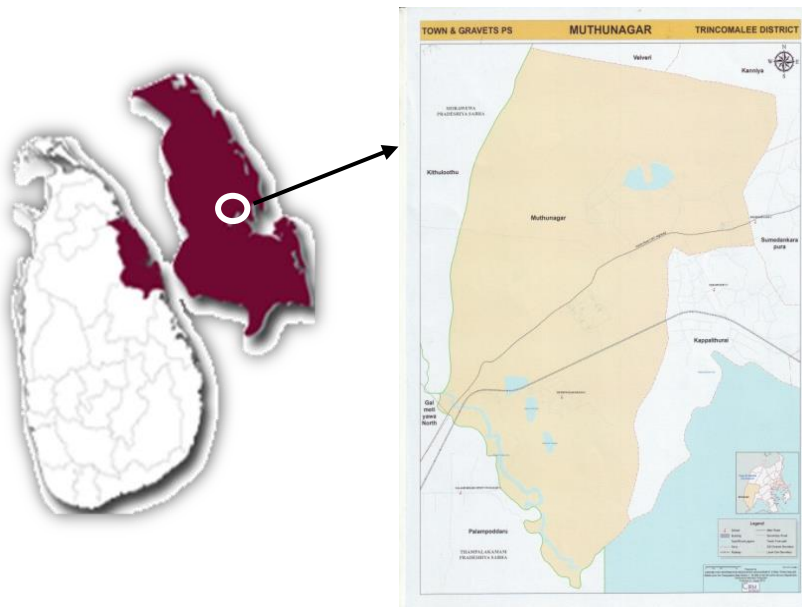


Figure 1: Map of the study area – Muthunagar Grama Niladhari division, Trincomalee district, Eastern province of Sri Lanka (Not to scale) - Grama Niladhari. Muthunagar

## Acknowledgement

The authors are thankful to Mr. A. Kirubaharan, Grama Niladhari, Muthunagar Grama Niladhari division, Trincomalee, to help us to conduct the survey and specially express our sincere thanks to Mr. A. R. M. Nasar, who was help us in this study to identify the medicinal plants and direct the paths in this area.

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# DETERMINATION OF FLOWER BIOLOGY OF POLLINATOR ATTRACTING UNDEREXPLOITED VEGETABLE, *Luffa cylindrica* (L.) GROWING IN HOME GARDENS OF JAFFNA

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## Abstract

*Luffa cylindrica* is an edible, underexploited, Cucurbitaceae vegetable crop in Sri Lanka. It has the potential to attract diverse pollinators and to sustain their visits to the home gardens. Conserving the pollinator diversity through the sustained contribution of potential plants that support pollinators in the home gardens were studied. Flower biology of *L. cylindrica* was investigated by assessing the flowering stages over time, corolla opening with time, maximum nectar production, anthesis, pollen shedding time and stigma receptivity. The rate of flower opening was at peak around 03.45 hr to 04.15 hr. Anthesis was prolonged for 2-2½ hours and stigma receptivity was 3-3½ hours after flower opening fully. The nectar volume appeared to be the highest around 6hr after flower opening. Of the *L. cylindrica* flower visitors, nine bee species (*Amegilla* sp, *Amegilla cingulata*, *Apis florea*, *Apis cerana*, *Ceratina binghami*, *Trigona iridipennis*, *Thyreus ramosellus* and *Lasioglossum vagans*, *Xylocopa fenestrata*), three butterfly species (*Catopsilia pyranthe*, *Telchinia violae* and *Appias paulina*) and an ant were found. These results confirm the potential of *L. cylindrica* to be grown as one of the pollinator conserving plant in the home gardens of Jaffna.

Keywords: *Luffa cylindrica*, home gardens, flower biology, anthesis, pollinator.

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## Introduction

*Luffa cylindrica*, known as sponge gourd, is a tropical and subtropical underexploited vegetable belongs to the family Cucurbitaceae. Though it is not commonly grown in home gardens, it has enormous potential to support pollinators by rewarding the pollens. These plants are growing in the wild and due to its multipurpose use people started domesticating it in their household however this crop has not been grown forever. Being a neglected crop growing in most of the households, this vegetable has enormous potential to attract pollinators. In general, plants in the family Cucurbitaceae possess potential to attract pollinators and *L. cylindrica* is one such noble crop used for salad preparation. This crop tolerates a wide range of climatic and soil conditions during flowering and fruiting period. Isolated cultivation of *L. cylindrica* is found in

home gardens in the dry zone of Sri Lanka. However, the information on the flower biology and flowering phenology of *L. cylindrica* is scarce. To explore the potential of its support to pollinators, the flower biology and flowering phenology of *L. cylindrica* needs to be understood.

## Materials and Methods

*Luffa cylindrica*, known as sponge gourd, is a tropical and subtropical underexploited vegetable belongs to the family Cucurbitaceae. Though it is not commonly grown in home gardens, it has enormous potential to support pollinators by rewarding the pollens. These plants are growing in the wild and due to its multipurpose use people started domesticating it in their household however this crop has not been grown forever. Being a neglected crop growing in most of the households, this vegetable has enormous potential to

attract pollinators. In general, plants in the family Cucurbitaceae possess potential to attract pollinators and *L. cylindrica* is one such noble crop used for salad preparation. This crop tolerates a wide range of climatic and soil conditions during flowering and fruiting period. Isolated cultivation of *L. cylindrica* is found in home gardens in the dry zone of Sri Lanka. However, the information on the flower biology and flowering phenology of *L. cylindrica* is scarce. To explore the potential of its support to pollinators, the flower biology and flowering phenology of *L. cylindrica* needs to be understood.

### Materials and Methods

The study was conducted at Thirunelvely area in Jaffna district located at 9° 40' 60N, 80° 1' 0E at an altitude of 8m above the mean sea level, which is in the Northern Province of Sri Lanka, falls in dry zone (DL3). *L. cylindrica* grown in a home garden was used to study time of anthesis; time taken to shedding of pollens; stigma receptivity; maximum nectar volume production; a measure of corolla opening with time; different stages of flower from bloom to cease. During the study number of pollinators visited was also investigated.

Fifty male and female flowers were observed and recorded for these studies. Anthers were observed under

the microscope to detect the opening and shedding time of pollens of *L. cylindrica* flower. Stigma was observed under the microscope to find out the receptivity.

Thirty six *Luffa* male flowers were covered by a net (2mm mesh size) separately in a plant before opening to prevent visiting of any pollinator in a day. On the following day after blooming the flower, nectar was collected from a flower with the help of micro-capillary tube in an hour interval from bloom (05:00 hr) to senesce (16:00 hr). The maximum volume of nectar were determined. Each flower stages were recorded with the help of series of photographs. Corolla length was measured with the help of scale from 03:30 hr to 16:00 hr. All the pollinators visited the flowers were recorded and collected between 03:30 hr to 16:00 hr on a day.

### Results and Discussion

The corolla width of fully opened male flower and female flower was ranged from 80-84 mm and 78-80 mm diameter, respectively. Fully opened flowers were yellow in colour. Further analysis of flowering phenology with age revealed that the stigma was receptive in two to three hours after flower opening. Rate of flower opening was at its peak around 03.45 hr to 04.15 hr. Anther opening was observed 2-2½ hours after flower opening.

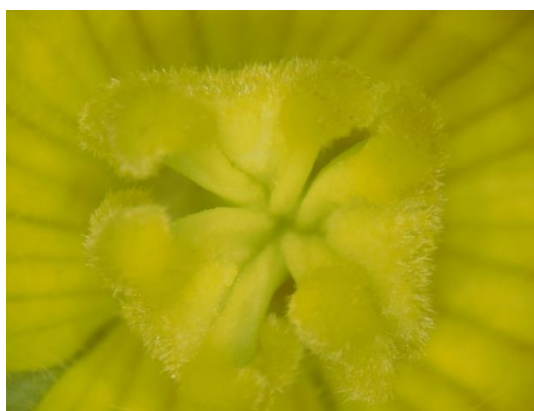


Plate 1: (A) Receptive stigma and (B) anther under 10×3.5 Stereo microscope

The nectar volume and nectar recovery appeared to be highest (2.8 ML) around 11.00 hr. and the lowest nectar volume (0) was measured in 04.00 hr and 17.00 hr.

Each flower stages were recorded. Plate 2: Flower stages with time showed that 1 – 9 stages were appeared between 03.00 hr and 09.00 hr. Stage 1 is

called as bud stage. And stage 9 was the fully opened flower. In stages 10, 11 and 12, flower was started to

senesce. Finally this flower was ceased at 16.00 hr (Stage 13)

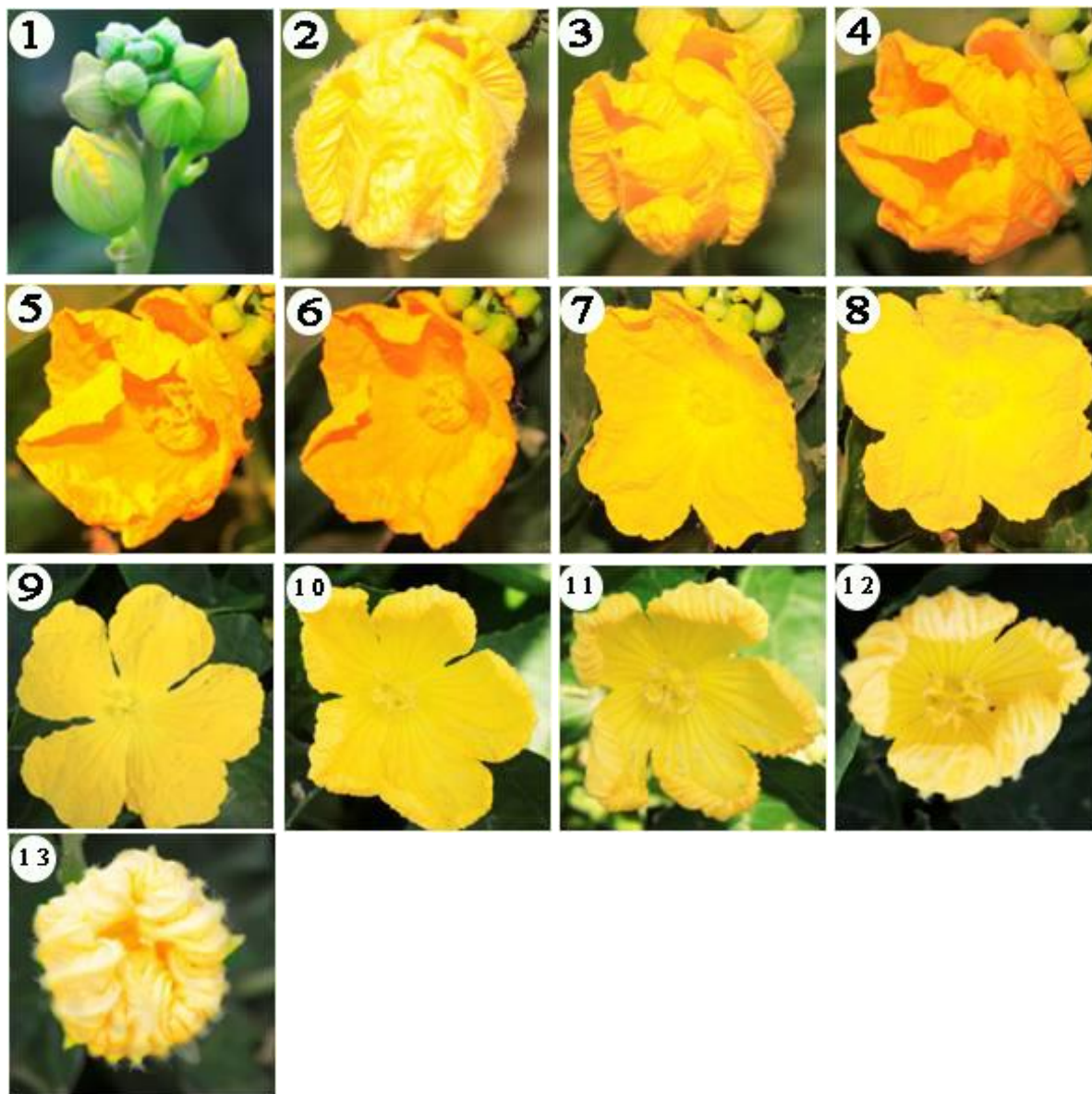


Plate 2: Flowering stages of *Luffa cylindrica* with time

The observation was during 15-25, July 2014 during the hours 03:00 to 16:00 for a total of 140 hours. (Throughout the study hours the day and night average temperature and relative humidity were 98°F, 79°F and 79.8 %.) Pollinators belonging to three orders were recorded on the *L. cylindrica* flower. These included ants and bees (Hymenoptera) and butterflies

(Lepidoptera). Ants were recorded as the most abundant floral visitors. There were nine bee species, three butterfly species and an ant recorded as floral visitors. Among those bee species, majority of bees belong to the family Apidae (66.67%). Three species of social bees, *Apis dorsata*, *A. cerana*, *A. florea* and *Trigona iridipennis* were visited. Six species of solitary bees were foraging on pollen collecting nectar of *Luffa*



flower at home gardens. *Amegilla* sp, *Amegilla cingulata*, *Ceratina binghami*, *Thyreus ramosellus* and *Lasioglossum vagans*, *Xylocopa fenestrata* were identified under solitary bees.

### Conclusion

From the observations of various flower stages, the maximum corolla width of male and female flowers was ranged from 80-84 mm and 78-80 mm diameter, respectively. The timing of anthesis was at the peak between 2-2½ hours after male flower opening and stigma was receptive in 2-2½ hours after female flower opening. Maximum nectar volume was 2.8 ML around 6hr after opening of flower. Nine bee species, three butterfly species and an ant were recorded as pollinators. Though *Luffa* is considered as an underutilized plant, it can be grown in the home gardens to preserve and protect the pollinators in the ecosystem.

### Acknowledgement

Wish to thank the farmers who supported for this studies and the NSF Food Security Project NTRP/2012/FS/PG05/P02 for their financial assistance.

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## Appendix

*Table 1: Corolla opening with time (Distance between two ends of petals) of Luffa flower*

Time	Diameter (cm)	Diameter (cm)	Diameter (cm)
3.30	0.0	0.0	0.0
3.40	0.1	0.2	0.2
3.50	0.3	0.4	0.4
4.00	0.6	0.7	0.7
4.10	0.8	0.9	0.9
4.20	1.0	1.3	1.2
4.30	1.6	1.8	1.7
4.40	1.9	2.1	2.0
4.50	2.5	2.8	2.7
5.00	2.8	3.0	2.9
5.10	4.1	4.5	4.3
5.20	5.0	5.1	5.1
5.30	5.8	6.0	5.9
5.40	6.4	6.6	6.5
5.50	6.9	7.0	7.0
6.00	7.0	7.1	7.1
7.00	8.0	8.2	8.1
8.00	8.0	8.2	8.1
9.00	8.1	8.4	8.3
10.00	8.0	8.2	8.1
11.00	6.1	6.2	6.2
12.00	4.9	5.2	5.1
13.00	4.5	4.9	4.7
14.00	4.0	4.4	4.2
15.00	1.5	2.0	1.8
16.00	1.0	1.0	1.0

*Table 2: Nectar total volume of Luffa flower*

Time	Day 1	Day 2	Day 3
4.00	0	0	0
5.00	2	2	3
6.00	8	7	8
7.00	13	12	10
8.00	17	22	19
9.00	21	25	23
10.00	24	26	26
11.00	25	27	31
12.00	24	23	28
13.00	14	13	19
14.00	11	12	15
15.00	11	11	11
16.00	4	8	6
17.00	0	0	0

# PRODUCTION, QUALITY ASSESSMENT AND SHELF LIFE EVALUATION OF PROTEIN – RICH BISCUITS MADE FROM BLENDS OF WHEAT AND DEFATTED COCONUT FLOURS

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## Abstract

Defatted coconut flour obtained from the whitish kernel residue left after the extraction of virgin coconut oil has potential application in high protein-fiber enriched food products. Therefore, a study was conducted to utilize coconut flour, a by-product of the Virgin Coconut Oil (VCO) industry for the partial substitution of wheat flour. In this study, wheat flour was substituted with defatted coconut flour in varying proportions of 0, 10, 20, 30, 40 and 50% w/w to prepare a series of blends for biscuit and the possibility of using coconut flour for the production of biscuits was investigate. Prepared biscuits were subjected to nutritional, physical, textural and organoleptic analysis to evaluate the suitability of biscuits for consumption. Nutritional analysis of coconut flour revealed that it contains 12.6% protein, 13.0% fiber, 9.2% fat, 13.7% sugar, 8.2% ash and 4.2% moisture. Protein, fiber and fat value of defatted coconut flour fortified biscuits increased with progressive increase in proportion of defatted coconut flour and 40% coconut flour added biscuits obtained values of 10.73%, 11.30% and 22.72% respectively, while the lowest values of 4.98%, 8.26% and 16.86% recorded for the wheat flour biscuits. The moisture and ash were increased with corresponding increase in the percentage of defatted coconut flour while showing the decrease in carbohydrate content. Defatted coconut flour incorporated cookies were found to be lesser harder than control cookies when tested for hardness with texture analyzer. 40% defatted coconut flour added biscuits scored the highest overall acceptable rating compared to other tested combinations and could be stored up to 5 months in aluminum package without significant changes in keeping quality.

Keywords: biscuit, defatted coconut flour, nutritional profile, sensory evaluation

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## Introduction

Utilisation of food by – products and wastes receive more attention in the food industry. These wastes would be minimized through the utilisation of available resources into various types of food products. Therefore, an effective effort was needed to solve those problems by developing high nutritional and industrial potential of by – products, wastes and utilised directly for human consumption.

Various attempts had been made for researching for the use of food by – products such as pomace of apple, citrus fruits, grape skin and seed, guava, mango and pineapple with a view to explore the potential

applications and physiological activities of that particular food by – products. The demand for by – products from fruits and vegetables increased due to their high content in nutritional quality, low in caloric content, strong in antioxidant capacity and high water retention capacity.

Virgin coconut oil is a recently emerging high demand product in the world. Defatted coconut flour is one of the major by – product generated from the virgin coconut oil industry. However, the defatted coconut flour is often discarded. The whitish residue remained after extracting virgin coconut oil can be milled into flour. The coconut flour can provide not only value added income to the country, but also a nutritious and

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healthy source of dietary fiber (Trinidad *et al.*, 2003). Coconut flour plays a role in controlling cholesterol and sugar levels in blood and prevention of colon cancer. Studies revealed that consumption of high fiber coconut flour increases faecal bulk (Arancon, 2009).

Biscuits, among the bakery products are the most significant snack foods in the world. These are an important food product used as snacks by children and adults (Hussain *et al.*, 2010). Biscuits differ from other baked products like bread and cakes because of their low moisture content, which ensures that they are free from microbial spoilage and confer a long shelf life on the product. Good eating quality makes the biscuits more attractive for fortification and other nutritional improvement.

The production of biscuits incorporated with defatted coconut flour led to the innovation of new product by utilising the by – products from coconut. Nowadays, people were aware about the consumption of healthier food in their daily life. The innovation was in coherent with consumers demand for a healthier choice of food product. Therefore, the present study was aimed to develop nutritionally enriched biscuits and to assess the nutritional, physical, microbial and organoleptic qualities in the formulated biscuits.

## Materials and Methods

### Raw materials

Refined wheat flour was purchased from Prima company (Pvt) Ltd, Trincomalee. Coconut gratings were defatted and powdered using 2 mm sieve. Wheat flour was mixed with defatted coconut flour and biscuits were prepared according to the following treatments using the recipe described below:

Treatments:

T<sub>1</sub> - 100% wheat flour (Control)

T<sub>2</sub> - 90% wheat flour + 10% de-fatted coconut flour

T<sub>3</sub> - 80% wheat flour + 20% de-fatted coconut flour

T<sub>4</sub> - 70% wheat flour + 30% de-fatted coconut flour

T<sub>5</sub> - 60% wheat flour + 40% de-fatted coconut flour

T<sub>6</sub> - 50% wheat flour + 50% de-fatted coconut flour

### Development of nutritionally enriched biscuits

The biscuits were prepared by using a Creamery method. Biscuits were made at the incorporation of defatted coconut flour with the replacement of refined wheat flour at the level of 10, 20, 30, 40 and 50% in the standardized formulations. 50 g low fat margarine and 50 g powdered sugar were creamed together by electric beater. All purpose wheat flour (250 g) and baking powder (5 g) were sieved twice together. The sieved flour was added to the creamed paste. As per the treatment, firm dough was prepared from all mixture. The dough was rolled out to 2.5 mm thickness in a baking tray and cut into round shape having 5 cm diameter with a biscuit cutter. The biscuits were placed in greased aluminium trays and baked in a pre – heated oven at 185 °C for 15 minutes. These biscuits were assessed for nutritional, physical, microbial and organoleptic qualities.

### Nutritional analysis

The moisture, ash, protein, fiber and fat of the biscuits were determined according to the standard AOAC (2000) methods. The carbohydrate content was determined by calculating the difference. Data were analysed by Analysis of Variance (ANOVA) and the difference between means was compared using Duncan's Multiple Range Test (DMRT), through Statistical Analysis System (SAS) software statistical package.

### Physical property

Biscuits were cooled for one hour for the determination of spread ratio as per the method described in AACC (2000). Six biscuits were taken randomly and placed them edge to edge and stacking for the estimation of diameter and thickness and finally by the subtraction of their average value spread ratios were obtained.

### ***Textural Property***

Texture attributes of biscuits like hardness and breaking strength were taken as quality parameters to check the textural properties of biscuits by using TAHD Texture analyser. Hardness and breaking strength were determined as mentioned by Singh (2003). The probe was calibrated and then a test was run by placing sample on the platform of the texture analyser. Hardness was measured using texture analyser as maximum peak force (N) required to break the sample. Breaking strength was also measured by using the same texture analyser.

### ***Sensory analysis***

The sensory attributes including taste, texture, colour, flavour and overall acceptability were evaluate by a trained 30 – member panel. The evaluation was held either 10 am for the morning session and at 3 pm for the afternoon session. The Seven – point hedonic scale was used to evaluate the degree of liking (7) and disliking (1) for preference of the biscuits. The mean scores were analysed using analysis of variance (ANOVA) method and difference separated using Fredmann Test.

### ***Microbial analysis***

The aerobic plate count was carried out using the method of Fawole and Oso, (1998). 10 g of each sample was taken aseptically and homogenized in 90 ml sterile distilled water using a blender (Philips Type HR 2815i) for 2 min. Serial dilutions (using 1 ml of homogenates)

were made in 9 ml sterile distilled water, dispensed in test tubes. One millilitre of each dilution was pour plated in sterile Petri dishes, using the plate count agar (PCA, oxoid), incubated at 37°C for 24 - 36 h. Counts of visible colonies were made and expressed as log CFU/g sample.

## **Results and Discussion**

### ***Composition of defatted coconut flour***

The nutritional composition of the coconut flour were moisture 4.2%, fat 9.2%, protein 12.6%, total sugar 13.7%, ash 8.2%, fiber 13.0% and soluble carbohydrate 39.1%. The values are in accordance with Marquez (1999). Composition of coconut flour depends on the retention components after the extraction of coconut oil from scraped coconut.

### ***Nutritional composition of wheat – defatted coconut flour biscuits***

The nutritional analysis of prepared biscuits indicated that all the samples contained favourable proportions of protein, fiber and fat.

### ***Protein content***

In this study, the partial substitution of wheat flour by defatted coconut flour increased the protein content of biscuits. The protein increased from 4.98 to 11.96% with the increase in the percentage of defatted coconut flour from 0 to 50%. Figure .1 shows the changes in protein and fiber content of developed biscuits.

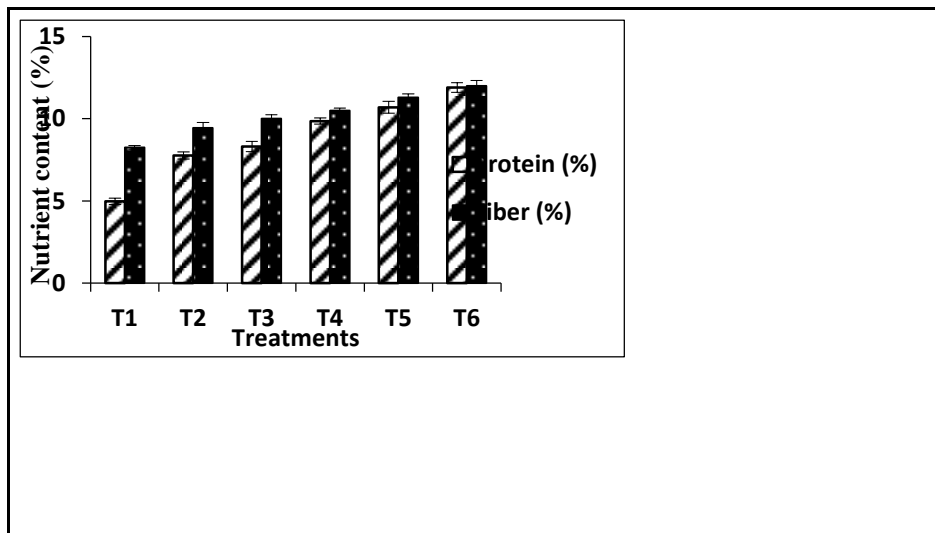


Figure.1: Changes in Protein and Fiber Content of Freshly Made Wheat- Defatted Coconut Flour Biscuits

The values are means of triplicates. The vertical bars indicate the standard errors

According to Bressani and Wilson (2012), cereal protein, including wheat, is limited in the essential amino acid lysine content and they also recommended that the biological value of wheat flour can be significantly improved by the addition of lysine. Coconut flour when incorporated into wheat flour increases the amino acid content, especially lysine. Therefore, incorporation of coconut flour into wheat flour improves the protein content of composite flour and thus improves the nutritional status of the biscuits.

According to DMRT, control biscuits made from 100% wheat flour has the lowest protein content of 4.98% whereas 50% defatted coconut flour added biscuits has the highest protein content of 11.96%.

#### **Fiber content**

Fiber content of biscuits ranged from 8.26 to 12.0% when the defatted coconut flour was incorporated from 0 to 50%. The increment in fiber content could be due to the increase in the proportion of defatted coconut flour in the composite flour blend. Figure 4.1 shows the changes in fiber content of the developed biscuits. Fibers in biscuits is mainly contributed by coconut fiber and wheat flour contains only 2.7% dietary fiber (Leelavathi and Rao, 1993). As fiber absorbs large

amount of water, it gives a sensation of fullness (having an appetite completely satisfied). Therefore, coconut fiber supplemented biscuits can be prepared with high fiber content.

According to DMRT, T<sub>6</sub> which was containing 50% defatted coconut flour has the highest fiber content of 12.0% and control biscuit made from 100% wheat flour has the lowest fiber content of 8.26%. There was no significant different between 20% and 30% defatted coconut flour added biscuits.

#### **Fat content**

The fat content of biscuits increased with an increase in the substitution level of defatted coconut flour. The fat content of biscuits increased from 16.8 to 24.5% with increase in the percentage (0-50%) of defatted coconut flour as shown in Figure.2 The refined wheat flour was having lower fat content whereas there was a slight increase in fat content with increase in defatted coconut flour incorporation. This may be due to the fat found in defatted coconut flour. Yalagama *et al.* (2013) reported that, fat, which is attached to the fibers, remains with cell wall components resulting in high fat content in coconut flour.

According to DMRT, biscuits made from 100% wheat flour has the lowest fat content of 16.8% and 50% defatted coconut flour added biscuits has the highest fat content of 24.5%. There is no significant difference

between T<sub>3</sub> (20% defatted coconut flour added biscuit) and T<sub>4</sub> (30% defatted coconut flour added biscuit) at 5% significant level.

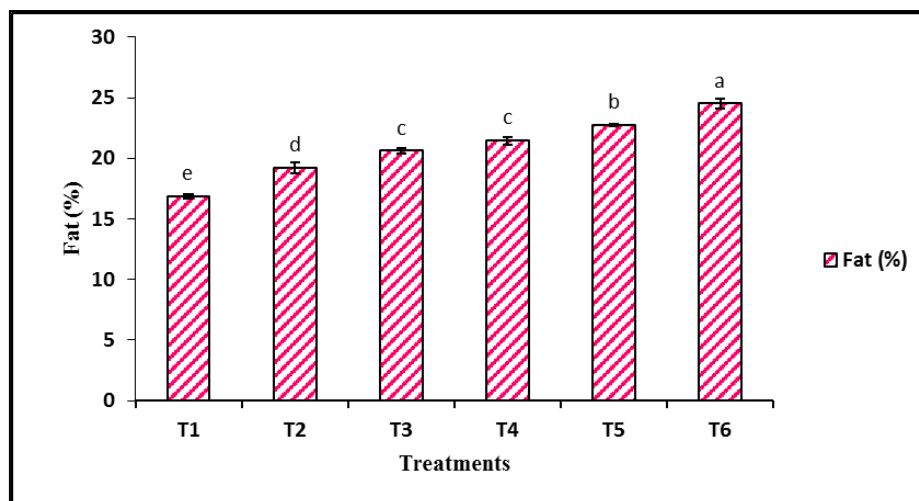


Figure.2: Changes in Fat Content of Freshly Made Wheat-Defatted Coconut Flour Biscuits

The values are means of triplicates. The vertical bars indicate the standard errors

### Moisture content

The moisture content of biscuits gradually increased from 4.33 to 5.92% with the incremental addition of

defatted coconut flour from 0 to 50%. Changes in moisture, ash and total soluble carbohydrate content of freshly made wheat-defatted coconut flour biscuits are shown in Table.1

Table.1: Moisture, Ash and Total Soluble Carbohydrate Content of Freshly Made Wheat- Defatted Coconut Flour Biscuits

Treatment	Moisture (%)	Ash (%)	Total Soluble Carbohydrates (%)
T <sub>1</sub>	4.33 ± 0.012 <sup>e</sup>	0.35 ± 0.08 <sup>d</sup>	65.71 ± 0.43 <sup>a</sup>
T <sub>2</sub>	4.39 ± 0.013 <sup>d</sup>	0.51 ± 0.014 <sup>c</sup>	59.29 ± 0.16 <sup>b</sup>
T <sub>3</sub>	4.48 ± 0.003 <sup>c</sup>	0.78 ± 0.04 <sup>c</sup>	56.30 ± 0.27 <sup>b</sup>
T <sub>4</sub>	5.49 ± 0.020 <sup>b</sup>	1.08 ± 0.012 <sup>b</sup>	52.37 ± 0.33 <sup>c</sup>
T <sub>5</sub>	5.79 ± 0.017 <sup>b</sup>	1.28 ± 0.023 <sup>b</sup>	48.96 ± 0.48 <sup>d</sup>
T <sub>6</sub>	5.92 ± 0.04 <sup>a</sup>	1.48 ± 0.032 <sup>a</sup>	44.71 ± 0.29 <sup>e</sup>

The values are means of triplicates ± SE.

Mean values with the same superscript letters within the same column do not differ significantly at 5% level

These values were within the range reported to have no adverse effect on the quality attributes of the product

(Kaur *et al.*, 1996). The increase in moisture content can be attributed to the increased protein content that

also increase the water binding capacity of biscuits with high levels of defatted coconut flour. Singthong *et al.* (2011) observed similar increase in moisture content with increasing levels of coconut flour.

According to DMRT, 50% defatted coconut flour added biscuits has the highest moisture content of 5.92% and 100% wheat flour added biscuit (control) has the lowest moisture content of 4.33%.

#### **Ash content**

The ash content of a food material could be used as an index of mineral constituents of the food (Sidorova *et al.*, 2007). The ash content of biscuits increased from 0.35 to 1.48% with increase in the percentage of defatted coconut flour from 0 to 50%. Changes in ash content of freshly made wheat-defatted coconut flour biscuits are shown in Table.1

The increasing trend observed in ash content might be due to the fact that defatted coconut flour contained higher amounts of materials compared to wheat flour. Srivastava *et al.* (2010) also reported that an increase in moisture and ash values with increasing percentages of defatted coconut flour substitution in wheat flour biscuits. According to the DMRT, T<sub>6</sub> which was containing 50% defatted coconut flour has the highest ash content.

#### **Total soluble carbohydrate**

The results showed that soluble carbohydrate content decreased from 65.7 to 44.7% with increase in the percentage (0-50%) of defatted coconut flour as shown

in Table.1 The variations in soluble carbohydrate among the biscuit samples may result from the difference in the level of protein, fat, ash and moisture content of wheat flour and defatted coconut flour.

According to DMRT, the control treatment (T<sub>1</sub>) has the highest total soluble carbohydrate of 65.7% and biscuit which contains 50% defatted coconut flour has the lowest soluble carbohydrate content of 44.7%. According to DMRT, the control treatment (T<sub>1</sub>) has the highest total soluble carbohydrate of 65.7% and biscuit which contains 50% defatted coconut flour has the lowest soluble carbohydrate content of 44.7%.

#### **Physical Analysis**

An increase in diameter was observed after baking of biscuits. The increase in diameter can also be attributed to the fat content since the defatted coconut flour also has a certain amount of fat that contributes to the total fat content of biscuits. As the fat increases an increase in expansion of biscuits was observed that increase in diameter. Thickness of the biscuits showed gradual decrease as the level of defatted coconut flour replacement. The reduction in thickness as the level of coconut flour increased which may be contributed to enhanced hydration capacity of flour after fortification. Figure.3 shows the changes in thickness and diameter of different biscuit samples with different treatments.



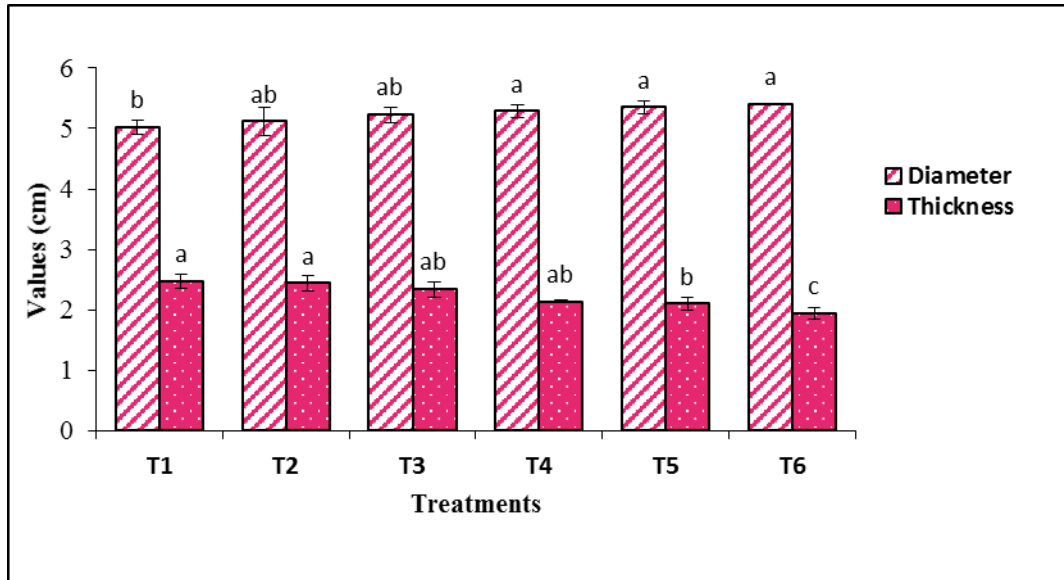


Figure.3: Thickness and Diameter of Biscuits Made with Different levels of Defatted Coconut Flour

The values are means of triplicates. The vertical bars indicate the standard errors

### Textural Analysis

Textural characteristics of biscuits containing wheat-defatted coconut flour biscuits is shown in Table.2 Biscuits made up with 100% wheat flour has the highest mean score for hardness (10.9 N) while the 50% defatted coconut flour incorporated biscuits gained the lowest score of 6.22 N. Decreasing trend in hardness on

addition of defatted coconut flour was also observed by Srivastava *et al.* (2010).

A declining trend was observed for breaking strength with increase in the level of defatted coconut flour. The values for breaking strength were ranging from 68.89 to 16.54 N. At high fat content, the lubricating function is high, thus less water is required and a softer texture is obtained (Yadav and Subramanyan, 2012).

Table 2: Textural Characteristics of Biscuits Containing Wheat-Defatted Coconut Flour Biscuits

Treatment	Hardness (N)	Breaking strength (N-mm)
T <sub>1</sub>	10.90 ± 0.21 <sup>a</sup>	68.89 ± 0.01 <sup>a</sup>
T <sub>2</sub>	9.98 ± 0.31 <sup>a</sup>	69.09 ± 0.19 <sup>a</sup>
T <sub>3</sub>	9.38 ± 0.17 <sup>a</sup>	63.32 ± 0.17 <sup>b</sup>
T <sub>4</sub>	9.49 ± 0.27 <sup>a</sup>	57.77 ± 0.34 <sup>c</sup>
T <sub>5</sub>	7.21 ± 0.13 <sup>b</sup>	31.84 ± 0.17 <sup>d</sup>
T <sub>6</sub>	6.22 ± 0.23 <sup>b</sup>	16.54 ± 0.27 <sup>e</sup>

Mean values with the same superscript letters within the same column do not differ significantly at 5% significant level. Values are means of triplicates.

Hence the hardness and breaking strength gradually decreased forming a softer biscuits with an increased level of defatted coconut flour biscuits. This shows that incorporation of defatted coconut flour has positive effect on textural properties.

### Sensory Analysis

The products developed were assessed for sensory evaluation and was compared with control biscuits. The results proved the worth of study and were found satisfactory with high value of overall acceptability. Data regarding the organoleptic evaluation is presented in figure.4

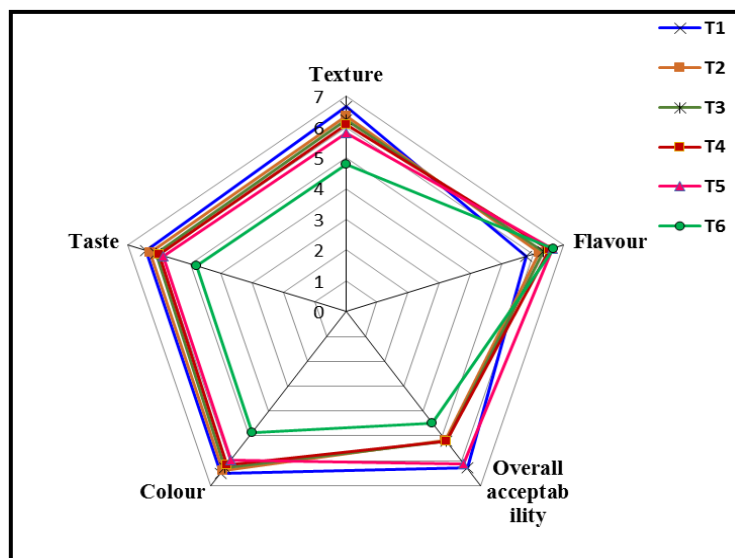


Figure.4: Sensory Properties of Biscuits Incorporated With Defatted Coconut Flour

The colour gets darker and when defatted coconut flour is further increased; more darkness takes place, which results in the reduction of quality score for the colour of the biscuits. As amino acids react with reducing sugars during baking and as a result Maillard reaction takes place. This was supported by Dhingra (2000).

More darkness in the colour was observed in the biscuits as the level of the supplementation of defatted coconut flour was increased. This may be due to the browning and caramelization of sugar present in coconut flour during baking. This decreasing trend of quality score for colour of the biscuits may be due to the high level of protein and sugar present in coconut flour.

The score for taste decreased from 6.17 to 4.80 on increasing the level of substitution of defatted coconut flour. The significant decreasing trend of taste may be due to the own taste of coconut flour which dominated

when used in high amount (Hussain, 2000). The control biscuits made with 100% wheat flour (T<sub>1</sub>) has the highest mean score of 6.17 for taste whereas 50% coconut flour added biscuit (T<sub>6</sub>) has the lowest score of 4.80.

The crust texture of biscuits was related to the external appearance of the biscuit top, which is the smoothness or roughness of the crust. Biscuit's texture analysis revealed that there was a significant effect on the texture of the biscuits when coconut flour was added. Decreasing trend was observed in the quality score for texture when the percentage of defatted coconut flour increased. The decreasing trend for texture of the biscuits may be due to the proteins present in coconut flour.

Flavour of biscuits increased from 5.81 to 6.66 with increasing in the substitution level of coconut flour. Quality score of the biscuits revealed that flavour of

biscuits varied significantly among the treatments. The results indicated that the biscuits prepared from T<sub>6</sub> significantly ( $p > 0.05$ ) got the highest score (6.66) for flavour. This is due to the flavour of coconut flour.

Overall acceptability includes many implications, which is the important parameter in sensory estimation. There was significant difference between control treatment (T<sub>1</sub>) and 40% coconut flour added biscuits (T<sub>5</sub>). Biscuits made with 40% defatted coconut flour added biscuits (T<sub>5</sub>) has the highest mean value for overall acceptability according to DMRT while 50% defatted coconut flour added biscuits (T<sub>6</sub>) has the lowest score.

### Conclusions

The developed biscuits showed good quality characteristics on all parameters considered. The nutritional analysis of defatted coconut flour revealed that it contains 12.6% protein, 9.2% fat, 13.0% fiber and 4.2% moisture. The addition of defatted coconut flour resulted in significant improvement in protein and fiber in wheat – defatted coconut flour biscuits.

From this study, it can be concluded that biscuits incorporated with 40% addition of defatted coconut flour were nutritionally rich and scored high sensory attributes than the other tested treatments. Biscuits substituted with 40% defatted coconut flour has 10.52% protein, 22.68% fat and 11.01% fiber. Textural property of biscuits clearly showed a decreasing trend in hardness and breaking strength. Hardness and breaking strength of the selected biscuit (40% defatted coconut flour biscuit) were 7.21 N and 31.84 Nmm respectively. Biscuit substituted with 40% defatted coconut flour has the diameter of 5.35 cm whereas its thickness is 2.10 cm.

From the overall acceptance rating, the 40% defatted coconut flour added biscuits has the highest mean value and there is no remarkable changes in organoleptic characters were observed up to 5 months of storage in ambient condition of average temperature 30°C and the RH of 75 – 80% indicating that the shelf life of 40%

defatted coconut flour added biscuits were shelf life stable up to 5 months.

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# BIOSURFACTANT PROCESS SYNTHESIS AND STABILIZATION OF SILVER NANOPARTICLES FOR MODIFIED PRESERVATION METHODS ON COMMON FERMENTED FOODS

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## Abstract

A biosurfactant produced by *Pseudomonas aeruginosa* PBSC1 cultivated in a low-cost Cashew Apple Juice medium was employed to synthesize and stabilize silver nanoparticles in the liquid phase. The particles were initially synthesized using NaBH<sub>4</sub> as reducing agent in biosurfactant reverse micelles and were extracted from the micellar solution to disperse in heptane. A silver particle size in the range of 11 nm was observed. The UV-vis absorption spectra proposed that silver nanoparticles could be formed in the reverse micelles and relatively stabilized for at least 3 months without passivator addition. The Transmission Electron Microscope (TEM) shows that the silver nanoparticles are of spherical form and relatively uniform. This method provided a simpler way for nanoparticle synthesis compared to existing systems using whole organisms or partially purified biological extracts, showing that the low-cost biosurfactant can be used for nanoparticle synthesis as a non-toxic and biodegradable stabilizing agent. Thus synthesized silver nano particles along with bacteriocin found to be very effective antimicrobial agent against food spoiling organisms such as *Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Pediococcus* and *Escherichia coli*. Antimicrobial activity of the silver nano particles and bacteriocin combination made a modification in the preservation methods in fermented foods such as pickled cucumbers, pickled beets and Sauerkraut. This study proved effective control and preservation of the selected common fermented foods. This modified method in food preservation not only improves the quality of fermented foods but also satisfy consumers.

Keywords: *Pseudomonas aeruginosa*, Cashew Apple Juice, Rhamnolipid, Silver nanoparticles, bacteriocin, Food preservation.

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## Introduction

Biosurfactants are surface-active secondary metabolites produced on living surfaces, mostly on microbial cell surfaces or excreted extracellular in the growth medium. Contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively. They hold numerous advantages compared to their chemical synthesized counterparts, for example, they can be

produced from renewable resources and are alive under extreme conditions pH and temperature and highly biodegradable [1, 7, 13].

The area of the biosurfactant mediated process of nanoparticle synthesis is emerging as part of green chemistry and they act as a potent stabilizer in the silver nanoparticles synthesis [9]. Currently, many techniques have been devoted to synthesizing nanosize silver particles, such as chemical reduction [15],

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photochemical reduction, reverse micelle based and lamellar liquid crystals approaches [10], aerosol techniques and an electrostatic spraying technique. Since reverse micelles system was used to form metal nanoparticles by Boutonnet *et al.* [2], these methods have been paid more and more attention. The antimicrobial property of the silver nanoparticles have been studied extensively and the used in the control of many potential pathogens that cause food spoilage.

In the present study the isolation of potent biosurfactant producers from mangrove ecosystem and extraction, purification and characterize biosurfactant was studied. The optimization of biosurfactant production using Cashew Apple Juice medium through Response Surface Methodology (RSM) and to synthesize and stabilize the silver nanoparticles using biosurfactants, exploring antimicrobial activity against food spoiling organisms for developing modified preservation methods for common fermented foods.

## Materials and Methods

### Microorganisms

*Pseudomonas aeruginosa* PBSC1 was isolated from Mangrove soil sediments after screening using drop collapse assay, haemolytic assay, oil displacement test and CTAB agar plate method. The isolate PBSC1 was identified by the 16S r RNA sequence as *Pseudomonas aeruginosa* PBSC1 and deposited under the accession number KJ920194 in the GenBank.

### Fermentation media

The sterilized MSM broth was added with various concentrations of cashew apple juice (2, 4, 6 and 8 per cent). To prove the effect of cashew apple juice on the biosurfactant production, cashew apple juice was used as such without inorganic mineral salts. For comparison, defined medium (MSM with 2 per cent glucose) was included in the study. To that 5.0 ml inoculum of *Pseudomonas aeruginosa* PBSC1 were inoculated to the respective flasks and incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ) for 5 days over an Orbital rotary shaker set at  $120 \text{ rpm min}^{-1}$ . To find out the

variation in the biosurfactant production, the reduction in surface tension was made using 20 ml of cell free culture broth recorded at zero and at 120 h in a duNouy Tensiometer (Kruss Digital-Tensiometer, Germany) at room temperature ( $28 \pm 2^\circ\text{C}$ ).

### Response Surface Methodology (RSM) for the optimization of biosurfactant production from *P. aeruginosa* PBSC1 using Cashew Apple Juice

To examine the interaction effect of different selected parameters (glycerol, sodium nitrate, pH and temperature) on biosurfactant production by *P. aeruginosa* PBSC1, the Central Composite Design (CCD), with 30 experiments were performed in duplicate. The reduction in surface tension of the medium is the direct measure of the biosurfactant production. Hence, the value of the dependent response surface tension reduction was the mean of two replications. The reduction in surface tension was determined. The second-order polynomial coefficients were calculated and analyzed using Design Expert software (version 8.0.7.1, Stat-Ease Inc., USA), Central composite design was conducted in the optimum vicinity to locate the true optimum conditions of Glycerol (A), sodium nitrate (B), pH (C), Temperature (D) for the production of biosurfactant (Table 1). For the four factors, this trial was a  $2^{5-1}$  factorial design augmented by six axial points (or called star points) coded  $\alpha \pm 2$  and two replicates of center points (all factors at level 0), resulting in a total number of 30 experiments.

Response surfaces were drawn to determine the individual and interactive effects of the test variable on the reduction in the surface tension of the medium. The optimal values of the test variables were first obtained in coded units and then converted to the uncoded units. The quality fit of the model equation was expressed with the coefficient of determination  $R^2$  and its statistical significance was determined by an F-test. The significance of the regression coefficients was tested by a t-test.

### ***Extraction of Biosurfactant***

The biosurfactant was extracted from the culture medium after cell removal by centrifugation at 5000g for 30 min. The supernatant pH was adjusted to 2.0 with 6.0 M HCl and an equal volume of CHCl<sub>3</sub>/CH<sub>3</sub>OH (2:1) was added. The mixture was vigorously shaken for 15 min and allowed to set until phase separation. The organic phase was removed and the operation was repeated twice. The product was concentrated from the pooled organic phases using a rotary evaporator. The viscous yellowish product obtained was dissolved in methanol and concentrated again by evaporation of the solvent at 45° C.

### ***Synthesis of Silver nanoparticles [16]***

For the synthesis of silver nanoparticles in situ in the water-in-oil microemulsion phase, a 0.05 mol/l aqueous AgNO<sub>3</sub> solution and a 0.1 mol/l aqueous NaBH<sub>4</sub> solution were separately used instead of water to form reverse micelles with the biosurfactant. NaBH<sub>4</sub> was used here to act as reducing agent. The first synthesis involved mixing 1.0 ml of 0.05 mol/l aqueous AgNO<sub>3</sub> solution, 0.1 g/l biosurfactant and 25 ml n-heptane together and stirred vigorously at room temperature until homogeneous reverse micelles formed and the same bulk of 0.1 mol/l aqueous NaBH<sub>4</sub> solution was used to replace aqueous AgNO<sub>3</sub> to form the other reverse micelles. The two samples were mixed under stirring for 60 min. Then, the particles were precipitated from the solution and isolated by centrifugation at 14,000 × g. Then, 0.5 ml ethanol was added for each 1 ml reverse micelles. Ethanol was added to the complete removal of the surfactant and n-heptane. The prepared silver nanoparticles could be readily redispersed to obtain a suspension in 10 ml n-butanol aided by sonication. The second microemulsion was prepared by dissolving 0.1 g/l of the biosurfactant in 6.25 ml of n-heptane and 1 ml AgNO<sub>3</sub>. Solution was added to the mixture with continuous stirring for 10 min at room temperature. Then, 1 ml NaBH<sub>4</sub> was added to the mixture which was agitated for 30 min. After agitation, 10 ml ethanol was added to break the reverse micelles, thus forming two phases. The precipitate was separated by centrifugation at a speed of 14,000 × g for 30 min

and 10 ml of n-butanol was added to obtain a suspension.

### ***Characterization of silver nanoparticles***

#### ***UV-Visible Spectroscopy***

The optical characterizations of the synthesized silver nanoparticles were analyzed through absorption spectra measured in room temperature in a UV Visible absorption spectrometer (ELICO SL 244) at the wavelength of 200 to 800 nm under dispersion mode.

#### ***Transmission Electron Microscopy (TEM)***

Transmission electron microscopy (TEM) is an imaging technique whereby a beam of electrons is focused onto a specimen causing an enlarged version to appear on a fluorescent screen or layer of photographic film, or to be detected by a CCD camera. The microstructure studied by use of the image mode. In our study the synthesized silver nanoparticles were lyophilized and dispersed in 100 per cent absolute ethanol. The ethanol dispersed particles were then sonicated to deposit on a copper grid. That was analyzed in Transmission Electron Microscope (JEM-2100F LaB<sub>6</sub>, USA) under 100,000 X magnification.

### ***Antimicrobial activity of silver nanoparticles against food pathogens***

The antimicrobial activity of the silver nanoparticles against food pathogens was studied using well cutting method. Two hundred and fifty ml of Muller Hinton agar plates was prepared and sterilized in an autoclave for 15 min at 121°C and the plates were swabbed with food pathogens viz., *Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*, *Pedococcus* sp. and *Escherichia coli* respectively and wells were made with steel cork borer (1 cm in diameter). The silver nanoparticles with various concentrations (10, 20, 30, 40, 50 µl), Bacteriocin obtained from *Lactobacillus plantarum* @ 1000 ppm, Bacteriocin obtained from *Lactobacillus plantarum* @ 1000 ppm combined with silver nanoparticles @ highest concentration was prepared and poured in the well and the plates were

incubated for 24 h at  $37 \pm 2^\circ\text{C}$  for bacteria and for 48 h at  $25 \pm 2^\circ\text{C}$  for yeast respectively. After the incubation period the diameter of the zone of inhibition was measured [3]. To ensure that, the results were reproducible, the average of five independent measurements was taken.

### Statistical analysis

This data was analyzed by the analysis of variance (ANOVA) technique to find out which factors had the most effective interactions for higher biosurfactant production [14].

### Results and Discussion

The biosurfactant from *P. aeruginosa* PBSC1 cultivated in a low-cost medium Cashew apple juice was produced during 120 h at  $37^\circ\text{C}$ . The medium surface tension was reduced to 31.1 mN/m at the end of fermentation and the isolated biosurfactant corresponded to a concentration of 12.54 g/l. The extraction using acidification with equal volume of Methanol: Chloroform showed highest emulsification activity (70.4 per cent) for the extracted aliquots of *P. aeruginosa* PBSC1. The brown coloured precipitate was observed after incubation, collected by centrifugation and pellet was resuspended with the solvent and stored at  $4^\circ\text{C}$ .

The experimental and predicted values of surface tension reduction of the media were represented in Figure 1 and Table 2. The statistical significance of the model equation was calculated by F-test for analysis of variance (ANOVA), which indicates that the regression was strongly significant at 99 per cent ( $P < 0.05$ ) confidence level (Table 3).

Biosurfactants have numerous advantages compared to chemically synthesized surfactants, but on the other hand, they have high production costs due to low yields and fastidious purification. In the present study, an attempt has been made to develop the biosurfactant production process economically attractive by using cheap renewable substrates from agro-industrial waste, optimized and efficient bio-processes for obtaining maximum productivity. An attempt was made to

synthesize silver nanoparticles in water-in-oil microemulsion stabilized by low cost biosurfactant synthesized using cheap renewable substrates [12, 1].

The optical absorption spectrum of the silver nanoparticles synthesis using biosurfactant from *P. aeruginosa* PBSC1 was shown in the Figure 2. From the figure, the optical absorption of the silver nanoparticles possess narrow band edge (432 nm), which originated from the uniform sized particle distribution of the sample. The absorption edge was shifted to the lower wavelength region confirmed nano-sized formation of the final product, which was caused by the quantum confinement effect. The optical band gap of the material was calculated using effective mass approximation was found to be 3.7 eV (432 nm). Light below this wavelength holds sufficient energy to excite electrons and hence absorbed by silver nitrate. On the other hand, light with longer wavelength which was higher than the band gap energy (towards the visible light) will not be absorbed.

UV-visible absorption spectrum of silver nanoparticles in n-heptane. A strong absorption peak at approximately 406 nm originates from the surface plasmon absorption of nanosized silver particles. Similar results were recorded in our study with the absorption spectrum of 432 nm for the SNPs synthesized using biosurfactant from PBSC1. Nano-scale silver can be synthesized in reverse micelles using glycolipid as stabilizer [9, 6, 17]

HR-TEM analysis was carried out on the silver Nano particles to observe the individual size and shape of it. HR-TEM micrograph of samples synthesized was shown in Figure 3a. A large number of smaller particles were distributed on the films in the size range of 15-32 nm of silver nanoparticles from PBSC1. This indicates that the distribution of silver nanoparticles stabilized by the biosurfactant was rather uniform. The typical TEM micrographs of the silver nanoparticles [10, 11] were obtained in this study. However, some larger particles on the films are observed. Two possibilities are there, one is that the nanometer-sized water layers limit the packing of the particles in the direction perpendicular to the water layers when the particles are growing in reverse micelles, the absorption of surfactant molecules

cannot totally prevent particles from aggregating and the thickness of the water layers cannot absolutely restrict the particle size due to the flexibility of the surfactant bilayers [10]. The other is that during the extraction and redispersion process a part of particles impact each other and aggregation.

The Selected Area Electron Diffraction (SAED) analysis showed those continuous ring patterns which originate from polycrystalline state or by the more crystallites attached to the surface of the single particles (Figure 3b). Bright ring pattern showed the high density of crystallites in the materials in the silver nanoparticle samples (PBSC1).

The silver nanoparticles synthesized using biosurfactant from *P. aeruginosa* PBSC1 after a day showed a relatively intense absorption peak around 432nm UV spectroscopy. On increasing the time from 1, 30 to 60 days the Plasmon absorption bands of three samples are quite similar for silver nanoparticles synthesized using biosurfactant from PBSC1 (Figure 4). No obvious changes in the position and symmetry of the absorption peak except for the decrease of the absorbance, indicating a little aggregation of silver nanoparticles. During the entire chemistry process, no passivator was added into the system. It proves that the silver nanoparticles solution prepared in such proportional reverse micelles can remain relatively stable for at least 2 months. The remnant rhamnolipid and lipopeptide in the solution was regarded as the stabilizer, which form a steric hindrance around the particles to preventing them aggregation greatly by electrostatic interactions. Xie *et al.* [16] reported that on increasing the time from 1 to 60 days, the Plasmon absorption bands are quite similar. The silver nanoparticles solution prepared in reverse micelles can remain relatively stable for at least 2 months. Kiran *et al.* [8] reported that the glycolipid biosurfactant produced from sponge-associated marine *Brevibacterium casei* MSA19 synthesized silver nanoparticles were uniform and stable for 2 months. Farias *et al.* [5] studied that the silver nanoparticles solution prepared in such proportional reverse micelles can remain relatively stable for at least three months. Similar results were obtained in the present study that the silver nanoparticles were stable for 2 months in the solution, hence it was proved that the biosurfactant act

as a stabilizing agent and prevented the formation of aggregates.

In the antimicrobial activity the concentration of 50  $\mu$ l was effective against all the tested food pathogens. The *S. aureus* growth was highly controlled by the silver nanoparticles. The results were found similar with the bacteriocin obtained from *Lactobacillus plantarum* @ 1000 ppm. The bacteriocin obtained from *Lactobacillus plantarum* @ 1000 ppm combined with silver nanoparticles @ 50  $\mu$ l concentration found very effective against all test specimens (Figure 5, 6).

## Conclusion

The present work demonstrates a simple eco-friendly method for synthesizing spherical silver nanoparticles by microemulsion technique. Silver nanoparticles were successfully synthesized using the biosurfactant from *P. aeruginosa* PBSC1. The synthesized nanoparticles were found to be spherical in shape with uniform distribution. The experimental observation was supported by UV spectroscopy and TEM analysis. The silver nanoparticles can be stabilized correspondingly for at least 3 months without passivator addition. The use of low-cost, renewable and biodegradable biosurfactants in replacement to toxic synthetic surfactants is a promising alternative for the synthesis of inorganic nanoparticles for industrial application. Antimicrobial activity of the silver nanoparticles and bacteriocin combination made a modification in the preservation methods in fermented foods such as pickled cucumbers, pickled beets and Sauerkraut. This study proved effective control and preservation of the selected common fermented foods. This modified method in food preservation not only improves the quality of fermented foods but also satisfy consumers.



Table - 1 Level of different process variables in coded and un-coded form for the reduction of surface tension (*P. aeruginosa* PBSC1)

Variables	Codes	Levels				
		-2	-1	0	+1	+2
Glycerol (g/l)	A	1.5	2	2.5	3	3.5
Sodium Nitrate (g/l)	B	1.5	3	4.5	6	7.5
pH	C	6	6.5	7	7.5	8
Temperature (°C)	D	20	25	30	35	40

Table - 2 Experimental conditions of 2<sup>4</sup> central composition design showing experimental and predicted surface tension reduction

Std	Run order	Glycerol (g/l)	Sodium Nitrate (g/l)	pH	Temperature (°C)	Experimental values *ST (mN/m)
6	1	1	-1	1	-1	40.2
4	2	1	1	-1	-1	40.4
27	3	0	0	0	0	31.3
18	4	2	0	0	0	38.8
13	5	-1	-1	1	1	38.9
22	6	0	0	2	0	38.1
24	7	0	0	0	2	36.7
7	8	-1	1	1	-1	39.8
20	9	0	2	0	0	38.7
23	10	0	0	0	-2	40.1
17	11	-2	0	0	0	38.6
10	12	1	-1	-1	1	38.9
21	13	0	0	-2	0	38.5
30	14	0	0	0	0	31.1
19	15	0	-2	0	0	40.1
1	16	-1	-1	-1	-1	39.7
25	17	0	0	0	0	31.3
9	18	-1	-1	-1	1	39.2
26	19	0	0	0	0	31.3
14	20	1	-1	1	1	38.8
29	21	0	0	0	0	31.0
11	22	-1	1	-1	1	38.9
15	23	-1	1	1	1	37.4
8	24	1	1	1	-1	39.6
12	25	1	1	-1	1	38.6
16	26	1	1	1	1	36.2

5	27	-1	-1	1	-1	40.2
3	28	-1	1	-1	-1	40.4
28	29	0	0	0	0	31.1
2	30	1	-1	-1	-1	39.9

Notes: \*ST=Surface Tension

Table - 3 ANOVA for Response Surface Quadratic Model Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob> F	
Model	322.03	14	23	164.89	< 0.0001	significant
A-A	0.094	1	0.094	0.67	0.4252	
B-B	2.22	1	2.22	15.92	0.0012	
C-C	1.35	1	1.35	9.7	0.0071	
D-D	16.83	1	16.83	120.67	< 0.0001	
AB	0.14	1	0.14	1.01	0.3313	
AC	0.076	1	0.076	0.54	0.4729	
AD	0.23	1	0.23	1.62	0.2228	
BC	2.03	1	2.03	14.56	0.0017	
BD	1.5	1	1.5	10.76	0.0051	
CD	0.86	1	0.86	6.13	0.0257	
A2	105.44	1	105.44	755.82	< 0.0001	
B2	125.1	1	125.1	896.77	< 0.0001	
C2	94.96	1	94.96	680.69	< 0.0001	
D2	97.52	1	97.52	699.1	< 0.0001	
Residual	2.09	15	0.14			
Lack of Fit	2	10	0.2	10.71	0.0087	significant
Pure Error	0.093	5	0.019			
Cor Total	324.13	29				

Notes: The Model F-value of 164.89 implies the model is significant. There is only a 0.01 per cent chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant.

Std. Dev.	0.37	R-Squared	0.9935
Mean	37.46	Adj R-Squared	0.9875
C.V. %	1.00	Pred R-Squared	0.9641
PRESS	11.65	Adeq Precision	34.901

The "Pred R-Squared" of 0.9641 is in reasonable agreement with the "Adj R-Squared" of 0.9875. "Adeq

Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 34.901

indicates an adequate signal. This model can be used to navigate the design space.

## Acknowledgement

The authors wish to express their gratitude for the support extended by the authorities of Department of Microbiology, Annamalai University, Annamalai nagar for carrying out the research work. I sincerely thank Dr. R. Parthasarathi and Dr. P. Anna Joice for the conception of this idea and subsequently carrying out research work with the help of Major Research Project University Grants Commission, India

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# IS FEED AND FODDER SHORTAGE A MAJOR IMPEDIMENT TO ACCELERATED LIVESTOCK DEVELOPMENT IN BHUTAN?

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## Abstract

Accelerated livestock production in Bhutan is constrained by perceived unavailability of feed/fodder both in quality and quantity. Consequently the domestic livestock are underfed and are less productive. To understand its detrimental effect, a study was conducted in major Agro-Climatic Zone (ACZ) of Bhutan. Availability and contribution of different feed and fodder resource to diet of domestic livestock was assessed to generate information on the extent of feed and fodder shortage at the different ACZ and explore availability of different feeding options to optimize production.

Study covered eight out of 20 districts in Bhutan. Within each dzongkhags 16 representative *geog* (sub-district) in three ACZ was selected and, ten households per *geog* were chosen for interview. Primary data is collected adopting Feed and Fodder Assessment Tools recommended by International Livestock Research Institute.

Results indicated that to supplement conventional fodder resources, farmers across ACZ cultivated improved fodder crops with average acreage of 2.14 acres per household. Some excess summer fodder was conserved as hay and silage. Besides, 79% of available crop residues are conserved to feed animals during lean season. Conserved fodder/straw contributed 25% while fodder trees supplied about 29% of fodder required during winter. Remaining fodder needs was met via forages collected from forests, gruel made out of grains and a little of concentrate feed purchased.

Study concluded that fodder shortage is localized and is not uniform across ACZ. In higher elevations, fodder shortage is acute due to longer winter which hampered animal production. However, the farmers in mid and low altitude did not face severe fodder/feed storage because of shorter winter and other feeding alternatives available. The study recommended that proper planning of fodder resource utilization in different ACZ, vigorous promotion of available technologies, nutrient enrichment of crop residues, research on frost resistant fodder varieties, and utilization of breweries waste can bring fodder shortage to near zero.

Keywords: ACZ , Bhutan, fodder shortage , livestock production Introduction

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## Introduction

Livestock is an integral part of the Bhutanese farming system with every rural household owning a few livestock of one type or other. Over 77% of households own cattle (DoL 2010). But only about three percent of cattle owners own improved temperate pasture and over

two percent of cattle owners own improved sub-tropical pasture (NFFDP, 2008). The other important fodder resources contributing to the fodder requirement are grazing on forest and fallow land, fodder collection from forest and fringes, fodder trees and crop residues. Of these, forest grazing and natural grassland contribute over 44% of the total fodder requirement (Roder, 1990).

Some of the pertaining issues that hinders livestock production is inadequate availability of feed and fodder both in quality and quantity. Further, overgrazing and deterioration of grazing resources limits fodder availability for ruminants (Roder, 2002). Lack of knowhow on the use and formulation of local feed are other limiting factors (Wangchuk & Dorji, 2009). Consequently the domestic livestock are often underfed, and therefore are less productive. There is a need for high year round supply of quality fodder and nutritious feed to optimize livestock production and get value from improved livestock breeds.

Owing to the apparent problem that has detrimental effect on livestock productivity, a nationwide study was conducted to understand root causes of the problem and devise measures to exploit harness existing

opportunities to ensure year round availability of feed and fodder. The specific objectives were to assess availability and contribution of feed/ fodder resources to diet of the livestock in major Agro Climatic Zone (ACZ) and understand the extent, cause and effect of feed/fodder shortage and explore availability of different agro-industrial by-product as livestock feed supplement.

### **Study location**

Study covered eight out of 20 Dzongkhags( districts) in Bhutan. Within each dzongkhags 16 representative geog (sub-district) were purposively selected based dominant livestock farming system that covers dairy, egg and chicken, pig/pork at all agro-ecological zones (low, mid and high altitude) (Table 1).

Table 1: Study location description

Agro Climatic Zone	Districts selected	Sub-district selected	Farming System
High Altitude(temperate and alpine zone) (>1800 <i>m asl</i> )	Bumthang	Chumi and Chokor Chokor	Dryland, pastoral based system
Mid altitude (dry and humid subtropical zone) (600-1800 <i>m asl</i> )	Samdrup	Orong	Dryland, maize based system
	Jongkhar	Deothang	
	Tashigang	Kanglung	
		Samkhar	Wetland rice based system
	Chukha	Darla	
	Punakha	Talo	
Low altitude (wet wubtropical zone(150-600 <i>m asl</i> )		Kabji	
	Tsirang	Kikhorthang	
		Tsholingkhor	
	Chukha	Samphelling	Dryland, maize based system
	Sarpang	Dekiling	Wetland, rice based system
		Sangpangkha	
	Samtse	Ugentse	
		Samtse	

### Data collection methods

Primary data is collected from each region, adopting Feed and Fodder Assessment Tools-a Participatory Rural Appraisal Method recommended by International Livestock Research Institute (ILRI) to understand the problems/ issues in livestock farming and assess feed and fodder situation in selected *dzongkhags/geogs*. Within the *geogs*, nine households (three each from each category poor/average/rich) were selected for interview. Categorization of interviewee was determined by their land holdings ( < 3 acre with few

livestock- low income group, 3-6 acres with moderate number of livestock- average or medium income group and >6 acres land with good quality livestock- higher income group). The feed and fodder availability in selected households was assessed through site visits, focused group discussion (Fig 1) and individual farmers interviews using tools such as problem ranking, seasonal calendar and semi-structured questionnaires prescribed by ILRI. The answers obtained are validated with key informants- village elderly (*tshogpa*). The secondary data is collected through review of published documents related to feed and fodder research done in Bhutan



Figure 1 Focus group discussion, Punakha

### Data Analysis

Quantitative variables were entered in spread sheet and analyzed using software SPSS 14. Qualitative data acquired through semi structured interview, answers in many cases that fall into patterns with the same answer appearing frequently were coded and entered in spreadsheet. The frequency of each answer was sorted, counted manually and, when appropriate, converted into percentage.

Qualitative data acquired through discussion with herders were summarized and described

### Results and discussions

#### *Farming system*

Farmers in high altitude (1800 to 2800 *m asl*) cultivate sweet and bitter buckwheat, wheat, barley and maize. The dairy farmers also cultivate oats for seed production and tuber crops such as turnips and radish for feeding the animals during winter. In the mid altitude (1200-1800 *masl*), the main cereal crops grown are maize, followed by rice, soya bean and mustard. In the low altitude (below 1200 *masl*), the major cereals cultivated are rice, maize and millet. In the warmer areas farmers also practice double cropping of maize in a year. These cereals left behind the valuable crop residues which could be fed to the livestock. Winter cereals cultivated includes wheat, barley and

buckwheat. Cattle were reared across all ecological zones followed by poultry while pigs were reared only in some few remote pockets owing to religious reasons

### ***Herd size of improved cattle***

Farmers in the high altitude reared more number of improved cattle than the farmers in the lower elevations. This could be attributed to the availability of other opportunities in the mid and low altitude. For instance, farmers in the lower altitude reared poultry owing to higher potentials for the trade. The average improved herd size is 7, 4 and 5 for high, mid and low altitude respectively.

Many farmers (72 %) across all agro-ecological zones are interested to increase the herd size of productive animals and move into semi-commercial mode (5-15 cows). Some farmers were even interested to go into commercial farming rearing up to 30 productive cows. However, marginal farmers (with little or no land and money) were restricted by limited resources despite their interest in expanding the farm. On the other hand, some farmers with larger herds wanted to decrease the herd size due to labor shortage.

### ***Livestock management system***

Majority of the farmers in mid altitude of Trashigang, Tsirang and Punakha (72%) and low altitude Samdrup Jongkhar (58 %) stall fed their cattle while the practice of day time grazing and night time stalling was common in the high altitude (41%). Open grazing and seasonal migration was practiced mostly for local cattle and buffaloes. The higher percent of farmers stall feeding in mid altitude could be because this area is mostly crop intensive area and letting animals loose destroys crops. The other probable reason could be the awareness among farmers who live in mid altitude the environmental benefits of rearing livestock in confinements.

### ***Fodder production***

Farmers across all AEZ cultivate improved pasture and fodder. Fodder under orchard and propagation of sub-

tropical grass mixture is common in low and mid altitudes. Improved fodder species like Napier, Guatemala and fodder trees that were planted along the difficult terrains served as good fodder while they also helped stabilize the soil (Fig 3). Broom grass (*Amliso*) was extensively cultivated by the farmers in mid altitude of Tsirang and Chukha due to its multiple uses which could be adopted by the farmers in other areas. Some farmers dedicated their second maize crops solely for fodder. In the higher elevations, farmers cultivated temperate mixture (Cocksfoot, Italian rye and White clover) for summer and turnips, radish and oats for winter and spring feeding.



Figure 2: Fodder production Whooling Samdrup Jongkharystem

Acreage of pasture varied with Average improved pasture owned by the farmers was 2.14 acres especially among the dairy groups with range from 0.52 to 5.4 acres (Table 2). Farmers with small landholdings, and a variety of agricultural practices, have always been constrained by availability of land. Similar finding have been reported from hills where limited land with low fertility potential is leading to socio-economic consequences (FAO, 1993). A slightly higher acreage of pasture at Samdrup Jongkhar is due to wider uptake of pasture development and dairy farming activities by the area farmers

Table 2. Acreage of improved pasture in sampled households.

ACZ	Improved pasture ( acres, mean)	Standard Deviation	Broom grass (acres, mean)	Standard Deviation
Mid altitude (Tsirang, Punakha, Trashigang)	0.52	0.05	1.29	0.47
High Altitude Bumthang	2.95	0.92		
Low altitude S/Jongkhar	5.40	2.7		

### ***Fodder conservation and fodder trees***

In high altitude temperate areas, excess fodder was conserved for winter in form of hay and straw while silage making was not popular due to religious reasons, inadequate infrastructure support such as silo pits. Available fodder conserved included grass hay and buckwheat straw wherever they are cultivated. Willow trees have been adequately grown but are largely underutilized due to lack of adequate awareness on the technology. In mid and lower elevations, the farmers conserved paddy straw, maize husks and millet straw. In dairy intensive areas farmers treat maize stover with urea and molasses resulting to minimal wastage which alleviated winter fodder shortage at large.

The fodder trees contributed about 29% of fodder requirement while 25 % of fodder requirement was met from conserved fodder. Some farmers also collected available fodder from the forests during the lean season

### **Crop residue utilization**

In high attitude areas about 50% of sweet buckwheat straw had been used for feeding livestock in winter. Other crop residues such as wheat, barley and oat straw were not even harvested from the field resulting to wastage. Further buckwheat straws were also not properly stored which were stacked on tree tops and temporary platforms exposed to weathering, leaching of nutrients and fungal infestations. In mid altitude, about 91 % of the available rice straw were used to feed livestock as bulk fiber along with tree fodder and were supplemented with cooked gruels of rice bran and flour. However, only about 65% of the crop residues were

being fed to the animals in the low altitude which could be attributed to the availability of other fodder resources such as tree fodder and fodder plantations. This could also be due to inadequate support on the enrichment technologies. The overall average utilization of the crop residues is about 79 % (Fig 3).

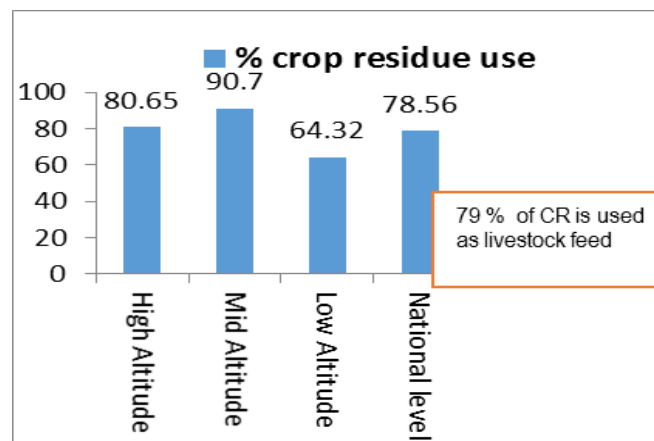


Figure 3 Crop residue use

Maize stover is not used beyond 50 % in maize based systems reportedly probably due to its coarseness and poor palatability. However, urea and molasses treated maize stover was more palatable and used extensively by the dairy groups in Trashigang. The finding is in line with use of crop residue s in other countries of Asia. For example, in Thailand, it was reported that 75% of the rice straw from rain fed upland farms and 82% in the lowland farms is collected by farmers for use as feed (Wanapat, 1990). In Bangladesh, Saadullah et al. (1991) found that 47% of rice straw is used as animal feed. However ILRI projected crop residue utilization between 20-50% (ILRI, 2013). Thus crop residue remains a substantial part of the daily ration of the livestock in mixed farming system.



### Availability of agro industrial byproducts

A substantial quantities (> 60 MT) of wet brewers grains (semi-liquid) is found to be produced from Army Welfare Project(AWP), Gelephu daily while 24 MT of the same was produced by Brewery Unit at Pasakha bi-monthly. Farmers around Gelephu however are not able to utilize these byproducts although AWP, Gelephu allowed them to take them for free owing to transportation and storage problems while 90 % of the brewery waste from Pasakha was exported to India. Hence, the AWP, Gelephu had installed a drying plant adjacent to the brewery plant and produces about 8 MT of Dry Distillers Grains Solubles (DDGS) which was exported to India. This indicates that there was a great potential to tap these resources for feeding the animals and operating a feed mill within Bhutan Though exact percentage of use in Bhutan is not known, feeding of DDGS upto 40% of the diet to growing dairy heifers achieve excellent growth rate and dairy cow 20 percent of the diet can be replaced without decreasing dry matter intake, milk production, and percentage milk fat and protein (Abdelqader, *et al.* 2006)

### Fodder shortage scenario

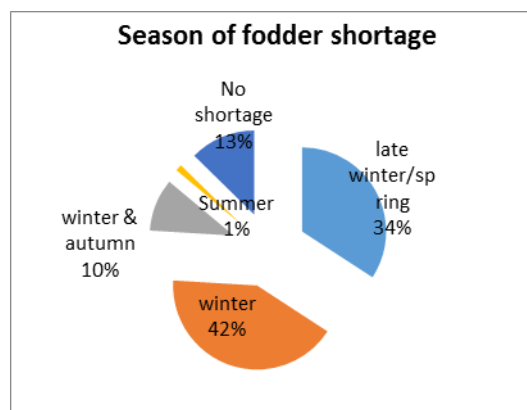


Figure 4 : Fodder shortage scenario in different season

The secondary data indicated that adequate fodder resources were available in the country. However, some cases of fodder shortage had been reported which were localized and regionalized. This could be attributed to under utilization of the available fodder resources. In higher elevations fodder shortage was experienced during late winter and early spring (mid February to

mid April) since the conserved fodder and crop residues got exhausted due to long winter season (Fig 4). However, the farmers in mid and low altitude did not face acute shortage of fodder since they cultivated oats which provided them three cuts (harvests) in spring while they also cultivated other fodder crops such as winter maize, broom grass and had ample fodder trees and crop residues. The farmers in low elevations stated that the subtropical pasture and fodder remained green till the end of December while new regeneration of grass began by April. This indicated very short lean period which was easily supplemented by crop residues and fodder trees during from January to March. However, the availability of fodder largely depended on careful planning and management of the available fodder resources.

### Feed milling, availability, affordability and quality

Karma feed produced at Phuntsholing Bhutan is extensively used by all farmers. Feed is reported to be available with all most all the feed agents. However, Majority (98%) of the farmers reported that ever rising price has drastically cut the profit margin to bare minimal level, not even enough to sustain the business thereby threatening the mere existence of on-going livestock enterprises. Farther the distance from feed mill in Phuntsholing higher is the transportation cost and resultant price. Thus far off *dzongkhags* are reportedly hard hit.

Among seven mini feed mills (of < 1MT capacity /hr) supplied across Bhutan only one mill at Tashigang is functional. Others, for want of spare parts, break down of motor and lack of feed ingredient had made it non functional. But the functional one also suffers serious problem in maintaining product quality, the feed is often not mixed properly and seems to be with lots of lumps and solid materials. Poor monitoring of production process, not following standard guidelines provided on use of ingredient are the main cause of quality deterioration. Thus as suggested by ILRI (2013) and as is true in other countries investing in feed manufacturing process needs to consider quality control regulatory framework.

### ***Production performances during winter***

In the high altitude areas milk production during winter is estimated to reduce by about 50% to what can be produced during summer. Farmers attributed this to cold weather condition, damage to fodder by frost, snow covering the grazing ground which resulted to fodder shortage. In mid and lower elevations too, milk production is reported to decrease during winter but production upset is marginal. Further body condition score is recorded to be below 2.5 (scale 0-5) for most of the animals during winter.

### **Conclusion and recommendations**

Fodder shortage is not so acute in mid hills and lower subtropical belt because of existing feeding alternatives owing to mild weather condition. But in high altitude area, fodder shortage is more pronounced and is having a deleterious effect on livestock production.

Across all ACZ proper planning of fodder resource utilization is non-existent and fodder conservation technologies is unheard by in some areas. Farmers' inadequate knowledge on effective use of available feeding resource warrants a greater attention and advocacy on various technologies and feeding options available during different seasons of the year.

Lack of knowhow on fodder production, feed formulation and feed milling are drawback that hinders concentrate feed availability and affordability to smallholder farmers

Therefore, to bring-down fodder shortage to near zero the study recommends:..

- Vigorous promotion of all available technologies, conservation and nutrient enrichment of crop residues is way forward to overcome fodder shortage
- Breweries waste though is a cheap source nutrition for livestock, potential of these resources for feeding the animals and use of DDGs as good protein sources for feed mill within Bhutan remained to be tapped
- To address issue of compound feed, a greater awareness on formulation of local feed resource and make optimal use of existing feed mills and installation of additional feedmills of higher capacity but with better quality materials
- Research has to immediately focus on frost resistant winter fodder varieties, improving yields of available fodder species and provide alternatives to farmers.

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# STUDIES ON PHENOLOGICAL BEHAVIOR OF TWO CASSIA SPECIES IN GIRNAR RESERVE FOREST, GUJARAT, INDIA

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## Abstract

Phenological cycle of two tree species viz. *Cassia siamea* Lam and *Cassia fistula* L. was studied in Girnar Reserve Forest, Gujarat, India from August-2008 to August-2011. Both species varied with different phenological behavior in all four phenological events studied such as new foliage, leaf fall, flowering and fruiting by showing significant variation (P value <0.05) in number of days. For *Cassia fistula* L., mean new foliage, leaf fall, flowering and fruiting days were 43, 52, 103 and 107 approximately but they were, 40, 162, 160 and 135 respectively for *Cassia siamea* Lam. Among different morpho-phenological characters studied, positive significant association was recorded between diameter of stem and branches per tree (0.84\*\*), flowers per branch and inflorescence per branch (0.91\*\*) for *Cassia fistula* L. However, in *Cassia siamea* Lam, positive correlations were recorded between inflorescence per branch and flowers per branch (0.93\*\*). Interestingly, there were negative correlations found between leaves per branch and inflorescence per branch (-0.55\*), also between leaves per branch and flowers per branch (-0.54\*) for same species. Climatic factors affected phenology of both species by showing direct association. In *Cassia fistula* L., wind speed showed positive association with inflorescence per branch (0.59\*), and with flowers per branch (0.56\*) whereas, rain pertained positive correlation with fruits per branch (0.64\*). In case of *Cassia siamea* Lam, positive correlation was observed between wind speed and fruits per branch (0.49\*) as well as between rain and fruits per branch (0.74\*). This kind of work can be highly useful in understanding adaptation mechanisms of plant species; can also be of immense use for different branches such as physiology, ecology and forestry.

Keywords: Phenology, Morpho-phenological characters, Girnar Reserve Forest, Climate

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## Introduction

Phenology is time of recurring phenomena in relation with climate. Phenological events such as new foliage, leaf fall, flowering and fruiting are influenced by abiotic factors like rain, temperature and wind speed. Phenological studies have importance in conservation of forest genetic tree recourses, furthermore; they can be utilized for making strategies for management system of forest in reforestation and in climate change studies. According to Molau (1993), timing of flowering is much crucial to reproductive success of all plants however in phenological analysis fruiting plays very important role too, especially in study against

climate change (Cortes Flores *et al* 2013). New foliage and leaf fall are useful characters, and were studied for phenology (Kikim and Yadava, 2001) in addition to reproductive characters.

General aspects studied for phenology for different species are very inspiring (Borchert, 1983; Daubenmire, 1972; Jyotinath, 2008), but there are few examples of published material for phenological studies for Girnar Reserve Forest, Gujarat, India (Nakar and Jadeja, 2009; Jadeja and Nakar, 2010; Nakar and Jadeja, 2014; Nakar and Jadeja, 2015). Present study aim (i) to analyze phenological behavior of two two tree species of Cassia

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viz. *Cassia siamea* Lam. and *Cassia fistula* L. (ii) to correlate data of climatic factors such as rain, temperature and wind speed with that of morpho-phenological data.

## Material and methods

### Study area and climate

Girnar Reserve Forest, is National Sanctuary in West Saurashtra region of Gujarat State of India. It has spread of 186 km, surrounded by Junagadh and Bhesan Talukas at both sides. It lies within 70°28'-70°27'N longitude and 21°30'-21°26' E latitude. Forest is mainly surrounded by Teak, which is in mixture of other species. There are different areas such as Bordevi forest, Jatashankar forest, Jinababa madhi forest, forest area near Prerarana dham, area near Gayatri Mandir etc. Climate of the Girnar Reserve Forest can be divided into mainly three sub seasons: summer (April to mid June), monsoon (mid June to September) and winter (November to February). Of the total rain fall, almost 95% occurs during rainy season.

### Plant phenology

Phenological characters for *Cassia siamea* Lam. and *Cassia fistula* L. such as new foliage, leaf fall, flowering and fruiting were studied using method of Opler *et al.* (1980) for three years from Aug-2008 to Aug-2011. Month wise numbers of days for each species were calculated for all four phenological events, which later on converted into mean approximate number of days.

### Morpho-phenological characters

Morpho-phenological characters such as diameter of stem, branches per tree, leaves per branch, inflorescence per branch, flowers per branch and fruits per branch were observed at 2 month regular interval. For that, a branch was selected on 5 tagged trees, and then individual traits were recorded monthwise.

### Statistical analysis

Simple parameters like average, minimum, maximum were calculated using MS-Excel 2010 whereas, for studying variation one way ANOVA was performed using software SPSS. Linear correlation was studied using PAST software, between climatic and phenological traits to find association between them.

## Results and discussion

During three years study, highest temperature was 44.5°C in May 2009, and lowest was 18.35°C, recorded in December 2010. Mean values for rainfall during 2008–09, 2009–10 and 2010–11 were 103.05, 69.18 and 102.85 mm respectively, while wind speed showed average values of 6.39, 6.34 and 4.60 km h<sup>-1</sup> for same respective years. There was significant negative correlation between minimum temperature and wind speed.

Phenology of two *Cassia* species showed significant variation in their phenological behavior (*P* value<0.5). Mean number of new foliage showed values of 36, 45 and 48 days for respective years 2008-09, 2009-10 and 2010-11 for *Cassia fistula* L. however, they were, 27, 43 and 49 days for *Cassia siamea* Lam. In leaf fall event, mean leaf fall were 58, 50 and 49 days for *C. fistula* L. whereas they were, relatively higher in *Cassia siamea* Lam. with values of 174, 154 and 159 days. For reproductive phenological events, mean number of flowering days were, 107 and 148 in 2008-09 for *C. fistula* L. and *C. siamea* L., while in 2009-10, they were 101 and 161 days for both species. During last year 2010-11, *C. fistula* L. and *C. Siamea* Lam. exhibited 100 and 170 days for flowering. On the other hand for fruiting, *C. siamea* Lam showed more fruiting days compared to *C. fistula* with respective values of 135 for 2008-09, 121 for 2009-10 and 148 for 2010-11 Lam compared to values of 96 for year 2008-09, and same value 113 days for both next years for *C. siamea* Lam. Mean values for three years study indicated that, among all phenological events, fruiting event dominated with mean of 107 days followed by 102 days for *C. fistula* L. Interestingly, in *C. siamea* Lam. there was highest leaf fall period with value of 162 days followed by 159 days for flowering.

Variation was significant for all morpho-phenological traits studied. *C.siamea* Lam. showed range of 41.20 to 41.28 cm for diameter of stem, 4 to 4.2 for branches per tree, 41.2 to 1056 for leaves per branch, 11 to 882 for inflorescence per branch, 14 to 642 for flowers per branch, and 0 to 158 for fruits per branch. In case of *C. fistula* L., they were, 33.24 to 33.28 cm or diameter of stem, 3.9 to 3.93 for branches per tree, 33.25 to 1328 for leaves per branch, 5 to 903 for inflorescence per branch, 13 to 1268 for flowers per branch and 0 to 580 for fruits per branch. In *C.fistula* L., positive significant correlation was recorded between BPT and DOS (0.841\*\*), between FLPB and IPB (0.92\*\*), between IPB and wind speed (0.59\*), between FLPB and wind speed (0.56\*), as well as between FRPB and rain (0.64\*). But on the other hand, for *C.siamea* Lam., positive significant association was recorded between IPB and FLPB (0.93\*), FRPB and wind speed (0.49\*), rain and FRPB (0.74\*). Additionally, there was negative significant correlation found between LPB and IPB (-0.55\*), and between LPB and FLPB (0.54\*). Analysis of Variance for three years study showed that variation between species was significant furthermore, there was also found significant interaction between phenological parameters studied and years.

In recently published study, there was positive interaction between year and phenology for selected taxa of herbs, shrubs undershrubs (Nakar and Jadeja, 2015). In addition, it was also pertained that there was strong correlation between climatic factors and phenological patterns of Girnar Reserve Forest which is in line of current work. Both *Cassia* species showed leaf fall from December to February which is peak season for most of the species for this study area, hence support earlier studies (Jadeja and Nakar, 2010; Nakar and Jadeja, 2013, 2014) In one interesting study, 64% woody species found to be in fruiting during pre-monsoon period in Northern Western Ghats, India (Tadwalkar, 2012). According to Singh and Khushwaha (2006), there is wide range of time lag around 1 to 8 months for vegetative phase and reproductive phase for deciduous species. Even here from vegetative to reproductive stage approximately 8 to 9 months were taken to complete whole process. They further stated that in most of the species flowering was recorded

during summer season, which is good evidence for current study. Earlier study on two Bombacean members at same site indicated very high variation for leaves per branch compared to other morpho-phenological traits, even in current one there was high range of leaves per branch for both species. Additionally, there was positive significant correlation between diameter of stem and branches per tree for bombacaceae member (Nakar and Jadeja, 2014) similarly here, strong positive significant correlation was recorded between both these traits for two species of Caesalpinaceae family.

## Conclusion

It is concluded that, phenology of two *Cassia* species showed significant variation in all traits studied viz. new foliage, leaf fall, flowering and fruiting. Positive association of climatic factors such as wind speed and rain advocated that, these factors affect phenological behavior of species directly, hence it is the plant adaptation in response to climate of the area. Interestingly, rain showed positive significant association for both species, which emitted that, if there was increased rain then, fruiting duration was elongated for both species of *Cassia*. Current work can be highly useful to workers of different fields but, still more work is required to put some concrete output.

## Acknowledgement

Authors are thankful to Dr. H.L.Dhaduk (Anand Agricultural University), Dr. Chetanaben Mandavia and Dr. V.P.Chovatiya (Junagadh Agricultural University), Dr. P.S. Nagar (M.S.University, Baroda). They also show gratitude to Forest Department of Junagadh and Gujarat for providing me necessary permission to work in Girnar Reserve Forest, Gujarat, India.

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Table 1. Phenological diversity in mean number of days from Girnar Reserve Forest

Year	New foliage		Leaf fall		Flowering		Fruiting	
	CS	CF	CS	CF	CS	CF	CS	CF
2008-09	36	27	58	174	107	148	96	135
2009-10	45	43	50	154	101	161	113	121
2010-11	48	49	49	159	100	170	113	148
Mean $\pm$ SD	43 $\pm$ 6	40 $\pm$ 11	52 $\pm$ 5	162 $\pm$ 10	103 $\pm$ 4	160 $\pm$ 11	107 $\pm$ 9	135 $\pm$ 14

Where, CS and CF are *Cassia siamea* Lam, and *Cassia fistula* L. respectively.

Table 2. Analysis of Variance (MS Values) for different phenological traits

EFFECT	DF	NF days	LF days	FL days	FR days	DOS	BPT	LPB	IPB	FLB	FRPB
Block	1	18.75	12.00	192.00	48.00	0.00	0.00	50897.79	30653.52	7252.08	520.08
Species	1	60.75	29601.33**	6816.33**	2028.00**	190.68**	0.05**	324647.33	46066.02*	330.75	2914.08
Year	2	254.33*	204.33	375.08	495.75*	0.00*	0.00*	180567.00	14138.40	592.02	18832.94*
Species x Year	2	109.00	30.33	300.08	452.25*	0.00*	0.00	82706.63	21545.90	597.56	11271.27
Residual	5	22.15	152.80	214.20	46.40	0.00	0.00	71898.08	6841.72	597.83	2397.63
Total	11	83.36	2804.24	857.24	382.18	17.34	0.01	114689.34	16572.43	1177.38	6875.52
CV		11.74	11.41	10.82	5.85	1.74	0.53	34.66	68.47	43.53	62.17
LSD (0.01) for sp.		10.95	28.77	34.07	15.85	0.015	0.049	624.21	192.48	56.92	113.97

Here, \* and \*\* indicate significance level at 0.5 and 0.1 (P value <0.5, 0.1) respectively. CV, DF and LSD show Coefficient of variation, Degree of freedom and Least significance difference. DOS, BPT,LPB,IPB,FLB,FRPB are Diameter of stem, Branches per tree, Leaves per branch, Inflorescence per branch, Flowers per branch and Fruits per branch respectively.

Table 3. Linear correlation between different phenological traits

No.	Correlation bet.	Cassia siamea Lam.		Cassia fistula L.	
		R Value	Result	R Value	Result
1.	BPT-DOS	0.84**	P < 0.01	0.22*	P < 0.05
2.	FLPB-IPB	0.94**	P < 0.01	0.93**	P < 0.01
3.	IPB-Wind sp.	0.59*	P < 0.05	-	NS
4.	FLPB-Wind sp.	0.56*	P < 0.05	-	NS
5.	FRPB-Wind sp.	-	NS	0.49*	P < 0.05
6.	FRPB-Rain	0.64*	P < 0.05	0.74*	P < 0.05
7.	IPB-LPB	-	NS	-0.55*	P < 0.05
8.	IPB-FLPB	-	NS	-0.54*	P < 0.05

NS exhibits Non-significant results, BPT-Branches per tree, DOS- Diameter of Stem, FLPB-Flowers per branch, IPB- Inflorescence per branch, FRPB-Fruits per branch, LPB-Leaves per branch respectively.

Figure 1. Photographs of selected tree species for phenological study



[A] *Cassia siamea* Lam. in flowering



[C] *Cassia siamea* Lam. fruiting/  
seeds



[B]. *Cassia fistula* L. in flowering



[D]. *Cassia fistula* L. in fruiting/  
seeds



# KEY ISSUES IN APPLICATION OF COMMUNITY USE ZONE (CUZ) TO CROCKER RANGE PARK FROM THE PERSPECTIVE OF SOCIAL WORK

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## Abstract

Throughout the world including Malaysia, parks and protected areas are commonly managed by strict top-down enactments that marginalize local people. This often has resulted in recurrent conflict between park management and communities living in and around these areas. To tackle the problem, Sabah parks in collaboration with Borneo Biodiversity and Ecosystems Conservation (BBEC) started implementing a program known as Community Use Zone (CUZ). The major aim of this program is to harmonize situation between Crocker Range Park (CRP) authority and the communities residing in CRP by balancing existing local community needs and conservation through participation and collaboration. The objective of this research is to assess implementation process of CUZ program in its two years span by using thematic analysis of data obtained through interviewing relevant stakeholders, i.e., communities and park management. The result indicated that land use issue, such as different perspective by CUZ community and parks management regarding restriction of planting, hunting, land expansion and no cutting trees policy in CUZ areas, to be the most salient frustrating proper implementation of the program. This study hope more concerted effort by parks management and CUZ committee in conducting discussions regularly from the issues identified. This will be useful in designing future CUZ programs in other regions.

Keywords: Social work, Community Use Zone, Park Conservation, Malaysia.

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## Introduction

Attention to issues related to the environment, ecology and the issue of sustainability has increased in the field of social work. Social work seen to have an orderly plan to engage in efforts to raise public awareness about the importance of agriculture and forest conservation for the future (Besthorn, 2013). Today social work has shown the uniqueness of this profession by helping others, this related because of the relationship between humans and the environment is a practice that cannot be separated (Compton, Galaway, & Cournoyer, 2005)

The objective of this research is to look on the key issue emerge in application of Community Use Zone (CUZ) to control agricultural activities especially in the areas of national parks or forest reserves. It is important to

avoid the destruction of habitat and avoids causing damage to the forest ecosystem. Disturbing forest ecosystems will directly lead to the extinction of many species of flora and fauna. Neutral policies with priority to the environment and human life are important to reduce agricultural activities in the National Park area. Local people especially the indigenous residents should also be educated in order to see the importance of the existence of forest ecosystems continue for future generations. Therefore scientific research and social studies are very important to control of agricultural activities continue to grow in the area of national parks (Tilman et al., 2001).

The main key point of this program is to make an agreement to enable the communities of Ulu Senangang village living in and around the park to continue their livelihood activities while taking into account the need to conserve and manage resources in the CUZ area. To achieve it the local community must also take the same responsibility in the conservation effort, the success of such programs often caught the attention of various stakeholders because it is depends on the attitude and the acceptable of local communities involvement with the management of the park (Daugstad et.al, 2006).

### **Problem Statement**

Crocker Range Forest Reserve was gazette in 1969 by the state authority, by 1984 the Forest Reserve were gazette to a park and rename as Crocker Range Park. Settlement began in Crocker Range Park in 1975 that is in between 1969 to 1984 and named the settlement area as Ulu Senangang Village. Originally communities in Ulu Senangang Village were migrating from Pensiangan district. Since the communities moved in to the park area in 1975, they have being trying to apply a legal land status with the Land and Survey Department and it's never being approved because of the land status and location as Crocker Range Park.

In 2001, communities in Ulu Senangang Village again requested the area they occupy to be removed and giving a legal status. However this action was not approved by Sabah Park. To neutralize the situation Sabah Park has allowed activities within the park area but any expansion of land are prohibited, this is due to the Parks Enactment 1984, stated they are no settlements or villages, and any activities or subsistence agriculture inside the park. By the way Sabah Parks granted the communities to continue farming only on the land that the community already cultivated before 1984 and communities of Ulu Senangang are subject to the Parks Enactment 1984.

Eventually after taking a consideration of International Law on Indigenous People (IUCN WCC Resolution 1.53) Crocker Range Park Management Plan has implemented a concept of Community Use Zone (CUZ) to allow the existence of current community continue their agricultural activities within the zone on mutually

agreed terms and conditions. Initially implementation of CUZ goes smooth for each party, problems arise when implementing of CUZ started to get dissatisfaction from the community in CUZ area based on strict rules on the allocation of those living in the zone and the communities see the rules are quite strict. Therefore, this study attempts to analyze the issues that led to the dissatisfaction of the community.

### **Methodology**

A qualitative approach was practiced in this study. Qualitative research provides an opportunity for researchers to explore in-depth views, to understand deeply the meaning of each respondent words and underlying belief that constructed over time through social relationships and reflection (Patton, 2002). The primary focus of the data analysis was on how the participants made sense on issues of application of CUZ at Crocker Range Park. Respondent interpretations and their experiences as well as researcher interpretations came together during data analysis. This approach means to highlight on a topic through in-depth inquiry to understanding similar problem that occur during research.

### **Sample**

Using purposive sampling technique, 15 respondents from Crocker Range Park CUZ participated in this study and based on the consent of their interview being recorded (Bernard, 2000). Criteria for the key informant selection were based on the criterion of key informant roles as a chief village, senior community members and Sabah Park staff of area that participates in the (CUZ) program in Crocker Range Park (CRP).

### **Data collection and data analysis**

We use a standardized open-ended interview also known as semi structured interview, and collected the data from each participant with separate interview ranging from 30- 50 minutes to each participant. Semi-Structured questionnaire used to each participant and all the interview are recorded by audio recorder tools (Creswell, 2013). Researcher directly doing the

interview using a set of semi structured questionnaire and asking the same set of questionnaire to all key informants that participate in this research (Patton, 2002). The data from participant is transcribed in to verbatim from audio tapes. Verbatim data then was analyzed and coded, researcher then look for basic emerge themes. Theme will be put in potential category and organized to main theme.

## Findings

According to most respondents interviewed, many of them consistently, states that the land use in implementing the CUZ was a main key issue emerge, for other issue it may be can consider but in term of land use issue it's hard to tolerate. Respondents criticized the issue in related to cutting trees rules, prohibited of hunting animal, issue on land expansion and freedom in planting tree.

### Cutting trees rules

There are respondents who feel troubled with cutting trees rules that really make them in difficulty in their livelihood style. As expressed by some respondents:

*"With the rules set up in CUZ, we are unable to chop tree anymore for general purpose, before this... we can find a good tree and we select only best and hard one...we not simply chop other small tree" ... (Respondent M)*

*"They only allow us chop tree that has been fall down...that type of tree is useless and the wood can't be use for build house"..., is only can be use as Woodstock ....( Respondent E)*

*"They said we can only allowed to cut or chop any of our commercial tree....why we must cut our own tree that we plant...that was not a logical point at all.." (Respondent A)*

*"I'm not understand at all why the CUZ management didn't give permission to cut tree if the tree mature to cut and we use it wisely"... (Respondent D)*

*"No chances to look for good wood to build house or for other command use anymore, if want then can look at sawmills only" ... (Respondent L)*

### Prohibited of hunting animal

According this two respondents there were no chances to hunting like before as hunting was a culture and activities for food supply. The Parks management should rearrange the rules at least a partially free hunting zone close to CUZ:

*"Now it's hard to hunting since we agree to the CUZ regulation ...it's a bit disappointed as hunting is our culture from our great-grandparent"... (Respondent F)*

*"I want have a fresh meet just go to buy in market, I can't not hunting here in CUZ it's prohibited now"... (Respondent O)*

Only animal that enter the CUZ and causing damage to their crop be able to be hunted. Other than that communities in the CUZ area are totally prohibited to hunting. As all animal are also being protected, villagers may have limitation to hunt other type of animal, here one of the respondent comment:

*"We only being allowed to hunt animal that destruct our agricultural product...that mean if a wild boar run out of CUZ we can't hunt them anymore"....(Respondent H)*

According in CUZ terms catching fish is only inside CUZ border. The communities must perform the local rules of catching fish season that may only be able to catch fish twice a year or only once a year, this rules may not have any trouble for villagers to follow. They worry if during raining season and heavy rain fall will cause flood that may flow all the fish out of CUZ, this may cause no fish anymore in CUZ river zone:

*"Catching fish only inside the CUZ, during heavy rain and flooding all the fish being flow far away, at last no more fish in CUZ compound river..." (Respondent B)*

### Issue on land expansion

Besides having issue in cutting trees and hunting activities, communities also have issue in term of land expansion, as the CUZ will be implemented they request a larger land for cultivation of differentness plant as one of the respondent stated below:

*"With larger land provided, so that we can plant different agriculture product to balance with other commercial*

*agriculture product that not productive” ... (Respondent G)*

*“Land that we use is now very small, sometimes planting in to other people's boundaries this situation not so nice, so through this CUZ it can prevent soil and land border conflict” ... (Respondent N)*

Land expansion issue is related because the community's population is getting more in future time, they will be not enough space for agriculture in the future if all the generation will continue to have cultivation activities. While one respondent stating as a big family one day everybody have their own family and own land for their children too:

*“We request larger land because our population are getting more, my son will growth up and they may need a land for their own cultivation, they also may build their own house, so if I have 4 sons that means the demand of land is crucial” ... (Respondent K)*

### **Freedom in planting**

Respondents also criticized the rules that not allowing them to plant oil palm in the area of CUZ. Planting oil palm for respondent may give high return due to prize of the commodity in producing oil palm. There are also rules that need villagers to request permission for plant that not consider as domestic or local plant to be cultivated in CUZ.

*“I don't know why they not allowed us to plant oil palm... as I know the oil palm tree can generate good income... what I know they said the land not suitable for planting oil palm...in my view there shouldn't be a problem at all” ..... (Respondent C)*

*“If we need to plant any other plant that not a domestic plant at CUZ....we need to get permission ...we need to fill up paper work and wait for approval” ... (Respondent I)*

*“If we plant different crops here we have a variety of food sources and not just rely on one type of crop, because there are some plants only seasonal fruits such as durian trees and deliver according to the season” ... (Respondent J)*

### **Discussion**

This research aimed to find out issue arise from the implementation of Community Use Zone (CUZ) in Ulu Senagang Village, this findings suggest that before the implementation of CUZ, it's important to address the concern from the communities in Ulu Senagang needs regarding of CUZ rules and regulation. For example the communities was not happy with the rules which ban tree cutting, because tree cutting purposes for making household bridges and other facilities.

Tree cutting rules should be considered for these communities to provide opportunities for them to obtain forest products for daily use. The perception of the respondents surveyed also indicated that members of the community are also not satisfied with the issues involved in hunting the animal CUZ zone, where only animal that can be hunted only destroy crops, this means that the animals are running out of the zone cannot be hunted or be penalized for failing to follow rules and regulations of CUZ (Blouch, 2010).

Another problem faced by the communities when implemented of CUZ is the problem of getting more land for crops and for the purpose of building future settlements. This is because the land there is now quite limited magnitude. Therefore attention should be given to CUZ community demand in more land could be provided to them. As the community argued the area of Sabah Parks is still wide when compared with the total area of land that they have requested. However the decision not to allow an oil palm plantation in the term and regulations of CUZ near the park area is logical to be considered. Looking on the terrain in Ulu Senagang circumstances, it is impossible not to damage the forest soil system, which of course has an impact on Sabah Park ecosystems. The issue of oil palm planting prohibited by the park is viewed correctly.

Indeed, the findings of this study indicate the important of concerted effort by parks management and CUZ committee in conducting discussions regularly from the issues identified. Participatory involving stakeholders in communities to address the current situation in CUZ are very important. Discussion that involve various parties is important, not just the party of government agencies,

the NGOs should also be involved in meetings involving CUZ rules, if everyone can understand the needs and considerate the feeling of the communities, issue of dissatisfaction among the people in this village can be easily avoided and reach a consensus to implement CUZ. Meeting and discussions should be held more frequently and have reached a consensus so that the next meeting and discussions between the management of the park and residents Ulu Senagang going smooth as well (Foley et al, 2005).

## Conclusion

This qualitative study identified many issues of implementation CUZ. The perception of the community about the terms and regulations are not happy because there are some things that are not satisfactory. Although they occupy an area of the parks, does not mean that they are denied the right for livelihood survival. Today economic situation will undoubtedly have an impact on the lives of this people. Both party needs a balanced cooperate so that conflicts do not arise with the people involved in the area of CUZ.

Overall, the study showed that respondents require the park management to be more appropriate to pay attention on the issues CUZ term and regulations that emphasis on the program more on conservation than the livelihood of the communities need. Its importance of looking after the welfare and needs of the community as important as protecting and conserving the environment of Crocker Range Park. Land use issues are very important to the communities that rely on

agriculture for their daily subsistence. Any implementation process should take into account the interests of subsistence farmers and in balance with forest care for watersheds and forest ecosystems.

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# ASSESSMENT OF THE VARIABILITY OF YIELD OF MAIZE IN LILONGWE DISTRICT IN RELATION TO CLIMATE CHANGE USING DSSAT MODEL

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## Abstract

Malawi is vulnerable to climate change and variability because agriculture production is rain-fed and dominated by smallholder farmers. Variability in rainfall affects crop productivity and calls for the need to generate information on seasonal rainfall and temperature characteristics to guide in decision making on adaptation strategies. In this study, analysis of historical data sets of daily rainfall and temperature was done to generate information on seasonal rainfall characteristics that would be used to understand climate variability and opportunities for adaptation of maize based cropping systems.

DSSAT model was used to run the crop simulations for the cropping season of 1996/1997 to 2007/2008 for growth, development and yields of hybrid Maize at Chitedze Agriculture Research Station, and to assess which agronomic management practices can help adapt to climate variability. The DSSAT model was used to provide information concerning management options such as the timing of planting, specifically the impact on the yield with reference to different planting dates at Chitedze Agricultural Research Station.

The results show that planting maize early December (15th December) increase yield other than late and early November (30th November and 15th November respectively), late December, and late and early January for Chitedze, supported by the Index of Agreement of 0.861 (d-stat) which signifies the closeness of the relationship between the observed and the simulated yield, and the efficiency of DSSAT model to simulate yield with little root mean square of error (220.69 kg/ha),  $R^2=0.770$ , mean difference of -99.61 kg/ha. The mean observed maize yield was 1350 kg/ha and the mean simulated being 1250 kg/ha through regression analysis were positively correlated,  $R^2=0.77$ .

Keywords: Climate variability, DSSAT model, crop modeling, maize, planting date

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## Introduction

(Department of Meteorological Services, 2009).

### *Climate of Malawi*

The climate of Malawi is sub-tropical, which is relatively dry and strongly seasonal. The warm-wet season stretches from November to April, during which 95% of the annual precipitation takes place. Rainfall is unimodal with yearly average varying from 725mm to 2,500mm with Lilongwe having an average of 900mm

### *Crop production in Malawi*

Agricultural production is under rain-fed conditions. Crops grown includes cereals (maize, rice, millet, and sorghum), legumes (soybean, common beans, and pigeon peas, cowpeas, and Bambara nuts), tobacco, cassava and sweet potatoes. As in other countries in southern Africa, cropping systems are dominated by

maize as the main staple food (Malawi Integrated Household Survey, 2005).

Maize production in Malawi amounted to 3,445,000 tonnes in 2007, (MOAFs 2007). The domestic production has fluctuated widely from one year to the next over the recent 16 years, with the average standing somewhere between 1 million and 2.5 million tonnes. Production below 1.5 million tonnes indicates a famine, which occurs every two or three years.

Soil moisture is one of the determinants to plant growth and development. There is a clear distinction between the rainy and dry seasons. Maize is grown in the rainy season. It is also cultivated during the dry season at small scale in areas where irrigation facilities are available. Frequent droughts seriously destabilize maize production, failing to meet the food needs of the population.

### ***Climate and agricultural production***

Climate is one of the important factors that affect agricultural production. Some of the climate variables that affect plant growth and development are light, rainfall and temperature. Plants require optimum levels of each of the climate variables for various plant physiological processes. Climate hazards caused by climate change and extreme weather events have negative impacts on agricultural production. Some of these climate hazards are droughts, excessive rainfall, high temperatures, floods, unpredictable onset of planting rains, early cessation of rainfall, (Malawi National Adaptation Programmes of Action 2006). Erratic rains results into acute crop failure that results into food insecurity and malnutrition due to low food production.

Droughts and floods, in particular adversely affect food, water, health, energy and the sustainable livelihoods with about 65% of the population living below the poverty line that most of the times do not have capacity to cope with, or adapt to the extreme impacts of climate change events.

The effects of climate variability have significant impacts on agricultural production and this is important

for countries like Malawi where agriculture is predominantly rain-fed. There is need therefore to identify cropping systems for adapting to climate change and variability (Malawi National Adaptation Programmes of Action 2006).

Climate change has vast effects on crop production. Water in all its forms plays a vital role in the growth of plants and the production of all crops (Ayoade, 2004). It provides the medium by which food and nutrients are carried through the plant. Ezedimma (1986) reported that water is the main constituents of the physiological plant tissue and a reagent in photosynthesis. Water is required for all metabolic reactions in plant. If the climatic conditions are not conducive plant growth is affected hence resulting into low crop production since germination is affected where rainfall is erratic. Intense temperatures also affect metabolic processes hence affecting the growth of the plants leading to low crop yield

### **Problem Statement and Justification**

Agriculture remains the only major source of income in terms of employment and foreign exchange in Malawi. Climate variability has been significantly destructive and disruptive to crops growing communities threatening food security in the areas. Proposed research addresses one of the core challenges, as identified by the governments Malawi Growth and Development Strategy (MDGs) 2006-2011 of making Malawi food self-sufficient.

There has been a decline in maize production in Malawi over the past few years due to recurrent droughts, which have resulted in high variability in timing (onset), distribution and amount of rainfall and season length. Consequently, the past planting dates for maize in Malawi are out of phase and needs update (Kumwenda *et al.*, 1998).

Crop modeling can help to predict productions under variable climate scenarios and this can help to develop strategies and technologies that can mitigate effects of climate variability. In Malawi, little research has been done on crop modeling, therefore DSSAT will be one of the quick decision making tool in this research.

## Materials and Methods

### Site

The study focused on Chitedze Agriculture Research Station in Lilongwe District of Central Malawi.

Chitedze Agriculture Research Station lies at 13°58'S and 33°58'S; and altitude of 1146m. Chitedze falls in the mid altitude areas with annual rainfall of 800mm-1200mm, well drained sandy loam soils classified as alfisols (Brown and Young, 1962).

Table 1: Agro ecological characteristics of Chitedze Agricultural Research Station, Lilongwe District

Characteristics	Value
Length of growing period (days)	150-165
Mean temperature during growing period(°c)	20.0-22.5
Mean annual precipitation(mm)	800-1200
Mean number of dry months	7-8
Mean annual temperature(°c)	20.0-22.5
Mean minimum temperature of coolest month(°c)	10.0-12.5
Plant-extractable soil Water(mm)	27.4
Available N, average of two samples(kg/ha)	60.0
Altitude	1146m
Soil class	CSA-Humid Sub Tropical Class from koeppen Map in DSSAT model software

(Source: Application of a maize crop simulation model in the central Region of Malawi. (Thornton *et al.*, 1995).

### Data collection

Secondary data were collected on climate, cropping systems and soil characteristics

#### *Cropping systems and soil characteristics data*

Secondary data on maize cropping systems and soil characteristics were reviewed from literature. The sources of information included Chitedze Agriculture Research Station, libraries (Bunda College and Chitedze Agriculture research Station), ICRISAT, Ministry of Agriculture and Food Security, selected Extension Planning Areas (EPAs) in Lilongwe; Lilongwe Agriculture Development Division, Lilongwe District Agricultural Office, and scholarly articles.

#### *Climate data*

Climate data for the previous 30 years were accessed from the Department of Climate Change and Meteorology Services of Malawi. Solar radiation, daily rainfall, relative humidity, sunshine hours, minimum and maximum daily temperatures data were accessed. This data was complemented by data from NASA web portal.



## **Data analysis**

### **Descriptive and Inferential Statistics**

Estimate of cumulative annual totals of rainfall, temperatures, and yield data of the crops was plotted against their corresponding years. Time series, correlation and multiple regression was used to analyze the relationship existing between the variables. The Index of Agreement (d-stat), coefficient of determination ( $R^2$ ) were calculated to find out the direct relationship existing between the simulated and the observed maize yields of the past 12 years of cropping seasons.

### **Method of manipulation of the data set**

The data for 12 years starting from 1996/1997 to 2007/2008 growing season was used to plot graphs. The yield data was in form of production and these were converted into tonnes per hectare by dividing total production of that season by area. The observed yields were plotted against the seasonal rainfall to check the consistency of the data and the correlation between the yield and seasonal rainfall.

### **Rainfall and yield of maize**

The daily rainfall data starting from 1997/98 to 2007/08 cropping season was analyzed. That is total wet days, total dry days, seasonal rainfall, annual rainfall were calculated using the daily rainfall data for Chitedze Agriculture Research Station. The methodology used in this study was adapted from Genesis *et al* (2010).

The total dry and wet days hence were derived within the growing season (taking advantage of the seasonal period that is considering the onset of rainfall and cessation of rainfall). Dry days is amount of rainfall less than 1mm, whilst wet days the rainfall amount is 1mm or greater than 1 mm of rainfall received daily.

### **DSSAT program**

DSSAT-CERES maize model was used to run the simulations for maize at Chitedze under specific

climatic conditions, soil characteristics, cropping systems and management practices. The Decision Support System for Agro-technology Transfer (DSSAT) Version 4.5 is a software application program that comprises crop simulation models for over 28 crops. Data base management programs for soil, weather, and crop management and experimental data, utilities and application programs support DSSAT. The crop simulation models simulate growth, development and yield as a function of the soil-plant-atmosphere dynamics (Hoogenboom *et al* 2003).

### **Input files creation**

#### **Weather file**

The weather data was entered in excel sheet that includes rainfall, solar radiation, wind speed, sunshine hours, relative humidity. Then DSSAT utility weatherman was used to import the weather data for Chitedze. The DSSAT has the capability to recalculate the missing values once commanded.

#### **Soil file**

The soil file was created using the physical and chemical soil data of the study site. Firstly, the soil data was manually added into the soils database. DSSAT recalculated the missing values once commanded.

#### **Experiment file**

Creation of experiment file involved input of crop management data into the DSSAT model. For missing values, you look at parameters of a crop in the DSSAT that is similar to the variety of focus in the study. Running of the model was successfully done using the weather data, soils file and the experiment that was created using the crop management data

## Results and Discussion

### *Historical rainfall patterns effects on yields of maize*

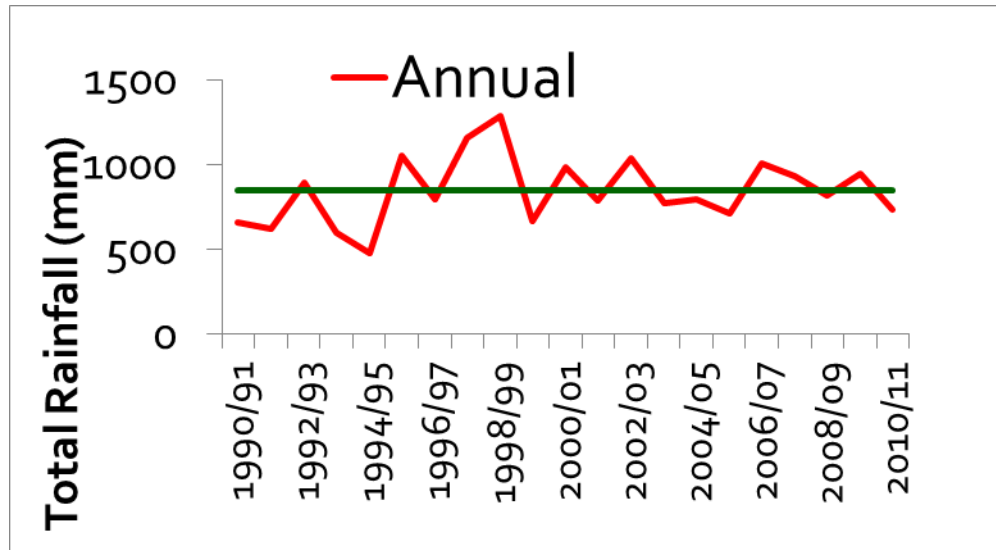


Figure 1: Seasonal rainfall variation from 90/91 to 10/11 cropping seasons at Chitedze Research Station

Figure 1 shows that rainfall at Chitedze Research Station is highly variable and is the most important variable affecting the yield. Maize requires a well-distributed, considerable amount of rainfall over an

appropriate numbers of days during its growing season for optimum yield, with largest rainfall of about 1285.1mm in the year 1998/1999 cropping season.

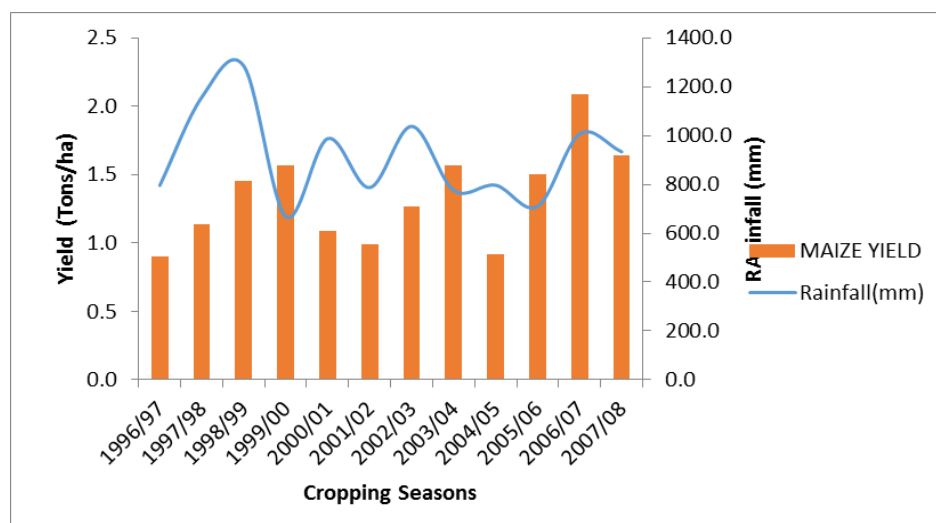


Figure 2: Maize grain yield and total annual rainfall for Chitedze Research Station from 1996/97 to 2007/08 cropping seasons

Figure 2 shows the trend of grain yield in Lilongwe District over 12 years from 1996/97 cropping season. Starting from 1996 there was an increase in yield but in 2000/2001, the yields decreased. The decrease in yield in 2000/01 coincides with the period Malawi experienced dry spells and a reduction in total annual rainfall. The results in Figure 2 shows that in 2000/2001 season and 2006/2007 period the rainfall amount was equal but yields were high in 2006/2007 season and this

could be due to introduction of farm input subsidy programme (FISP). The FISP programme solved the input problems like scarcity of seed and fertilizer which led people have sources of inputs (MOAFs 2006) for maize production. Cicek *et al* (2005) found out that of all the climatic parameters affecting crop production and yield, moisture is the most important parameter. Moisture is primarily gotten from rainfall that in the tropics usually cyclic and highly dependable.

Table 2: Total dry days, total wet days, annual rainfall (mm) seasonal rainfall amount (mm), rainfall onset date, rainfall cessation date, observed maize yield (kg/ha) and the length of the growing season

Cropping season	Total wet days	Total dry days	Length of growing season (days)	Annual rainfall (mm)	Seasonal rainfall (mm)	Rainfall onset day	Rainfall cessation day	Observed yield (kg/ha)
1996/1997	65	53	118	845	634	11 Dec	8 April	1100
1997/1998	70	63	133	1157	879	8 Dec	20 Mar	1100
1998/1999	64	37	101	1285	912	22 Dec	2 Apr	1500
1999/2000	56	53	109	669	498	22 Dec	10 Apr	1600
2000/2001	29	90	119	988	424	21Dec	21 Apr	900
2001/2002	34	59	93	787	577	21Dec	23 Apr	1000
2002/2003	48	59	107	1038	768.5	10 Dec	27 Apr	1300
2003/2004	53	43	96	776	713	10Dec	15 Mar	1600
2004/2005	47	47	94	845	680	8Dec	12 Mar	900
2005/2006	53	85	138	710	689	25Nov	12 Apr	1500
2006/2007	60	71	131	1007	927	27Nov	7 April	2100
2007/2008	79	23	102	933	873	7 Dec	19Mar	1600

Table 2 shows results of annual and seasonal rainfall distribution, number of wet and dry days, onset and cessation of rainfall, length of growing season and observed maize yields from 1996/97-2008/08 seasons. There is variation in weather variables as observed in the number of wet days (20-79), dry days (23-90), and length of growing period (93-118). 2006/2007 cropping season optimum maize grain yield were obtained because of less number of dry days.

cropping seasons having high dry days are associated with low yields. The results are also supported by high seasonal rainfall amounts of 927mm which is within the recommended levels for cropping season 2006/2007, according to Kumwenda (1998) between 800mm-1200mm hence high yields obtained, whilst in the cropping season 2000/2001 the seasonal rainfall amount was 424mm which is very low and below the recommended rainfall amount

The trend in table 2 shows that high yields are highly correlated to high values of wet days, whilst those

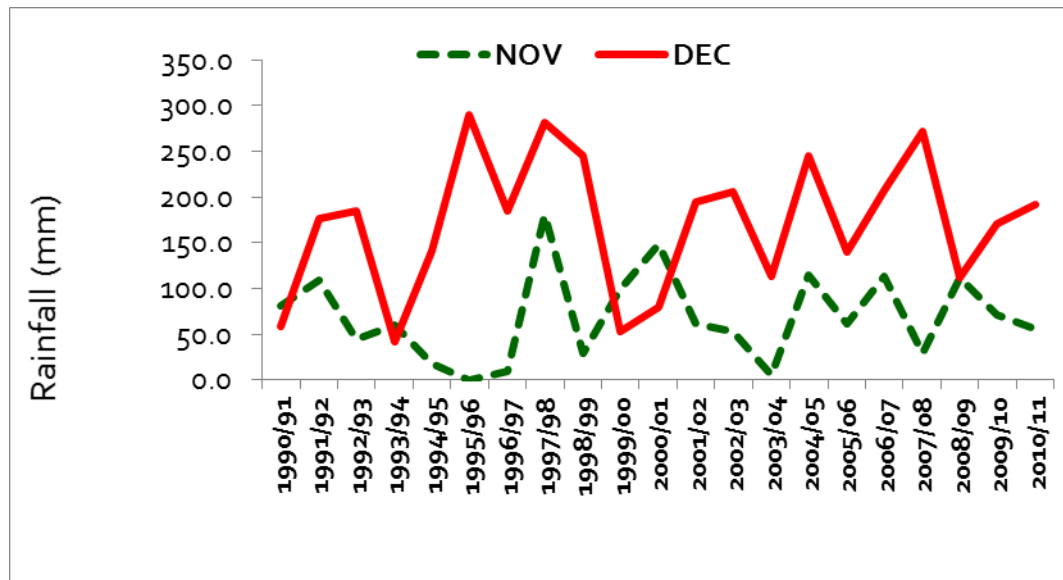


Figure 3: Monthly rainfall (mm) for November and December for Chitedze Research Station

Figure 3 clearly shows that December month, rainfall reached its peak than November. Rain days have the greatest influence on maize yield in the study area. This means that despite the fact that maize plant requires a

considerable amount of rainfall for effective growth and yield, it is still highly sensitive to excessive rainfall and continuous daily rainfall pattern typical of the tropics during raining season

Table 3: Results on number of wet and dry days for the month of November, December and January from 96/97 to 07/08 cropping seasons

Month	Mean		Standard deviation		Total days	
	Wet days	Dry days	Wet days	Dry days	Wet days	Dry days
November	5	25	4	4	65	325
December	27	16	3	3	189	214
January	18	13	4	4	235	168

Table 3 shows results on number of wet and dry days. December month received equitable amount of rainfall supporting the growth and development of maize plant because it had the largest mean wet days of  $27 \pm 3$ . This shows that maize planted in November and January suffers a moisture deficient and excessive wet condition respectively that affects maize yields. In January, the number of wet days were excessive than dry days, whilst November month the wet days were just too little ( $5 \pm 4$ ) compared to dry days ( $25 \pm 4$ ) which implies that if maize is planted this month, seed emergence and

establishment is affected by the dry spells and this has a negative implication on maize growth and yield generally that affects maize growth. For the month of December, the wet days were at recommended level ( $27 \pm 3$ ) versus dry days ( $16 \pm 3$ ) which is not very bad hence supporting maize growth. December could be the best time to plant maize as throughout there is an equitable amount of rainfall received in a number of days hence supporting the vegetative, flowering stages of the plant leading to efficient biomass partitioning hence high yields other than the January and December month.

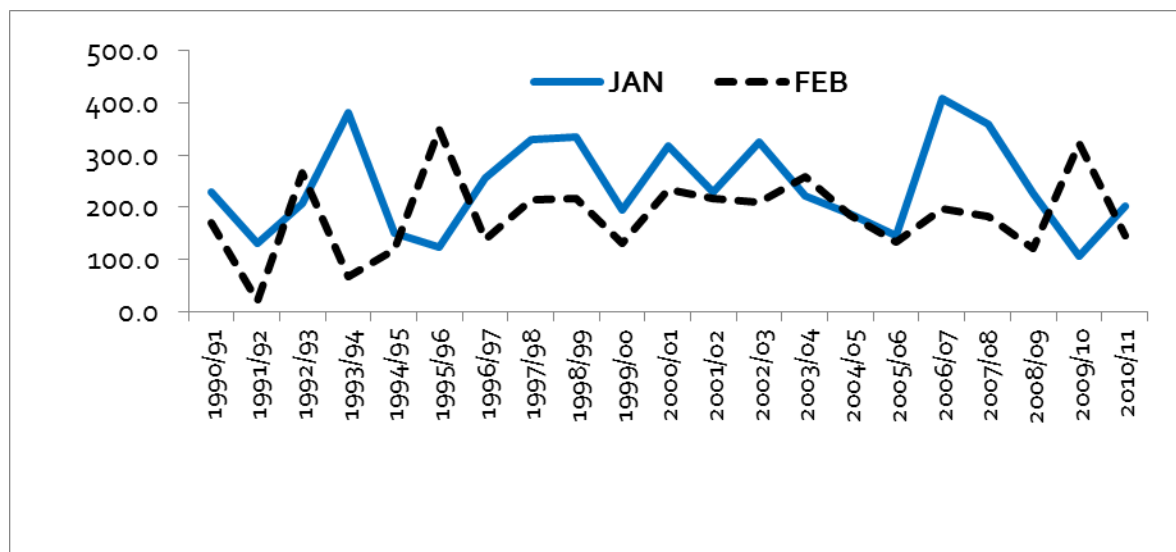


Figure 4: Monthly rainfall, January, February for Chitedze

Figure 3 and 4 shows the trend in rainfall patterns for the months where most farmers' plant maize crops during the period of December, January and those farmers who plant late, around January and February in Lilongwe District. Overall, the trends indicate variability of rainfall patterns over a 12 year period and this can affect planting dates hence all these changes in planting dates and months are partly due to continuous shifting of the rainfall patterns with later rainy onset in most of the areas in the country and earlier cessation in all areas.

In general, climate models have shown that in Malawi the trend in rainfall patterns will continue into the future (Malawi Vulnerability Assessment, 2013). With the average monthly rainfall expected to decrease during the months of December and January and increase during the months of February, March and April. Overall, the rain day frequency is expected to decrease slightly while dry periods are expected to increase. Therefore, since different areas are affected by rainfall variability at different levels, hence the research

should be conducted in a number of districts to find out how the yields respond to different rainfall amounts.

#### ***DSSAT application to simulate yields of maize***

The cropping management, weatherman and soils files were created to help in running the model. The main aim of using the DSSAT model was to check the model efficiency in simulating the maize yields for Chitedze Research Station. The simulation for this run was on 1<sup>st</sup> November of every cropping season, but the rest of the scenarios the simulation started 10 days before planting, so that the model could simulate and be able to model the available moisture in the soil.

Fig 5 shows results by control simulation. There is a positive correlation ( $R^2=0.788$ ) between the simulated and the observed maize yield which signifies that the DSSAT model was able to predict the yield of the historical data set. The simulated yields somehow substantially over predicted observed yields (mean=1429 kg/ha vs. 1350 kg/ha).

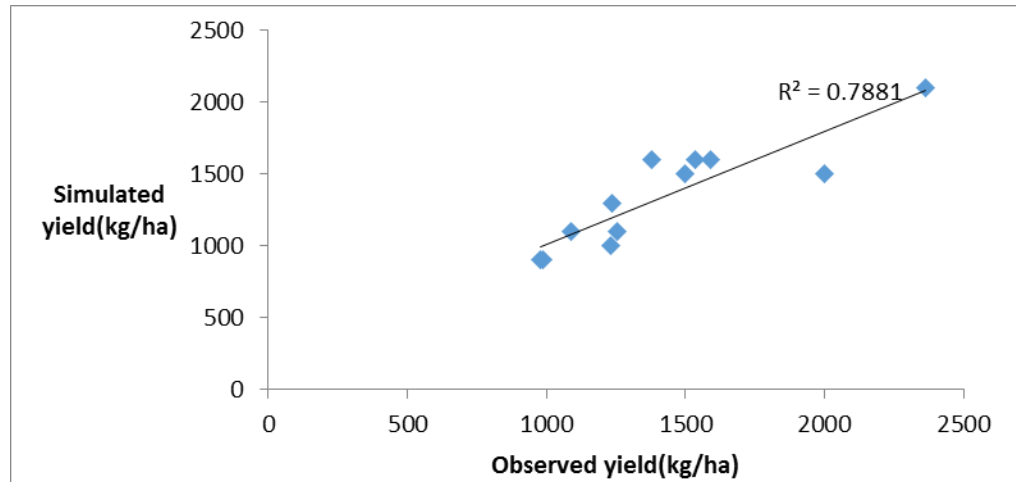


Figure 5: Simulated maize grain yields against observed maize grain yields

#### *Assessment of the effect of time of planting on maize yield in relation to climate variability*

This objective aims at applying different management scenarios to the model to check the effect on yield of maize. In this study, the effect of planting dates on the yield of maize was modelled. The planting dates were Early November (15 day of the month), late November planting (30<sup>th</sup> day), early December planting(15 December), late December planting(30 December), and

Early January(15<sup>th</sup> day), and late January (30<sup>th</sup> day). The assumption was that all agronomic parameters were kept constant for example soil fertility management, weeding only changing the planting dates. Rainfall being received, its impact on plant growth and development can be quantified easily compared to other climatic parameters like solar radiation, hence this study was focusing on the rainfall variability and how changing the planting dates affected the yield of the maize.

Table 4: Simulated and observed maize grain yield (kg/ha) at different planting dates

Planting dates	Mean observed	Mean simulated	Mean difference	R square ( $R^2$ )	RMSE (kg/ha)	d stat (Index of Agreement)
	(kg/ha)	(kg/ha)	(kg/ha)			
Standard run	1350	1429	78.93	0.788	198	0.888
15-Jan	1350	901	-448.84	0.525	519	0.534
30-Jan	1350	848	-501.58	0.479	491	0.463
15-Nov	1350	1102	-247.94	0.526	520	0.563
30-Nov	1350	1166	-183.64	0.678	292	0.663
15-Dec	1350	1250	-99.61	0.77	221	0.861
30-Dec	1350	1207	143.41	0.751	314	0.763

Table 4 shows that planting maize at Chitedze in early (15<sup>th</sup> December) produces high yield of both simulated and observed maize yield. The highest mean simulated yields are obtained using the DSSAT model on this date other than the standard run which has coefficient of determination ( $R^2$ ) of 0.788; RMSE is 197.90 and d-stat which is the index of agreement being 0.888 which signifies the capability of the DSSAT model to simulate maize yield at Chitedze, as the simulated and the observed maize yield are highly correlated.

The mean simulated yield for early December being 1250.39 kg/ha other than to the ideal situation. Planting in early December has many advantages because during this period the rain reaches its peak. From the results, the planting date of early December has got a coefficient of determination ( $R^2$ ) of about 0.770 which shows that the simulated maize yield and the observed yield are highly correlated implying the capability of the model or the model performed very well followed by 30 December then 30 November, 15 November, 15 January, 30 January having the  $R^2$  being 0.751, 0.678, 0.526, 0.525 and 0.479 respectively. The results show that planting on 30<sup>th</sup> January tends to reduce the yields. Planting on 15 December, from the table 3 it can be seen that the index of agreement is 0.861 which is very close to 1, it means it was a good simulation (Kihara *et al.*, 2010).

The root mean square of Error being 220.689 kg/ha which is small compared to the rest of the dates hence it signifies that little error of about 220.689 kg/ha of grain loss can occur if planted on 15 December whilst about 520.087kg/ha of maize could be lost when you plant your maize early November, that is you expect more grain loss or errors when you plant maize on 15 November, 30 January and 15 January because they have greater RMSE values being 520.09kg/ha, 491 kg/ha and 518.95 kg/ha respectively compared to 15 December because during the end January most of the soils have reached the field capacity already and also since during the end January most of the times the wet days are too much compared to dry days hence plant growth is inhibited. The concentration of rain days during rainy season has a great influence on maize yield annually such that the higher and less evenly distributed

the number of rain days, the lower the maize yield. This is in agreement to Cicek *et al* (2005) who found out that rainfall, sunshine, temperature, evaporation, are closely interrelated in their influence on maize. However, of all the climatic parameters affecting crop production and yield, moisture is the most important parameter. Moisture is primarily gotten from rainfall that in the tropics is cyclic and dependable. During period of heavy rain, the interception of light from the sun is not absorbed to the maximum point hence some physiological and biological processes are affected which affects the development and growth of maize crop for example canopy cover which when not sufficient tend to reduce the dry matter.

In terms of early November, generally the dry days were greater than wet days hence this affects the water uptake by the plants affecting the yield/dry matter accumulation at the end of the season. Early November here in Malawi, rainfall is not yet at its peak, hence planting around November could be the waste of grain as germination is also affected. Despite the fact that maize plant requires a considerable amount of rainfall for effective growth and yield, it is still highly sensitive to excessive rainfall and continuous daily rainfall pattern typical of the tropics during raining season.

The mean difference of simulated and observed yield is small, that is the observed yield deviate by about 99.61 kg/ha which is a small difference compared to the other five dates which have a larger value, which signifies how close the simulated yield is to the observed yield which signifies how good the DSSAT model is in simulating. Planting around early December is in agreement with the fewer dry days as shown in figure 9 experienced at Chitedze, meaning the soil had good soil moisture condition.

The results show that planting maize early December increase maize yields with low reduction (9.59%). Overall, the results show that planting early December (15<sup>th</sup> December) and late December (30<sup>th</sup> December) there is no much difference in terms of yield. Smallholder farmers if they plant on early and late December optimal grain yields are produced.

The results from this study recommend smallholder farmers to plant in December other than November and January month. January and November month are associated with high grain losses of 15.98% and 35.2 % respectively. It is the role of scientists to mainstream good management practices like encouraging

smallholder farmers to plant early to make full use of the rainfall that is equitably distributed during the December month.

Reduction % =  $\frac{\text{Average observed yield} - \text{Average simulated yield}}{\text{Observed yield}} \times 100$

Table 5: Reduction of maize yield planted in December, November and January

MONTH	TOTAL SIMULATED YIELD (kg/ha)	Average simulated yield (kg/ha)	Average observed yield (kg/ha)	Yield reduction (%)
DECEMBER	2456.98	1228.49	1350	9.59
NOVEMBER	2268.42	1134.21	1350	15.98
JANUARY	1749.58	874.79	1350	35.2

## Conclusion

DSSAT has the capability to simulate crop yield. However, there is requirement to downscale at farm level. It was observed that the model simulated some high yields than observed from the field experiments. Generally, this could be so because the model itself can be off by one day as it does not clearly indicate when during the day the rainfall occurred and at what time the measurements of rainfall were taken. There was no consistent relationship between the trends in the observed annual rainfall and the observed yield. Rainfall distribution within the growing season affects the maize yield responses. The critical rainfall variables affecting the maize yield were seasonal rainfall, total wet days and total dry days.

## Acknowledgement

Dr W. Mhango and Dr. L. Fiwa (Supervisors)

FANRPAN- SECCAP project for the financial support through Lilongwe University of Agriculture and Natural Resources (LUANAR).

### AWARD

Department of Climate Change and Meteorology, Malawi for the accessing climate data.

Ministry of Agriculture and Food Security, Lilongwe ADD, Chitedze Agriculture Research Station; CGIAR Centres (ICRISAT, SARNETT, CIAT, CIMMYT), Lilongwe, Malawi.

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## Appendix

*Table 6: Soil moisture % characteristics data for Chitedze Research Station*

Soil depth	Early season		Mid season	
0-20	11.1	13.6	29.1	24.1
20-40	25.0	16.3	29.1	22.2
40-60	25.0	22.0	26.4	47.2
60-80	25.0	28.2	24.8	26.5
80-100	42.9	16.3	28.2	7.3
100-120	25.0	16.3	29.9	27.1
0-20	16.3	13.6	28.4	32.1
20-40	25.0	22.0	25.5	9.2
40-60	25.0	19.0	26.1	14.3
60-80	25.0	22.0	27.7	35.2
80-100	25.0	28.2	26.6	35.1
100-120	25.0	22.0	26.5	12.3
0-20	11.1	16.3	26.4	23.6
20-40	25.0	22.0	22.6	29.7
40-60	11.1	22.0	24.3	53.6
60-80	25.0	22.0	24.3	23.9
80-100	25.0	28.2	23.3	13.7
100-120	25.0	31.6	23.0	23.2
0-20	25.0	28.2	24.4	22.8
20-40	11.1	22.0	19.7	24.8
40-60	25.0	28.2	26.1	43.6
60-80	25.0	28.2	24.1	22.0
80-100	25.0	28.2	26.0	22.8
100-120	25.0	28.2	24.7	23.8
0-20	11.1	22.0	15.1	24.5
20-40	11.1	19.0	17.4	28.3
40-60	25.0	19.0	17.9	24.1
60-80	25.0	22.0	17.3	23.2
80-100	25.0	28.2	16.8	22.5
100-120	25.0	35.1	17.2	24.6

*Table 7: Soil moisture % at Chitedze Agricultural Research Station*

DEPTH(CM)	Soil moisture			
	EARLY SEASON	CALIBRATED	MID SEASON	CALIBRATED
0-20	14.92	18.74	24.68	25.42
20-40	19.44	20.26	22.86	22.84
40-60	22.22	22.04	24.16	36.56
60-80	30	24.48	23.64	26.16
80-100	28.58	25.82	24.18	20.28
100-120	25	26.64	24.26	22.2

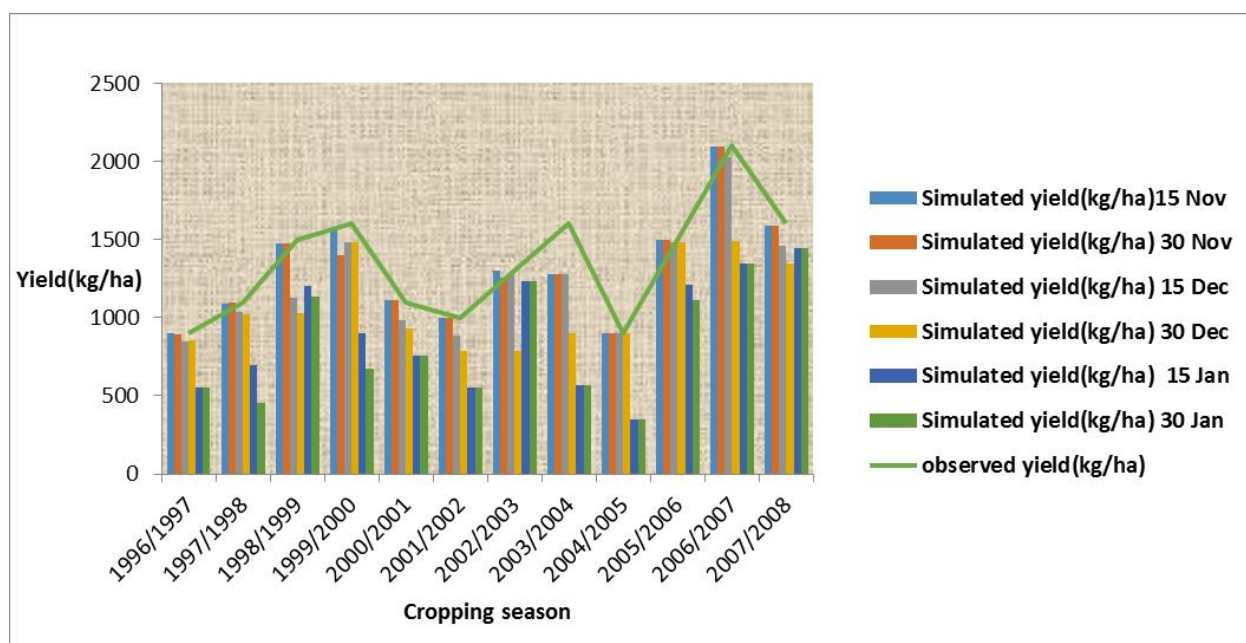


Figure 6: Simulated and observed maize grain yield for different planting dates against cropping seasons.

# PEASANT CLIMATE KNOWLEDGE, APPLICATION ON CROPS OF ONION BULB (*Allium cepa* L.)

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## Abstract

In agricultural areas where there is no irrigation water, the farmers scheduled crops based on their climate knowledge, which plays a key role. This knowledge is only recorded in the memory and is transmitted from generation to generation orally. Although its utility is known, however are unknown, the factors influencing farmer's prediction skills and how this knowledge enables them to schedule crops. The present study was carried out in the province of Tungurahua, south western area, which is characterized by the practice of the rain fed agriculture and its tradition in the cultivation of bulb onion (*Allium cepa* L.) since 40 years ago. The objective was to analyze the peasant climate knowledge used in the cultivation of onion bulb. For which, 50 informants were selected using the methodology "snowball", who conducted semi-structured interviews. In addition there was geographic coordinates into 140 plots of onion. Data analysis was carried out with descriptive statistics and inferential. The results show that local knowledge is related to among other factors, the level of education and gender. Depending on the plantings and harvests, identified three groups of farmers, of which the largest considers the rainy periods to start planting, while the others consider the climatic cycles and market prices. The results can be useful in the development of agricultural climatic calendars. It is suggested to consider climatic particularities in technology transfer

**Keywords:** traditional knowledge, onion crop, climate change, peasants, climatology

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## Introduction

In vast areas of the Andean region, agriculture is based only on the rainfall, which requires a deep understanding of the local climate by the peasants. Agriculture based on climatic cycles, presents a high risk for the peasants who, to reduce applied strategies, such as the use of species that are resistant to drought or frost (onion bulb) (Riera & Graciela Pereira, 2013). According to Nara, Mao, and Yen (2014) climatic factors, are determinants for crop growth. The excess or shortage of rains, affect the prices of agricultural products and in the presence of diseases. Rainfall and temperatures, plays a key role in agricultural development by what their knowledge is very

important. (Holzkämper, Calanca, & Fuhrer, 2011).

Given that the farmers to plan its cultivation, base in its personal experiences (Altieri, 1997), rather than in official climatic reports, the intuition of the peasant plays an important role to achieve good harvests (Kolawole, Wolski, Ngwenya, & Mmopelwa, 2014). On the other hand, farmers do not have the habit of recording in written form their ancestral knowledge, only do so in his memory and transmitted orally from generation to generation (Altieri, 1997). In addition, the low generational renewal in agriculture limits the transfer of local climate knowledge from adults to young farmers, while the changing climate environment constitutes a new learning scenario

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Despite the usefulness of climate knowledge in agricultural production, this has been rarely addressed in scientific research. In this regard are unknown factors that influence the abilities of the peasants for predicting the weather and how this knowledge allows them to plan their crops.

Preliminary investigations indicate that of onion crop is sensitive to brightness since its presence or absence affects the amount of quercetin (to sweeten) (Ko, Nile, Sharma, Li, & Park, 2015) and that the amount of water supplied to the crop, influences the quality of the bulbs and in the time of storage after harvest (Fan, Wang, & Nan, 2014). Which shows that the rainfall in one way or another affect the onion, so that their crop demand a broad knowledge of the local climate. On the other hand Kolawole *et al.* (2014) were identified that the peasants are experts in assessing seasonal weather patterns through their experience in the field. In the Province of Tungurahua (South-western area), the cultivation of onions from 40 years ago is one of the major items of the peasant economy. The research is based on the assumption that farmers in this region retain broad climate knowledge for the practice of seasonal agriculture. The objective of the research was to analyze the local climate knowledge related to the cultivation of bulb onion (*Allium cepa*), in the region south-west of the province. The information gathered contributes to the structuring of calendars agricultural

climate, which can be supplemented by similar studies in other regions of the province of Tungurahua. The results also reflect the great contribution that can offer the peasant knowledge to understand climate cycles in agriculture. In a changing climate environment, this knowledge can contribute to reducing the vulnerability of the agricultural sector (Gunasekera, 2010) (Hiwasaki, Luna, Syamsidik, & Shaw, 2014).

## Method

### Description of the study area

The study area is located in the province of Tungurahua (South West), comprising the cantons of Quero and Mocha, located between the geographic coordinates of  $01^{\circ} 22' 35''$  of South latitude and  $78^{\circ} 36' 21''$  of length West, between altitudes of 2700 to 3600 meters above sea level. In this territory, information was collected in the following sectors: Yayurwi, Chocalo, Rumipamba, Pilco, Guangalo, Yanayacu (without irrigation areas) and the lower area of Mocha (Figure 1), which have in common the presence of crops of onion bulb (Banco Central del Ecuador, 2014). The climate of this region is temperate-cold (annual average  $13^{\circ}\text{C}$ ) with moderate rainfall (589.31 ml/year). The soils vary according to the altitude, in the lower part are sandy and in the upper parts are clayey loamy.

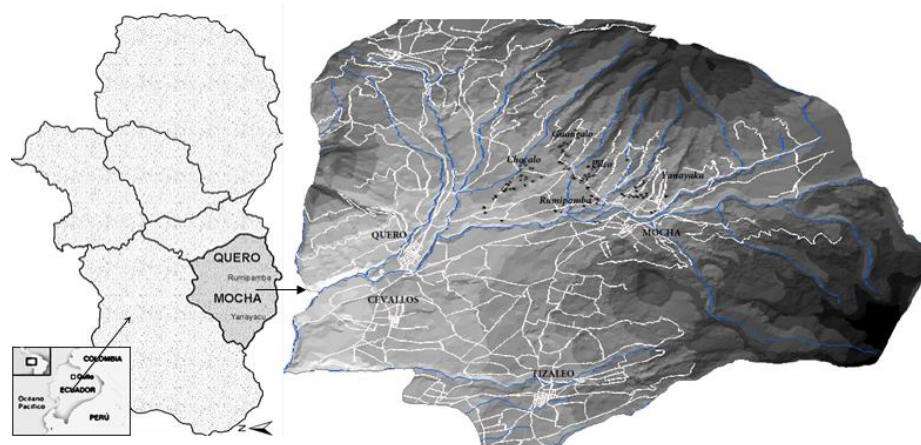


Figure 1: Location of the study area

### ***Climate analysis of the area***

This was done on the basis of two sources: (i) Climate information recorded between 1986 and 2014 by the weather station Quero-chaca (part of the network of the National Institute of Meteorology and Hydrology), average temperatures in degrees Celsius (°C), precipitation (ml/year) and heliofania (light-hours a day) and (ii) field information collected through semi-structured interviews and in depth, according to the methodology suggested by Pedraz, Zarco, Ramasco, and Palmar (2014). The informants' selection was realized in base in the methodology "snowball" proposed for (Sandoval, 1996), for which were considered two criteria: (i) have crops of onions and (ii) be located in the delimited territory. Fifty peasants were interviewed, between April and May of 2015, the content of the interview treatment on: the experience of peasants to predict rain throughout the year, the use of bio-indicators for the prediction of rain, the transfer of knowledge and the skills of observation of clouds and stars to predict rainfall (Kolawole et al., 2014).

### ***Market analysis,***

Databases of the wholesale market of Ambato, of the years 2010 to 2014, and statistics of the Central Bank of Ecuador, were analyzed to find out the price fluctuations of the onion bulb of the province of Tungurahua. It is analyzed the movements of prices per month for four years expressed in dollars, for which the statistical program SPSS 19 was used.

### ***Spatial analyses of crops***

Geographical coordinates were recorded in 140 cultivated plots of onion, through travel on the territory selected. The selection of the plots was carried out considering the criteria of horizontal and vertical location of the crops in the territory, that according to Altieri and Nicholls (2009) in the Andean region vary according to the altitude. The data were processed by the ArcGIS program 10.1(Figure 1)

## **Results and discussion**

### ***Conditions of the farmers***

The average age of farmers who are dedicated to the cultivation of onions is 45 years (standard deviation - DT - 11.09). Of the total (n = 50) of interviewees: 52% were women, who on average have 46.8 years and 48% men with an average of 43.2 years. The participation of women in agriculture is higher than the reported by FAO (2011) 43%. The presence of a higher number of women working in agriculture, confirms to Lastarria-Cornhiel (2008) who said that Latin America, presents a process of feminization of agriculture, among other things by the migration of the male population, the development of non-agricultural activities in the rural areas. The 64% of the farmers have between 32 and 50 years of age, only 8% are between the ages of 21 and 30 years of age. According to Zagata and Sutherland (2015,1) in developed countries where the farms are small, is also reported shortage of young people in the agricultural activity. These data show a low level of succession between the onion growers in the studied area.

The 16% of the farmers interviewed, reported not knowing how to read and write, this value is higher than the national rate (13.5%) (Instituto Nacional de Estadísticas y Censos, 2012).

### ***Crop Conditions***

In terms of varieties of onion bulb cultivated: The 90.3% corresponds to *Allium cepa* variety Aggregatum (spread by bulbs) and the 9.7% *Allium cepa* varieties Burguesa, Red Star y Perla (spread by seeds). The first crops of the variety Aggregatum, were established by 1975, while the first crops of seed varieties, by 1995. Despite the introduction of new varieties, the majority

of farmers cultivate the variety Aggregatum, to which they attach greater resistance to rotting of the root caused by *Sclerotium cepivorum* Berk, which contradicts to Granados (2005) who stated that there are no varieties of onion bulb resistant to this fungus. The first crops of onions propagated by seeds produce good

yields, however in the following years decrease because of the root rot. The development of rot, according to Walker 1969, referred to in Granados (2005) is favored by soil moisture. In the rainy months this disease can be more aggressive than in the dry months. From there, the knowledge of the cycles of rainfall can help reduce risks and to select the most suitable varieties.

As regards the irrigation system, 96.8% of plots are cultivated in grounds without irrigation water, this indicates that the agriculture of this area is highly determined by the rains cycles.

### *Use of peasant climate knowledge*

The development of agriculture in this region, demand a broad knowledge of the climatic cycles to schedule agricultural activities during the year. The 63% of the farmers interviewed mentioned that the rainiest month is May, and between the 36 and 46% believe that the rainy months are also: March, April and June. And the months more dry are November and December (30%), August (28%) and September (26%). While that for 50% of interviewed the coldest month is August, for the 8 and 10 per cent are also very cold July and September, based on this information, the farmers scheduled sowings. Three groups of farmers have been identified in this respect: A first group (G1) (21 %) than planting between September and November; a second group (G2) (49.4 %) than planting between December

and January and a third group (G3) (26 %) than planting between February and mid-March, it is clear that the times of planting influence in the months of harvests. Even though all farmers know the traditional climate calendar, among them there are three groups of farmers, which reflects a more climatic cycles, is also considered the market prices (Retnowati, Anantasari, Marfai, & Dittmann, 2014).

The knowledge also depends on the skills of the peasants for forecasting the weather; in this respect it was found that 70% are able to predict rainfall through the observation of groups of clouds (Table 1). In applying the Chi square test to the variables: ability to predict rainfall by observation of clouds and levels of education was obtained a high significance (0.826) which suggests that there is a strong relationship between these variables. For the prediction of rainfall the farmers also based on observation of bio-indicators such as the flight of swallows (*Hirundo rustica*) and the sound of the Toad (*Chaunus spinulosa*) which is also reported by Gómez (2014).

On the other hand the gender analysis revealed that the women are able to predict the presence of rain through the clouds (80%) more than men (58%). Which can be attributed to the intuition, which according to Sadler-Smith (2011) in women is more developed than in men, this capability would also associated with the ability of women in the observation.

*Table 1: Climatic knowledge of the peasants*

Preguntas	Totally agree (%)	Agreed! (%)	indeciso (%)	In disagreement (%)
Based on personal experience, you can predict if there will be enough rain or not in agricultural year?	6,0	44,0	46,0	4,0
Through the songs of some birds and toads sounds, can predict whether it will rain or not?	12,0	66,0	18,0	4,0
It takes decisions necessary for overcoming any problem of the time required and appropriate?	14,0	66,0	18,0	2,0
A través de ciertas plantas, puede predecir si va a llover o no?	14,0	22,0	58,0	6,0
Their ancestors of those who acquired the agricultural knowledge had broad and proven experience to predict the time from which you learned?	18,0	36,0	42,0	4,0

Can you through the observation of groups of clouds in a certain direction in the sky predict whether it will rain or not?	22,0	48,0	28,0	2,0
Can you predict the abundance or scarcity of rainfall based on the pattern of early rain in agricultural year?	10,0	28,0	60,0	2,0
Through the observation of star constellations and/or the moon, you can predict whether it will rain or not?	10,0	44,0	42,0	4,0

### ***Cultivation of onion, rainfall and temperatures***

According to reports from the meteorological station of Quero-Chaca, the months of highest precipitation are: April, May and June (66.5; 57.6; 62.8 ml) respectively. While the driest months are: January, September and December (35.8; 35.3; 39.8 ml) respectively (Figure 2a). To compare this information with the appreciation of the peasants there is match both in the dry and the rainy months. According to Estudios e Investigaciones Meteorológicas (2014), in November in Quero-chaca and Riobamba was presented a water deficit, which affected the development of agriculture and livestock due to their water needs were not met.

As the G1 begins between September-November sowings, the crop cycle in this case extends to February, March and April. According to Schwartz and Cramer (2011) the onion bulb presents two defined phases: the first that starts with the growth of root and stem and ends with the growth of the prebulbo (90 and 110 days after planting); the second phase begins with the development of the bulb (2.5 to 4.0 cm) and ends with the formation of the bulb (over 170 dds), considering these phases, the crops of the G1 traverse two critical months of rainfall (December and January) and benefit from the light showers (October and November), however the restriction of irrigation for long periods can have negative effects on the conservation of the onion bulbs stored (Rattin, Assuero, Sasso, & Tognetti, 2011). While the crops grown by the G2 (December and January), ending its cycle between May and June, in this case the crop takes advantage of high precipitation and the final phase of the culture coincides with the decline of rainfall, however, this may cause increased incidence and severity of diseases to foliage, such as the macha purple (*Alternaria porri*), or more attack of pathogenic fungi to the bulb, due to the

high humidity of the soil (Osuna-Canizales & Ramírez-Rojas, 2013). And finally the crops grown by the G3 (February and March) are harvested between July and August, in this case the first phase of the crop benefits from the rainy months and in the final stage coincides with the reduction of precipitation, this enhances the quality of the bulb. According to Martín de Santa Olalla, Domínguez-Padilla, and López (2004) crops exposed to high volumes of water in the stages of growth and maturation, produce larger bulbs. While that the scarcity of water in the time of bulb formation, it leads to a greater number of small bulbs.

Since in the area, drops heavy rainfall in the months of April, May and June, it is necessary to implement systems to capture rain-water, to reduce soil erosion, store and use in precision irrigation, for which there is a need for climate information in real-time (Leite, Martínez-Romero, Tarjuelo, & Domínguez, 2015).

On the other hand, the lowest temperatures according to the weather statistics are recorded in July and August (11.5 oC) and the highest in November, December and January (13.8 oC) (Figure 2b).

The appreciation of the peasants on the colder months, agrees with data from the weather station. However the presence of frost is not a limiting factor for the crop. In the months of frost most of onion crops are in the final stage.

While the information of the weather station of Quero-chaca, reveals that between the years 1986 and 2014 there was an increase of the temperature (Figure 2c), which can have a positive or negative effect on the crops (Ashardiono & Cassim, 2014). To raise the temperature there is most likely to increase the onion crops and other crops to higher altitude, which



would adversely affect the paramo ecosystem. According to Bobojonov and Aw-Hassan (2014) in a scenario of climate change, the effects depend on agro-ecological zones as well as the social conditions of the population. As well in the medium term some farmers may benefit by the more favorable conditions for the growth of the crops, however, in the long run these expectations can be unfavorable due to scarcity of water for irrigation. A strategy for mitigating climate change may be to introduce new crops better suited to warmer climates as proposed Dosery, Mathew, Suresh, and Al-Menaie (2012).

The months of more light-hours are October, November, December and January. According to Ko et al. (2015), the light influences the amount of quercitina (glucose) that is one of the major flavonoids present in the onion. Given that the onion plants only receive natural light, the months most appropriate for the final stage of the crop would be January, October and November however, in these months the majority of crops are in the early stages.

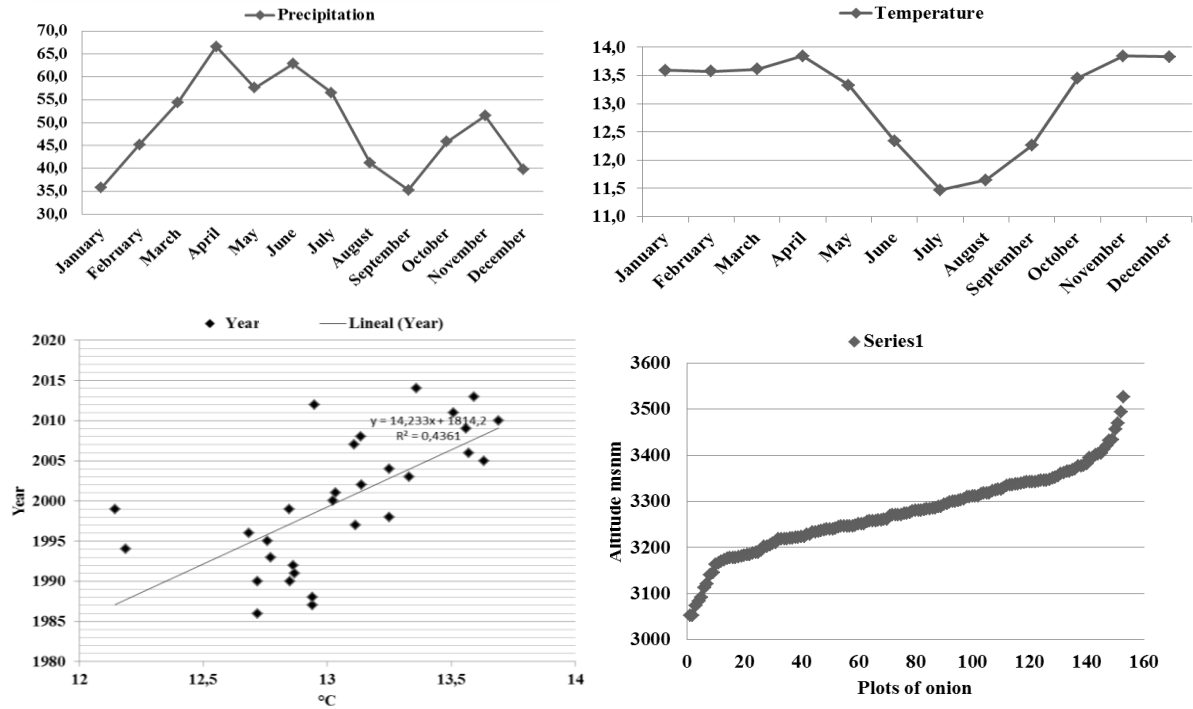
#### ***Onion crops and height above the sea level***

Crops of onion bulb in this region, were found from 3052 meters above sea level, up to the 3526 meters above sea level, the greater amount of plots (75.7%) were located between 3200 and 3400 meters above sea level (Figure 2d). According to Bach *et al.* (2003) the evapotranspiration decreases at higher altitudes, and when the evapotranspiration is low, affects the dynamics of water in the soil and the infiltration increases. On the other hand, each species grows in certain climatic conditions, so that its spatial location of the crops, also serves as a baseline for tracking of the species in the future (Holzkämper *et al.*, 2011). The expansion of agricultural areas, is explained by the advance of urbanization in the lowland areas, a phenomenon that also occurs in other regions of the world (Lin, Huang, & Budd, 2013).

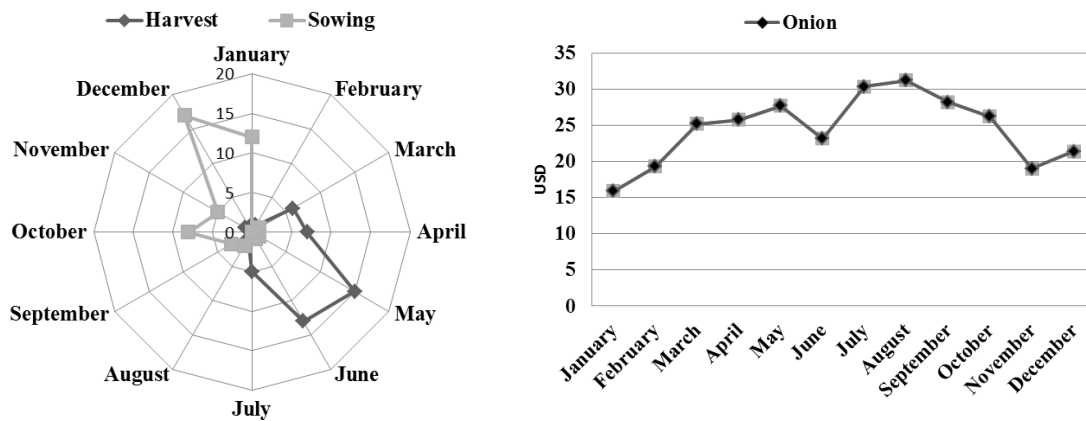
#### ***Periods of crop and market prices***

As mentioned before, the time of the harvest depend on the time of planting. In this context was found: the G1

sells their crops between February and April, when the average price on the wholesale market of Ambato is between 19.3 and 25.2 USD; the G2 sold between May and June (27.7 and 23.2 USD) and the G3 sold between July and September (30.3; 31, 28.2 USD) (Figure 3a), these costs relate to a package of 45 kg. It is clear that the G3 sells in the best prices, while the G1 sells at lower prices; this suggests that farmers decide when to plant based on market prices and rainy seasons. While prices (2013) highest at the national level, were reported between April and May (Ministerio de Agricultura Ganaderia y Pesca, 2013). Although prices in the wholesale market of Ambato presented a common pattern during the year (Figure 3b), these vary from one year to another. The prices in this market in addition to the climate, depends on other factors such as: the commercial opening of the Ecuador with neighbor countries and the increase in consumption of onions in certain months of the year. In this regard, the peasants are dissatisfied with the imports of onion that affects the prices of the national onion. In areas traditionally of onion crops as Yanayacu, it was noted that little plots of this crop (10). In the Canton of Mocha considered as an area of high production of onion found little plots of the crop. According to the peasants, the cultivation of onions is ceasing to be profitable, this indicates that the trade agreements for the import of onion affect domestic production (Jayne, Chamberlin, & Headey, 2014).



**Figure 2:** a) Precipitation overage 1986-2014; b) Temperature overage 1986-2014; c) Temperature rise 1986-2014; d) Plots of onion and altitude



**Figure 3:** a) Cycles of sowing and harvests of Onion; b) Average prices of onion (variety aggregatum) 2010-2014, in the wholesale market of Ambato.

## Conclusions

The local climate knowledge in this region is the basis for the establishment of the crop (onion), which depends on the skills of the farmers who have acquired through experience and by oral transmission of their ancestors. Among the peasants this knowledge is related to the level of education and gender. The peasant knowledge compared to meteorological statistics has high coincidence in relation to the periods of rainfall, drought and frost. Farmers take advantage of the dry months (November December) to start the plantings of onion, which enables them to take advantage of the rains in April, May, June, when the majority of crops are in full development. Times of crops of the majority of lots do not coincide with the months of high onion prices, which show that crop planning is not only depending on the market, but rainfall cycles.

## Acknowledgement

Thanks to the Faculty of Agricultural Sciences and the authorities of the Technical University of Ambato

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## POTATO PRODUCTION UNDER BRACKISH WATER AND COMPOST USE

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### Abstract

The non-linear transition to adulthood of today's youth signaled by numerous theoreticians has consequences on the forms of political participation favoured by young people. Nevertheless, citizenship education continues to play a significant role in shaping the political behaviour of future adults. After highlighting the characteristics of citizenship education in Europe as outlined in the most recent Eurydice study (2012) and briefly presenting the alternative forms of political and social participation embraced by the young generation, the present paper aims to determine the level of interest and involvement of Romanian teenagers in political and social activities at both national and European level. The research method employed is a quantitative one, pupils from the 11<sup>th</sup> and 12<sup>th</sup>- grade being the subjects of a survey conducted in six Romanian high schools. Findings reveal that although teenagers are willing to make their voice heard by various means, including alternative forms of participation, they still trust the effectiveness of electoral forms of participation in influencing the decision-making process and are eager to exercise the democratic right of voting.

Keywords: Keywords: potato, brackish water, compost, salt accumulation

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### Introduction

Many potato production areas in Jordan are characterized by the availability of brackish water that can be used for irrigation. The obstacle of using such water in irrigation is that the yield has been known to decrease as salinity level increases. Salinity reduces water availability in a similar manner for all types of plants; however there is a variation upon crop responses to salinity. The effect of salinity level of irrigation water depends on the potato cultivar (Zhang et al., 1993). The other obstacle of using saline water in irrigation, is the possibility that water can be a source of salts and lead to soil salinity accumulation. Potatoes are moderately sensitive to salinity compared with other crops; soil salinity level of 2 ds/m reduces the yield up to 50% (Maas and Hoffman, 1977, FAO, 1989), particularly at the early growth stages (Levy 1992, Nadler and Heuer, 1995). About 74% of the normal yield of a cultivar can be obtained under surface irrigation with water salinity levels of 2-4 ds/m (Paliwal and Yadav, 1980). According to FAO (1989), the

potato yield ranges between 540 and 3300 kg/du and the salinity threshold (100% yields) for brackish irrigation water is 1.1 dS/m. The highest yield (3300kg/du) was obtained with fresh water irrigation (average EC 2.2 dS/m) followed by (3000 kg/du) which was obtained with an average water salinity of 3.8 dS/m. Bustan et al., (2004) reported that drip irrigation with saline water up to 6.2 dS/m had no significant effect on potato production in an arid environment, but the interaction between saline irrigation and prolonged heat wave events that occur during crop growth caused a decline in potato yield. The accumulation of soluble salts in the soil is directly related with the salt content of the irrigation water (Somani, 1991). Over the years, soil salinity level increased linearly with time for the soils irrigated with saline water, this case has been seen in some areas of intensive agriculture in the Jordan Valley that are becoming saline soils (JVA, 2003). The salinity problem related to water quality occurs if the total quantity of the salts in the irrigation water is high which leads to salt accumulation in the root zone. In

areas where annual rainfall is less than 250 mm, saline water EC more than 4 dS/m will cause salt toxicity for most crops, while in areas where annual rainfall exceeds 500 mm, water salinity up to 16 dS/m can be utilized for some crops (Tesdeschi and Menenti, 2002). Potato is one of the most important vegetable crops grown in Jordan and Jordan's water availability ranks among the lowest in the world. The use of saline water in potato production could be an important strategy in sustainable agriculture in Jordan, however potatoes are very sensitive to their growth environment and not all potato cultivars are adapted to the Jordan zone and water qualities. The effect of salinity level of water depends on the potato cultivar and farmers are more interested in the cultivars producing high yields under their growth conditions without taking into consideration the impact of accumulated salts that can lead to soil degradation. The main objective of this research is to study potato production irrigated with brackish water by minimizing salt accumulation by adding organic matter.

## Materials and Methods

This experiment was conducted at the Agricultural Research Station of Al-Balqa' Applied University, Jordan during the 2014 growing season to examine the sensitivity of some local potato cultivars to saline irrigation water, to study the impact of saline water on soil quality and to investigate the impact of adding compost on salt accumulation in the soil. The brackish water was transported from a local spring in the Jordan Valley. Water was mixed with different amounts of fresh water to obtain the required water salinity levels. The three potato cultivars; Spunta, Faluka and Ambition were tested for salt sensitivity and compost use to reduce the impact of salinity. Treatments were arranged in a Randomized Complete Block Design (RCBD) with a Split- Split Plot arrangement with three replicates. The potato cultivars were arranged in the main plots, the brackish water treatments were in the sub-main and the soil amended treatments were in the sub-sub plots. Compost of EC of 0.72 dS/m and pH of 7.4 was used. The three cultivars (one tuber seed/ pot) were planted in 15 L pots with different soil and compost percentage as the following: T1 100 % Soil and 0% Compost, T2 80 % Soil and 20% Compost,

T3 60 % Soil and 40% Compost, T4 40 % Soil and 60% Compost, T5 20 % Soil and 80% Compost, T6 0% Soil and 100 % Compost. All pots were drip irrigated equally with three water salinity levels: fresh water (as the control) 1.25 dS/m, 5 dS/m and 10 dS/m. Average root zone salinity (dS/m) was measured by preparing a 1:1 oven dried soil and distilled water (50 gm of soil mixed with 50 gm of distilled water), the electrical conductivity of the soil water solution was measured after 5 minutes by an EC meter. Tubers weight (g/pot) was determined for each cultivar under each water salinity in the three replicates. The drained water was collected and salinity was measured at weekly intervals. All statistical analyses were performed using SAS/STAT Version 9.2 and Analysis of Variance was conducted by the PROC GLIMMIX procedure.

## Results and Discussion

### *Effect of brackish water on potato yield:*

The effect of water salinity on the yields of potato cultivars was evaluated at the end of the experiment (June 2014). As presented in Table 1, the highest yield (g of tubers/pot) was recorded for Spunta cultivar. It was significantly higher than that of Ambition and Faluka. This indicates that this cultivar possesses less salinity sensitivity than the two other cultivars. In addition, the greatest yield reduction, as a result of saline water treatment, was recorded for plants that received water with salinity level 10 dS/m (Table 2 and Figure 1). The yield reduction was significant when compared to the reduction caused by other water salinity treatments. It is worth mentioning that, in contrary to Spunta and Ambition, the yield of Faluka cultivar was higher in plants that received water with salinity level 5 dS/m than those irrigated with water of EC 1.25 dS/m (Figure 1).

Table 1. Yield of three potato cultivars at the end of the experiment (June 2014).

Cultivar	Yield per pot Mean (g)
Spunta	298 a
Ambition	182 b
Faluka	190 b

\*Means followed by the same latter are not significantly different according to LSD test at 0.05 level of probability.

Table 2. Effect of water salinity on potato yields.

Water Salinity	Yield per pot Mean (g)
1.25 dS/m	294 <sup>a</sup>
5 dS/m	236 <sup>a</sup>
10 dS/m	99 <sup>b</sup>

\*Means followed by the same latter are not significantly different according to LSD test at 0.05 level of probability.

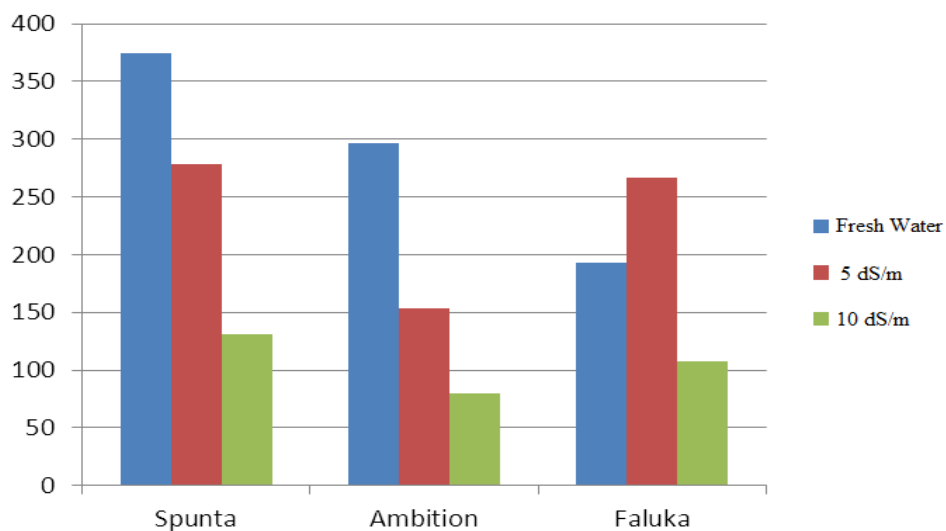


Figure 1. Effect of water salinity on the yield of three potato cultivars

### ***Effect of compost on salt accumulation in drainage water and planting media:***

Data presented in tables 3 and 4 show the effect of compost addition to the planting media on the salinity of drainage water collected from each treatment and on the accumulation of salt in the planting media. A positive correlation between compost percentage in the planting media and the salinity of drainage water was

observed (Table 3). The highest drainage water salinity was recorded for treatments that had highest compost level. This clearly indicates that compost addition improves drainage, which in turn will reduce the impact of brackish irrigation water. Therefore, less salt was accumulated in the planting media with increasing the compost percentage (Table 4).

*Table 3. Salinity of drainage water as affected with compost percentages collected from the three potato cultivars.*

compost %	Treatment		
	1.25 dS/m	5 dS/m	10 dS/m
<b>100</b>	28 <sup>a</sup>	41 <sup>a</sup>	43 <sup>a</sup>
<b>80</b>	26 <sup>a</sup>	35 <sup>a</sup>	37 <sup>a</sup>
<b>60</b>	16 <sup>b</sup>	30 <sup>a</sup>	31 <sup>b</sup>
<b>40</b>	15 <sup>b</sup>	21 <sup>b</sup>	24 <sup>b</sup>
<b>20</b>	13 <sup>b</sup>	12 <sup>c</sup>	19 <sup>c</sup>
<b>0</b>	4 <sup>c</sup>	8 <sup>c</sup>	11 <sup>c</sup>

*\*Means followed by the same latter are not significantly different according to LSD test at 0.05 level of probability.*

*Table 4. Salinity level of planting media (at the end of the growing season).*

		Salinity of irrigation water		
		1.25 dS/m	5.0 dS/m	10 dS/m
Compost %	<b>100</b>	0.3 <sup>d</sup>	1.1 <sup>c</sup>	3.5 <sup>b</sup>
	<b>80</b>	0.4 <sup>cd</sup>	1.25 <sup>c</sup>	3.5 <sup>b</sup>
	<b>60</b>	0.4 <sup>cd</sup>	1.25 <sup>c</sup>	3.7 <sup>b</sup>
	<b>40</b>	0.5 <sup>bc</sup>	1.4 <sup>b</sup>	3.6 <sup>b</sup>
	<b>20</b>	0.6 <sup>ab</sup>	1.7 <sup>a</sup>	4.1 <sup>a</sup>
	<b>0</b>	0.75 <sup>a</sup>	1.8 <sup>a</sup>	4.0 <sup>a</sup>

*\*Means followed by the same latter are not significantly different according to LSD test at 0.05 level of probability.*

The results of this study are in agreement with many studies that clearly proved the impact of soil amendments that improve vegetable growth and yield. For example, Sahrawat et al., (2009) reported that crops and cultivars within a crop differ in their sensitivity to

salts and may show variable response to salt concentration in the growing medium. Moreover, they stated that compost is a common practice for growing plants in pot culture studies and that EC should be



measured because it provides a simple and rapid measure of the salt concentration.

The knowledge of the threshold of the crop to salinity is very important. In their study, Cai et al., (2010) indicated that when salt content was appropriate, composted sewage sludge can be used alone as a vegetable seedling growth medium without the need for grinding or blending with other materials. In our study, brackish water, up to 5 dS/m can be used to irrigate potato specifically, if organic amendments are added to the soil to promote plant growth, yield and reduce salt accumulation.

### Conclusion

Brackish water of up to 5 ds /cm can be used in production of some potato cultivars in Jordan. Although salts were accumulated in the soil as a result of using saline water in irrigation, organic matter can reduce salts accumulations and promote plant growth. Compost in the planting media can significantly reduce salt accumulation in the planting media. Based upon the results of this study, farmers are advised to add compost to the soil where potatoes will be grown to reduce the accumulation of salt in the soil. This practice will give an opportunity for farmers to cultivate their farms for longer periods when relatively saline water is used for irrigation.

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# INFLUENCE OF SUPPLEMENTAL LIGHTING ON REDUCING PRE-MATURE FRUIT DROP AND INCREASING FRUIT YIELD OF GREENHOUSE GHERKINS

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## Abstract

The gherkin industry in Sri Lanka has been experiencing a problem of greater pre-mature fruit drop, leading to lower crop production, hence this research was designed to investigate the influence of supplemental lighting on reducing pre-mature fruit drop and increasing fruit yield of greenhouse gherkins. The study was conducted at a commercial scale production greenhouse in the Low Country Wet Zone of Sri Lanka during Yala season (May - July), 2012 (Experiment I) and early Maha season (September - November), 2014 (Experiment II). In Treatment 1 and Treatment 3, supplemental lighting was provided to extend day length from 5.00 a.m. to 7.00 a.m. and 5.00 p.m. to 7.00 p.m. while in treatment 2, supplemental lighting was provided only under rainy/cloudy weather condition from 7.00 a.m. to 5.00 p.m. In Experiment I, a combination of fluorescent lamps and incandescent lamps were used at the ratio of 2:1, while the same source of lights were used at the ratio of 5:1 for treatment 3 and treatment 1. In Experiment II the light source of treatment 1 and treatment 2 were replaced with LED (light emitting diodes) while treatment 3 was kept similar to treatment 3 in Experiment I. The treatments were laid according to Completely Randomized Design (CRD) with three replicates per treatment, assigning 20 plants per each replicate. Results revealed that fruit drop in both experiments (Experiment I and II) with respect to T1 (40; 109 fruits/plant), T2 (38; 105 fruits/plant) and T3 (44; 111 fruits/pant) has significantly reduced through supplemental lighting when compared to that of control (51; 159 fruits/plant); however, no significant difference was observed among the supplemental lighting treatments related to fruit drop. Grade I fruit yield was significantly ( $P \leq 0.05$ ) greater in T2 (392.8; 885.3 g/plant) as a result of increased overall fruit yield (498.6; 993.3 g/plant) and reduced fruit drop, compared to that of T1 (304.5; 747.5 g/plant) and T3 (324.3; 709.4 g/plant). Given T2 was more cost effective than T1 in Experiment II, we conclude that T2 as the most appropriate supplementary lighting solution to reduce the pre-mature fruit drop and to increase fruit yield of greenhouse gherkin in the low country wet zone of Sri Lanka.

Keywords: Fruit drop, gherkin, grade I yield, greenhouse, supplementary lighting.

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## Introduction

Gherkin (*Cucumis sativus*) is an important vegetable crop cultivated extensively in sub-tropical and tropical countries. It is a popular commercial cash crop, recently introduced to greenhouse vegetable sub sector in Sri Lanka (Jayaweera *et al.*, 2013). Protected culture could be successfully used to overcome environmental limitations in agriculture enhancing high quality, fresh production while maintaining production stability

(Weerakkody, 1998). However, semi-intensive scale protected culture is being practiced in most developing countries aiming to minimize cost factors.

Furthermore, gherkin industry in Sri Lanka has been experiencing a problem of greater pre-mature fruit drop, resulting lower yield/crop production. The reasons would be undesirable external environmental conditions that partially/indirectly affect on growth of indoor

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plants and competition between sources and sinks of gherkin plant. In addition, lower light intensity levels in greenhouse than the open field condition could be another reason since cucurbits are warm season crops which require plenty of light (Dana and Lerner, 2000). Several attempts have been taken on resolving the problem such as use of exogenous plant growth regulators under controlled environmental condition (Dissanayake *et al.*, 2011); different fertilizer formula under controlled environmental condition (Hettiarachchi, 2012; Jayaweera *et al.*, 2013) and testing the effect of pruning, fruit thinning and stimulant application (Subasinghe, 2005) on reducing pre-mature fruit drop of gherkin; however, limited progress have been shown so far.

For an optimum plant production light intensity, light spectrum and photoperiod have to be met with the requirement of the crop while optimizing other growth factors. Therefore this research was designed to investigate the influence of supplemental lighting on reducing pre-mature fruit drop and increasing fruit yield of greenhouse gherkins.

## Materials and Methods

This study was conducted under greenhouse (40×10 m) conditions at Sunfrost (Pvt.) Limited, Alawwa in Low Country Wet Zone (LCWZ) of Sri Lanka during *Yala* season (May - July), 2012 (Experiment I) and early *Maha* season (September - November), 2014 (Experiment II). The research was laid out according to Completely Randomized Design (CRD) with three replicates per treatment, assigning 20 plants per each replicate. The gherkin variety “Vertina” was planted in drip-fertigated grow bag culture at the spacing of 45 (within a row) × 90 cm (in between rows).

Three supplemental lighting treatments (treatment 1, 2 and 3) were applied 4 weeks after sowing (WAS) with natural light as the control treatment (treatment 4). In treatment 1 and treatment 3, supplemental lighting was provided to extend day length during 5.00 a.m. to

7.00 a.m. and 5.00 p.m. to 7.00 p.m. In treatment 2, supplemental lighting was provided only under rainy/cloudy weather condition from 7.00 a.m. to 5.00 p.m. In Experiment I, a combination of fluorescent lamps and incandescent lamps at a ratio of 2:1 was used for treatment 3, while the same source of lights at a ratio of 5:1 was used in treatment 1. In Experiment II, the light source of treatment 1 and treatment 2 were replaced with LED (light emitting diodes) while treatment 3 was kept similar to treatment 3 in the Experiment I.

The lighting was provided at the rate of 120 W×3/plot (in case of LEDs) at the canopy height in Experiment II and 120 W×2/plot (in case of fluorescent lamps and incandescent lamps) in Experiment I. Black polythene was used to blackout against the boarder effect between lighting treatments. The improved technologies adopted in the protected culture were also used in this experiment such as containerized transplants, black polythene mulch, fertigation, drip irrigation, pruning and training of vines. After harvesting, fruits were graded according to their diameter. In Experiment II, fruits having diameter between 17-21 mm were categorized to Grade I and beyond 22 mm to a grade called CRS. Nevertheless the harvesting standards followed in Experiment I, were slightly different from Experiment II since 12-14 mm diameter fruits were categorized to Grade I while fruits with diameter >15 mm were categorized to CRS grade in Experiment I. Vegetative parameters (plant height, internodal length, leaf area, number of leaves and number of branches) were measured weekly until vines reached upper horizontal crop support, established 1.75 m from ground level. As reproductive parameters, yield per vine, Grade I fruit yield per vine, number of fruits dropped per vine and crooked fruit yield per vine were measured. Daily light intensity, temperature and relative humidity inside the greenhouse were also measured. Net assimilation rate was measured once using portable photosynthetic machine (Licor 6400) in Experiment II. The results were statistically analyzed using the software, SAS.



## Results

### Environmental parameters

The variation of the weekly temperature and relative humidity are shown in Figure 1. The mean day temperature in Experiment I was  $30.0 \pm 0.4$  °C and it was comparatively similar to the Experiment II, which

exhibited  $31.0 \pm 2$  °C. The average relative humidity (RH) in Experiment I was also similar ( $83.0 \pm 2.4\%$ ) to that in Experiment II ( $83.0 \pm 4.2\%$ ). Furthermore, the number of supplemental lighting hours provided at Experiment I (67 hrs) was lower than the Experiment II (120 hrs). In Experiment I, use of supplemental lighting was highest during 9 WAS, while in Experiment II it was during 10 WAS.

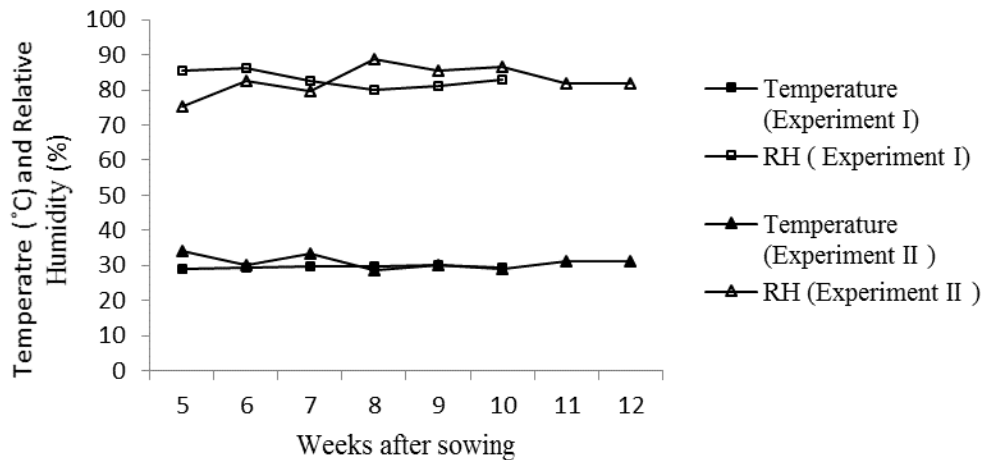


Figure 1: Variation of weekly temperature (°C) and Relative Humidity (%)

### Vegetative parameters

There were no significant differences ( $P \leq 0.05$ ) among light treatments with respect to any of the vegetative growth parameters (plant height, internodal length, leaf area, number of leaves and number of branches), except for plant height at 4 WAS and number of branches at 6 WAS in Experiment II. Experiment I reported slightly higher plant height ( $230 \pm 22$  cm), but a lower leaf number ( $24 \pm 2.5$  per plant), and similar internodal length of ( $12 \pm 0.4$  cm), when compared to Experiment II at 6 WAS. In contrast Experiment II reported a mean plant height of  $175 \pm 13$  cm, LAI of  $44.5 \pm 3.0$ , number of leaves of  $33.5 \pm 6.0$  per plant and internodal length of  $11.4 \pm 0.1$  at 6 WAP.

### Reproductive parameters

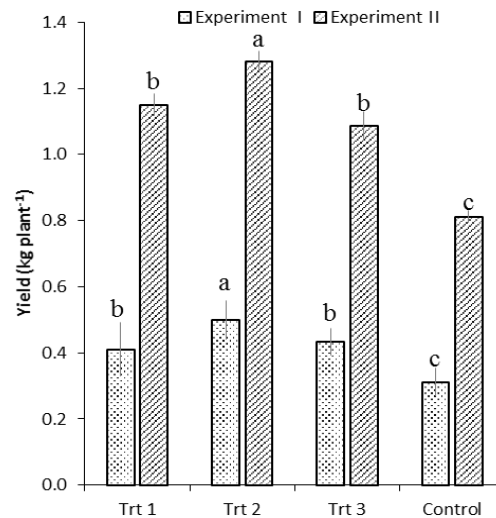
#### Yield per plant

Treatment effects on yield per plant were statistically significant (at  $P \leq 0.05$ ) in both Experiment I and Experiment II. Both supplemental lighting during cloudy conditions and supplemental lighting provided to increase day length have significantly increased the total yield per plant when compared to that of control (Figure 2). In both experiments, treatment effect of supplemental lighting under cloudy conditions (treatment 2) has shown a significant effect on yield per plant, compared to other treatments. However, there was no significant difference between two light treatments, used to increase the day length using the same light source at different ratios in Experiment I and

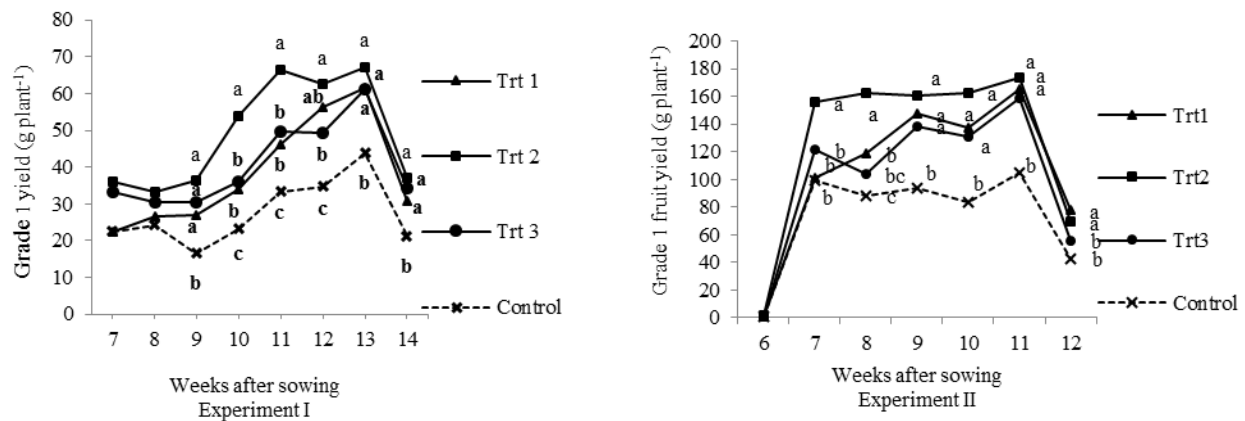
by using different light treatments (i.e., LEDs and combination of incandescent and fluorescent lamps at the proportion of 2:1) in Experiment II. The overall yield of Experiment II has increased when compared to the Experiment I, potentially due to slightly different harvesting standards followed in each case.

Treatment effect on Grade I yield per plant was very similar to the treatment effects on the total yield per plant. Grade I yield per plant in control has shown

drastic reduction when compared to supplemental lighting treatments at 9<sup>th</sup> WAS in Experiment I (Figure 3). Furthermore, in Experiment II control exhibited significantly lower yield when compared to remaining 3 treatments (treatment 1, 2 and treatment 3). Though treatment 1 and 3 demonstrated slighter reduction in Grade I yield per plant when compared to treatment 2 at 10 WAS, the difference was statistically non-significant at  $P \leq 0.05$ .



**Figure 2:** Effect of supplemental lighting on yield per plant in Experiment I and Experiment II. Different letters indicate a significant difference at ( $P \leq 0.05$ ).

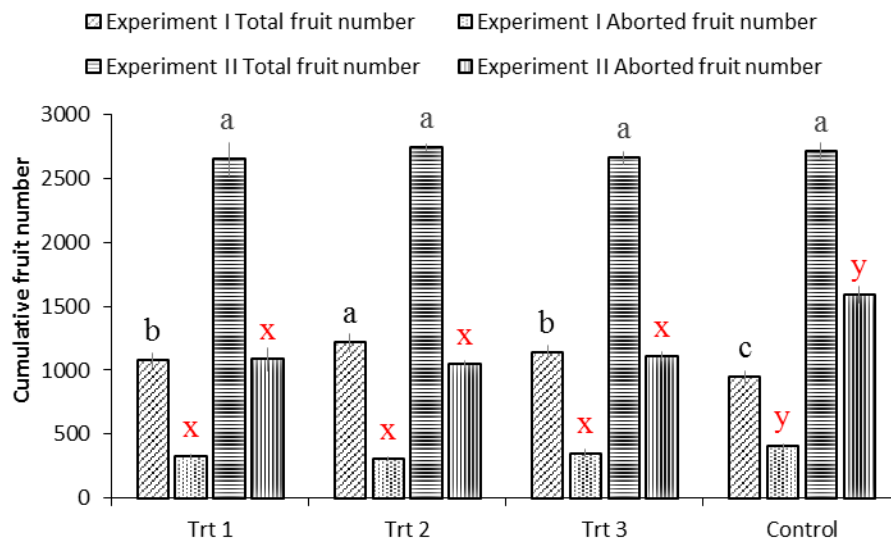


**Figure 3:** Variation of weekly Grade I fruit yield per plant under different light treatments in Experiment I and Experiment II. Different letters indicate a significant difference at ( $P \leq 0.05$ ).

## Fruit drop

Supplemental lighting treatments (treatment 1, 2 and 3) in both Experiment I and II have shown significantly reduced fruit drop at ( $P \leq 0.05$ ) (Figure 4). However, there were no significant differences observed among the treatments on total fruit number in Experiment II. Furthermore, supplemental lighting have significantly increased the total fruit number when compared to the

control in Experiment I. However, there was no significant difference observed between Treatment 1 and Treatment 3, where the supplemental lighting was provided in order to increase the day length using same combination of lighting sources at different ratios.



**Figure 4:** Effect of supplemental lighting on cumulative fruit number. Different letters indicate a significant difference at ( $P \leq 0.05$ ).

## Net assimilation rates (Experiment II)

There was a significant difference ( $P \leq 0.05$ ) in net assimilation rates (NAR) between treatment 2 and control under cloudy conditions, indicating that supplemental lighting under cloudy condition increased

the NAR, compared to that of control. Further, there was a significant treatment effect on NAR between 5.00 p.m. to 6.00 p.m., in treatment 1 and treatment 3, where day length was increased by different light sources. However, net assimilation rates recorded during 5.00 p.m. to 6.00 p.m. were very lower and positive NAR were not exhibited after 6.00 p.m.

*Table 1 Impact of supplemental lighting on net assimilation rate in different treatments*

Treatment	Net assimilation rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )
Treatment 2 (under cloudy)	$10.6 \pm 1.1\text{a}$
Treatment 4 (under cloudy)	$4.9 \pm 1.3\text{b}$
P<	0.0001
CV	18.4
Treatment 1 (between 5.00 p.m.to 6.00 p.m.)	$0.9 \pm 0.3\text{a}$
Treatment 3 (between 5.00 p.m.to 6.00 p.m.)	$0.6 \pm 0.1\text{b}$
Control (between 5.00 p.m.to 6.00 p.m.)	$0.1 \pm 0.3\text{c}$
P<	0.0001
CV	39.1

## Discussion

The higher RH and lower sunshine hours at Alawwa, could be the result of continuous rain that existed during the *Maha* season in Experiment II. However, both experiments maintained relatively high temperature during day time ( $31.0 \pm 2.0$  °C). Light intensity recorded during experiment was relatively 25-35% lower when compared to open field condition. Further different light intensity levels were found at different canopy heights due to mutual shading. Cloudy/rainy conditions demonstrated further reduction in an incident light levels, affecting assimilation rates thereby reducing dry mater partitioning to fruit sink. Therefore these light limitations can be overcome by providing light supplementation wherever necessary.

The influence of supplemental lighting (treatment 1, treatment 2 and treatment 3) in both Experiment I and II for greenhouse gherkin may have caused formation of more fruits leading to higher Grade I yield and lower fruit drop, compared to that of control. Therefore, both supplemental lighting under rainy /cloudy condition and supplemental lighting provided to increase day length have significant effect on increasing fruit yield and reducing fruit drop. However, no significant difference reported between treatment 1 and treatment 3, where supplemental lighting was provided to increase the day length in both Experiment I and II.

Incandescent lamps radiate higher red light intensity than the blue light hence incandescent lamps are not usually effective sources of radiation for supplemental lighting (Anon., 2010). However, fluorescent lamps are cooler, more light efficient than incandescent lamps and radiate higher blue light than red light (Anon., 2010). Therefore, in order to produce fairly balanced spectrum in the photosynthetically active radiation (PAR) zone two ratio combinations of fluorescent lamps and incandescent lamps (5:1 and 2:1) were used in Experiment I.

The plants in Treatment 2 maintained their NAR at a considerable level, even under cloudy conditions, when compared to the control through the supplemental lighting (Table 1). The higher NAR of Treatment 2 under rainy/cloudy weather compared to of the control under rainy/cloudy weather could have contributed to greater yield per plant and lower fruit abortion of Treatment 2 in both experiments. This point has also been very clearly demonstrated by variation of weekly Grade I fruit yield per plant under different light treatments in Experiment I and Experiment II (Figure 3). During 9<sup>th</sup> and 10<sup>th</sup> weeks in Experiment I and Experiment II, (when supplemental lighting was highest), Treatment 2 maintained the consistency in Grade I yield even under cloudy conditions, without exhibiting a yield drop. In Experiment II, LEDs were used for supplemental lighting under cloudy conditions



(Treatment 2). With LED lighting, spectral output can be tuned, consequently makes it possible to apply the optimum 'light recipe' at every stage of the crop growth (Berstrand and Schussler, 2012).

There was no significant difference ( $P \leq 0.05$ ) among the light treatments on any of the vegetative growth parameters (plant height, internodal length, leaf area, number of leaves per plant, number of branches per vine and stem thickness) under greenhouse conditions in Experiment I (Chamindika, 2012). The same trend was evident with the results of Experiment II as well.

In order to reach to a sustainable and economically viable production, cost effectiveness of supplemental lighting has to be maximized. For commercial greenhouse production, supplemental lighting is most

beneficial in areas that receive less than 4.5 hours of average daily sunshine. But in tropical region, artificial lighting is not cost effective due to high cost of production (Anon., 2010). However, most of gherkin farmers in Sri Lanka are practicing semi-intensive levels of protected culture in order to minimize the cost of production. Therefore supplemental lighting should be cost effective in order to reach economic benefits under Sri Lankan context. The capability of LEDs to provide optimum 'light recipe' at every stage of the crop growth, with its additional advantages on effective heat management, long lifetime, high luminous efficiency and energy efficiency has indicated the potential benefits on greenhouse crop production.

Table 2 Cost related to supplemental lighting over the season

Treatment	Investment cost/plant (LKR)	Electricity cost/plant (LKR)	Supplemental cost/plant (LKR)	Additional yield/plant (kg)	Additional income/plant (LKR)	Additional profit/plant (LKR)
Experiment I	-	-	99.45	0.10	13.85	-85.60
Treatment 1 (Fluorescent/incandescent)	-	-	27.06	0.19	24.99	-2.07
Treatment 2 (Incandescent)	-	-				
Treatment	Investment cost/plant (LKR)	Electricity cost/plant (LKR)	Supplemental cost/plant (LKR)	Additional yield/plant (kg)	Additional income/plant (LKR)	Additional profit/plant (LKR)
Experiment II						
Treatment 1 (LEDs)	4.21	5.06	9.27	0.23	24.50	+15.23
Treatment 2 (LEDs)	2.95	3.22	6.17	0.34	35.95	+29.78

According to the cost analysis (Table 2), supplemental lighting during rainy/cloudy conditions would be more productive (Anon., 2010). Supplemental lighting in treatment 1, where the day length was increased using

LEDs and treatment 2, in which LEDs were used for supplemental lighting under rainy/cloudy in Experiment II were cost effective due to the lower running cost of LEDs. Among the two treatments, treatment 2 was the most cost effective due to the significant yield increase

when compared to treatment 1. Given above, we conclude that supplemental lighting under cloudy condition can be a viable option in reducing pre-mature fruit drop and increasing fruit yield of greenhouse gherkins in LCWZ of Sri Lanka.

## Acknowledgement

This research was funded by the Sunfrost Limited (Hayleys), Alawwa, Kegalle, Sri Lanka. Our special thanks goes to the staff of Sunfrost Limited (Hayleys) and staff of Department of Crop science, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

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# ORIENTATIONAL EFFECT OF AQUEOUS LEAF EXTRACT OF *CITRUS AURANTIFOLIA* ON HOUSEFLY, *MUSCA DOMESTICA* (DIPTERA:MUSCIDAE)

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## Abstract

Housefly, *Musca domestica* (L.) is a major public health pest act as vector for human and animal disease causing organisms therefore the control of housefly is vital to human health. Products obtained from certain medicinal plants have proved as alternatives to synthetic chemicals. In this view the present study was aimed to study the repellent activity of aqueous leaf extract of *Citrus aurantifolia* (lime) in comparison with neem leaf extract and additive Turmeric rhizome powder using self made six-armed olfactometer. Five gram of meat pieces soaked separately for one minute in 1:10 (w/v) aqueous citrus leaf extract (aq.CLE), 1:10 (w/v) aqueous neem leaf extract (aq.NLE), 1:10 (w/v) aqueous turmeric rhizome powder (aq.TRP), mixture of 1:10 (w/v) [aq.CLE + aq.NLE], mixture of 1:10 (w/v) [aq.NLE + aq.CLE + aq.TRP] and one ml of distilled water for control and were tested for repellent activity against 1-2 days old adult flies based on the number of adults oriented towards each treatment. Orientation of housefly towards extracts was significantly less in numbers ( $P < 0.05$ ) in all treatments than the water control after 10 – 60 minutes and 24 hours of adult exposure. From the LSD analysis, Aq.CLE showed significantly ( $P < 0.05$ ) high repellent activity as much as Aq.NLE, mixture of Aq.NLE+Aq.CLE and mixture of Aq.NLE+Aq.CLE +AqTRP after one hour and 24 hours of exposure. After 24 hours of exposure, the percentage repellency range from 74 % (for TRP) to 96 % (for mixture of CLE+NLE+TRP). Citrus leaf extract alone showed 83% repellency. It was concluded that the aqueous citrus leaf extract at 1:10 (w/v) has potential repellent effect against house fly and could be considered for integration with other control options in the control of housefly and also pave the way for its use as eco friendly control measure..

Keywords: Housefly, *Musca domestica*, *Citrus aurantifolia*, neem, repellent, orientation

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## Introduction

Housefly, *Musca domestica* (L.) is a major domesticated pest act as a vector of human and animal disease and also cause nuisance to human so control of housefly is vital to human health. The usage of conventional insecticide pose a substantial hazard to human and environment and also cause pest resistance to insecticides, hence the rising drawbacks of synthetic chemical substances have stimulated the search for ecofriendly and biodegradable insecticides. In this view, previous studies revealed the possibility of using

plant extracts as insecticidal on different stages of housefly (Malik *et al.* 2007, Nabawy *et al.*, 2011, Shalaby *et al.*, 1998) and repellent effect against adult fly (Liao, 1999 & 2010, Mathanraj and Rajan, 2012, 2013). Locally and freely available citrus plant parts such as leaves, peel powder and peel oil found to be effective insecticidal and repellent against stored product pests, vegetable pests and flies. Most of the studies carried out using crude extracts of various parts of the plants mostly peel powder and peel oil and used chemical solvents for extract preparation (Siskos *et*

al.,2007) A few studies were carried out against housefly using mostly peel powder and peel oil (Samarasekara,2006) but fresh leaves are easily obtainable at any time. In order to introduce most effective, with low input technology which can be utilized by anybody at anytime without any cost and environmentally safe control method this study was aimed to evaluate the repellent effect of aqueous leaf extract of *Citrus aurantifolia* (lime) and compare the effectiveness with neem leaf extract and additive turmeric rhizome powder against house fly using self made six- armed Olfactometer.

### Materials and Methods

Olfactometer setup was used to test the repellent effect of aqueous neem leaf extract against house fly.

#### *Rearing of house fly*

Adult houseflies were collected from local shop by sweeping net. They were transferred into a breeding cage (30 x 30 x 30 cm). The cage made of three mesh metal sides and a wooden bottom. On front side a muslin sieve (70cm) long was fixed to an opening. Foods for adults were provided in Petri dishes. 10% (w/v) multivitamin syrup solution and five gram fresh pork liver meat as a food source and oviposition site. Third petri dish contained a piece of cotton wool soaked in water to maintain the humidity (Bisseleua et al, 2008).

#### *Preparation of leaf extract*

To prepare the aqueous extracts of citrus and neem; fresh leaves of citrus and neem were collected from the home garden and washed in the distilled water separately and one gram of leaves crushed using motor and pestle. 10ml of distilled water added into the leaf paste and then filtered through muslin cloth separately. The resulting filtrate 1:10 (w/v) used for the experiment.

Olfactometer was made using six plastic bottles. One bottle used as center bottle in which 8cm diameter 6 holes made and connected to 1000ml plastic bottles separately with the help of 32cm long card board tube

with equal gap between them. The end of each arm is fitted with a plastic bottle(1000 ml).

Five gm of meat piece soaked in 1 ml of (i) aqueous citrus leaf extract(aq.CLE) , 1:10 (w/v) (ii) aqueous neem leaf extract (aq.NLE), (iii) 1:10 (w/v) aqueous turmeric rhizome powder (aq.TRP) , (iv) mixture of 1:10 (w/v) [aq.CLE + aq.NLE] , (v) mixture of 1:10 (w/v) [aq.NLE + aq.CLE + aq.TRP] and (vi) one ml of distilled water for control separately for one minute then placed into watch glass separately and kept into each plastic bottle of olfactometer finally closed with lid. Fifty numbers of one to two days old adults from laboratory culture were introduced into the center bottle and covered with muslin cloth.

Number of houseflies each bottle counted in 10 minutes interval up to 60 minutes and then after 24 hours of adult introduction. This experiment was repeated five times. All experiments were carried at the temperature  $26\pm 2^{\circ}\text{C}$  and relative humidity (RH) 70-75%. Control experiment was conducted concurrently along with the experimental trials. Data was analysed using ANOVA, T- test and LSD. The percentage of repellency (R %) was determined in accordance with the method of Champbell (1983).

The percentage Repellency (R%) was calculated as follows

$$R \% = [100 \times \frac{(C-T)}{C}]$$

Where, C- The number of flies trapped in the control bottle

T- The number of flies trapped in the treated bottle

### Results and Discussion

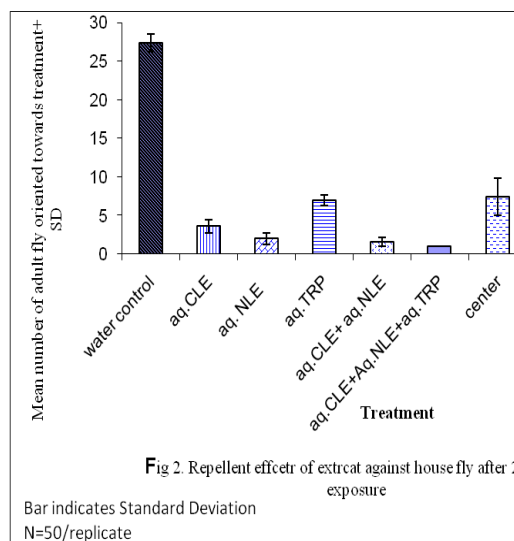
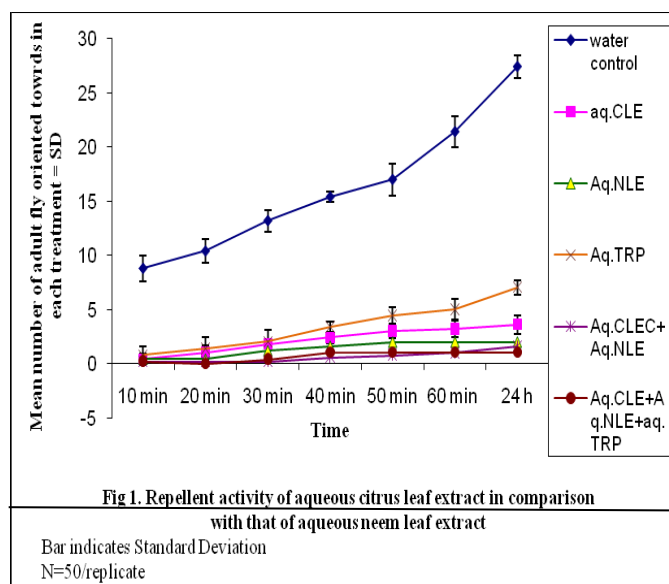
Number of flies' moves towards the treatment bottles containing different extract formulations was compared with that of water control.

The data pertaining to the repelled activity of leaf extract was presented in Figure 1-3. In general all extract formulation tested showed repellent activity against housefly. Orientation of housefly towards extracts was significantly less in numbers ( $P<0.05$ ) in all treatments than the water control after 10 – 60 minutes and 24 hours of adult exposure. From the LSD

analysis, after one hour of adult exposure number of flies in each treated as follows; (aq.NLE+Aq.CLE +aqTRP) =(aq.NLE+Aq.CLE) = aqNLE < aq.CLE =aq.TRP <water control while after 24 hours of exposure, the number of adult flies in each treatment as follows; aq. NLE= (aq.NLE+Aq.CLE) = (aq.NLE+Aq.CLE +aqTRP) = aq.CLE < aqTRP < water control. Citrus leaf extract showed significantly ( $P<0.05$ ) high repellent activity as much as aq.NLE, mixture of aq.NLE+aq.CLE and mixture of aq.NLE+aq.CLE +aqTRP after 24 hours of exposure.

In the olfactometer study, ater 24 hours of exposure, the percentage repellency range from 74 % (for TRP) to 96

% (for mixture of CLE+NLE+TRP) (Table 1). Citrus leaf extract and neem leaf extract alone showed 83.2% and 92.7 % repellency respectively. Therefore the reduction in number of flies oriented or repellent activity may be due to the potentiality of the compounds present in the leaf extracts at 1:10 (w/v). which are enough to repel the house flies . In previous study, aqueous neem leaf extract at1:1 w/v showed 94% of repellency against housefly (Mathanaraj & Rajan, 2013). This is confirmed by this study. Citrus leaves are freely available in plenty, biodegradable, eco friendly moreover resistant does not develop in insects so the aqueous leaf extract could be used as repellent against housefly.



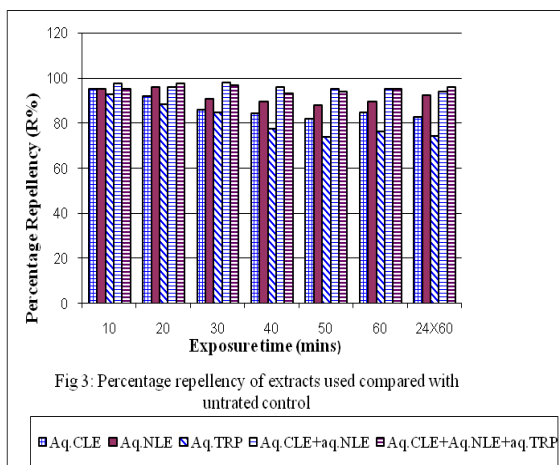


Table 1. Percentage repellency effect of citrus leaf extract compared with water control

	Aq.CLE	Aq.NLE	Aq.CLE+AqTRP	aq.NLE+Aq.CLE	Aq.CLE+Aq.NLE+Aq.TRP
After 1 hr	85.04	89.72	76.64	95.32	95.32
After 24 hrs	83.2	92.7	74.45	94.16	96.35

## Conclusion

In this present study a small scale attempt is made to screen locally available plant and using easy application method. It was concluded that the aqueous citrus leaf extract at 1:10 (w/v) has potential repellent effect against house fly and could be considered for integration with other control options in the control of housefly.

No comparable data with aqueous formulation of citrus leaf extract on repellency against house fly therefore further study may be carried out to confirm the present data.

## Acknowledgement

Author express acknowledge to Ms.S.Neelaja for her great assistance in this research.

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# EVALUATION OF GROWTH OF *Listeria monocytogenes* WITH CINNAMON OIL AND POWDER UNDER DIFFERENT TEMPERATURES BY INDIRECT CONDUCTIMETRY

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## Abstract

Cinnamon oil and cinnamon powder apply as a flavouring agent and to suppress the growth of acid tolerant food borne pathogens. The attempt was taken to evaluate the growth of *L. monocytogenes* under two temperature regimes by adding cinnamon oil and powder and to select the form of cinnamon having antibacterial properties. The growth rates of *L. monocytogenes* in tryptone soy broth plus yeast extract (TSBYE) were estimated using indirect conductimetry by Don Whitley RABIT system under 25oC and 37oC. Concentrations 0.05%, 0.10% and 0.15% (v/v) cinnamon oil and 0.01%, 0.03% and 0.05% (w/v) cinnamon ground were added to TSBYE as treatments. Treated samples with inoculated *L. monocytogenes* were incubated for 48hrs using Don Whitley RABIT system at 25oC and 37oC. Detection solution (KOH + 0.1% Agar) with conductivity more than 6000  $\mu$ S was used throughout the investigation. Conductivity measurements of the detection solution were taken for every 6 mins over the period of 48 hrs and recorded by detection software. Time to detection (TTD) was recorded as 15hrs for 25oC and 14hr and 6 mins for 37oC in the cinnamon oil treated samples. Results indicate cinnamon oil exerted significant effect on TSBYE media against *L. monocytogenes* in both 25oC and 37oC. Cinnamon oil has the ability to suppress the growth of *L. monocytogenes*. Growth suppression of *L. monocytogenes* was not observed from ground cinnamon in TSBYE at 25oC and 37oC. Therefore, cinnamon oil can be used as flavoring agent with antimicrobial to suppress the growth of *L. monocytogenes*.

Keywords: *L. monocytogenes*, indirect conductimetry, cinnamon oil, cinnamon powder.

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## Introduction

Food-borne disease associated with *Listeria monocytogenes* is considered as lethal diseases in the world. Stricter controls in food manufacturing processes are essential to decrease the incidence of food infections caused by *L. monocytogenes*. Antimicrobials have been tested by many scientists to inactivate *L. monocytogenes* in food products without alteration of fresh sensory quality and nutrient content of it. Essential oils from plants used as antimicrobials and have also been investigated due to the demand for food

products free from synthetic preservatives. Cinnamon oil and powder both have shown to be of value in this regard and cinnamon alone or in combination with other antimicrobial treatments or agents has been documented [1, 11]. *L. monocytogenes* was found to be more sensitive to cinnamon bark oil than *E. coli* and *Salmonella enteritidis* [6]. Gill and Holley [4, 7] observed cinnamon oil and cinnamaldehyde cause a decrease in the intracellular ATP by ATPase activity without obvious changes to the cell membrane of *L. monocytogenes*.



The antibacterial activity of cinnamon depends on the intrinsic factors and extrinsic factors of the product. Storage temperature is an important extrinsic factor that influences the antibacterial activity of cinnamon. Temperature is affecting the efficacy of essential oils because; the permeability of microbial cell membrane increased at high temperatures and could explain as an increased cellular diffusion of antimicrobial substances [2]. Yuste and Fung [11] reported a 4 to 6 log CFU/ml reduction of *L. monocytogenes* inoculated in pasteurized apple juice with 0.1%, 0.2%, and 0.3% (w/v) of cinnamon after 1 hr of incubation at 5 and 20 °C. Moreover they found no growth of any microorganism during 7 days of storage. An immediate 2 log CFU/ml reduction of *E. coli* in unpasteurized apple cider maintained at 42 °C by adding 2% (w/v) cinnamon powder was observed [5]. Hence the optimum temperature for effective antimicrobial activity of cinnamon oil/ powder against the pathogenic microorganisms may be of interest to industry.

However, the effects of these factors on the antimicrobial activity of cinnamon oil and powder have been assessed using classical plate count methods. Determinations of quantitative data on microbial growth rates, under a wide range of conditions, are essential for mathematical modeling studies, which are seen as the basis for improved food safety measures [11]. The conventional cultural methods for the detection of *L. monocytogenes* are slow, typically 3–5 days and labour intensive. The conductimetric technique is now a well-established method and has been legally accepted in the UK for the detection of *Salmonella* in processed animal protein for some time [1].

In the indirect conductimetric technique, the electrodes are immersed in a separate solution (usually a potassium hydroxide solution) instead of the inoculated growth medium. The indirect conductance method of growth detection for bacteria relies on the production of carbon dioxide (CO<sub>2</sub>) by the bacteria as they metabolise carbohydrates and amino acids. The gas dissolves in the aqueous phase of the growth medium and can then diffuse into the headspace from where it can be absorbed into a solution, or gel, of potassium hydroxide (KOH). Electrodes placed in the KOH

measure the electrical changes caused as the CO<sub>2</sub> produced by the bacteria reacts with the KOH to form bicarbonate ions whose conductivity is less than that of the original solution. Impedance systems measure the relative or absolute changes in conductance, capacitance or impedance at regular time intervals during growth of bacteria at a given temperature. The measured electrical signals are then graphically plotted on the ordinate against the incubation times on the abscissa, producing impedance growth curves. In a typical impedance growth curve, the first region has quite a stable impedance value and then starts decreasing. The time corresponding to a point at which the decrease in impedance value exceeds a threshold value is called as the detection time (td). The detection time does not appear until the bacterial number reaches 10<sup>6</sup>–10<sup>7</sup> cfu/ml. The impedance value finally levels off when the bacteria have reached a concentration of 10<sup>8</sup>cfu/ml or more, and all the nutrients in the medium have been metabolized to end products [10].

This investigation sought to determine growth rates for *L. monocytogenes* using indirect conductimetry with the treatments of cinnamon oil and cinnamon powder under temperatures of 25°C and 37°C which can be used as a alternative method to determine the effects of antimicrobial compounds under wide range of conditions and to select the form of cinnamon which can be used to suppress the growth of *L.monocytogenes*.

## Materials and Methods

### *Microorganisms and culture conditions*

*Listeria monocytogenes* (NCTC 10357) were obtained from National Collection of Type Cultures (NCTC), Health Protection Agency, United Kingdom and used throughout the investigation.

Stock cultures of *L. monocytogenes* were grown on Tryptone Soy Broth (TSB) (Oxoid, CM1065) with 0.6% (w/v) Yeast Extract (YE) (Oxoid, CM0019). Cultures were incubated at 37 °C for 24 hrs to obtain cells in early stationary growth phase. Maximum growth (10<sup>9</sup>) of bacteria was expected.

Universal containers each containing 9 ml of Maximum Recovery Diluent (MRD) were taken and labelled as 10-1 to 10-6 to prepare dilution series. 1 ml aliquots of bacterial suspension of each stock culture were transferred in to the 10-1labelled container under aseptic conditions and shook well. 1 ml from 10-1 labelled container was then transferred to 10-2 labeled container. Process was repeated for others to obtain the required densities of cells of pathogen. A 10-5 dilution of the stock culture was prepared and 1 ml of this dilution was inoculated into 100 ml pre-warmed TSBYE to give an initial count of approximately 10<sup>3</sup> CFU ml<sup>-1</sup>; this is used as the working culture. At the same time 1 ml aliquots of bacterial suspension of each stock culture were poured on Nutrient agar plate and incubated for 24 hrs at 37oC. Colony counts were taken in each plate to make sure the initial count is 10<sup>3</sup> CFU ml<sup>-1</sup>.

#### ***Sample preparation***

A volume of 0.025ml, 0.050ml and 0.075ml of cinnamon bark oil was added to 300 ml of TSBYE in individually bottled TSBYE containing sterilized screw cap bottles to obtain 0.05% (v/v), 0.1% (v/v) and 0.15% (v/v) concentrations respectively under horizontal laminar flow air cabinet. 0.050g, 0.15g and 0.25g of cinnamon ground will be mixed with 300 ml of TSBYE samples in individually bottled sterilized screw cap bottles in order to obtain 0.1% (w/v), 0.3% (w/v) and 0.5% (w/v) concentrations respectively under horizontal laminar flow air cabinet. 300 ml TSBYE samples in sterilized screw cap bottles without adding cinnamon bark oil (0% (v/v) and cinnamon ground 0% (w/v) served as controls. 2 replicates of samples were prepared from each concentration of cinnamon bark oil and cinnamon ground.

#### **Determination of antimicrobial activity of cinnamon bark oil and cinnamon powder at 25oC and 37oC through indirect conductimetry**

A Don Whitley Scientific RABIT system (Don Whitley Scientific, Shipley, UK) was used for determination of the antimicrobial activity of cinnamon powder and

cinnamon oil. 0.35 g of KOH was dissolved in 50 ml of deionised water. 1g of Bacteriological Agar was dispersed in 100 ml sterilized screw cap bottle and dissolved by boiling. At 70oC temperature of Agar, cold KOH solution was added and mixed thoroughly. 700µl of equal volumes of KOH bacteriological Agar was dispensed to the clean sterile RABIT tubes, allow solidifying for about 15 mins and were tightly stoppered and allowed to stabilized for 2 days and tubes were chosen for experiment only if their conductance was over 6000µS at 30oC.

The growth medium (Cinnamon oil / cinnamon ground) with TSBYE (4.5 ml) was contained in sterile glass test tubes (12×75 mm) and inoculated (100 µl) with the working culture then placed within the RABIT tubes, which were resealed and loaded into the conductimetric incubator block. Throughout the investigation, readings were taken every 6 min over a 24 h period and a detection criterion of -20 µS was chosen to ensure this value was reached only when cultures were showing abundant growth. The value is the difference between successive conductance measurements and was entered into the RABIT growth detection software. When this criterion was reached for three successive readings, the time taken to detect this threshold was automatically recorded by the RABIT software and this is referred to as the time to detection (TTD).

#### **Results And Discussion**

##### ***Effect of cinnamon oil on growth of L .monocytogenes in TSBYE***

Effect of cinnamon oil and ground cinnamon on the growth of *L. monocytogenes* was examined in TSBYE media. The growth of food borne pathogen was examined through indirect conductrimetry under 25oC and 37oC temperatures. Using conductimetry, the growth of *L.monocytogenes* was detected when the culture had grown enough to produce sufficient CO<sub>2</sub> to cause a detectable fall in the conductance (µS) of the detecting solution.

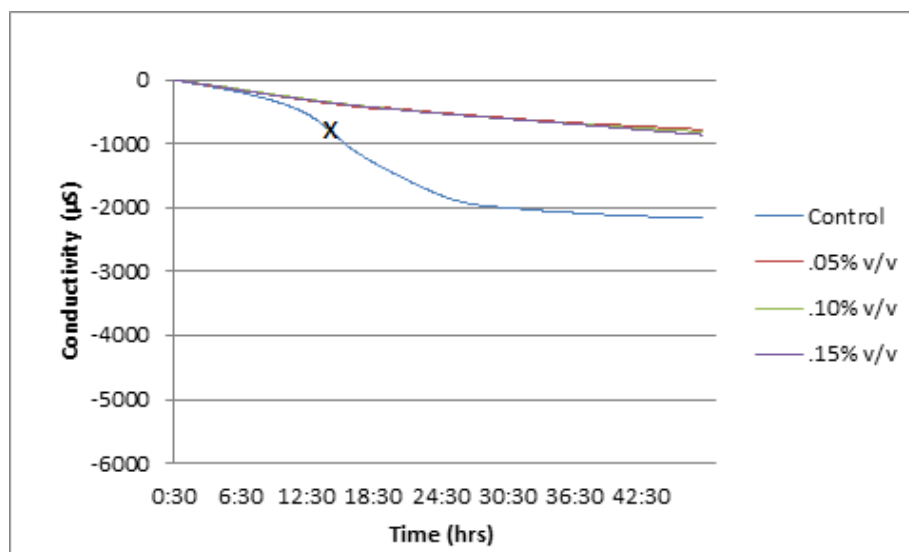


Figure 1. Fall of conductivity in detection solution for TSBYE containing cinnamon oil against *L. monocytogenes* at 25°C. Key: (X) TTD

A major fall in conductance corresponds to late log/early stationary phase (Fig. 1), when the highest numbers of metabolically active cells are present. According to the graph after 15hrs of incubation *L. monocytogenes* showed detectable conductivity fall in the control, which indicates that *L. monocytogenes* had

grown in TSBYE without cinnamon oil at 25 °C. But no observed growth of *L. monocytogenes* was detected in 0.05% (v/v), 0.10% (v/v) and 0.15% (v/v) cinnamon oil treated TSBYE at 25°C after 15 hrs. Therefore no conductivity fall observed in treated samples with compared to control after 15 hrs of incubation.

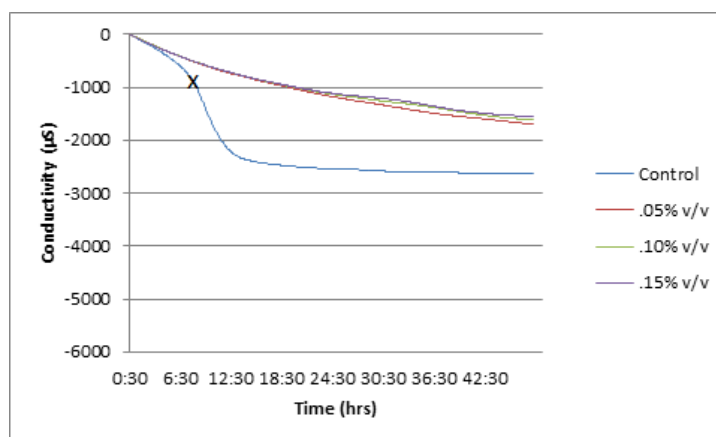


Figure 2. Fall of conductivity in detection solution for TSBYE containing cinnamon oil against *L. monocytogenes* at 37°C. Key: (X) time to detection (TTD)

Results reported in Figure 2 show a TTD value of 7hrs 36mins in the control, which indicates *L. monocytogenes* had grown in TSBYE at 37°C. Growth of *L. monocytogenes* was not detected in 0.5% (v/v),

0.1% (v/v) and 0.15% (v/v) cinnamon oil treated TSBYE samples.

Results from the study indicate that growth of *L. monocytogenes* occurred in TSBYE at 25°C and 37°C with no cinnamon oil. But, samples treated with 0.05%

(v/v), 0.1% (v/v) and 0.15% (v/v) concentrations of cinnamon oil shows promising inhibitory activity against pathogen. Similar results have been made by Prabuseenivasan [9]. This result let us to believe that cinnamon oil might be involved in cell death occurring. Paparella [8] reported further that at 37°C *L. monocytogenes* showed growth suppression in treated with 0.05% (v/v) and 0.02% (v/v) cinnamon oil in apple juice. Therefore, result reported in experiment points out 0.05% (v/v), 0.10% (v/v) and 0.15% (v/v) cinnamon

oil had a similar effect on suppressing growth of *L. monocytogenes*. Minimum concentration of cinnamon oil used (0.05% (v/v)) in experiment exerted an antimicrobial effect. *L. monocytogenes* shows there would be a potential to assess cinnamon oil as an antimicrobial agent.

#### *Effect of cinnamon ground on growth of L. monocytogenes in TSBYE*

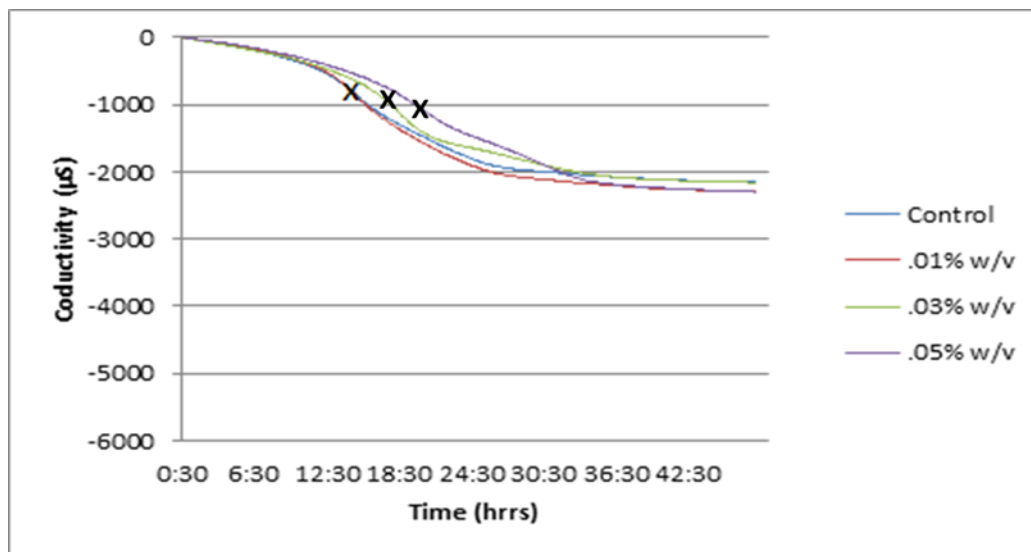


Figure 3. Fall of conductivity in detection solution for TSBYE containing with cinnamon ground against *L. monocytogenes* at 25°C Key: (X) TTD

The TTD value was observed as 14 hrs 6 mins in control which indicating *L. monocytogenes* had shown growth in TSBYE (Figure 3). TTD values for 0.01(w/v) cinnamon ground treated sample was observed at the

same time. Growth of *L. monocytogenes* were detected in 0.03% and 0.05% ground cinnamon treated samples of TSBYE. Therefore TTD is recorded.

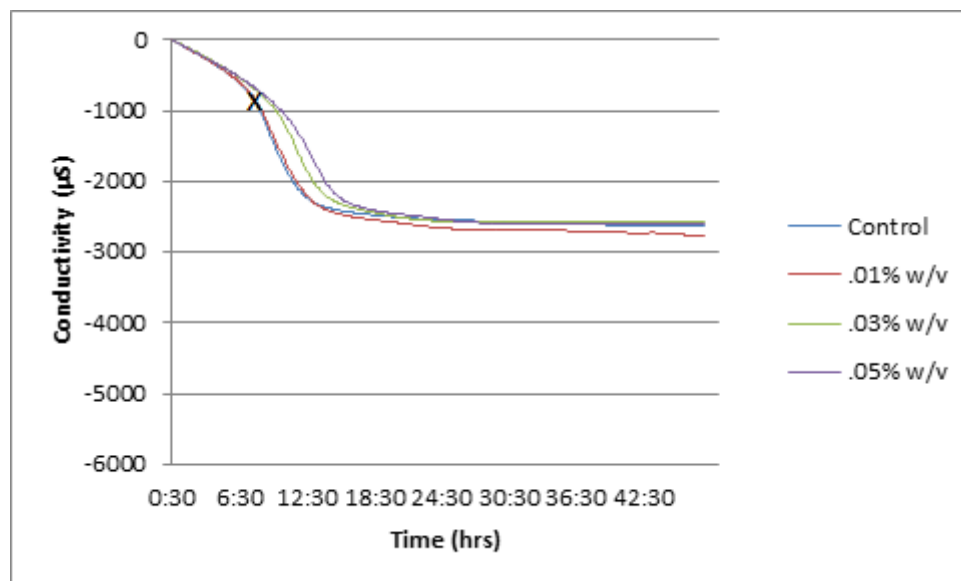


Figure.4. Fall of conductivity in detection solution for TSBYE containing ground cinnamon against *L.monocytogenes* at 37 oC Key: (X) TTD

TTD value was observed as 7hrs 36 mins in control which indicating *L.monocytogenes* has been shown growth in TSBYE (Figure 4). Growth of *L. monocytogenes* was detected in 0.01%, 0.03% and 0.05% ground cinnamon treated samples of TSBYE.

Results obtained from the experiment shows there was no antibacterial activity of ground cinnamon against *L. monocytogenes* at 25oC and 37oC in TSBYE. Yuste and Fung [11] showed that ground cinnamon in apple juice reduces populations of *L. monocytogenes* [1, 5,11]. Thus, the experiment had not given the same results. According to the results of the investigation, cinnamon oil has ability to suppress *L. monocytogenes* growth at both 25oC and 37oC perhaps cinnamon ground did not show suppression in *L. monocytogenes* both 25oC and 37oC. Therefore, it can conclude cinnamon oil can be used as an antimicrobial and as a flavoring agent to suppress the growth of *L. monocytogenes* and cinnamon ground can only used as a flavoring agent.

## Acknowledgement

Buddhika Wijamunige thanks entire academic staff members and the people who helped in numerous ways to success this project in the Natural resources Institute, University of Greenwich, United Kingdom.

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# A POTENTIAL BIOSURFACTANT MEDIATED CONTROL OF FUSARIUM WILT IN TOMATO CROP AND A NOVEL METHOD FOR CLEANING PESTICIDE RESIDUES IN TOMATOES

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## Abstract

The biosurfactant produced by *Serratia rubidaea* SNAU02 (NCBI accession number KC560769, has rhl gene KF 835609) was isolated from hydrocarbon-contaminated soils of Cuddalore district, Tamilnadu, India. The molecular characterization of the biosurfactant revealed the presence of rhamnolipid. The strain exhibited antifungal activity and demonstrated no toxicity against the seeds of *Brassica oleracea* and *Artemia salina* employed as a bio-indicator. The concentration of 250 µg/ml biosurfactant application controlled the *Fusarium* wilt of tomato crop. The feasibility of pesticide residues cleaning in tomatoes was studied five times using HPLC analysis. One kg of each tomato was treated with the 100ppm of Monocrotophos solution and allowed to stand for 24 h. After that, the tomatoes were collected and air dried for 1 hour. Further, the tomatoes were soaked for 30 min in the following treatments, T<sub>1</sub> (1000 ml distilled water), T<sub>2</sub> (1000 ml luke warm water), T<sub>3</sub> (2 per cent NaCl<sub>2</sub> in 1000 ml of distilled water), T<sub>4</sub> (2 per cent NaCl<sub>2</sub> in 1000 ml of luke warm) and T<sub>5</sub> (10 mg biosurfactant in 1000 ml distilled water) respectively. Followed that the tomatoes were carefully washed with 100ml of double distilled water and the washings were then collected and subjected to UV/Vis detector HPLC analysis. The treatment washings revealed that, there was no change in peak observation for T<sub>1</sub> and T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. Whereas, T<sub>5</sub> showed three broad peaks with the retention time of 2.432, 2.784 and 2.955 respectively, which denote the cleanup of pesticide into intermediate products which may be non-toxic.

Keywords: *Serratia rubidaea*, rhamnolipid, biocontrol, *Fusarium* wilt, Pesticide, Tomato

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## Introduction

Biosurfactants are amphiphilic compounds, produced by variety of microorganisms such as bacteria, fungi, and yeast and can reduce surface and interfacial tension of the liquids [14]. The biosurfactants have several advantages over chemical surfactants including high ionic strength tolerance, high temperature tolerance, higher biodegradability, lower toxicity, lower critical micelle concentration (CMC), and higher surface activity [2].

Biosurfactant producers can only be effective when

they are maintained at their optimal ambient conditions required for its growth and activity. In this regard, one of the best methodologies for optimization experiment, response surface methodology (RSM) is an empirical technique employed for multiple regression analysis by using quantitative data obtained from properly designed experiments to solve multivalent equations simultaneously [3]. In the past few years, numerous microorganisms with antifungal activities and their antifungal factors have been identified. In addition, the mechanisms by which microorganisms inhibit growth

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of potentially pathogenic fungi have been demonstrated [13]. Among lipopeptide and rhamnolipid biosurfactant, several species of *Bacillus* genus has been reported as bacterial biocontrol agents. There are limited reports on rhamnolipid biosurfactant as biocontrol agents. Rhamnolipid have found to have important antagonistic effect on economically important zoospore plant pathogen, thus opening their use as biocontrol agents [13]. Rhamnolipids have demonstrated inhibition of zoospore forming plant pathogens that have acquired resistance to commercial chemical pesticides [10] and another investigation has shown that rhamnolipid can stimulate plant immunity which is considered as an alternative strategy to reduce the infection by plant pathogens [15].

In agriculture production, various pesticides have been used for protection against plant diseases and insect pests with the result, these chemicals persist for longer period in fruits and vegetables which causing harm to human beings. Concern over the pesticide residues in fruits and vegetables have led to the development of many clean up and analysis methods. Taking these into account and considering the need of potential biosurfactant producers, economic production processes using agro-industrial wastes, controlled the *Fusarium* wilt of tomato crop and cleanup of pesticide into intermediate products which may be non-toxic.

## **Materials and Method**

### ***Microorganism***

*S. rubidaea* SNAU02 (accession number KC560769) ([http:// www.ncbi.nlm.nih.gov/nuccore/KC560769.1](http://www.ncbi.nlm.nih.gov/nuccore/KC560769.1)), a potent biosurfactant producer was used for the present study [6]. The strain was grown in nutrient agar (NA), sub-cultured each month and stored at 4 °C.

### ***Substrate***

Cashew apple juice was used as substrate for the present study.

### ***Biosurfactant production***

100 ml of Mineral salt medium (MSM) broth was sterilized in an autoclave at 121°C for 15 min. The clarified cashew apple juice as such without inorganic mineral salts to prove the effect of CAJ on the biosurfactant production. For the comparison, defined medium (MSM with 2% glucose) was included in this study. The sterilized MSM broth was inoculated with 5ml of the isolate SNAU02 and incubated at room temperature over an orbital rotary shaker set at 129 rpm min<sup>-1</sup> for 3 days.

### ***Response surface methodology (RSM) for the optimization of biosurfactant production***

In the experimental model, factors such as Inoculum size (g l<sup>-1</sup>), Peptone(g l<sup>-1</sup>), Initial pH, temperature and Shaker (rpm) were optimized by RSM. The specific codes for each independent variable and range of the variables used for this experiment are given in Table 1. The experiment was performed using central composite design (CCD) for which a total of 50 treatment combinations were generated using designer expert 7.0 software (Stat-Ease Inc. Minneapolis, USA).

From the experimental data according to this design, a second order polynomial regression model equation was derived as below:

Surface tension reduction of the media =

$$46.97482 + 0.28023A - 0.35236B - 0.29234C - 0.43173D + 0.40777E - 0.20312AB - 0.57188AC - 0.090625AD - 0.45938AE + 0.13437BC + 0.065625BD - 0.17812BE + 0.65937CD + 0.30312CE - 0.15313DE - 2.29423A^2 - 2.29423B^2 - 2.29423C^2 - 0.230307D^2 - 2.29423E^2$$

Where A: Inoculum size; B – Peptone; C - Initial pH; D – Temperature; E – Shaker; \* - 72. The RSM experimental model was employed in order to study the interaction between the factors for the optimization of the production of biosurfactant. Every level was included in the run matrix for the study on effect of various independent variables on the production of



biosurfactant by *S. rubidaea* SNAU02. Here, each experiment was done in three sets.

### **Statistical analysis**

This data was analyzed by the analysis of variance (ANOVA) technique to find out which factors had the most effective interactions for higher biosurfactant production [5].

### **Biosurfactant isolation**

The cell free supernatant was acidified with 6 N hydrochloric acid solutions to pH 2.0. The precipitate contained biosurfactant was allowed to settle down and kept overnight at 4 °C. The precipitated biosurfactant was collected by centrifugation at 15,000 rpm for 20 min. The precipitate was neutralised and recentrifuged at 12000 rpm for 10 min. The precipitate was freeze dried and stored.

### **Characterization of biosurfactant**

#### **Thin layer chromatography**

The extracted biosurfactant was characterized by thin layer chromatography (TLC) using silica gel plate (Silica gel 60; Merck, Darmstadt, Germany) and chloroform-methanol-water (65:15:2, v/v/v) as solvent system. The spots separated were visualized by placing the silica gel plate in iodine vapour in a glass chamber.

#### **Fourier transform infrared spectroscopy**

The extracted biosurfactant was subjected to Fourier transform infrared spectroscopy (FT-IR) analysis to identify the chemical bonds or the functional groups present. One milligram (freeze dried) purified biosurfactant was ground with 100 mg KBr pellet and pressed with 7,500 kg for 30 seconds to obtain translucent pellet. For this study, AVATAR-NICOLAT FT-IR system was used with a spectral resolution and wave number accuracy of 4 and 0.01cm<sup>-1</sup>, respectively. All the measurements consisted of 500 scans and a KBr pellet was used as background reference.

### **Gas chromatography-mass spectrometry (GC-MS)**

The GC-MS analysis was performed on a THERMO GC - TRACE ULTRA VER: 5.0, THERMO MS DSQ II. The column used was ZB 5 - MS capillary standard non - polar column. The oven temperature program was as follows: 70 °C raised to 260 °C at 6 °C /min. The electron impact ion source was maintained at 200°C. The electron energy was set at 70 eV.

### **HPLC analysis**

The derivatization and HPLC analysis were carried out using a modified method described by [9] and [4] rhamnolipid. The TLC fraction was further tested for their purity by a HPLC analysis. One mg of extract of *Serratia rubidaea* SNAU02 was dissolved in 1ml distilled water and 1ml of acetonitrile containing 2-bromoacetophenone ;Et<sub>3</sub>N) and heated for 1h at 80°C. The mixture was filtered through 0.22 µm syringe filter to remove particulate materials. Monocrotophos was used as standard.

### **Detection of rhl gene (rhamnosyltransferase) and sequencing**

Primers used for rhlAB were based on the gene sequences reported from *Pseudomonas aeruginosa* PA01 (Ochsner et al.1994). The amplification of rhl gene was performed using Rhlabf (5'-CAGGCCGATGAAGGGAAATA-3') and RhlAbr (5'AGGACGACGAGGTGGAAATC-3') primers. The reaction mixture was carried out in a (50µl) volume containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM each deoxy nucleoside triphosphate, 2 U *Taq* DNA polymerase (Invitrogen), 0.2 µM forward primer, reverse primer and 0.1 µg of template genomic DNA. The PCR condition were as follows: (i) 5min at 95 °C (ii) 30 cycle with 1 cycle consisting of 30s at 95°C, 1 min at 50 °C, and 2min at 72 °C, and (iii) a final extension step of 10min at 72 °C. The PCR product was separated and by

agarose gel electrophoresis and visualized, purified and sequenced. The sequence was submitted in the GenBank database. The gene bank accession number (KF835609) for rhlAB gene was obtained from NCBI.

#### ***Antifungal activity against plant pathogens***

Antifungal activity was screened by agar well diffusion method (11). The various concentration of biosurfactant (0,100, 250 and 500 µg/ml) were tested against plant pathogens, viz. *Fusarium oxysporum*. The PDA medium was poured in to the sterile Petri plates and allowed to solidify. The test fungal cultures were evenly spread over the PDA using sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. Each well was filled with various concentration of 200µl of biosurfactant solution and distilled water served as control. The plates were incubated at 27°C for 72 h and the plates were observed for formation of clear inhibition zone around the well and zone of inhibition was measured.

#### ***Biosurfactant toxicity assay***

##### ***Phytotoxicity assay***

The phytotoxicity assay for biosurfactant was performed according to Tiquia et al., (11). The seed germination and root elongation were carried out using cabbage (*Brassica oleracea*). Different concentration of extracted biosurfactant solutions (0,100, 250 and 500 µg/ml) were prepared with distilled water. The toxicity was assessed in sterilized Petri plate containing Whatman No.1 filter paper. The cabbage seeds were pretreated with 1% sodium hypochlorite for 15min. The Petri dish with 10 cabbage seeds was inoculated with 5.0ml of the biosurfactant solution and incubated at 27°C for five days. Then the number seed germination, root elongation and germination index (GI) were determined as follows:

Relative seed germination = (%) x 100

Relative root length (%) = x 100

$$GI = \frac{\text{Seed germination} \times \text{Root length of treatment}}{\text{Seed germination} \times \text{Root length of control}} \times 100$$

Controls were prepared with distilled water. The analysis was performed in triplicate.

#### ***Artemia assay***

The toxicity assay was performed with different concentration of the biosurfactant (0,100, 250 and 500 µg/ml) using brine shrimp (*Artemia salina*). Brine shrimp eggs were obtained from the Center of Advance Study in Marine Biology, Annamalai University, Parangipettai, India and the larvae were used within one day of hatching. Biosurfactant solution with saline water (33.3 g/l) was taken in penicillin tube containing 10 brine shrimp larvae in 5.0 ml of saline water per tube. The brine shrimp larvae in each penicillin tube were tested using 5.0 ml per different concentration of isolated biosurfactant solution and were observed for 24 h to calculate the mortality. The toxicity threshold concentration, expressed as biosurfactant concentration per 100 ml of saline water, was defined as the lowest concentration that killed all brine shrimp within 24 h. Each test was run in triplicate and saline water was used as the control. **Cleaning pesticide residues in tomatoes.**

One kg of each tomato was treated with the 100 ppm of Monocrotophos solution and allowed to stand for 24 h. After that, the tomatoes were collected and air dried for 1 hour. Further, the tomatoes were soaked for 30 min in the following treatments, T<sub>1</sub> (1000 ml distilled water), T<sub>2</sub> (1000 ml luke warm water), T<sub>3</sub> (2 per cent NaCl<sub>2</sub> in 1000 ml of distilled water), T<sub>4</sub> (2 per cent NaCl<sub>2</sub> in 1000 ml of luke warm) and T<sub>5</sub> (10 mg biosurfactant in 1000 ml distilled water) respectively. Followed that the tomatoes were carefully washed with 100ml of double distilled water and the washings were then collected and subjected to UV/Vis detector HPLC analysis.

#### **Result and Discussion**

The isolate *Serratia rubidaea* SNAU02 was identified as potential biosurfactant producer. The study resulted in a novel bacterial isolate, characterized as *Serratia rubidaea* SNAU02 and found possessing rhl gene. The detection of rhl gene confirmed the ability of *S. rubidaea* SNAU02 for the production of rhamnolipid type of biosurfactant. The expected size 777bp (Fig.1)

for rhamnosyltransferase gene, which eventually showed biosynthetic pathway of rhamnolipid in *S. rubidaea* SNAU02. The strain exhibited antifungal activity (Fig.2) and demonstrated no toxicity against the seeds of *Brassica oleracea* and *Artemia salina* employed as a bio-indicator [6,7].

RSM is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariate equations simultaneously [8]. RSM is not only used for optimization of culture parameters in the fermentation process, but also for studying the combined effects of medium components [1]. The model was designed with the result of 50 treatments (runs) presented, actual and predicted value of ST reduction in Table 1. The ANOVA analysis results showed that Inoculum size ( $\text{gl}^{-1}$ ), Peptone ( $\text{gl}^{-1}$ ), Initial pH, temperature and Shaker (rpm) had a significant effect on biosurfactant production (Table 2). The fit of the model was expressed with the coefficient of determination  $R^2$  that was 0.9471, indicating that 94.71% of variability in the response could be explained by this model. The adjusted  $R^2$  value of the model was 0.9106 and predicted  $R^2$  value was 0.8112. These results showed that application of RSM enhanced the biosurfactant production with the combination of inputs and proved that cashew apple juice could be effectively used as substrate for the production of biosurfactants.

The results of the HPLC chromatograms of all the five treatments (Table 3) on pesticide washing in the tomato were presented in Fig. 4.a-d. There was no change in peak observation for the treatments of  $T_1$  and  $T_2$ . Whereas a slight bend was observed in the retention time of 2.98 in  $T_3$ . In treatment  $T_4$  a single peak was observed in the retention time 3.0 which indicated the presence of pesticide residues in a little quantity.

In treatment  $T_5$  which was treated with 10 ppm concentration of biosurfactant solution showed three broad peaks with the retention time of 2.432, 2.784 and 2.955 which denote the cleanup of pesticide as well as

the breakdown of parent compound i.e. Monocrotophos, into intermediate products which may be non-toxic. The peak observed with the retention time of 3.147 for the standard was used in the comparative study for cleaning up pesticide residues. Thus, it was found that, biosurfactants were able to disintegrate the Monocrotophos compound sediment in tomato vegetables and it was evidenced by the different peak values for the treatment  $T_5$ .

## Conclusion

The biosurfactant produced by *Serratia rubidaea* SNAU02 isolated from hydrocarbon-contaminated soils of Cuddalore district, Tamilnadu, India. The molecular characterization of the biosurfactant revealed the presence of rhamnolipid. The strain exhibited antifungal activity and demonstrated no toxicity against the seeds of *Brassica oleracea* and *Artemia salina* employed as a bio-indicator. The concentration of 250  $\mu\text{g/ml}$  biosurfactant application controlled the *Fusarium* wilt of tomato crop.

The treatment washings revealed that, there was no change in peak observation for  $T_1$  and  $T_2$ ,  $T_3$  and  $T_4$ . Whereas,  $T_5$  showed three broad peaks with the retention time of 2.432, 2.784 and 2.955 respectively, which denote the cleanup of pesticide into intermediate products which may be non-toxic. Based on the present findings, the use of agro-industrial wastes as substrates would be an ideal process for the production of rhamnolipid biosurfactant. Considering the growing concern over the waste management challenges, the using of agro-industrial waste can be used as a cleaner bioprocess for the utilization of industrial waste as alternate substrate and the use of rhamnolipid in washing of pesticide indicates that it will be an efficient agent for cleaning of pesticide residues in tomato.

*Table-1 Experimental conditions of 2<sup>5</sup> factorial central composition design showing experimental and predicted surface tension reduction*

Run No.	Run order	Inoculum size (gl <sup>-1</sup> )	Peptone (gl <sup>-1</sup> )	Initial pH	Temperature (°C)	Shaker (rpm)	Experimental ST reduction (mN/m)	Predicted ST reduction(mN/m)
1	16	4	7	8	45	120	34.200	34.777
2	39	3	5	7	30	150	34.100	34.962
3	13	2	3	8	45	120	34.200	34.571
4	50	3	5	7	40	150	47.00	46.973
5	9	2	3	6	45	120	34.200	33.568
6	18	4	3	6	35	170	38.100	38.557
7	25	2	3	6	45	170	34.200	34.746
8	11	2	7	6	45	120	34.200	33.489
9	46	3	5	7	40	150	47.000	46.973
10	40	3	5	7	50	150	34.100	32.907
11	23	2	7	8	35	170	38.200	36.245
12	31	2	7	8	45	170	37.300	36.707
13	44	3	5	7	40	150	47.000	46.973
14	22	4	3	8	35	170	35.200	35.847
15	17	2	3	6	35	170	39.100	35.847
16	21	2	3	8	35	170	37.500	37.184
17	26	4	3	6	45	170	37.500	36.762
18	30	4	3	8	45	170	37.500	35.756
19	14	4	3	8	45	120	36.200	35.684
20	20	4	7	6	35	170	37.500	35.131
21	42	3	5	7	40	200	34.100	36.690
22	2	4	3	6	35	120	39.200	34.955
23	1	2	3	6	35	120	35.200	38.604
24	4	4	7	6	35	120	39.400	35.394
25	7	2	7	8	35	120	32.100	37.450
26	38	3	5	9	40	150	34.200	33.955
27	33	1	5	7	40	150	34.100	33.289
28	49	3	5	7	40	150	32.100	33.318
29	35	3	0	7	40	150	47.000	46.973
30	8	4	7	8	35	120	34.100	34.823
31	43	3	5	7	40	150	34.200	34.065
32	5	2	3	8	35	120	47.000	46.973
33	24	4	7	8	35	170	34.200	33.759
34	34	5	5	7	40	150	34.200	34.518
35	32	4	7	8	45	170	34.200	34.657
36	41	3	5	7	40	100	33.100	34.617
37	29	2	3	8	45	170	34.200	33.014
38	3	2	7	6	35	120	34.100	35.052
39	45	3	5	7	40	150	47.000	46.973
40	12	4	7	6	45	120	34.200	35.524
41	36	3	9	7	40	150	34.200	35.524
42	10	4	3	6	45	120	34.200	33.146
43	47	3	5	7	40	150	47.000	46.973
44	15	2	7	8	45	120	34.200	35.029
45	19	2	7	6	35	170	34.100	36.129
46	28	4	7	6	45	170	34.200	34.152
47	6	4	3	8	35	120	34.100	34.682
48	27	2	4	6	45	170	34.100	33.954
49	48	3	5	7	40	150	47.000	46.973
50	37	3	5	5	40	150	34.100	34.680

Table-2 ANOVA: Effect of five variables on biosurfactant production\*

Source	Sum of squares	Df	Mean square	F value	p-value Prob > F
Model significant	1025.13	20	51.26	25.94	< 0.0001
A-A3.40	1	3.40	1.72	0.1998	
B-B5.38	1	5.38	2.72	0.1098	
C-C	3.70	1	3.70	1.87	0.1816
D-D	8.07	1	8.07	1.09	0.0526
E-E7.20	1	7.20	3.65	0.0662	
AB1.32	1	1.32	0.67	0.4203	
AC10.47	1	10.47	5.30	0.0287	
AD0.26	1	0.26	0.13	0.7180	
AE6.75	1	6.75	3.42	0.0747	
BC0.58	1	0.58	0.29	0.5928	
BD0.14	1	0.14	0.070	0.7936	
BE1.02	1	1.02	0.51	0.4792	
CD13.91	1	13.91	7.04	0.0128	
CE2.94	1	2.94	1.49	0.2323	
DE0.75	1	0.75	0.38	0.5425	
A <sup>2</sup> 292.49	1	292.49	148.03	< 0.0001	
B <sup>2</sup> 292.49	1	292.49	148.03	< 0.0001	
C <sup>2</sup> 292.49	1	292.49	148.03	< 0.0001	
D <sup>2</sup> 294.74	1	292.74	148.17	< 0.0001	
E <sup>2</sup> 292.49	1	292.49	148.03	< 0.0001	
Residual	57.30	29	1.98		
Lack of fit	57.30	22	2.60		
Pure error	0.0000	7	0.0000		
Cor Total	1082.42	49			

Table-3 Cleaning of pesticide residues in Tomato using different washing solution

Treatments	Washing solutions
T1	1000ml distilled water
T2	1000ml lukewarm water
T3	2 percent sodium chloride in 1000ml of distilled water
T4	2 percent sodium chloride in 1000ml of lukewarm water
T5	10ppm of biosurfactant solution(10mg in 1000ml distilled water)

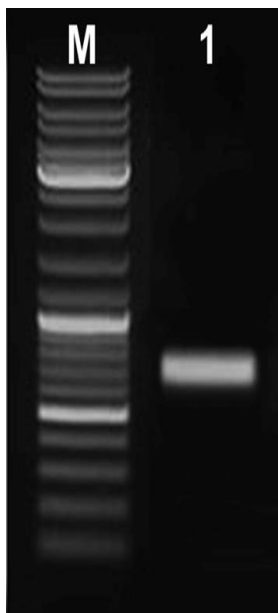


Figure 1: Agarose gel electrophoresis for PCR product of rhamnosyltransferase gene in *S. rubidaea* SNAU02 (Lane M(Marker)-1kb DNA ladder, Lane 1- rhamnosyltransferase gene - 777bp).

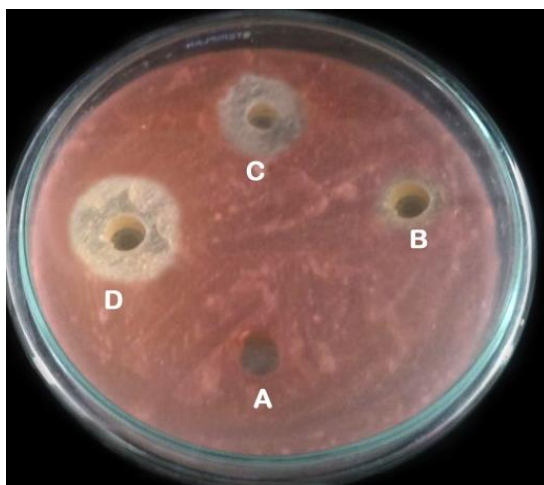


Figure 2: Antifungal activity of biosurfactant at various concentrations (A-control, B-100µg/ml, C-250µg/ml, D- 500µg/ml) against *Fusarium oxysporum*

<Chromatogram>

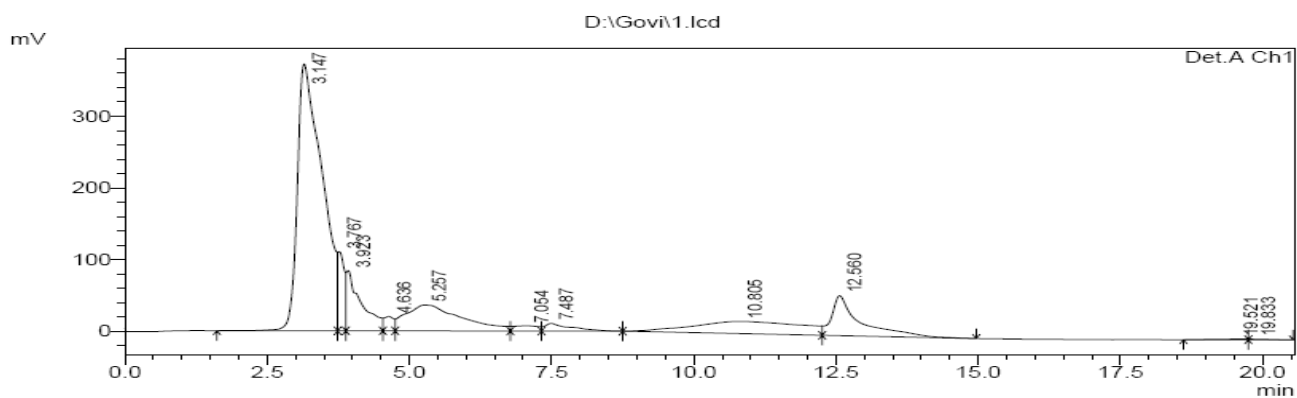


Figure 4: a. Monocrotophos Standard

<Chromatogram>

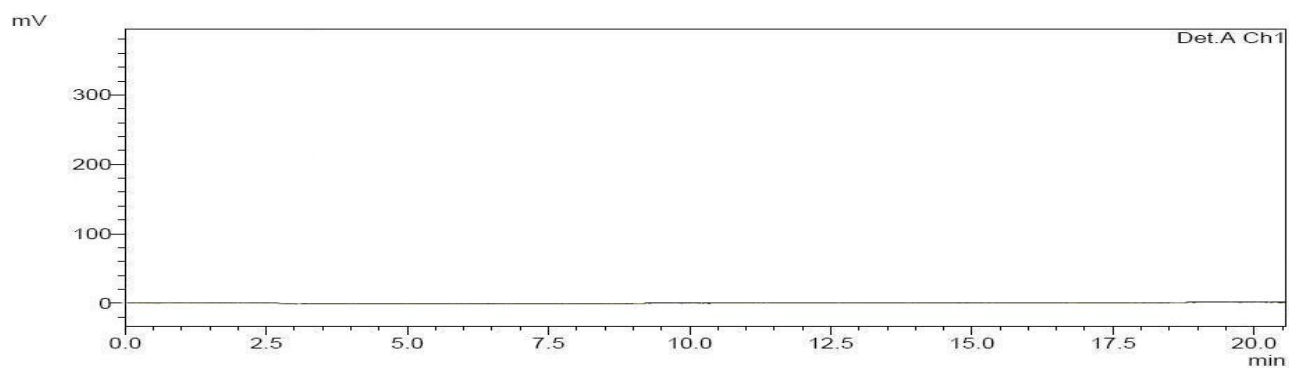


Figure 4: b. T1- 1000 ml distilled water

<Chromatogram>

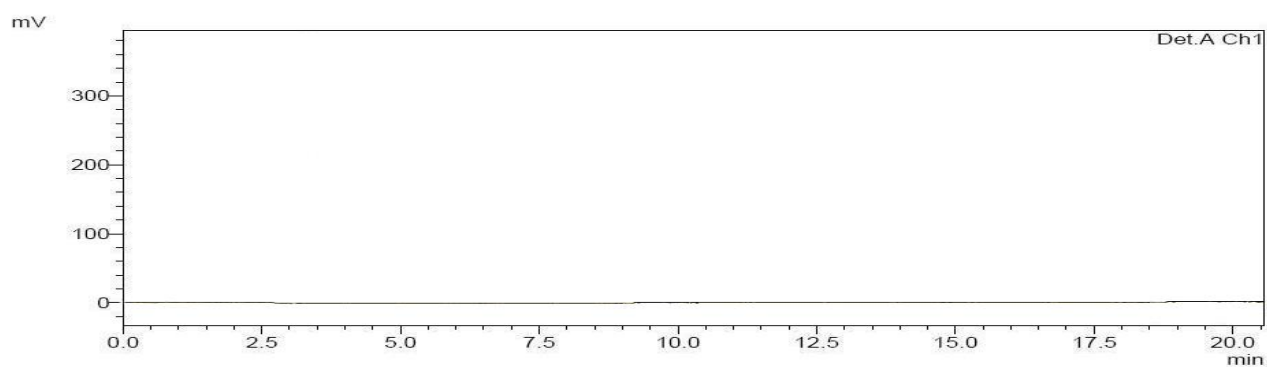


Figure 4: c. T2. 1000 ml lukewarm distilled water

# <Chromatogram>

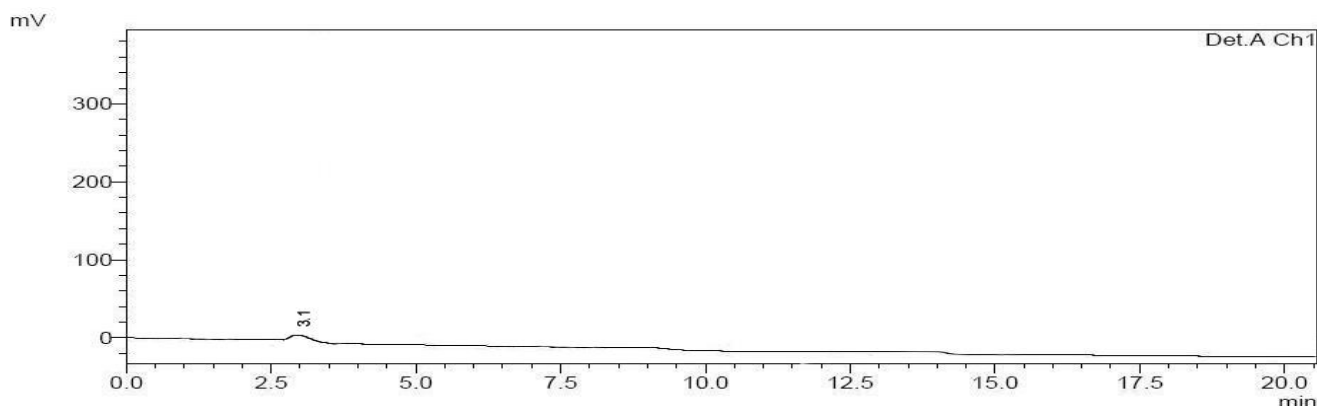


Figure 4: d. T3. 2 per cent NaCl in 1000 ml distilled water

## Acknowledgement

The authors wish to express their gratitude for the support extended by the authorities of Department of Microbiology, Annamalai University, Annamalai nagar for carrying out the research work. The corresponding author place on record that the work was executed with a help of major research project funding from UGC, India

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# INTERACTION EFFECT OF AM FUNGI AND SALT STRESS ON THE GROWTH OF CURCUMA LONGA L. GROWN UNDER GREEN HOUSE CONDITION

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## Abstract

Arbuscular Mycorrhiza is a ubiquitous fungus which is associated with root system of higher plants. The distribution and abundance of AM fungi vary greatly among different sites including natural and manmade ecosystems. *Curcuma longa*, L. is a herbaceous perennial plant belongs to the Family: Zingiberaceae, commonly called as turmeric which is a native of South Asia particularly India. India is a leading producer and exporter of turmeric in the world. It is used as condiment, dye, drug and cosmetic in addition to its use in religious ceremonies. The present study dealt with the interactive effect of AM fungi and salt stress on the vegetative growth of turmeric plants grown under green house condition. The study reported that the low concentration of sodium chloride does not affect the plant growth when they treated with AM fungi compared to control plants. Thus this AM fungi stimulated the salt tolerance in turmeric plants against the low concentrations of sodium chloride.

Keywords: AM fungi, Turmeric, Nutrient mobilization and salt stress.

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## Introduction

Arbuscular Mycorrhiza is an endophytic fungi which is associated with root system of higher plants. Turmeric (*Curcuma longa* L), the ancient and sacred spice of India known as 'Indian saffron' is an important commercial spice crop grown in India. Andhra Pradesh, Tamil Nadu, Orissa, Karnataka, West Bengal, Gujarat, Meghalaya, Maharashtra, Assam are some of the important states cultivates turmeric, of which, Andhra Pradesh alone occupies 35.0% of area and 47.0% of production. It is used in diversified forms as a condiment, flavouring and colouring agent and as a principal ingredient in Indian culinary as curry powder. It has anti cancer and anti viral activities and hence finds use in the drug industry and cosmetic industry. 'Kum-kum', popular with every house wife, is also a by-product of turmeric. It finds a place in offerings on religious and ceremonial occasions. A type of starch is also being extracted from a particular type of turmeric. The increasing demand for natural products as food additives makes turmeric as ideal produce as a food

colourant. The present study deals with the assessment of various concentrations of Sodium chloride influence the growth of *Curcuma longa* under green house condition.

## Materials and methods

Open pot culture was used for the mass production of AM fungi. A layer of 100g of collected soil samples (inoculum) was spread over pot mixture (sterilized soil and sand=1:3; about 3 kg) in earthen pots (20 cm height and 25 cm diameter). These pots were used for the experimentation. The pot without AM fungal inoculum was used as control. The 2cm size rhizome of *Curcuma longa* was propagated at the depth of 5cm in pot soil. The plant was assigned for the following treatments: T1- 0.1, T2- 0.2, T3 - 0.3, T4 - 0.4 and T5 - 0.5%. Sodium chloride treatment for 7 days period after 90<sup>th</sup> day of plant growth. The vegetative growth of *Curcuma longa* analysed by Chlorophyll and carotenoid

estimation method (Arnon, 1949) and free proline content (Bates *et al.*, 1973) was estimated at regular interval of 30 days. The data collected in this study was subjected to analysis of variance (ANOVA) and means comparison has done using Duncan's multiple range test (DMRT) (Little and hills, 1978).

## Results and discussion

Soil salinity is a major abiotic stress adversely affecting plant growth and crop production worldwide. Microbes like AM fungi are able to inoculate plants, in their natural environment. Some beneficial microbes like bacteria and fungi can improve the plant growth under stress environments and also enhance yield (Evelin *et al.*, 2009).

In the present study such AM fungi inoculation in *Curcuma longa* plant significantly enhanced the vegetative growth (increase the chlorophyll and carotenoid pigments) and synthesis of physiologically protective compounds (proline accumulation) when compared to control plants. Initial period of plant growth the chlorophyll and carotenoid content were normal (Table: 1&2; Fig: 1) and were decreased during stress period induced for 7 days except in case of proline accumulation (Fig:2), it was higher when the plants grown under various concentrations of sodium chloride. But the lower concentrations 0.1% does not affect the plant growth when they grown under salt stress. These plants were recovered faster than the other higher concentrations of salt induced plants. It is concluded that the AM fungi inoculated plant showed significant growth and showed mild salt tolerance against low concentrations of sodium chloride when compared to non- AM fungi inoculated plants.

AM fungi are associated with the roots of over 80% terrestrial plant species (Smith and Read, 2008). AM fungi have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by utilizing various mechanisms, such as enhancing nutrient uptake (Evelin *et al.*, 2012), producing plant growth hormones, improving rhizospheric and soil conditions (Asghar *et al.*, 2005), improvement in photosynthetic activity or water use efficiency (Hajiboland *et al.*, 2010), accumulation of

compatible solutes (Evelin *et al.*, 2013) and production of higher antioxidant enzymes (Manchanda and Garg, 2011).

The present investigation showed that the inoculation of AM fungi and low concentration of sodium chloride on *Curcuma longa* plant enhanced the vegetative growth by means of increase in chlorophyll and carotenoid content and tolerance for few days of salt stress. But the higher concentrations affected the growth of *Curcuma longa* plants grown under green house conditions. It is also suggested that the soil application of such Am fungi not only enhanced the vegetative plant growth but also increased the soil fertility and reduced the risk of application of chemical fertilizers in the agricultural field. They are generally termed as eco-friendly fertilizers and do not cause any environmental pollution.

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## Appendix

Table: 1 Interaction effect of AM fungi and Salt stress on the Chlorophyll a (mg/g fresh leaf) content of *Curcuma longa* under green house condition

Sl No	Treatments	60 <sup>th</sup> Day	90 <sup>th</sup> Day	97 <sup>th</sup> Day	120 <sup>th</sup> DAY	150 <sup>th</sup> Day	180 <sup>th</sup> Day	210 <sup>th</sup> Day	240 <sup>th</sup> Day
1	Control	0.1132 <sup>a</sup> ±0.020	0.3212 <sup>c</sup> ±0.012	0.1054 <sup>a</sup> ±0.010	0.2104 <sup>b</sup> ±0.102	0.5261 <sup>e</sup> ±0.027	0.4203 <sup>d</sup> ±0.020	0.4015 <sup>d</sup> ±0.020	0.3209 <sup>c</sup> ±0.010
2	T1	0.3845 <sup>a</sup> ±0.040	0.7532 <sup>b</sup> ±0.012	0.7911 <sup>b</sup> ±0.020	1.1043 <sup>c</sup> ±0.116	3.0241 <sup>d</sup> ±0.020	5.0241 <sup>e</sup> ±0.200	8.1253 <sup>f</sup> ±0.140	5.0126 <sup>e</sup> ±0.122
3	T2	0.3845 <sup>a</sup> ±0.040	0.7532 <sup>b</sup> ±0.032	0.7221 <sup>b</sup> ±0.011	0.8242 <sup>c</sup> ±0.113	1.1023 <sup>d</sup> ±0.103	3.0221 <sup>f</sup> ±0.110	5.0012 <sup>g</sup> ±0.520	2.0134 <sup>e</sup> ±0.310
4	T3	0.3845 <sup>e</sup> ±0.040	0.7532 <sup>b</sup> ±0.021	0.6713 <sup>b</sup> ±0.023	0.8013 <sup>c</sup> ±0.025	1.0242 <sup>d</sup> ±0.102	3.1325 <sup>f</sup> ±0.053	4.1370 <sup>g</sup> ±0.240	2.0143 <sup>e</sup> ±0.126
5	T4	0.3845 <sup>a</sup> ±0.040	0.7532 <sup>c</sup> ±0.010	0.6201 <sup>b</sup> ±0.031	0.7804 <sup>c</sup> ±0.031	1.1432 <sup>d</sup> ±0.028	3.4572 <sup>f</sup> ±0.125	4.0321 <sup>g</sup> ±0.210	2.1187 <sup>e</sup> ±0.031
6	T5	0.3845 <sup>a</sup> ±0.040	0.7532 <sup>c</sup> ±0.040	0.5023 <sup>b</sup> ±0.025	0.7724 <sup>c</sup> ±0.009	1.2314 <sup>d</sup> ±0.027	3.2144 <sup>f</sup> ±0.052	4.1452 <sup>g</sup> ±0.220	2.1658 <sup>e</sup> ±0.113

Values are mean of five replicates ± SD The mean difference is significant at the 0.05

Table: 2 Interaction effects of AM fungi and Salt stress on the Chlorophyll b (mg/g fresh leaf) content of *Curcuma longa* under green house condition

Sl No	Treatments	60 <sup>th</sup> Day	90 <sup>th</sup> Day	97 <sup>th</sup> Day	120 <sup>th</sup> DAY	150 <sup>th</sup> Day	180 <sup>th</sup> Day	210 <sup>th</sup> Day	240 <sup>th</sup> Day
1	Control	0.0072 <sup>c</sup> ±0.010	0.0095 <sup>d</sup> ±0.023	0.0064 <sup>b</sup> ±0.002	0.0070 <sup>c</sup> ±0.002	0.0061 <sup>b</sup> ±0.017	0.0103 <sup>e</sup> ±0.030	0.0100 <sup>e</sup> ±0.021	0.0009 <sup>a</sup> ±0.003
2	T1	0.0934 <sup>a</sup> ±0.022	0.2394 <sup>b</sup> ±0.026	0.2441 <sup>b</sup> ±0.007	0.3233 <sup>c</sup> ±0.106	0.4241 <sup>d</sup> ±0.030	0.6231 <sup>e</sup> ±0.030	0.6853 <sup>e</sup> ±0.023	0.4023 <sup>d</sup> ±0.008
3	T2	0.0934 <sup>a</sup> ±0.022	0.2394 <sup>b</sup> ±0.026	0.2100 <sup>b</sup> ±0.011	0.2205 <sup>b</sup> ±0.013	0.3123 <sup>c</sup> ±0.011	0.4125 <sup>d</sup> ±0.033	0.5002 <sup>e</sup> ±0.010	0.3032 <sup>c</sup> ±0.010
4	T3	0.0934 <sup>a</sup> ±0.022	0.2394 <sup>c</sup> ±0.026	0.2101 <sup>b</sup> ±0.063	0.2113 <sup>b</sup> ±0.125	0.2245 <sup>b</sup> ±0.020	0.4226 <sup>e</sup> ±0.062	0.5200 <sup>f</sup> ±0.020	0.3140 <sup>d</sup> ±0.024
5	T4	0.0934 <sup>a</sup> ±0.022	0.2394 <sup>d</sup> ±0.026	0.2013 <sup>c</sup> ±0.004	0.1808 <sup>b</sup> ±0.021	0.2232 <sup>c</sup> ±0.018	0.3802 <sup>e</sup> ±0.015	0.4821 <sup>f</sup> ±0.040	0.3017 <sup>d</sup> ±0.024
6	T5	0.0934 <sup>b</sup> ±0.022	0.2394 <sup>e</sup> ±0.026	0.0900 <sup>a</sup> ±0.025	0.1621 <sup>c</sup> ±0.012	0.2114 <sup>d</sup> ±0.017	0.3704 <sup>f</sup> ±0.022	0.3955 <sup>g</sup> ±0.032	0.2057 <sup>d</sup> ±0.015

Values are mean of five replicates ± SD The mean difference is significant at the 0.05

Figure: 1 Interaction effect of AM fungi and Salt stress on the Carotenoid content (mg/g fresh leaf.) of *Curcuma longa* under green house condition

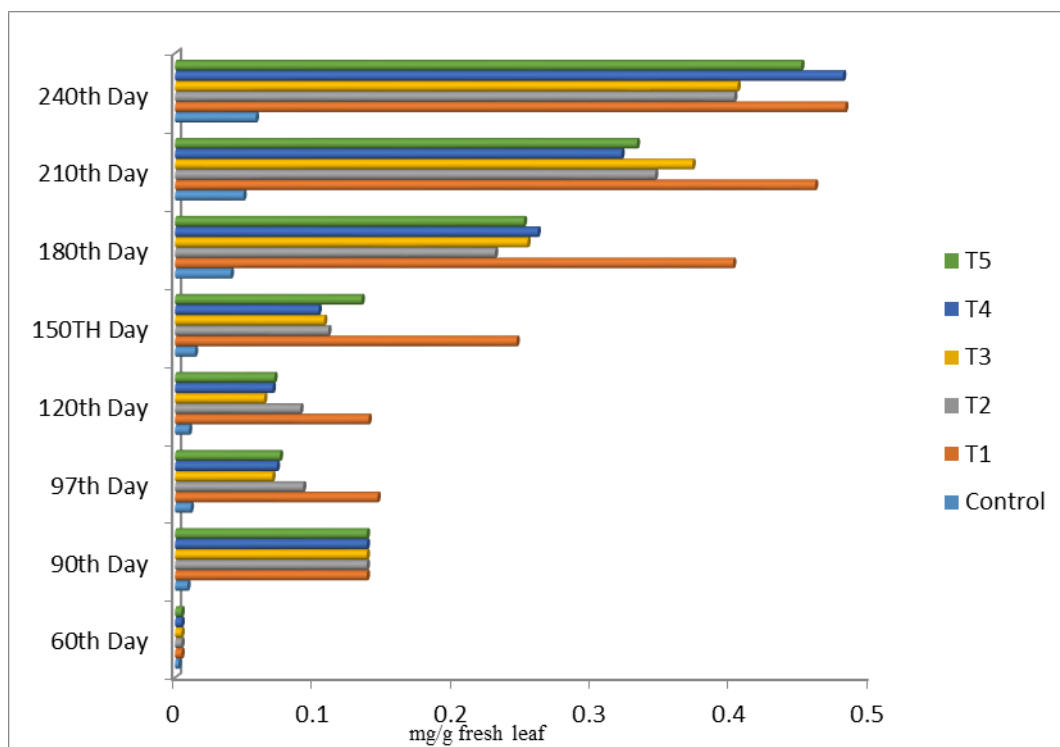
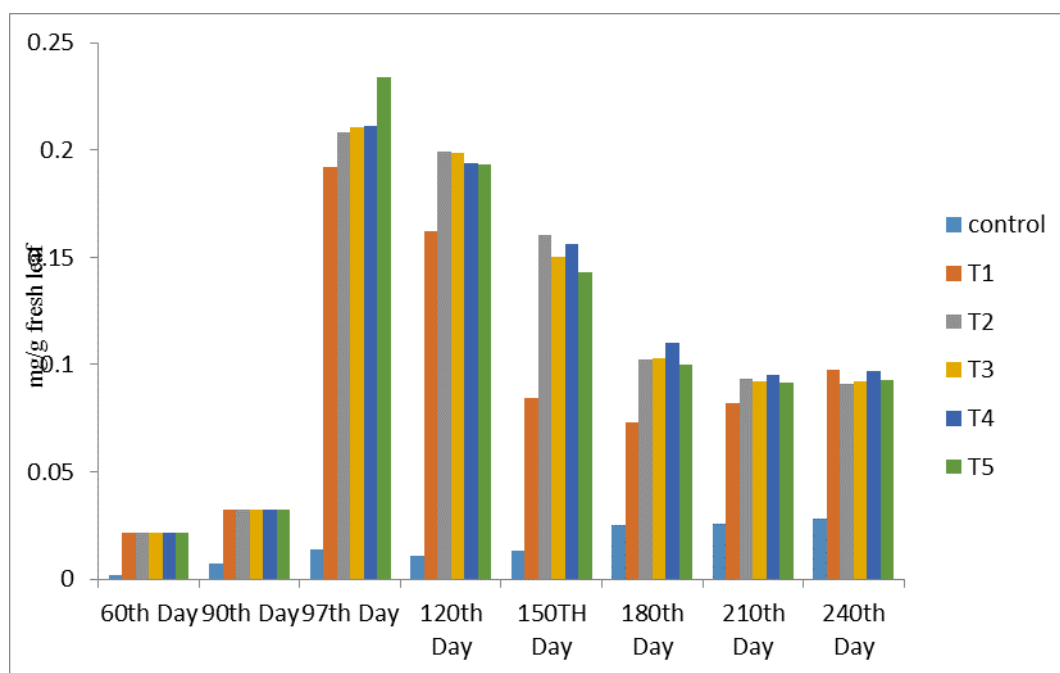


Figure: 2 Interaction effect of AM fungi and Salt stress on the proline content (mg/g fresh leaf.) of *Curcuma longa* under green house condition



# BIOCHEMICAL CHARACTERIZATION AND INSECTICIDAL ACTIVITY OF DIFFERENT SOLVENT CRUDE EXTRACTS OF *LANTANA* *CAMARA* L. ON DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LINN.)

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## Abstract

A study was made to evaluate the insecticidal action of *Lantana camara* L. against diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Sixty to seventy five grams of ground plant material were successively extracted using eight organic solvents viz., acetone, benzene, chloroform, ethanol, ethyl acetate, petroleum ether, hexane, and methanol in Soxhlet apparatus for 24 hrs and tested against *P. xylostella* at six concentrations (1, 2, 4, 6, 8 and 10 per cent). Bioassay results indicated that the toxicity increased proportional to the concentrations of the all extracts. Among the extracts of different solvents experimented, hexane extract proved to be the best solvent followed by others; we also have compared these extracts with neem oil of same concentrations. In all solvent extracts eight and ten per cent concentration of *L. camara* had stronger ovicidal and oviposition deterrent effects with low larvicidal activities. Plant metabolites are highly diverse, having distinct functions according to their structure and Gas chromatography Mass Spectrometry (GC-MS) analysis of hexane extract revealed the presence of 19 major phytochemicals including caryophyllene, caryophyllene oxide, 2-hexadecen-1-ol, benzene, hexatriacontane, tetrapentacontane, 1, 3-cyclohexadiene-1-carboxaldehyde, 6S-2,3,8,8-tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene, 3-nonanone, phytol and squalene etc.

Keywords: *Lantana camara*, GC-MS analysis, contact toxicity, ovicidal effect

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## Introduction

Cabbage and cauliflower are important cash crops for farmers often produced under small holder conditions throughout tropical and subtropical areas of Asia, Africa, Latin America and the Caribbean countries. In India, vegetables play important role in nutritional security, economic viability and source of remunerative income for many small and marginal farmers under intensive farming system. The cole vegetables are cultivated in 4.00 and 4.34 lakh ha producing 9039.00 and 8573.00 MT with an average yield was 22.6 and 19.80 t ha<sup>-1</sup> of cabbage and cauliflower respectively in India. During 2013-2014, India produced 162.19 million tonnes of vegetables and exported worth of Rs.

5462.93 crores (Indian Horticulture Database, 2013).

The production share of cruciferous vegetable crops (2013-14) was to the extent of 5.5 and 5.3 per cent of cabbage and cauliflower respectively and the yield loss estimated upto 17- 99 per cent of both (Uijtewaal, 2006; Uthamasamy *et al.* 2011 and IHD, 2013). In Tamil Nadu, it occupies an area of 24000 and 9500 ha with an annual production of 130.42 and 209.17 MT and productivity is 50 and 22T ha<sup>-1</sup> of cabbage and cauliflower respectively. The economic loss due to this pest has been estimated worldwide to be US\$ 4-5 billion (Zalucki *et al.*, 2012) and US\$ 16 million annually in India (Mohan and Gujar, 2003). Several

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applications of synthetic insecticides on crucifers are common among commercial farmers (FAO, 2005). *Lantana* (Verbenaceae) is mostly distributed in approximately 50 countries in the world. Introductions of non-native or agricultural plants into novel habitats without their co-evolved counterparts may induce novel plant-insect interactions (Strong, 1979).

Many kinds of monoterpenes from plant sources have been evaluated as feeding deterrents against insects (Koul, 1982). The major secondary substances found among plants are organic compounds such as alkaloids, terpenes, saponins, phenol, cardiac and cyanogenic glycosides, nitro-containing compounds, resins and certain proteins and acids (Lewis and Elvin-Levis, 1977). Oil extracted from *L. camara* are known to exhibit ovicidal, insecticidal, antifeedant, attractant, repellent, antiviral and anti-juvenile hormone activities (Rejesus, 1986). However, information on the efficacy of *L. camara* on cruciferous vegetables is very scarce. Keeping these in view, the present study was made to evaluate the efficacy of different solvent crude extracts of *L. camara* against diamondback moth *P. xylostella* in comparison with commonly used botanical neem oil.

## Materials and Methods

The study focused on the effectiveness of different organic solvent extractions from the aerial part of *L. camara* tested against *P. xylostella* in cole vegetables. Experiments were conducted at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, from August 2014 to December 2014.

### Collection of test plant materials

Aerial parts of the test plant *L. camara* were collected from Thondamuthur and Aalandurai block of Coimbatore district during November 2013. The samples were air-dried for 15-20 days under shade. After complete shade drying, the plant parts were pulverized into powder with the help of local motor grinder. The plant material was extracted by Soxhlet extraction method. The aqueous extract of these plant materials was prepared on per cent basis as per Sharma *et al.* (1997) for which a stock solution of 10 per cent

concentration was prepared by dissolving 10 gram of plant material in 90 ml of distilled water and used at different concentrations.

### Soxhlet Extraction

The plant material was subjected to Soxhlet extraction suggested by Sukthamrong *et al.* (1981) and Sharma and Gupta (2009) in order to extract more active principles. Known amount (50 - 75g) of plant material of each solvent was filled into the Soxhlet apparatus. A cotton plug was used at the place of thimble to stop the entry of the crude material into the siphoning tube. The required organic solvents *viz.*, acetone, benzene, chloroform, ethanol, ethyl acetate, petroleum ether, hexane, and methanol were filled up five times more than total amount of the sample material into the flask of the apparatus. The apparatus was then connected with the water supply to the condenser. The temperature of the heating mantle was maintained according to the boiling point of organic solvents. The process was carried out for 24 hrs for each sample of the solvent.

The pooled extract was then filtered using a Whatmann filter paper no.1 and concentrated by rotary evaporation at 40°C. After drying in desicator, crude extracts were weighed, stored in stock vials and kept in refrigerator (0 - 4°C) for further use. It was further purified by flash or column chromatography (anhydrous Na<sub>2</sub>SO<sub>4</sub> + silica gel + dehydrated charcoal) for GC MS analysis.

### Gas Chromatography – Mass Spectrometry analysis of *L. Camara* leaf extract

A shimadzu QP – 2010 plus GC-MS was used in pesticide Toxicology laboratory, Department of Agricultural Entomology and method described by Shettima *et al.* (2013). The GC-MS was equipped with a split injector and an ion-trap mass with 220°C spectrometer detector together with a fused silica capillary column (RXI-1MS) having a thickness of 0.25µm, dimensions of 30m x 0.25mm and temperature limits of 60°C to 260°C. The column temperature was programmed between 60°C and 260°C at a rate of 3.0ml/min. The mass range and temperature of the

injector and detector were at 500 M/z of 220°C and 200°C respectively. Helium gas was used as a carrier gas at a flow rate of 0.95 ml per minute.

### **Laboratory rearing of the diamondback moth, *Plutella xylostella* (L.)**

#### ***Raising of mustard seedlings***

The soil mixture required for raising mustard seedlings were prepared by mixing soil, compost and leaf litter at a ratio of 1:1:1. Plastic tea cups (6 x 3cm) with holes at the bottom were filled with soil mixture to a height of 2.5 cm. Mustard seeds, treated with carbendazim were sown evenly over the entire soil surface and watering was done once in two days. The seeds germinated in about three days at a room temperature of 28±5°C. The seedlings, at a height of 4 - 5 cm, were used for oviposition by female DBM.

#### ***Adult oviposition and collection of eggs***

The DBM culture was initiated with the larvae collected from the field of Aalandurai Narasipuram and Thondamuthur areas of Coimbatore. The collected larvae were reared on young cauliflower leaves. When they pupated, 50 nos. were transferred to adult emergence cages (30x30x30 cm). Sugar solution (10%) fortified with multi-vitamin drops was provided as adult diet for the moths. A day after the emergence of adults, mustard seedlings were provided for oviposition. The moths laid eggs on both the surfaces of mustard leaves as well as on petioles. Fresh seedlings were provided once in two days until all the adults died.

#### ***DBM larval rearing***

Larval rearing was carried out in cages with the size of 30x30x30 cm. The first instar larvae hatched in about 3 to 4 days were initially fed by mining into the mustard leaves and later on the entire leaves. For second instar larvae, tender cauliflower leaves were provided as feed material. Most of the larvae migrated to cauliflower leaves within a day and the larvae were provided with fresh leaves every day. To meet the daily requirement of leaves, cauliflower plants were grown continuously

in pots and field. The larval stage lasted for 12 to 14 days and pupation mostly occurred on the lower surfaces of the leaves. The larvae pupated during different dates were collected by using a camel hair brush. To synchronise the emergence of moths, the collected pupae were stored in a refrigerator. When all the larvae were pupated, they were taken out from the refrigerator and kept in the adult emergence cage. The pupal period lasted for 5 to 6 days. DBM were rearing under laboratory conditions at a photoperiod of 12:12 (light: dark) and temperature of 28±4.0°C with RH of 65±5 per cent.

### **Bioassays (No Choice Test)**

#### ***Larval contact toxicity***

The contact toxicity of *L. camara* crude extracts was evaluated using Potter's tower (Potter, 1952). One ml of each concentration (1, 2, 4, 6, 8 and 10%) was delivered on the ten larvae were placed in a petri plates per replication including the untreated leaf disc. The crude extracts were sprayed through a Potter tower (Burkard, Rickmansworth, UK) on cauliflower leaf discs (9.0 cm diameter) at 0.34 bar (34 kPa) pressure with APSA 80 0.1 ml + one ml spray aliquot. The treatment using organic solvent + APSA 80 0.1 ml served as the control of respective extraction. After complete evaporation, the leaves were transferred to clean bioassay containers over a moistened filter paper. The leaf discs were placed slantingly to rest on side of the container so that larvae can move on either side. Ten larvae were released in each petri dish and three replicates were maintained per treatment. Per cent mortality was observed 24, 48 and 72 h after treatment. All the experiments were carried out in a room temperature with a photoperiod of 12:12 (L: D) and experiments with control mortality more than 20 per cent were discarded and repeated. The mortality data were recorded at different interval till adult emergence. The total per cent mortality data were calculated and corrected using Abbott's formula (Abbott, 1925).



### ***Pupal contact toxicity***

The contact toxicity of *L. camara* crude extracts in pupae was evaluated by direct dip bioassay described by Idris and Grafius (1993). About fifty pupae used in each concentration of *P. xylostella* were placed in a 4 cm tea filter and dipped in the defined time for 30 sec. In the control treatment the test insect were tipped in tap water. The pupae were removed and blotted dry on filter paper and transformed onto untreated cabbage leaf discs in 9 cm petri dishes. The mortalities were recorded every 24 hrs upto seven days or died as dark pupae and all the experiments were replicated three times.

### ***Ovicidal toxicity***

Ovicidal toxicity was evolved as per Kumar *et al.* (2009). 6 - 8 week old cabbage/ cauliflower seedlings were placed in a screened insect cage (40 x 40 x 40 cm) where 20 pairs of 2 day old moths were placed; after 24 h, the eggs deposited on the seedlings were collected and counted. Fifty eggs deposited on the leaves were dipped into 1 ml of each solution of six concentrations (1, 2, 4, 6, 8 and 10%) for 30 sec and placed in a petri dish. The number of unhatched eggs was counted on the fifth day after treatment. Each treatment, including the control, was replicated three times.

### ***Oviposition deterrent activity***

Oviposition deterrent activity was evaluated as per Soontorn and Rejesus (2005). *L. camara* crude extracts of 1, 2, 4, 6, 8 and 10 per cent were sprayed for 6 - 8 week old cabbage and cauliflower seedlings, which were then placed inside a cage. Three seedlings represented in each treatment, including a control that was sprayed with distilled water. Adult *P. xylostella* females were tested for oviposition in a no-choice test on treated and untreated cauliflower plants in a screened cage (30 x 30 x 30 cm) kept in a laboratory at 25°C with 60-70 per cent R.H. For each replicate, mated females (within 24 h) was released and eggs on each leaf per live plant were counted after 24 hrs and mean counts expressed in oviposition deterrent indices (ODI) as: Oviposition

deterency index =  $(C-T)/(C+T) \times 100$ ; where C = number of eggs on control plants and T = number of eggs on treated plants.

### ***Statistical analysis***

The laboratory experiment was conducted in completely randomized design. The raw data were subjected to square root transformation and the data on percentage were transformed into arc sine values before statistical analysis. Observed mortality data were converted to percentage and were subjected to probit analysis (Finney, 1971) for obtaining regression equations for dosage mortality response and to determine the LC50 and LC95 values. The mean values were separated using LSD through ANOVA.

## **Results and Discussion**

The active principles from aerial parts of *L. camara* were extracted using eight solvents of different polarity. Among all the solvents used, extracts from petroleum ether, hexane, methanol and chloroform retain their natural appearance. The amount, temperatures of extraction and appearance of each solvent fraction colours are presented in (Table 1). Phytochemical compounds are believed to deter invertebrates from plants, either by acting as antifeedants or by being toxic through hormonal disruption upon ingestion. Most of plants were reported to be insecticidal without specifying the type of action. In these contexts, this experiment assumed importance that the results would compartmentalize toxicity either as contact and/or stomach poisons.

### ***Larval and pupal contact toxicity***

The toxicity of different solvents extracts of *L. camara* and neem oil at every 24 hrs is illustrated in (Figures 2 a - j). Significant differences were found among the treatments ( $P < 0.05$ ); and corresponding probit curves/mortality of second instars are represented in (Figure 1). Among the eight solvent crude extracts tested, hexane extract showed greater performance in terms of contact toxicity of larval and pupae, ovicidal toxicity, oviposition deterrent activity as it are evident

from the data. On the basis of probit analysis (Table 2) the efficiency (LC<sub>50</sub> and LC<sub>95</sub>) of extracts was as follows: hexane > chloroform> methanol> ethyl acetate> acetone> petroleum ether> ethanol> benzene.

Among the larval stages second and third instars are highly vulnerable rather than fourth instar and pupal stage Figure 5.

Table 1 Physical characteristics of fractions obtained from leaf of *Lantana camara* L.

S.No.	Organic Solvents and their quantity (Lit.)	Temp. (°C)	Sample Weight(g)		Texture	Colour	
			Leaf	Crude			
1	Acetone	2.0	55-57	270	30.00	Jelly	Dark green
2	Benzene	1.0	80.10	230	30.00	Gummy	Light yellow
3	Chloroform	1.8	60-62	400	30.00	Gummy	Green
4	Ethanol	1.4	78.37	220	30.00	Oil	Pale green
5	Ether Petroleum	1.0	40-60	345	30.00	Gummy	Straw yellow
6	Ethyl acetate	1.2	76-77	240	30.00	Crude oil	Dark green
7	Hexane	1.2	65-70	230	30.00	Clear oil	Yellow
8	Methanol	1.1	64-66	200	30.00	Oil	Dark green
9	Aqua.*					Semi solid	Dark brown

Table 2 Contact toxicity of different solvent crude extracts of *Lantana camara* against the second instars of diamondback moth, *Plutella xylostella*

Solvents used for extraction	$\chi^2$ at $p \leq 0.05$ (n=30)	Regression equation	LC <sub>50</sub> %	95 per cent fiducial limit		LC <sub>95</sub> %	95 per cent fiducial limit	
				LL	UL		LL	UL
Acetone	4.060	Y =1.422x+3.041	23.43	9.58	57.35	513.33	48.29	5456.45
Benzene	1.597	Y =1.353x+2.880	40.07	11.72	136.98	1496.1	64.35	34786.4
Chloroform	0.656	Y =1.631x+4.075	3.665	2.71	4.94	43.045	16.86	109.87
Ethanol	0.511	Y =1.413x+2.952	32.13	10.14	101.80	989.28	50.30	19454.3
Ethyl acetate	0.222	Y =1.686x+3.398	8.869	6.04	13.01	95.770	28.92	317.08
Hexane	1.514	Y =1.072x+4.597	1.567	0.55	4.45	266.56	8.85	8020.62
Methanol	3.619	Y =1.405x+3.744	7.673	5.24	11.22	103.78	27.09	397.58
Petroleum ether	0.330	Y =1.251x+3.240	31.44	8.96	110.22	1356.0	42.64	43116.9

Being compounds of natural origin, no problems with persistence in the environment is anticipated (Gebbinck *et al.*, 2002). Thus, products based on plant extracts, phyto-oils and purified substances of plant origin can be an alternative to the conventional pesticides (Isman, 2001). The crude plant extract consists of complex mixtures of active compounds. The complex mixtures act synergistically (Berenbaum, 1985) and show greater overall bioactivity compared to the individual components (Chen *et al.*, 1995). Also, there is less preference for insect to develop resistance against such mixtures (Shukla and Toke, 2013).

#### **Ovicidal toxicity and oviposition deterrent activity**

The studies reveal that the aqueous extract of *L. camara* leaf at 10 per cent concentration gave minimum egg hatch of 73.20 per cent, whereas at one per cent concentration, the egg hatch was maximum (81.20) and was at par with control (90.20) (Figure 3). However the hexane extract of this plant resulted in 15.33 per cent egg hatch at 10 per cent concentration, whereas it was 62.54 per cent at 1 per cent and 93.23 per cent in the untreated control. The neem oil resulted in 15.55 and 86.66 per cent egg hatch, respectively at 10 and 1 per cent concentrations in comparison to 91.11 per cent egg hatch in control, whereas the petroleum ether extract of this plant gave 13.33 and 77.50 per cent egg hatch at the respective concentrations in comparison to 90.83 per cent in control.

Among different solvent plant extracts of *L. camara* hexane extract was found to be more effective in detergency recording 2.33 and 15.00 egg/female/day at one and ten per cent respectively when compared to control (54.00 eggs). This was followed by ethyl acetate where mean no. eggs laid in leaf extract 1 and 10 per cent of *L. camara* was 0.00 and 20.67 respectively. When the comparison of aqueous and hexane extract was made, the hexane extract was found to be effective next to the neem and was significantly different with the other extract (Figure 4). An earlier study the used different solvents for preparation of test material against storage insect pest, *Cadra cautella* (Walker) of

wheat; the seed protection activity of *L. camara* extract from hexane was best reported by (Gotyal *et al.*, 2010) is supported for present study.

Azadirachtin, active neem constituent, has been reported to interfere with ecdysis of insects (Singh and Bhathal, 1994) and moulting disruption due to neem constituents has been observed in *Spodoptera frugiperda*, *Pectinophora gossypiella*, *Heliothis virescens* and *H. zea* (Kubo and Klocke, 1982), *S. aexampta* (Tanzubil and McCaffery, 1990), *S. littoralis* (Martinez and Van Emden, 2001). Elumalai *et al.* (2007) reported that oviposition deterrent activity of mentha and neem oils found to have more deterrent activity against the gravid moths of *S. litura* and their significance are apparent. It may be due to the consequence volatiles present in the oils which makes malfunctioning of the ovariole in female moths.

#### **GC-MS Analysis**

The phytochemical components present in the all crude extracts of *L. camara* in different solvents were identified by GC-MS. The results showed the presence of alkaloids, tannins, flavonoids, saponins, steroids and reducing sugar in the plant. Table 3 - 9 revealed the groups of secondary metabolites detected in each solvent fraction. The chromatogram (Figures 6 -12) and phytochemical components with their retention time, molecular weight and percentage of composition in different solvents extracts presented in (Tables 3 - 9). Six to nineteen major components elucidated viz., caryophyllene, caryophyllene oxide, selina-6-en-4-ol, 2-hexadecen-1-ol, hexatriacontane, tetrapentacontane, 1, 3-cyclohexadiene-1-carboxaldehyde, 6 S-2,3,8,8-tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene, benzene, 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester, 2,6,10-Trimethyl,14-ethylene-14-pentadecne, 3-nonanone, phytol and squalene were identified in various crude extracts. Caryophyllene oxide has been reported as having analgesic, anti-inflammatory activity and antifungal activity against dermatophytes (Chavan *et al.*, 2010). It is also well known as a preservative in food, drugs and cosmetics (Yang *et al.*, 1999).

## Conclusion

The use of persistent synthetic insecticides on vegetables and fruits is a concern due to practical limitations of the pre-harvest interval. Hence, the scientific communities working in the field of insect pest management have academic interest in the discovery and development of new bioinsecticides that are environmentally friendly to be integrated, in combination or rotation, with biopesticide segment. Experiment conducted to evaluate the insecticidal activity of *L. camara* leaves extracts against diamondback moth revealed high larval mortality, ovicidal effects and oviposition deterrence. Among the various solvents tested hexane crude extract showed maximum efficiency that was on par with neem oil.

The present study is the significant result of the extraction of *L. camara* leaves by soxhlet apparatus. The crude extracts are known to possess insect growth regulatory and strong oviposition deterency and further investigation have to be carryout to find the activity of crude form and to incorporate the IPM schedule. This trend is in line with the requirements of new regulations on Integrated Pest Management.

## Acknowledgment

The authors express sincere thanks to the University Grants Commission, New Delhi for the financial support by providing fellowship for Ph.D. research to the first author and to the Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore for valuable logistic support during research period.

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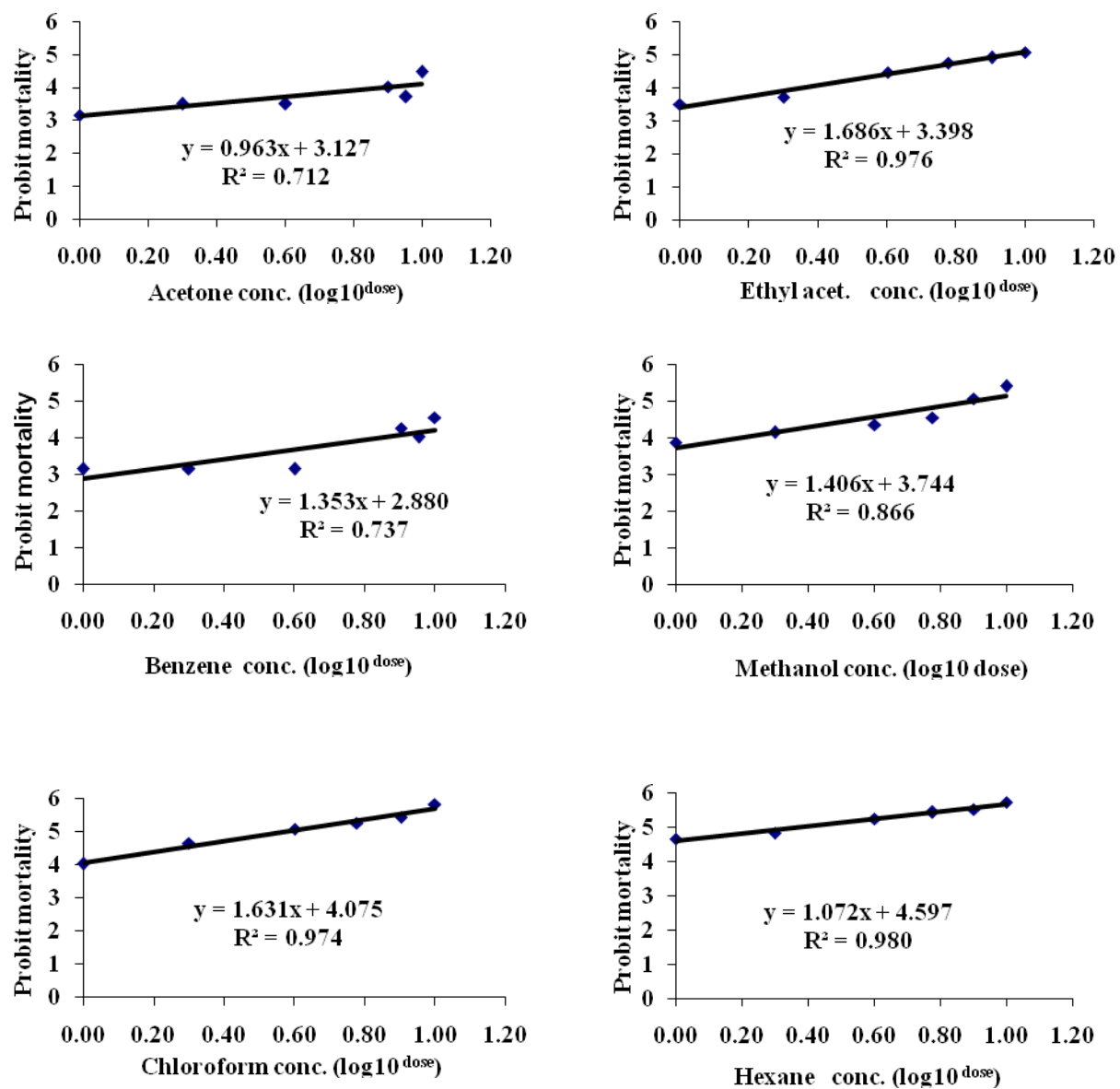
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## Appendix

Figure 1. Concentration mortality response of *Lantana camara* L. crude extraction in different solvents against second instars of DBM



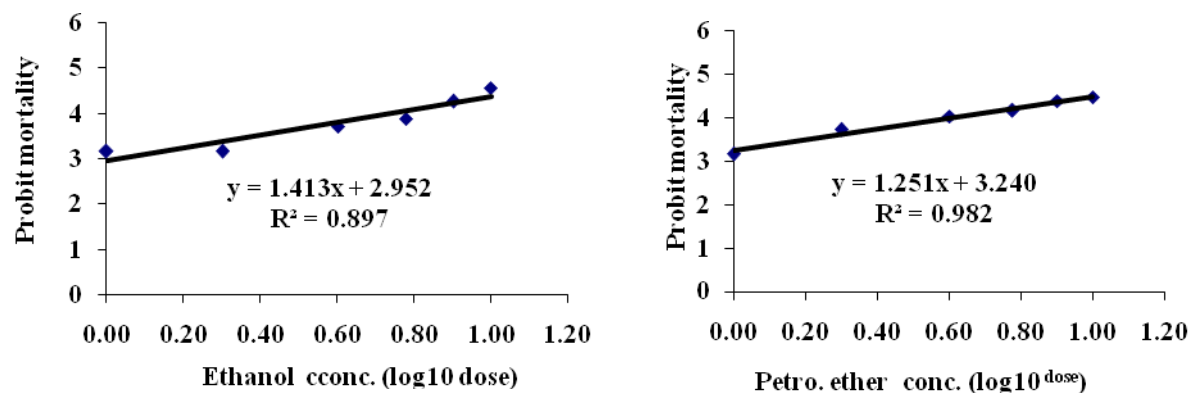
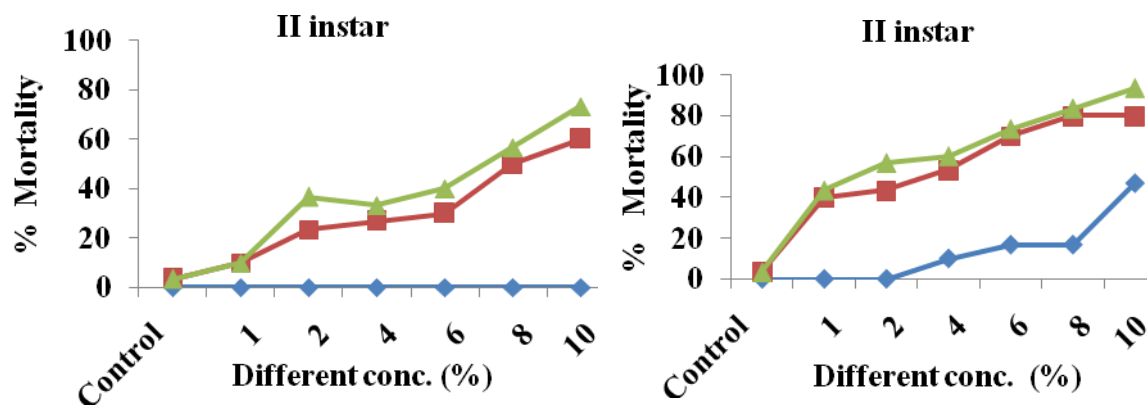


Figure 2. Toxicity of crude extracts of *Lantana camara* against diamondback moth, *Plutella xylostella*

a. Aqueous Extract

b. Acetone Extract



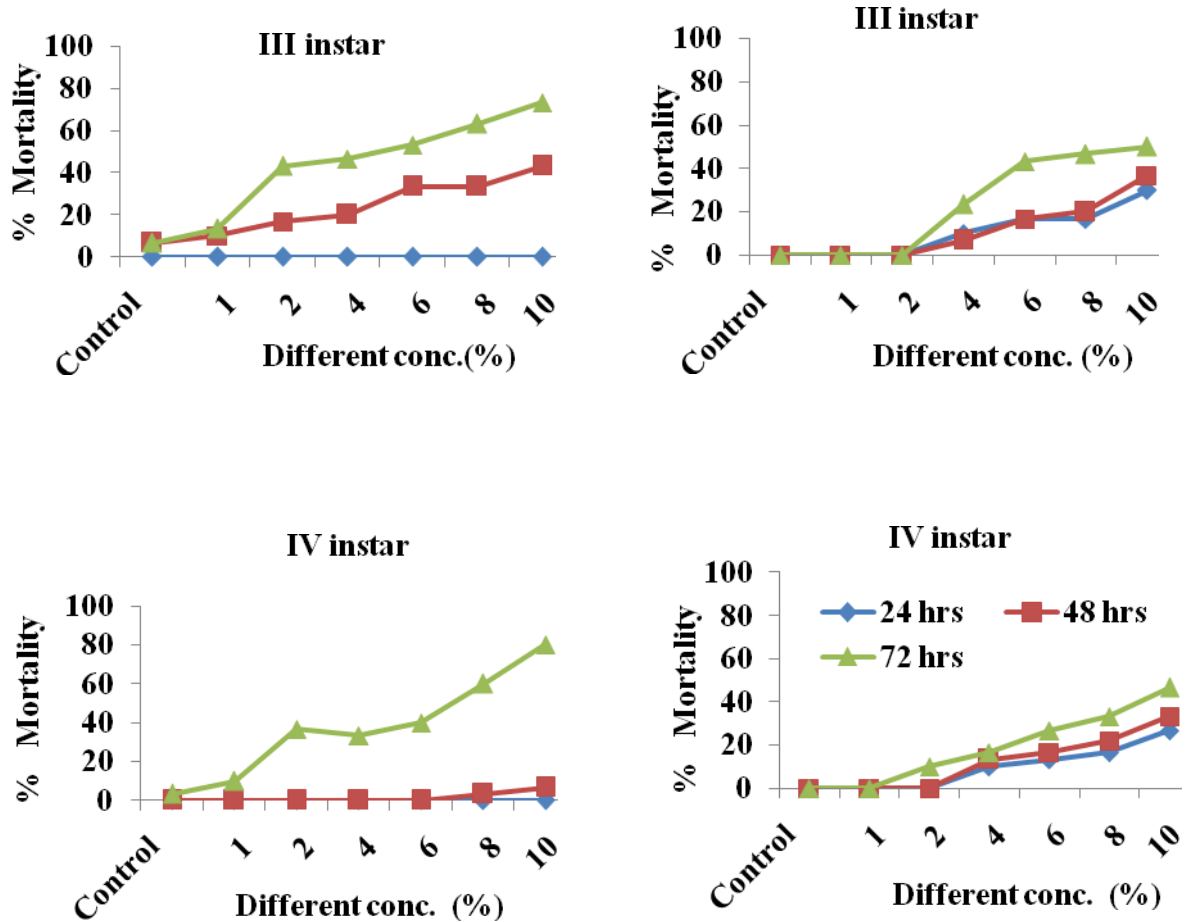
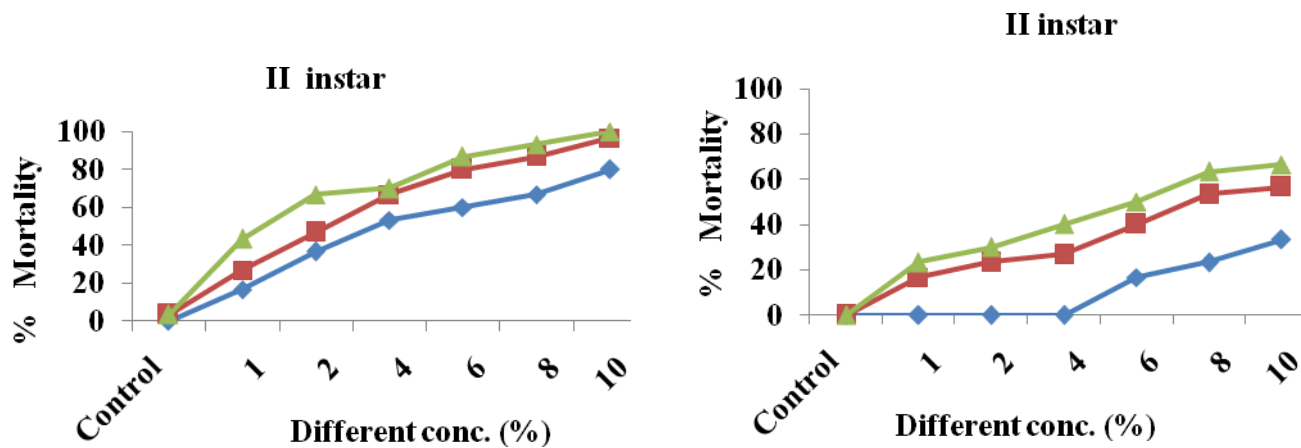


Figure 2. Toxicity of crude extracts of Lantana camara against diamondback moth, Plutella xylostella

c. Chloroform crude Extract

d. Benzene crude Extract





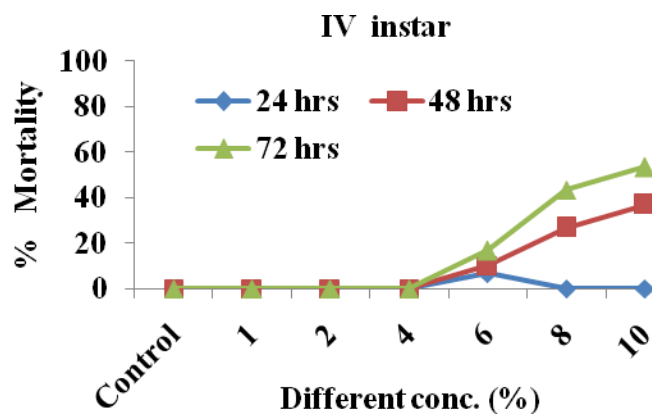
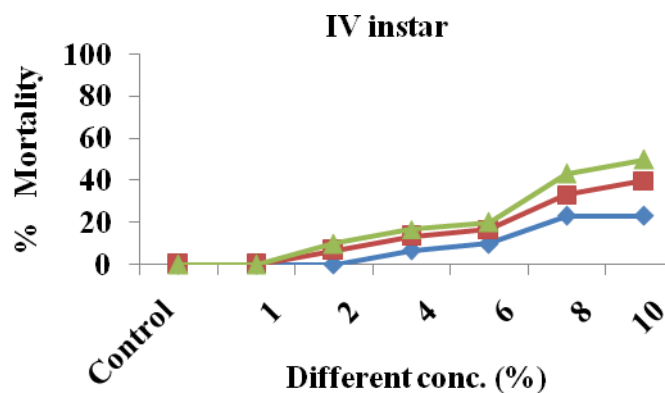
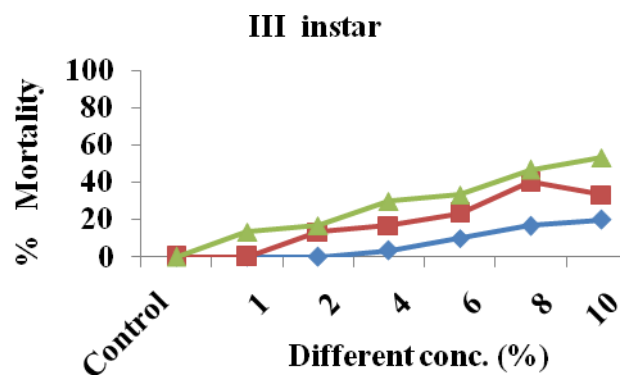
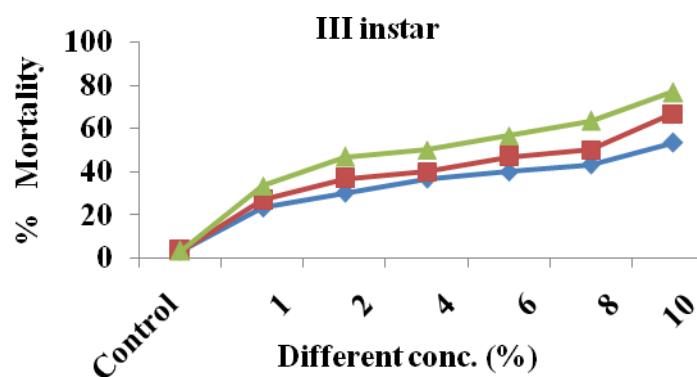


Figure 2. Toxicity of crude extracts of *Lantana camara* against diamondback moth, *Plutella xylostella*

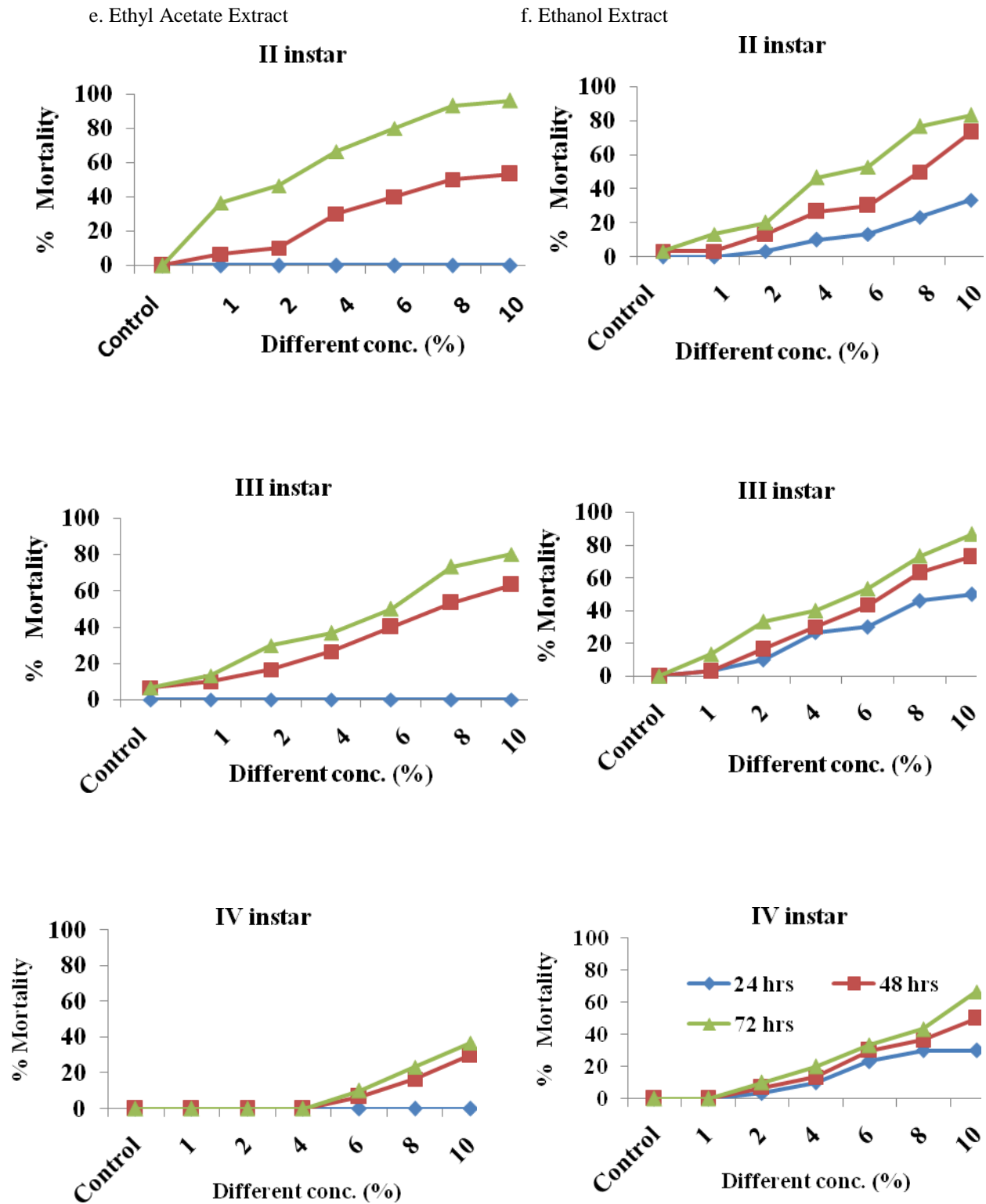
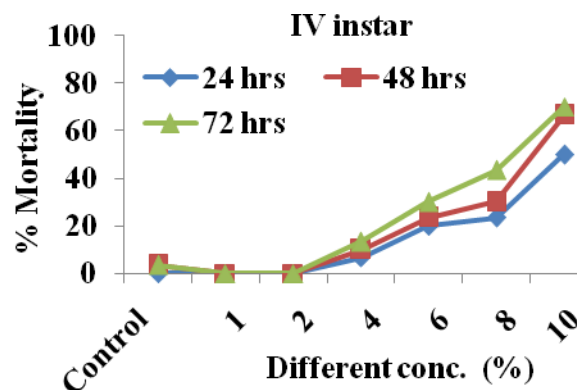
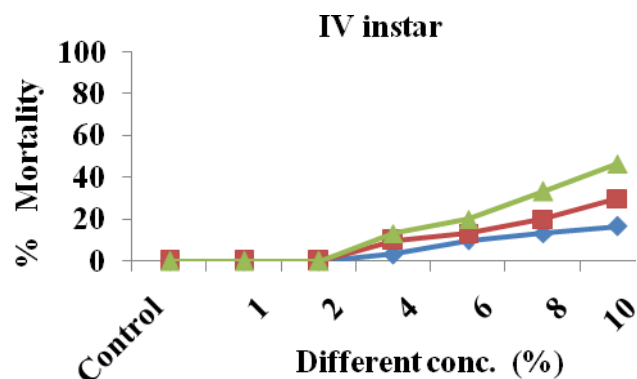
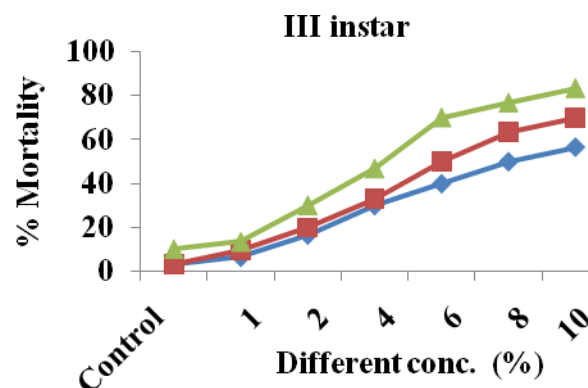
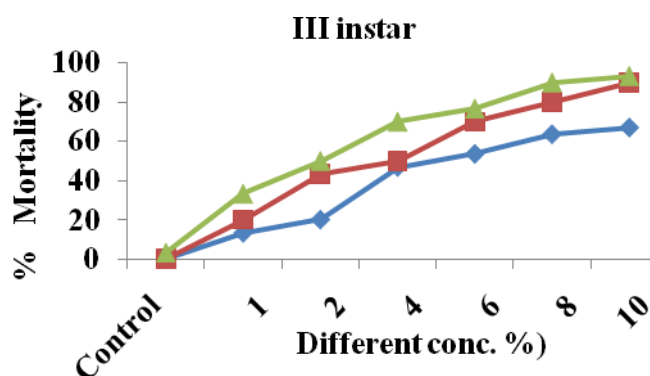
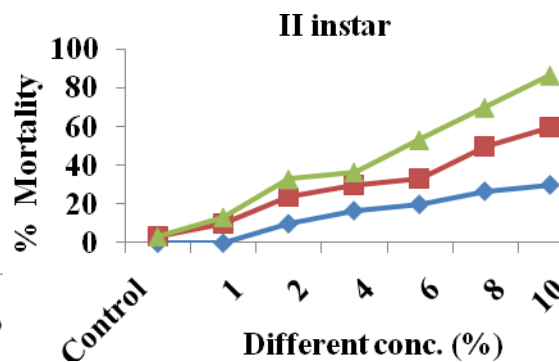
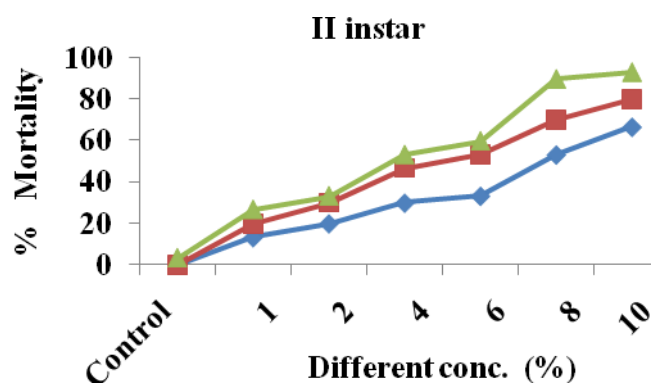


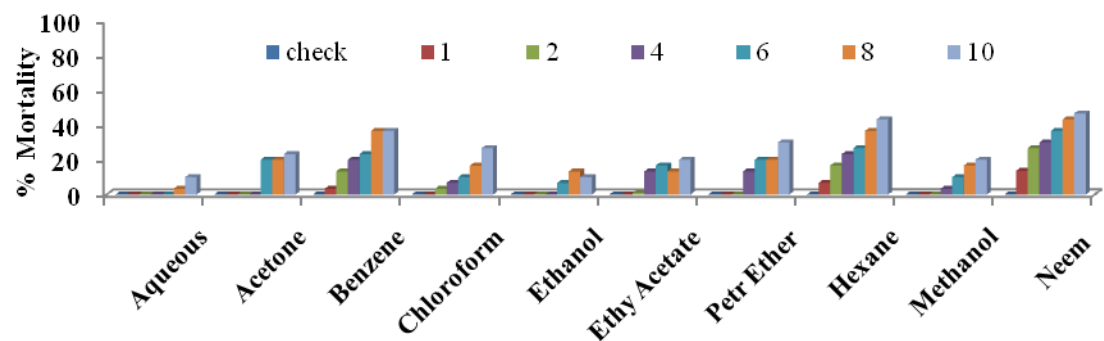
Figure 2. Toxicity of crude extracts of *Lantana camara* against diamondback moth, *Plutella xylostella*

g. Methanol Extract

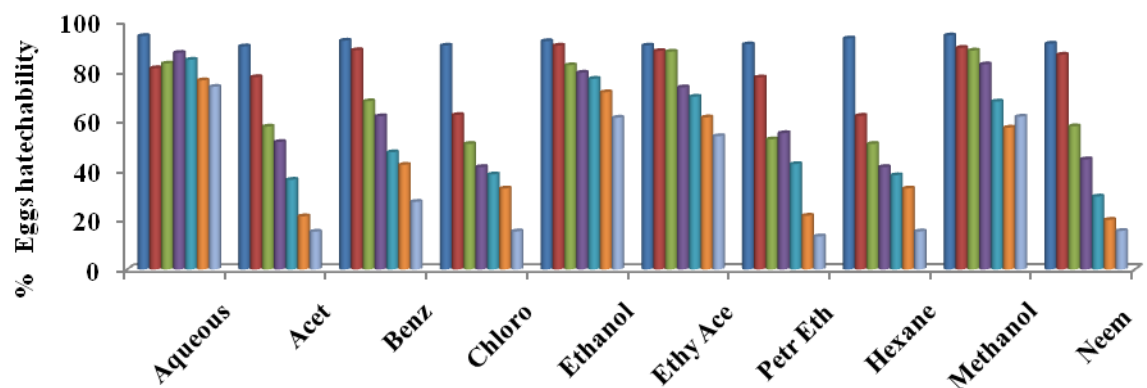
h. Petroleum ether Extract



**Figure 3. Insecticidal effects of different concentration with solvent crude extracts against the pupae of DBM, *Plutella xylostella* (L.)**



**Figure 4. Ovicidal effects of different concentration with solvent crude extracts against the DBM, *Plutella xylostella* (L.)**



**Figure 5. Oviposition deterency of different concentration with solvent crude extracts on 24hrs old adult DBM , *Plutella xylostella* (L.)**

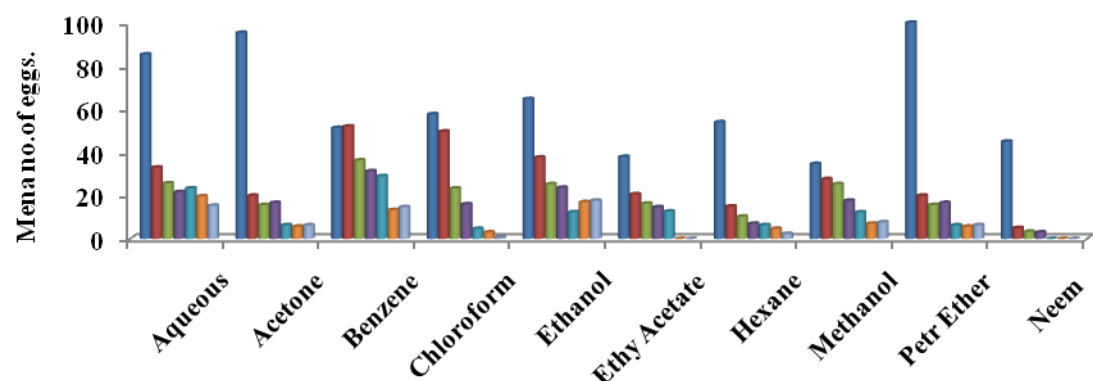


Table 3 Major phytochemical components identified in the acetone crude extract of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	5.412	69556	8.74	142	3- Nonanone
2	6.297	84859	10.67	98	3-Hexen-2-one
3	18.395	241744	30.39	204	Caryophyllene
4	28.437	118298	14.87	296	4-Hexen-1-ol
5	38.152	63813	8.02	268	Octadecane
6	39.288	217229	27.31	410	Squalene

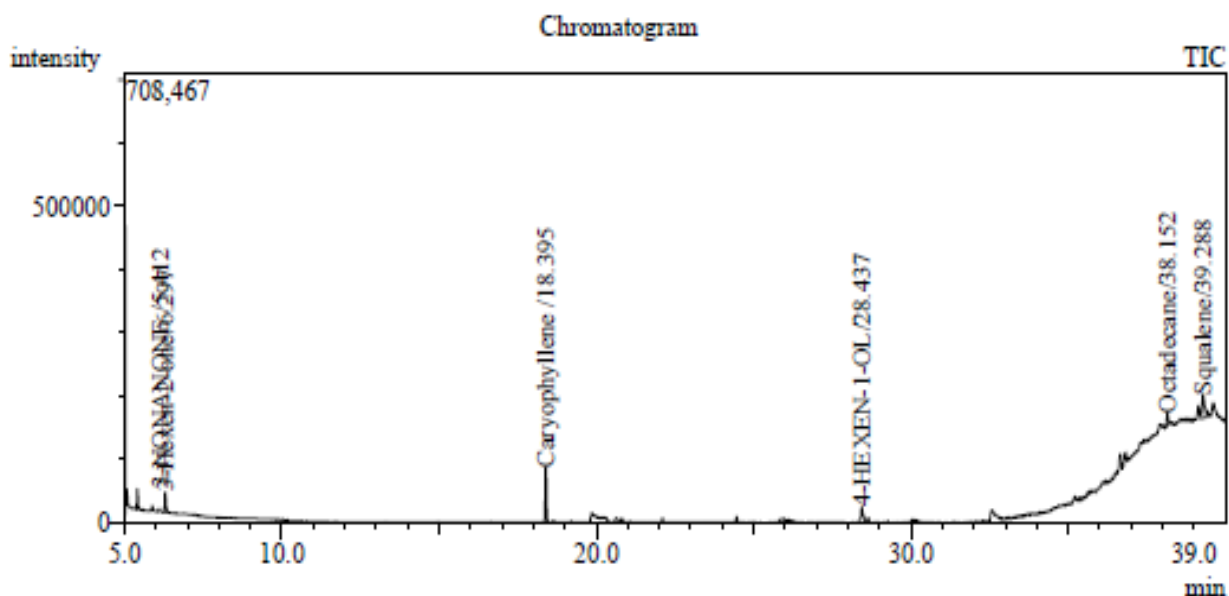


Figure 6. Gas chromatograph of *L. camara* acetone leaf extract

Table 4 Major phytochemical components identified in the benzene crude extract of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	5.413	129315	1.50	142	3- Nonanone
2	6.298	149176	1.73	98	3-Hexen-2-one
3	18.398	124393	1.44	204	Caryophyllene
4	33.168	1104850	12.78	408	6S-2,3,8,8-Tetramethyltricyclo[5.2.2.0(1,6)]undec-2-ene
5	34.860	1504455	17.41	404	Stigmast-5-en-3-ol
6	36.588	2874871	33.27	758	Tetrapentacontane
7	38.805	2755230	31.88	376	Bicyclo[4.1.0]Heptane

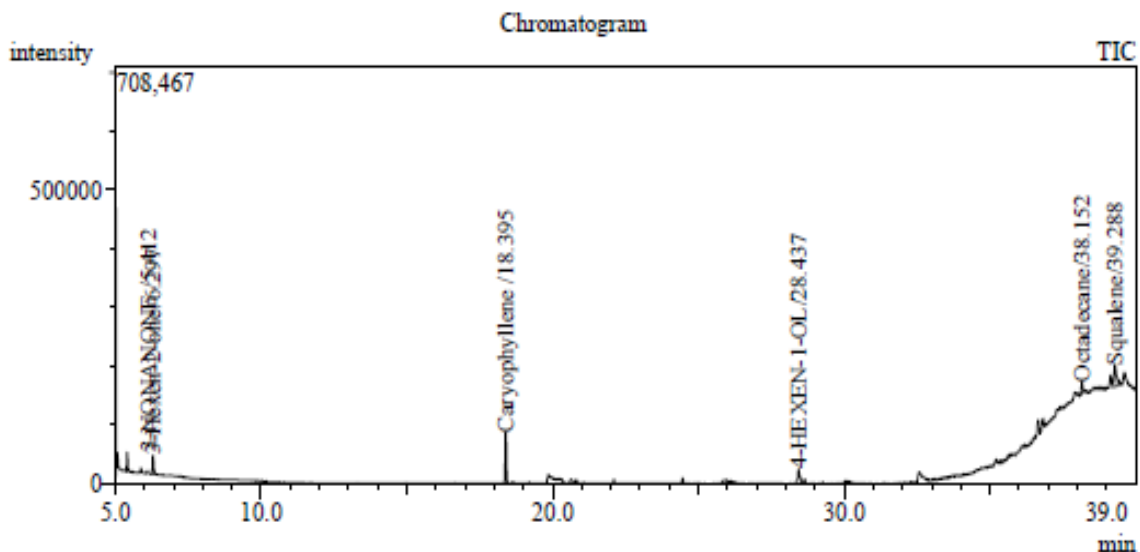


Figure 7. Gas chromatograph of *L. camara* benzene leaf extract

Table 5 Major Phytochemical components identified in the chloroform crude extract of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	5.411	84838	2.09	142	3- Nonanone
2	6.297	100721	2.48	98	3-Hexen-2-one
3	18.395	703969	17.34	204	Caryophyllene
4	18.636	34815	0.86	288	1,6-Cyclodecadiene
5	19.796	99508	2.45	202	1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene
6	22.096	134177	3.31	220	(-)-5-Oxatricyclo [8.2.0.0(4,6)] dodecane
7	24.450	107785	2.66	190	3,7-Cyclodecadien-1-one
8	28.416	529755	13.05	278	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
9	29.249	104342	2.57	278	2,6,10-Trimethyl,14-ethylene-14-pentadecne
10	32.539	203174	5.00	296	Phytol
11	36.542	473621	11.67	278	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
12	37.579	1482762	36.53	758	Tetrapentacontane

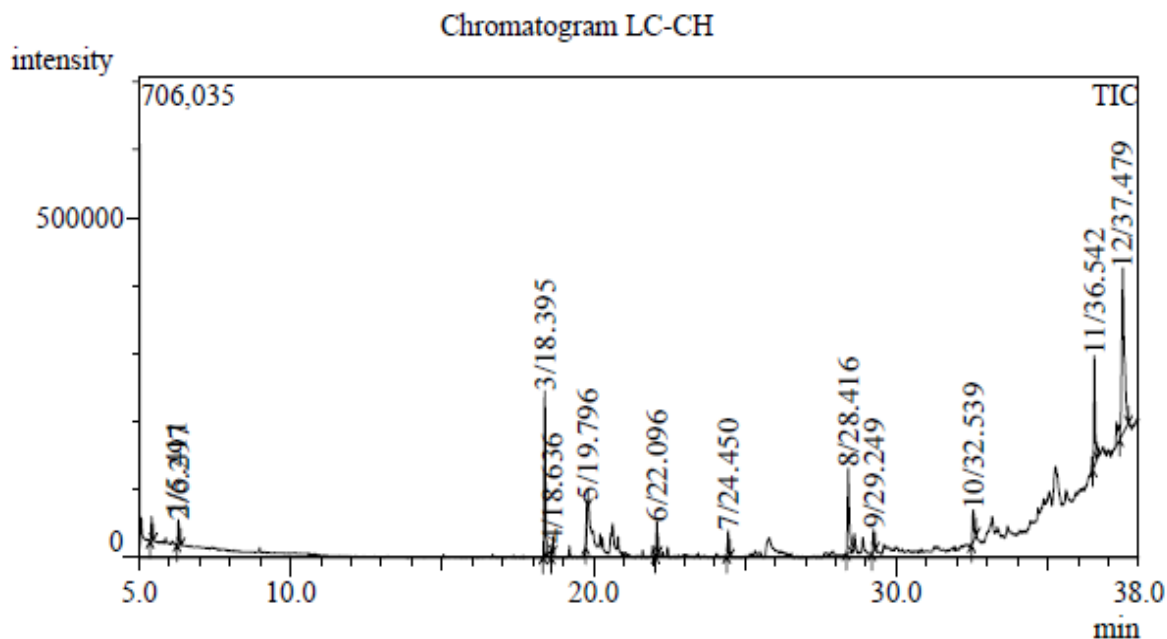


Figure 8. Gas chromatograph of *L. camara* chloroform leaf extract

Table 6 Major phytochemical components identified in the ethyl acetate crude extract of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	5.412	126557	2.47	116	3- Nonanone
2	6.298	141429	2.76	98	3-Hexen-2-one
3	18.400	224689	4.38	204	Caryophyllene
4	20.814	31406	0.61	218	A-Copaene
5	22.100	42748	0.83	220	2-Naphthaleneethanol
6	24.452	36104	0.70	150	1,3-Cyclohexadiene-1-carboxaldehyde
7	28.422	183526	3.58	278	2,6,10-Trimethyl,14-ethylene-14-pentadecne
8	32.544	106342	2.07	296	2-Hexadecen-1-ol
9	33.702	250132	4.88	278	2,6,10-Trimethyl,14-ethylene-14-pentadecne
10	35.235	1206182	23.53	758	Tetrapentacontane
11	36.547	340604	6.64	278	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
12	37.477	2436513	47.53	758	Tetrapentacontane

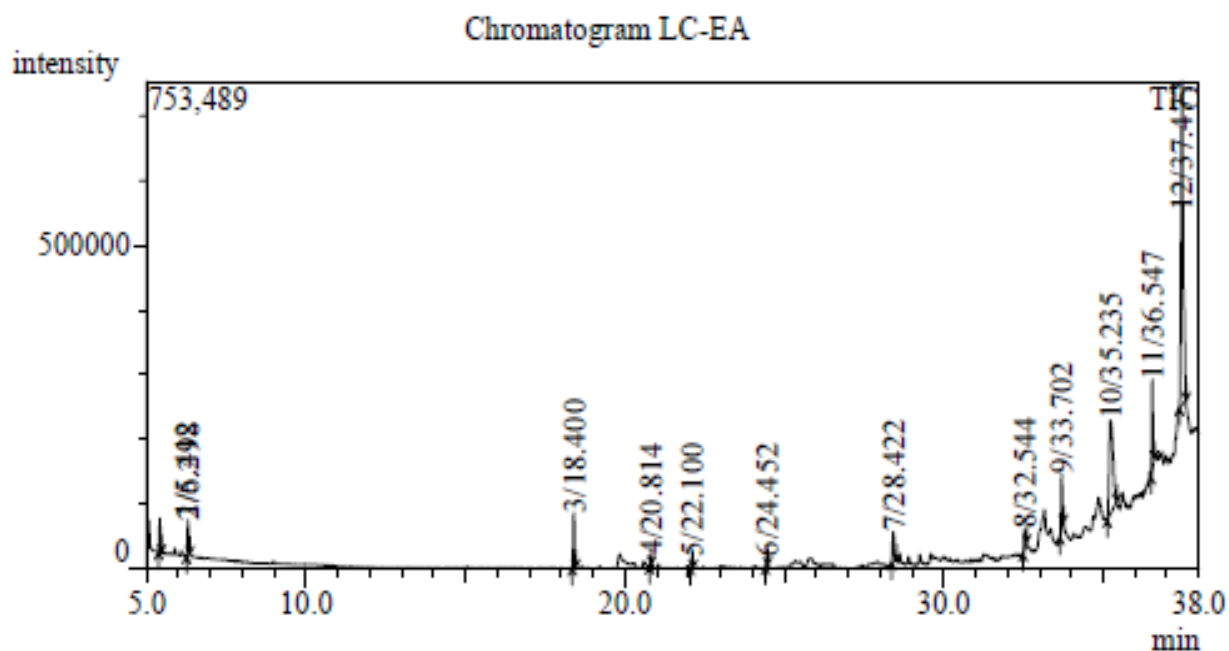


Figure 9. Gas chromatograph of *L. camara* ethyl acetate leaf extract

Table 7 Major phytochemical components identified in the hexane crude extract of *Lanatan camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	7.416	629223	3.72	142	Decane
2	8.026	289124	1.71	142	Octane
3	10.054	364146	2.15	170	Dodecane
4	18.395	2460437	14.54	204	Caryophyllene
5	19.776	2463832	14.56	202	Benzene,
6	20.561	577326	3.41	204	1,3-Cyclohexadiene
7	20.636	792782	4.69	272	1H-Benzocyclohepten-7-ol,
8	21.953	269683	1.59	220	1H-Cycloprop[e]azulen-7-ol,
9	22.096	596924	3.53	220	Caryophyllene oxide
10	22.275	301239	1.78	178	2-Cyclohexen-1-one,
11	22.447	268953	1.59	218	3,7-Cyclodecadien-1-one,
12	24.449	607914	3.59	190	3,7-Cyclodecadien-1-one,
13	25.706	2424352	14.33	204	1,5,5,9-Tetramethylspiro [5.5]undeca-1,8-dien
14	32.529	832065	4.92	296	Phytol
15	36.461	191438	1.13	338	Tetracosane
16	37.272	435561	2.57	506	Hexatriacontane
17	38.137	886886	5.24	506	Dotriacontane
18	39.117	1082388	6.40	450	Dotriacontane
19	39.271	1442021	8.52	450	2,6,10,14,18,22-Tetracosahexaene,



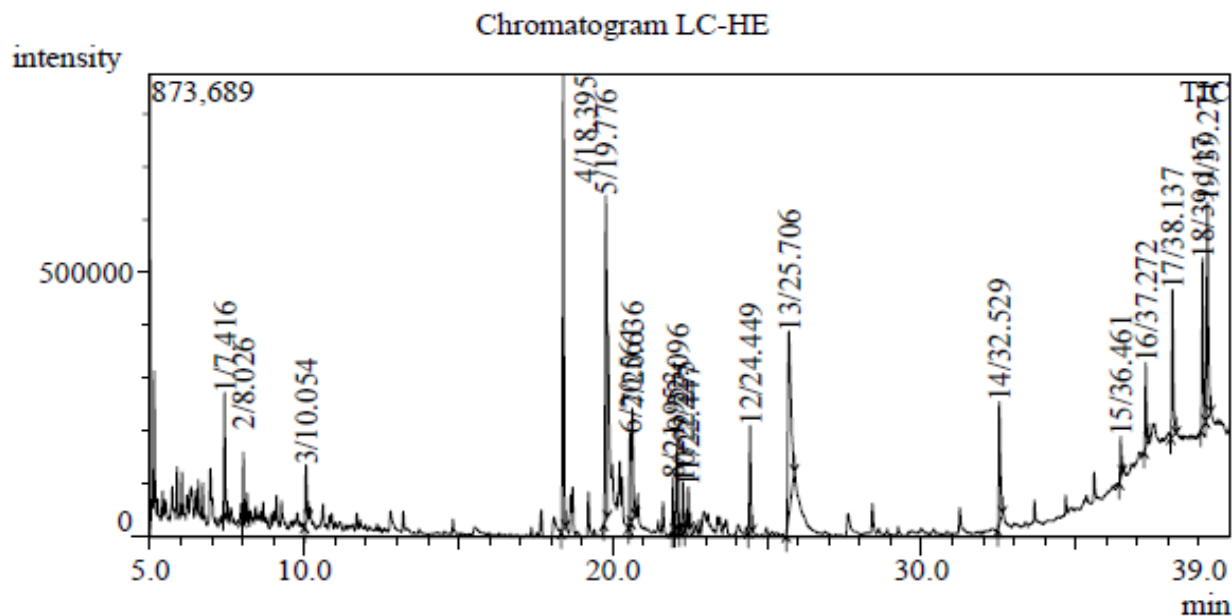


Figure 10. Gas chromatograph of *L. camara* hexane leaf extract

Table 8 Major Phytochemical components identified in the methanol crude extracts of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	5.408	128942	1.13	116	1-Pentanol,
2	6.293	156637	1.38	98	3-Hexen-2-one
3	18.391	867985	7.63	204	Caryophyllene
4	19.779	944108	8.30	208	Benzene
5	21.183	228871	2.01	222	Selina-6-en-4-ol
6	21.948	143428	1.26	220	1H-Cycloprop[e]azulen-7-ol,
7	22.091	363490	3.19	220	(-)-5-Oxatricyclo [8.2.0.0(4,6)] Dodecane
8	24.442	365690	3.21	190	3,7-Cyclodecadien-1-one
9	25.693	1702224	14.96	218	Phenol
10	28.410	254676	2.24	208	2,6,10-Trimethyl
11	32.523	488416	4.29	296	2-Hexadecen-1-ol
12	33.137	866945	7.62	204	6S-2,3,8,8-Tetramethyltricyclo [5.2.2.0(1,6)]undec-2-ene
13	34.852	1761406	15.48	376	Stigmast-5-en-3-ol, (3.beta.)
14	36.544	1236104	10.86	414	Tetrapentacontane

15                      38.792                      1870578                      16.44                      758                      Cyclopentane-3'-spiropentacyclo

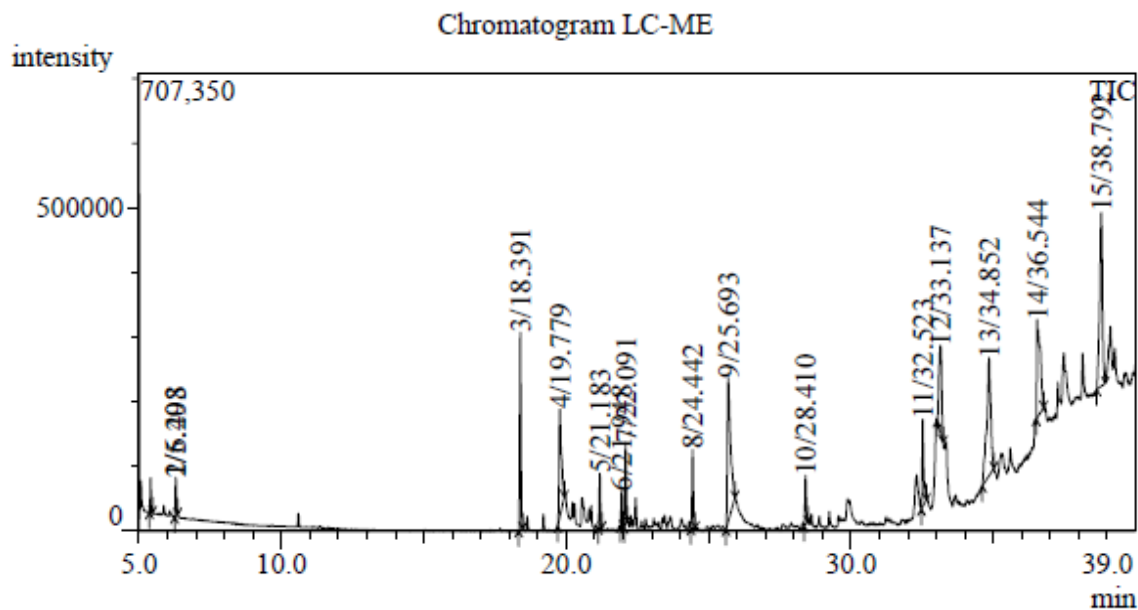


Figure 11. Gas chromatograph of *L. camara* methanol leaf extract

Table 9 Major Phytochemical components identified in the petroleum ether crude extract of *Lantana camara*

Peak no.	R. Time	Area	Area %	Molecular wt.	Components
1	18.396	1294144	15.99	204	Caryophyllene
2	19.790	747228	9.23	202	Benzene
3	19.850	475404	5.88	204	1,4-Methanoazulene
4	20.637	168648	2.08	204	1,4-Methanoazulene
5	22.097	296501	3.66	220	(-)-5-Oxatricyclo [8.2.0.0(4,6)] dodecane
6	24.451	258861	3.20	190	3,7-Cyclodecadien-1-one
7	25.720	1256391	15.53	296	Spiro[5.5]undeca-1,8-diene
8	32.532	328410	4.06	278	2-Hexadecen-1-ol,
9	36.542	1546546	19.11	506	1,2-Benzenedicarboxylic acid,
10	38.137	338233	4.18	410	Eicosane
11	38.786	450307	5.57	204	2-Cyclohexen-1-ol
12	39.120	409638	5.06	282	Hexatriacontane
13	39.271	521326	6.44	220	2,6,10,14,18,22-Tetracosahexaene,

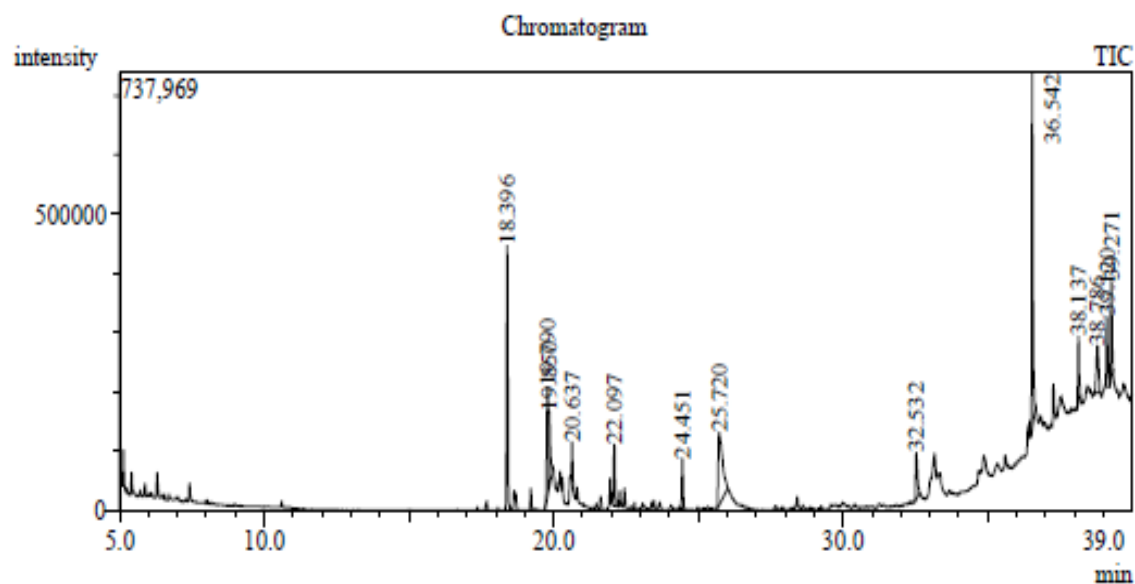


Figure 12. Gas chromatograph of *L. camara* petroleum ether leaf extract

## INCREASING THE YIELD OF ONION THROUGH IMPROVED PRODUCTION TECHNOLOGY IN KALABURAGI DISTRICT OF KARNATAKA

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### Abstract

Onion is extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the fruits and vegetables. Krishi Vigyan Kendra, Kalaburagi has made 60 demonstrations in farmers' field at different villages of Kalaburagi district during the period from 2009-10 to 2013-14 to enhance the yield of onion through improved production technology and sustainable development. The result showed that, on an average the highest yield achieved by adopting improved production technology was 247.10 quintals ha<sup>-1</sup> whereas the corresponding yield ranges under farmers practices was to 202.60 quintals ha<sup>-1</sup> of onion. Adoption of improved production technology will increase the yield 20.30% over farmer practices. The average technological gap, extension gap and technological index were noticed 196.66 quintals ha<sup>-1</sup>, 44.6 quintals ha<sup>-1</sup> and 43.54 % respectively. The economics of data indicated that an average of Rs. 2,26,437 ha<sup>-1</sup> was recorded net profit under recommended practices while it was Rs 1,74,750 ha<sup>-1</sup> under farmer practices. Cost benefit ratio was 7.09 under demonstration, while was 5.79 under farmer practices. Practicing of improved production technology and sustainable development will improve the farmer socio-economical level and sustain the fertility of soil.

Keywords: Onion, technology gap, technology index, extension gap, economics

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### Introduction

Onions are an essential ingredient in many dishes and virtually indispensable for winter stews and casseroles. Onion (*Allium cepa* L) is extremely important vegetable crop not only for internal consumption but also as highest foreign exchange earner among the vegetables. It occupies an area of 1064 thousand ha, with production of 15118 thousand tonnes (Indian Horticulture Database, 2011). The export of onion during 2011 -12 was 13,09,863.26 thousand tons with a value of Rs 1,722.85 crores. It is used either fresh as a salad or in preserved form (Islam *et al*, 2007). Leading countries in onion production are China, India, Pakistan, Bangladesh, Indonesia and Turkey.

The key factors in the successful growing of onions are,

planting at the right time, fertilizer application and keeping the weeds down as onions need their full growing season and resent competition from weeds (Kumar *et al*, 2001). Improper methods of farmers' practices, insect pests and diseases are among the major constraints to enhancing production and productivity of onion. In recent years, farmer incomes have been declining particularly due to the rising costs of inputs for plant protection. For each disease, good crop nutrition is mentioned as a means of reducing disease levels. This nutrition generally involves ensuring adequate calcium and probably boron levels to reduce the ease with which fungi can penetrate host tissue, generally adequate nutrients for steady non-stressed growth and the avoidance of excess nitrogen which can increase the easy food value for the fungi as well as contributing to a softer cell wall structure. Crucifers, cucurbits and chrysanthemum are highly attractive to

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International Conference on Agriculture and Forestry, 10-12 June 2015, Colombo, Sri Lanka

onion thrips. Using a trap crop involves planting small strips or patches of the alternative crop within an onion field to attract thrips.

Studies to evaluate and compare farmers' practices strategies are necessary to provide farmers options to manage the crop with improved production technologies through integrated crop management (ICM) according to their preferences and particular situation (Juan *et al*, 2009). The objective of this study was to determine transfer of technology to the farmers and increasing the yield and quality of onion through sustainable production technologies with the use of organic manures and trap crop.

## Material and methods

The present study was conducted by the Krishi Vigyan Kendra, Kalaburagi under University of Agricultural Sciences, Raichur. Based on "seeing is believing" concept, the aim of the increase the yield of onion by adopting improved production technology and sustainable development of land. Improved production technology demonstrate the impact of research emanated production technologies that varieties most suitable the agro climatic conditions and befitting to the existing cropping sequence. The adoption of improved technologies and innovations are the most important tools for enhancing the agricultural production at faster rate and hence it is a crucial aspect under innovation diffusion process. The main objective was to demonstrate the productivity, potential, profitability and sustainability of the soil fertility through latest improved production technology in real farm situation under different and aberrant weather situations to address the following problems were identified.

1. Use of high seed rate with improper method & planting geometry.
2. Lack of concept of crop rotation.
3. Heavy reliability on traditional varieties coupled with inappropriate sowing time.
4. Low use of organic matter and bio-fertilizers.
5. Lack of application of secondary nutrients and rare use of micro nutrients.
6. Improper time of fertilizer application.

7. Heavy infestation of weeds in onion.

8. Lack of intercultural operations by cultivators adopting chemical weed control.

The field trials were conducted for consecutive years during the period from 2009-10 to 2013-14 at different villages of Kalaburagi district of Karnataka. The Kalaburagi District situated between 17° 19' North and 76° 54' East longitude. The soil of farmers field is medium black soil with pH (6.8-7.5) EC (0.24-0.36 dSm<sup>-1</sup>), available N (250-24 kg ha<sup>-1</sup>), available P (75 kg ha<sup>-1</sup>) and available K (390 kg ha<sup>-1</sup>). The treatments T1 (Farmers practices) and T2 (Improved production technology). The whole package approach demonstrated to the farmers through improved production technology field trails included the components like High yielding variety (Bhima super), seed treatment, seedlings are treated with biofertilizers, spacing, integrated nutrient management (Farm yard manure, vermicompost, neem cake, biofertilizers, urea, phosphors, potash, sulphur, zinc, boron and vegetable special) integrated pest and disease management. Vegetable special sprayed 3 times at 20 days with concentration of 2 gm lit<sup>-1</sup> of water. Radish and chrysanthemum are grown as trap crop. The data generated, in farmers practices and improved technology was utilized for calculating the technological index, technology and extension gaps using the formulae given by Kadian *et al*, 1997.

(1)% YIOFP\* = Average demonstration yield - farmer's average plot yield

-----X 100  
Farmer's avg. plot yield

\*YIOFP= Yield increase over farmers practice

(2) Technological gap = Potential yield-Demonstration yield

(3) Extension gap= Demonstration yield-Farmers yield

(4) Technology index = Potential yield –Demonstration yield

----- X 100  
Potential Yield

## Result and discussion

A comparison of productivity levels between improved production technology in demonstration trials and farmers' practices is shown in table 1. During the study period it was observed that the adoption of improved production technologies in demonstration trials has increased the yield over the farmers' practices.

**Crop performance and yield:** The performance of onion owing to the adoption of improved technologies assessed over a period of five years and presented in table 1 reveal that, the effect of improved technologies earmarks the productivity sustainability of onion production in the black soils region of southern India of Karnataka state. The data in table 1 revealed that the average yield level of 247.10 q ha<sup>-1</sup> and 20.30 % yields increased over farmers practice. Similar findings were reported by Funda et al. (2011). Integrated nutrient management (INM) in onion bulb crop improved the quality and yield of onion bulbs through integration of chemical fertilizers along with organic manures (Singh et al., 2001). Increasing the yield of onion may be attributed due to adoption of improved production technologies.

**Yield gaps:** The data on table 2 indicates that technological gap, extension gap and technological index of onion through improved technologies. The yield gaps in the present study were categorized into technological gap and extension gaps. The average technological gap and extension gap was noticed 196.66 q ha<sup>-1</sup> and 44.6 q ha<sup>-1</sup> respectively, during the period from 2009-10 to 2013-14 (Ranjeet Singh et al, 2011). Generally, the technological and extension gap appears even if the improved production technology is conducted under the strict supervision of the scientists in the farmers' fields. This may be attributed mainly due to lack of high yielding varieties, irrigation facilities, integrated nutrient management, variation in the soil fertility status, non congenial weather conditions and local specific management problems faced for the attainment of potential and demonstration yields. Therefore, location specific recommendations are necessary to a bridge the gap besides strengthening of irrigation facilities, integrated nutrient management, use of high yielding varieties, in the region. The higher

extension gap indicates that there is a strong need to motivate the farmers for adoption of improved technologies over their local practices.

**Technology index:** Technology index indicates the feasibility of the evolved technology in the farmers' fields. Lower the value of technology index, higher is the feasibility of the improved technology. Technology index varied from 50.30 to 11.60 % in the onion during the period from 2009-10 to 2013-14 five years of the study. This indicates that a strong gap exists between the generated technology at the research institution and disseminated at the farmer's field. The findings of the present study are similar with the findings of Hiremath and Nagaraju (2009). Introduction of high yielding varieties and integrated nutrient management through improved technology on onion would eventually lead to higher adoption among farmers in the region (Hiremath, 2007).

**Economics of cultivation:** Data in table 3 reveal that the cost involved in the adoption of improved technology in onion varied and was more profitable. The average net return of demonstration field is 2,26,437 Rs ha<sup>-1</sup> where as 1,74,750 Rs ha<sup>-1</sup> under farmers practices. The mean B: C ratio was 7.09 and 5.79 for the demonstration field and farmers' practices respectively, were similar findings of Rameez et al. (2014). The economic analysis indicates that use of improved technology in onion would substantially increase the income as well as livelihood of the farming community of Kalaburagi district.

## Conclusion

It is concluded from the study that there exists a wide gap between the potential and demonstration yields in onion mainly due to technology and extension gaps and also due to the lack of awareness about growing of onion in Kalaburagi district of Karnataka. The study highlights that by adoption of improved production technology, onion productivity and soil fertility can be improved greatly even under farming situations and adverse weather conditions of Kalaburagi district of Karnataka. The improved production technology has also shown potential to increase the yield of onion. It is further suggested that sincere extension efforts are

required to educate the farmers for adoption of improved production technology besides strengthening improved technologies, so that resource poor farmers could improve their livelihood, providing employment to their local peoples, diversify their farming systems, and fertility of soil. (3-4):1-10

**Table 1:** Impact of improved production technology on realization of productivity and potential of onion under real farm situation

Year	Area(Ha)	No. of demonstrations	Yield q ha <sup>-1</sup>			% Increase in yield over farmer practice
			Potential yield	Demonstration yield	Farmers practices	
2009-10	5	12	450	193.40	169.0	14.43
2010-11	5	12	450	195.50	170.0	15.00
2011-12	5	12	450	203.00	172.0	18.02
2012-13	5	12	450	247.30	202.7	22.00
2013-14	5	12	450	397.50	300.0	32.50
Average			450	247.10	202.6	20.30
Total	25	60				

**Table 2:** Technological gap, Extension gap and Technological index of the respondents

Year	Area(Ha)	Technological gap (q ha <sup>-1</sup> )	Extension gap (q ha <sup>-1</sup> )	Technological index (%)
2009-10	5	226.6	24.4	50.30
2010-11	5	254.5	25.5	56.50
2011-12	5	247.0	31.0	54.80
2012-13	5	202.7	44.6	45.04
2013-14	5	52.5	97.5	11.60
Average		196.66	44.6	43.54

**Table 3:** Impact of improved production technology on economics of onion under real farm situation

Year	Cost of cultivation (Rs ha <sup>-1</sup> )		Gross return (Rs ha <sup>-1</sup> )		Net return (Rs ha <sup>-1</sup> )		BCR	
	Demo	Farmer practice	Demo	Farmer practice	Demo	Farmer practice	Demo	Farmer practice
2009-10	28000	28500	187500	162500	159500	134000	1:6.6	1:5.7
2010-11	29000	29600	173250	170600	144250	141000	1:5.97	1:5.6
2011-12	30000	32000	304500	258000	274500	226000	1:10.15	1:8.06
2012-13	40000	42000	266437	216750	226437	174750	1:7.09	1:5.84
2013-14	70000	70200	397500	268200	327500	198000	1:5.67	1:3.75
Average					226437	174750	1:7.09	1:5.79

## **Acknowledgement**

This work was carried out at different villages under the jurisdiction of Krishi Vigyan Kendra Kalaburgi (Karnataka state). The authors are grateful to the Director of Extension, UAS Raichur for the constant support and help in carrying out the research work and Zonal project Director –Zone VIII, Bangalore for the financial assistance. The authors also express the gratitude to late Smt. Rajeshwari, Karadi who carried out the experiment for one year.

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## **Appendix**







Figure 1 General view of field trail in Kalaburagi District of Karnataka

# INTER-RELATIONSHIP OF ENVIRONMENTAL AND MANAGEMENTAL PARAMETERS ON BULL SEMEN EVALUATION

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## Abstract

Sri Lanka is being with the tropical and sub tropical nature and also the usage of cattle and buffalo for milk, meat and draught purpose is in an advance. Quality semen is the key indicator towards the successful breeding program. The present study was designed to identify the effect of environmental and management conditions on semen evaluation (volume, concentration, motility and production). Four stud bulls (Friesian, Sahiwal, AFS and Murrah) have been used at the Artificial Insemination Centre, Polonnaruwa, Sri Lanka. Semen from those stud bulls was collected twice per week using artificial vagina. Environmental temperature was ( $p < 0.05$ ) influenced with the relative humidity ( $r = 0.76$ ) and temperature humidity index ( $r = 0.76$ ). The mean volume (ml) of Friesian, Sahiwal, AFS and Murrah were  $4.1 \pm 0.87$ ,  $6.0 \pm 1.78$ ,  $8.3 \pm 1.96$  and  $4.5 \pm 2.09$ , respectively. Mass motility of the fresh semen was  $83.2 \pm 2.5\%$ ,  $80.5 \pm 1.46\%$ , and  $80.3 \pm 1.28\%$  and  $80.0 \pm 0.00\%$  for Friesian, Sahiwal, AFS and Murrah, respectively. The mean value of semen concentration was 1785.3, 1411.5, 438.1 and 735.0 million/ml for Friesian, Sahiwal, AFS and Murrah, respectively. The production performance of Friesian, Sahiwal, AFS and Murrah reached 183.8 ml, 203 ml, 105.6 ml and 97.9 ml, respectively which need to be enhanced with the best quality semen in future.

Keywords: Semen, Stud bulls, volume, concentration, motility.

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## Introduction

The cattle and buffalo are playing a major role in socio-economic and livelihood development process in Sri Lanka. Population of cattle and buffalo in Sri Lanka in 2012 has been recorded as 1.235 and 0.414 million, respectively (Source: Department of Census and Statistics, 2012). Domestic milk production was recorded with the growth of 13.69% in year 2012 compared to year 2011. Contribution of the livestock sector to the Agriculture component was around 1.2%, while total contribution of the livestock sector to the national GDP was around 7.14% in 2012. However, overall contribution of Agriculture sector to the national GDP in 2012 was 11.5%. (Source: Department of Census and Statistics, 2014).

On the other hand, Sri Lanka is being a tropical and sub tropical nature with the long hot period that extends

from March to September and average ambient temperature range is 26-32 °C. This hot condition can adversely affect reproductive efficiency of the high dairy potential animals. Studies have shown that the male reproductive performance was influenced by environmental conditions which affect on conception rates in cows by producing poor quality of semen during hot period (Barth and Waldner, 2002). Environmental factors such as environmental temperature, relative humidity and rain fall are specially considered to improve the semen quality (Colas *et al.*, 1988). Quality of the semen is also affected by both health and nutritional status of the bulls (Soeparna Soeparna *et al.*, 2013).

The continuous evaluation of their semen quality and quantity is required, to achieve higher non return rates. In tropical countries, low reproductive performance is a

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major problem which is associated with semen quality of the stud bull (Annual report of DAPH, 2011). The breeding programme is a useful tool to improve the quality of the semen for the successful breeding performance by considering the external factors towards the success in good quality semen production (Folch, 1983 and Annual report of DAPH, 2011). The information on semen quality and freezability are lacking in dry zone of Sri Lanka. Therefore, the present study was designed to analysis the semen quality during processing and storage of selected stud bulls being reared in the Artificial Insemination Centre, Polonnaruwa, Sri Lanka.

### **Materials and Methods**

Study was conducted in the Artificial Insemination (AI) Centre, located in Polonnaruwa district in the Northern Central province of Sri Lanka. In this study, Australian Friesian Sahiwal (AFS), Sahiwal, Friesian and Murrah (Buffalo) were used for at the Artificial Insemination centre, Polonnaruwa during study period

#### ***Materials***

Processing and evaluation of the collected semen were conducted using electron microscope, electronic analytical balance, artificial vagina, sterilization oven, artificial vagina (AV oven) oven, electronic water bath, diluter, magnetic agitator, bovine electro photometer, electronic filling, sealing and printing machine and cold handling cabinet. In addition to that, tris, citric acid monohydrate, D-fructose, streptomycin sulphate, benzyl penicillin, glycerol, double distilled water, sodium citrate and egg yolk also were used in semen evaluation.

#### ***Environmental parameters***

Temperature, relative humidity and rain fall data were collected from the Meteorology Department located in the Polonnaruwa region.

### ***Management procedures***

Head-head housing system was developed in the east-west direction where the stud bulls were fed by cut and carries system with the supplementary of concentrates. Vaccination and preventive measures were considered as the health issues during the rearing period.

### ***Semen collection procedure***

Collection process has been already scheduled on Tuesday and Friday (twice per week) at 4.30 a.m. Bulls were undergone to preparatory measures before the semen collection. The semen was collected using artificial vagina (AV) and collected semen was dispatched to laboratory immediate after collection.

### ***Preparation of diluter (Calibration)***

Electronic Diluter (Micro Lab, 500A series, Germany) was used to mix the semen with sodium citrate in a cavity. Diluter itself consists two suction ends. One end for sodium citrate (concentration 0.9%) suction and another end for the semen suction. Sodium citrate was solely used to initiate (zero level) the Electronic Bovine Photometer at 546 nm wave length.

### ***Laboratory parameters***

The volume of collected semen was measured using collection tube in milliliter (ml).

### ***Concentration in million and motility***

Cavity with sodium citrate and semen was placed in Electronic Bovine Photometer. Identification number, ejaculate volume, motility and concentration of semen (in million) were obtained.

### ***Production***

Production of specific bull was calculated by multiplying the number of filled straw with the known volume of the single straw.

### Method of data analysis

Data were undergone to the correlation and regression analysis by using the 22<sup>nd</sup> version of SPSS package.

## Results and Discussions

### Environmental parameters

The mean  $\pm$  SD of ET during the period of semen collection was  $29.57 \pm 1.59$  °C (ranges: 25.05 °C - 34 °C). The value of RH and THI during the collection period was  $72.80 \pm 9.19\%$  (range: 54% - 94%) and  $70.73 \pm 2.31$  (ranges: 61.41 - 75.39), respectively. The value of rain fall was recorded as mean 11.6 mm (ranges: 00.00 - 39.80 mm) during the semen collection period at the Polonnaruwa AI Centre.

### Management procedures

Animals are keeping under semi intensive system with suitable cattle shed. Animal are fed by chopped fresh fodder according to the body weight (10% of body weight in fresh matter basis) with individual manger. Adequate fresh clean water was providing for drinking

purpose. In addition, concentrate feed was given around 2.5 kg per stud bull per day.

### Relationship between environmental and semen parameters

In this study, there was a negative ( $p < 0.05$ ) correlation between environmental temperature and RH while ET was positively ( $p < 0.05$ ) correlated with THI. The results were agreed with Thankachan (2007) he was reported that the increasing of environmental temperature was positive relationship between THI and the physiological parameters of cattle. On the other hand, RH was negatively ( $p < 0.05$ ) correlated with THI. However, ET, RH and THI had no any ( $p > 0.05$ ) effects on rain fall during the period of semen collection period.

Table 1 is shown that ET and THI did not show any ( $p < 0.05$ ) influence with the semen parameters of any of the stud bulls. The present results agreed with the results recorded by Hussain *et al.* (1985), Tomar and Gupta (1984) and Sarder *et al.* (2000) who reported that volume of semen is not much differed with .environmental temperature and THI.

Table: 1 Relationship of stud bull semen parameters

Breed	Parameters	Temperature	THI	Volume	Concentration
Sahiwal	Volume	-0.12	-0.04	-	-
	Concentration	0.27	0.04	0.21	-
	Motility	0.23	0.15	0.47*	0.49*
Friesian	Volume	0.12	0.09	-	-
	Concentration	-0.05	-0.31	0.49	-
	Motility	-0.10	-0.24	-0.22	0.26
AFS	Volume	-0.13	0.18	-	-
	Concentration	0.02	0.20	0.57*	-
	Motility	-0.01	0.33	0.83*	0.54*
Murrah	Volume	0.12	0.13	-	-
	Concentration	-0.22	-0.20	0.23	-
	Motility	-0.03	-0.01	0.50*	0.32

### **Relationship between Environmental and semen parameters**

#### **Volume and motility of stud bulls**

Present study showed that there was a decreasing trend in motility observed with the increasing volume of Sahiwal ( $r=0.3984$ ) and Friesian ( $r=0.2177$ ) stud bulls. However motility of the semen was increased with the increasing volume in AFS ( $r=0.1593$ ) stud bull. Besides affecting the volume of bulls, scrotal circumference also had certain influence to the sperm motility. However, the effect on sperm motility was not as high as that on semen volume (Ha *et al.*, (2012).

#### **Concentration and motility of the stud bulls**

While the concentration increases the motility of the semen was increased in both Sahiwal ( $r=0.2455$ ) and Friesian ( $r=0.2569$ ) stud bulls. On the other hand, AFS

( $r=0.1516$ ) showed the decreasing trend on its concentration and motility. These results partially agree with the study of the semen concentration of Sahiwal and AFS stud bull having the positive ( $p<0.05$ ) relationship with its motility (Mathevon *et al.*, 1998).

#### **Volume and concentration of stud bulls**

In this study, while volume increases concentration showed slightly deduction in Sahiwal, AFS and Murrah stud bulls. In contrast, The similar responses has been reported by Hossain *et al.* (2012) as while the volume of the semen increasing, concentration of the semen decreased Friesian ( $r=0.4924$ ) showed the increasing trend in an acceptable way.

Table 2: Mean value of semen parameters

	<b>Friesian Mean</b>	<b>Sahiwal Mean</b>	<b>AFS Mean</b>	<b>Murrah Mean</b>
<b>Volume (ml)</b>	4.1	6.0	8.3	4.5
<b>Motility (%)</b>	83.2	80.5	80.3	80.0
<b>Concentration (in millions)</b>	1785.3	1411.5	438.1	735.0
<b>Production (ml)</b>	183.8	203	105.6	97.9

In this study, the highest volume (7.3 ml) was ejaculated from the AFS stud bull and the lowest volume (3.9 ml) was ejaculated in buffalo (Murrah) during the period of semen collection. Similar results were reported by Shaha *et al.* (2008) has indicated that mean volume of the ejaculate in adult buffalo stud bull

was 1.5 to 3.7 ml. The ejaculate volume of the semen varied from breeds to breed. It is influenced by a number of factors such as age, breed, weight and environment. Laing (1988) reported that higher fertility bull produced large volume of semen than of lower fertility bull. Thus, ejaculated volume is a one of the

reason to improve the fertility in cow (Djimde and Weniger (1986).

Highest percentage of motile sperm was observed in Friesian stud (83.2%). These results agree with those of Mukherjee and Banerjee (1980); Saxena and Tripathi (1981). In general this variation could be caused by the age of animals, environment, nutrition, animal physiological status and management.

Concentration is also one of the semen parameter at the time of semen collection. Friesian stud showed the higher concentration 1785.3 million/ml compared to other stud bulls. It may be the concentration had not highly influenced by the age of the stud bull (Kollalpitiya *et al.*, 2012).

Higher environmental temperature remarkably reduced the production level of all stud bulls which were used for the semen collection. During the hot period (January to July, 2014) number of frozen semen doses per bull reduced in Friesian breeds. The previous study indicated that the hot season deteriorated semen quality in terms of mass motility, individual motility and number of doses in Friesian breeds (Fiaz *et al.*, 2009). Because of the environmental adaptation, Sahiwal stud bull took place the highest production and number of doses in AI centre, Polonnaruwa.

## Conclusions

Present study showed that the environmental temperature (ET) had the positive relationship with THI and had the negative relationship with RH. However, the RH was negative relationship with THI. Sahiwal and AFS stud bulls showed the significant relationship in between its volume and motility. In the overall, volume was higher in AFS stud bull. In this study mean value of motility and concentration were higher in Friesian stud bull and lower in AFS stud bull. The production was higher in Sahiwal stud bull than the other stud bulls at the semen collection centre. Good quality of semen production make successful breeding program.

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# EFFECT OF NaCl AND ROOT-MEDIA SELECTION ON YIELD ATTRIBUTES, OIL COMPOSITION AND MINERAL COMPOSITION OF ROSE GERANIUM

(*Pelargonium graveolens* L.)

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## Abstract

Rose geranium is an essential oil crop that is commercially produced worldwide for use in perfumery, medicinal and aromatherapy industries. A study was carried out in a greenhouse tunnel at the University of the Free State to evaluate the effect of salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>) and root-media (sand and sawdust) on the yield, mineral and oil composition of rose geranium. Treatments were laid out in a split plot design which was arranged in a randomised complete block. Salinity levels were allocated to the main plots and root-media to the subplots replicated three times. Salinity at 4.0 mS cm<sup>-1</sup> level significantly reduced the yield attributes and also oil quality which is ascribed to C:G ratio. Sawdust root-media had better yield attributes compared to sand root-media. Significant interaction was found between salinity at 4.0 mS cm<sup>-1</sup> and sand root-media for the Na content. Mineral contents of P, K, Mg and Cl were significantly affected by salinity levels. It is evident from the study that rose geranium should be grown using nutrient solution with salinity below 4.0 mS cm<sup>-1</sup> for better yield and oil quality using sawdust as a root-media.

Keywords: Mineral composition, root-media, rose geranium, salinity levels

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## Introduction

Recent reports of most studies indicate that medicinal and aromatic plants have been receiving much attention in perfumery, pharmaceutical, cosmetic, food and agroalimentary industries [15; 20; 31]. Medicinal and aromatic plants contain essential oil compounds which are a complex mixture of volatile compounds. Biosynthesis of essential oils is highly dependent on the biotic and abiotic environmental stress factors [20]. Beside climate and nutrients abiotic stress factors effects, salinity has posed as one of the most common essential oil yield and quality reducer. This has been shown on peppermint (*Mentha piperita* L.), where high salinity stress reduced the stem length, root length, shoot fresh mass, root fresh mass and dry mass and also the oil yield [20]. Ashraf et al. [3] also reported the

reduction of yield and yield attributes when salinity concentration was high. In the same study, fresh mass and dry mass of both shoots and roots of snapdragon (*Ammolei majus* L.) was reduced significantly. Moreover, on the study conducted by [15], high salinity reduced the methyl chavicol biosynthesis and accumulation of basil (*Ocimum basilicum* L.). It was also found that high salinity stress significantly reduced the growth parameters of chamomile (*Matricaria chamomile* L.) [15].

Contrary to the reduction of yield and yield attributes of essential oil plants, high salinity concentrations have been found to improve quality of most essential oil plants instead. This has been shown on basil and coriander (*Coriandrum sativum* L.) where linalool content was increased by 57% and 45%, respectively [4; 25]. Moreover, improved oil quality was reported on

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sage (*Salvia officinalis* L.), where salinity increased oxygenated monoterpene by 48% [43]. Hendawy and Khalid [16] also stated the effects of salinity conditions on the plants physiological and biochemical potentials, which in turn affect the plants primary and secondary metabolism.

According to the report of [45], around 20-30% of the cultivated land is affected by the salinity globally and this inhibits plant production. Razmjoo et al. [29] indicated that the correction of these affected areas is a main issue in agriculture. In South Africa, the demand for good quality water is escalating due to the presence of specific concentrations of ions such as Na and Cl and this made the water quality to become a key factor [36; 45]. Hassanpouraghdam et al. [15] also reported that saline sodic being a primary restraining problem for plants physiological process limiting field and horticultural crops particularly in arid and semi-arid region with NaCl being the principal salts sources. Moreover, these excess soluble salts contribute to the sodicity of the soil and increased salinity of the feeding water leading to osmotic stress, specific ion toxicity and ionic imbalances [23]. It has been noted that high salinity in the feeding water induces problems relating to metabolic activities in plants from many studies focusing on salinity stress. The effect of salinity varies between plants species and also according to the type of production system used [18; 40]. Said-Al Ahl and Omer [33] reported that accumulation of salts in the root media was caused by rates of evapo-transpiration and poor leaching of water.

Rose geranium oil is produced commercially worldwide for use in perfumery, medicinal and aromatherapy industries [11; 47]. Rose geranium is characterised by lobed leaves, small pink flowers and a rosy aroma [21]. Leaves, flowers and branches are important parts of the plant because this is where most of the oil bearing trichomes are found [1]. Sedibe and Allemann [35] and [12] reported that the yield of rose geranium is affected by nutritional requirements and other environmental factors. Therefore, an integrated nutrient solution management regime with efficient root-media is necessary to ensure proper growth and normal development of rose geranium to improve the yield and essential oil quality. The aim of the experiment was to

evaluate the effect of NaCl (salinity) and root-media selection on yield attributes, oil composition and mineral composition of rose geranium.

## Material and Methods

### *Site and climate descriptions*

This experiment was carried out in a greenhouse at Bloemfontein, campus of the University of the Free State located in the semi-arid area with coordinates of 29°10'S and 26°17'E at an altitude of 1395 m above sea-level. The experiment was conducted during summer season and the temperature was kept at maximum 26°C using two axial fans and a wet-wall of the greenhouse which were triggered by a climate adapter (Climate adapter Johnson A419 series USA).

### *Experimental field plan and crop management*

The experiment was laid out in a split plot design, where the main plots consisted of four different salt levels (1.68, 2.40, 3.20 and 4.0 mS cm<sup>-1</sup>) and the sub-plots consisted of two root media types (silica sand [with a diameter of 2 mm] and sawdust) and the units were replicated three times, arranged in a randomised complete block design. Each salinity concentration level contained 12 potted plants that were split-plotted into two cultivation units (Figure 1). In each unit, six potted plants were grown in different root media, sand and sawdust.

Nutrient composition was prepared according to the levels described by [9] with varying concentrations of Na and Cl (Table 1). The pH of the nutrient solution was maintained at 5.5 in all the experimental units. Salinity shock was reduced by initiating NaCl application gradually in a weekly sequence in the third month after transplanting at 25, 50, 75 and 100% until constant levels were met in all treatment levels.

Rooted cuttings ( $\pm 10$  cm) Bourbon cultivar of rose geranium was obtained from a commercial grower (Pico-gro RSA). One rooted cutting was planted per each pot of 5 L used [19]. Aphids and red spider mites were problematic during the experimental period. With no registered insecticide for rose geranium, aphids and

red spider mites were controlled by a full cover spray of malasol at 1.75 mL L<sup>-1</sup> and abamectin at 1.20 mL L<sup>-1</sup>. These applications were repeated for three to six days at four-week intervals.

#### ***Description of irrigation systems used***

A customized small-scale growing units (450 x 800 x 215 cm) adapted from the unit used by [37] was used to grow rose geranium (Figure 1). The irrigation systems had six-dripper tubing with a flow rate of 4 L hour<sup>-1</sup>; these drippers were allocated to six potted plants. An irrigation pump with a flow rate capacity of 700 L hour<sup>-1</sup> was mounted to a 20 mm tubing pipe distributed to the pot-holding tank of the growing unit. All nutrient solutions used were recirculated and replaced with fresh solution every 14 days. Three irrigation system cycles were scheduled for one hour per cycle at 7:00, 11:00 and 15:00.

#### ***Parameters measured***

Number of leaves, plant height, number of branches and foliar fresh mass (FFM) were determined at harvest following techniques of [27].

#### ***Stomatal conductance***

Stomatal conductance was determined twice at 10:00 and 14:00 for four days before harvesting. Data were collected on clear days, using a leaf porometer (Decagon SC-1 USA). Measurements were taken on six selected mature leaves [7].

#### ***Relative water content***

Three leaves on each plant were collected to determine relative water content (RWC). Leaf fresh mass (LFM) was determined immediately at harvesting and subsequently the leaves were immersed in distilled water for 12 hours to determine leaf turgid mass (LTM). Afterwards these samples were dried in an oven set at 60°C for 24 hours to determine the leaf dry mass (LDM). The relative water content was determined by procedure described by [38];

$$\text{RWC}\% = (\text{LFM} - \text{LDM}) \div (\text{LTM} - \text{LDM}) \times 100$$

#### ***Chlorophyll content and leaf area***

Chlorophyll content was determined according to the procedure described by [8] before harvest using portable non-destructive chlorophyll meter (Optisciences CCM 200 USA). Readings were taken randomly from the upper six mature leaves on the crop. Leaf area was measured using a portable leaf area meter (CI-202 USA) used by [14]. Six mature leaves were harvested from the crop in the following sequence; two from the top, two from the middle and two at the bottom of the plant. Measurements were taken immediately after harvest.

#### ***Essential oil extraction and oil composition analysis***

Rose geranium oil was extracted from the leaves and stems using a custom-built steam distillation unit [38]. About  $\pm 5$  kg of fresh plant material was distilled for oil at a temperature of  $\pm 98^\circ\text{C}$  for one hour. The mass of the oil volume (yield) was determined by weighing the oil volume using PGL 2002 Adam scale (USA) immediately after extraction as described by [42].

Essential oil compounds were primed by comparing the retention times of the chromatogram peaks. Key oil components determined for oil composition were citronellol, geraniol, linalool, iso-menthone, citronellyl formate, geranyl formate and guaia-6,9-diene. The retention indices were computed from a gas chromatogram that was logarithmically interpolated between the n-alkanes. A homologous series of n-alkanes (C8-C22 Polyscience USA) was used as a standard. The oil concentration data were obtained by electronic integration of peak areas as described by [17].

#### ***Mineral analysis***

Leaf nitrogen content was determined using the Dumas combustion nitrogen analyzer [22]. Phosphorus, Zn, Fe, Cu and B were measured using a high resolution atomic absorption spectrometer as described by [38], [24] and [32], respectively. Sulphur content was measured following the procedure of [38]. Potassium and Na concentrations were measured by flame photometry [2], while Ca and Mg were determined using a Laser

induced breakdown spectroscopy [24]. Chlorine was estimated by silver ion-titration with a Corning 926 chloridometer [30]. Molybdenum was determined using ultraviolet-visible spectrophotometer [5].

### **Data analysis**

Data was analyzed with a SAS statistical software version 9.2 [34]. Significant differences were compared using Tukey's least significant difference (LSDT) at 5% level of significance [41].

## **Results and Discussion**

### **Yield and yield attributes**

The results on the number of leaves, plant height, number of branches, leaf area, RWC, chlorophyll content, FFM, oil yield and oil content are shown in Table 2. No significant effect of salinity was found on RWC, FFM (herbage yield), oil yield and oil content as tabulated in Table 2. However, the number of leaves ( $p < .01$ ), plant height ( $p < .05$ ), and number of branches ( $p < .05$ ), leaf area ( $p < .01$ ) chlorophyll content ( $p < .05$ ) and FDM ( $p < .01$ ) were significantly affected by salinity. This significant parameters are attributed to yield attributes. Moreover, there was a strong polynomial relationship between number of leaves ( $r^2=0.99$ ), plant height ( $r^2=0.88$ ), number of branches ( $r^2=0.97$ ), leaf area ( $r^2=0.99$ ), chlorophyll content ( $r^2=0.92$ ) and the salinity levels shown in Figure 2, 3, 4, 5 and 6, respectively. Yield attributes of rose geranium were significantly reduced at 4.0 mS cm<sup>-1</sup> salinity level and this is associated with the toxicity effects of NaCl within the plant cell. High salinity levels in feeding water induces severe ion toxicity by depositing high levels of Na in plant cells, causing the plant membrane to disorganise and thereafter inhibit cell division and cell expansion [48]. Vacuole is an antiporter and it regulates Na uptake; excess Na ions will be transported and stored in the vacuole [6; 40]. In high levels of Na ions also disrupt the activities of enzymes in the plant cell. According to [26], plants can tolerate salinity up to a certain threshold without any reduction in yield and in this case rose geranium could not withstand high salinity level at 4.0 mS cm<sup>-1</sup>. High

salinity conditions has been shown to reduce the number of branches, plant height and number of flowers of chamomile [10].

Plant height, number of branches, RWC, chlorophyll content, oil yield and oil content were also not significantly affected by the root-media (Table 2). However, root-media affected the number of leaves ( $p < .01$ ), leaf area ( $p < .01$ ) and FFM ( $p < .01$ ) of rose geranium. The number of leaves, leaf area and FFM were better when using sawdust root-media with an increase of 451.83 plant<sup>-1</sup>, 799.08 cm<sup>2</sup> and 511.24 g plant<sup>-1</sup>, respectively, compared to the sand as shown in Table 2. No significant interactions were found in all yields and yield attributes parameters. The effect of salinity varies between plants species and also according to the type of production system used [18; 40]. Said-Al Ahl and Omer [33] reported that accumulation of salts in the root-media was caused by rates of evapo-transpiration and poor leaching of water. In this case, attributes of sawdust results compared to sand are due to better leaching of water although the parameter was not measured. Sawdust has better and sufficient root-zone moisture, temperature and lower bulk density compared to sand as root-media. According to [38] and [28], sawdust offers better drainage and good aeration and it also provides plants with sufficient moisture, aeration and an optimum ratio between elements in the root-zone. Softwood cuttings (*Ficus binnendijkii* L.) and gypsophila (*Gypsophila paniculata* L.) developed better sprouts when sawdust was used as a substrate [39; 46].

### **Mineral composition**

Figure 7 illustrate the interactions between the salinity levels and root-media. The results found on the data not presented showed that salinity levels, root-media and the interactions were all found significant at  $p < .01$ . Significant interaction was found between salinity at 4.0 mS cm<sup>-1</sup> and using sand as a root-media. Furthermore, leaf mineral content of P ( $p < .05$ ), K ( $p < .01$ ) and Mg ( $p < .01$ ) were significantly high where salinity levels were low at 1.6 mS cm<sup>-1</sup> and consequently gradually reduced when salinity levels increased up to 4.0 mS cm<sup>-1</sup> (Figure 8). Dissimilar to P,

K and Mg; the leaf mineral content of Na ( $p < .01$ ) and Cl ( $p < .01$ ) were significantly increased at high salinity level (Figure 8). The interaction between salinity at 4.0 mS cm<sup>-1</sup> and sand root-media is ascribed to the high application of salinity at 4.0 mS cm<sup>-1</sup> and also the accumulation of Na in sand with poor water leaching compared to sawdust [33]. The current study results are in agreement with [13] study on eucalyptus species, whereby high salinity concentration increased the Na and Cl leaf-tissue content. The decrease in K and Mg content is associated to the cation uptake competition between Na, K and Mg which will consequently be followed by the mineral deficiency [9].

### ***Oil composition***

As shown in Table 3, salinity had no significant effects on the linalool, iso-menthone, citronellol, geraniol, citronellyl formate and guaia-6,9-diene contents of rose geranium. However, significant results were found on geranyl formate ( $p < .05$ ) and C:G ratio ( $p < .01$ ) where salinity level were high, 4.0 mS cm<sup>-1</sup>. Furthermore, root-media had no significant on most oil composition content except iso-menthone ( $p < .05$ ). Geranyl formate content was reduced at a high salinity level of 4.0 mS cm<sup>-1</sup>; consequently the C:G ratio was increased, thus lowering the oil quality based on literature. No significant interaction between salinity and root media was recorded either.

In most salinity studies, it has been shown that salinity affects the biosynthesis of essential oils [37]. Furthermore, it has also been shown that these biosynthesis activities occur inside the palisade cells and are stored in the vacuole. Sodium at high levels tends to accumulate in the plant cell and directly interferes with the biosynthesis of essential oils in the vacuole [6; 37]. Literature was not found that explains in details how salinity affects the synthesis of geranyl formate. However, since salinity is involved in the biosynthetic activities associated with those of essential oil biosynthesis, the significant effects of salinity on geranyl formate and C:G ratio is ascribed to directly interferes with the biosynthesis of essential oils in the vacuole [6; 37]. Beside geranyl formate; other essential oil compounds has been shown to be affected by

salinity. Linalool content of basil and coriander was increased to respectively 57% and 45% at high salinity levels [4]. Good oil quality was reported on sage, where high salinity increased oxygenated monotepernes [43].

### **Conclusion**

Rose geranium yield attributes were found to be significantly reduced by salinity at 4.0 mS cm<sup>-1</sup> and also using sand as a root-media although no interactions were found. Oil yield was found statistically non-significant but the trend showed that at salinity at 4.0 mS cm<sup>-1</sup> reduced oil yield. Furthermore, essential oil quality was reduced by the increase in salinity levels compared to industrial requirements of 1:1 and 2:1 C:G ratio for Bourbon cultivar. It is evident from the study that rose geranium should be grown using nutrient solution with salinity below 4.0 mS cm<sup>-1</sup> for better yield and oil quality using sawdust as a root-media

Table 1 Nutrient solution concentrations used to study the response of rose geranium to salinity and root-media (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>)

Salinity (mS cm <sup>-1</sup> )	Ions															
	Micro-nutrients (mmol L <sup>-1</sup> )						Micro-nutrients (mg L <sup>-1</sup> )									
	Na <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	K <sup>+</sup>	Ca <sup>+</sup>	Mg <sup>2+</sup>	NO <sub>3</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Fe <sup>2+</sup>	Mn <sup>3+</sup>	B	Cu <sup>2+</sup>	Mo <sup>2+</sup>	Zn <sup>2+</sup>
<b>1.6</b>	<b>1.31</b>	1.00	5.5	6.50	2.5	11.04	0.10	3.80	<b>1.31</b>	0.40	1.12	0.54	0.03	0.02	0.05	0.18
<b>2.4</b>	<b>8.49</b>	1.00	5.5	6.50	2.5	11.04	0.10	3.80	<b>8.49</b>	0.40	1.12	0.54	0.03	0.02	0.05	0.18
<b>3.2</b>	<b>16.5</b>	1.00	5.5	6.50	2.5	11.04	0.10	3.80	<b>16.5</b>	0.40	1.12	0.54	0.03	0.02	0.05	0.18
<b>4.0</b>	<b>24.5</b>	1.00	5.5	6.50	2.5	11.04	0.10	3.80	<b>24.5</b>	0.40	1.12	0.54	0.03	0.02	0.05	0.18

Table 2 The effect of salinity and root-media on the number of leaves, plant height, number of branches, leaf area, relative water content, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Notes. Means followed by the same letter in the same column are statistically non-significant with ns = not significant at  $p < .05$ .

\*  $F$ -ratio probability of  $p < .01$ .

Treatments	Parameters								
	Number of leaves (plant <sup>-1</sup> )	Plant height (cm)	Number of branches (plant <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Relative water content	Chlorophyll (%)	Foliar fresh mass (g plant <sup>-1</sup> )	Oil yield (g plant <sup>-1</sup> )	Oil content (%)
NaCl level (mS cm <sup>-1</sup> )									
1.6	448.44 <sup>a</sup>	43.44 <sup>a</sup>	38.88 <sup>a</sup>	931.16 <sup>a</sup>	84.56 <sup>a</sup>	28.11 <sup>a</sup>	490.83 <sup>a</sup>	1.49 <sup>a</sup>	.31 <sup>a</sup>
2.4	482.11 <sup>a</sup>	42.83 <sup>a</sup>	33.33 <sup>ab</sup>	811.05 <sup>ab</sup>	81.27 <sup>a</sup>	26.05 <sup>ab</sup>	430.27 <sup>a</sup>	1.44 <sup>a</sup>	.35 <sup>a</sup>
3.2	425.44 <sup>a</sup>	43.49 <sup>a</sup>	32.11 <sup>b</sup>	686.11 <sup>b</sup>	86.57 <sup>a</sup>	25.87 <sup>ab</sup>	502.50 <sup>a</sup>	1.87 <sup>a</sup>	.37 <sup>a</sup>
4.0	249.94 <sup>b</sup>	36.27 <sup>b</sup>	30.50 <sup>b</sup>	459.44 <sup>c</sup>	83.13 <sup>a</sup>	22.92 <sup>b</sup>	373.33 <sup>a</sup>	1.28 <sup>a</sup>	.35 <sup>a</sup>
LSD <sub>(0.05)</sub>	88.64*	4.75**	5.91**	155.09*	ns	3.22**	ns	ns	ns
Root-media									
Sand	351.13 <sup>b</sup>	40.10 <sup>a</sup>	32.66 <sup>a</sup>	644.80 <sup>b</sup>	83.62 <sup>a</sup>	24.92 <sup>a</sup>	387.22 <sup>b</sup>	1.34 <sup>a</sup>	.35 <sup>a</sup>
Sawdust	451.83 <sup>a</sup>	42.91 <sup>a</sup>	34.75 <sup>a</sup>	799.08 <sup>a</sup>	84.15 <sup>a</sup>	26.55 <sup>a</sup>	511.24 <sup>a</sup>	1.70 <sup>a</sup>	.34 <sup>a</sup>
LSD <sub>(0.05)</sub>	62.68*	ns	ns	109.66*	ns	ns	85.62*	ns	ns

\*\*  $F$ -ratio probability of  $p < .05$ .

Table 3 Effect of salinity-induced nutrient solution and root-media on oil composition of rose geranium

Notes. Means followed by the same letter in the same column are statistically non-significant with ns = not significant at  $p < .05$ .

Treatments	Oil composition (%)							
	Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6, 9-diene	C:G ratio
NaCl level (mS cm <sup>-1</sup> )								
1.6	1.23 <sup>a</sup>	1.78 <sup>a</sup>	31.51 <sup>a</sup>	13.71 <sup>a</sup>	21.21 <sup>a</sup>	7.98 <sup>a</sup>	9.37 <sup>a</sup>	2.35 <sup>a</sup>
2.4	1.46 <sup>a</sup>	1.95 <sup>a</sup>	31.84 <sup>a</sup>	12.70 <sup>a</sup>	21.55 <sup>a</sup>	7.61 <sup>a</sup>	9.37 <sup>a</sup>	2.54 <sup>a</sup>
3.2	1.48 <sup>a</sup>	2.18 <sup>a</sup>	32.48 <sup>a</sup>	12.62 <sup>a</sup>	21.66 <sup>a</sup>	7.63 <sup>a</sup>	9.51 <sup>a</sup>	2.60 <sup>a</sup>
4.0	1.10 <sup>a</sup>	1.48 <sup>a</sup>	35.19 <sup>a</sup>	11.10 <sup>a</sup>	22.73 <sup>a</sup>	6.53 <sup>b</sup>	9.49 <sup>a</sup>	3.18 <sup>b</sup>
LSD (0.05)	ns	ns	ns	ns	ns	.94**	ns	.40*
Root-media								
Sand	1.04 <sup>a</sup>	2.54 <sup>a</sup>	32.58 <sup>a</sup>	12.57 <sup>a</sup>	21.97 <sup>a</sup>	7.53 <sup>a</sup>	9.71 <sup>a</sup>	2.66 <sup>a</sup>
Sawdust	1.39 <sup>a</sup>	1.66 <sup>b</sup>	32.98 <sup>a</sup>	12.50 <sup>a</sup>	21.60 <sup>a</sup>	7.32 <sup>a</sup>	9.16 <sup>a</sup>	2.68 <sup>a</sup>
LSD (0.05)	ns	.79**	ns	ns	ns	ns	ns	ns

\* *F*-ratio probability of  $p < .01$ .

\*\* *F*-ratio probability of  $p < .05$ .



## Acknowledgement

Authors would like to acknowledge CUT, FS and NRF for funding and support throughout the experiment

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## Appendix

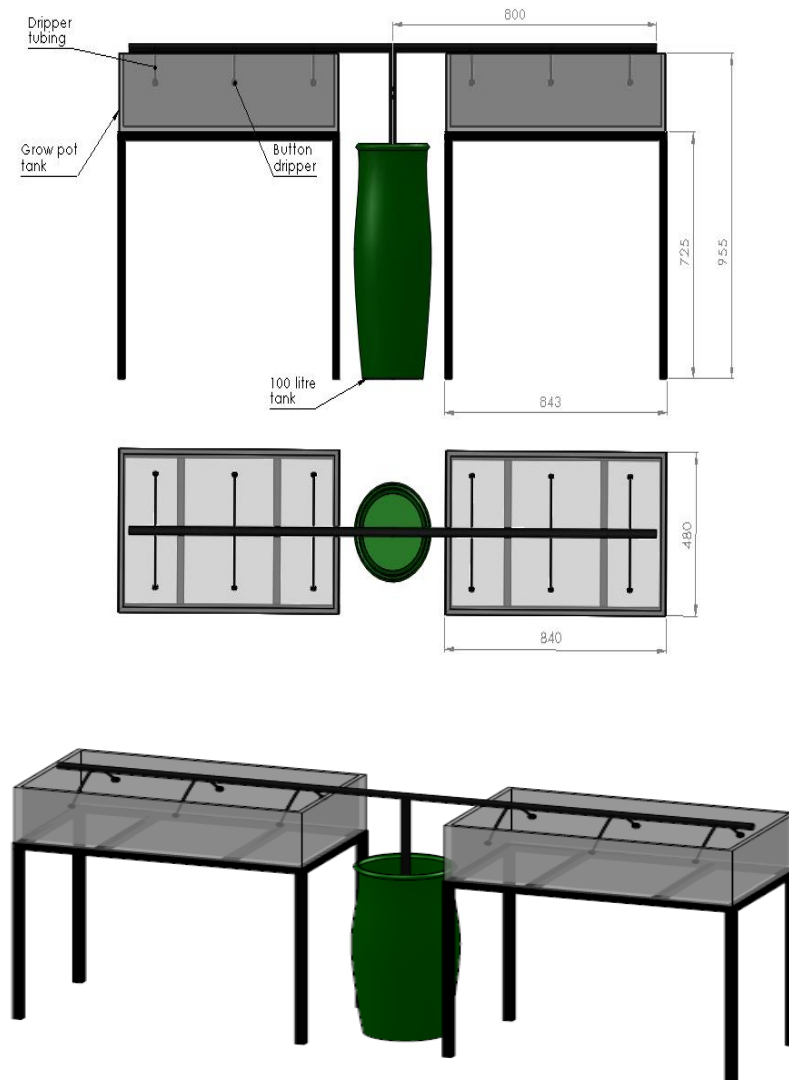


Figure 1. Schematic representation of small-scale growing unit and irrigation system used to grow rose geranium plants

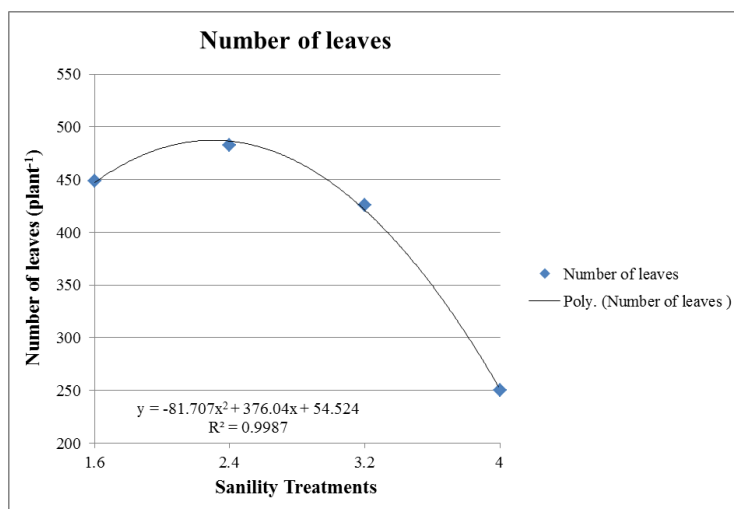


Figure 2. Polynomial relationship between number of leaves and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>)

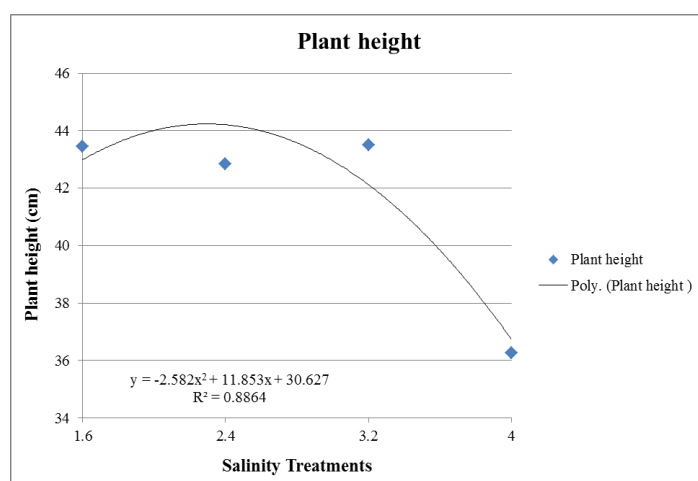


Figure 3. Polynomial relationship between plant height and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>)

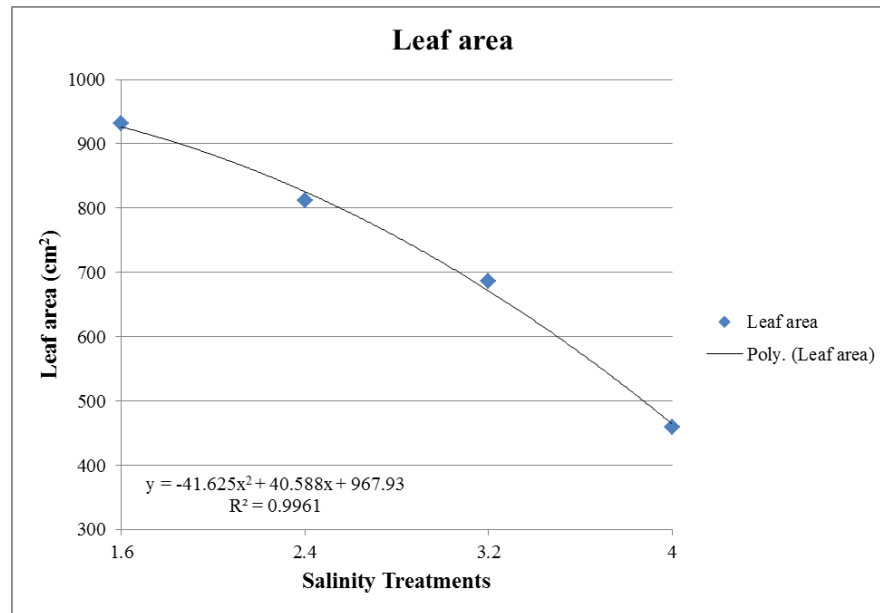


Figure 4. Polynomial relationship between leaf area and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>).

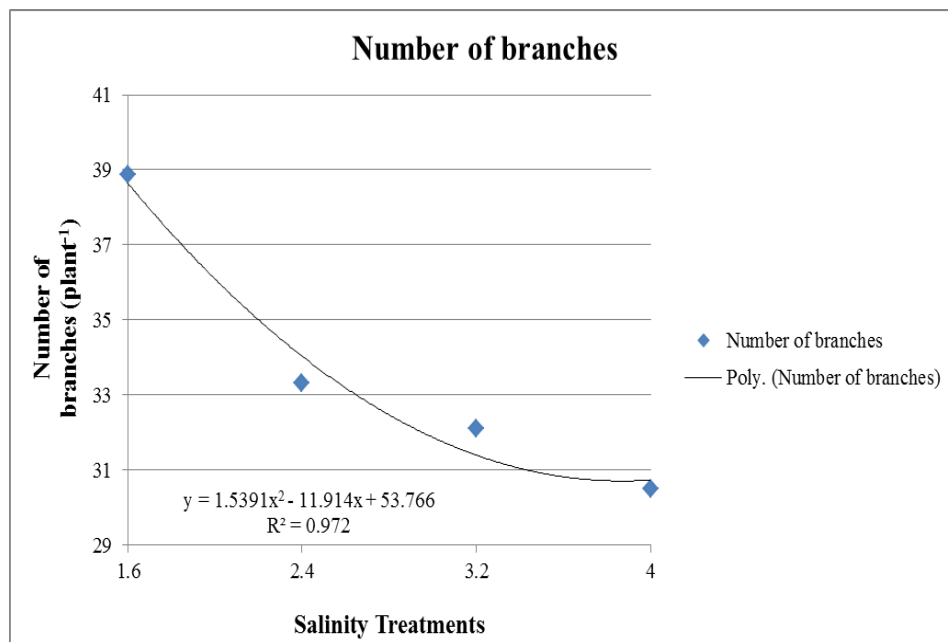


Figure 5. Polynomial relationship between number of branches and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>).

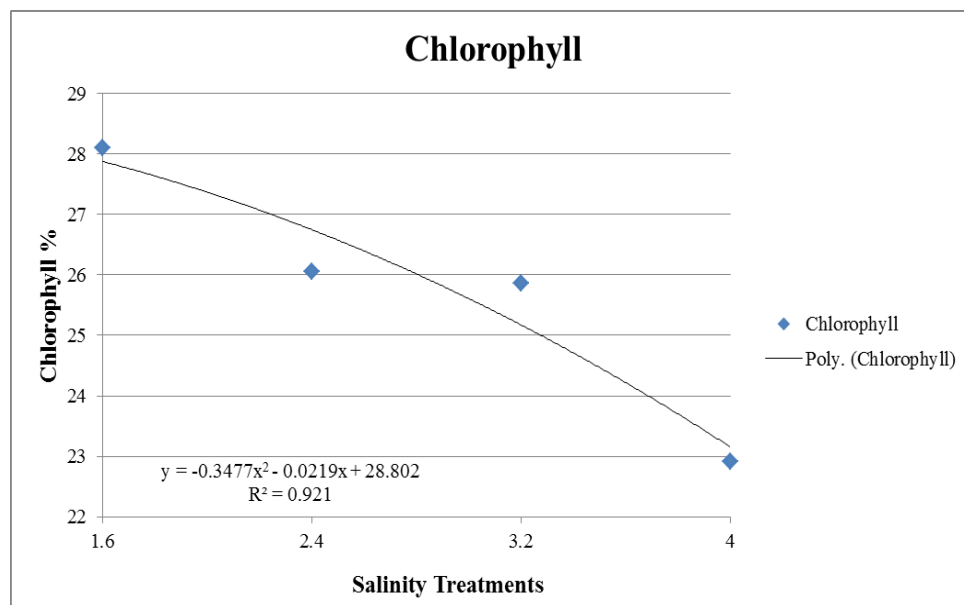


Figure 6. Polynomial relationship between chlorophyll content and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm<sup>-1</sup>).

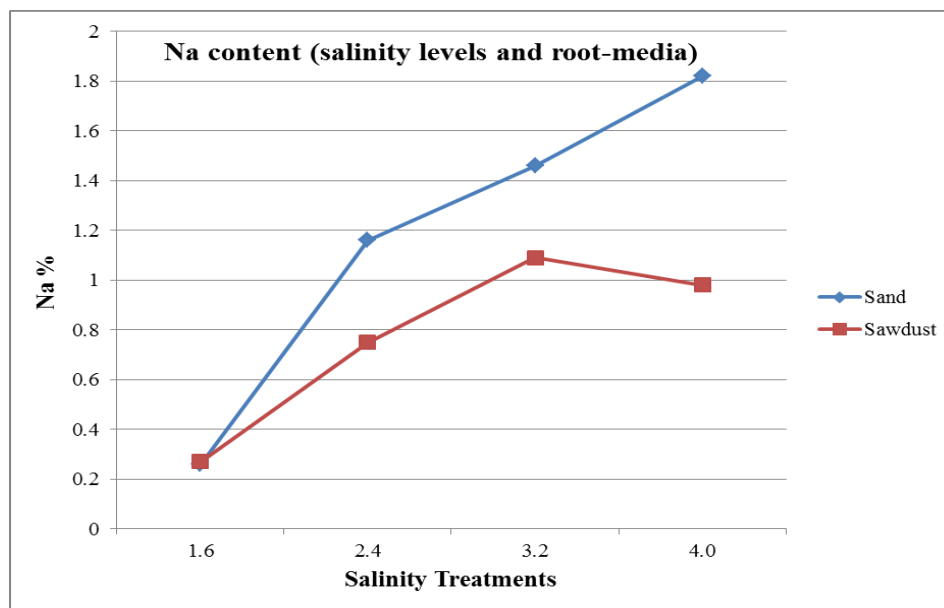


Figure 7. Interaction effects of Na content between salinity concentrations and root-media of rose geranium.

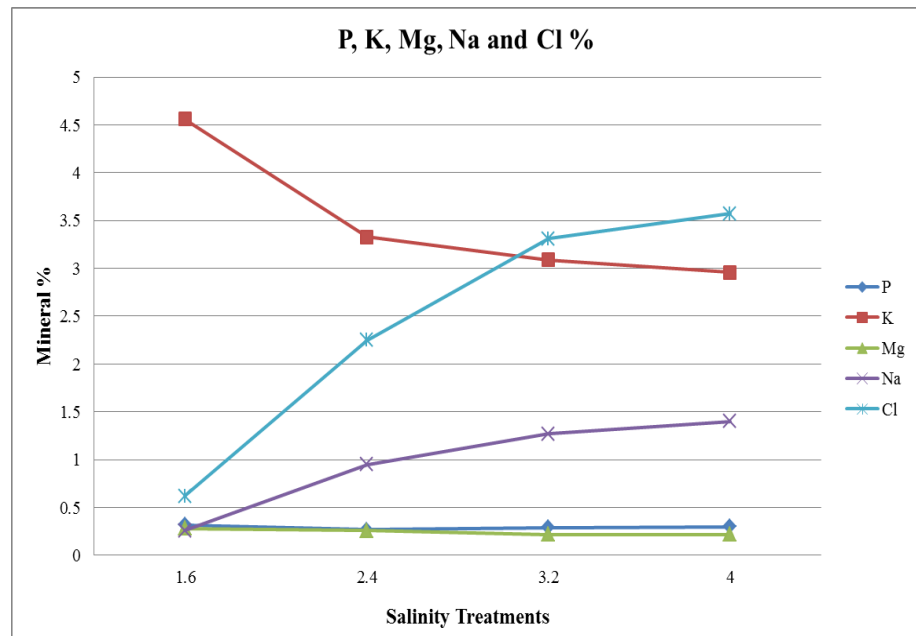


Figure 8. Effects of salinity-induced nutrient solution and root-media on P, K, Mg, Na and Cl content of rose geranium

# USE OF COMPUTER MODELS IN AGRICULTURE: A REVIEW

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## Abstract

Computer models are being highly used in agriculture sector to increase efficiency of decision making, and to find out the best cropping and management options. However, to get good output from those models, need to select best models for particular crops and good data source for calibration and validation process. Otherwise outputs of the model do not address the real situation in the field. In this study select three crop models used in rice cropping system and discussed about present data requirement, their application in rice cultivation and model limitations and future potentials. APSIM, ORYZA2000 and DSSAT models were evaluated in this research. However those available models are highly depended on the technical data such as climate, soil, crop and management data and those models do not significantly consider economic and social-cultural factors in agriculture systems. Therefore, simulation results by models do not match with the observed values. Due to this Limitation there is a mandatory requirement to make necessary adjustment or improvements in those models by considering farmers' socio-economic and cultural indicators. In addition, most of the computer models are concentrating in the same crops. When consider the Sri Lankan context, Sri Lanka cultivate comparatively considerable amount of other field crops mainly vegetables with rice where most of these computer models have not been developed to capture the management of vegetables with rice. Hence these limitations in current computer models create an opportunity for researchers to think about new computer models which can capture local conditions and resulting with better model outputs.

Keywords: Efficiency, computer models, socio-economic and cultural data

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## Introduction

Rice is the staple food of most of the Asian as well as other developing countries. Two third of the world population is concentrated in Asia and land extend used in agriculture is continuously decreasing due to high population growth, urbanization and industrialization in most of the developing countries including Asia. There is a crucial need to increase the land and water productivity in order to feed the increased population (FAO, 2013). In agriculture systems, decision making has become a difficult task due to uncertainty in climate, various types of inputs, different types of management practices and environmental conditions. Application of computer models in decision making

will be a potential solution to find out the best cropping and management systems and computer models need comparatively less time and low cost than field trials, can run for several field trials at a given time duration, has a possibility of incorporating the changes in climatic parameters to predict the yields. On the other hand, the generation of new data and knowledge through traditional agronomic research methods and its communication are not sufficient to meet these increasing challenges. In addition, these field trials are time consuming and expensive.

The current challenges in crop production with the context of continuously increasing demand for higher

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crop yields while reducing inputs such as fertilizer, water and pesticide, have created an increasing demand for agronomic knowledge and enhanced decision support guidelines. In considering the present context, crop models are increasingly being used to improve cropping techniques and cropping systems (Uehera and Tsuji, 1993; Penning de Vries and Teng, 1993; Boote et al., 1996). Crop modeling has resulted through combination of mechanistic models designed by crop physiologists, agronomists, soil scientists, hydrologists and meteorologists. Crop models make it possible to identify, very rapidly, the adaptations required to enable cropping systems to respond to changes in the economic, environmental or regulatory contexts (Rossing et al., 1997). The overall objective of this study was to identify the present data requirement in computer models, limitations and future potentials to increase accuracy of model output and efficiency of decision making process.

## **Methodology**

Three crop models (APSIM, ORYZA 2000 and DSSAT) were selected and one application of each models in paddy cultivation were reviewed based on what are the crops and what are the data can be used to model calibration and validation (In here APSIM model application were based on the raw data collected from field). Finally discussed what the limitations of each crop models and what are the future challenges scientists have to face when developing better crop models related to particular region or country.

## **Results and Discussion**

### ***Different Types of Models used in Crop and Water Management in Agricultural System***

Crop growth models have been used since 1970s (Hoogenboom, 2003). Crop growth is a very complex phenomenon and a product of a series of complicated interactions of soil, plant and weather. In addition to climatic factors, there are large number of edaphic, hydrologic, biotic and agronomic factors that influence crop growth and productivity (Nagarjuna, 2009). In the past decade, the dynamics of crop growth models have made substantial progress (Gerdes, 1993) and many

crop models are available on the market. Some of models are, ORYZA1 (Kropff et al., 1994), CERES-Rice (Singh et al., 1993), SIMRIW (Simulation Model for Rice-Weather relations) (Matthews et al., 1994), rice-weed competition model (Graf et al., 1990), ORYZA2000 (Bouman, 2006), DSSAT (Decision Support System for Agrotechnology Transfer) (Jones et al., 2003), APSIM (Agriculture production Simulator) (Keating et al., 2003), AquaCrop (Raes et al., 2009) and STICS model (Simulateur Multidisciplinaire Pour Les Cultures Standard) (Bruno et al., 1998). Also scientists have developed some specific models to soil and water management such as SPAW (Soil-Plant-Air-Water) model (Saxton and Willey, 2006). Each model has its specific objective(s) and, its own set of assumptions and complexity. Computer models have achieved various degrees of success in application. They all have their weaknesses and failed under certain circumstances, therefore authors of models should clarify the limitations of their models and ranges of applications (Ma and Schaffer, 2001).

### ***Crop Models Applications in Rice Cultivation to Increase Productivity***

#### ***APSIM Model***

APSIM is a software tool that enables sub-models to be linked to simulate agricultural systems (McCown et al., 1996). APSIM has various modules grouped and categorized as plant, environment and management. APSIM was developed (APSRU, 1991) to simulate biophysical process in farming systems, in particular where there is interest in the economic and ecological outcomes of management practices in the face of climatic risks. APSIM has been used in a broad range of applications, including support for on-farm decision making, farming systems design for production or resource management objectives, assessment of the value of seasonal climate forecasting, analysis of supply chain issues in agribusiness activities.

**Model application-** Modeling the Effect of Water Stress on Paddy Yield Using APSIM (Kumara et al., 2013)

A study was done in Kadaweramulla area of the Kurunegala district in Sri Lanka. Seven farmers who were cultivating Bg 358 rice variety in the 2012/2013 Maha season were selected. APSIM crop model was used to evaluate paddy yield under different water management options (Irrigation only, Rainfall only and Irrigation+Rainfall conditions) to identify water stress condition for paddy cultivation. Also the model was used to simulate rice yield under number of dates irrigation supply and simulated water content in the root zone under different water management options for each farmer and graphically showed.

The following data were used in the modeling exercise

Crop data: leaf area index, number of panicles, dates of the main phenological stages, and genetic coefficients.

Management data: starting date of ponding, end date of ponding, maximum depth of ponding, start date of irrigation

Weather data: rainfall, maximum and minimum temperatures, sunshine hours

Soil data: pH, organic matter, N-N03, N-NH4, bulk density, texture, moisture content

According to the results of different water management options, the simulated yield was higher under the irrigation+rainfall option compared to other two options because of less water stress conditions (Fig. 1). According to the simulated data, after increasing no of irrigation days for paddy field, paddy yields continuously increases in the low soil moisture fields (Fig. 2) and after some point, yield starts to decrease. The fields which had comparatively higher soil moisture conditions showed opposite results (Fig. 3). Those two phenomena mainly happened due to low and high water stress conditions, respectively. (in the diagram, IRRI+RF =Irrigation+Rainfall, NRF =No rainfall, NIRRI=No irrigation, OBSERVED =Observed yield in each field).

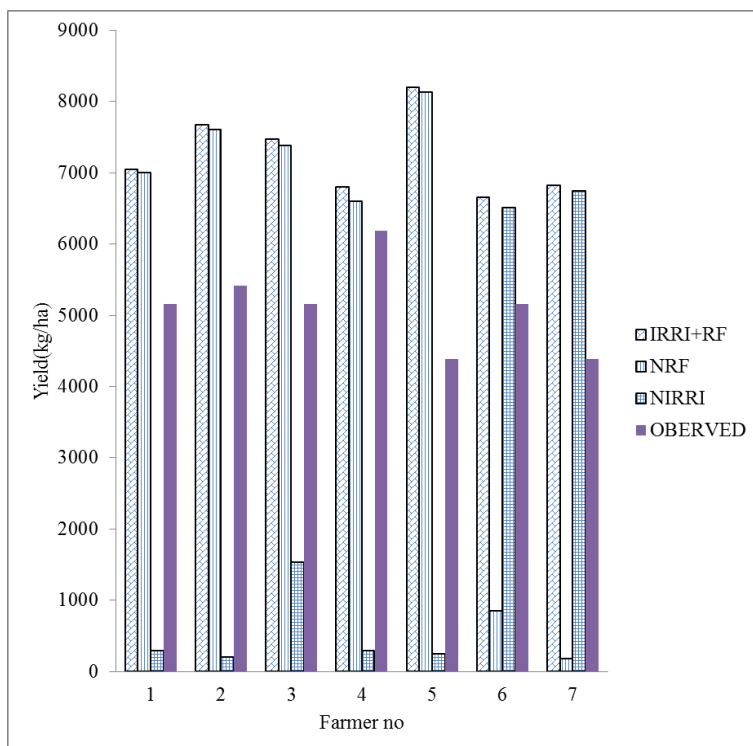


Figure 1: Yield variation with different water management methods for each farmer

Simulated yield is high under irrigation + rainfall water management method. Farmer no

6 and 7 rice fields have high yields under without irrigation. These two fields were

located in lower part of the catena with poor drainage conditions. This location has comparatively higher soil moisture conditions compared to other fields studied.

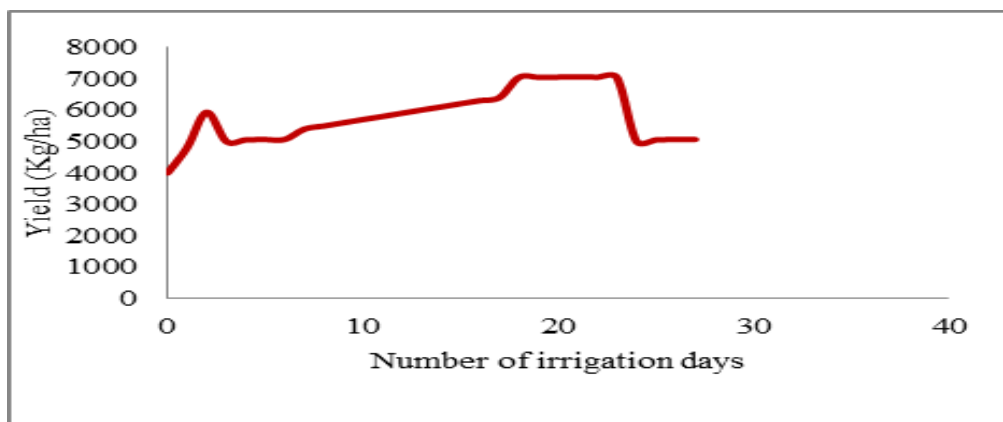


Figure 2: Yield variation with number of irrigation dates provided for farmer no 1

Field belongs to farmer 1 had low moisture condition according to analyzed results. The simulated yield was gradually increased with increasing number of irrigation days for that field and the highest yield was obtained at 23 number of irrigation dates.

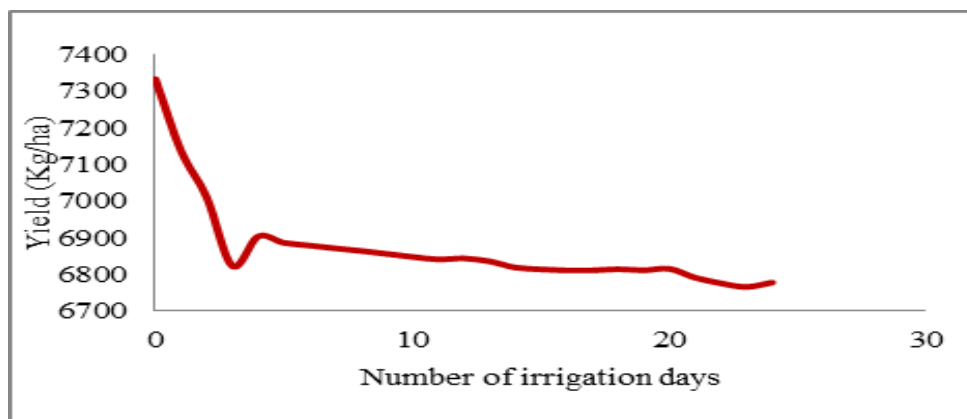
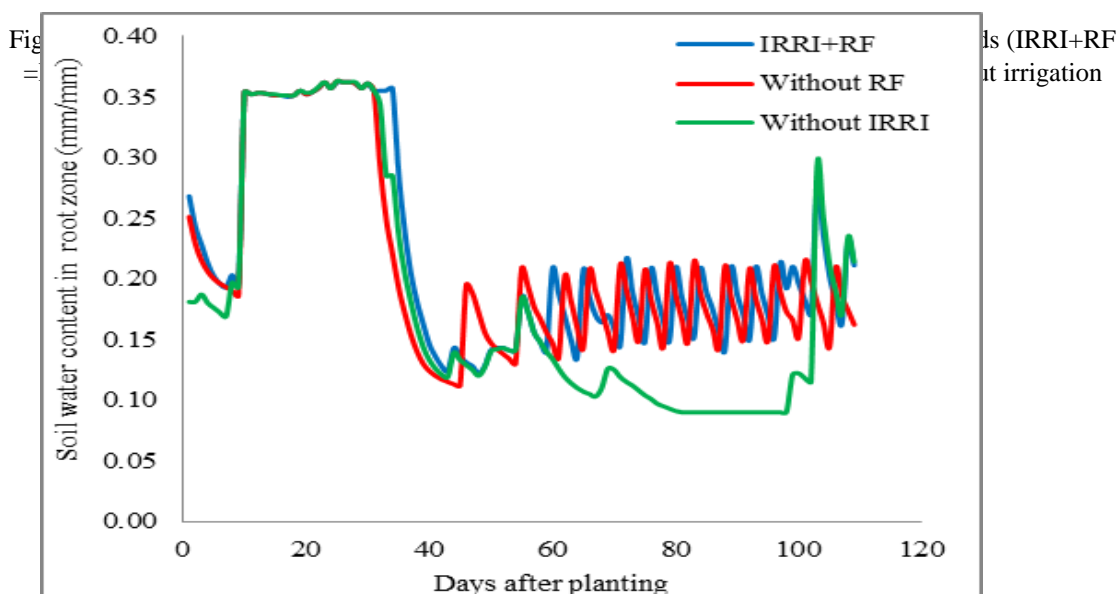


Figure 3: Yield variation with number of irrigation dates provides for farmer no 7

The field of farmer no 6 has high moisture content and therefore, without any irrigation, that field can provide higher rice yield. When increasing the number of irrigation days, yield starts to decreased due to higher moisture stress.



According to results of Fig. 4, soil water content in the root zone is varied in whole life cycle of the rice plant. During the initial stages of the season, the high soil water content is due to land preparation which uses high amount of water. After 10-14 days, farmers start irrigation and maintain standing water in the field for about 14 days.

### ORYZA2000 Model

ORYZA2000 is the successor to a series of rice growth models developed during the 1990s under the project “Simulation and Systems Analysis for Rice Production” (SARP). It is an update and integration of the models ORYZA1 for potential production (Kropff et al., 1994), ORYZA\_W for water-limited production (Wopereis et al., 1996), and ORYZA-N for nitrogen-limited production (Drenth et al., 1994). It simulates the growth and development of a rice crop in situations of potential production, water limitations, and nitrogen limitations. A detailed explanation of the model and program code is given in Bouman et al. (2001), and the key modules for potential- and water-limited-production are well explained in the literature (Arora et al., 2006; Boling et al., 2007; Feng et al., 2007).

**Model application-** Evaluation and application of ORYZA2000 for irrigation scheduling of puddled transplanted rice in North West India (Sudhir et al., 2011).

This study tested the ability of the ORYZA2000 model to simulate the effects of water management on rice growth, yield, water productivity (WP), components of the water balance, and soil water dynamics in north-west India.

Following data were used.

Crop data: leaf area index, dates of the main phenological stages, genetic coefficients etc.

Management data: starting date of ponding, last date of ponding, maximum depth of ponding, starting date of irrigation

Weather data: rainfall, maximum and minimum temperatures, sunshine hours

Soil data: pH, organic matter, bulk density, texture, moisture content

The scenario analysis for 40 rice seasons always indicated some yield penalty when changing from continues flooding (CF) to alternate wetting and drying

(AWD) (Humphreys et al., 2008). ORYZA2000 performs well in predicting the effects of irrigation scheduling on crop growth, yield, water balance components and water productivity of puddled transplanted rice when calibrated for the range of stresses  $\times$  seasonal conditions. The results of the simulations are consistent with the findings of field studies that AWD has great potential for large irrigation water savings resulting increased irrigation water productivity in comparison with CF. The main cause of the irrigation water saving in AWD is greatly reduced drainage with a relatively small (60 mm) decrease in ET. The effects of the irrigation treatments (AWD) on ET are small, and drainage water is likely to be internally recycled in this region. Thus, results suggest that no impact of changing irrigation management from frequent to less frequent AWD on groundwater depletion resulting a regional sustainability of water resources.

## DSSAT

DSSAT was developed using CERES-Rice model by International Benchmark Systems Network for Agrotechnology Transfer (IBSNAT) (Tsuji et al., 1998). The model encompasses process-based computer models that predict growth, development and yield as a function of local weather and soil conditions, crop management scenarios and genetic information. The crops that are covered include grain cereals, grain legumes, tuber crops, cotton, sugarcane, and various other species. DSSAT includes a basic set of tools to prepare the input data, as well as application programs for seasonal, crop rotation and spatial analysis. This model not only predicts crop yield, but also resource dynamics, for water, nitrogen and carbon, and environmental impacts, such as nitrogen leaching. In addition, the DSSAT includes an economic component that calculates gross margins based on harvested yield and byproducts, the price of the harvested products, and input costs (Simone, 2012).

**Model application-** Assessment of the effect of climate change on boro rice production in Bangladesh using DSSAT model (Basak et al., 2010).

Following data were used.

Weather data: solar radiation, maximum and minimum air temperature, rainfall,

Crop data: leaf area index, dates of the main phenological stages, genetic coefficients etc.

Management data: seeding rate, fertilizer applications, irrigations Water, planting date, planting depth, row spacing, plant population

Soil data: soil pH, surface runoff, evaporation from the soil surface, drainage etc.

The yield of BR3 and BR14 boro varieties for the years 2008, 2030, 2050 and 2070 have been simulated for 12 locations (districts) in Bangladesh. The model predicted significant reduction in yield of both varieties of boro rice due to climate change. The average yield reductions of over 20% and 50% have been predicted for both rice varieties for the years 2050 and 2070, respectively. Increases in daily maximum and minimum temperatures have been found to be primarily responsible for reduction in yield. Increases in incoming solar radiation and atmospheric CO<sub>2</sub> concentration have increases rice yield to some extent, but their effect is not significant compared to the negative effects of temperature. Variations in rainfall pattern over the growing period have also been found to affect rice yield and water requirement. Increasing temperatures and solar radiation have been found to reduce the duration of physiological maturity of the rice varieties. Model results also suggest that in addition to reducing yield. DSSAT modeling system could be a useful tool for assessing possible impacts of climate change and management practices on different varieties rice and other crops.

### Comparison of model input data

Table 1: Data requirements for each model

Model		APSIM	DSSAT	ORYZA2000
Data requirement	Weather Data	✓	✓	✓
	Soil data	✓	✓	✓
	Crop data	✓	✓	✓
	Management data	✓	✓	✓
	Economic data	✓	✓	✗
	Social data	✗	✗	✗
	Cultural data	✗	✗	✗

As illustrates in table 1 each model required different types of data. Basically all three models totally depend on the technical data such as crop data, climate data, soil data but APSIM and DSSAT the newly developed models now consider on economics data and those models do not significantly consider about socio-economic and cultural backgrounds of farming society.

When considering the Sri Lankan situation, population growth, urbanization, and industrialization create problems and fresh water availability for irrigation in agriculture is becoming a major issue. In village level, more than 12000 minor tanks systems are available for crop cultivation, but those water sources are not being efficiently used. Hence, the use of computer models to increase crop and water productivity under these minor tank systems will help the sustainability of rural agriculture while guaranteeing the water and food security. In Sri Lankan context, we can't use those models directly under the village based agriculture systems since these models have been made in developed countries according to their context. Hence,

it is a mandatory requirement to make necessary adjustment or improvements in those models because those models do not consider farmers' socio-economic situation, attitudes, farmer organization decisions,

irrigation water quality, irrigation water allocation and political influence in agriculture. Therefore, simulation results generated without considering socio-economic and cultural factors do not match with the observed values. When model is run only by using technical data results failing failure in the final output of computer models. As an example, during the APSIM model application, simulate results and observed values are found to be highly different because of farmers decision making and model inputs are completely different.

In addition, most of the crops simulating computer models are concentrating in the same crop (mono culture) such as rice, wheat, maize, and some legumes. However, in Sri Lankan situation, Sri Lankan farmers cultivate comparatively large amount of other field crops (OFC) mainly vegetables (Brinjal, Okra, Tomato, Cabbage, Carrot, Pumpkin etc) with rice where most of these computer models have not been developed to capture the management of vegetables with rice. In the Maha season, generally the entire field is cultivated with rice crop while during the Yala season, rice+OFC cultivation is a common practice of farmers in Sri Lanka. These situations create an opportunity for researchers to think about new computer models which can capture Sri Lankan conditions resulting to increased

agricultural and water productivity. The development of a computer model to select the best cropping system considering water availability, climate, soil, management, economic, social, and farmer organization decisions will help to increase water, land and crop productivity in Sri Lanka.

## Conclusion

APSIM model applications gave evidence on how scarce water resources can be managed efficiently for rice cultivation and increasing water productivity. ORYZA2000 model application depicts how computer models can be used in irrigation scheduling and how those computer models analyze under field conditions. In addition, DSSAT model application depicts what are the future challenges to paddy cultivation and how those challenges can be predicted using past data and computer models. All computer models govern by technical data and gave less priority to socio-economics and cultural aspects. Also all models are concentrating in same crops. Therefore simulated results by models do not compatible with the observed data. Hence there is a future potential to develop new crop models considering all data forms and possible combination of crops cultivate in the particular country.

## Acknowledgements

This work was carried out with the aid of a grant from the International Development Research Centre, Ottawa, Canada.

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# GENDER AND PRODUCTIVITY DIFFERENTIALS AMONG RICE FARMERS IN NIGER STATE, NIGERIA

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## Abstract

The study compared the differences in the productivity of male and female rice farmers in Niger State, Nigeria. Data used for the study were obtained from primary source using a multi-stage sampling technique with structured questionnaires administered to 150 randomly selected male and female rice farmers from the study area. Descriptive statistics such as means, standard deviations and percentages were used to summarize the variables used in the analysis while input– oriented data envelopment analysis (DEA) was used to empirically determine the total technical, pure technical and scale efficiency with respect to gender in the study area. The DEA results revealed that the male rice farmers were more scale efficient than their female counterparts with mean scale efficiency scores of 0.71 and 0.63 for male and female rice farms respectively. The results also showed that about 77 % and 83% of male and female rice farms operated at increasing returns to scale level respectively. This implies that the two farm groups could achieve higher efficiency level by increasing the production scale. The comparison test for significant differences in mean technical efficiency among the two farm categories confirmed that the mean total and pure technical efficiency with scale efficiency are statistically and significantly higher on male rice farms than on female rice farms. The implication of these findings is that male rice farmers are fairly efficient in utilizing their resources than their female counterparts and any expansion in the use of resources would bring more than proportionate increase in their outputs. The study therefore recommended that research efforts directed towards the generation of new technology, especially for rice farmers, should be encouraged in the study area.

Keywords: Gender, Productivity, scale efficiency, rice production

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## Introduction

Rice is a major staple food in Nigeria, but its domestic production has never been able to meet the demand. It has been estimated that annual rice production needs to increase from 586 million metric tonnes in 2001 to meet the projected global demand of about 756 million metric tonnes by 2030 (Kueneman, 2006). The crop is commonly consumed even as a food crop for household food security. The average Nigerian consumes about 24.8 kg of rice annually, representing 9 per cent of the total annual calories intake and 23 per cent of total annual cereal consumption (Fakayode, 2009). Although rice production in Nigeria has boomed over the years, there has been a considerable lag between production

and demand level with imports making up the shortfall. Domestic productions of this commodity have been inadequate and unable to bridge the increasing demand-supply gap (Idiong, 2007). The Government's goal of achieving self sufficiency in rice production to a large extent will depend on the level of farmers' productivity.

In Nigerian agriculture, rice farming is practiced by both genders (men and women) which bring about differences in farmers' productivity. Gender in agriculture focuses on the relationship between men and women with regard to their roles, access to and control of resources, division of labour and needs. In agricultural production, women have been found to be

more constrained in accessing production resources than their male counterparts. This has often been reflected in women having less access to information, technology, inputs and credit resulting in women having more depressed productivity than men counterpart (Shultz, 1988 quoted from Ojo *et al*, 2013). The gender yield differential apparently is caused by the difference in the intensity, with which measured inputs of labour, manure, and fertilizer are applied on plots controlled by men and women, rather than by difference in the efficiency with which these inputs are used (Adeleke *et al*, 2008).

The subject on whether men are more resource-use productive than women has been extensively discussed in literatures, while some reported that women are as productive as men, others found that women are less productive than men. Agricultural productivity of production unit, defined as the ratio of its output to its input varies due to differences in production technology, differences in setting in which production occurs and differences in efficiency of the production process (Tewodros, 2001). Currently, policy makers have started to believe that an important source of growth in agricultural sector is efficiency gain through greater technical, economic and allocative efficiency by producers in response to better education and information. Efficiency is an important factor of productivity growth especially in developing agricultural economies where resources are meagre and opportunity for developing and adopting better technologies have lately started dwindling (Ali and Chaudhry, 1990). The role of increased efficiency and productivity of rice farms across genders is no longer debatable but a great necessity in order to reverse the low resource productivity of small holder farms in Nigeria. The main objective of this paper is to compare the resource productivity level between men and women rice farmers in the study area. This will help in providing information that may be useful in designing effective policies toward agricultural productivity in the nation at large.

### **Analytical Framework**

The terms productivity and efficiency are often used interchangeably but these are not precisely the same

things. Productivity is an absolute concept and is measured by the ratio of outputs to inputs while efficiency is a relative concept and is measured by comparing the actual ratio of outputs to inputs with the optimal ratio of outputs to inputs. Productivity could be measured in terms of marginal physical product (MPP) in which case, the interest is in the addition to total product resulting exclusively from a unit increase in the use of that input i.e., total factor productivity (TFP) growth, which is measured using the frontier and non-frontier approaches. It therefore suffices to say that productivity can only be measured and ascertained from farm-level efficiency (Udoh and Falake, 2006). According to Arthur *et al.*, (2001), an important concept of productivity analysis is technical efficiency. Productivity is generally measured in terms of the efficiency with which factor inputs, such as land, labour, fertilizer, herbicides, tools, seeds and equipment etc are converted to output within the production process (Umoh and Yusuf, 1999). Generally, there are two approaches to measure efficiency estimates of a firm i.e. parametric approach and non-parametric approach. Parametric approach involves the use of stochastic frontier analysis (SFA) while non-parametric approach involves the use of data envelopment analysis (DEA). DEA approach was preferred over parametric approach for the estimation of efficiency in this study because it provides means of decomposing total technical efficiency into pure technical and scale efficiency (SE). Technical efficiency scores can be obtained by running a constant returns to scale DEA model or variable returns to scale (VRS) DEA model. Technical efficiency scores obtained from constant returns to scale (CRS) DEA model are called total technical efficiency and from variable returns to scale DEA model as pure technical efficiency. Total technical efficiency of a firm can be decomposed into pure technical and scale efficiency. Pure technical efficiency relates to management practices while scale efficiency relates to the residuals. This would enable better understanding of the nature of technical efficiency of farms and would assess the possibilities for productivity gains by improving the efficiency of farmers in the study area. The key construct of a DEA model is the envelopment surface and the efficient projection path to the envelopment surface (Charnes *et al.*, 1978). The

envelopment surface will differ depending on the scale assumptions that underline the model. The efficiency projection path to the envelopment/surface will differ depending on if the model is output-oriented or input oriented. The choice of model depends upon optimization production process characterizing the firm. Input oriented DEA determines how much the mix for a firm would have to change to achieve the output level that coincides with the best practice frontier. Output-oriented DEA is used to determine a firm's potential output given its inputs mix if operated as efficiently as firms along the best practice frontier. For this study input-oriented DEA was used to determine how much input mix the farmers would have to change to achieve the output level that coincides with the best practice frontier. For this study, technical efficiency was used to estimate the resource productivity of the farmers in the study area. Measurement of technical efficiency is important because it is a success indicator of performance measure by which production units are evaluated (Ajibefun, 2008).

DEA is a relative measure of efficiency where the general problem is given as:

$$\text{Max TE} = \frac{\sum_{r=1}^s \alpha_r Y_{ro}}{\sum_{i=1}^m \beta_i X_{io}} = \frac{q}{q^*} \quad (1)$$

Subject to :

$$\frac{\sum_{r=1}^s \alpha_r Y_{rj}}{\sum_{i=1}^m \beta_i X_{ij}} \leq 1, j = 1, \dots, n \quad (2)$$

$$\alpha_r, \beta_i \geq 0; r = 1, \dots, s; i = 1, \dots, m$$

Where  $X_{ij}$  and  $Y_{ij}$  respectively are quantities of the  $i^{\text{th}}$  input and  $r^{\text{th}}$  output of the  $j^{\text{th}}$  firm and  $\alpha_r, \beta_i \geq 0$  are the variable weights to be determined by the solution to this problem. Scale efficiency can be obtained residually

from CRS and VRS technical efficiency scores as follow:

$$\text{SE} = \text{CRSTE} / \text{VRSTE}$$

SE = 1 indicates scale efficiency or constant return to scale (CRS) and SE < 1 indicates scale inefficiency. Scale inefficiencies arise due to the presence of either increasing returns to scale or decreasing return to scale.

## Methodology

### Description of Study Area

The study was conducted in Niger State of Nigeria. Niger State is located between latitudes 8°11'N and 11° 20' N and longitude 4° 30'E and 7° 20'E. It is bordered on the north-east by Kaduna state and on the South-east by the Federal Capital Territory, Abuja. It is also bordered on the North, West, South West and South by Zamfara, Kebbi, Kogi and Kwara States respectively (see figures 3.1 and 3.2). It shares a foreign border with the Republic of Benin in the North West. The state covers an estimated land area of 76,363 square kilometers and a population of 4,082,558 people (Wikipedia, 2011). The state is agrarian and well suited for production of arable crops such as rice, cassava, cowpea, yam, and maize because of favourable climatic conditions. The annual rainfall is between 1100mm – 1600mm with average monthly temperature ranges from 23 ° C and 37 ° C (Wikipedia, 2010). Kaduna State and Federal Capital Territory (Abuja) are her borders to the North-East and South-East respectively; Zamfara state borders the North, Kebbi State in North-West, Kogi State in South and Kwara State in South-West. The vegetation consists mainly of short grasses, shrubs and scattered trees.

### Sampling Technique and Sample Size

Primary data for this study were collected using multi-stage sampling technique. The first stage involved the random selection of 2 Local Government Areas (LGAs) in the study area. The second stage involved random selection of five villages in each LGA and 75 rice farmers (male and female) in each LGA totalling 150 farmers (eighty-six males and sixty-four females)

altogether in the selected two LGAs in the study area. The selection was based on the proportion of male and female registered farmers at the State Agricultural Development Programme (ADP).

### **Method of Data Collection**

Primary data for study were collected with the use of a structured questionnaire that administered to the respondents. Data that were collected include total rice output produced per annum in kg, while the inputs included the size of farm land in hectare, quantity of seeds as planting materials in kg; quantity of fertilizer used in kg; quantity of herbicides used in litres and total labour in man-days which include family and hired labour utilised during pre and post planting operations and harvesting; unit price of the rice in naira; total production cost per year; average wage rate per man days of labour, price per kg of planting materials, average price of agrochemicals, average price of fertilizer and average price of farm tools.

### **Empirical Model specification**

The empirical model is as specified in equations 1 and 2. The output variable used for estimating efficiency scores was total rice output (kg) (Y). The inputs used included farm size (ha), labour (man-day), planting materials (kg), herbicides (litres), fertilizer (kg) and capital Input (Naira).

### **Results and Discussion**

The summary statistics of the variables for the data envelopment analysis (DEA) for rice production in the study area are presented in Table 1. They include the sample mean and the standard deviation for each of the variables. The results from Table 1 shows that the average output of rice for men farmers is 452.50 kg obtained from about 3ha while their female counterparts recorded an average output of 167.89kg from about 2ha. This is an indication that the study

covered small scale family managed farm units in the study area. This finding agrees with the findings of Oladeebo and Fajuyigbe, (2007) and Ojo *et al.*, (2013), who reported that food crop production is mostly carried out by small scale farmers in Nigeria.

The total technical, pure technical and scale efficiency scores of tuber crop production in the study area are presented in Table 2. Decomposition of technical efficiency shows that, on average, the male rice farmers are more scale efficient than their female counterparts. The mean scale efficiency of both male and female rice farms are 0.71 and 0.63 respectively. The result further revealed that the mean total technical efficiency of the male and female rice farms are 0.57 and 0.45 respectively, implying that the male and female farmers would have to reduce the level of inputs by 43% and 55% respectively if they were operating at the frontier. All these findings indicate that male rice farmers are more resource-use productive than their female counterpart. This results agree with the findings of Ojo *et al.*, (2010) and Ogunniyi *et al.*, (2012), who reported that male farmers are more resource-use efficient than their female counterpart.

Table 3 presents mean efficiency estimates for the male and female rice farms in the study area. The results show the overall technical inefficiency ranges from 43% on male rice farms to 55% on female rice farms, suggesting that male rice farms are more technically efficient than female rice farms. The decomposition of technical efficiency into pure technical efficiency and scale efficiency further reveals that male rice farms are pure technically more efficient (0.79) than female rice farms (0.68). The high level of technical efficiency observed on male rice farms was mainly due to scale efficiency.

Table 1: Summary statistics of the variables in data envelopment analysis for rice production in the study area

Male farmers				
Variables	Mean	Standard Deviation	Minimum	Maximum
Rice output(kg)	452.50	232.82	60.00	900.00
Farm size(ha)	2.82	1.69	1.00	9.00
Labour(manday)	145.49	145.68	62.50	1375.00
Fertilizer(kg)	283.78	109.79	50.00	500.00
Herbicide(litres)	7.75	3.46	2.00	16.00
Seed(kg)	99.36	40.34	30.00	200.00
Depreciation(Naira)	2118.43	1924.97	200.00	10800.00
Female farmers				
Rice output(kg)	167.89	73.42	60.00	400.00
Farm size(ha)	2.10	1.41	0.53	5.00
Labour(manday)	211.23	288.33	12.50	1250.00
Fertilizer(kg)	261.02	117.55	25.00	500.00
Herbicide(litres)	5.95	2.97	1.00	16.00
Seed(kg)	83.00	32.73	10.00	200.00
Depreciation(Naira)	3205.03	2709.98	150.00	13500.00

Source: Field survey, 2014

Table 2: Summary statistics of efficiency estimates in rice production by sex in the study area.

Statistics	Male (Efficiency Measures)			Female(Efficiency Measures)		
	crste	vrste	scale	crste	Vrste	Scale
Mean	0.57	0.79	0.71	0.45	0.68	0.63
Standard Deviation	0.28	0.18	0.27	0.27	0.20	0.22
Minimum	0.06	0.40	0.11	0.13	0.33	0.21
Maximum	1.00	1.00	1.00	1.00	1.00	1.00

Source: Field survey, 2014

The comparison test for significant differences in mean technical efficiency among the two farm categories, summarised in Table 4, confirms that mean total and pure technical efficiency with scale efficiency are statistically and significantly higher on male rice farms than on female rice farms.

Table 3. Estimated mean efficiency measures and proportion of efficient farms

Efficiency Measures	Male farmers		Female farmers	
	Mean	%	Mean	%
CRS Technical Efficiency	0.57	0.10	0.45	0.13
VRS Technical Efficiency	0.79	0.29	0.68	0.16
Scale Efficiency	0.71	0.10	0.63	0.13

Source: Field survey, 2014

Table 4. Comparison tests for the differences in mean efficiency estimates between male and female farmers

Efficiency Measures	Male versus Female	
	Mean difference	Sig
CRS Technical Efficiency	0.123	0.000***
VRS Technical Efficiency	0.112	0.000***
Scale Efficiency	0.081	0.050**

Note: \*\*\* and \*\* denote significance at 0.01 and 0.05 probability level respectively

Source: Field survey, 2014

Table 5 further reveals that the highest share (16.28%) of scale efficient farms lies in the group of male rice farms. The results also showed that about 77 % and 83% of male and female rice farms operated at increasing returns to scale level respectively. This implies that the two farm groups could achieve higher efficiency level by increasing the production scale.

Table 5: Share of farms under CRS (scale efficient), IRS (increasing returns to scale) and DRS (decreasing returns to scale) by gender in rice production in the study area

Gender	Scale efficient farms	%	Farms under IRS	%	Farms under DRS	%
Male	14	16.28	66	76.74	6	6.98
Female	8	12.50	53	82.81	3	4.69

Source: Field survey, 2014

Table 6 shows slack inputs for rice farms in the study area. A slack variable represents the amount of excess expenditure on an input, i.e., the amount by which the expenditure on a particular input could be reduced without altering the production level. It is evident that 18 male rice farms and 8 female rice farms could reduce total expenditures on the farm land by 9.18% and 11.75% respectively, without reducing their current level of production. Similarly, excess expenditures on labour (8.74% and 1.57%), fertilizer (20.50% and 9.11%), herbicide (20.50% and 11.60%), seed (14.33% and 16.60%), and capital inputs (29.20% and 34.92%) are estimated for male and female farms, respectively.

Table 6. Input slacks and number of rice farms using excess inputs in the study area

Male farmers				
Inputs	Number of farms	Mean slack	Mean input used	Excess input use (%)
Farm size(ha)	18	0.259	2.82	9.18
Labour(manday)	29	12.722	145.49	8.74
Fertilizer(kg)	65	58.189	283.78	20.50
Herbicide(litres)	61	1.589	7.75	20.50
Seed(kg)	36	14.234	99.36	14.33
Depreciation(Naira)	55	618.656	2118.43	29.20

Female farmers

Farm size(ha)	8	0.24	2.10	11.75
Labour(manday)	5	3.31	211.23	1.57
Fertilizer(kg)	24	23.79	261.02	9.11
Herbicide(litres)	31	0.69	5.95	11.60
Seed(kg)	41	13.78	83	16.60
Depreciation(Naira)	51	1119.06	3205.03	34.92

Source: Field survey, 2014

## Conclusion and Recommendation

The study examines the productivity differentials between male and female rice farmers in Niger State, Nigeria. The findings in this study showed that the overall technical inefficiency ranges from 43% on male rice farms to 55% on female rice farms, suggesting that male rice farms are more technically efficient than female rice farms. The findings further revealed that most of the rice farms (both male and female farms) operated at increasing returns to scale level, implying that the two farm groups could achieve higher efficiency level by increasing the production scale. The implication of these findings is that male rice farmers are fairly efficient in utilizing their resources than their female counterparts and any expansion in the use of resources would bring more than proportionate increase in their outputs. It is therefore recommended that research efforts directed towards the generation of new technology, especially for rice farmers, should be encouraged in the study area.

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# NATURAL RUBBER PRICES FORECASTING USING SIMULTANEOUS SUPPLY-DEMAND AND PRICE SYSTEM EQUATION AND VECM MODEL: BETWEEN THEORY AND REALITY

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## Abstract

Malaysia is the third largest producer of natural rubber (NR) in the world. NR price fluctuations become the world debt crisis and global economic slowdown with rubber price-related factors. Meanwhile, most of buyers in China were unwilling to commit themselves due to falling prices and uncertainties of market. In these situations, NR price forecasts are necessary to help in decision-making. The objectives of the study were conducted to investigate the inter-relationships between production, consumption and prices in the Malaysian NR market, to explore a simultaneous supply-demand and price system equation model compared with Vector Error Correction Method (VECM) model between theory and reality of the current market situation, to forecast a short term (ex-post forecast) and long term NR future price (ex-ante forecast) and to make recommendation this study is more efficient and wider applicability in the future. The price forecasting models will be utilized using quarterly data from 1990 Q1 to 2013 Q with providing a total of 96 observations and will be carried out for the period of 2013 Q1 to 2013 Q4 on the short-term and until to 2020 Q1 to 2020 Q4 on the long-term investment decisions. As such, an accurate estimation method of NR price forecasting is vital, to help in the decision-making process of economic planning for the NR sustainable production and the world market economy as well. .

Keywords: Natural Rubber, Forecasting, Supply-Demand and Price, VECM, Malaysia

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## Introduction

Rubber (*Hevea brasiliensis*) can only be grown in areas of Amazon rain forests, which effectively restricts production to regions 15 to 20 degrees latitude north or south of the equator. It takes 5 to 8 years for a rubber tree to mature to the girth at which it can be tapped and its economic life will then be 20 to 30 years. At the end of its life rubberwood provides a valuable end product as a medium density tropical hardwood. Actually, rubber is a polymer with the property of elasticity which, is known as a ‘thermoset elastomer’. There are two main types of rubber,

natural and synthetic. The natural rubber (NR) is made from the latex derived ‘naturally’ from the rubber tree, while the synthetic rubber (SR) is synthesized from chemicals sourced from petroleum refining (IRSG, 2014). Almost 60% of global consumption is by the world’s tyre manufacturing industry, with the remainder going into the ‘general rubber different goods and products’ are manufactured by this sector, serving many industries, including transport, construction, health, mining etc.

Malaysia is the third largest producer of NR in the world and the NR price depends on the world debt

crisis, global economic slowdown and rubber-related factors. In the last decade, the world NR industry has undergone very rapid and fundamental changes with the appearance of many new players, in particular the growth of some traditional suppliers and the emergence of new ones. Much of the changes and consequent challenges, both internally and externally, have impacted on Malaysia's comparative and competitive advantage in NR production. Moreover, the Malaysian rubber industry has produced positive

net trade flows and consistent earnings for the government. Malaysian rubber planted area from the estate sector accounted for only 7 percent (77 thousand hectares) while planted area from the smallholder sector accounted for 93 percent (980 thousand hectares) of total rubber planted area (1057 thousand hectares) in 2013 and other NR producing countries total planted area in Figure 1 (MRB, 2014).

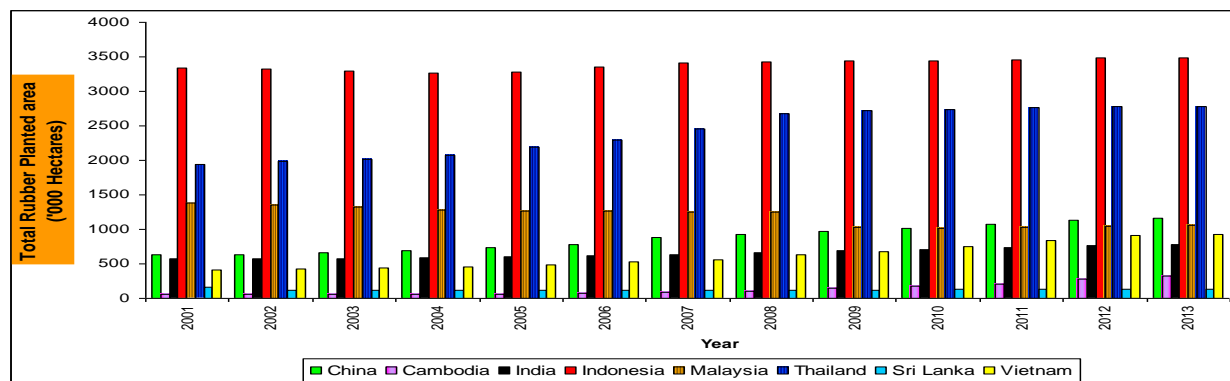


Figure 1. NR producing countries total planted area from 2001-2013 (MRB, 2014).

Domestic total NR production (dry and latex) recorded a decrease from 2010 (939 thousand tonnes) to 2013 (826 thousand tonnes; i.e. 6.8 percent of world NR production). Domestic total NR consumption (dry and latex) decreased from 2010 (458 thousand tonnes) by 79 percent to 2013 (434 thousand tonnes; i.e. 3.8 percent of world NR consumption). Global rubber production (both NR and Synthetic Rubber (SR)) was 28.5 million tonnes and global rubber consumption (both NR and Synthetic Rubber (SR)) was 28.6 million tonnes in 2014 (Figure 2). The latest available

data from IRSG (2014) indicated that with a relatively strong recovery in the world economy, world rubber consumption (both NR and Synthetic Rubber SR) is forecasted to reach 30.4 million tonnes by 2019, with world NR production of 34.0 million tonnes. NR Standard Malaysia Rubber Grade 20 (SMR20) price in Kuala Lumpur market was declined considerable to USD 2300 per ton in 2013 December and also remained low again at USD 1400 per ton in 2014 December. However, the price was high at USD 1600 per ton in 2015 June (MRB, 2015).

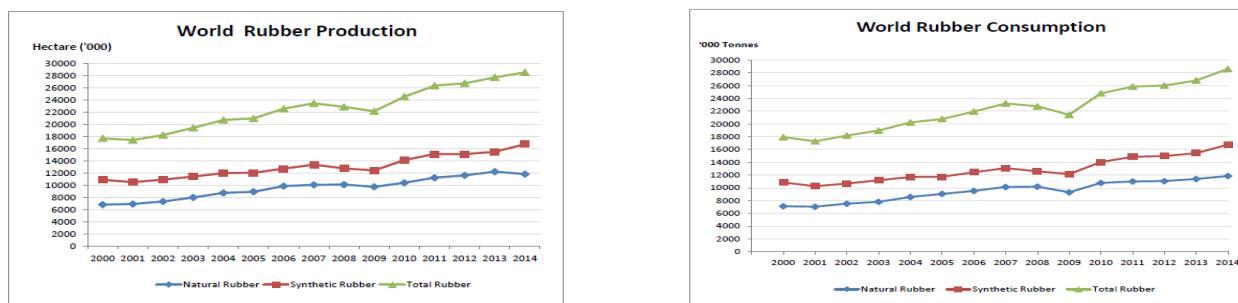


Figure 2. World rubber production and consumption from 2000-2014 (IRSG, 2014).

Moreover, Thailand, Indonesia, Vietnam, Malaysia, Myanmar and Cambodia accounted for 76 % of the world's production of NR in 2013. Malaysia is also the world's third NR producer of latex gloves, catheters and latex thread after Thailand and Indonesia in 2012 and now the seventh largest consumer of NR in the world after China, India, the USA and Japan in 2014. Since then South East Asia namely Thailand, Indonesia and Malaysia have become the world's largest producer of NR and Thailand has become the world's largest producer at 4.2 million tonnes (35 percent of World's NR production), followed by Indonesia at 3.1 million tonnes (26 percent) and Malaysia at 0.8 million tonnes (7 percent) in 2013

(MRB, 2014).

In 2014, the Malaysia exported about 45.9 percent, 13.9 percent and 5.6 percent to China, Germany and Iran of total NR exports volume 721 thousand tonnes, respectively. In comparison, the Malaysia imported about 48.7 percent, 25.4 percent and 6.5 percent from Thailand, Vietnam and Philippines of total NR imports volume 905 thousand tonnes, respectively. Rubber industry's contribution to national exports earnings was 30.94 billion in Figure 3. However, trade balance of export and import was increased to 10.3 RM Million in 2014 from 9.9 RM Million in 2013 (MRB, 2014).

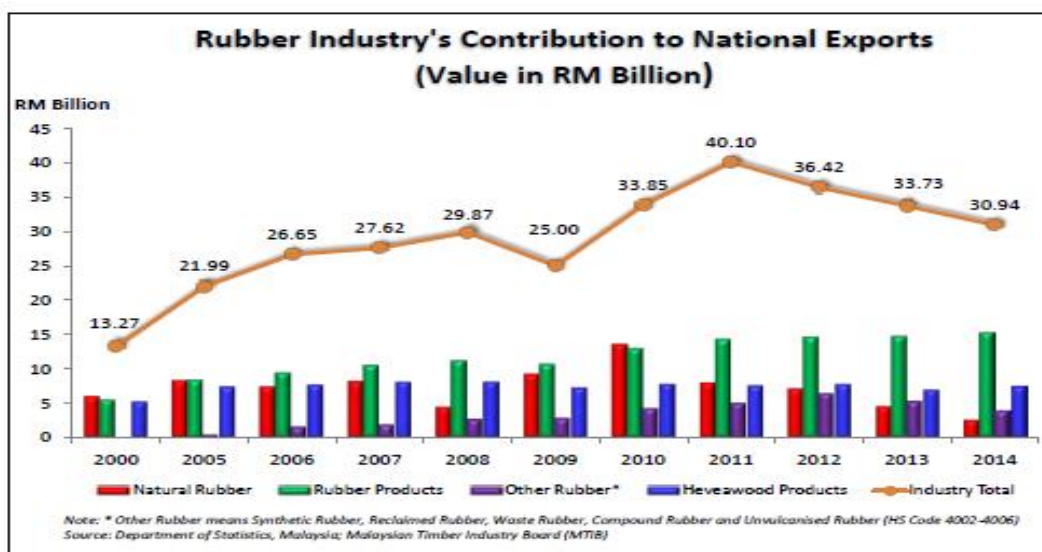


Figure 3. Rubber industry's contribution to national exports earnings in Malaysia (MRB, 2014).

In fact, if the producers' and buyers' expect price to continue to increase or decrease, they might be attracted to produce or buy when NR price is high or low. The response of producers and consumers in the NR market depends on their expectation of future movements in the prices. If their anticipation are incorrect and future prices fluctuate, then such behavior can lead to substantial losses. In these situations of considerable uncertainty and high risk, NR price forecasts are necessary to help in decision-making (Burger and Smit, 2000). Generally, NR prices were strong fundamentally influenced by external factors of ongoing Euro Zone debt crisis, global economic slowdown and rubber-related factors, including crude petroleum oil prices, exchange rates, time-lag, stock, demand and supply situation and slowing growth in agricultural productivity, as well as government policies (MRB, 2014). Accordingly, on December 2011, prices started to drift down due to persistent rains and flood in Thailand and uncertainties over the effectiveness of the steps taken by European leaders to settle the region's debt crisis, coupled with global economic slowdown. Meanwhile, most of buyers in China were unwilling to commit themselves due to falling prices and uncertainties of market. Despite the steady crude oil prices, market players (producers and buyers) remained cautious about prospects for economic growth in both Europe and China.

Moreover, ASEAN Rubber Business Council (ARBC) reported that rubber producers were rejected requests to renegotiate contracts with buyers and a number of buyers had cancelled contracts due to remain low again prices of NR in 2011. Therefore, International Rubber Consortium (IRC) would help to producers' trade with more transparent and reliable prices to take "specific measures", to forecast and support future prices after a meeting of representatives from the three governments (Thailand, Indonesia and Malaysia) (ARBC, 2011). Malaysian Rubber Board (MRB) expects that some RM 275 million will be invested under the rubber National Key Economic Area (NKEA), mainly involving replanting and new planting of rubber trees nationwide, i.e. active replanting activities would cover about 38,000

hectares per year focusing mainly on Peninsular Malaysia while new planting would cover about 5,000 hectares each in Sarawak and Sabah in 2012. Under the rubber NKEA, the rubber industry is targeted to contribute about RM 90 billion of gross national income by 2020. Furthermore, rubber's performance in 2012 is expected to stay robust with growth coming from the Asia-Pacific, mainly from China and India. These two economies with their huge domestic markets, low cost and abundant domestic labour, offer vast market opportunities for NR (MRB, 2011).

Krichene (2005) has argued that a relationship exist between crude oil prices, changes in the nominal effective exchange rate of the US\$, and the U.S. interest rates. The study used the simultaneous equations model for world crude oil and natural gas markets and found that both interest rates and the nominal effective exchange rate were shown to influence crude prices inversely. The result explained that demand and supply for both crude oil and natural gas were highly price inelastic in the short run, leading to excessive volatility in crude oil and natural gas market. From the study, a simultaneous equations model estimation methodology could provide realistic and relevant information for this paper.

Abdul Rahim et al. (2010) also analyzed the short run and long run effects of the world crude oil prices on the Malaysian NR price and palm oil export price. The results reveal that there was evidence of cointegrating relationship between world crude oil prices and both commodities prices. Romprasert, S (2011) studied the forecasting of NR future price and the market efficiency by using the time series data of the spot price of Thailand. The results indicated that, the daily futures prices served as unbiased estimators of future spot prices and there was independence on daily price changes. This result showed that Thailand's rubber futures market was efficient and aided the process of price. The analytical model was shown to be applicable and would be facilitated and related studies in forecasting the futures prices of other commodities.

Khin et al. (2011) developed a short-term econometric model of world NR price. This study used the Vector Error Correction Method (VECM) with cointegration

characteristics and was utilized using monthly data from 1990 to 2008. The results indicated that the price of NR is highly and positively dependent on total production of NR and highly and negatively dependent on total consumption of NR. The cointegration approach was used directly to test long-term variables and indirectly to know imbalance in short-term by using parameter estimate from long-term relationship variables. Estimations revealed that the explanatory variables, namely total production of NR and the total consumption of NR and synthetic rubber, were the most important explanatory variables in the cointegration equation of price forecasting VECM model with significance at 0.01 level.

Therefore, in these situations, NR price forecasts are necessary to help in decision-making. Besides, the objectives of the study were conducted to investigate the inter-relationships between production, consumption and prices in the Malaysian NR market, to explore a simultaneous supply-demand and price system equation model compared with Vector Error Correction Method (VECM) model between theory and reality of the current market situation, to forecast a short term (ex-post forecast) and long term NR future price (ex-ante forecast) and to make recommendation this study is more efficient and wider applicability in the future. The price forecasting models will be utilized using quarterly data from 1990 Q1 to 2013 Q4 with providing a total of 96 observations and will be carried out for the period of 2013 Q1 to 2013 Q4 on the short-term and until to 2020 Q1 to 2020 Q4 on the long-term investment decisions.

## **Materials and Methods**

### ***NR Supply Forecasting Model***

The research earlier examined and reviewed the supply, demand and price relationship based on models developed by (Tan, 1984), (Barlow et al., 1994), (Arshad and Zainalabdin, 1994), (Goodwin, 1994), (Ferris, 1998), (Burger and Smit, 2000), (Enders, 2004), and (Khin et al., 2011). A quarterly model of the Malaysia NR market is formulated comprising of three behavioral single-equations and

identified first the supply of NR (TPNR) as a function of related factors (in logs) as follow:

$$TPNR_t = a_0 + a_1 NRP20_{t-1} + a_2 TPNR_{t-1} + e_t \quad (1)$$

### ***NR Demand Forecasting Model***

The demand of NR (TCNR) as a function of related factors (in logs) can be specified as follow:

$$TCNR_t = b_0 - b_1 NRP20_t + b_2 TCNR_{t-1} - b_3 RSS1_t + e_t \quad (2)$$

### ***NR Price Forecasting Model***

The NR price (NRP20) equation, which was derived, based on related factors (in logs) and however, the study will only be tested to forecast the NR price forecasting model which can be stated as follows:

$$NRP20_t = c_1 + d_1 TPNR_{t-1} - d_2 TCNR_{t-1} - d_3 STONR_{t-1} - d_4 RSS1_{t-1} + d_5 COPT_{t-1} + d_6 REERT_{t-1} + d_7 NRP20_{t-1} + \varepsilon_6 t \quad (3)$$

### ***Model Specification for NR Price Forecasting Models***

#### **(1) Simultaneous Supply-Demand and Price Equation Model**

The simultaneous equation model is a two-equation model based on the market demand and supply where price and quantity are both endogenous variables (Ferris, 1998), (Pindyck and Rubinfeld, 1998) and (Gujarati, 2003). The model deals with directly to the interaction of supply and demand in establishing prices without separately using the single-equations of supply, demand and price. Price and supply are endogenous also; jointly determined price and demand are endogenous variables. Others are exogenous variables. Therefore, the simultaneous equations model will be substantially compared to the single-equation of VECM price forecasting model are considered in this study. Following is the model (in logs) with price dependent supply and demand illustrating the dynamics of such models.

Assuming the sign on as follow  $a_1$  and  $a_2$  are positive,  $b_1$  and  $b_2$  are negative and  $a_0$  and  $b_0$  are intercepting. Therefore, we can write for the price dependent equation for supply based on supply equation (1) as.

$$NRP20_{t-1} = a_0 + (a_1 + a_2) (TPNR_{t-1}) + e_t \quad (4)$$

Moreover, the model with price dependent equation for demand based on demand equation (2) and then we can write as follows:

$$NRP20_t = b_0 - (b_1 + b_2) (TCNR_{t-1}) - b_3 RSS1_t + e_t \quad (5)$$

If exports and imports are negligible, Supply = Demand. Therefore, supply equation (1) and demand equation (2) will be

$$a_0 + a_1 NRP20_{t-1} + a_2 TPNR_{t-1} + e_t = b_0 - b_1 NRP20_{t-1} - b_2 TCNR_{t-1} - b_3 RSS1_t + e_t$$

Therefore, we can write the price simultaneous equation (in logs) on equation (6) as follows:

$$NRP20_t = (a_0 + b_0) / (a_1 + b_1) + a_2 TPNR_{t-1} - b_2 TCNR_{t-1} - b_3 RSS1_t \quad (6)$$

## (2) Vector Error Correction Model (VECM)

Co-integration is a statistical concept within the regression theory framework that explains the long run equilibrium in economic theories. Engle and Granger (1987) pointed out that the residual has a pattern and if residual are stationary, the two variables are co-integrated and there is a long run relationship between the two variables. It is called the error correction model (ECM). If residuals are random walk, the two variables are not co-integrated and there is not a long run relationship between the two variables. In the ECM model, the only right-hand side variable is the error correction term and this term is zero. And also co-integrating equation is no lagged difference terms. Besides, ECM models have no vector of intercept terms ( $\alpha_i$ ) and the disturbance terms ( $\epsilon_i$ ). The co-integration equations for Malaysian NR price long-term forecast based on equation (3) is:

$$CointEq: NRP20_t + TPNR_t - TCNR_t - STONR_t - RSS1_t + COP_t + REER_t = 0 \quad (7)$$

Vector error correction method (VECM) is developed in two stages. First, a general autoregressive distribute lag equation is specified, which explains an endogenous variable by its current and own lagged exogenous variables. Second, this equation is manipulated to reformulate it in terms that are more easily interpreted, producing a term representing the extent to whether the long-term equilibrium is met. The last term, is called an error-correction term since it reflects the current "error" in achieving long-run equilibrium. According to Engle and Granger (1991) a linear combination of two or more non stationary series might be stationary. Therefore, the Malaysian NR price forecasting model for short-term forecast the VECM model based on equation (3) is:

$$\Delta NRP20_t = c_1 + d_1 TPNR_{t-1} - d_2 TCNR_{t-1} - d_3 STONR_{t-1} - d_4 RSS1_{t-1} + d_5 COP_{t-1} + d_6 REER_{t-1} + d_7 NRP20_{t-1} + \epsilon_6 t \quad (8)$$

where

$NRP20$  = Standard Malaysia Rubber Grade20 NR Export FOB price (USD/ton)

$RSS1$  =  $RSS1$  NR Export FOB price (USD/ton)

$TPNR$  = Malaysian Total Production of NR (Total Supply) ('000 tonnes)

$TCNR$  = Malaysian Total Consumption of Rubber (Total Demand) ('000 tonnes)

$STONR$  = World NR stocks ('000 tonnes)

$COP$  = Crude petroleum oil yearly price (USD/barrel)

$REER$  = Real effective exchange rate in foreign currency per RM (USD/RM)

$T$  = Time trend 1990 to 2013 quarterly data

$t$  and  $eti$  = Time period and error terms respectively

### Model Simulation and Model Evaluation

Significantly, the models are needed to do for the error checking with the classical assumptions for ordinary least squares (OLS) estimators. The classical assumptions must be met in order for OLS estimators to be the best available (Studenmund, 2011). For instance, heteroskedasticity takes account of correcting the standard errors and it has a constant variance (White, 1980). Therefore, H<sub>0</sub>: residual are not heteroskedasticity (the error term has a constant variance) and H<sub>A</sub>: residual are heteroskedasticity (the error term has not a constant variance). If in the White test, sig p-value >  $\alpha$  0.05, then fail to reject H<sub>0</sub>. There is no heteroskedasticity. Therefore, the forecasting model is satisfactory and no need to revise.

The model simulation time horizon is based on (Pindyck and Rubinfeld, 1998) and as short term and long term price forecast. If the study will be based on the quarterly data from 1990 to 2013, the ex-post forecast (short term price forecast) would be from 2013 Q1 to 2014 Q4 and followed by ex-ante forecast (long term price forecast) would be from 2010 Q1 to 2020 Q4. Accuracy is generally being accepted as the most important factor in evaluating a forecasting technique, but there is no consensus as to how accuracy should be measured. Indeed, one of the difficulties in dealing with the criterion of accuracy in forecasting situations is that there is no one single universally accepted measure of accuracy (Makridakis et al., 1998) and (Pindyck and Rubinfeld, 1998).

The simultaneous supply-demand and price system equation model and VECM model of the NR price would be in terms of their modelling accuracy based on Root Mean Square Error (RMSE), Mean Absolute Error (MAE), and Mean Absolute Percent Error (MAPE) and (U-Theil) criteria. The values of RMSE and MAE are all small, the values of the Theil's inequality coefficient (U-Theil) are all nearly zero which is that the forecasting performance and accuracy of the forecasting model is satisfactory and the model is no need to revise.

### Results & Discussions

This research is conducted in stages. In each stage, a set of analysis is applied, and the findings in the respective stage determine the next stage. Upon collection of data, which is extracted during 1990 Q1 to 2013 Q4 quarterly, stationary analyses are to be tested. Hence, Augmented Dickey Fuller Test (ADF) and Phillip-Perron Test (PP) are to be conducted on data to test for existence of unit root in Table 1. If data are non-stationary, they are to be treated to become stationary. This is done by means of differencing from data. After that (Khin et al., 2011) suggested that cointegration test should be done before conducting VECM Model.

Table 1 The Results of Unit Root test of NRP20, TPNR, TCNR, STONR, RSS1, COP and REER

Variables	Augmented Dickey Fuller Test			Phillip-Perron Test		
	Level	1 <sup>st</sup> Difference	2 <sup>nd</sup> Difference	Level	1 <sup>st</sup> Difference	2 <sup>nd</sup> Difference
NRP20	-1.372	-7.242***	-7.824***	-1.401	-7.903***	-54.024***
TPNR	0.301	-16.000***	-9.069***	-2.788*	-25.093***	-43.306***
TCNR	-0.428	-5.300***	-19.088***	-0.631	-38.751***	-91.197***
STONR	-0.987	-3.874***	-7.150***	-0.859	-9.769***	-28.676***
RSS1	-1.282	-8.065***	-8.049***	-1.025	-7.999***	-56.249***

COP	-0.386	-9.258***	-7.313***	-0.690	-11.126***	-44.694***
REER	-1.672	-7.278***	-6.741***	-1.523	-7.281***	-40.371***

Note: \*, \*\*, \*\*\*: statistically significant at respectively 0.10, 0.05, and 0.01 acceptance levels

Results of unit root tests presented in Table 1 which indicates NR price and variables with TPNR, TCNR, STONR, RSS1, COP and REER are stationary only after the 1st difference and 2nd difference. Results of ADF and PP tests confirm each other.

*Simultaneous Supply-Demand and Price Equation Model:* In order to test the model, long-term relationship between NR price with other variables TPNR, TCNR, and RSS1 are identified and showed by price simultaneous equation (9).

$$\text{NRP20t} = 1.031 + 0.118 \text{TPNRt-1} - 0.174 \text{TCNRt-1} - 0.514 \text{RSS1t} + 0.352 \text{et} \quad (9)$$

$$t \text{ statistic} = \begin{matrix} [2.175**] & [-2.927**] & [-45.028***] \end{matrix}$$

$$R^2 = 0.514 \quad \text{Adjusted } R^2 = 0.501$$

Heteroskedasticity Test: White

$$F\text{-statistic} \quad 1.0075 \quad \text{Prob. } F(3,92) \quad 0.3931$$

In Equation (9), it based on the results of simultaneous supply-demand and price equation (6) about NR price model. The result reveals that the relationship between NR price and production, consumption and RSS1 price. In other words, it shows the forecasting power of NR price with other variables' lag selection is on one period (lag) ahead in time. Results are significant at 0.05 and 0.01 acceptance levels with R-Square value of 0.514, indicates that up to 51.4 percent of variation in short term changes of NR price of Malaysia are explained by variation in the lagged variables as well as TPNR, TCNR, and RSS1. Therefore, this is a concrete model in predicting and

explaining long term movement of NR price of Malaysia. Moreover, existence of such relationship is statistically supported. T-statistic of 2.175 indicates that the NR price is positively affecting TPNR production significantly at 0.05 acceptance level. Moreover, t-statistics of 2.927 and 45.028 suggest that NR price is negatively affecting the TCNR, and RSS1 are statistically significant at 0.05 and 0.01 acceptance level. Moreover, in the White test, sig p-value  $0.3931 > \alpha 0.01$ , then fail to reject  $H_0$ . There is no heteroskedasticity of residuals. Therefore, the forecasting model is satisfactory and no need to revise.

*Vector Error Correction Model (VECM):* In order to test the model, long-term relationship between NR price with other variables TPNR, TCNR, STONR, RSS1, COP, and REER are identified by means of co-integration tests based on the equation (7). Results of Johansson co-integration test on the model (co-integration rank) is presented in Table 2. It provides Johansson co-integration results obtained from both methods of Trace and Maximum Eigenvalues. Results of trace method suggest existence of two co-integration equations; similarly, maximum Eigenvalue suggests there are also two co-integration equations. In other words, both methods confirm each others that there are two long-run equilibrium equations between NR price and other variables TPNR, TCNR, STONR, RSS1, COP, and REER exists within a multivariate framework.



Table 2 Results of Johansson Co-integration Test on NR Price Forecasting VECM Model

Hypothesized No. of CE (s)	Eigenvalue	Trace Statistic	0.05 Critical Value	Prof. **
None *	0.664543	204.9670	125.6154	0.0000
At most 1 *	0.399541	102.2945	95.75366	0.0164
At most 2	0.251596	54.34871	69.81889	0.4466
At most 3	0.129996	27.10638	47.85613	0.8502
At most 4	0.099696	14.01615	29.79707	0.8397
At most 5	0.042821	4.144047	15.49471	0.8916
At most 6	0.000321	0.030177	3.841466	0.8620
Hypothesized No. of CE (s)	Eigenvalue	Max-Eigen Statistic	0.05 Critical Value	Prof. **
None *	0.664543	102.6725	46.23142	0.0000
At most 1 *	0.399541	47.94579	40.07757	0.0053
At most 2	0.251596	27.24233	33.87687	0.2506
At most 3	0.129996	13.09024	27.58434	0.8799
At most 4	0.099696	9.872102	21.13162	0.7566
At most 5	0.042821	4.113870	14.26460	0.8471
At most 6	0.000321	0.030177	3.841466	0.8620
Trace and Max-Eigen statistics indicate 2 cointegrating equations at the 0.05 level.				
* denotes rejection of the hypothesis at the 0.05 level.				
** Mackinnon-Haung-Michelis (1999) p-values				

As illustrated by Equation (10), the long run relation based on co-integration equation (7), which is the horizontal equation in first row in VECM model, suggests that long-term relationship between NR price and TPNR, TCNR, RSS1, and REER are statistically significant. This is due to respective t-statistics of 7.347, 3.247, 2.016 and 2.242, which suggest a significant relationship between TPNR, TCNR, RSS1 and REER at 0.01, 0.05 and 0.10 acceptance level. On the other hand, t-statistic of 1.644 and 1.879 fails to support any form of relation between STONR and COP and NR price in Malaysia. The sign of coefficients of production, consumption and stock variables are right signs with NR price. Hence, one may infer a direct long-term relationship between NR price with other variables TPNR, TCNR, RSS1 and REER.

#### Co-integration Equation

$$-0.084 \Delta \text{NRPt-1} + 0.161 \Delta \text{TPNRt-1} - 0.037 \Delta \text{TCNRt-1} - 0.034 \Delta \text{STONRt-1} + 0.066 \Delta \text{RSS1t-1}$$

$$t \text{ statistic} = [-2.326^{**}] \quad [7.347^{***}] \quad [-3.247^{**}] \\ [-1.644] \quad [-2.016^{*}]$$

$$+ 0.082 \Delta \text{COPt-1} + 0.019 \Delta \text{REERt-1} = 0 \quad (10)$$

$$t \text{ statistic} = [1.879] \quad [2.242^{**}]$$

#### VECM Equations of PSMR20 Model

$$\Delta \text{NRPt} = 0.015 + 0.325 \Delta \text{TPNRt-1} - 0.419 \Delta \text{TCNRt-1} - 0.563 \Delta \text{STONRt-1} - 0.149 \Delta \text{RSS1t-1} +$$

$$t \text{ statistic} = [3.832^{**}] \quad [-3.053^{**}] \quad [-2.991^{**}] \\ [-0.628]$$

$$0.123 \Delta \text{COP}_{t-1} + 1.189 \Delta \text{REER}_{t-1} + 0.156 \Delta \text{NRP}_{20t-1} + 0.003 \text{ et} \quad (11)$$

$$t \text{ statistic} = [1.215] \quad [2.656^{**}] \quad [0.751]$$

$$R^2 = 0.818 \quad \text{Adjusted } R^2 = 0.810$$

Heteroskedasticity Test: White

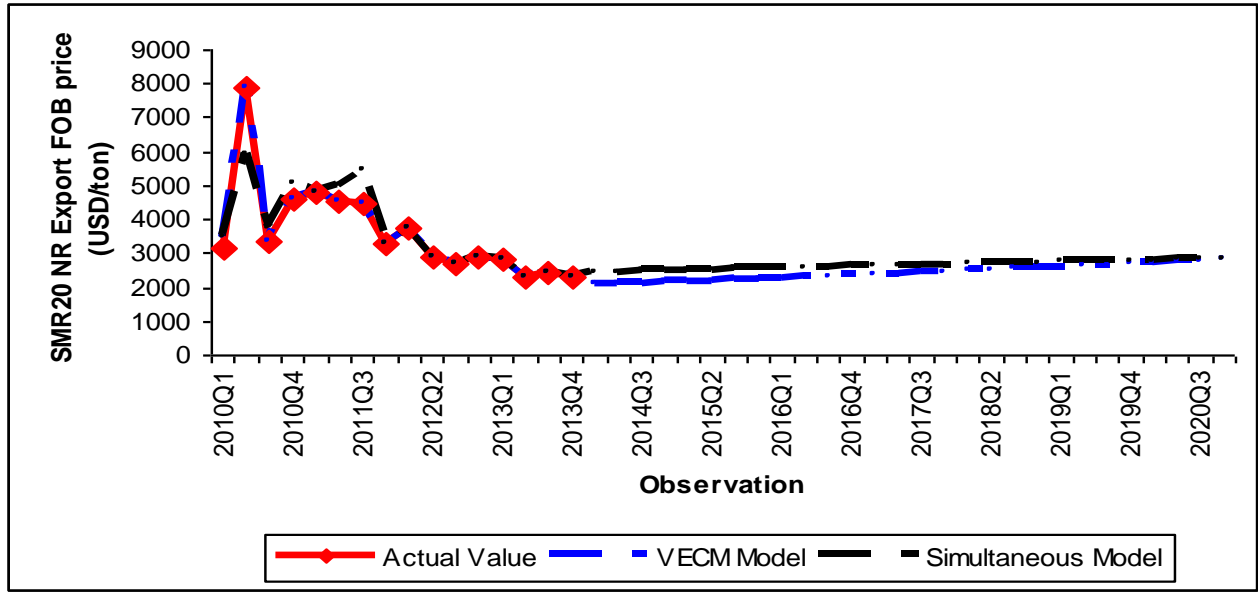
$$F\text{-statistic} \quad 2.722 \quad \text{Prob. } F(6,89) \quad 0.0179$$

In Equation (11), it provides the results of VECM equation about NR price based on equation (8). The result reveals that the short run relationship between NR price with only production, consumption, stock and real exchange rate. In other words, it shows the forecasting power of NR price with other variables' lag selection is on one period (lag) ahead in time. Results are significant at 0.05 acceptance levels with R-Square value of 0.818, indicates that up to 81.8 percent of variation in short term changes of NR price of Malaysia are explained by variation in the lagged variables as well as TPNR, TCNR, STONR, RSS1, COP, and REER. Therefore, this is a concrete model in predicting and explaining short term movement of NR price of Malaysia. Moreover, existence of such relationship is statistically supported. T-statistic of 3.832 indicates that the NR price is positively affecting TPNR production significantly at 0.05 acceptance level. Moreover, t-statistics of 3.053, 2.991 and 2.656 suggest that the TCNR and STONR are negatively and REER is positively statistically significant at 0.05 acceptance level. Moreover, in the White test, sig p-value  $0.0179 > \alpha 0.01$ , then fail to reject  $H_0$ . There is no heteroskedasticity of residuals. Therefore, the forecasting model is satisfactory and no need to revise.

Khin and Thambiah (2014) advocated selecting a forecasting model if it significantly contributed to the forecasting accuracy of a combined forecast using a

simultaneous supply-demand and price system equation model and univariate model of the ARIMA of Malaysia NR price. Both models utilized data from 1990 Q1 to 2013 Q4 as estimation period and data were estimated as a short term price forecast was to 2014 Q1 to 2014 Q4. The result showed that the one lagged of NR price and RSS1 price were the most important explanatory variable with statistically significance at  $\alpha 0.01$  level in the NR price model.

In Equation (9) and (11), the results of both NR price forecasting models show that NR price is significantly both short-term and long-term relationship between TPNR production and TCNR consumption. In Figure 4, it is to select a forecasting model if it significantly contributes to the forecasting accuracy of a combined forecast using a simultaneous supply-demand and price system equation model and VECM model of NR prices. Both models used the quarterly data from 1990 Q1 to 2013 Q4 as estimation period, and data from 2010 Q1 to 2014 Q4 was estimated as an ex-post forecast short term price forecast and followed by ex-ante long term price forecast was to 2010 Q1 to 2020 Q4. The results showed that the comparative forecasting powers criteria' values of VECM model of cointegration equation for short term and long term price forecast were smaller than the values estimated by the simultaneous supply-demand and price system equation model. It meant that the forecasting performance of VECM model of cointegration equation model was satisfactory and thus, a revision of the model was not necessary. These statistics suggested that the forecasting performance of VECM model of cointegration equation model is more efficient than the simultaneous supply-demand and price system equation model.



Forecasting powers criteria' values	VECM Model	Simultaneous Model
RMSE	0.0736	0.0767
MAE	0.0579	0.0604
MAPE	0.8165	0.8502
U-Theil	0.0051	0.0053
Bias Proportion	0.0000	0.0000
Variance Proportion	0.0041	0.0045
Covariance Proportion	0.9959	0.9955

Figure 4. Short-term (Ex-post) forecast and Long-term (Ex-ante) forecast of NR price forecasting models from 2010 Q1 to 2020 Q4

The price trend of the Malaysian NR shows that NR price is predicted to increasing trend from 2016 Q1 until to 2020 Q4 long term price forecast and however, at the end of 2014 Q4, the prices of NR were down for short term price forecast in Fig 3. Therefore, it would be most effective for the long-term investment decisions which results in the greatest increase in demand. For short term, it may be weather, seasonal factors, currency movements, futures markets activities, market interventions and irregular demand ensured a brief interruption to the downward trend. If some of the major automobile manufacturers could be planned to boost their production in coming year as a result of low inventories, which would also aid price level stability (MRB, 2015).

## Conclusion

World Bank (2015) also supported for this study and rubber prices are volatile and are influenced by many factors also. Over the past decade NR prices have increased from US\$1 per kg to US\$4.5 per kg. The elevated price volatility following the 2005 to 2008 commodity booms caused concern to both international organizations and policymakers. The volatility of NR prices, as a general rule, tends to be more volatile than other mainstream commodities. Following the news of an Ebola outbreak in West Africa at the beginning of the 2015, share prices of rubber gloves have risen by 5 to 10%. Any significant development in the situation or a full-scale Pandemic

would further boost worldwide healthcare awareness and could potentially spark a surge in demand for NR examination gloves, given that they remain as the most affordable and fundamental form of protection against diseases. The World Bank commodities price forecast in (nominal) US\$ indicates that Malaysian rubber price will reach US\$2.40 per kg in 2015 and US\$2.44 per kg in 2016. As such, an accurate estimation method of NR price forecasting is vital, to help in the decision-making process of economic planning for the NR sustainable production and the world market economy as well.

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