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Proceedings of Ninth National Symposium "Noni for Everyone"

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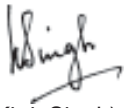
FOREWORD

The World Noni Research Foundation (WNRF) is the only global organisation committed to undertake research and development on Noni (*Morinda citrifolia* L) with the ultimate objective of providing human wellness, nutrition, health, prosperity and empowerment. WNRF besides taking up in-house research encourages research organizations to take up need-based research programmes on Noni by providing appropriate funding and also collaborate and coordinate with the research activities carried out elsewhere on all aspects of Noni. So far WNRF has outsourced 29 research projects on Noni-crop Improvement and Management, Crop Protection, Clinical Science, Pharmacology and Food Science research. Some of the completed projects have given very informative results. To disseminate the research results, International Society for Noni Science (ISNS) and World Noni Research Foundation (WNRF) besides publishing journals like International Journal of Noni Research , organize theme based symposium every year since 2006 as "Noni Search" and published the proceedings. The Ninth National Symposium, 'Noni for Everyone' is scheduled on 27 and 28 September 2014 at International Centre Goa, Goa. The Noni Search 2014 is organized by World Noni Research Foundation (WNRF), International Society for Noni Science (ISNS) and Noni BioTech, Chennai in collaboration with ICAR Research Complex for Goa, Ela, Goa and Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. This symposium is the fourth of its kind after the formation of the Society. Renowned scientists from various research and development institutes, research organizations and universities around the country are participating and presenting papers in seven Technical Sessions dealing with (i) Noni Biodiversity and Improvement, (ii) Good Noni Production Practices (iii) Good Noni Protection Practices (iv) Product Development, Value addition and By-product (v) Clinical and Pharmacological Studies, (vi) Trade and Marketing of Noni, vii) Success Stories of Divine Noni Gold.

There will be an exhibition with all Noni Products and Publications. The Pre-Symposium Proceedings of the Ninth Noni Search-2014 is being brought out for the benefit of the participants and others who are interested in Noni.

I take this opportunity to thank Dr .P. Rethinam, Member, Research Advisory Board and the Editor as well and Dr .T. Marimuthu, Additional Director and Dr. K. V. Peter, Director, WNRF for their help in bringing out this useful publication. I also thank Prof. P.I. Peter, Chairman, Noni BioTech for his guidance and whole hearted support in organizing the symposium.

My appreciation to Mr. T. Thanigai Kumar, Mr. K. R. Krishnan, Mr. K. Ramanathan, Mr. A. Arunachalam and Ms. A. Ammu who assisted in bringing out this publication on time.



Chennai - 600 096
September, 2014

(Kirti Singh)
Chairperson

PREFACE

I have great pleasure in compiling, editing and bringing out this Pre- symposium Proceedings of Ninth Noni Search -2014 which, I am sure will enable participants and delegates attending the symposium to read the research papers well in advance and participate more effectively in the discussions. This symposium has seven technical sessions. I am very much thankful to all the presenters who had spared their valuable time in preparing the full papers along with abstracts and sending in time. This proceeding is yet another mile stone to Noni information wealth brought out by WNRF and ISNS from time to time.

I am very much grateful to Dr. Kirti Singh, Chairperson, WNRF for involving me in editing the Proceedings. My sincere thanks are due to Prof. P. I. Peter, Chairman, Noni BioTech for his continued support, encouragement and appreciation. Special thanks are due to Dr. K. V. Peter, Director, WNRF and Dr. Brahma Singh, Member, Research Advisory Board for their help extended at all the stages. I specially thank Dr. T. Marimuthu, Additional Director, WNRF for helping me in editing the proceedings as Joint Editor. I acknowledge the help rendered by Mr. T. Thanigai Kumar, Mr. K. R. Krishnan, Mr. K. Ramanathan, Mr. A. Arunachalam, Ms. A. Ammu for their technical help extended in bringing out this publication in time.

(P. Rethinam)
Editor

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September, 2014

I-OP-1

Leaf and fruit production in Noni (*Morinda citrifolia* L.) germplasm of Konkan coast of Goa and Maharashtra

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Abstract

Morinda citrifolia L. commonly known as Noni is a widely used source of traditional medicine. Present study investigates the leaf and fruit production potential of the Noni populations from Konkan Coast of India. A total of 131 Noni plants of 20 accessions collected from 16 locations of Goa and Coastal Maharashtra were analyzed for traits such as, number of leaves, number of fruits, leaf yield and the fruit yield produced by the plant. One year old plants were studied for the leaf and fruit production traits over a time span of one year. The plants were grouped into three categories, North Goa, South Goa and Coastal Maharashtra according to the place of collection for data comparison and the range, mean, standard deviation and Coefficient of Variation was calculated for each individual trait. Nagaon population (IC0598515) recorded number of leaves (413 leaves/plant/year) and also had highest annual leaf yield (2.8 Kg/plant). MPT 3 population (IC0598516) produced lowest (122) leaves/ plant/year) and had lowest leaf yield (1.05 Kg/plant/year). Loutolim population (IC0598229) recorded highest fruit yield of 2.77 Kg/plant/year. The highest number of 67 fruits/plant/year was seen in Nagaon population (IC0598515). MPT3 population recorded lowest fruit yield (11 fruits or 0.46 Kg/plant/year). The Shannon-Weaver Diversity Index was calculated for comparison of the yield. Diversity was high (0.81) for the number of fruits produced by the South Goa plants (mean=38, CV%=55.3). North Goa plants gave a lowest diversity value (0.57) in terms of fruit yield (mean=1090.4g, CV%=67.8). Coefficient of variation ranged from 49.3% for number of fruits to 73.6% for fruit weight.

Keywords : juvenile yield, Rubiaceae, foliar, variability

Introduction

Morinda citrifolia L. known as Indian Mulberry or commercially known as Noni is a widely used medicinal plant and its use as a botanical dietary supplement has been increasing tremendously in the recent years (Alison and Douglas, 2007). This plant grows naturally where it is relatively wet to moderately wet, from sea level to about 1500 feet elevation(Nelson,2003).Traditionally, Noni has been used as herbal cure for human diseases throughout Southeast Asia, the Polynesia and other Pacific Islands, and also in some parts of India, Africa and the Carribean Islands (Waki *et al.,* 2008). It is also prominent among the increasing number of botanicals currently promoted by the herbal and healthy food supplements industry (Anna *et al.,*1999). The Noni is a remarkable tree that deserves recognition for its ability to survive in harsh environments. It is extremely salt resistant and can tolerate drought for several months (Julia, 1992). Noni grows as a small tree or perennial bush bearing fruits throughout the year, one year after planting. Fruits of different stages of maturity can be found on the same plant at the same time(Yanine*et al.,*2006). Due to its adaptability to wide range of climatic conditions, the Noni plant grows even in infertile, acidic and alkaline soils and in the dry and wet areas. In India, Noni is widely grown under natural conditions and as fence and roadside plant in coastal and island locations. The fruit of Noni is a drupe wherein the whole inflorescence connate to form a fleshy or hard syncarp. The fruits are yellowish white and fleshy, 5-14cm long, about 3-7.5cm in diameter soft and fetid when ripe(Jules and Robert,2008). The leaves are glossy, membranous, and elliptic to elliptic-ovate 20-45cm long, 7-25 cm wide (McClatchey, 2003). Both the leaves and fruits are used for medicinal purpose, the fruit juice mainly in high demand in alternative medicine. The leaves of Noni are used as vegetable and forms part of the diets of the Pacific Islands and in Southeast Asia. They are also known to be a significant source of protein and Vitamin A, making the leaves an important food resource in the tropics (West and Bing-nan, 2008). The fruit yield per year depends on the variety or genotypes of the plant and also on the environment. The plant produces fruits throughout the year though the fruit production is lowered in winter. Annual yields may be only a few pounds per year for tall plants growing under forest shade to 80,000kg/hectare or more for large fruited genotypes in monoculture in full sun with heavy fertilization (Nelson, 2001). The present study aims to evaluate the fruit and leaf yielding potential of the Noni accessions of Konkan coast of India collected during our previous study (Ashwita and Arunachalam, 2013).

Materials and Materials

A total of 131 Noni plants from 20 accessions of Goa and Maharashtra already growing in the ICAR RC farm and were assessed for the leaf and fruit biomass production by the plant in a time span of one year. Each plant was studied individually for the number and weight of leaves and fruits produced by the plant.

To obtain the total number of leaves produced by the plant, the number of leaves on each branch of the plant, were counted manually and was multiplied by the total number of branches present on the plant. Ten leaves from each plant were removed and weighed using the Kern weighing balance. The average weight of these leaves was calculated and on multiplying with the total number of leaves, the total leaf weight (kg) produced by the plant was obtained.

To obtain the fruit yield, the number of fruits on each branch was counted manually and was multiplied by the total number of branches present on the plant. Thus the total number of fruits produced by the plant was obtained. Five ripe fruits from each plant were harvested and weighed using Kern weighing balance. The average weight was calculated and when multiplied by the total number of fruits of the plant, the total fruit weight (kg) produced by the plant per year was obtained.

The age of plants was calculated and the values of leaf and fruit yield were adjusted according to the amount produced in a one year time span depending on the age of the plant. The data was fed into the Microsoft Excel spreadsheet and the range, mean, standard deviation and CV% were calculated for each trait.

For data analysis purpose, the plants were distributed in three groups namely North Goa, South Goa and Coastal Maharashtra according to their place of collection (Ashwita and Arunachalam, 2013). Shannon-Weaver Diversity Index was calculated for each trait using the frequency values of class intervals. The accessions were also separated and the Shannon-Weaver Index was calculated for each trait. Proportion (P) of occurrence of each character states was used in each trait (i) and the Shannon-Weaver estimates have been worked out using the formula where n is the number of character states, pi = proportion of individuals in the i^{th} state of character. Each H' estimate is normalized by dividing $\log_2^n$

Results and Discussion

Morinda citrifolia L. (Noni) plants growing in the ICAR RC farm were studied for leaf and fruit yield produced. A total of 131 Noni plants from 20 different accessions of Goa and Maharashtra collected from 16 different locations were studied individually.

Table1 : Extent of variation in Noni accessions of Konkan coast for leaf and fruit production

Character	Maximum	Plant ID	Minimum	Plant ID	Mean	SE	CV%
Total number of leaves/ plant/year	681.00	Bicholim plant 3	34.00	MPT3 plant 84	292.90	11.30	43.90
Total number of fruits/ plant/year	103.00	Nagaon plant 124	8.00	MPT3 plant 46, 84 and 98	41.20	1.79	49.60
Total leaf weight (kg)/ plant/year	4.22	Malvan1 plant 129	0.17	Cacra1 plant 109	1.88	0.08	49.80
Total fruit weight (kg)/ plant/year	5.26	Nagaon plant 74	0.15	Bogmalo plant 90	1.38	0.08	73.80

Variation was seen in all the traits studied. The number of leaves ranged from 34 in MPT3 plant84 to 681 in Bicholim plant3 (Mean=292.9±11.3, CV%=43.9). The lowest number of fruits were observed (Table-1) in MPT3 plants which had only 8 fruits whereas the highest number offruits was observed (Table-1) in Nagaon plant124 (Mean=41.2±1.79, CV%=49.6). Lowest leaf weightwas recorded for Cacra1 plant109 (Table-1) whereas the highest value of was recorded (Table-1)for Malvan1 plant129 (Mean=1886.7±89.9, CV%=73.6). Bogmalo plant90 gave the lowest fruit

yield of (Table-1) and the highest value (Table-1) was observed in Nagaon plant74 (Mean=1391.8±89.9, CV%=73.6).

Maximum diversity was observed in the total number of fruits in South Goa Noni plants (Table-2) whereas the lowest diversity value was also observed for the total number of fruits in South Goa plants (Table-2). The highest mean value was observed for the total leaf weight (kg) (Table-2) and a lowest total fruit weight (Table-2). The Noni plants were then separated depending on the accessions and the Shannon-Weaver diversity was calculated for each of the trait separately.

Table-2: Shannon-Weaver Diversity of the traits in Noni of Konkan coast

Character	North Goa (n=45)	South Goa (n=37)	Coastal Maharashtra (n=49)	Mean
Total number of leaves/ plant/year	0.70	0.79	0.72	0.74
Total number of fruits/ plant/year	0.65	0.81	0.78	0.74
Total leaf weight(kg)/ plant/year	0.77	0.77	0.78	0.77
Total fruit weight(kg)/ plant/year	0.57	0.73	0.76	0.69

Nagaon population (IC0598515) recorded the highest number of leaves (Table-3)and also the highest leaf yield (Table-3) whereas the MPT3 population recorded the lowest number of leaves (Table-3) and also the lowest leaf yield (Table-3). Nagaon population also had the highest number of fruits (Table-3) whereas the MPT3 population showed the lowest number (Table-3). Loutolim population recorded the highest fruit yield (Table-3) and MPT3 plants with the lowest fruit yield (Table-3).

Table-3: Total leaf and fruit production in the Noni accessions

Accession	Total number of leaves/ plant/year	Total number of fruits/ plant/year	Total leaf weight (kg) / Plant/year	Total fruit weight (g)/ Plant/year
Baina(IC0595273)	315	48	2.08	1.86
Bicholim(IC0598223)	326	37	1.95	1.14
Bogmalo(IC0595276)	217	23	1.02	0.90
Cacra(IC0595275)	242	41	1.39	1.12
Harihareshwar(*)	185	0	0.49	0
Loutolim(IC0598229)	371	64	2.44	2.76
MPT (Vasco) (IC0598231)	269	36	1.92	1.49
MPT3(IC0598516)	121	11	1.05	0.46
Sancoale(IC0595272)	288	47	1.75	1.62
Shirdona(IC0595274)	271	33	1.81	0.93
Vagator(IC0595277)	221	30	1.61	0.84
Vazare(IC0598230)	369	46	2.14	1.55
Dapoli(IC0598235)	327	52	1.82	1.90
JMU(*)	341	45	2.09	1.56
Malvan(*)	331	24	2.35	0.76
Mal3(IC0598232)	359	38	2.80	0.96
Mal4(IC0598233)	216	36	1.18	0.69
Malvan Fort(IC0598234)	303	39	1.99	1.34
Nagaon(IC0598515)	413	67	2.81	2.71
Velneshwar(*)	288	46	2.08	1.69

(*) IC Numbers awaited.

The total number of fruits/plant/year ranged from 11-67 fruits/plant/year and the total fruit weight(kg)/plant/year ranged from 0.46-2.76 kg/plant/year which was higher than the yield obtained in previous studies (Basavaraju and Prashant, 2013) which was reported to be 26.6 fruits/plant/2 years and a fruit weight of 0.15 kg/plant/2 years. The yield obtained was similar to the yield obtained for a 3 year old plant, the total number of fruits being 41.4 fruits/plant/3 years and the total fruit weight being 0.85kg/plant/3 years(Basavaraju and Prashant, 2013).

Conclusion

Based on the assessment of the different accessions of the Noni (*Morinda citrifolia* L.) the Nagaon population (IC0598515) of the Noni plants was found to be the highest yielding leaf and fruit yielding plant in terms of number of leaves and the leaf weight and also the number of fruits. Loutolim population of (IC0598229) Noni plants were found to yield the most in terms of fruit weight. These accessions of Noni plants can be further evaluated for yield under field conditions, best performing germplasm selected and tested under multilocation (MLT) before commercializing these accessions.

References

Alison, D.P. and Douglas, K. 2007. Review of ethnobotany, chemistry, biological activity and safety of botanical dietery supplement *Morinda citrifolia* (Noni). *Journal of Pharmacy and Pharmacology*, (59):1587-1609.

Anna, R.D., Heather, M., Nina, L.E. 1999. Ferment this: The transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). *Economic Botany*, (53):51-68.

Ashwita, N. and Arunachalam, V. 2013. Phenotypic diversity of quantitative traits in Noni (Morinda citrifolia, L.) in Konkan Coast of India. *In: (Eds) P. Rethinam and T. Marimuthu. Proceedings of Eight National Symposium on Noni for Sustainable Wellness, Oct., 29-30, pp:1-9.*

Basavaraju, T.B. and Prashant, M. 2013. Performance of Noni (*Morinda citrifolia* L. as a mixed crop in coconut garden. *In: (Eds) P. Rethinam and T. Marimuthu. Proceedings of Eight National Symposium on Noni for Sustainable Wellness, Oct., 29-30, pp:36-39.*

Jules, J. and Robert, E. 2008. The Encyclopedia of Fruits and Nuts, CABI, p.765.

Julia, F. 1992. The Ocean-going Noni, or Indian Mulberry (*Morinda citrifolia*, Rubiaceae) and some of its colorful relatives. *Economic Botany*, 46:241-256.

Nelson, S.C. 2001. Noni Cultivation of Hawaii. CTAHR-Cooperation Extension Service. PD-19.

Nelson, S.C. 2003. Noni Cultivation and Production in Hawaii. In: Proceedings of the 2002 Hawaii Noni Conference, University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, Hawaii.

Waki, J., Okpul, T. and Komolong, M.K. 2008. Assessing the extent of diversity among Noni (*Morinda citrifolia* L.) genotypes of Morobe Province, Papua New Guinea. *The South pacific Journal of Natural Sciences*, (26):11-20.

West, B.J. and Bing-nan, Z. 2008. Identification of major aroma compounds in the leaf of *Morinda citrifolia* Linn. *Journal of Natural Medicines*, (62):485-487.

McClatechy, W. 2003. Diversity of Growth Forms and Uses in *Morinda citrifolia* L. Complex. *In:(Eds) Nelson, S.C. Proceedings of the 2002 Hawaii Noni Conference, University of Hawaii at Manoa, Hawaii, USA,5-10.*

Yanine, C-B., Fabrice, V., Ana, M-P., Max, R., Jean-Marc, B. and Pierre, B. 2006. The Noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutrition and therapeutic properties. *Journal of Food Composition and Analysis*, 645-654.

I-OP-2

Evaluation of *Morinda tomentosa Heyne ex* Roth genotypes for physico-biochemical attributes in rainfed semi-arid environment of Western India

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Abstract

A total of 31 elite genotypes having desirable horticultural traits were collected and established under field condition at Experimental Farm of Central Horticultural Experiment Station (CIAH-ICAR), Vejalpur, Panchmahals (Godhra), Gujarat and evaluated for physico-biochemical attributes under rainfed conditions of western India during the years 2013-2014. These genotypes showed wide range of variations in their physical and chemical composition. The quantitative fruit characters in terms of number of pyrines, number of seed/fruit and fresh weight of seed ranged between 10.91-23.79, 25.27-49.50 and 0.06-0.12g, respectively. Variations in values of TSS, pH, vitamin C, acidity, phenols, Ca, K, Na, and Zn ranged between 7.00 - 11.50 brix, 3.54-7.50, 21.00 -40.25 mg/ 100g, 1.15-1.53 per cent, 11.17-20.25 mg, 90.20-101.90 mg, 36.12-50.32mg, 78.16-93.00 mg and 0.12-0.34 mg among the genotypes characterized for their biochemical composition. Based on the overall observations, CHESN-1, CHESN -16 and CHESN -31 were found to better with respect to most of the qualitative and quantitative characters than rest of the genotypes evaluated under rainfed conditions of western India.

Keywords : *Morinda tomentosa*, evaluation, genotypes, qualitative

Introduction

M. tomentosa, a close ally of *M. citrifolia*, is ever green, small and hardy tree, having capacity to adapt itself successfully in a wide range of habitat from arid, semi-arid to mesophytic conditions (Bermer and Menon, 2002). It is known to bear flowers and fruits throughout the year, but flowering is more prominent during rainy season. Majority of this fruit trees are growing wild and have adapted to acidic, alkaline and saline conditions. Plants of *M. tomentosa* are found every part of Gujarat in forest, road sides and nearby gullies. It is also not exacting in its climatic requirement, adaptable to tropic to subtropics, heavy rainfall to drought prone areas and tolerates water logging, severe drought and heavy winds (Jenson *et al.,* 2002). Its roots are applied as paste as antidote for venom of poisonous creature. Its decoction is used to cure chronic skin infections like boils (Cook, 1994). Ash of its leaves is also made into paste in oil base and applied on boils. Its fruits, after discarding seeds, are used as poultice. Its bark and wood are used to make yellow pigment and wood is used as timber for making stakes, pillars and handles of farm implements. Its fragrant flowers are offered to Lord Ganesha. Medicinal properties of *M. citrifolia* are being exploited similarly *M. tomentosa* may also be used as alternative after evaluation of its physico-chemical properties. In Gujarat and Madhya Pradesh, individuals of *M. tomentosa* are found scattered in patches in almost all over the state mainly in forest areas having wide range of diversity in their growth, flowering and fruiting behavior and quality attributes. The objective of this investigation is to evaluate various genotypes of *M. tomentosa* for their qualitative characters.

Materials and Methods

The location of the experiment is 113 m above msl on latitude 22° 41' 38" N and longitude 73° 33' 22" E and is characterized by hot semi-arid climate. The mean summer temperature is 34.9°C while the mean winter temperature is 21.3° C indicating that the area falls under hyperthermic soil regime. The area is characterized by semi-arid hot climate. The annual water needed or potential evapotranspiration of the area ranges between 1500 to 1600 mm, whereas actual mean usual precipitation is about 831 mm thus causing an annual water deficit of nearly 769 mm, rain is confined to three months (July to September) with average rainy days about 31. The mean monthly maximum temperature ranges from 26 and 40°C, while the minimum

monthly temperature varies between 09°C and 26°C. The soil depth of experimental field ranged from 0.50 to 0.70 m derived from mixed alluvial basalt, quartzite, granite and layers of limestone, and falls under semi-arid hot climate. Sixteen mature fruits were randomly collected from all the direction of each genotype to record the qualitative characters. The extent of variation in physico-chemical traits of fruits from different genotypes was also recorded. Total soluble solids and p[H] of fruits were measured with the help of hand refrectometer and pH meter, respectively. Biochemical compositions like vitamin C, phenols, tannins acidity were determined by the methods outlined in A.O.A.C (1980). The pooled data were statistically analyzed as per method given by Gomez and Gomez (1984).

Results and Discussion

Physico-chemical attributes

It is evident from the data that the fruit attributes vary greatly in different genotypes (Table 1). The number of pyrines/fruit was found maximum in CHESN-1 (23.79) followed by CHESN-16 (23.50) and CHESN-31(22.56) and the same was observed minimum in the genotype CHESN-22 (10.91) followed by CISHN-13 (12.28). Total number of seed per fruit was counted the highest in CHESN-19 (49.50) followed by CHESN-1 (48.78) and CHESN-30 (47.29), whereas lowest number of seeds were recorded in CHESN-12 (25.27) followed by CHESN-27(26.10) and CHESN-13(27.72).Total fresh seed weight was recorded maximum in CHESN-12 and CHESN-26 (0.12 gm) and it was minimum in CHESN-2, CHESN-4 and CHESN-29 having similar weight *i.e.* 0.06 gm. Combi and Ash (1994) also reported variations in the physico-chemical attributes in Fijian medicinal plants.

Table 1: Physico-chemical attributes of different *M. tomentosa* genotypes

Genotypes	No. of pyrines/ fruit	Number of seed / fruit	Fresh seed weight(g)	TSS(%)	pH of fruit juice	Acidity (%)	Vitamin C g/100 ml juice
CHESN-1	23.79	48.78	0.07	11.50	5.03	1.30	40.25
CHESN-2	17.15	37.50	0.06	08.05	4.71	1.36	33.18
CHESN-3	15.19	30.28	0.10	09.10	5.00	1.23	32.07

Genotypes	No. of pyrines/ fruit	Number of seed / fruit	Fresh seed weight(g)	TSS(%)	pH of fruit juice	Acidity (%)	Vitamin C g/100 ml juice
CHESN-4	16.29	36.21	0.06	10.00	4.71	1.15	30.23
CHESN-5	15.38	38.44	0.08	09.00	4.88	1.39	36.00
CHESN-6	17.24	36.15	0.09	10.00	4.59	1.22	31.41
CHESN-7	18.14	45.11	0.08	07.11	3.54	1.41	36.23
CHESN-8	14.59	36.03	0.10	09.07	4.67	1.49	35.62
CHESN-9	13.10	38.14	0.07	10.14	7.50	1.36	28.60
CHESN-10	13.72	28.23	0.08	08.51	5.27	1.30	29.16
CHESN-11	17.79	41.13	0.10	07.11	4.74	1.24	31.49
CHESN-12	12.39	25.27	0.12	11.07	5.04	1.39	30.79
CHESN-13	12.28	27.72	0.07	08.53	4.65	1.44	26.02
CHESN-14	14.12	34.13	0.07	08.50	4.23	1.43	38.17
CHESN-15	20.29	45.08	0.08	07.00	4.63	1.29	37.16
CHESN-16	23.50	42.24	0.09	11.36	4.23	1.24	38.89
CHESN-17	17.13	36.05	0.10	10.00	4.29	1.33	30.14
CHESN-18	17.16	41.78	0.08	11.28	4.55	1.27	34.00
CHESN-19	17.20	49.50	0.10	09.28	4.24	1.41	35.26
CHESN-20	14.24	32.14	0.08	09.18	4.72	1.46	37.01
CHESN-21	15.65	41.13	0.10	09.16	5.57	1.33	30.05
CHESN-22	10.91	29.35	0.07	10.17	4.66	1.15	21.00
CHESN-23	17.46	41.74	0.08	11.50	4.33	1.53	22.17
CHESN-24	16.38	36.05	0.07	08.50	4.54	1.29	32.14
CHESN-25	17.29	34.28	0.09	10.18	4.51	1.29	32.36
CHESN-26	15.69	29.05	0.12	08.50	4.35	1.39	22.26
CHESN-27	14.46	26.10	0.07	10.18	3.56	1.46	30.14
CHESN-28	14.85	29.05	0.08	07.11	4.31	1.32	35.16
CHESN-29	14.87	30.13	0.06	10.17	4.64	1.34	35.17
CHESN-30	19.54	47.29	0.09	9.07	5.43	1.42	34.06
CHESN-31	22.56	40.46	0.07	11.38	3.93	1.31	37.03
C.D at 5%	1.38	3.62	0.006	0.98	0.47	0.13	03.49

Results of study indicated that the quality attributes of *M. tomentosa* genotypes showed significant differences in their values. The total soluble solids were recorded the maximum in CHESN-1 and CHESN-23 (11.50°brix) followed by CHESN-31 (11.38°brix) and CHESN-16 (11.36 °brix) while the minimum value for the same was noted in the genotype CHESN-15 (7°.00 brix) followed by CHESN-7, CHESN-11 and CHESN-28 having similar values i.e. 7.11. Fruit acidity was estimated maximum in CHESN-23 (1.53%) followed by CHESN-8 (1.49%) and CHESN-27 (1.46%) and it was minimum in CHESN-4, CHESN-22 i.e. 1.15% followed by CHESN-3(1.23%) and CHESN-11,CHESN-16 (1.24%). pH of fruit juice was observed highest in CHESN-9 (7.50) followed by CHESN-21 (5.57) and the lowest was recorded in CHESN-7 (3.54) followed by CHESN-27 (3.56). Vitamin 'C' content was estimated the maximum in CHESN-1 (40.25mg/100g) followed by CHESN-16 (38.89 mg/100 gm) and the same was recorded the minimum in CHESN-22 (21.00 mg/100 gm) followed by CHESN-23 (22.17 mg/100gm). Variations in the chemical composition of different underutilized fruits have been reported by Singh and Singh (2005) in tamarind, Singh and Singh (2005) in mahua, Su *et al.* (2005) in noni and Singh *et al.* (2006) in chironji genotypes under different agro-climatic conditions.

Biochemical constituents

The biochemical composition of *Morinda* fruits consisted of Ca, K, Na, Zn and phenol varied between 90.20 -101.90 mg, 36.12 -50.32 mg,78.16-93.00mg, 0.11-0.34 and 11.17-20.25 mg respectively in different genotypes of *Morinda* (Table 2). The minimum calcium content was observed in CHESN-14 (90.20mg) and it was maximum in CHESN-31 (101.90 mg), whereas the maximum potassium content was recorded in CHESN-1 (50.32 mg) followed by CHESN-16 (46.88 mg) and CHESN-31 (45.11mg) whereas the minimum of the same was recorded in CHESN-26 (36.12 mg) followed by CHESN- 27(36.28 mg) , CHESN- 18 (37.76 mg) and CHESN-21(37.96mg). The Na content was recorded highest in CHESN-24 (93.00 mg) and the same was lowest in CHESN-1 (78.16 mg), respectively. The highest content of Zn was found in CHESN-1 (0.34 mg) followed by CHESN-31 (0.27mg), whereas the minimum was estimated in CHESN-14 (0.12 mg).Total phenols were recorded highest in CHESN-29 (20.25mg) and lowest value for the same was observed in CHESN-2 (11.17mg). Differences in the various biochemical constituents of fruits of *M. tomentosa* genotypes may be genetic in nature rather than due to edaphic or other environmental factors. These results in close agreement with

findings as reported by Singh and Singh (2005) in jamun and Shah *et al.* (2006), Wang (2002) and Singh *et al.* (2007) in case of *Morinda citrifolia* under different edaphoclimatic conditions.

Table 2: Biochemial variations in *M. tomentosa* genotypes

Genotypes	Ca (mg)	K (mg)	Na (mg)	Zn (mg)	Phenol (mg)
CHESN-1	101.61	50.32	79.14	0.34	13.32
CHESN-2	93.94	40.11	82.41	0.17	11.17
CHESN-3	94.18	39.86	84.34	0.20	11.41
CHESN-4	95.44	39.41	85.73	0.24	12.56
CHESN-5	98.16	41.18	86.81	0.23	15.69
CHESN-6	97.73	42.09	87.29	0.17	17.09
CHESN-7	97.12	44.13	88.39	0.18	14.21
CHESN-8	93.44	42.19	82.99	0.20	13.07
CHESN-9	92.46	40.10	92.09	0.24	13.43
CHESN-10	99.13	40.93	90.11	0.17	12.91
CHESN-11	94.68	41.87	89.22	0.16	16.88
CHESN-12	95.71	42.75	87.18	0.18	13.71
CHESN-13	97.70	41.09	88.34	0.17	12.83
CHESN-14	90.20	39.91	89.90	0.12	14.25
CHESN-15	92.73	40.09	87.36	0.16	14.87
CHESN-16	99.26	46.88	81.92	0.23	15.12
CHESN-17	92.25	42.48	84.07	0.22	17.36
CHESN-18	94.68	37.76	90.45	0.17	13.42
CHESN-19	97.13	38.83	84.39	0.12	12.93
CHESN-20	96.40	39.93	86.82	0.14	17.13
CHESN-21	96.43	37.96	84.90	0.25	15.00
CHESN-22	96.54	41.11	91.14	0.17	16.29
CHESN-23	99.03	42.17	92.67	0.19	14.41

Genotypes	Ca (mg)	K (mg)	Na (mg)	Zn (mg)	Phenol (mg)
CHESN-24	97.04	41.05	93.00	0.15	11.74
CHESN-25	96.14	38.00	91.28	0.22	16.61
CHESN-26	97.88	36.12	82.67	0.20	15.73
CHESN-27	94.14	36.28	84.42	0.21	18.26
CHESN-28	93.86	39.16	82.43	0.21	19.13
CHESN-29	93.73	38.52	88.70	0.24	20.25
CHESN-30	93.79	37.94	89.93	0.28	16.11
CHESN-31	101.90	45.11	80.13	0.27	13.14
C.D at 5%	09.45	03.19	08.66	0.03	01.44

Based on the observations, it may be inferred from the study that the genotypes viz., CHESN-1, CHESN-16 and CHESN-31 were found to be superior with respect to biochemical constituents and they may be used for herbal formulation and in breeding programme for better offspring.

References

A. O. A. C. 1980. Official Methods of Analysis, Association of Official Agricultural Chemists, Benjamin Franklin Station, Washington, D.C.

Bermer, B. and Manen, J. F. 2000. Phylogeny and classification of subfamily Rubioideae (Rubiaceae). *Plant Systematic and Evolution,* 225:43-72

Cambie, R. and Ash, J. 1994. Fijian Medicinal Plants, CSIRO, Australia.

Cooke, T.H. 1908. The Flora of Presidency of Bombay. Vol II, Calcutta, pp.43.

Gomez, K. A and Gomez, A. 1984. *Statistical Procedure for Agricultural Research* (2nd Edn.), John Wiley and Sos Inc., New York.

Jenson, S., Robbrecht, E., Beeckmasn, H., Kanomi, A. and Puff, C. 2002. A survey of the systematic wood anatomy of Rubioidae (Rubiaceae). *Journal of Economic and Taxonomic Botany*, 7:389-397.

Shah, A., Kumar, S. and Gupta, D K. D. 2006. Value added products from *Morinda citrifolia* (Indian Noni) - An Overview. *National Seminar on Production, Utilization and Export of Underutilized Fruits with Commercial Potentialities, B. C. K.V. Kalyani, Nadia, West Bengal, India, November 22-23, pp. 91-92*.

Singh, D. R., Srivastava, R. C and Sudha, R. 2007. Studies on shelf life and biochemical changes of *Morinda citrifolia* L. (Noni) fruits during storage. *Proc. National Symposium on Noni for Health and Wellness, Chennai, 27th and 28th October, pp. 84-91.*

Singh Sanjay and Singh, A. K. 2005. Genetic variability in Jamun (*Syzygium cumini*) in Gujarat. *Progressive Horticulture,* 37(1): 144-148.

Singh Sanjay and Singh, A. K. 2005.Genetic diversity in mahua (*Bassia latifolia*) under semi-arid ecosystem of Gujarat. *Indian Journal of Agricultural Sciences* 75(8): 519-23.

Singh Sanjay and Singh A. K. 2005. Studies on variability in tamarind (*Tamarindus indica* L) from Gujarat. *Progressive Horticulture,* 37: 280-284.

Singh Sanjay, Singh, A. K. and Appa Rao, V. V. 2006. Genetic diversity in *Chironji* (*Buchanania lanzan* Spreng) under semi-arid ecosystem of Gujarat. *Indian Journal of Agricultural Sciences*, 76: 695-698.

Su, B. N., Pawlus, A. D and Jung, H. A. 2005.Chemical constituents of fruit of *Morinda citrifolia* (Noni) and their antioxident activity. *Journal of Natural Products,* 68: 592-595.

Wang, Y., West, B., Jenson, J., Nowicki, D., Su, C., Palu, A. and Anderson, G. 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. *Acta Pharmacological Sinica,* 23 (12): 11-27.

I-OP-3

Morphological, anatomical and genetic diversity in *Morinda citrifolia* L.

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Abstract

Noni, Morinda citrifolia L. is consider as a wonder plant species with numerous medicinal properties. The genus Morinda is pan tropical in its distribution with around 80 species with no clear sub populations bearing unique characteristics. Species diversity is highest in the indo-pacific region in near Oceania with attenuation into Remote Oceania. Among the species of Morinda, M. citrifolia is distributed throughout the islands countries in indo-pacific region. One of the distinctive adaptations or niches of M. citrifolia seems to be its ability to colonize new islands and new terrain. It is frequently identified as a part of primary forests or shrub vegetation on small atolls, new lava flows and other newly emergent surfaces near the ocean. Although the species M. citrifolia is having unique morphological features, there are distinct variations observed in many of its ecotypes/varieties. Further, it is confusing to categorize them as different ecotype/variety/ sub species or a separate species itself with the already available literature. Therefore, the present is initiated to investigate the morphological, anatomical and genetic diversity of M. citrifolia. Nine different ecotypes/varieties of M. citrifolia were taken to analyze in detail about their morphological, anatomical and molecular level similarities and dissimilarities. The marker compound scopoletin was also considered in the analysis. The leaf and stem of the various samples were collected and studied anatomically. Genetic diversity of the cultivars was determined using amplified fragment length polymorphism (AFLP) markers. Different characteristics were observed, among all the varieties and the variety with big bracts showed distinct morphological and molecular features from the other samples. Importantly, M. citrifolia samples have shown sufficient polymorphism.

Keywords : *Morinda citrifolia,* morphology, anatomy, amplified fragment length polymorphism, scopoletin.

II-LP-1

Crop production and Good Crop Production Practices of Noni (*Morinda citrifolia* L.): A review

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Abstract

Morinda citrifolia L., popularly known as Indian Noni or Indian mulberry, belonging to family Rubiaceae is an ever green small tree which flowers and fruits through out the year and naturally spread in the tropical regions of the world, generally found from sea level to 400 m above MSL, although it adapts better to coastal regions. In India it is widely grown under natural conditions in Andaman and Nicobar Islands and also seen throughout the coastal region along of Kerala, Karnataka, Tamil Nadu and many other places like fences , road sides and forests due to its wider adaptability to hardy environment. Systematic cultivation in large area is very much limited to about 200 ha, in Karnataka, Kerala, Maharashtra, Tamil Nadu and the bulk of the fruit supply comes from natural wild growth.

A global review made has indicated that Noni is relatively easy to propagate from seeds.. Though it is easy to propagate by seeds vegetative propagation through cuttings made from stem verticals is preferred for uniform performance. Micro propagation using tissue culture is the other possibility of multiplication of planting material. Seed germination technique, use of hormones for rooting has been attempted but large scale technology standardization is yet to be done. Vegetative propagation through 2 or 3 noded cuttings treated with cow dung slurry, hormone, tender coconut water etc. had shown promising results. Location specific nutrient and water management studies through drip system are still in infant stage only and presently adhoc recommendations are being made. Organic cultivation is advocated but still

cost effective technology development is also in infant stage. Noni as a mixed crop in coconut has performed well in many Centres in different states under Al India Coordinated Research Projects on Palms. The Good Production technologies for this crop including germination of seeds, standardization of nursery techniques, nutrient and water management, cropping/ farming system, pruning and training, standardization of harvest and post harvest technologies need to be perfected for its successful cultivation through organics..

Introduction

Morinda citrifolia, L., Noni loves tropical humid island/costal/marine climate for its natural growth and development including of flowering and fruit setting round the year (Nelson, 2002 and Lu"berch and Hannes, 2001**).** It is an ever green small tree bearing flowers and fruits throughout the year and belongs to family *Rubiaceae*. It grows in tropical regions of the world from sea level to 400 m above MSL, although it adapts better to coastal regions (Lu "berch and Hannes, 2001). The tropical humid climate is very much suitable for its cultivation (Singh *et al*., 2005). It is believed that about 2000 years ago, Polynesian sailors took this plant from South East Asia and used for many centuries as food and medicine (Wang *et al.*, 2002). It is often found growing along lava flows. Bulk of the crop is wild growing in hardy environment and adopts to acidic, alkaline and saline soil conditions (Camba and Ash, 1994 and Singh et.al., 2007). The genus *Morinda* is present worldwide predominantly in tropical countries. It occurs in Africa, Australia, Barbados, Cambodia, Caribbean, Cayman Islands, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French West Indies, Guadeloupe, Guam, Haiti, Hawaii, India, Jamaica, Java, Laos, Malaysia, Marquesas Islands, Philippines, Polynesia, Puerto Rico, Raratonga, Samoa, Seychelles, Solomon Islands, Southeast Asia, St. Croix, Surinam, Tahiti, Thailand, Tonga, Trinidad and Tobago and Vietnam (Rethinam and Sivaraman, 2007).

It is seen throughout the coastal region along fences and road sides due to its wider adaptability to hardy environment. In the main land of India, it is found along the coastal areas of Kerala, Karnataka, Tamil Nadu and many other places. The fruits collected from these wild trees are used for medicinal purposes and as food supplement. Recently, farmers in India have started taking up the cultivation of Noni as irrigated crop since this crop has the capacity to change the socio

economic status of the farmers through its high yield and returns. In Andaman and Nicobar Islands after Tsunami this crop has been projected as lively hood security crop for Bay Islands (Srivastava and Singh, 2008) and all efforts are being made to popularise this crop as small holders crop (Singh *et al*., 2007 and Subash Chand *et al*., 2008(a)). The economic appraisal had also been made to show the profitability of this crop (Subash Chand and Singh, 2007) and technological interventions for enhancing the productivity has been identified and implemented (Srivastava and Singh, 2007). The agro climatic requirements and production potential for Noni were also worked out for A& N Islands (Singh and Srivastava, 2006 and 2007; Singh and Singh 2010).

Morton (1992) reported that the fruits of this tree have a history of use in the pharmacopoeias of pharmaceutical unit in the universe because it has more than 150 nutraceuticals, several vitamins, minerals, micro and macro nutrients that help the body in various ways from cellular level to organ level. Noni is one of the important traditional folk medicinal plants that have been used for over 2000 years in Polynesia. It has been reported to have a broad range of therapeutic and nutritional value. The ancestors of Polynesians are believed to have brought many plants with them, as they migrated from Southeast Asia about 2000 years ago (Tabrah and Eveleth, 1966; Gerlach, 1996). Of the 12 most common plants they brought, Noni was the second most popular plant used in herbal remedies to treat various common diseases and to maintain overall good health (Krauss, 1993; Gerlach, 1996). While lot of information are available on medicinal and nutraceuticals effect of Noni not much literature are available on cultivation side. More than 200 commercial entities across the globe sell these products and enjoy enormous market share (Nina *et al*., 2002). A Review of agricultural research, nutritional and therapeutic properties of the Noni Fruit (*Morinda citrifolia*, L. was made by Blanco et al., (2006), Mathivanan et al., (2005), Rethinam and Sivaraman (2007) and Rethinam (2008).

The importance of Noni in the livelihood security, economic appraisal as well as popularization on Noni have been reported (Subash Chand and Singh, 2007; Subash Chand *et al*., 2008(a) and Subash Chand *et al*., 2008(b)). It is now cultivated in Andhra Pradesh, Andaman and Nicobar Islands, Karnataka, Kerala, Chattisgarh, Gujarat, Meghalaya, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu and West Bengal under organic contract farming. Rosanglura,2010, indicated the possibility of growing Noni in Mizoram.

2. Planting Material Production

Noni is relatively easy to propagate from seeds, stem, or rooted cuttings and air layering. The preferred methods of propagation are by seeds and cuttings made from stem verticals (Nelson, 2001). It was also found out that vegetative propagated planting materials maintain uniformity and productivity (Wagner *et al*., 1999; Nelson, 2001; Hartmann *et al*., 2002). Micro propagation using tissue culture is the other possibility of multiplication of planting material.

2..1. Seeds

It is best to collect *Morinda* fruits after they are fallen from the tree for seed purpose (Nelson, 2005). Seeds are extracted from the fruits and sowing can be done immediately after extraction. The seeds possess woody water coat which enables the germination (Wagner *et al*., 1999). The seed requires scarification to reduce the time to germinate and uniformity. To scarify the seed is to cut, scratch or soften its outer coat to allow ready penetration of water and air. Unscarified seed usually take at least 60 days and usually much longer (up to six months or more) for germination than scarified (3 to 4 weeks) depending upon the condition. Scarifying can be done by a blending machine, nail cutter etc., (Nelson, 2005). Seeds can be planted in the seedling flats, trays, or directly in the light medium containers immediately after extractions from fruits and requires hot and wet conditions for optimum germination. Under green house condition raising seedling in the warmest part of land provide better environment for better seed germination (Singh *et al*., 2007). Noni seed has dormancy due to hard seed coat (water repellant) thus delaying the germination and need 43 days to germinate (Mathivanan *et al*., 2005). Seeds after drying in shade for 3 or 4 days can be stored in air-tight containers at room temperature. Preliminary seed storage experiments have shown that seeds have low viability but are orthodox in nature (Veena Gupta and Sharma, 2008). However, detailed storage studies are yet to be taken up (Singh *et al*., 2007). The treatment with hot water at 40°C for a period of 24 hours and a treatment with sulphuric acid at 50% concentration for 5 minutes were able to overcome the seed dormancy (Ponnaiyan and Vezhavendan, 2005). The highest germination of seeds was obtained where the seeds were nicked and then treated with Gibberllic acid (GA) at 1000ppm for a period of 24 hours (Ponnaiyan and Vezhavendan, 2005). Seeds treatment with hot water at 40°C combined with sea weed (*Ascophyllum nodosum*) extract Biozyme and the treatment with sulphuric acid 50% for 5 minute combined with Biozyme were able to break seed dormancy as well as give

better health and vigour to the germinated seedlings (Muthu and Mathan, 2006). Seed germination studies of soaking seeds for 24 hours with Gibberllic Acid (GA) at 800 ppm increased the germination percentage to 91.06% as against mere water treatment (51.4%). The interaction of seed soaking and treatment by GA 800 ppm increased high percentage of seedlings and number of leaves (Singh and Rai, 2005 and Singh et al., 2007). Pre treatment of seeds with $NaHClO_3$ (5% available chlorine for 30 minutes) increased the germination up to 84%. However, the growth parameters were good in KNO_3 (150 ppm) (Sudha and Singh, 2007). Seasonal influence on seed germination was also reported by Singh *et al*., 2007. Singh *et al*., 2009 while reviewing the planting materials production had indicated that hulling, hot water, sulphuric acid treatments are economical and easily applicable in developing quality planting materials over the costly plant growth regulators and associated technicalities, Scarifying the hard seed coat by gently hulling it significantly reduces germination time, improves germination percentage and promotes uniform sprouting.

2.2.2. Vegetative Propagation

The importance of vegetative propagation is to get best planting materials with highest genetic quality (Nanda, 1970; Wright, 1975; Hatman and Kester, 1983). Singh and Rai (2005) and Singh *et al.,* (2007) have suggested the use of growth regulators like Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) for quick and better rooting in vegetative propagation. Vertical and lateral stem cuttings with sap flow at the time of cutting with vigorous growing points are the best suited for vegetative propagation. Vezhavedan and Ponnaiyan (2005) compared different types of cuttings viz., tip, semi hard, and hard wood cuttings with different number of nodes (2,3 and 4) and reported that hard wood cuttings with 4 nodes performed better and gave more success percentage and healthy planting materials. It is better to avoid the cuttings without sap flow for vegetative propagation. It is also better to avoid hollow stem cuttings since the percentage of recoverable seedlings are low and take 5 days more than non hollow cuttings which took only 15 days and survival was 79.4% (Singh et al., 2007). In another study Sudha et al., (2007) and Sudha and Singh (2007) had found that both in hollow and non hollow cuttings, IBA 6000 ppm showed significantly higher rooting values than IBA 2000 ppm. Root initiation and percentage of sprouting were higher in non-hollow cuttings compared to hollow cuttings. In a preliminary study with single, double and triple node cuttings dipped with cow dung solution and IBA solution it was observed encouraging results at COIN which should be further studied (Personal Communication).

Singh *et al.* (2009) whilereviewing the propagation indicated that vegetative propagation of non hallow soft wood cutting should be preferred for better performance. They further indicated that the cutting treated with 4000 ppm of IBA performed better and enhanced with closed poly house . If IBA is not available with the farmer ,cow dung can be used. *Morinda tomentosa* can be propagated successfully through softwood cutting treated with IBA @ 300 ppm under semi arid ecosystem of Western India (Singh *et al.*, 2011).

Wedge grafting method was used in noni grafting. Healthy non- flowering, non-hallow green mature shoot of current growth was used as scion. Root stock was raised through the seedlings of noni plants of IC 0598235. A healthy root stock seedling of 3-4 months old was used. A transverse downward cut was given in the soft wood portion on the stem to make a cleft lips at the top. The wedge shaped scion stick was inserted in to the cleft of the root stock and tied in with polythene strip taking care that no water enters the grafted portion. Grafting was attempted during rainy season June-July 2013. About 82 grafts were made out of which, 35 survived which showed 42.68% success rate. Twelve grafts of six months old are planted in the field and observations are recorded. Grafted noni plants flowered in 6 months after planting. The grafted plant showed good growth after 6 months of planting with 30 fruits/plant and 193 leaves/plant. (Personal Communication).

Root hormones may help to promote the vegetative growth of cutting. Soaking of the cuttings with 4000 ppm Indole Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA) separately and in combination promoted root and shoot growth and establishment besides increasing percentage of rooting, length and number of roots, length of longest primary root (Singh *et al.*, 2007). At high level of auxins reduced root growth is recorded due to nutritional imbalances (Spiegel, 1954; Nanda et al., 1974). The sprouting of cuttings under closed poly house was earlier (15 days) and survival of cuttings was 83.3% while in covered poly house it was 20 days with 60 % sprouting at 33.5°C and 80% RH. Under open conditions with optimum light intensity of 44382 lux and maximum temperature of 31.9°C, minimum of 27.03°C and RH 77.1%. The growth of vegetative propagated plants under open grew faster and put up 4 branches in 32 days and reached reproductive stage (Singh *et al.*, 2007). Since vegetative propagated materials will have uniformity and this area of research needs immediate attention. Organic promoters and growing media on vegetative propagation of Noni studied by Asha Sankar et al. 2014 indicated that dipping the

cuttings in 75% cow dung slurry, pulse dip in Charcoal slurry, dipping in Noni fruit juice ,etc. supplemented with Azospirillum, Plant growth promoting Rhizobacterium (PGPR 1) and Arbuscular mycorrhizal fungi (AMF) in the pot mixture promoted good rooting system. Maheswarappa *et al*., 2010 observed higher number of fruits in cuttings rather than that of seedlings and tissue culture plants

2.2.3. Micro propagation / Tissue Culture

The varying effect of cytokinin and auxin combinations were studied on *Morinda citrifolia* for effective in-vitro induction of shoots from nodal explants and it clearly indicated that irrespective of basal media used (either MS or WP), it is the hormonal combinations which are very vital for the in-vitro response, BAP alone for shoot initiation, kinetin along with BAP for multiple shoot formation. Calli with roots produce shoot(s) in BAP with IBA medium (Antony Selvaraj etal., 2006). Further studies showed that the rooted plants have established well with 95-98.5% survival under green house conditions while hardening. Further better growth with zero per cent mortality was observed at nursery stage (Subramani *et al*., 2006). Now micro propagated plants have gone for field test. Presently cell culture study is being carried out (Subramani *et al*., 2007 a & b). Studies on the cell suspense culture of *Morinda citrifolia*, L., is under progress (Subramani, 2008). Basseth and Tramper (1995) have reported the use of non conventional media in *Morinda citrifolia*, L., cell culture. The number and weight of fruits/plant was higher in tissue culture planted field compare to seedlings planted (Basavaraju and Prasanth, (2013).

2.3. Plantation Management

Information on plantation management like pruning and training and canopy management is lacking but the preliminary observational studies gave encouraging results which should be persuaded.

2.3.1. Planting season

Ideal season for planting is from June to October. It can also be planted during January to March (Singh *et al*., 2007).

2.3.2. Pitting for planting

A pit of 2 x 2 x 2 feet is dug a few days before planting and allowed to dry. Then fill the pit with top soil, 2 kg of Compost or Farm Yard Manure, 2 kg of vermicompost and 1 kg if Neem cake along with Pseudomonas sp., Tricoderma, VAM and *Azospyrillum* and mix thoroughly. Now the pit is ready for planting. Irrigation channels to be formed or drip system need to be installed (Rethinam, 2007 and Singh *et al*., 2009).

2.3.3. Spacing

The recommended spacing is 12 x 12 feet or 4 x 4 m with a plant population of 700 plants per hectare (Singh *et al*., 2009).

2.3.4. Weed Management

It is suggested to take up proper weed management till the plants are established and later adopt repeated mulching along with inter cropping with indigo, cow pea, horse gram or fodder crop (Peter, 2007). In Hawaii weeds like Guinea grass and sensitive grass (*Mimosa pudica*) compete with Noni. It is also attacked by dodder (*Cuscuta sandwihensis*), a parasitic weed. Manual removal of weeds from the field is recommended in case of Noni (Nelson, 2001).

2.4. Nutrient Management

Noni has a deep tap root system and extensive surface feeding root system. The tree may not compete well in a landscape with plants that have aggressive, surface-feeding lateral roots, such as grasses. So it can absorb the fertilizers on the surface which is racked well coupled with irrigation. A fertilizer application of 10-20-20 kg NPK/ha will be sufficient (Nelson, 2005). The strategy to apply nutrients is similar to that of other fruit crops like citrus and coffee. Young non fruiting plants are encouraged to produce lush vegetative growth with balanced fertilizers (14:14:14 or 16:16:16), whereas more mature and fruit bearing trees are encouraged to produce many large fruits by application of high phosphorus fertilizers such as 10:20:20 or 10: 45: 19 (Singh et al., 2009). Since organic cultivation is promoted in India, the above nutrient dose may be given in the organic form from the approved organic sources. A tentative manurial application for Organic Noni Cultivation has been suggested as shown below till scientifically the technology is developed:

At 6th month : 2 kg. vermi compost or compost + ½ kg Neem cake.

At 12th month : 2kg. vermi compost or compost + 1 kg neem cake + Bio fertilizers.

At 18th month : 2kg. vermi compost + 1kg neem cake.

At 24th month : 5kg. vermi compost + 5kg wood ash + 1kg neem cake + Bio fertilizers including *Tricoderma viridis, Azatobacter, Phosphobacterin, Psudomonas sp*. etc,.

After 24 months : the above dose repeated every 6 months (Rethinam, 2007).

Noni is reported to display abnormal foliar symptoms for nitrogen, iron, and phosphorous deficiencies. Inter veinal chlorosis, scorching of leaf margins, leaf curling, purpling and marginal necrosis are some of the distinct deficiency symptoms. The leaf nutrient content, uptake of nutrients at different stages of the crop critical level of nutrients etc., are to be assessed. This presentation made by Palanichami 2008,highlights the achievements / gains in COIN and in a few cultivators farm about specific benefits / gains achieved in terms of soil testing, vermi composting, Panchakavya, cow urine, Jeevamirtham, pruning and plant architecture, labour saving equipments, certification and new monitoring system in Indian Noni Cultivation Council.

2.5. Irrigation Management

Nelson (2005) reported that the plant can thrive well with moderate irrigation and can survive extended drought once established and matured. In dry condition when the plants are less than two to three years, irrigation once or more per week with 10 gallons of water per plant and for older plant more water with less frequency were suggested. How ever over watering is not recommended. There is not much literature available on irrigation and quantity of water to be irrigated. However, in India irrigated Noni is advocated for getting better yield and hence an adhoc recommendation has been given as shown below.

Irrigating the planting pits to completely wet the soil for three consecutive days to wet the pit before planting the seedling. After planting drip irrigation everyday for half an hour to supply 4 liters of water/plant/day for first three months, 8 liters of water/plant/day for next nine months and there after 12 liters/plants/day up to 2 years are suggested. For adult plants after 2 years 15 liters/plant/day was suggested. With good mulching at the base this quantity of water should be adequate depending upon the climatic, soil conditions and canopy growth (Rethinam, 2008)

VishalNath et al.,2009 found out that .Irrigation at 50 to 75% Pan Evaporation Replenishment (PER) along with paddy straw and coco-coir pith mulching found most efficient in terms of plant height ,canopy spread and trunk cross sectional area of plant.

2.6. Green Manure crops

Growing green manure crops like sun hemp, daincha, Indigo and green leaf manure crops like Cassia, Gliricidia, agathi (*Sesbania sp*.), subosavi, kubdool etc., can produce adequate biomass which can be recycled. Growing green manure crops in the planted area for a period of 2 to 3 months will provide shade as well as produce bio mass besides creating an ideal micro climate to the plants during summer. These crops also fix atmospheric nitrogen. If these are cut and put at the base it becomes mulching. Mulching will reduce evaporation losses, prevents weed growth and conserve moisture. Up on slow decomposition in the basin the physico chemical and biological properties of soil improve and increase water holding capacity and the over all soil health (Rethinam, 2008).

2.7. Inter and mixed crops

Even though systematic studies on inter/mixed cropping have not been con ducted, there is lot of scope for such crops to increase the unit income in unit time and also to provide bio mass for organic recycling. Growing green gram, black gram, cowpea, horse gram, ground nut, rain fed millets etc., during monsoon season utilizing the available moisture has been suggested. In case if irrigation facilities can be extended to the intercrops, the interspaces can be used for growing vegetables, flowers, medicinal plants, fodder crops etc., which have good market potential in that area. Noni crop can be well fitted in coconut and arecanut plantation as mixed crop. In mango and cashew plantations also noni can be planted in between the rows. However, crops like banana, sugarcane, papaya, rose and other crops which require more water should be avoided as they may promote the nematode problem (Rethinam 2008). In a study on the influence of different types of planting materials and planting geometry on growth and yield of Noni grown as mixed crop in coconut garden under Litoral sandy soil, Maheswarappa et. al.,(2010) indicated that Noni can be successfully under coconut with out affecting the yield of coconut. Single hedge row of Noni was comparatively better than double hedge system (Maheshwarappa and Dhanapal, 2011). Amitabha Kar *et al*., (2011) suggested that to support

the farmer's investment, traditional vegetables suitable to the area such as brinjal and okra can be grown as intercropping to fetch regular income utilizing minimal water through drip irrigation. Noni as a mixed crop in coconut garden in Arsikere, Karnataka added 1.21 to 1.48 t/ha of leaf litter and also increased the coconut yield also.(Basavaraju and Prasanth, 2013)

3. Harvest and Post Harvest Processing

Noni bears fruits in about nine months to one year after planting. Harvesting can be done throughout the year although there are seasonal patterns in flowering and fruiting (Nelson, 2001 and 2003). However, the commercial harvest can be done from 20-24 months after planting and it is suggested to remove all flower buds up to one year and six months and then allow for flowering so that the plant can be studied. Depending on the post-harvest technology programme adopted, the fruits may be harvested at different stages of development. After harvesting, the fruit ripens within a week at ambient temperature and also because of its short storage life the fruits cannot be transported to the distant places even with in the country. To over come the problem harvesting the fruits with pedicel helps to maintain better quality and market acceptability as the highest spoilage of fruits was observed in fruits harvested without pedicel (Fruits with pedicel performed well in terms of keeping quality, ascorbic acid and TSS. Among the accessions, SPG-2 recorded minimum loss of weight (2.90%) followed by Pbay-7 (3.74 %) in 9 days during storage (Singh *et al*., 2007). Nonetheless, most of the processors buy the fruits harvested at the ''hard white'' stage for juice production, as the fruits become soft too quickly once this stage is reached (Nelson, 2001 and 2003). The change from stage 4 to stage 5 occurs very quickly (few hours) and the pulp practically liquefies and turns from green to white, as well as develops the characteristic butyric smell. The fruits are individually selected on the tree and harvested by hand. At the ''hard white'' stage, they are well able to withstand being transported in baskets or containers, and exposure of the fruits to light or high temperatures immediately after harvest does not affect their overall quality. Before processing, fruits are ripened at room temperature for a day or more, depending on the end product (tea, juice, pulp, dietetic products, etc. (Nelson, 2003)). Recently a protocol for packaging and shipment of noni fruits have been developed which needs further scaling up (Singh *et al*., 2008).

4.Yield

Morinda citrifolia is a perennial bush and it is possible to find fruits at different stages of maturity on the same plant at the same time. Under favorable conditions, the plant bears fruit about nine months to one year after planting. At this stage, the fruits can be harvested, but they are generally small and the yield per tree is low. Some producers choose not to harvest in the first year, and they prune in order to let the bush grow stronger. In Hawaii, Noni fruits are harvested throughout the year, although there are seasonal patterns in flowering and fruit bearing (meteorological factors, fumigation, and irrigation) (Nelson, 2001 and 2003). In India the plants are allowed to grow for two years without any side growth by periodical pruning so as to make the plant sturdy. It is reported that Noni plant is capable of giving yield up to 250-300 kg after 7-8 years of planting under better cultivation conditions. However, in the initial stages yield may range from 30-40 kg per plant and the well grown tree will produce an average of 90 – 100 kg. It is also reported that the productivity of the trees will be up to 40-50 years and the harvest can be done more than 6 - 7 times in a year (Nelson, 2001 and 2003).

In Hawaii, Noni fruits are usually harvested two or three times per month, although fruit production is lower during winter. With a density of 638 plants per hectare with good soil fertility, drainage, and irrigation and appropriate pest, disease and weed control, along with an appropriate fertilization plan, it is possible to obtain yield from 7 tons/ha/year in the second year after planting to approximately 70 tons/ha/year after the fifth year (Nelson, 2001and 2003). With a juice extraction rate of approximately 50% (w/w), one hectare can thus, yield around 35 tons of juice. However, many factors may affect these yields, and most producers do not obtain such good results because of diseases or poor agricultural practices (grown wild plants). In Hawaii, an average annual yield of 50 tons/ha is generally attained (Nelson, 2001 and 2003).

The cost of cultivation per acre under Andaman conditions has been worked out to Rs.42,425 per ha (Singh *et al.*, 2007). It was observed that five years plantations in Bay Islands gave a gross income of Rs. 4,68,750 /- with a net income of Rs.2,00,731/- (Subhash Chand and Singh, 2007).

5. Storage

Ripened fruit can be stored up to 9 days and juice need to be extracted before 9 days of maturity (Peter, 2007). Singh *et al.,* (2007) studied the effect of storage of different quality parameters and reported that the physiological loss of weight was highest (28.26%) and spoilage was higher in non pedicillate fruits. Fruit harvested with pedicel had maximum Ascorbic acid and less spoilage.

6. Future Research Programmes in Production

The overall scanning of available literature have shown that very little research work has been done on Noni on the production of crop including selection of varieties while bulk of the research have been done on the pharmaceuticals and health aspects of Noni and its products. Keeping in view of its immense potential for this under utilized crop, the research needs given below have been suggested for future research.

6.3. Standardisation of Nursery Techniques

1. Standardisation of nursery techniques using seeds-
2. Standardisation of Vegetative propagation using stem cuttings (hard/semi hard/ tip cutting)
3. Propagation through air-layering/soft wood grafting and cutting with the aid of growth regulators.
4. Micro propagation for mass multiplication

6.4. Development of sustainable production technology

1. Standardization of planting geometry and plant canopy architecture.
2. High Density Cropping system.
3. Standardization of appropriate dose of nutrients for *Morinda citrifolia L.,* through organic sources.
4. Development of drip irrigation scheduling and moisture conservation techniques.
5. Developing DRIS norms for recommending fertilizer/manure application.

6. Root development studies at various stages of Noni crop growth right from seedling stage.
7. Studies on the nutrient deficiency symptoms for the major, secondary and micro nutrients.
8. Assessment of uptake of nutrients and critical leaf nutrients of Noni at different stages of crop growth..
9. Evaluation of different organic sources on the growth and yield which are cost effective for different agro climatic zones.
10. Evaluation of the role of micro nutrients, identifying deficiency symptoms and developing correction measures..

6.5. Noni Based Cropping/ Farming Systems

Inter cropping with annual crops, medicinal and aromatic crops, vegetables etc.

6.6. Noni Based Mixed Farming System –

Growing grasses and rearing animals and recycling bio mass to the plantations.

6.7. Evaluation of *Morinda citrifolia, L.* in challenged environment - viz., marshy areas, waste land, drought situations, saline/alkaline soils, sloppy land etc., and developing suitable management practices.

6.8. Harvest and Post Harvest Studies.

1. Development of pre and post technologies including maturity indices.
2. Storage, packaging including cold storage, transport, juice extraction, product development diversification and self life studies.
3. Possibilities of using the extract of various parts of Noni as Noni based plant products for management of pest and diseases as well as growth hormones.
4. Exploring the possibilities of using by product after extraction juice such as cattle, poultry and fishmeal.
5. Enriching functional foods with Noni fruit juice for infant foods, food for convalescents.

References

Antony Selvaraj, S.L. Manu Kamath and Subramani, J. 2006. Micro propagation of *Morinda citrifolia* L., *Int. J. of Noni Research* 1 (2):4-9.

Amitabha Kar, Tribhuwan Singh, Ashwini Kumar Singh and Ashok Tripathi 2011.Cultivation of Noni with Vegetable Intercropping by Adopting Drip Irrigation in Jaunpur District. *Noni Search 2011.*

Asha Sankar, M, Varna Vival, Krishnan, S. and Nybe ,E.V 2014.Influence of Organic growth promoters and growing media on Vegetative propagation of Noni, *Morinda citrifolia, L . Noni Search 2014.* (in Press).

Basavraju,T.B.and Prasanth,M.2013 .Performance of Noni(*Morinda citrifolia, L)* as a mixed crop in Coconut garden. *Proceedings of Noni Search 2013. Eight National Symposium on Noni for Sustainable Wellness. pp:36-39.*

Basseth , L. and Tramper, J. 1995. Use of non conventional media in *Morinda citrifolia* L., Cell culture. *Plant cell Tissue and Organ culture,* 43(2):93-95.

Blanco, Y. C., Vaillant, F., Perez, A.M., Reynes, M., Brill, J.M and Brat, P. 2006. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. *Journal of Food Composition and Analysis,* 19:645-654.

Brett J West, Claude Jarakae Jensen , Johannes Westendorf, 2008. A new Vegetable Oil from Noni (*Morinda citrifolia* L.) seeds. *Int. J. of Food Science & Technology,* 43(11):1988-92.

Cambie, R.C and Ash, J. 1994. *Fijian Medicinal Plants. CSIRO, Australia.*

Cardon, D. 2003. Le Monde des Teintures Naturaelles, Berlin, Paris. Chemistry 49, 4478-4481.

Chapin Melany, H., 1990 Noni. Hawaii Plant Conservation Centre of the National Resource in Tropical Botany.

Dixon, A. R., Maxmillan, H and Etkin, N. L. 1999. The transformation of Noni, a Traditional Polynesian medicine (*Morinda citrifolia* L., Rubiaceae). *Economics and Botany*, 53 (1):51-68.

Duke, J.A. 1992. Hand book of phytochemicals. Boca Raton, FL: CRC publishing.

Duncan, S.H., Flint, H.J., Stewart, C.S., 1998. Inhibitory activity of gut bacteria against *Escherichia coli* O157 mediated by dietary plant metabolites. F*EMS Microbiology Letters,* 164 : 258-283.

Elkins, R. 1997. Hawaiian Noni (*Morinda citrifolia* L.), Pleasant Grove, UT, Woodland Publishing.

Elkins, R. 2000. Polynesian Noni (*Morinda citrifolia* L.), Pleasant Grove, UT, Woodland Publishing.

Gerlach, J. 1996. Native or introduced plant species? *Phelsuma*. 4: 70-74.

Hartman, H.T. and Kester, D.E. 1983. Plant Propagation-Principles and Practices. Prentice Hall, Englewood chiffs, New Jersy.

Hartmann, H.T., Kester, D.E., Davies, I.T. and Genere, R.L., 2002. Plant Propagation : Principles and Practices, Seventh Practices Hall Inc. USA. pp: 362.

Johanssen, J.T. 1994. The genus *Morinda*, Rubiaceae in New Caledonia : Taxonomy and phylogeny, *Opera Botanica*, 46 : 241- 256.

Krauss, B. 1993. Plants in Hawaiian culture. Honolulu: University of Hawaii press. p103.

Mabberly, D. J. 1997. The Plant–Book : A Portable Dictionary of Vascular Plants., Secon Edit. Cambridge University press. Cambridge, UK.

Maheswarappa, H.P., Dhanapal,R., and Geetha, P.,2010.Influence of different types of planting materials and planting geometry on growth and yield of Noni, *(Morinda citrifolia, L)*grown as mixed crop in coconut garden under Littoral soil. *Noni Search 2010-Proceedings of Fifth National Symposium on Noni for Global Wellness, Chennai, October 1-3 , 2010, pp:142-*

149.

Maheswarappa, H.P., Dhanapal,R.,2011.Effect of typesof planting material and planting geometry on growth and yield of Noni *(Morinda citrifolia, L)*grown as mixed crop in coconut garden under Littoral sandy soil. *Proceedings of sixth National Symposium on Noni A Panacea for wellness, Chennai, October 1-2 ,pp.396.*

Marimuthu, T. and Nakkeeran, S. 2007. Cues for ISR and Trophic Interactions of PGPR and their Possible applications for the Management of Pests and Diseases of Noni (*Morinda citrifolia* L.) - *Souvenir and Abstracts - Noni Search 2007: 40.*

Mathivanan, N., Surendiran, G., Surendiran, K., Sagadevan, E and Malarvizhi, K. 2005. Review on the current scenario of Noni research; Taxonomy, distribution, chemistry, medicinal and therapeutic values of *Morinda citrifolia* .L Int.J.Noni Res.1 (1):1-16.

Mathivanan. N 2007. Pest control strategies for Noni (*Morinda citrifolia* L.) present and future perspectives - Souvenir and Abstracts - Noni Search 2007.

Mathivanan. N and Surendiran, G. 2006. Chemical and biological properties of *Morinda* spp. - Proc. *Nat.Sym.on Noni Research*: 48 - 62.

McClatchey, W. 2002. From Polynesian healers to health food stores: Changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integral Cancer Therapy* 1, 110-120.

McClatchey, W. C., 2002 a. Diversity of Growth Forms and Uses in the *Morinda citrifolia* L., Complex:Proc. of the 2002 Hawaii Noni Conference, S.C.Nelson (ed) University of Hawaii at Manoa, College of Tropical Agriculture and Human Resources, pp.5-10.

Morton, J.F. 1992. The ocean-going Noni, or Indian Mulberry *(Morinda citrifolia*, Rubiaceae) and some of its 'colorful' relatives. *Economic Botany*. 46: 241-56.

Murdiatia, T.B., Adiwinata, G. and Hildasari, D. 2000. To trace the active compound in Menkudu (*Morinda citrifolia*) with Anthelmintic activity against Haemonchry contortus. *Journal ilmu termak dan veteriner*, 5: 4,255-259.

Muthu, G and Mathan, K. 2006. Effect of Sea weed *Ascophyllum nodosum* extract (Bio zyme) along with conventional treatments, on seed germination of *Morinda citrifolia L . Int. J. Noni Res.* 1(2) :10-13.

Nath ,V., Pandey, V.,Verma R.K.Misra , A., and Singh D.R.2009. Effects of irrigation levels and mulching on growth of young noni plants under light soil conditions. Proceedings of Fourth *National Symposium on Noni for Empowerment and Prosperity, Chennai, October24-25, 2009. pp:268.*

Nanda, K.K., Kumar, P and Kocher, V. K. 1974. Role of auxins, antiauxins and phenols in the production and differentiation of callus on stem cuttings of Populus robusta. *N.Z.J.sci. 4 (2); 338-346.*

Nanda. K.K. 1970. Investigation on the use of auxins, antiauxins in the vegetative propagation of forest plants. *Final report of PL480 Research Project. A7-FS-11:1-215.*

Nelson, S. C. 2005. *Morinda citrifolia* L., Species profile for Pacific Island Agro forestry Version 1.2., (link active May 23,2005).

Nelson, S.C. 2001. Noni cultivation in Hawaii. Fruit and Nuts 4, 1-4.

Nelson, S.C. 2002. Noni cultivation in Hawaii. Uni. of Hawaii CTAHR-Cooperative Extension Service.

Nelson, S.C., 2003. Noni Cultivation and Production in Hawaii. In: Proceedings of the 2002 Hawaii Noni Conference. University of Hawaii at Nanoa. College of Tropical Agriculture and Human Resources. Hawaii.

Nishikimi, M., Rao, N.A. and Yagi, K. 1972. The occurrence of super oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. *Biophysics. Res. Commun., 46: 849-54.*

Palanichame, Ma 2008. Organic noni path – Progress and prospects . Noni Search 2008. *Proc. Third National Symposium on Noni for Health and Wellness. pp.45-48.*

Pan, C, Zhan, R., Ding, R., and Xu, H. 2002. Effect of various organic fertilizers on growth and mineral nutrient absorption of *Morinda officinalis. OCT. 25(10):699-701.*

Peter, K. V. 2007. Under utilized and under exploited Horticultural Crops.Vol.1.edited by K.V. Peter, New India Publishers , New Delhi.pp.378

Ponnaiyan, C and Vezhavendan, S. 2005. The effect of hot water and sulphuric acid treatment on seed germination of *Morinda citrifolia* L. *Int. J. Noni Res.,* 1(1):37.

Ponnaiyan, P. and Vezhavendan, S. 2005a. The effect of growth regulators and its combination with nicking on the germination of Indian Mulberry (*Morinda citrifolia* L.) *Int. J. Noni Res.,* :23-26.

Rethinam, P and Sithanantham, S. 2008. Sustainable Management of Bio diversity and Productivity of Medicinal Plants: A Case Study of Noni. (Abst). *In Souvenir and Abstract. World Congress of Integrated Medicines, 23-26, March, 2008. Chennai:p62.*

Rethinam, P and Sivaraman, K. 2007. Noni (*Morinda citrifolia* L.) - A Holistic Review. *Int. J. Noni Res.* 2 (1-2) : 1-34.

Rethinam, P. 2008. Noni (*Morinda citrifolia* L.) Production-A Global Review. *Paper presented in Noni Search 2008, Oct. 18 and 19, NewDelhi.*

Rethinam, P. 2008. Noni Training Manual. Personal Communication,

Singh ,D.R., Srivatsava, R.C., and Damodaran, T. 2007. Effect of Growth regulators on rooting of hollow and non hollow cuttings of *Morinda citrifolia* L. *Noni Search 2007. Proc. Second National Symposium on Noni for Health and Wellness. pp.57-61.*

Singh D.R., Srivastava, R.C., and Subhash Chand, 2008. Noni, *Morinda citrifolia* L. A review of agricultural research, Noni Search. *Third National Symposium, 18-19 October, 2007 pp.81.*

Singh D.R., Srivastava, R.C., Subhash Chand and Abhay Kumar Srivastava, 2006. *Morinda citrifolia* L., - An Evergreen plant for diversification in commercial horticulture. *Proc. Nat. Sym. on Noni Research pp: 9 - 27.*

Singh D.R., Srivastava, R.C., Subhash Chand and Abhay Kumar Srivastava, 2007. *Morinda citrifolia* L., - an evergreen tree for diversification in Commercial Horticulture Monograph on Noni (First Published in 2007), published by WNRF Chennai pp.18-33.

Singh, D.R., Rai, R.B and Singh, B. 2005. The Great Morinda- a potential under utilized fruit for Tsunami affected areas in Bay Islands .UTS's Voice, Port Blair, April 16-30 pp 21.

Singh, D.R..,Srivatsava, R.C.and George,Z.2009.Production of quality planting materials for *Morinda citrifolia*, L. *Fourth National Symposium on Noni Research 2009Noni for Empowerment and Prosperity, October, 24-25, 2009. pp.43-60.*

Singh, A. K., Singh, S., Joshi, H. K., Bagle, B. G., More, T. A., 2008. Survey, identification, collection and evaluation of Morinda species under semi-arid ecosystem of Gujarat. *Noni search, Third National Symposium, 18-19, October, pp. 57-58.*

Singh,A.K., Singh,S.,Joshi, H.K.and Sisodia, P.S. 2011. Standardisation of propagation technique in *Morinda tomentosa* Meyne exRoth. *Proceedings of sixth National Symposium on Noni A Panacea for wellness, Chennai, October 1-2 ,pp.396.*

Singh, D. R., Sudha, R., Pandey, V.B., Jayakumar, V., Srivastava, R.C and Damodaran, T. 2007. Influence of Noni (*Morinda citifolia* L.) herbal extracts on growth and production of chillies, *Capsicum annum* var. Arka Lohit. *Souvenir and Abstracts – Second National Symposium on Noni Research, October, 27-29, 2007 pp.39.*

Singh, D.R , Medhi, R.P., Manju, S and Alvira D' Souza. 2006. Seed germination studies on *Morinda citrifolia* L. *Int. J. Noni Research*, 1 (2) :23-28.

Singh, D.R and.Rai, R.B. 2005. Effect of growth regulators in rooting of stem cuttings of *Morinda citrifolia,*L. *var.citrifolia* in Bay Islands. *Int. J. Noni Res.* 1(1):17-22.

Singh, D.R. and Rai, R.B. 2005. Influence of Gibberellic acid on seed germination in Noni (*Morinda citrifolia* L.) of Andamans. *Int. J. Noni Res.* 1(1):31-35.

Singh, D.R. and Srivastava, R.C. 2007. Studies on growth and development stages of *Morinda citrifolia* (Abstract Poster). *Second National Symposium on Noni Research, October 27-28, 2006, pp.53.*

Singh, D.R., 2006. Commercial production of Noni possible in A & N Islands. UTS Voice, Sept. 1-15. pp.6.

Singh, D.R., 2007. Under utilized fruit crop of Andaman and Nicobar Islands, Under utilized and under exploited horticultural crops, Published by New India Publishing Agency, New Delhi (India) pp: 11-54.

Singh, D.R., Amit Srivastava, and Srivastava, R.C. 2008. Genetic diversity and analysis of *Morinda citrifolia* accession across Andaman and Nicobar using RAPD primer and marker. *Noni search, Third National Symposium, 18-19 October, pp.57.*

Singh, D.R., and Rai, R.B. 2007. *Morinda citrifolia* – An important fruit tree of Andaman and Nicobar Islands. *Nat. Pro. Rad. Vol 6(1), National Inst. of Science. Comm. And Inform. Reso. CSIR., New Delhi. Pp 62-65.*

Singh, D.R., Medhi, R.P., Manju, S and Alvira D' Souza. 2006. Morphological and compositional studies of *Morinda citrifolia* grown in Andaman and Nicobar Islands. *International J. Noni Res. 1 (2): p: 14-17.*

Singh, D.R., Medhi, R.P., Manju, S and Prasant kumar Sikdar. 2006. Floral characters of *Morinda citrifolia* in Bay Islands., *International. J. Noni Res. 1 (2): 1-3.*

Singh, D.R., Rai, R.B and Singh, B. 2005. Great Morinda- A potential under utilized fruit grown in Bay Islands. *The Daily Telegrams*, Port Blair. pp:2.

Singh, D.R., Srivastava, R.C and Jai Sunder. 2009. Bibiliography on Noni. Pub. Central Agricultural Research Institute, Port Blair, Andaman & Nicobar Islands, India.

Singh, D.R., Srivastava, R.C and Raja, R. 2007. *Morinda citrifolia* – A viable alternative for Tsunami affected agricultural lands (Abstract-poster). *Second National Symposium on Noni Research, October 27-28, 2006, pp.53.*

Singh, D.R., Srivastava, R.C and Raja, R., and Manju, S. 2008. Salt tolerance of *Morinda citrifolia* by using sodium chloride as irrigation water during seedling growth, *Noni Search, Third National Symposium, 18-19 October, 2007, pp.81.*

Singh, D.R., Srivastava, R.C., 2008. The Noni. *Published by Central Agricultural Research Institute, Port Blair, pp.1-40.*

Singh, D.R., Srivastava, R.C., and Jai Sunder. 2009. *Bibiliography on Noni. Central Agricultural Research Institute. pp.144.*

Singh, D.R., Srivastava, R.C., George, Z., and Subhash Chand, 2008. Development of protocol for packaging and shipment of *Morinda citrifolia. Noni search, Third National Symposium, 18-19 October, 2007, pp.68.*

Singh, D.R., Srivastava, R.C., Sudha, R., and Abhay K. Srivatsava. 2007. Studies on shelf life and bio chemical changes of *Morinda citrifolia* L. (Noni) fruits during storage. *Noni Search 2007. Proc. Second National Sym. on Noni for Health and Wellness. Edit. P.Rethinam:84-92.*

Singh, D.R., Srivastava, R.C., Sudha, R'and Damodaran, T. 2007. Effect of pre-sowing treatment on seed germination and seedling vigour in *Morinda citrifolia* L.var. *citrifolia* an indigenous medicinal plant suitable for Tsunami affected land. *Noni Search 2007. Proc.Second National Sym. on Noni for Health and Wellness. Edit. P.Rethinam. pp.62-70.*

Singh, D.R., Sudha, R., Pandey, V.B., Abhay Kumar Srivastava, 2007. Studies on shelf life and Biochemical changes of *Morinda citrifolia* L., fruits during storage (Abstract). *Second National Symposium on Noni Research, October 27-29, 2006, pp.42.*

Singh, D.R., Zamir Ahmad, S.K. and Srivastava, R.C. 2007. A study involving the production of *Morinda citrifolia* (Noni) by small farmers in A & N Islands (Abstract). *Second National Symposium on Noni Research, October 27-28, 2006 pp.27-35.*

Singh,D.R., Srivatsava,R.C.and George, Z.2009.Production of quality planting materials for *Morinda citrifolia L. Proceedings of Fourth National Symposium on Noni for Empowerment and Prosperity, Chennai, October24-25, 2009. pp: 268.*

Singh, J. and Tiwari, R.D. 1976. Flavones glycosides from the flowers of *Morinda citrifolia. J. Indian Chem. Soc., 53: 424.*

Singh, R. S., Singh, D. R and Srivastava, R. C. 2006. Agro-climatic requirements and Potential for Noni (*Morinda citrifolia* L.) cultivation in Andaman and Nicobar Islands of India (Abstract). *First National Symposium on Noni Research, October 7-8, 2006, pp.9-17.*

Singh, R. S., Singh, D. R and Srivastava, R. C. 2007. Climatic suitability for cultivation of Noni (*Morinda citrifolia*) in different parts of India (Abstract). *Second National Symposium on Noni Research, October 27-28, 2006, pp.55.*

Singh, R. S., Singh, D. R., Mall, R. K and Indrasen Singh, 2008. Emergence and growth of Noni Seedlings under sub-tropical and dry sub-humid climate of Varanasi (UP) in India, Noni search, *Third National Symposium, 18-19 October, 2007, pp.82.*

Singh, R.S and Singh, D.R. 2010.Growth nad fruit yield in relation to ambient temperature and relative humidity under different natural light and shading treatments of Noni (*Morinda citrifolia*) in sub tropical climatic regions of India. *Proceedings of First World Noni Congress. , 1-3 October , 2010, pp:544.*

Spiegel, P. 1954. Auxins and inhibition in canes of vitis. *Bull. Res. Council. Israel.*

Srivastava, M and Singh, J. 1993. A new anthraquinone glycoside form *Morinda citrifolia* L. *Journal of Pharmacology,* 31, 182-184.

Srivastava, R. C and Singh, D. R. 2007. Creating livelihood through technological interventions in enhancing productivity of *Morinda citrifolia* L. Noni search, *Proceedings of the second National Symposium on Noni for Health and wellness, pp.7-17.*

Srivastava, R. C and Singh, D. R. 2008. Cultivate *Morinda citrifolia* (Noni) as livelihood option in Bay islands. *The Daily Telegraph*. Sunday, April 20, 2008 pp.3.

Subash Chand, Singh, D.R and Srivastava, R. C and Kanakalata, 2008. Popularization of *Morinda citrifolia* cultivation in Bay islands : efforts of CARI, Port Blair, *Noni search, Third National Symposium, 18-19 October, pp.83.*

Subash Chand, Singh, D.R and Srivastava, R.C. 2007. Economical Appraisal of *Morinda citrifolia*, L. (Noni) cultivation in Bay Islands. *Noni Search 2007. Proc. Second National Sym. on Noni for Health and Wellness. Edit. P.Rethinam. WNRF .Publication : 36-43.*

Subramani, J., Antony Selvaraj, S., Vijay, D and Sakthivel, M. 2007. Micro propagation, field evaluation and cell culture study of *Morinda citrifolia* L. - Souvenir and Abstracts - Noni Search 2007 : 31.

Subramani, J., Antony Selvaraj, S., Vijay, D., Saktivel, M and Umashanthi, M. 2006. Micro propagation of *Morinda citrifolia* L. noni Search 2006- Proc.First National Symposium on Noni Research, Hyderabad, pp. 40-47.

Subramani,J. 2008. Callus and cell suspension studies of *Morinda citrifolia*, Noni search, Third National Symposium, 18-19 October, pp.58.

Sudha R and Singh, D.R. 2007. Effect of growth regulators on rooting of hollow and non hollow cuttings of *Morinda citrifolia (*Abstract) in Second National Symposium on Noni Research. pp.52-53.

Sudha, R and Singh, D.R. 2007. Effect of pre sowing Treatment on Seed Germination and Seedling Vigour of *Morinda citrifolia* L. In Noni Search 2007. Souvenir and Abstracts:pp:54

Surendiran. G and Mathivanan, N. 2006: An overview of the potentials of *Morinda citrifolia* L. - Proc. *Nat. Sym.on Noni Research 2006:1 - 8.*

Tabrah, F.L and Eveleth, B. M. 1966. Evaluation of the effectiveness of ancient Hawaiian medicine. Hawaii Med.J.25:223 30.

Veena Gupta and Sharma, S. K. 2008. Genetic Resources of Noni, *Morinda citrifolia* L. Abstract. Noni Search 2008. *Third National Symposium on Health and Wellness. Souvenir and Abstract :56-57.*

Vezhavendan, S and Ponnaiyan, P. 2005. An approach to obtain true Noni through cuttings. *Int. J. Noni Res.1(1):27-30.*

Vishal Nath,Pandey,V. , Rajesh KumarVerma, Ajay Misra and Singh,D.R. 2009.Effectofdrip irrigation level and mulching on growth of young Noni plants under light soil conditions. Proceedings of Fourth National Symposium, Noni for Empowerment and Prosperity, *Noni Search 2009*.:pp.65-72.

Vishal Nath, Pandey, V., Rajesh Kumar Verma, Ajay Misra and Singh, D.R. 2011. Response of drip irrigation and organic mulching on growth of *Morinda citrifolia, L (*Noni) plants. *Proceedings of Sixth National Symposium, Noni – A Panacea for Wellness, Noni Search 2011.:pp.396.*

Wagner,W.L., Herbst, D.H. and Sohmer, S.H. 1999. Manual of flowering plants of Hawaii (Revised Edition), University of Hawaii Press, Honolulu.

Wang, M.Y., West, B. J., Jensen, J., Nowicki, D., Su, C., Palu, A and Anderson, G.2002. *Morinda citrifolia* L. (Noni):A literature review .

Wright, R. C. M. 1975. The Complete Hand Book of Plant Propagation, Mc Milan, New York.

II-OP-1

Influence of organic growth promoters and growing media on vegetative propogules of noni (*Morinda citrifolia* L.)

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Abstract

In an experiment to ascertain the type of cuttings to be used as vegetative propogules in noni and the influence of organic growth promoters and growing media on their performance, it was observed that five noded semi hardwood cuttings and 2-3 noded hollow stem cuttings performed with better respect to the parameters studied. Dipping in seventy percent cowdung slurry for ten minutes recorded significantly higher percentage of sprouting in semi hardwood cuttings and hollow stem cuttings. In semi hardwood cuttings, pulse dip in charcoal slurry registered a sprouting percentage, on par with that of cowdung slurry dip. Basal dip in sugar solution registered significantly lower values for shoot parameters. Longest sprouts and highest number of leaves after sprouting, were produced on basal dip in cowdung slurry. Dipping in noni fruit juice recorded values on par with the best treatment, with respect to length of sprout, in hollow stem cuttings. Dipping in cowdung slurry and charcoal slurry was superior to control and rest of the treatments, in root characteristics. Supplementing Azospirillum to standard potting mixture and vermicompost based potting mixture and enriching standard potting mixture with the plant growth promoting rhizobacterium, PGPR-1, recorded significantly higher sprouting percentage in both type of cuttings. Standard potting mixture supplemented with Azospirillum in PGPR-1 singly and PGPR-1 along with Arbuscular mycorrhizal fungi recorded significantly higher percentage of establishment. Significantly higher mean number of roots was produced by cuttings raised in standard potting mixture supplemented with PGPR-1 and AMF, while longest roots were produced in potting mixture enriched with PGPR-1 singly and along with AMF.

Keywords : *Morinda citrifolia,* semi hardwood cuttings, hollow stem cuttings, organic growth promoters, Azospirillum, plant growth promoting rhizobia.

Introduction

Morinda citrifolia commonly known as Indian Noni is one of the most therapeutically effective medicinal species. About 160 phytochemicals have been identified in the plant, xeronine being the principal alkaloid (Heinicke, 1985). Noni can be propagated by seeds, stem cuttings and air layering. Noni seeds have a problem of dormancy due to hard seed coat, thus limiting its commercial cultivation. Hence vegetative propagation of the species assumes significance not only to generate true to type propagules from elite plants, but also as a quick method of regeneration. Available reports suggest the use of synthetic growth regulators like NAA and IAA for quick and better rooting of noni cuttings (Singh and Rai, 2005).

Agricultural practices are moving towards a more sustainable and environmental friendly approach. When increasing environment damage is considered, it is necessary to reexamine many of these existing approaches in agriculture that include use of the synthetic growth hormones in propagation of crop plants. Employing organic growth promoters and microbial associations has become an important consideration in low input organic agriculture. Bioaugmentation of potting mixture by appropriate microbes and organic amendments has been employed with varying degrees of success in vegetative propagation of crop plants (Jeffries *et al*., 2003). Keeping in tune with the global shift towards organic agriculture, the present study was taken up to assess the influence of organic growth promoters and growing media on vegetative propagation of this versatile medicinal species, leading to the generation of quality planting materials.

Materials and Methods

The experiments were conducted at Regional Research Centre for Noni, Parlikkad, Thrissur, Kerala of World Noni Research Foundation and Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during June 2013. The study was conducted in three sub experiments.

Sub experiment 1: Standardisation of type of cuttings to be used as vegetative propagules.

To identify the best propagules of noni for vegetative propagation, softwood, semi hardwood and hardwood shoots of eight year old *Morinda citrifolia* trees were used to raise cuttings. The sub experiment was laid out in a Completely Randomized Design with nine treatments as given below, in three replications, with 10 plants in each replication.

Treatments

T1 - Two noded softwood cuttings

T2 - Three noded softwood cuttings

T3 - Three noded semi hardwood cuttings

T4 - Four noded semi hardwood cuttings

T5 - Five noded semi hardwood cuttings

T6 - Three noded hardwood cuttings

T7 - Four noded hardwood cuttings

T8 - Five noded hardwood cuttings

T9 - 2-3 noded hollow stem cuttings

Cuttings were planted in black polythene bags of size 8" x 7" filled with potting mixture, comprising of sand, soil and farmyard manure in the ratio1:1:1 and kept under 50 per cent shade in a shade net house. Cuttings were watered daily. Periodic weeding was carried out. Five plants under each treatment, in each replication, were selected as observational plants. Observations were recorded on the following parameters.

1. Earliness in sprouting : Date of emergence of first sprout was recorded and the mean calculated.

2. Percentage of sprouting : Out of the total number of cuttings planted, the number of cuttings sprouted was counted and expressed as percentage.

3. Length of sprout : Length from the base of the sprout to apex was taken and mean length expressed in centimeters.

4.	Number of leaves	:	Total number of leaves produced, 14 days after sprouting, under each treatment. was counted and the mean calculated.
5.	Percentage of establishment	:	Number of sprouted cuttings established. were counted and expressed as percentage.
6.	Number of leaves after establishment	:	Total number of leaves per cuttings, after establishment were counted. in each treatment and the mean calculated.

Sub experiment 2: Standardization of basal dip treatments of cuttings of noni using organic growth promoters.

The type of cuttings identified as best in Sub experiment 1 were subjected to various basal dip treatments by dipping the basal 2 cm of cuttings in various organic growth promoters as given below. The trial was laid out in a Completely Randomized Design with eight treatments and three replications, with ten cuttings per replication, in each treatment.

Treatments

T1- Dipping in cow dung slurry (75 per cent) for 10 min

T2- Dipping in tender coconut water (100 per cent) for 30 min

T3- Dipping in tender coconut water (50 per cent) for 30 min

T4- Dipping in panchagavya (100 per cent) for 10 min

T5- Dipping in Noni fruit juice (100 per cent) for 10 min

T6- Dipping in charcoal slurry (75 per cent) for 10 min

T7- Dipping n sugar solution (2 per cent) for 10 min

T8- Control

After basal dip treatments cuttings were raised as detailed in Sub experiment 1. Observations on the following parameters were taken after selecting 5 observational plants in each treatment.

Observations recorded

1 to 6	:	As given in Sub experiment 1
7. Length of roots	:	Length of longest root was measured for each observational plant and the mean calculated.
8. Number of roots	:	Total number of roots emerged in each plant was recorded and the mean calculated.

Sub experiment 3 : Standardisation of organic growing media for raising vegetative propagules of noni

The type of cuttings identified as best in sub experiment 1 were planted without any basal dip in black polythene bags of size 8" x 7", filled with various growing media as given below. The sub experiment was conducted in a completely randomized design with 11 treatments and three replications with 10 cuttings per treatment in each replication. Cuttings were raised as detailed in sub experiment 1.

Treatments

T1- Standard potting mixture (sand+soil+FYM - 1:1:1)

T2- Standard potting mixture+Azospirilum, (10 g per bag)

T3- Standard potting mixture+neem cake (sand+soil+FYM+neem cake – 1:1:1:0.25)

T4- Standard potting mixture+neem cake+ Azospirilum (10 g per bag)

T5- Vermicompost based potting mixture (sand+soil+vermicompost – 1:1:1)

T6- Vermicompost based potting mixture (sand+soil+vermicompost – 1:1:0.5)

T7- Vermicompost based potting mixture + Azospirilum (10 g per bag)

T8- Standard potting mixture+ Plant Growth Promoting Rhizobacteria (PGPR 1) (10 g per bag)

T9- Standard potting mixture + Arbuscular Mycorrhizal Fungi (AMF) (6 g per bag)

T10- Coir pith compost based potting mixture (soil + coirpith compost + FYM - 1:1:1)

T11- Standard potting mixture + PGPR 1 (10 g per bag) + AMF (6 g per bag)

Commercial formulations of microbial inoculants were used in the study. The microbial inoculants were applied at the centre of polybag at 5 cm depth, in the respective treatments. Five plants under each treatment in each replication were selected as observational plants and observations were recorded on parameters as detailed in Sub experiment 1 and 2

Statistical analysis:- MSTAT package was followed for statistical analysis. Data were analysed by technique of Analysis of Variance and significance was tested by Duncans Multiple Range Test.

Results and Discussion

Sub experiment 1: Standardisation of type of cuttings to be used as vegetative propagules in noni

Significant variation was noted among type of cuttings, with respect to per centage of sprouting (Table 1). Two noded softwood cuttings recorded significantly higher percentage of sprouting (80 per cent) followed by three noded softwood cuttings and 2-3 noded hollow stem cuttings, which recorded 76.67 per cent sprouting, all values being on par with one another. Five noded semi hardwood cuttings recorded 73.33 per cent sprouting on par with best treatment. Three noded softwood cuttings were significantly earlier to sprout (10 days) followed by two noded softwood cuttings and 2-3 noded hollow stem cuttings (11 days and 11.33 days respectively). However, softwood cuttings failed to sustain the sprout and rotting commenced. Hardwood cuttings sprouted late, registering 14 days for 4 noded and 13.33 days for 5 noded cuttings.

Table 1: Standardization of type of cuttings to be used as propagules in noni

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots at 6 MAP (cm)	No. of roots at 6 MAP
Soft wood two noded	80.00a	11.00cd	Nil	Nil	Nil	Nil	Nil	Nil
Soft wood three noded	76.67a	10.00d	Nil	Nil	Nil	Nil	Nil	Nil

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots at 6 MAP (cm)	No. of roots at 6 MAP
Semi hardwood three noded	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Semi hardwood four noded	50.00b	12.00bc	1.03c	2.07b	36.67c	Nil	Nil	Nil
Semi hardwood five noded	73.33 a	12.00bc	1.51a	2.40ab	70.00a	10.27b	16.59b	9.53b
Hardwood three noded	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Hardwood four noded	30.00c	14.00a	0.8d	2.00b	30.00c	Nil	Nil	Nil
Hardwood five noded	53.33b	13.33ab	1.22b	2.27ab	50.00b	6.10c	13.01c	7.20c
Hollow two-three noded	76.67a	11.33cd	1.25b	2.53a	73.33a	10.87a	21.32a	13.67a

Treatement means with similar alphabets in superscript, do not differ significantly

Regarding sprout length, five noded semi hardwood cuttings recorded a significantly higher sprout length of 1.15 cm, 14 days after sprouting, followed by 2-3 noded hollow stem cuttings (1.25 cm). Hardwood cuttings were inferior with respect to sprout length (Table 1). Two to three noded hollow stem cuttings were significantly superior with respect to number of leaves (2.53), 14 days after sprouting. Four noded cuttings, both semi hardwood and hardwood, were inferior with respect to number of leaves produced. None of the softwood cuttings sustained to produce leaves.

Dawson and King (1994) observed that type of cuttings employed, influenced the success of vegetative propagation of crop plants. In noni, Chandra and Gnanasagar (2013) reported that hardwood cuttings with four nodes performed better and registered higher per centage of success during vegetative propagation of noni. However, in the present study, 2-3 noded hollow stem cuttings and 5 noded semi hardwood cuttings were superior as vegetative propagules of noni, with respect to the parameters studied.

Sub experiment 2: Standardization of basal dip treatments of cuttings of noni using organic growth promoters

Basal dip treatments of semi hardwood and hollow stem cuttings of noni, with organic growth promoters, recorded significant variation among one another, with respect to the parameters studied (Table 2a). Dipping in cowdung slurry recorded significantly superior performance with respect to sprouting percentage in both semi hardwood (93.33 per cent) and hollow stem (96.67 per cent) cuttings of noni. Cowdung slurry dip registered earlier sprouting in both semi hardwood (8.67 days) and hollow stem (9 days) cuttings as well. The same treatment produced significantly higher number of leaves 14 days after sprouting as well as greater sprout length in both semi hardwood (3.67 and 2,83 cm respectively) and hollow stem (3.53 and 2.17 cm respectively) cuttings. Significantly higher percentage of establishment was obtained on cowdung slurry dip in semi hardwood (90 per cent) and hollow stem cuttings (93.33 per cent).

Table 2a: Effect of basal dip treatments on performance of semi hardwood stem cuttings of noni

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Control	73.33b (8.59)	11.67 a (3.49)	1.41b	2.4 d	70.00 b (8.39)	7.93b	9.35e	9.00 d
Cow dung slurry (75 %)	93.33a (9.68)	8.67 b (3.02)	2.83a	3.67a	90.00 a (9.51)	9.87a	19.94a	12.47 b
Tender coconut water (100%)	86.67 ab (9.33)	10.00 ab (3.24)	1.72b	2.93b	81.67 a (9.06)	8.33b	9.8e	9.80 c
Tender coconut water (50 %)	85.00 ab (9.24)	10.00 ab (3.24)	1.85b	2.93b	80.00 ab (8.97)	8.20b	9.45e	9.40 c
Panchagavya (100%)	86.67 ab (9.33)	11.33 a (3.43)	1.51b	2.47cd	78.33 ab (8.88)	8.07b	9.31e	8.93 d
Noni fruit juice (100%)	90.00ab (9.51)	10.33 ab (3.29)	1.94 b	3.00b	83.33 a (9.15)	8.20b	15.17c	10.00 c
Charcoal slurry (75%)	93.33 a (9.68)	9.33 ab (3.13)	1.53 b	3.07b	90.00 a (9.51)	8.20b	18.09b	13.87 a
Sugar solution (2 %)	76.67 b (8.73)	10.00 ab (3.24)	1.42b	2.87bc	58.33 c (7.63)	8.07b	10.77d	10.00 c

Treatement means with similar alphabets in superscript, do not differ significantly; values in parentheses indicate transformed values.

Variation was noted among basal dip treatments with respect to root characteristics in both type of cuttings (Tables 2a and 2b), wherein basal dip in cowdung slurry was significantly superior with respect to length of roots (19.94 cm in semi hardwood cuttings and 19.83 cm in hollow stem cuttings). Cowdung is traditionally used as organic manure and microbial composition of cowdung includes several bacteria like *Bacillus subtilis* and fungi like *Aspergillus* (Swain *et al*., 2007). In the same study, firm evidence was reported that indole acetic acid is produced by cowdung microflora, particularly, *Bacillus* spp, which contributes to the growth promoting activities of cowdung slurry. Beneficial effect of plastering of cut ends of sugarcane with cowdung slurry has been reported by Kesavan (2006) as well.

Table 2b. Effect of basal dip treatments on performance of hollow stem cuttings of noni

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Control	76.67^{c} (8.78)	11.33^{a} (3.44)	1.22^{c}	2.53^{c}	73.33^{cd} (8.59)	7.86^{c}	10.17^{f}	9.07^{d}
Cow dung slurry (75 %)	96.67 a (9.85)	9.00 c (3.08)	2.17 a	3.53 a	93.33 a (9.69)	11.67 a	19.83 a	12.73b
Tender coconut water (100%)	86.67 abc (9.33)	9.33 bc (3.13)	1.46 bc	3.00 b	80.00 bc (8.97)	8.33 c	11.69 d	10.20c
Tender coconut water (50 %)	86.67 abc (9.33)	9.33 bc (3.13)	1.53 b	2.87 bc	80.00 bc (8.97)	8.00 c	10.93 e	9.20 d
Panchagavya (100%)	83.33 bc (9.15)	10.00 bc (3.24)	1.46 bc	2.87 bc	78.33 bc (8.88)	7.93 c	9.92 f	9.07 d
Noni fruit juice (100%)	93.33 ab (9.68)	10.67 ab (3.34)	2.05 a	3.13 b	81.67 bc (9.06)	8.27 c	15.99 c	10.73 c
Charcoal slurry (75 %)	86.67 abc (9.33)	9.33 bc (3.13)	1.59 b	3.13 b	85.00 b (9.24)	9.40 b	18.13 b	17.80 a
Sugar solution (2 %)	80.00 c (8.97)	11.33 a (3.44)	1.42 bc	2.80 bc	68.33 d (8.30)	8.47 c	11.93 d	10.67 c

Treatement means with similar alphabets in superscript, do not differ significantly; values in parentheses indicate transformed values.

Pulse dip in charcoal slurry proved to be a promising treatment, registering significantly superior performance with respect to sprouting percentage and percentage of establishment in semi hardwood cuttings, on par with that obtained by cowdung slurry dip (Table 2 a). Charcoal slurry dip was the second best treatment with respect to earliness in sprouting in semi hardwood cuttings (9.33 days) and percentage of establishment in hollow stem cuttings (85 per cent). Regarding root characteristics, charcoal slurry dip produced significantly higher number of roots in both semi hardwood cuttings (13.87) and hollow stem (17.80) cuttings. Stimulatory effect of activated charcoal on shoot multiplication and elongation has been reported in black wattle by Quoirin *et al.* (2001), as well. Root promoting action of activated charcoal is evident in *Pinus sylvestris* (Gronroos and Arnold, 1985) and peas (Eliasson,1981), wherein activated charcoal was observed to stimulate wound tissue growth, producing short tracheids with many pores from which roots developed.

Basal dip of cuttings in tender coconut water (50 and 100 per cent), as well as noni fruit juice (100 per cent) produced values significantly superior to that of untreated control with respect to sprouting percentage, earliness in sprouting, number of leaves after sprouting in both type of cuttings and number of roots in semi hardwood cuttings (Tables 2a and 2b).

Use of coconut water as a growth promoting component can be traced to more than a half a century, wherein it was introduced as a component of nutrient medium for callus culture of crop plants. Nutritional composition of coconut water has been studied at large and it is reported to contain proteins, amino acids and sugars, vitamins, minerals and growth hormones which contribute to its growth promoting activity (George and Sherrington, 1993). They further observed that dipping stem cuttings of *Bongainvillea spectabilis* in coconut water for one hour stimulated root initiation and development.

The present study revealed the ineffectiveness of sugar solution as an organic growth promoter in vegetative propagation of noni, wherein basal dip in 2 per cent sugar solution registered significantly lower values for the parameters as compared to the rest of the treatments and values even lower than the untreated control with respect to per centage of establishment (Tables 2a and 2b). Soaking of *Salix nigra* cuttings in dilute sucrose solution before planting resulted in increased aerenchyma development, adventitious rooting and root length (Baud and Pazeskhi, 2013). In the same study it was reported that as the major photoassimilate, sucrose is transported to root

elongation zone, where it provides energy via respiration. However, shifting of resource allocation to roots in response to sucrose soaking is species dependant as observed by Baud and Pazeshki (2013), which explains the comparative non responsiveness of noni cuttings to basal dip in sucrose solution in the present study. The organic growth promoter, panchagavya, also did not prove to be promising, registering values on par with that of control with respect to root characteristics (Tables 2a and 2b) contrary to observations emphasizing the beneficial effects of panchagavya in pulses as reported by Sangeetha and Thevanathan (2010).

Sub experiment 3: Standardisation of organic growing media for raising vegetative propagation of noni

Various organic growing media employed to raise vegetative propagation in noni exhibited significant variation among one another with respect to the parameters studied (Tables 3a and 3b). Supplementing Azospirillum to standard potting mixture and vermicompost based potting mixture recorded significantly highest percentage of sprouting (96.67 per cent) in semi hardwood and hollow stem cuttings. Both semi hardwood cuttings and hollow stem cuttings registered percentage of sprouting on par with the highest value, in standard potting mixture supplemented with PGPR-1 and standard potting mixture enriched with PGPR-1 and AMF as well, in the latter. Enriching standard potting mixture with Azospirillum resulted in significantly early emergence of sprouts (8 days each) in both type of cuttings. The same treatment registered significantly longer sprouts in semi hardwood (2.49 cm) and hollow stem (2.27 cm) cuttings, the latter registering a sprout length (2.15 cm) on par with the best treatment in Azospirillum enriched vermicompost based potting mixture as well.

In semi hardwood cuttings, number of leaves, 14 days after sprouting, recorded significantly higher values in standard potting mixture supplemented with Azospirillum (3.6), PGPR-1 (3.47) and PGPR-1 along with *Arbuscular mycorrhizal* fungi (3.53), while addition of Azospirillum in standard potting mixture alone recorded significantly higher number of leaves in hollow stem cuttings (3.67). In both type of cuttings, significantly higher establishment percentages were obtained in standard potting mixture supplemented with Azospirillum and PGPR-1 singly and PGPR-1 along with AMF (Tables 3a and 3b). Regarding root characteristics, supplementing PGPR-1 singly and in combination with AMF registered significantly longer roots in semi hardwood (10.91 cm and 11.2 cm respectively) and in hollow stem cuttings (16.7 cm and 16.77 cm

respectively). Standard potting mixture supplemented with both PGPR-1 and AMF recorded significantly higher mean number of roots in semi hardwood (10.67 cm) and hollow stem (12.47 cm) cuttings.

Promotion of plant growth by Azospirillum has been reported in field and nursery plants, due to its biological nitrogen fixing capacity in rhizosphere and its efficiency in absorption of water and nutrients (Bashan and Hoguin, 1997), which explains the increased growth characters exhibited by noni cuttings raised in Azospirillum supplemented media in the present study, especially with respect to shoot characteristics. Positive response of vegetative propagules to Azospirillum has been well documented in many crops. In black pepper cuttings Azospirillum isolates significantly enhanced growth parameters, recording 67 per cent more plant height (Sangeeth *et al*., 2008). Inoculation of Azospirillum had significant positive effects on growth characteristics in Jatropha propagules as well (Ravikumar *et al*., 2011).

The other beneficial microbial supplement, Plant Growth Promoting Rhizobacteria is credited with various beneficial mechanisms, which include facilitation of resource acquisition, especially nitrogen and phosphorus by nitrogen fixation and phosphate utilization by synthesis of gluconic acid and citric acid. The bacteria mineralize organic phosphorus through synthesis of phosphates, catalyzing hydrolysis of phosphoric esters to which the beneficial effect of PGPR-1 in enhancing mean root number and root length could be attributed (Glick, 2012).

In the present study, incorporation of Vesicular Arbuscular Mycorrhizal fungi(VAM) singly, into standard potting mixture, registered values, significantly inferior than the superior treatments, with respect to the parameters observed. Contrary to this result, benefit of mycorrhization in semi hardwood cuttings of crop plants was evident in black pepper cuttings (Anandraj and Sarma, 1994) and olive cuttings (Sidhoun and Fortar, 2011). Harrig (1986) observed that mixing AMF into rooting medium changes composition of protein and amino acid during root formation in cuttings exposed to VAM inocullum. AMF is also reported to have hormonal effects in crops (Anandraj and Sarma, 1994). In the present study, however, positive effect of AMF when applied along with PGPR-1 was noted with respect to percentage of establishment and root characteristics (Tables 3a and 3b). Synergistic effect of PGPR-1 and AMF as witnessed in the present study has also been reported by Parlade *et al*. (1999).

The study revealed that noni cuttings planted in standard potting mixture without any organic supplements was significantly inferior with respect to shoot characteristics, while addition of neem cake to standard potting mixture resulted in significantly lower percentage of establishment, mean root length and mean number of roots in both semi hardwood and hollow stem cuttings. Replacing sand with coir pith compost also did not register any significant improvement in any of the parameters studied (Tables 3a and 3b).

Table 3a : Effect of potting mixture combinations on performance of semi hardwood stem cuttings of noni

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Sand +Soil+ FYM	73.33^{d} (8.59)	11.67^{ab} (3.49)	1.41^{e}	2.6^{c}	70.00^{bc} (8.39)	6.33^{d}	8.96^{cd}	7.2^{e}
Sand +Soil+ FYM + Azospirillum	96.67^{a} (9.85)	8.00^{a} (2.91)	2.49^{a}	3.6^{a}	85.00^{a} (9.24)	8.47^{ab}	9.73^{b}	9.33^{c}
Sand +Soil+ FYM + Neem cake	76.67^{cd} (8.78)	12.33^{a} (3.58)	1.83^{c}	2.6^{c}	66.67^{c} (8.19)	5.33^{e}	7.09^{e}	6.6^{f}
Sand +Soil+ FYM + Neem cake + Azospirillum	86.67^{abc} (9.33)	10.33^{cd} (3.29)	2.13^{b}	3.27^{ab}	73.33^{bc} (8.59)	7.25^{cd}	9.26^{bc}	8.73^{d}
Sand +Soil+ Vermicompost 1:1:1	80.00^{bcd} (8.96)	10.33^{cd} (3.29)	1.59^{d}	2.93^{bc}	75.00^{b} (8.68)	7.00^{cd}	9.53^{b}	7.6^{e}
Sand +Soil+ Vermicompost 1:1:0.5	76.67^{cd} (8.78)	11.00^{bc} (3.39)	1.51^{de}	2.73^{c}	73.33^{bc} (8.59)	6.54^{d}	8.75^{d}	6.73^{f}
Sand +Soil+ Vermicompost + Azospirillum	96.67^{a} (9.85)	9.33^{d} (3.13)	2.1^{b}	3.2^{ab}	83.33^{a} (9.15)	8.03^{bc}	9.55^{b}	8.8^{d}
Sand +Soil+ FYM +PGPR 1	93.33^{a} (9.68)	9.33^{d} (3.13)	2.09^{b}	3.47^{a}	86.67^{a} (9.33)	9.27^{a}	10.91^{a}	10.00^{b}

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Sand +Soil+ FYM+AMF	73.33^{d} (8.58)	10.33^{cd} (3.29)	1.45^{de}	2.6^{c}	70.00^{bc} (8.40)	7.07^{cd}	9.68^{b}	8.80^{d}
Sand + Soil + Coir pith compost	80.00^{bcd} (8.96)	10.33^{cd} (3.29)	1.77^{c}	2.73^{c}	73.33^{bc} (8.59)	6.80^{d}	9.52^{b}	7.40^{e}
Sand +Soil+ FYM +PGPR 1+ AMF	90.00^{ab} (9.51)	9.33^{d} (3.13)	2.05^{b}	3.53^{a}	85.00^{a} (9.25)	9.00^{a}	11.2^{a}	10.67^{a}

Treatement means with similar alphabets in superscript, do not differ significantly; values in parentheses indicate transformed values.

Table 3b Effect of potting mixture combinations on performance of hollow stem cuttings of noni

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Sand +Soil+ FYM	73.33^{bc} (8.59)	11.67^{ab} (3.49)	1.45^{d}	2.6^{d}	70.00^{bc} (8.40)	7.93^{e}	11.00^{e}	8.4^{f}
Sand +Soil+ FYM + Azospirillum	96.67^{a} (9.85)	8.00^{d} (2.91)	2.27^{a}	3.67^{a}	86.67^{a} (9.33)	10.33^{b}	14.61^{b}	11.27^{bc}
Sand +Soil+ FYM + Neem cake	73.33^{bc} (8.59)	12.33^{a} (3.58)	1.63^{cd}	2.73^{cd}	65.00^{c} (8.09)	5.93^{g}	7.99^{g}	7.6^{g}
Sand +Soil+ FYM + Neem cake + Azospirillum	76.67^{b} (8.78)	10.33^{bc} (3.29)	1.87^{bc}	3.07^{bc}	68.33^{c} (8.29)	9.67^{c}	13.51^{c}	9.47^{de}
Sand +Soil+ Vermicompost 1:1:1	81.67^{b} (9.06)	11.33^{ab} (3.43)	2.05^{ab}	3.00^{bcd}	73.33^{b} (8.59)	8.93^{d}	11.13^{e}	8.8^{ef}
Sand +Soil+ Vermicompost 1:1:0.5	78.33^{b} (8.88)	12.03^{a} (3.53)	1.71^{c}	2.87^{bcd}	71.67^{bc} (8.49)	7.13^{f}	9.64^{f}	8.00^{fg}

Parameters	Percentage of sprouting	Earliness in sprouting (days)	Length of sprout (cm)	No. of leaves	Percentage of establishment	No. of leaves after establishment	Length of roots (cm)	No. of roots
Sand +Soil+ Vermicompost + Azospirillum	96.67^{a} (9.85)	9.00^{cd} (3.08)	2.15^{a}	3.20^{b}	83.33^{a} (9.15)	9.93^{bc}	13.87^{c}	10.67^{c}
Sand +Soil+ FYM + PGPR 1	96.67^{a} (9.85)	9.00^{cd} (3.08)	2.08^{ab}	3.13^{bc}	86.67^{a} (9.33)	11.33^{a}	16.7^{a}	12.00^{ab}
Sand +Soil+ FYM+AMF	78.33^{b} (8.87)	10.33^{bc} (3.29)	1.76^{c}	2.73^{cd}	73.33^{b} (8.59)	8.99^{d}	12.73^{d}	9.72^{d}
Sand + Soil + Coir pith compost	66.67^{c} (8.19)	12.67^{a} (3.63)	1.65^{cd}	2.73^{cd}	66.67^{b} (8.19)	8.8^{d}	11.23^{e}	8.60^{f}
Sand +Soil+ FYM + PGPR 1+AMF	93.33^{a} (9.69)	9.33^{c} (3.13)	2.07^{ab}	3.07^{bc}	86.67^{a} (9.33)	9.87^{bc}	16.77^{a}	12.47^{a}

Treatement means with similar alphabets in superscript, do not differ significantly; values in parentheses indicate transformed values

The results obtained by Singh *et al*. (2012) in *Coleus forskohlii* cuttings wherein significant increase in shoot length was observed on incorporation of neem cake in potting mixture and Reghuvaran and Raveendranath (2010) in *Piper longum* who identified coir pith compost as an effective potting medium do not support the observations of the present study. However the results obtained in the present study confirm to the results reported by Venkatesan *et al*. (2010), who observed inferior performance of *Gymnema sylvestre* cuttings in neem cake incorporated growing medium.

Also, effectiveness of coir pith compost is dependent on the microbial species used for degradation of coir pith as observed by and Reghuvaran and Raveendranath (2010), which explains the ineffectiveness of coir pith compost as a potting medium in the present study. The present study standardized a sustainable technology for generation of quality planting materials in noni, employing organic growth promoters and amendments and utilizing beneficial plant microbe interactions, eliminating the use of synthetic growth hormones.

References

Anandraj, M. and Sarma, Y.R. 1994. Effect of Vesicular Arbuscular Mycorrhiza on rooting of black pepper (*Piper nigrum* L) *Journal of Spices and Aromatic crops,* 3(1): 39-42.

Bashan, Y. and Holguin, G. 1997. Azospirillum – plant relationships environmental and physiological advances. *Canadian Journal of Microbiology,* 43: 103-121.

Baud, D.R. and Pazeshki, S.R. 2013. Adventitious rooting response in *Salix nigra* and *Acer negundo* cuttings to exogenous sucrose. *Environment Ecology and Management*, 2(1): 44-45.

Chandra, K.J. and Gnanasagar, D.D. 2013. A review on the propagation methods of a miracle fruit *Morinda citrifolia*. *Indian Journal of Plant Sciences,* 2(2): 78-84

Dawson, I.A. and King, R.W. 1994. Propagation of some woody Australian plants from cuttings. *Australian Journal of Experimental Agriculture,* 34:1225-123.

Eliasson, H.1981. Factors effecting the inhibitory effect of indolyl acetic acid on root formation in pea cuttings. *Physiologia Plantarum,* 51(1): 23-26.

Glick, S. 2012. Plant growth promoting bacteria: Mechanisms and Applications. *Scientifica Article* ID 963401.

Gronroos, R. and Arnold, S. 1985: Initiation and development of wound tissue and roots on hypocotyls cuttings of *Pinus sylvestris Physiolgia Plantarum,* 64(3): 393-401.

Hassig, B.E. 1986. Metabolic process in adventitious rooting of cuttings. In Jackson M.B (ed.) *New root formation in plants' own cuttings*. Martins Nijholf Publication, Lancaster pp 141-159.

Heincke, R.M. 1985. The pharmacologically active ingredients of noni. *Bulletin of the National Tropical Botanical Garden,* 15: 10-14.

Jeffries, P., Gianinazzi, S., Peretto, S., Turnan, K. and Barea, J. 2003. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils,* 37(1): 16.

Kesavan, P.C. 2006 from green revolution to evergreen revolution: pathways and terminologies. *Current Science,* 90: 45-46.

Parlade, J., Pera, J. Alvarez, I. F., Bouchard, G., Genero, B. and Tacon, F. 1999. Effect of inoculation and substrate disinfection method on rooting and ectomycoorhiza formulation of Douglas fir cuttings. *Annals of Forest Science*, 56: 35-40.

Ravikumar, S, Syed Ali, M. and Valliammal, N. 2011. Biofertiliser effect of halophilic azospirillum on growth of *Jatropha curcus* L seedlings. *Annals of Biological Research,* 2(2): 153-157.

Reghuvaran, A. and Ravindranath, A.S. 2010. Efficiency of biodegraded coir pith for cultivation of medicinal plants. *Journal of Scientific and Industrial Research,* 69: 554-559.

Sangeeth, K.P., Susheela Bhai, R. and Srinivasan, V. 2008. Evaluation of indigenous Azospirillum isolates for growth promotion in black pepper (*Piper nigrum* L) rooted cuttings. *Journal of Spices and Aromatic crops,* 17(2): 128-133.

Sangeetha, V. and Thevanathan, R. 2010. Biofertilizer potential of traditional and panchagavya amended with sea weed extract. *The Journal of American Science,* 6(2): 61-67.

Sidhoun, W. and Fortas, Z. 2011. Effect of Arbuscular mycorrhizal fungi on growth of semi woody olive cuttings of the variety "Sigoise" *Algeria-American Journal of Research Communication,* 1(11): 244-257.

Singh, D.R. and Rai, R.B. 2005 Effect of growth regulators in rooting of stem cutting of *Morinda citrifolia* var. *citrifolia* in Bay Islands. *International Journal of Noni Research,* 1(1): 17-22.

Singh, R., Kalra, A., Ravish, B.S., Divya, S., Parameswaran, T. N., Srinivas, K.V.N.S. and Bagyaraj, D.J. 2012. Effect of potential bioinoculants and organic manures on root rot and wilt, growth, yield and quality of organically grown *Coleus forskohlii* in a semi arid tropical region of Bangalore (India). *Plant Pathology,* 61: 700-708.

Swain, M.R., Naskar, S.K. and Ray, R.C. 2007 Indole -3-Acetic Acid production and its effect on sprouting of Yam (*Dioscorea rotundata* L) minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. *Polish Journal of Microbiology,* 56(2): 103-110.

Venkatesan, S., Sudhagar, R. and Shakila, A. 2010. Effect of potting media on rooting of Gymnema sylvestre cuttings. *Asian Journal of Horticulture,* 5(2): 260-262.

II-OP-2

Grafting for propagation of Noni, *Morinda citrifolia* L.

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Abstract

Grafting is an important vegetative propagation to multiply elite planting material in fruit crops. In our recent study, we attempted grafting to propagate noni (Morinda citrifolia L). Wedge grafting method was used in noni grafting. Healthy non- flowering, non-hallow green mature shoot of current growth was used as scion. Root stock was raised through the seedlings of noni plants of IC 0598235. A healthy root stock seedling of 3-4 months old was used. A transverse downward cut was given in the soft wood portion on the stem to make a cleft lips at the top. The wedge shaped scion stick was inserted in to the cleft of the root stock and tied in with polythene strip taking care that no water enters the grafted portion. Grafting was attempted during rainy season June-July 2013. About 82 grafts were made out of which, 35 survived which showed 42.68% success rate. Twelve grafts of six months old grafts were planted in the field and observations are recorded. Grafted noni plants flowered in 6 months after planting. The grafted plant shows good growth after 6 months of planting with 30 fruits/plant and 193 leaves/plant. Noni seedlings of the same variety took about 7-8 months for flowering and fruiting.

Keywords : clonal, Morinda, Rubiaceae, propagation, bearing

Introduction

Noni (*Morinda citrifolia L.*) having a medicinal background finds a great place in the pharmacological industry. Not only its fruits but also its leaves and bark are very much useful. It has traditionally been used for cold, flu, diabetes, anxiety, and high blood pressure, as well as for

depression and anxiety. Being of commercial importance its continuous propagation on large scale is necessary. Noni is mostly propagated through seeds and cuttings. Scarification of seeds increases the rate of germination. To scarify a seed is to cut, scratch, or soften its outer coat to allow ready penetration of water and air which on the other hand, can germinate in 3–4 weeks, depending on the conditions (Nelson, 2005). Vertical and lateral stem cutting with sap flow at the time of cutting with vigorous growing points are best suited for vegetative propagation (Rethinam and Sivaraman, 2007).The disadvantage of producing plants vegetatively from cuttings is that it may not be as strong and disease resistant as seedlings (Nelson, 2003). There is only one report of grafting in seedless noni at Indonesia (Raharjdo *et al.,* 2013). In this report, we describe the attempt of grafting to propagate noni plants.

Materials and Methods

A total of 4 accessions were chosen for grafting, IC 0598229 (Lo), IC 0598516 (MPT 3), IC 0595274 (Shi), IC 0595277 (V), IC 0598230 (Va). The stems of noni plants are hollow inside. Therefore non- flowering, non-hollow, healthy green, mature shoot of successive growth was used as scion. Root stock was raised through the seedlings of noni plants of IC 0598235. A healthy root stock seedling of 3-4 months old was used for grafting. On the root stock seedling very tender portion of the terminal shoot was nipped off using the grafting knife. A transverse downward cut was given in the soft wood portion on the stem to make a cleft lips at the top (6-7cm). A scion stick matching to the cleft of root stock was selected and cut in wedge shape by giving smooth inward slant cuts at one stroke longitudinally downwards on opposite sides on the scion stick(6-7cm). The wedge shaped scion stick was inserted in to the cleft of the root stock and tied in with polythene strip taking care that no water enters the grafted portion. Grafting was attempted during rainy season June-July 2013. Polythene sheets were removed after 5 months to avoid girdling on graft union. The side shoots arising from the root stock region should be removed periodically.

Results and Discussions

Grafting made in noni with hallow stem, the union was not proper as the stems were mostly hallow. When the non-hallow non flowering shoots were used the success rate was 43 %. High humidity (80-97 %) and moderate temperature

(20-28 °C) during the period favoured graft union. Detail of graft success in different accessions is listed as Table.1. In January 2014, the grafted plant materials were ready for planting. Twelve grafts were planted in ICAR RC Goa experimental farm on 17th January, 2014. After 7 months of planting, vegetative characters and yield data were recorded in each plant (Table.2 and 3). Grafted noni plants are found to grow normal and flower and bear fruits. Raharjdo *et al.,* (2013) has tried different grafting techniques for propagation of seedless noni. Grafting is successful in Rubiaceaeae plants such as coffee (Cramer, 1934). Scions of commercial coffee cultivars are grafted on the rootstocks resistant to nematode pests and hence useful for managing the nematode problems (Bertrand *et al.,* 2001). The results demonstrate the feasibility of using grafting technique to multiply elite genotypes of noni for commercial cultivation. Large number of grafts are to be produced for confirmation of success of grafting. Attempts are also to be made to increase the success percentage of grafts from the present level of 43 per cent.

Table 1. Details of graft success in noni accessions

IC numbers	Place	No of grafts made	No. of grafts surviving	No. of grafts planted
IC 0598229	Loutolim	6	2	2
IC 0598516	MPT, Vasco	10	4	2
IC 0595274	Shirdona	51	23	2
IC 0595277	Vagator	10	4	4
IC 0598230	Vazare	5	2	2

Table 2. Vegetative characters in grafted plants of noni

IC numbers	Place	Height (cm)	Trunk diameter (cm)	Internodal length (cm)	Leaf length (cm)	Leaf Breadth (cm)
IC 0598229	Loutolim	155.00	3.66	14.67	28.5	14.17
IC 0598229	Loutolim	111.50	3.03	7.50	25.50	12.00
IC 0598516	MPT	130.00	3.34	6.00	29.83	14.50

IC numbers	Place	Height (cm)	Trunk diameter (cm)	Internodal length (cm)	Leaf length (cm)	Leaf Breadth (cm)
IC 0598516	MPT	121.5	3.18	5.83	29.00	15.33
IC 0595274	Shirdona	108.5	2.71	10.67	25.33	12.33
IC 0595274	Shirdona	103.00	2.55	9.00	27.00	12.50
IC 0595277	Vagator	113.5	2.71	6.00	25.83	12.00
IC 0598230	Vazare	108.00	2.87	6.67	29.17	13.67
IC 0598230	Vazare	81.50	1.59	3.83	27.5	12.33
IC 0595277	Vagator	100.50	3.34	7.17	28.33	14.33
IC 0595277	Vagator	103.00	2.55	8.33	26.83	12.17
IC 0595277	Vagator	100.00	1.91	6.83	28.33	13.00

Table 3. Branches, leaves and fruits produced by noni grafts

IC numbers	Place	No. of leaves	No. of branches	No. of fruits
IC 0598229	Loutolim	193	30	15
IC 0598229	Loutolim	185	27	16
IC 0598516	MPT	90	13	7
IC 0598516	MPT	88	25	8
IC 0595274	Shirdona	143	18	16
IC 0595274	Shirdona	129	16	7
IC 0595277	Vagator	98	21	8
IC 0598230	Vazare	117	25	9
IC 0598230	Vazare	37	6	3
IC 0595277	Vagator	87	18	9
IC 0595277	Vagator	121	17	10
IC 0595277	Vagator	85	13	7

References

Cramer P.J.S. 1934. Early experiments on grafting coffee in Java. *Empire Journal of Experimental Agriculture*, 2: 200-204.

Bertrand, B., Etienne, H., ESkes, A. 2001. Growth, production, and bean quality of *Coffea arabica* as affected by interspecific grafting: consequences for rootstock breeding. *Hort Science*, 36(2):269–273.

Raharjdo, M., Djauharia, E., and Darwati, I. 2013. The effect of rootstock age on growth of grafted seedless noni. *event-archives.litbang.depkes.go.id*. 24 (1-3). http://balittro.litbang.deptan.go.id/ind/images/publikasi/bul.vol.24.no.1/3-Mono%20Raharjo-Umur%20Batang%20Bawah-rafting%20Mengkudu.pdf

Rethinam, P. and Sivaraman, K. 2007. Noni (*Morinda citrifolia L.*) -the Miracle Fruit - A Holistic Review. *International Journal of Noni Research*, 2(1-2):1-34.

Nelson, S.C. 2003. Noni Cultivation and Production in Hawai'i. In : Nelson, S.C. (ed.). Proceedings *of 2002 Hawaii Noni Conference. University of Hawaii CTAHR Cooperative University of Hawaii, Honolulu, Hawaii, pp:33-50*

Nelson, S.C. 2005. Noni seed handling and seedling production. http://www.ctahr. hawaii.edu/noni. UH–CTAHR, F&N-10 — July 2005 University of Hawaii, Manoa.

III-LP-1

Do plant protection techniques to manage pests of Noni (*Morinda citrifolia* L.) require a paradigm shift from conventional approaches ?

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Abstract

Noni (Morinda citrifolia), a well known medicinal plant used for its pharmaceutical and nutraceutical properties, is reported to succumb to several biotic and abiotic stresses. Biotic factors like bacteria, fungi, viruses, algae and nematodes; sucking insect pests, non-insect pests, defoliators, stem borers, stem gridlers; abiotic factors like deficiencies of major and minor nutrients, cold injuries etc. are likely to cause economical loss to the cultivators when the crop is intensively cultivated on commercial basis, although in natural populations of noni the incidences are not above economical thresholds.

Development of Plant Protection technology and delivery need to be a continuum and should be interactive. If we trace the development of protection technology over the past several decades, it was continuous and many a times not interactive with the other components of production technology. Commodity based production is giving way to system based production and there is a paradigm shift using farming system to production to consortium system of operation. Diversification of production is fast happening along with widespread dietary evolution. The major recommendations of the National Commission on Farmers in its third report are soil health, technology and inputs, which have bearing on plant protection. Under optimized soil health the Biocontrol Agents (BCA) or the beneficial microorganism can do their job more efficiently. In the fast changing scenario of crop production owing to compulsion due

to the globalization and environmental concerns, an ecologically sustainable method for the management of pests and diseases is highly desirable.

Understanding the ecological niche of pests and pathogens and the epidemiological parameters that determine their growth and multiplication to a level that could cause damage to plants/crops, soil ecology with special reference to rhizosphere where beneficial and harmful organisms compete for their survival, interaction of plant, environment, biotic factors and the various inputs used for crop production and the beneficial / harmful effects on crop / plant per se and various defense strategies plants adapt under different conditions of stresses are crucial to develop newer ecofriendly approaches to manage pests / diseases of a given crop under different environmental conditions of crop growth.

Keywords : plant protection, paradigm shift, epidemiology, soil ecology, rhizosphere, biocontrol

III-OP-1

Recent research on fruit damage by pests in noni (*Morinda citrifolia L.*) and scope for their eco-friendly management

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Abstract

Our current research on pest damage to fruits of noni (Morinda citrifolia) has provided significant findings on the variability pattern in fruit damage levels across locations, seasons and noni ecotypes, the indirect effects of fruit infestation on the levels of some phytochemicals in fruits, besides confirming the identity of the fruit fly species causing damage and also the scope for using two repellant botanical biopesticides for minimizing the noni fruit damage. Studies on the spatio-temporal characterization of pest-caused fruit damage at NRCN, Chengalpattu, and T.N. showed monthly damage levels to range from 0 to 46% in 2012-13 and 5 to 45% in 2013-14, with greater damage levels occurring mainly during August-December in both years. The inter-locational variation in fruit damage during November 2013 to April, 2014 was seen as 0 to 45% at NRCN, against 3 to 19% and 7 to 17%, respectively in the noni farms at Mandya and Obulapura (in Karnataka). Internally damaged fruits were significant reaching up to 60% of the overall fruits damaged at NRCN and were reckoned as priority for control actions. It was found that internal fruit damage was caused by the oriental fruit fly, Bactrocera dorsalis, so confirmed by conventional and molecular taxonomic tools, being the first confirmed record of this Tephritid species as naturally infesting noni fruits and completing its life cycle on this host plant anywhere in the world. The monthly catches of adult fruit flies in para-pheromone traps kept at NRCN during the two years showed a seasonal pattern similar to the percent fruits damaged internally by pests. Monthly assessment of internal fruit damage among the two noni ecotypes over 24 months showed

significantly greater extent of damaged fruits was observed in Mangalore ecotype than Calicut ecotype, possibly due to greater preference for and/or favorable factors the for survival by fruit flies. Biochemical studies showed that secondary effect of both internal and external damage to fruits significantly reduced the levels of phytochemicals like scopoletin and flavonoids, besides proteins and carbohydrates, more clearly in Mangalore ecotype.

Exploratory field trial for eco-friendly control of fruit infestation with botanical biopesticide products showed promising results with spraying of a neem product (Sun Agro Neem-1500 ppm) and a sweet flag product (from TNAU) ,which could reduce the fruit damage from 20.0 in no-spray plots to 3.7% and 3.2%, respectively. Also, laboratory bioassays confirmed that both the treatments had repellant action against fruit fly adults. These promising spray application treatments were also found to be mostly neutral to the micro flora load in phyllosphere and on fruits, except that sweet flag sprays showed some reduction in overall microbial load, which needs further investigation. Further studies are being pursued to optimize the dose regimes of the biopesticides and to integrate them with a promising consortium of disease control bioagent strains, towards evolving a cost-effective and safe protection package against noni fruit damage.

Keywords : *Noni, fruit damage, seasonal pattern, fruit fly, control method, indirect effect*

Introduction

The wonder plant-noni (*Morinda citrifolia*), has been widely used as local medicine and also supportive food among Asia-Pacific communities for many centuries (Mathivanan *et al.* 2005). Its nutraceutical properties have become popular globally and efforts are being made to cultivate them for augmenting the use of their fruits on a wider scale (Singh *et al.* 2007). When moved from natural habitat to be grown under input intensive monoculture cultivation system, the noni plant tends to be more prone to damage by pests (Santhakumar *et al.* 2008). WNRF-supported pest survey projects visits in Andhra Pradesh, Karnataka, Kerala and Tamil Nadu (Jayakumar et al., 2009; Malarvannan *et al.,* 2009) and overseas visits to Fiji, Tahiti and Hawaii (Mathivanan and Sithanantham, 2008) have provided valuable information on the range of fruit damage symptoms, besides listing of the known and new pests of local importance in India and in the Pacific region.

Preliminary studies made by Mathivanan and Sithanantham (2010) and Mathivanan *et al.* (2011) had shown that the extent of both external and internal fruit damage caused by pests tended to be greater in Mangalore ecotype than in Calicut ecotype. More recent studies on noni fruit-damage by pests in South India (Venkatachalam *et al*., 2013) had shown that Tephritid fruit flies were widely caught parapheromone traps kept in noni fields, while extent of natural fruit fly infestation and their completion of life cycle using noni as host were being examined.

The present paper covers the latest research findings on spatio-temporal pattern of fruit damage by pests, differential fruit damage severity among noni ecotypes, parapheromone trap monitoring of fruit fly adults in noni ecosystem, natural infestation and confirmation of identification of the fruit fly species occurring on noni ,besides exploratory field and laboratory studies on promising eco-friendly pest management technology options and the scope for minimizing the loss in marketable fruit yield and quality in cultivated noni.

Materials and Methods

1. Seasonal fruit damage by pests at NRCN

The observations were initiated at the National Research Centre for Noni (NRCN) farm located near Chengalpattu, TN, in four blocks of the farm, with 5 trees per block chosen at random and tagged to enable successive monthly observations in two noni ecotypes-Calicut and Mangalore . The numbers of fruits damaged by pests (insects and or other animals like birds) were assessed monthly from August 2012 till July2014. by examining both intact and fallen fruits in each tree, also recording those with either external damage (by peeling/chopping) or with internal damage (bore holes only).The details of sampling and the parameters adopted were described elsewhere by Venkatachalam *et al.*(2013) .

2. Location effects on noni fruit damage by pests

Monthly visits were made to two noni farms, one each in Mandya and Obulapura in Karnataka during Nov-2013-April-2014 to estimate the fruit damage by pests in these two locations. The protocol for the assessment of the damage category was the same as adopted for monthly assessment at NRCN.

3. Fruit fly trap monitoring

Standard fruit fly traps (described by Sithanantham, 2012) were kept with two alternative lures- Methyl Eugenol (ME) or Cue lure (CL) for monitoring the seasonal occurrence and relative abundance of main fruit fly species adults in noni farms during August 2012-July 2014. The traps were kept in six blocks of NRCN and the lures replaced alternate weeks; trap catches were recorded weekly.

4. Fruit fly species infesting noni- confirmation studies

Samples of maggot collected from naturally infested noni fruits were reared to adult stage and found to belong to the Tephritid family, *Bactrocera* genus and *dorsalis* species according to taxonomic keys described by David and Ramani 2013. Samples of *Bactrocra dorsalis* so collected were also subjected to molecular taxonomic study to reconfirm their species status.

5. Exploratory testing of promising eco-friendly control practices

At NRCN, an exploratory field trial was taken up on botanical products, with five replications, as generic control options for reducing the fruit damage by pests. The botanical products included Sun Agro Neem 1500ppm, besides Neem soap + Pungam soap mix and sweet flag (Vasambu) as sprays the sprays were applied at alternate weeks totaling six times. Fruit damage assessments were made during 5 successive months in each treatment as per procedure adopted by Venkatachalam *et al.* (2013) for fruit damage studies at NRCN.

6. Indirect effects of the botanical biopesticide products

The indirect effect of the botanical foliar application treatments was assessed on fruit content of phytochemicals like scopoletin, flavonoids,protein and carbohydrates, besides phyllosphere microbial load using standard procedures (Harborne, 1998 and Evans, 1998). The repellant action of sweet flag on fruit fly adults was also verified in laboratory assays (as per the method adopted by Junaid ur Rehman *et al.* (2009).

Results and Discussion

1. Spatio-temporal characterization of pest damage to noni fruits:

1. 1. Seasonal pattern and major categories of pest-caused fruit damage at NRCN

The monthly assessment of fruits damaged by pests was completed for the 2 year period (2012-14) at NRCN showed significant differences in levels of overall fruit damage (Fig.1). The percent fruit damaged by pests was found to be mostly greater during August-December in both the years. Such apparently consistent trend in seasonal pattern of damage severity observed at NRCN could provide a local basis to time the pest control actions suitably. This result has provided a more holistic base-line data for NRCN and is complementary to the earlier limited studies on noni fruit damage seasonality reported by Mathivanan *et al.* (2012) and Venkatachalam *et al*. (2013a).

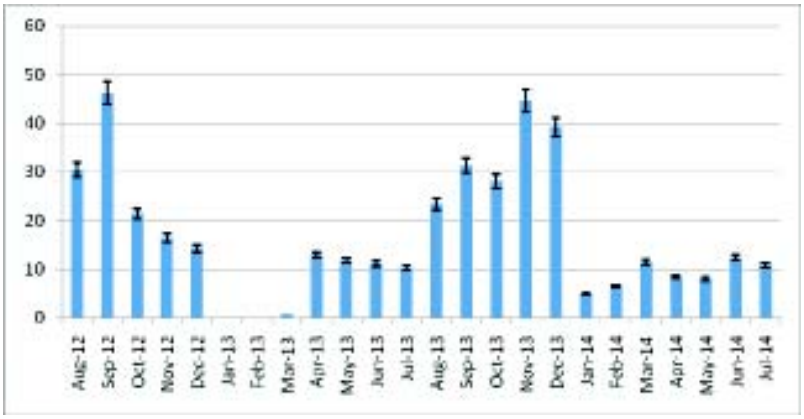


Fig.1: Monthly overall fruit damage by pests at NRCN Aug2012-July2014

The two major categories of fruit damage by pests observed in seasonal monthly assessments at NRCN were those with external damage (mainly by birds and different beetles) showing varying extents of removal of the fruit pulp and associated rotting due to external injury. The other category did not show any marked external symptoms, but had internal damage by larval stages, mostly of fruit flies. This internal damage usually led to slow rotting and/or pre-mature senescence of fruits. Adequate attention was paid to include both intact and fallen fruits to estimate the extent of fruits damaged in the two categories.

The monthly trend of fruits damaged internally versus externally at NRCN for the 2 year period (2012-14) is summarized in Fig.2. In general, the levels of damaged fruits in the two categories were mostly comparable, both seasonally and overall. Of course, from the point of view of preventive management of such pest damage to fruits, the internal damage to fruits may be more amenable for corrective interventions, since the pest has to develop and complete its life cycle within the fruit, whereas the external damage is mostly caused by brief landing/feeding by more mobile pests like birds, which does not permit prior timing of control action. Therefore, it is logical to focus future R&D for preventive control action to minimize noni fruit damage on internally damaging pests, especially fruit flies.

The scope for utilizing the information on spatial and temporal pattern of major insect pest of noni as foundation for evolving suitable monitoring and management practices for major pest of noni, as pointed out by Sithanantham *et al*. (2010) should be born in mind for suitably guiding the noni farmers in both preventive and curative pest control.

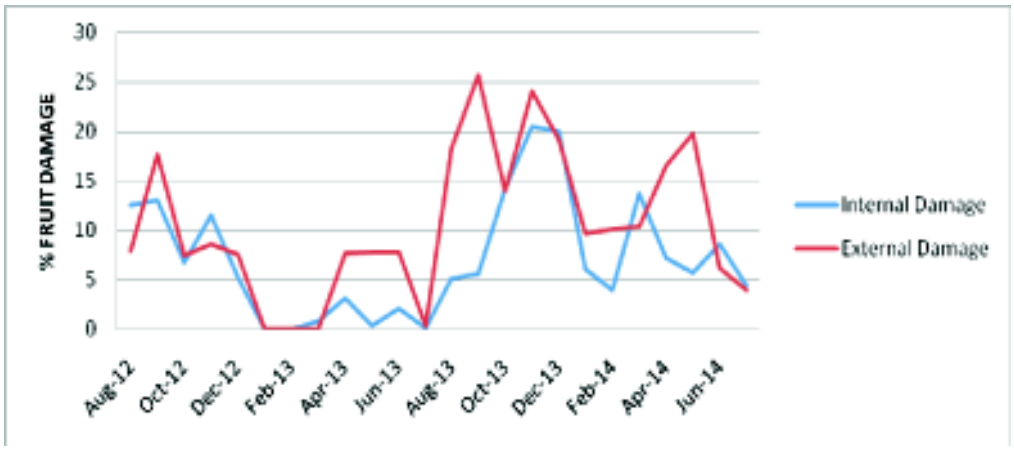


Fig.2: Monthly pattern of internal vs. external damaged noni fruits, NRCN, 2012-2014

1.2. Locational effects on overall fruit damage by pests

The results of monthly assessments of overall fruit damage comparing NRCN with three noni farms during 2013-14 are summarized in Fig.3. It was evident that location effects on fruit damage severity could be significant. For instance, while NRCN recorded above 40% fruit damage during

in November-December, the damage in mandya and Obalapura ranged 10 to 20%, the trend was the reverse during February-April, 2014, when in damage levels at NRCN were negligible/none whereas the fruit damage in the other two locations reached upto about 15%.

These results have thereby indicated the variability in trend of location to location differences in fruit damage by pests, indicating the need for local monitoring/ experience to also be taken into account for strategising the management interventions for noni fruit damage from pests, especially from fruit flies.

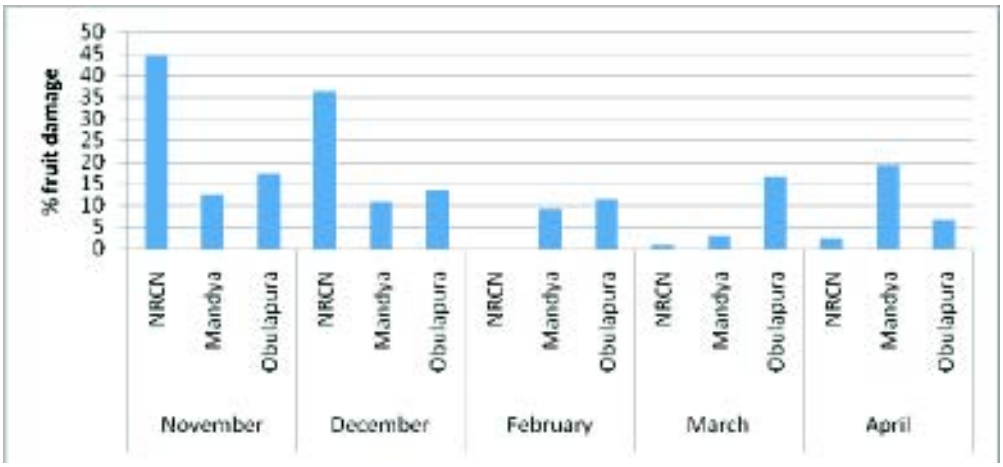


Fig. 3: Variation in noni fruit damage by pests among three locations: November 2013-April -2014

1.3. Relative fruit fly catches in two trap types

In both the years the monthly fruit fly catches were mostly greater in ME traps than in CL traps otherwise the trap catches in both trap types were somewhat higher during August-September in both years. Effectively ME trap accounted for the majority of fruit fly adults over the two year period. In addition, it is significant that the predominant fruit fly species in ME traps at NRCN was *Bactrocera dorsalis* (Venkatachalam *et al* 2013b). Our present study has also confirmed this trend at NRCN in both the years (Fig.4).

1.4. Seasonal pattern in fruit fly catches compared with trend in fruits damage internally

The overall monthly fruit fly catches at NRCN during 2012-14 showed a comparable trend to percent fruits damage internally (mainly by fruit flies) at NRCN (Fig. 5). The overall correlation between peaks in trap catches and peaks in internally damaged fruits suggests that monitoring of the fruit flies in traps (with ME as attractant) can provide an indication of the likely levels of internally damaged fruits in the same or subsequent months. This trend may be assessed for working out prediction formula towards short term forecasting of internal fruit damage severity by keeping fruit fly traps in noni farms. His expectation is justified since researchers in other crops like mango have shown the scope for associating fruit fly trap catches with fruit damage severity (Balasubramaniam *et al.,* 1971, Lakshmanan *et al.,* 1972).

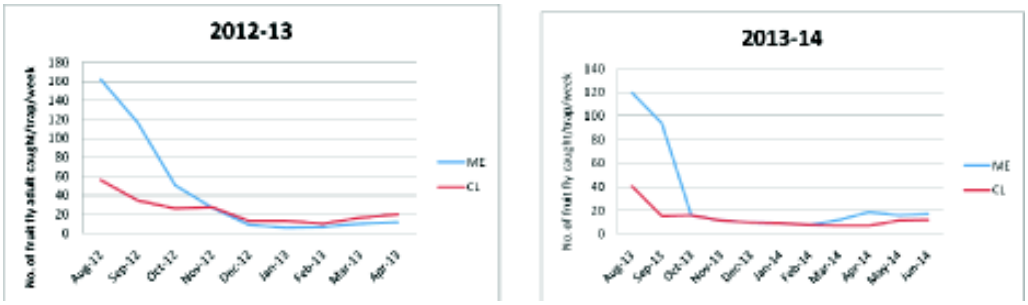


Fig.4: Monthly fruit fly catches in two trap types (ME and CL), NRCN 2012-14

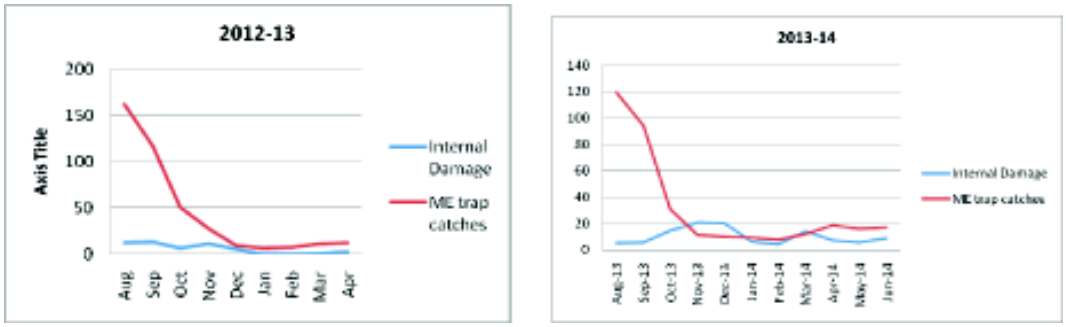


Fig. 5: Trend in fruit fly catches (ME traps) and fruits with internal damage, NRCN, 2012-14

1.4. Relative dominances of fruit fly species caught in ME trap

The relative numbers of three major fruit fly species caught in ME traps (in descending order) were *Bactrocera dorsalis* > *B. correcta* > *B. zonata* while in CL traps *B. cucurbitae* alone was caught. The overall proportion of the three species in trap catches was 65.6% for *B. dorsalis*, 19.3% for *B. correcta* and 8.03% for *B. zonata* (Fig.6).

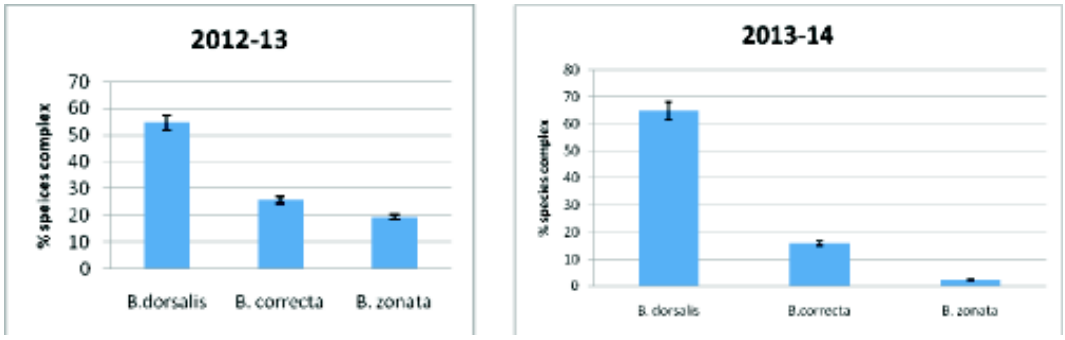


Fig.6: Relative dominance of three fruit fly species caught in ME traps, NRCN, 2012-14

2. Fruit fly incidence among two noni ecotypes

The relative monthly levels of fruit damage types among the tow noni ecotypes observed at NRCN during 2012-14 and the statistical anlysis results are presented in Table-1. It was found that in ecotype, the differences between damage catgegories and samling months as well as their interaction was found to be highly significant.

Table 1. Comparison of months and fruit damage types in 2 noni ecotypes, NRCN, 2012-14

Comparison	F value	Significance	CD value
Calicut			
Months	9.569	**	2.568
Damage type	16.073	**	1.482
Months x Damage types	3.064	**	5.136

Comparison	F value	Significance	CD value
Mangalore			
Months	7.742	**	4.802
Damage type	27.749	**	2.772
Months x Damage types	4.355	**	9.604

** = Highly significant

2.1. Overall fruit damage pattern in two ecotypes at NRCN

The monthly overall damage levels were found to be greater in general in Mangalore ecotype compared to Calicut ecotype in both the years at NRCN (Fig.6). Evedently this trend sugest that Mangalore ecotypes may be more preferred or susceptible to damage by pests including fruit fly. It is therefore useful to advise noni farmers growing Mangalore ecotypes to be more vigelant since they appear to be more known to fruit damage by pests. The intra species variability in noni (*Morinda citrifolia*) should be more systematically evoluated for their differential response or otherwise to insect pests especially those damaginf fruits includig fruit fly the occurnce of intra species variability to damage by pest and diseases, besides nematodes and the utilization of this knowledge for evolving suitable preventive control methods has already been pointed out (Sithanantham and Mathivanan, 2008, Mathivanan et al 2009).

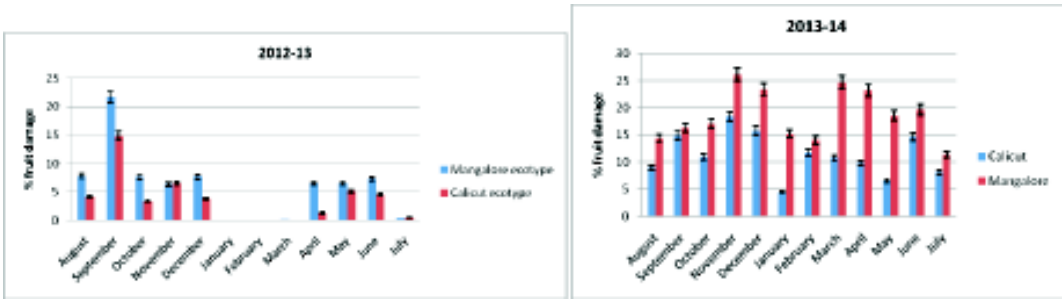


Fig.6: Relative severity of overall fruit damage in Mangalore and Calicut ecotypes at NRCN, 2012-14

2.2. *Studies on different fruit damage categories in two noni ecotypes.*

Our studies on the noni ecotypes at NRCN provided specific information on monthly damage among fruits, both with external and internal damage categories. Further for these two categories we also estimated the roportion of such damage in both intact fruits on the plants and recently fallen fruits on the ground as such we had four categories of damage – intact vs. fallen and internal vs. external. Our 24 month study of these four categories at NRCN offer interesting information about their relative occurance. It may be seen from Table- 1 that the differences among the four categories were significant over the 24 months period while the interaction of these categories with sampling month wa aslo significant, indicating that the relative occurance of the four damage types did not follow a similar pattern across the 2 months. Further the particining of fruit damage by pests in to damage sources and damge sites for each of the two noni ecotypes can be a very useful baseline information in statergesing pest control options in each ecotype.

3. Status of noni as host for fruit fly and indetification of the comonly infesting species

3.1. Confirming the status of noni as host for fruit fly

It was demonstrated that infested noni fruits collected from Periyakulam in TN provided adequate support for the larval stages to complete their development and emerge as adults without any extra energy source. The adults which emerged from noni fruits were also able to lay eggs in fresh noni fruits when offered in the laboratory, which also hatched in to maggots.

3.2. Confirming the associated fruit fly species

The fruit fly adults which emerged from naturally infested noni fruits were examined taxonomically, first by adopting the keys developed by David and Ramani (2013), then under the overall taxonomic guidance of and confirmation by Prof.V.V. Ramamurthy at IARI, New Delhi. Further, molecular taxonomic confirmation of the species, as per the procedures adopted by Dai *et al.* (2004) re-confirmed that the commonly infesting fruit fly species was *Bactrocera dorsali*s. This belonged to the family Tephritidae. This finding is significant not only for Indian scenario, but even globally this is apparently the first record of Tephritid fruit flies naturally infesting noni. So far, the only fruit fly group clearly recorded to damage noni fruits naturally belong to the family Drosophilidae,

the species being *Drosophila sechellia* in Seychelles (Amlou *et al*.,1997; Legal *et al*.,1992; Tsacas and Bachli,1981).This finding needs to be followed up to ascertain if the *B. dorsalis* populations emerging from noni are evolved as a local strain and adapted to re-infest only noni or can also infest other hosts like mango or guava and vice versa, while being part of the local polyphagous population.

4. Indirect effects of fruit damage categories on phytochemical levels in fruits

While the extent of fruits damaged provides an indication of the economic loss caused by fruit damage the indirect effects of such damage on the levels of beneficial phytochemicals like scopoletin, flavonoids, carbohydrates and protein are presented in this section.

4.1. Scopoletin levels: The overall scopoletin levels were slightly higher in Mangalore ecotype compared to Calicut ecotype Fig.7. There was significant reduction in scopoletin levels in internally or externally damaged fruits compared to healthy fruits. In Mangalore ecotype the levels of scopoletin were slightly reduced in externally damaged fruits while more drastically reduced in internally damaged fruits. In Calicut ecotype such market reduction was only seen in internally damaged fruits. It is evident that in both ecotypes protection from internal damage (caused by fruit flies) can help in retaining satisfactory levels of scopoletin in the harvest fruits. Which appear to be more important in Mangalore ecotype were the overall scopoletin levels are higher and the lasso in scopoletin due to internal damage is much greater. Considering the importance of scopoletin in remedying human blood pressure the protection of the fruits from in internal damage becomes more justified.

4.2. Flavonoids: The content of flavonoids was slightly greater in Calicut than Mangalore ecotype. The two damage categories (internal, external) caused significant reduction in flavonoid levels in both the ecotypes, as such protection from internal and external damage could benefit in preserving the flavonoids levels in the harvest fruits.

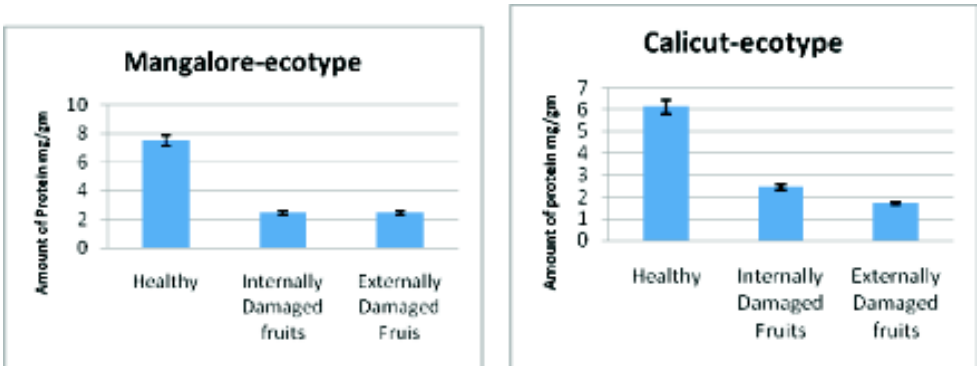
4.3. Total Protein levels: The content of total protein was slightly higher in Mangalore ecotype in the healthy fruits than Calicut ecotype. There was significant reduction in protein levels in both the ecotypes in both internal and external damage categories of fruits as such protection from either category of damage can benefit in preserving adequate levels of proteins in the harvested fruits.

4.4. Total Carbohydrate levels: the healthy fruits of Mangalore ecotype have slightly higher level of carbohydrate than the Calicut ecotype. Nevertheless, both internal and external damage with fruits caused significant reduction in carbohydrate levels in both the ecotypes, the extent of reduction being greater in internally damaged fruits. Therefore the expected benefit of safeguarding the carbohydrate content in harvest fruits would be more in protecting from internal damage than from external damage.

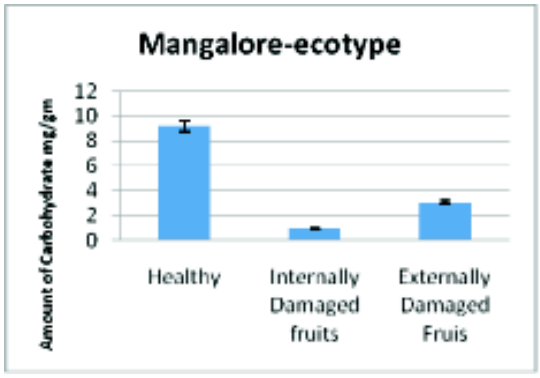
Based on all these results it is evident that the content of scopoletin and flavonoids besides proteins and carbohydrates trend to be significantly reduced due to pest damage in general. Further the reduction in the levels of in this phytochemicals appear to be greater in fruits with internal damage, suggesting that greater socio-economic importance be assigned for internally damaged fruits as means of safeguarding the quality of fruits of value in human health.

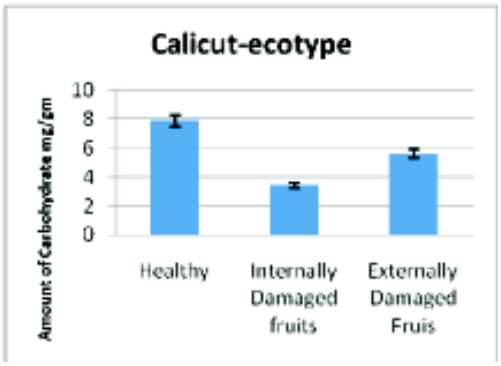
Fig.7: Effect of fruit damage by pests (2 categories) on phytochemical levels in two noni ecotypes

1. Total Protein level

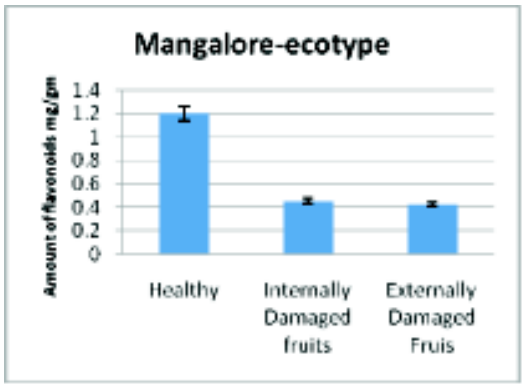


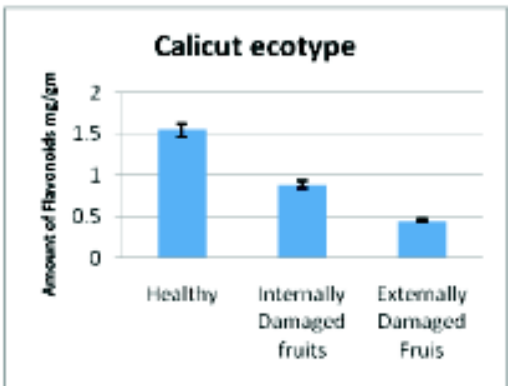
2. Total Carbohydrate level



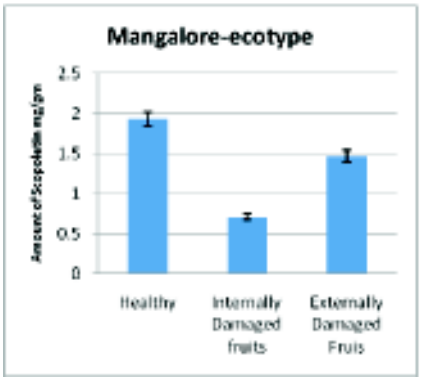


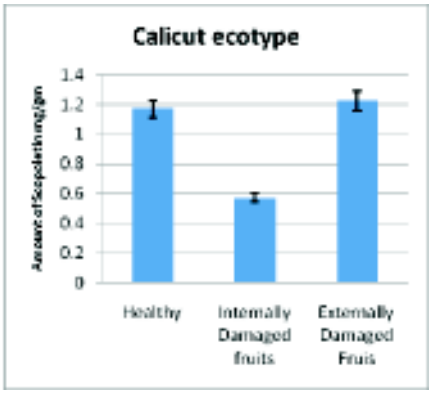
3. Total Flavonoids level





4. Total Scopoletin level





5. Scope for eco-friendly biopesticides products for minimizing fruit damage by pests

The need and scope for evolving organic crop protection options for noni have been pointed out by Sithanantham and Mathivanan(2008) and especially for protecting the fruits (Mathivanan *et. al.,* 2012). As part of this thrust, pilot studies were taken up in the laboratory and field to select promising botanical bipesticides products.

5.1. Lab study on sweet flag repellence to fruit fly

We tested a sprayable formulation of sweet flag (Vasambu) obtained from TNAU in the lab for verifying the reppelent mode of action. The recommended spray concentration (2g/lit) was sprayed on detached noni fruits braough from NRCN and kept for assessing kept in the laboratory cage in which adults of the fruit flies (*Bactrocera dorsalis*) were kept. The relative landing by the fly on the fruits sprayed with sweet falg were compared with the fruit kept wihout a spray as control in the same cage.The proportion of fruit fly landings on sweet flag sprayed fruits was mostly nil or limited during three successive dates of study is illustrated in Fig.8. These results confirmed that this formulation could be included for further testing as repellent botanical towards minimizing fruit fly infestation in noni fruis. Our results on this repellent effect of sweet flag is in conformity with earlier results on the repellant mode of action of sweet falg as a botanical biopestide in lab. study by Nair and Thomas(2001) on cucurbit fruit fly,*Bactrocera cucurbitae* and in field by Malik *et.al.*,(1996) on linseed bud fly,*Dasyneura lini.*

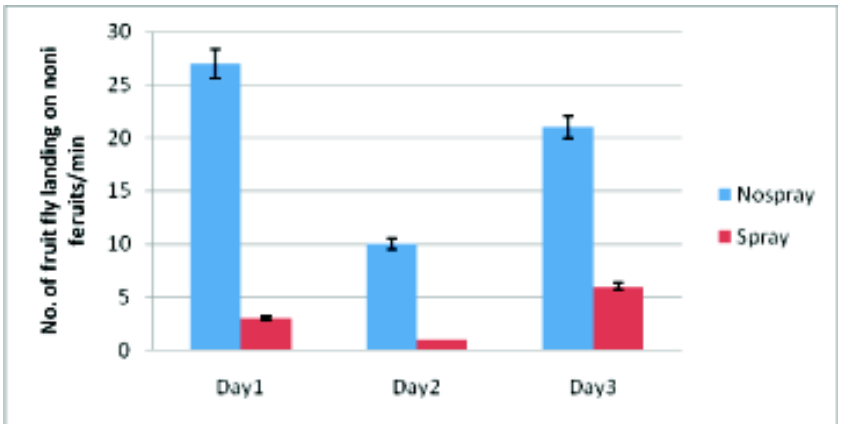


Fig.8: Repellency effect of Sweet flag spray on fruit fly adult landings, lab assay, 2014

5.2. Field study comparing promising botanical sprays

All the three candidate botanical products tested were found to significantly reduce the fruit damage in both Calicut and Mangalore ecotypes. Among them, the sweet flag formulation and Sun Agro Neem (1500 ppm) were found more promissing and on par compared to the combination of Pungam soap+Neem soap(Fig-9). These results have shown the potential for neem and sweet flag formulations.It is significant that these two eco-safe products have shown promise for use in noni .Their field efficacy for conferring such protection on pests of fruit crops have been demonstrated by other researchers (Pongnarin Chuenwong, 2006),while this is the first study confirming their potential in noni,apparently based on their repllent effects by preventing landing / feeding/ovipotition by phytophagous insects including fruit flies.

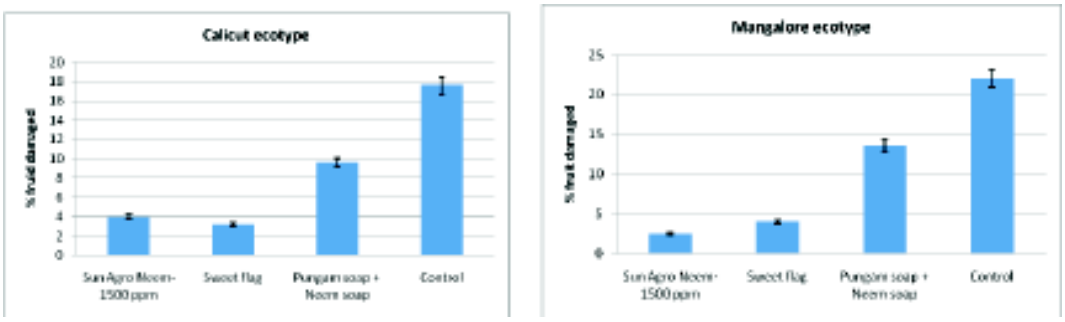


Fig.9: Effect of botanical sprays on internal fruit damage in 2 noni ecotypes at NRCN – December 2013-April 2014

5.3. Indirect effect of promising treatments on phyllosphere and fruit microflora

Both in leaves and fruits, there was no appreciable difference in microbial load due to neem product spray since it was comparable to control.In the case of sweet flag spray ,the phylosphere micoflora load appeared to be somewhat reduced (Fig.10). Furthure studies are being planned to distingusih whether the observed negative effect of sweet flag affected the benificial or phytopathogenic microflora groups,so to understand the overall impact of the botanical sprays. Further, the secondary (non-target) effect of these botanicals needs to be confirmed for different sub groups such as bacteria, fungi and actinomycetes and at different intervls after spray to determine the duration of effect of sprays and whether they are beneficial or neutral or negative.

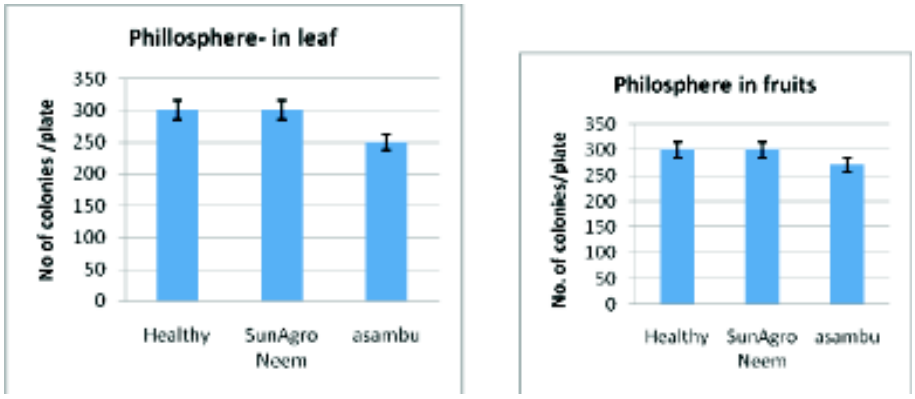


Fig.10: Indirect effect of botanical spray treatments on phyllosphere and fruit microflora

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References

Amlou, M.E., Pla, E., Moreteau, E., and David, J.R.1997. Genetic analysis by inter-specific crosses of the tolerance of *Drosophila sechellia* to major aliphatic acids of its host plant. *Genet.Sel.Evol.*, 29:511-522.

Balasubramanian, M., Abraham, E.., Vijayaraghavan, S., Subramanian, S., Santharaman, T.R.G and Gunasekaran, C.R. 1972. Use of male annihilation technique in the control of Oriental fruit fly, *Dacus dorsalis* Hendel. *Indian Journal of Agricultural Sciences*, 42:975-977.

Dai, S. M., C. C. Lin and C. Chang. 2004. Polymorphic microsatellite DNA markers from the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Molecular Ecology,* 4: 629–631.

David, K.J. and Ramani, S. 2011. An illustrated key to fruit flies (Diptera: Tephritidae) from Peninsular India and the Andaman and Nicobar Islands. *Zootaxa*, 3021: 1-31.

Evan, W.C. 1998. Trease and Evans Pharmacology. 14th edition, WB Saunders Company ltd, pp. 15-15.

Harborne, J.B. 1998. Phytochemical Methods, 3rd Edn. pp. 203-214.

Jayakumar, M. 2010. Pests and their natural enemies of *Morinda* in South India. WNRF Technical Bulletin-04., World Noni Research Foundation, Chennai, India.p-29.

Junaid ur Rehman, Ghulam Jilani, Mir Ajab Khan, Rafiq Masih and Sadia Kanvil. 2009. Repellent and oviposition deterrent effects of indigenous plant extracts to Peach fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae). *Pakistan J. Zool.*, 41(2): 101-108.

Lakshmanan, P.L., Balasubramaniam, G. and Subramaniam, T.R. 1973. Effect of methyl eugenol in the control of the oriental fruit fly *Dacus dorsalis* Hendel on mango. *Madras Agricultural Journal*, 60(7): 628-629.

Legal, L., David, J.R. and Jallon, J. M. 1992.Toxicity and attraction effects produced by Morinda citrifolia fruits on the *Drosophila melanogaster* complex of species. *Chemecology*, 3:125-129.

Malik,Y.P., Singh,S.V. and Pandey,N.D.1996. Field evaluation of some plant extgracts and insecticides aginst linseed budfly,*Dasyneura lini.Indian Journal of Entomology*, 58(1):323-35.

Mathivanan, N. 2007. Pest control strategies for Noni (*Morinda citrifolia*): Present and future perspectives. Second National Symposium on Noni for Health and Wellness, 27-28 October, 2007. (Abstr.) pp: 38-39.

Mathivanan, N. and Sithanantham, S. 2008. Noni mission- report of visit/ observations in Fiji, Hawaii and Tahiti. World Noni Research Foundation, Chennai. p. 20.

Mathivanan, N. and Sithanantham, S. 2010. Symptomology of fruit diseases and assessment of rots and other fruit damage patterns in noni (*Morinda citrifolia* L.). *In: (Eds.) P. Rethinam and T. Marimuthu, Proceedings of First World Noni Congress, Noni for Global Wellness, 1-3, October, Chennai. India. pp: 290-305.*

Mathivanan, N. and Sithanantham, S. and Marimuthu, T., 2009. Development of organic options for the management of disease of *Morinda citrifolia*. *Proceedings of fourth National Symposium on noni for empowerment and prosperity, Chennai. 24-25, October, 2009. (abstr) p.42.*

Mathivanan, N., Sithanantham, S., Marimuthu, T., Ramkumar, D. and Venkatachalam, A. 2012. Field studies on fruit damage patterns due to pests and diseases of noni (*Morinda citrifolia* L.). p.76. *Proceedings of Seventh National Symposium on 'Noni-The Tool for Wellness' (ISNS 2012).*

Mathivanan, N., Surendiran, G., Srinivasan, K., Sagadevan, E. and Malarvizhi, K. 2005. Review on the current scenario of Noni research: Taxonomy, distribution, chemistry, medicinal and therapeutic values of *Morinda citrifolia*. *Inter. J. Noni Res.,* 1: 1-9.

Nair, S. and Thomas, J. 2001. Oviposition deterrence of *Acorus calamus* on melon fly, *Bactrocera cucurbitae* Coq. *Journal of Tropical Agriculture*, 39:149-150.

Pongnarin Chuenwong, 2006. Biological control of oriental fruit flies (*Bactrocera dorsalis* (Hendel)) by the extracts of neem, sugar apple and mintweed. Ph.D. thesis, *Environmental Biology,* Suranaree University of Technology. pp: 101-103.

Shanthakumar, S.P., Malarvannan, S., Prabavathy, V.R. and Sudha Nair, M.S. 2008. Surveillance of insect pests of *Morinda citrifolia* and *M. tinctoria* in west coast of Kerala. *Third National Symposium noni,* 18-19 Oct. *New Delhi*, p. 87-88.

Singh, D.R, Srivatsava, R.C. and Damodaran, T. 2007. Studies on growth and development stages of *Morinda citrifolia* fruits. *In: Abstract of the Second National Symposium on Noni for Health and Wellness, 27-28 October, Chennai p.53.*

Sithanantham, S. and Mathivanan, N. 2008. Insect pests of noni: A global review. *Souvenir and Abstracts. Third National Symposium, New Delhi. 18-19, October, pp: 61-62.*

Sithanantham, S. and Mathivanan, N. 2008. Need and scope for evolving holistic and organic crop protection options for Noni (*Morinda citrifolia*). *Proceedings of Second National Symposium on Noni for Health and Wellness. 27-28, October, Chennai, pp. 103-120.*

Sithanantham, S., Mathivanan, N. Udhayalekha, Vatsala, V. and Suresh Kumar, K. 2008. Recent improvements in monitoring the insect pests and diseases on noni. *In: (Eds) P. Rethinam and T. Marimuthu, Proceedings of Third National Symposium, Noni for Nutrition and Health 18-19, October, New Delhi. pp: 84-85.*

Sithanantham, S., Mathivanan, N., Suresh Kumar, K., and Marimuthu, T. 2010. Studies on spatial and temporal pattern of major pests and diseases to evolve standard sampling and monitoring protocols in a medicinal plant noni. *Proceedings of First World noni Congress and Fifth National symposium on noni for empowerment and prosperity, October1-3, P.217-237.*

Venkatachalam, A., Sithanantham, S., Mathivanan, N. Ramkumar, D. and Marimuthu, T. 2013a. Investigations on fruit damage by pests in *Morinda citrifolia* L. (noni*). Proceedings in National Symposium on noni, 28-29 October 2013, GKVK, Bangalore, India.*

Venkatachalam, A., Sithanantham, S., Ramamurthy, V. V. Sakthi kumar Singh, Mathivanan, N. Ramkumar, D. Janarthanan, S. and Marimuthu, T. 2013b. Variability in fruit fly adult catches in traps with two lure types in noni (*Morinda citrifolia*) ecosystem in Tamil Nadu, India. *Proceedings in International Conference BIOCICON 2013, 26-29 November 2013, Palayamkottai, Tamil Nadu, India.*

III-OP-2

Eco-friendly managment of dry fruit rot disease of noni (*Morinda citrifolia* L.) using bacterial biocontrol agents

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Abstract

Systematic filed survey on noni diseases was carried out from August 2012 to July 2013 in National Research Centre for Noni (NRCN) located at Salavakkam, Kanchipuram District, Tamil Nadu, India. During the survey, foliar diseases such as leaf spot, shot hole, leaf blight, were commonly observed however, their severity was low. Importantly, the prevalent and major disease was found to be the dry fruit rot. It is the major limiting factor for the successful cultivation of noni as the fruit yield loss is economically significant. Considering the severity, research has been focused to develop a suitable eco-friendly control measures for the dry fruit rot disease. A total of 160 rhizosphere and 30 phyllosphere bacteria were isolated from the soil and leaf samples collected from Noni farms and screened them for antagonistic activity against dry rot pathogen. Among them, two rhizosphere and a phyllosphere isolates exhibited remarkable antagonistic activity against dry rot pathogens. The short listed isolates were screened in in vitro experiments for biocontrol mechanisms such as production of fungal cell wall lytic emzymes, hydrogen cyanide and siderophore. In addition, their ability to produce indole acetic acid and ammonia and also phosphate solubilisation was investigated. Based on the results, the isolates, MML2663 and MML2715 were selected for development of powder and liquid formulations. The compatibility between these isolates and also with a neem product (Sun Agro Neem-1500) was tested in different in vitro assays. Further, field trials are to be conducted at NRCN and in a farmer's farm to evaluate the efficacy of the bacterial bioformulatiuons for the management of dry fruit rot of noni.

Key words : Noni, *Morinda citrifolia*, dry fruit rot, rhizosphere, phyllosphere, biocontrol agents

Introduction

Morinda citrifolia L. under the family Rubiaceae, commercially known as "Noni" or "Indian Mulberry" is one of the traditional folk medicinal plant that has been used for over 2000 years. It is a small tropical evergreen shrub or tree growing naturally in India, Paciûc Islands, Southeast Asia and various other tropical areas. The fruit of *M. citrifolia* has a long history of use as a food and for treatment of colds, ûu, diabetes, anxiety, hypertension, cancer, and other health issues in folk medicine for thousands of years in tropical regions throughout the world (McClatchey, 2002; Wang *et al.,* 2002). In recent years, Noni has increased in popularity as a dietary supplement in western and other modern societies, and has attracted much attention from researchers (Hirazumi and Furusawa, 1999; Kamiya *et al.,* 2004).

Noni is susceptible to attack by a wide range of insect pests and disease causing pathogens. Diseases caused by fungal pathogens are a main obstacle to obtain a high yield during commercial cultivation of Noni. A severe foliar blight (Black flag) and fruit rot diseases of noni were found on the islands of Hawai'i (Nelson, 2004). Several leaf spot diseases caused by fungal pathogens were reported in *M. citrifolia* L. from most of the Pacific Islands, including American Samoa, Guam, Palau, and the Federated States of Micronesia (Sithanantham and Mathivanan, 2010; Fred Brooks, 2012). These foliar diseases caused by fungi may significantly affect fruit development. However, fruit rots are becoming the most important diseases of Noni, which lead to high yield loss and low quality of fruits.

In order to control the fungal diseases, various types of chemical fungicides are applied in general. However, application of chemical fungicides has resulted in accumulation of toxic compounds potentially hazardous to human and environment and there is also a high risk of development of fungicide resistant pathogens' strains. Therefore, biological control of plant pathogens is considered as an alternate control strategy and eco-friendly approach. Beneficial microorganisms with the ability to antagonize the fungal phytopathogens and thus prevent plant diseases as biocontrol agents represent a realistic alternative to chemical fungicides. Biological control is not only a

green approach to protect plants from infection by phytopathogenic organisms, but also to promote their growth.

Integrated cultural and preventive methods such as pruning, sanitation, and an appropriate cropping system would keep the noni fruit diseases in check. However, the growing demand for noni health products needs a modern mono cultural farming system with an eco-friendly disease management. In this scenario, the use of beneficial plant growth promoting rhizobacteria (PGPR) and phyllosphere bacteria for the control of fruit diseases and for the plant growth promotion of noni could be a promising strategy, which remains unexplored. Keeping these points in view, the present investigation was undertaken.

Materials and Methods

Isolation of rhizobacteria

Soil, leaf and fruit samples were collected from different noni fields in Tamil Nadu and Karnataka and total of 160 rhizosphere and 30 phyllosphere bacteria were isolated using serial dilution technique. The details of soil samples and morphological characteristics of all the culture were documented. Each culture was designated with three letter MML followed by four digits of Arabic numeral.

Screening of rhizosphere and phyllosphere bacteria for antagonistic activity against fruit rot fungal pathogen

Classical dual culture plate assay was performed to screen the rhizobacteria and phyllosphere bacteria against noni fruit rot fungal pathogen. Mycelial discs of fruit rot pathogen measuring 8 mm were placed separately in the centre of Nutrient agar with Potato Dextrose Agar plates and four different rhizobacteria or phyllosphere bacteria were placed at equidistance in the periphery of the plate leaving 1 cm gap from the plate rim. All the 160 rhizobacteria and 30 phyllosphere bacteria were screened for antagonistic activity against the fungal fruit rot pathogen. The plates were incubated for five days at room temperature (30 ± 2°C) after which, the antagonistic activity of rhizobacteria / phyllosphere bacteria against fruit rot pathogen was observed as zone of inhibition (ZOI), if any.

Selection of promising bacteria for antagonistic activity against fruit rot pathogen

Some isolates exhibited consistent antagonistic activity against fungal fruit rot pathogen. However, some antagonists showed reduced ZOI against the fungal pathogens. The isolates that showed appreciable amount of antagonistic activity against fungal fruit rot pathogens alone were selected for further studies on biocontrol mechanisms.

Mechanisms of biocontrol

Mechanisms of antagonistic activity exercised by five selected bacterial isolates on the suppression of fungal fruit rot pathogen were determined by various *in vitro* experiments. Competition for nutrition, lytic enzymes and antibiosis were investigated. Besides, the plant growth promotion properties such as production of IAA, ammonia and phosphate solubililzation by the antagonists were also studied.

Chrome Azurol S (CAS) assay for siderophores production (Schwyn and Neilands, 1987)

The CAS assay was done for selected antagonists. The CAS reagent was prepared described below: CAS (Sigma Chemicals Co., USA), 60.5 mg was dissolved in 50 mL of glass-distilled water and mixed with 10 mL of iron (III) solution (1 mM $FeCl_3.6H_2O$ in 10 mM HCl). This was added to 72.9 mg of hexadecyl trimethyl ammonium bromide (HDTMA) in 40 mL of water. The dark blue colored CAS reagent was then autoclaved for 15 min. This reagent was added to PIPES agar medium (30.24 g of PIPES buffer dissolved in 750 mL of distilled water + 18 g of agar) and the pH was adjusted to 6.8 using required amount of NaOH pellets.

Nutrient Agar (NA) medium was prepared and poured into Petriplates and allowed to solidify. One half of the solidified medium was cut and carefully removed. Molten PIPES agar medium was then mixed with CAS reagent and poured into the cut half and allowed to solidify. Bacterial strains were streaked separately onto one half of the plate containing the NA medium. The cultures were streaked away from the other half as far as possible. The plates were incubated in dark at 28 ± 2°C for 5 days. Plates were then observed for a change in the color of CAS PIPES agar medium from blue to brownish orange that indicate the production of siderophores by bacterial strains.

Production of lytic enzymes

Preparation of colloidal chitin

The colloidal chitin was prepared from the chitin flakes (Sigma chemicals Co, USA) by the methods of Skujins *et al*., (1965). A known amount of chitin was boiled in 2% potassium permanganate for 30 min followed by a brief oxalic acid (1%) treatment. The flakes were treated with 5 N HCl for 12 h, washed successively in water, ethanol/ether and dried. The product was now free of protein and ground to powder. This crude chitin powder was suspended in 5-10 times the volume of concentrated HCl and kept for 12 h at 4°C. This syrupy liquid was poured into 50% aqueous ethanol that was stirred vigorously by a magnetic stirrer. The precipitate was collected by centrifugation at 12,000g for 20 min. the pellet was repeatedly washed with distilled water and dialyzed against distilled water until the pH of the chitin suspension reached the pH of distilled water. Finally the pellet was lyophilized and stored at 10°C.

Qualitative assay of lytic enzymes

Nutrient agar (NA) medium was supplemented with respective substrate for the assay of lytic enzymes *viz.,* protease and cellulase, gelatin and carboxymethyl cellulose at a concentration of 0.5%, for chitinase, 0.1% colloidal chitin (prepared by the method of Skujins *et al.,* 1965) was used. The media were sterilized and then poured into Petriplates. Selected antagonistic bacteria were streaked on respective medium and incubated for 2 days at room temperature. Cellulase and chitinase production was visualized by a translucent zone around the bacterial patch after staining with 0.3% Congo Red and destained with 1 N NaCl for 15 min each. Protease activity was visualized as a clear zone around bacterial patches, after the plates were flooded with saturated ammonium sulfate solution prepared in 0.1 N HCl.

Production of volatile metabolite: Hydrogen cyanide (HCN)

Nutrient sucrose agar (NSA) medium was used to detect the production of HCN by antagonistic bacteria as described by Lorck (1948). Filter paper strips saturated with 0.5% picric acid in 2% aqueous sodium carbonate solution were placed on the lid of test isolate inoculated NSA plates. The plates were sealed with the help of parafilm to trap the HCN production. After two days of

incubation, the production of HCN was determined by the change of color of the filter paper from yellow to reddish brown.

Plant growth promotion traits

Production of Indole Acetic Acid (IAA)

The selected antagonistic bacterial strains were grown in 5 mL nutrient broth medium in test tubes for 24 h. After incubation period, bacterial cultures were harvested and centrifuged at 10000xg for 15 min at 4^0 C. Two drops of *o*-phosphoric acid were added to 2 mL of cell free supernatant and the development of color was observed. The presence of a pink color showed the production of IAA.

Phosphate solubilization

Modified Basal Sperber (1958) Medium (MBSM) was prepared, sterilized and poured in sterile Petriplates. Selected bacterial antagonists were streaked on the solidified MBSM separately and incubated for two days at room temperature. Production of a halo zone around the bacterial growth indicated the phosphate solubilizing ability of the respective bacterium.

Production of ammonia (Cappuccino and Sherman, 1992)

Ten bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 mL peptone water (0.5 g of peptone in 10 mL) in each tube and incubated at 30 ± 2°C for 48-72 h. Nessler's reagent (0.5 µL) was added in each tube. Development of brown to yellow color is a positive test for ammonia production.

Results

Screening of rhizobacteria and phyllosphere bacteria for antagonistic activity against fruit rot fungal pathogen

All the 160 rhizobacteria and 30 phyllosphere bacteria were screened for antagonistic activity against fungal fruit rot pathogens of *M. citrifolia*. Among them, the isolates, 33 showed antagonistic

activity against fruit rot pathogen. However, five isolates, MML2663, MML2666, MML2707, MML2715, MML2723 were selected for further studies.

Production of lytic enzymes

Among five antagonists, all the isolates except MML2723 produced cellulase enzyme in Carboxy methyl cellulose (CMC) amended agar medium. Two bacterial isolates (MML2663, MML2715) were able to produce protease enzyme in gelatin amended agar medium and chitinase enzyme in colloidal chitin amended agar medium.

Production of volatile metabolite: Hydrogen cyanide (HCN)

Among five selected antagonists, none of the antagonists changed the color of sodium picrate saturated filter paper from yellow to brown. This indicated that all these five isolates do not have cyanogenic activity.

Production of siderophores

Among five selected antagonists, four were able to produce siderophore excluding MML2715.

Plant growth promotion traits

Production of Indole Acetic Acid (IAA)

Interestingly all the five selected antagonistic bacteria were able to produce IAA. However, the isolate, MML2715 produced high amount of IAA compared to other isolates.

Phosphate solubilization

Among the selected antagonists, only one MML2663 could produce halo zone when it was grown in Modified Basal Sperber medium for 2 days. This indicated that this antagonist was able to solubilize tricalcium phosphate.

Production of ammonia

Among the five selected antagonists, three isolates were able to produced brown color when Nessler's reagent was added. This indicated that these three isolates were able to produce ammonia.

Discussion

Noni (*M. citrifolia)* health products especially, noni fruit juice is in high demand at present as an nutrient supplement and also as an alternative medicine for different kinds of illness and as health tonic. Therefore, the production of noni fruits warrants special attention. It is a matter of concern to maximize the fruit yield, their quality and reduction in the fruit losses due to diseases. In the recent past, the dry fruit rot is emerged as the most important diseases of noni caused by fungal pathogen (Sithanantham and Mathivanan, 2010; Manjunath *et al.*, 2010). Therefore, it is very important to develop suitable eco-friendly control measure to manage this fruit rot. Biological control of plant diseases using bacterial antagonists has always attracted researchers across the world as an eco-friendly method. The bacterial biocontrol agents have many advantages such as faster growth rate, easy to handle, amenable for genetic manipulation, rhizosphere competence, etc. (Meyer and Roberts, 2007).

Plant growth promoting rhizobacteria (PGPR), which exert positive effects on plant growth by direct mechanisms such as solubilization of nutrients, nitrogen fixation, production of growth regulators, etc., or by indirect mechanisms such as stimulation of systemic resistance, competitive exclusion of pathogens, or removal of phytotoxic substances that are produced by deleterious bacteria and plant roots under stress condition (Bashan and de-Bashan, 2010). With this background, investigations were carried out to identify potent biocontrol bacteria for the development of suitable formulation for field application. Among 160 rhizobacteria and 30 phyllosphere bacteria, five were showed efficient antagonistic activity against fruit rot pathogen and hence selected to study their mechanisms of biocontrol and plant growth promoting activities. The present investigation suggested that the antagonistic ability of the selected bacterial strains involve multiple biocontrol mechanisms. Importantly, the present study was helpful in identification of two most promising beneficial bacteria for further development of bioformulation.

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References

Bashan, Y. and de-Bashan, L.E. 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth–a critical assessment. *Adv. Agron*., 108: 77–136.

Cappuccinio, J.G. and Sherman, N. 1992. Microbiolgoy: A loboratory manual. Pearson Education, New Delhi, India.

Fred Brooks. 2012. Leaf spot diseases on noni/nonu. In: *Pacific Pest Detector News* (Ed. Barry Brennan), Hawaii. pp:1–8.

Hirazumi, A. and Furusawa, E. 1999. An immunomodulatory polysaccharide-rih substance from the fruit juice of Morinda citrifolia (noni) with antitumour activity. *Phytother. Res.* 13: 380–397.

Kamiya, K., Tanaka, Y., Endanq, H., Umar, M. and Satake, T. 2004. Chemical constituents of *Morinda citrifolia* fruits inhibit copper-induced low-density lipoprotein oxidation. *J. Agric. Food Chem.* 52: 5843–5448.

Lorck, H. 1948. Production of hydrocyanic acid by bacteria. *Physiologia Plantarum,* 1: 142–146.

McClatchey, W. 2002. From Polynesian healers to health food stores: Changing perspectivies of *Morinda citrifolia* (Rubiaceae). *Integ. Cancer Ther.,* 1: 110–120.

Meyer, S.L.F. and Roberts, D.P. 2007. Combinations of biocontrol agents for managent of plant parasitic nematods and soil-borne plant pathogenic fungi. *J. Nematol.,* 24: 1-8.

Nelson, S.C. 2004. Black Flag of Noni (*Morinda citrifolia*) caused by *Phytophthora* species. *Plant Dis.* 19: 1–4.

Schwyn, B. and Neilands, J.B. 1987. Universal chemical assay for the deltection and determination of siderophores. *Anal. Biochem.,* 160: 47–55.

Sithanantham, S., Mathivanan, N., Marimuthu, T., Kannaiyan, J., Jayakumar, M. and Nakkeeran, S. 2010. Identification of Pests and Diseases of Noni- A Hand Book. WNRF Technical Bulletin: 6, World Noni Research Foundation, Chennai, India, p.59.

Skujins, J.J., Patgieter, H.J. and Alexander, M. 1965. Dissolution of fungal cell walls by a *Streptomycete* chitinase and â-1,3 glucanase. *Arch. Biochem. Biophy.,* 111: 358-364.

Wang, M.Y., West, B.J., Jensen, C.J., Nowicki, D., Chen, S.U., Palu, A. and Anderson, G. 2002. Morindia citrifolia (Noni): A literature review and recent advances in Noni research. *Acta. Pharmacol. Sin.*, 23: 1127–1141.

III-OP-3

Essential oil phytochemistry of Morinda citrifolia for its insecticidal property against mealy bug, *Maconellicoccus hirsutus*

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Abstract

Essential oil extracted from leaves of medicinal plant, Morinda citrifolia was subjected to GC-MS analysis and subsequently identified major and minor compounds such as monoterpenes 8.28 % and sesquiterpene 3.81% respectively. A total profile of 60 compounds was identified. Among them, ?-Iso-methyl ionone (15.84%), 6-acetyl-1, 1, 2, 4, 4, 7-hexamethyltetralin (10.63%),Galaxolide (10.02 %), methyl dihydrojasmonate (8.31%), Lilial (7.68%) and Diethyl phthalate (7.63%) are the major compounds.The important aspect of the essential oil is that it can be applied as an effective insecticide against mealy bug Maconellicoccus hirsutus. The results of the bioassay have confirmed its insecticidal potential with LC50 values of 152.55 ppm and the LC90 value calculated as 357.12 ppm. To the best of our knowledge, the present study is the first report on the M. citrifolia leaf oil biochemical profile, which can either be used as a chemo taxonomical tool or insecticidal product as well.

Keywords: monoterpenes, *M.hirsutus*, insecticidal, GC-MS, *M.citrifolia,* dihydrojamonate.

Introduction

About 45000 species of plants and fungi occur naturally in India, representing 11% of the world flora and particularly it is the place known for its richness in diversity of medicinal plants. Not many of these medicinal plants were ventured and even though some have been explored, they were not recorded, and the information has not been passed on for conservation and development.

Among them, *Morinda citrifolia* L. (Family: Rubiaceae) is aplant species with high medicinal and therapeutic value against many human diseases such as gastric ulcers, gingivitis, headaches, heart diseases, hypertension, immune weakness, indigestion, intestinal parasites, kidney disease, malaria, menstrual cramps, mouth sores, respiratory disorders (Elkin, 1997). Unfortunately, this plant was under anthropogenic threat and most of the vegetation was degraded. Essential oils extracted from the plants have shown good insecticidal activity and was found to be safer repellent and fumigants (Petrakis *et al*., 2005; Papachristos *et al*., 2009). Many compounds isolated and reported from the *M.citrifolia* fruits, such as scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin- 1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, Alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine (Simonsen, 1920; Moorthy and Reddy, 1970; Singh and Tiwari, 1976; Levand and Larson, 1979; Heinicke, 1985; Budavari *et al*., 1989; Daulatabad *et al*., 1989; Peerzada *et al*., 1990; Higa and Fuyama, 1993; Legal *et al*., 1994; Farine *et al*., 1996). But only a negligible quantity of work has been carried out to venture the biochemical constituents of *M. citrifolia* leaf essential oil, which was neglected by most of the phytophagous arthropods and mammals gains remarkable attribute to ponder.Many chemical compounds were extracted from various plant species and their biological properties have been identified. To the best of our knowledge essential oil analysis of *M.citrifolia*leaveshas not been attempted previously. In the present study, for the first time, we have analysed the essential oil compositions of *M. citrifolia* leaves and its insecticidal property against the pink hibiscus mealy bug, *Maconellicoccus hirsutus.*

Materials and Methods

Plant material and oil extraction

The fresh matured leaf material was collected from matured trees of *M. citrifolia*, from the moist evergreen coastal belt in Allepey District, Kerala, India. Fresh leaves of *M. citrifolia* (500g) were coarsely ground and subjected to hydro-distillation for 4 h using a Clevenger's apparatus following published protocol (Clevenger, 1928) and essential oil was extracted using hexane. The extracted essential oil was stored at 4°C until GC-MS analysis.

Larval toxicity test

A laboratory colony of *Maconellicoccus hirsutus* third instar nymph was used for the insecticidal activity. The insect was collected from the natural habitat of *M. citrifolia* orchard present in Malur, Kolar district of Karnataka. Hundred numbers of third instar nymphs collected from the field was introduced into sleeved insect cage containing fresh pumpkin and reared for bioassay. After days, the first generation third instar nymphs of the laboratory population was released into pumpkin and different concentrations (100, 200, 300, 400 and 500ppm) of 10 ml essential oil of *Morinda citrifolia* was sprayed on the nymphs with hand atomiser. Three replicates of nymphs were maintained per treatment. The control mortalities were corrected by using Abbott's formula (Abbott's 1925). LC50 and LC90 were calculated from toxicity data by using probit analysis (Finney, 1971).

Gas Chromatography-Mass Spectrometry Analysis

The fractions of *Morinda citrifolia leaf* extract was analysed by GC-MS in order to examine their chemical structure. The GC (Agilent 6890) conditions were as follows, DB-5 column (30 m X 0.25 mm X 0.25 ¼m), stationary phase (5 % Phenyl Methyl Siloxane, 95 % Dimethyl po1ysiloxane), injection in split mode (50:1), the injector and detector temperatures were maintained at 250°C, helium was used as the carrier gas at a flow rate of 1 ml/min; the oven temperature was initially maintained at 100°C for 5 min and then raised to 220°C at a rate of 10°C/ min and held for 18 min. The MS (Agilent 5973 inert MSD) electron multiplier 2188.2 V, mass spectra data were acquired in the scan mode in m/ z range 58-550. The compounds of the oil were identified by comparing their retention indices (RI), with NIST (National Institute of Standards and Technology) library and by comparing their mass spectra with data already available in literature (Adams, 2007).

Results and Discussion

Maconellicoccus hirsutus treated by *M. citrifolia* essential oil was evaluated and observed differential mortality according to the concentration gradient. The LC50 values analysed for the essential oil was 152.55 ppm whereas the LC90 value was calculated as 357.12 ppm (Fig. 1). The survival rate of the mealy bugs was observed higher (3.2 days) in lower concentration (100 ppm)

and the survival rate was lower (1 day) in higher concentration (500 ppm) (Fig. 2). The essential oil was viscous and pale yellow in appearance. A total of 60 compounds were identified (Table. 1) in the essential oil following GC-MS analysis accounting for 100 % (Table 1). The major compound identified was ²-iso-methyl ionone (15.84 %), beta-iso-methyl ionone (Fig. 3) is a class of cyclic terpenoids present in *M. citrifolia* which also occur in other plant based essential oils exhibiting a sweet floral scent reminiscent of violets (Sharma *et al*., 1977). Most of the terpenoids were generally considered for their insecticidal efficiency and repellent activity (Zhang *et al*., 2011).

Table 1: Essential oil composition of *M. citrifolia*

S.No	Compound	Area (%)
1	±- Pinene	0.04
2	Sabinene	0.03
3	²- Pinene	0.28
4	Limonene	1.42
5	c- Terpinene	0.14
6	2,6-Dimethyl-7-octen-2-ol	5.41
7	Cis-linaloloxide	0.04
8	3-Tert-Butyltoluene	0.01
9	Linalool	2.43
10	Iso –Camphane	0.03
11	Camphor	0.03
12	± –Terpineol	0.06
13	L-Citrulline	0.02
14	Nerol	0.03
15	Acetaldehyde propyl hydrazone	0.03
16	Propylhydrazone acetaldehyde	0.12
17	Linalyl anthranilate	2.45
18	Geraniol	0.10

S.No	Compound	Area (%)
19	2-(4-tert-butylphenyl)acetaldehyde	0.14
20	3,7-Dimethyl-7-hydroxyoctanal	0.04
21	Terpinolene	0.08
22	Geranyl acetate	0.03
23	Isovanillin	1.68
24	4-t-Butyl propiophone	0.29
25	Coumarin	0.51
26	±-Patchoulene	0.15
27	3-Penten-2-one, 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)	1.29
28	cis-²-Guaiene	0.07
29	± –Bulnesene	0.37
30	Cyclohexane	1.44
31	Lilial	7.68
32	o-Cresol	0.16
33	‘lpha-ethyl-8. Beta-hydroxy-2,6-dimethyl bicyclo(4.4.0)dec-1-ene	2.77
34	Proponoic acid	0.94
35	Cyclohexanol	0.07
36	4-Methoxybenzenesulfinothioic acid S-phenyl ester	0.10
37	Diethyl phthalate	7.63
38	2-tert-Butyl-6-methylphenol	0.11
39	Citronellyl formate	0.56
40	Decahydro-4,4,8,9,10-pentamethylnaphthalene	0.63
41	Cycloheptyl N.N-Dipropylphosphoramidocyanidate	0.15
42	² -iso-methyl Ionone	15.84
43	² -N-Methylionone	4.44
44	Methyl dihydrojasmonate	8.31

S.No	Compound	Area (%)
45	Patchouli alcohol	0.60
46	Diphenylmethoxyphosphine	0.10
47	2-Allyl-1,4-dimethoxy-3-vinyloxymethylbenzene	1.17
48	3-tert-Butylphenol	2.59
49	Ornithine	0.17
50	7-Octylidenebicyclo(4.1.0)heptanes	0.09
51	Ambrox	1.11
52	3,4,4'-triaminodiphenyl ether	0.16
53	Traseolide	0.10
54	6,7-diethyl-1,1,4,4-tetramethyltetraline	0.47
55	2-(5-nitro-2-furyl)-1H-benzoimidazole	0.08
56	Galaxolide	10.02
57	6-acetyl-1,1,2,4,4,7-hexamethyltetralin	10.63
58	N-Acetyltyramine	0.65
59	Nordextromethorphan	0.09
60	Phthalic acid mono-2-ethylhexyl ester	0.79

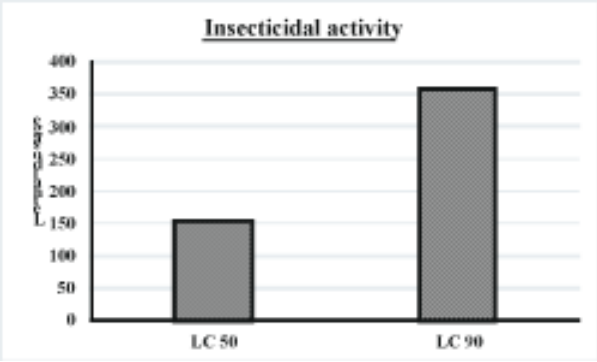

Fig. 1: Insecticidal activity of *M. citrifolia* essential oil against *Maconellicoccus hirsutus*

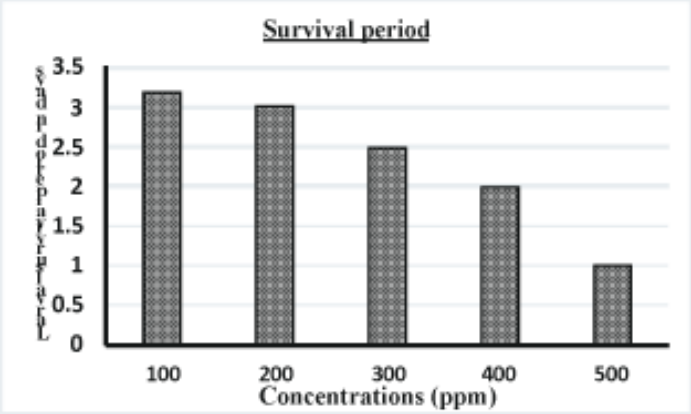

Fig. 2: Survival rate of Maconellicoccus hirsutus treated with *M. citrifolia* essential oil

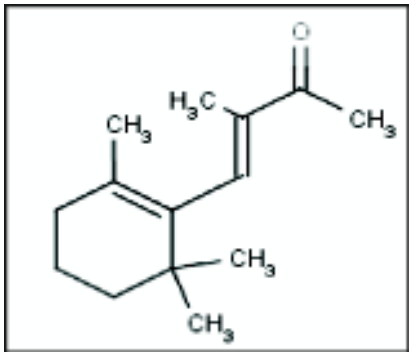

Fig. 3: β-Iso-methyl ionone

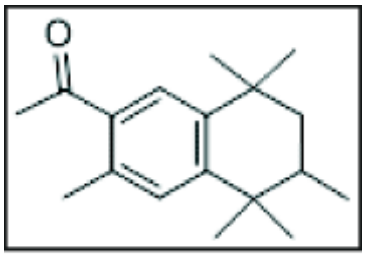

Fig. 4: 6-acetyl-1, 1, 2, 4, 4, 7-hexamethyltetralin

6-acetyl-1, 1, 2, 4, 4, 7-hexamethyltetralin (10.63 %) (Fig.4) is an aromatic compound found within the essential oil cascade of *M. citrifolia* which is widely used as a main source for perfume and deodorant (Roosens *et al*., 2007). Galaxolide (Fig. 5) (10.02 %) found in oil extract of *M. citrifolia* was reported to have insecticidal activity against *Chironomus riparius* (Artola *et al*., 2003), methyl dihydrojasmonate (Fig. 6) was found to present 8.31% in the *M. citrifolia* oil extract. Mann *et al*. (2010) has evaluated the toxicity level of semiochemical methyl jasmonate indicative of insecticidal activity determined against house fly and stable fly adults. Lilial (Fig.7) (7.68 %), Diethyl phthalate (Fig. 8) (7.63 %), 2, 6 Dimethyl-7-octen-2-ol (5.41 %), ²-N-methyl ionone (4.44), Alpha-ethyl-8.²-hydroxy-2,6- dimethyl bicycle (4.4.0) dec-1-ene (2.77%) and Linalyl anthranilate (2.45%) are other differential components of extracted oil whereas linalool (2.43%)present in the *M. citrifolia* extract was reported to have significant increment in the nymphal duration in German cockroach, *Blattella germanica* when fed through artificial diet (Karr and Coats, 1992).

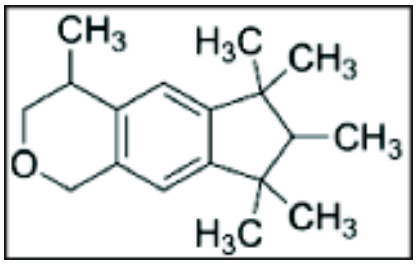

Fig. 5: Galaxolide

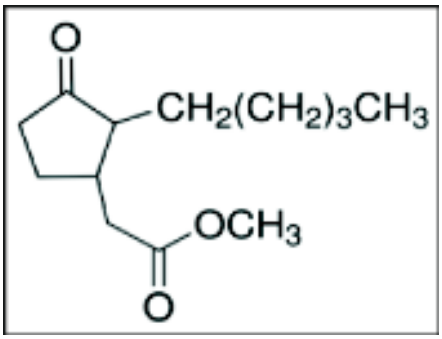

Fig. 6: Methyl dihydrojasmonate

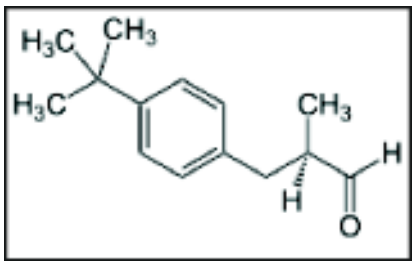

Fig. 7: Lilial

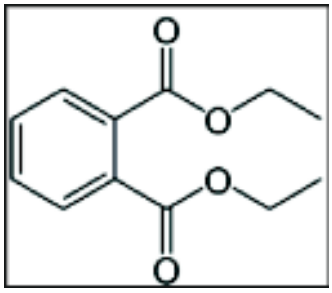

Fig. 8: Diethyl phthalate

Limonene (1.42%) an effective insecticidal plant based compound is identified as a component of *M. citrifolia* oil, limonene was reported to pertain lethal effect against mealy bugs and scale insects (Hollingsworth, 2005). Geraniol (0.10%) which is reported as an effective plant based mosquitocidal compound (Barnard and Xue, 2004) is found to be a component of *M. citrifolia* oil extract. Monoterpenes and triterpenes constituted approximately 8.28 % and 2.18 % respectively. Also the essential oil mixture included components of alcohol at 5.48 %, fatty acids 8.43 % and alkane 1.45 %. The presence of sesquiterpene compounds (3.81 %) in *M. citrifolia* oil extract were reported to possess insecticidal activity (Park *et al*., 2005; Nerio *et al*., 2010).

Conclusion

The present study highlighted the application of essential oil of *M. citrifolia*leaves which possessed higher composition of monoterpenoids and sesquiterpenoid compounds which in turn makes the oil an effective insecticide and repellent. We observed a functional response by third instar larvae

of *M. hirsutus* to the natural larvicidal product, the crude oil extracts of *M. citrifolia*. Therefore, this study provides first report on essential oil on the larvicidal activities against the mealy bugs from India. Thus it could serve as a new eco-friendly alternative for the management of mealy bug species.

Acknowledgement

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References

Abbott, W.S. 1925. A method of computing the effectiveness of insecticides. *J Econ Entomol.,* 18: 267–269.

Adams, R. P. 2007. Identification of Essential Oil Components by Gas chromatography/Mass spectrometry, 4th Ed., Allured Publishing, Carol Stream, Illinois.

Artola-Garicano E., Sinnige, T.L., Van Holsteijn,Vaes W.H. and Hermens, J. L. 2003. Bioconcentration and acute toxicity of polycyclic musk's in two benthic organisms (*Chironomus riparius* and *Lumbriculus variegatus*). *Environ Toxicol Chem.,* 22(5):1086-1092.

Bakker, J., Gommers, F. J. Nieuwenhuis, I. and Wynberg, H. 197). Photo activation of the nematicidal compound alpha-terthienyl from roots of marigolds (Targets species). A possible singlet oxygen role. *J. Biol. Chem*., 254: 1841-1844.

Barnard, D. R., and Xue, R. 2004. Laboratory evaluation of mosquito repellents against *Aedes albopictus, Culex nigri palpus,* and *Ochlerotatus triseriatus,* (Diptera: Culicidae). *J. Med. Entomol.,* 41 (4): 726–730.

Budavari, S, O'Neil, M.J., Smith, A. and Heckelman, P.E. 1989. In: The Merck Index. An encyclopaedia of chemicals, drugs, and biologicals, 11thed. Merck & Co Inc, Rathway, New Jersey.

Clevenger, J. F. 1928. Apparatus for the determination of volatile oil. *J. Ameri. Pharm. Assoc.* 17(4): 345-349.

Daulatabad, C.D., Mulla, G.M. and Mirajikar, A.M. 1989. Riconoleic acid in *Morinda citrifolia* seed oil. Oil Technologists' Association of India, 21:26–27.

Elkins, R. 1997. Hawaiian Noni (*Morinda citrifolia*). Woodland Publishing.

Farine, J.P., Legal, L., Moreteau, B. and Le Quere, J.L. 1996. Volatile components of ripe fruits of *Morinda citrifolia* and their effects on *Drosophila*. *Phytochem* ., 41:433–438.

Finney, D.J. 1971. Probit analysis. Cambridge University, London, pp 68–78.

Heinicke, R. 1985. The pharmacologically active ingredient of Noni. Bulletin of the National Tropical Botanical Garden.

Higa, I. and Fuyama, Y. 1993. Genetics of food preference in Drosophila sechellia. 1. Responses to food attractants. *Genetica,* 88:129–136.

Hollingsworth, R.G. 2005. Limonene, a citrus extract, for control of mealy bugs and scale. *Insects. Ecotoxicology,* 98: 772–779.

Karr, L. L. and Coats, J. R. 1992. Effects of four monoterpenoids on growth and reproduction of the German cockroach (Blattodea: Blattellidae). *J. Econ. Entomol*., 85: 424-429.

Legal, L., David, J.R. and Jallon, J. M. 1994. Molecular basis of *Morinda citrifolia* (L.): toxicity on *Drosophila*. *J. Chem. Ecology*, 20:1931–1943.

Levand, O. and Larson, H.O. 1979. Some chemical constituents of *Morinda citrifolia*. *Planta Med.*, 36:186–187.

Mann Rajinder, Kaufman, S., Phillip, E. and Butler, Jerry F. 2010. Evaluation of semi chemical toxicity to houseflies and stable flies (Diptera: Muscidae). *Pest. Manag. Sci.,* 66 (8): 1526-4998.

Moorthy, N.K. and Reddy, G. S. 1970. *Preliminary phytochemical and pharmacological study of Morinda citrifolia L. Antiseptic, 67:167–171.*

Nerio, L.S., Olivero-Verbel, J. and Stashenko, E. 2010. Repellent activity of essential oils: a review. *Bioresour Technol.*, 101: 372-378.

Papachristos, D.P., Kimbaris, A.C., Papadopoulos, N.T. and Polissiou, M.G. 2009. Toxicity of citrus essential oils against *Ceratitis capitata,* (Diptera: Tephritidae) larvae. *Annals of Applied Biology,* 155: 381– 389.

Park, B.S., Choi, W.S., Kim, J.H., Kim, K.H. and Lee, S. E 2005. Monoterpenes from thyme (Thymus vulgaris) as potential mosquito repellents. *J. Am. Mosq. Control Assoc*., 21: 80-83.

Peerzada, N., Renaud, S. and Ryan P. 1990. Vitamin C and elemental composition of some bush fruits. *J Plant Nutrition*, 13:787–793.

Petrakis PV, Roussis V, Papadimitriou D, Vagias C, Tsitsimpikou C, 2005. The effects of terpenoid extracts from 15 pine species on the feeding behavioral sequence of the late instars of the pine processionary caterpillar *Thaumetopea pityocampa*. *Behavioral Processes* 69: 303–322.

Roosens L, Covaci A, Neels H. 2007. Concentrations of synthetic musk compounds in personal care and sanitation products and human exposure profiles through dermal application. *Chemosphere* 69: 1540-1547.

Sharma R.V., Mathur S.N., Dmitrovskii A.A., Das R.C. and Ganguly J. 1977. Studies on the metabolism of beta-carotene and apo- beta-carotenoids in rats and chickens. *Biochemical and Biophysical Acta*, 486: 183-194.

Singh J, Tiwari RD, (1976). Flavone glycosides from the flowers of *Morinda citrifolia*. *J Indian Chem Soc* 53:424.

Zhang, Q., Margaryan, A., Rodeny G, and Schneidmiller. (2011). Methods for repelling insects using sesquiterpene hydrocarbons and their derivatives. US patent http://www.freepatentsonline.com/8057829.pdf.

IV-OP-1

Influence of different methods of extraction on phytochemicals and antioxidant activity of *Morinda citrifolia* L. juice

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Abstract

Morinda citrifolia L. commonly called Noni, has a long history as a medicinal plant and its use as a botanical dietary supplement has grown tremendously in recent years. Noni juice, and fruit extracts have functional biological compounds exhibiting properties such as scavenging of free radicals, anti-mutagenicity, anti-carcinoma activity, anti-clastogenic activity, inhibition of low density-lipoprotein oxidation, anti-inflammatory activity, blood purification, stimulation of the immune system, regulation of cell function, and regulation of cholesterols. The present study was carried out with an aim to investigate the method with higher juice recovery, better shelf life, physical and biochemical characteristics of Morinda citrifolia juice. For this, parameters such as phenotypic characters, proximate components, phytochemicals and anti-nutritional factors were examined. Results revealed that traditional fermentation practice and room-temperature storage dramatically decreased its free radical-scavenging activity. Refrigeration and freezing storage significantly retarded the decrease in (radical scavenging activity) RSA. Dehydration of pure juice of Noni resulted in only a limited reduction of RSA. For providing maximum potential health benefits of Noni products to consumers, processing of Noni powder or refrigeration and freezing of Noni juice are strongly recommended.

Keywords: antioxidant, extraction loss, *Morinda citrifolia* L., Noni juice, phytochemicals, RSA.

Introduction

Since fruits are generally difficult to be stored for a considerable length of time, ripe fruits are utilized either as fresh fruit processed into juice or as specialty products. Most of the fruits are perishable in nature and hence, need proper post harvest management as deterioration sets in almost immediately after harvest due to metabolic activities which continue even after harvest. However the perishable nature makes it difficult to store extract and the juice without loss of flavor and nutritional values. Large quantities of fruits are produced and wasted globally, thus Noni is one such fruit which needs prompt processing after its harvesting.

Fruit juice is a nutrient dense beverage choice that is rich in many of the nutrients found naturally in whole fruit and it is ready and rich source of vitamins, fibre and mineral salt for human consumption due to its uses as medicine, food and appetites (Ashurt, 1991). On smart addition to any well-balanced diet, fruits provide vitamins and minerals like potassium, vitamin C and folate. Fruit juice is also a convenient way for adults and children to help reach the recommended number of daily servings of fruits and vegetables. Better nutrition means stronger immune systems, less illness and better health. Consumers are now more conscious of nutritional value, and safety of food ingredients especially for plant or natural food and its products. Similarly, it is important to believe to be protected from disease and make strengthened to the body (Kellen et al., 2007). Most of the plants have been good sources of rich in nutritional value and medicinal characteristics.

In addition to the nutritive value of juices and the additional health benefits from phytochemicals, recently recognized components of many fruit juices will the popularity of such products. Even flavorful juices, that may not be balanced nutritionally or which lack nutrients or phytochemicals, can be blended or act as effective carriers for other natural or synthetic nutrients such as vitamins, minerals and fluid food products, including juices are easier to process, to heat, cool, freeze, standardize, transport, etc. Thus processing efficiency, safety and quality norms are easier to meet, the ease of mixing fluids promotes development of unique blends of juices with other products and practical combinations not found naturally, highly flavored juices are the basis for a range of juice co-products, such as ice cream and confectionery flavors, smoothies, bakery ingredients, etc. Modern processing, packaging, ingredient technology and distribution systems insure safe, stable and appealing juice and beverage products in a convenient, economical form far from the raw material source or season.

Material and Methods

Chemicals and Reagents

The analytical grade chemical reagents were used in the present study and 1,1-diphenyl 2 picrylhydrazyl (DPPH), gallic acid, anthrone reagent, aluminium chloride, boron tri fluoride (BF_3), heptane, formic acid, acetonitrile, acetic acid and tri fluoro acetic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tannic acid, ascorbic acid, conc. HCL, sodium acetate buffer and sulphuric acid were purchased from Himedia (Himedia Laboratories Pvt. Ltd., Mumbai). Methanol, rutin, folin-ciocaulteu reagent, KCl, copper sulphate and sodium hydroxide were purchased from Merck (Merck, Darmstadt, Germany) and sodium acetate, orthophosphoric acid, acetone and sodium carbonate solution were obtained from Rankem (Rankem, RFCL Ltd., New Delhi, India).

Noni fruit

Fruits were collected from single plant grown in germplasm block at Central Island Agricultural Research Institute, Port Blair, Andaman & Nicobar Islands (India). The collected fruits sample were cleaned with double distilled water and stored at -20º C for analysis.

Juice extraction methods

Fresh Noni juice was prepared by different methods *viz.,* direct squeezing adopting Nelson (2006), boiling method in which the Noni fruits were kept for boiling water for hours, then to make it a juice and freezing and thawing method in which the Noni fruits kept inside the freezer for a long time one hour interval and took outside then thawed in room temperature. This process was repeated at least four or five times. This fruit was used for juice extraction process.

Sample preparation

Samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The collected samples were washed with running tap water and chopped into small pieces. The 5 g of each sample was ground with 10ml methanol using pestle and mortar and the extract was added to 40ml of methanol and kept overnight in dark room for incubation. The mixture was filtered through a filter paper (Whatman No. 1) and evaporated under reduced

pressure using rotary evaporator. An aliquot of the concentrated extract was used for spectrophotometric observations and remaining sample was stored in dark glass bottles for further analysis.

Phytochemical compounds

The polyphenol content in the sample was analyzed by Folin-Ciocalteau reagent method (10%, v/v) (Singleton & Rossi, 1965) with some modifications. Gallic acid was used as standard and results were expressed as mg of gallic acid equivalent (mg/100g fresh weight). In brief, each sample extract was diluted with methanol in 1:100 ratio. The diluted extracts were mixed with Folin-Ciocalteau reagent and 7.5% $NaHCO_3$ in 0.5ml:2.5ml:2 ml ratio and kept for incubation in dark chamber for 30 min. Absorbance from samples was measured at 765 nm using UV-spectrophotometer (Elico SL-164, Pvt. Ltd., Hyderabad, India). The polyphenol content was calculated using following equation based on the calibration curve: $y = 0.147x$, $R^2 = 0.974$, where x is the gallic acid equivalent (mg/100g) and y is the absorbance expressed as gallic acid equivalents (GAE) in mg per gram of fresh weight basis.

Flavonoid content in sample of *M. citrifolia* was determined spectrophotometrically using Aluminum chloride reagent method (Chang et al. 2002). Briefly, 500mg sample extract mixed with equal volume of 2% ethanol solution of Aluminum chloride and kept for 1 h incubation and absorbance was taken at 415 nm by UV-spectrophotometer. The flavonoid content in samples was expressed as mg rutin equivalent (mg/100g fresh weight). Tannin content (TAE mg/100g fresh weight) in sample of *M. citrifolia* was estimated using AOAC method (1995) and concentration was determined using the equation based on the calibration curve: $y = 0.045x$, $R^2 = 0.961$, where x is the tannic acid equivalent (mg/100g) and y is the absorbance.

The anthocyanin in the sample was determined by pH differential method (Fulecki & Francis, 1968) and concentration was calculated using the following equation: TAC= "A x MW × DF x 1000)/å, where TAC is total anthocyanin content, "A is difference in OD value of extract at pH 1.0 and pH 4.5, MW is molecular weight of cynidine-3-glucoside, DF as dilution factor, å is molar absorbance coefficient of cynidine-3-glucoside. Total carotenoids content in plant parts was determined using the procedure given by Lichtenthaler and Buschmann (2001). Ascorbic acid was estimated using volumetric method and expressed as mg/100g fresh weight (Sadasivam and

Manikam, 1992). Phytate, oxalate and nitrate content in various parts of *M. citrifolia* samples were determined according to the method suggested by Hassan et al. (2008). Saponin content was determined following the AOAC (1995) method.

DPPH free radical scavenging activity

The antioxidant activity of sample extracts was estimated using methanol, acetone and aqueous solvents by DPPH (1,1-diphenyl-2- picrylhydrazyl) method as described by Singh et al. (2011). The sample extracts (0.1 ml) were added to 3 ml of methanol solution of DPPH (0.001M). Stock solution of extracts were diluted to 20, 40, 60, 80 and 100 µg/ml and immediately transferred to dark chamber. The absorbance readings of samples were recorded at 0, 30, 60, 90 and 120 min of incubation at 517 nm. The antioxidant activity (%) was calculated as $[(A_0 - A_E)/A_0] \times 100$ (A_0 = absorbance without extract; A_E = absorbance with extract), whilst IC_{50} values were estimated using a linear regression analysis.

Statistical analysis

Three replications of each treatment were maintained for all experiments. All the statistical analysis was carried out using Microsoft excel software.

Results

Phenotypic characters

Details corresponding to the phenotypic characters are summarized in Table 1. Fruit weight was maximal in fresh fruits (before freezing and thawing), corresponding to 357.9 ± 9.9 followed by fermented fruit and it was found minimal in freezing and thawing. However, fresh pulp weight was maximal in fermented fruit (107.9), whereas it was found significantly lower in other two methods. No significant difference in fresh seed weight is observed between freezing & thawing and fresh fruits, whereas it corresponded to 30.8 in fermented fruit. Dry pulp weight was maximal (142.3g) in fermented fruits and it was found well below par from other two methods. Seed weight was maximal (47.4g) in freezing and thawing while maximal fruit weight (357.9g), dry seed weight (14.8g) and seed powder weight (14.8g) were observed in fresh fruits. Fruit pulp

weight, juice weight and dry pulp weight were maximal (107.9g, 163.3g and 142.3g respectively) were observed in fermentation.

Table1 : Phenotypic characteristic of *Morinda citrifolia* juice using different extraction method

Phenotypic characters (gm)	Techniques		
	Freezing & thawing	Fresh Fruits (before freezing & thawing)	Fermented fruit
Fruit weight	258.32±3.9	357.9±9.9	339.4±15.8
Fresh pulp weight	51.21±2.8	75.2±11.6	107.9±6.7
Fresh seed weight	47.4±12.9	46.2±2.9	30.8±6.4
Juice weight	148.3±30.1	142.3±19.7	163.3±15.3
Dry pulp weight	10.6±5	10.8±1.1	142.3±6.3
Dry seed weight	13.1±2.9	14.8±0.8	11.8±1.9
Pulp powder weight	10.5±5	10.9±1.1	13.9±2.4
Seed powder weight	13.4±2.5	14.8±0.8	11.8±1.9

Proximate components

Details corresponding to the proximate characters are summarized in Table 2.Freezing and thawing yielded maximal juice recovery percentage of 57.3%, TSS 4.5 °Brix with acidity percentage of 0.2%., which was found to be comparatively higher. Further, non reducing sugar content was also found to be higher in this method (910.1 mg/100 g) along with total sugars (630.9 mg/100 g). However, reducing sugars and amino acids were reportedly higher in fermented juice method (395.9 mg/100 g) and in direct squeezing method (283.1 mg/100 g) respectively.

Table 2 : Proximate components of *Morinda citrifolia* juice using different extraction method

Proximate components	Techniques		
	Freezing & thawing	Direct squeezing	Fermented Juice
Juice (%)	57.3±9.9	39.7±4.4	48.2±4.4
TSS (°Brix)	4.5±0.3	4.4±0.3	4.4±0.2
Acidity (%)	0.2±0	0.1±0	0.2±0
Reducing sugar (mg/100g)	279.3±24.4	172.7±26.4	395.9±10.5
Non-reducing sugar (mg/100g)	910.1±17.7	383.7±38.1	65018.9
Total sugar (mg/100g)	630.9±24.1	2111±3.4	254.11±6.6
Free amino acids (mg/100g)	162.7±7.4	283.1±15.6	1311±9.2

Table 3 : Phytochemicals and antioxidant activity of *Morinda citrifolia* juice using different extraction methods

Phytochemicals	Techniques		
	Freezing & thawing	direct squeezing	Fermented juice
Phenol	165.9±5.9	294.3±24	267.9±11.2
Flavonoid	291.6±7.8	462.7±17.4	69.5±15.3
Tannin	374.4±11.7	201.5±21.4	874.8±20
Carotenoid	604.2±0.1	282.6±0	-683.1±0
Anthocyanin	67.7±0	68.6±0	102.2±0
Ascorbic acid	84.8±0.1	600.1±	108.9±0.5
Antioxidant activity			
Antioxidant activity	74.9±5.3	76.5±5.3	75.3±5.3
IC_{50} value	3.8±1.1	4.1±1.1	3.9±1.1

Phytochemicals

Details corresponding to the phytochemical characters are summarized in Table 3.Carotenoids were reportedly higher (604.2 µg/100 g) in freezing and thawing method while direct squeezing method yielded higher amounts of ploy phenol (294.3 mg/100 g), flavonoid (462.7 mg/100 g), ascorbic acid (600.1 mg/100 g), antioxidant activity (76.5%) and IC_{50} value of 4.1 µg/ml. However, tannin and anthocyanin contents were reportedly higher with 874.8 mg/100 g and 102.2 mg/100 g respectively in fermented juice method.

Anti-nutritional factors

Details corresponding to the phenotypic characters are summarized in Table 4.Three of the antinutritional factors *viz.,* phytate, oxalate and saponin were observed to be higher by direct squeezing method, wherein the values correspond to 844.6 mg/100g, 501.2 mg/100 g and 316.7 mg/100g respectively. On the other hand, nitrate was found to be higher (117.9 mg/100 g) by Freezing and thawing method.

Table 4 : Anti-nutritional factors of *Morinda citrifolia* juice using different extraction methods

Anti-nutritional factors	Techniques		
	Freezing & thawing	**Direct squeezing**	**Fermented juice**
Nitrate	117.9±4	57.1±4	77.5±4.5
Phytate	5034±5.8	844.6±96.9	621.6±21.7
Oxalate	30±1.5	501.2±2.5	53.1±3
Saponin	206.7±17.6	316.7±36.9	136.7±34

Discussion

Commercial Noni juice is traditionally made by fermentation of Noni fruits in sealed containers for 2 months at ambient temperature (Newton, 2002; Nelson, 2006). Oxidation reactions result

in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics. Such changes may be beneficial (as is the case with black tea) or harmful (browning of fruit) to consumer acceptability. Storage of wheat flour results in marked loss of phenolic acids. After 6 months of storage, flours contained the same phenolic acids in qualitative terms, but their concentrations were 70% lower. Previous studies documented that cold storage, in contrast, did not affect on the content of polyphenols in apples, pears or onions. At 25° C, storage of apple juice for 9 months results in a 60% loss of quercetin and a total loss of procyanidins, despite the fact that polyphenols are more stable in fruit juices than is vitamin C.

Conclusions

Fresh Noni fruit is a good source of antioxidants and phenolics, but traditional fermentation practice and room-temperature storage dramatically decreased its free radical-scavenging activity. Refrigeration and freezing storage significantly retarded the decrease in RSA. Dehydration of Noni pure juice produced only a limited reduction of RSA. For maximum potential health benefits of Noni products to consumers, processing of Noni powder or refrigeration and freezing of Noni juice are strongly recommended.

References

AOAC. 1995. Association of Official Methods of Analysis. 16th ed. Washington DC, USA.

Ashurt, P.R. 1991. History of fruit drinks and food flavouring. Rumbold, New York. pp: 9-35.

Chang, C.C., Yang, M.H., Wen, H.M. and Chern, J.C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal*., 10: 178-182.

Fulecki, T. and Francis, F.J. 1968. Quantitative methods for anthocyanin extraction and determination of total anthocyanin in cranberries. *J. Food Sci*., 33: 72-77.

Hassan, L.G., Dangoggo, S.M., Umar, K.J., Saidu, I. and Folorunsho, F.A. 2008. Proximate, minerals and antinutritional factors of *Daniellia oliveri* seed kernel. *Chem. Class J*., 5: 31-36.

Kellen, E., Zeegers, M.P., Hond, E.D. and Buntix, F. 2007. Blood cadmium may be associated with bladder carcinogenesis: the Belgian case-study on bladder cancer. *Cancer Detect Prev*., 31:77-82.

Lichtenthaler, H.K. and Buschmann, C. 2001. Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy. *In: (Ed.) R. E. Wrolstad, Current protocols food analytical chemistry. New York: Wiley. p F4.3.1–F4.3.8.*

Nelson, S. C. 2006. *Morinda citrifolia* (Noni). Species profiles for Pacific Island agroforestry. Available from www.tradtionaltree.org.

Sadaasivam, S. and Manikam, A. 1992. Biochemical methods. 3rd ed., Madras: Wiley Eastern Ltd. pp:246–248.

Singh, S., Singh, D.R., Salim, K.M., Srivastava, A., Singh, L.B. and Srivastava, R.C. 2011. Estimation of proximate composition, micronutrients and phytochemical compounds in traditional vegetables from Andaman and Nicobar Islands. *Intl. J. Food Sci. Nutr.,* 62(7): 765–773.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Eno Viticul*., 16:144–158.

IV-OP-2

Investigation of safety assessment of Noni juice - *Garcinia gambogia* mix

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Abstract

Morinda citrifolia L Noni is a divine medicinal plant and nature gift for human wellness. Noni traditionally used for treatment of diseases and food supplement over 2000 years. Review of literature revealed that clinical study data of Noni Juice on human clinical studies demonstrated for safe and effective food supplement. The present studies aimed at the investigation of the toxicological analysis for assessment of the safety aspect of Noni Juice contain Morinda citrifolia and Garcinia gambogia fruit juice mix. Safety Assessment of noni evaluated by acute oral toxicity study, sub acute toxicity, genotoxicity and allergenicity in animal models. The results of the single and repeated dose toxicity studies indicated no mortalities and no significant changes recorded in physical, physiological, hematological, biochemical and histopathological observations during the study period. There were no allergenic reactions in allergenicity test and no mutagenic potential in the Ames test under experimental conditions. Morinda citrifolia L., Noni fruit juice-Garginia cambogia mix investigated for safety analysis such as toxicology, genotoxicity, allergenicity and all other test results demonstrated that Noni juice is found to be safe and non toxic.

Keywords : safety assessment, noni juice, *Garginia cambogia*

Introduction

Morinda citrifolia L Noni is a versatile medicinal plant and nature gift for human wellness. Noni traditionally used for treatment of diseases and food supplement over 2000 years. High medicinal and nutritional benefits of noni due to the presence of enriched potential biomolecules and essential Nutraceuticals contents (Wang et al., 2002). Whole plant of noni used

as source for the food and therapeutic purpose by ancestors evidenced through etthnopharmacology studies (McClatchey W, 2002; Charles et al., 2006). Noni documented for broad-spectrum pharmacological activity due to the naturally gifted bioactive molecules such as anthroquinione, flavanoids, glycoside, alkaloids and irrioids etc (Potterat O, and Hamburger M.2007). The nutraceuticals value of Noni is also due to the presence of essential amino acids, vitamins and mineral composition (Pawlus AD and Kinghorn D.2002; Brett et al., 2011; Simla Basar, Johannes Westendorf.2012). Noni reported to possess immunomodulary, antioxidant and anticancer activity (Palu et al., 2008). Intraperitoneal injection of an ethanol-insoluble material (Noni-precipitate), obtained from the juice of the *Morinda citrifolia* fruits, has been shown to prolong the lifespan of mice implanted with Lewis lung carcinoma. It was suggested by the authors that the suppression of tumor growth was due to the enhancement of the host immune system by a partially identified polysaccharide (Hirazumi et al., 1994, 1996 and 1996). Noni fruit juice commercial used as food supplement throughout the world. Recently EU approved Noni fruit juice as a novel food supplement. *Morinda citrifolia* L Noni fruit juice investigated for safety analysis such as toxicology, genotoxicity and allergenicity and all the test results confirm the Noni juice is very safe and non toxic for human use (West et al., 2006). Review of literature revealed that clinical study data of Noni Juice in human trials demonstrated for more safe and effective food supplement (Brett et al., 2009). The present study aimed at investigation of the chemical composition and toxicological analysis for confirmation of the safety aspect of Noni Juice (DNJ) contains *Morinda citrifolia* and *Garcinia gambogia* fruit juice mix.

Materials and methods

The following tests were performed:

1. Acute oral toxicity study
2. Sub acute Toxicity
3. Genotoxicity
4. Allergenicity

1. Acute oral toxicity study

A safety factor of 100 was applied to convert the NO Observed Adverse Effect Level (NOAEL) of 80 ml/kg body weight/day determined in a 13-week average study with rats into an Acceptable

Daily Intake (ADI) in a man of 0.8 ml/kg body weight/day. Based on body weights from 20 to 80 kg, this corresponds to intakes ranging from 16 ml/day to 64 ml/day per person. Recommended dose 10-60 ml/day. Acute oral toxicity study of Original Noni *Garcinia gambogia* mix was investigated in rat and Mouse models.

A. Rat model (Single dose 14-day oral toxicity study)

10 rats were randomized groups (5 per group five male and five female). The test group received a single oral dose of 19 ml/kg of DNJ, Noni juice *Garcinia gambogia* Mix via gastric intubation. All the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days following vehicle or DNJ administration. Body weights were recorded once a week. On day 15, the overnight fasted animals (water allowed) were euthanized using CO2 euthanasia chamber and subjected to a gross pathological examination of all the major internal organs such as brain, heart, lung, liver, kidney, spleen, adrenals and sex organs.

B. Mice model (Single dose 14-day oral toxicity study)

10 mice were randomized into two groups (5 per group five male and five female). The group received a single oral dose of 19.5 ml/kg of Noni juice *Garcinia gambogia* Mix via gastric intubation. All the experimental animals were observed for mortality and clinical signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture) at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days following vehicle or Noni juice *Garcinia gambogia* Mix administration. Body weights were recorded once a week. On day 15, the overnight fasted animals (water allowed) were euthanized using CO2 euthanasia chamber and subjected to a gross pathological examination of all the major internal organs such as brain, heart, lung, liver, kidney, spleen, adrenals and sex organs.

2. Sub acute Toxicity (Repeated dose 28-day oral toxicity study)

Sub acute oral toxicity study of original Noni *Garcinia gambogia* mix was investigated in rat models. A 28-day repeated oral toxicity study was performed according to the OECD guideline,

TG 407 (Revised–18 December 2007) with minor modifications. In 10-40 ml /day of DNJ was recommended in adult humans. The rat dose of 270 mg/kg was arrived from the human dose based on body surface area conversion. In the present study, Noni juice *Garcinia gambogia* Mix was administered at three dose levels i.e., at 300, 600 and 900 mg/kg/day. Both sexes of Sprague Dawley rats (140-160g) were divided into 6 groups with 10 animals (5 males + 5 females) in each. Group I served as control and received 0.5% CMC as vehicle orally via gastric intubation at a dose volume of 10 ml/kg body weight. Group II, III and IV received NK in 300, 600 and 900 mg/kg/day, p.o, respectively (10 ml/kg body weight. at 0.5% CMC), for a period of 28 days via gastric intubation. In order to determine the reversibility or recovery from toxic effects, if any, the satellite groups were present. Group V served as satellite control (received vehicle) and group VI served as a treatment satellite group which received Noni juice *Garcinia gambogia* mix at 900 mg/kg/day, p.o for a period of 28 days.

The satellite groups were scheduled for follow-up observations for the next 14 days without vehicle or Noni juice *Garcinia gambogia* mix administration. All the experimental animals were observed for mortality and morbidity twice a day, till the completion of treatment. Clinical observations were made once daily to detect signs of toxicity, preferably at the same time(s) in each day (1 h after vehicle or NK administration). The focus of the observations was the same as described above for the acute toxicity study. Body weights of the animals were recorded once in a week. The amounts of food and water given and their remnants on the next day were measured to calculate the difference, which was regarded as daily consumption and the data were expressed as 7 days cumulative value.

A. Biochemical and hematological studies

At the end of the stipulated treatment period, the overnight fasted (water allowed) animals were anaesthetized, blood samples were collected by retro-orbital puncture in heparinised (for haematological and biochemical analysis) and non-heparinised tubes (for serum electrolytes). Haematological parameters such as haemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), Hematocrit (HCT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV), Plateletcrit (PCT) and red cell indices were measured using fully automated haematology analyzer (PE 6000). Plasma biochemical parameters such

as glucose, total cholesterol (TC), triglycerides (TG), total protein (TP), albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), ã-glutamyl transpeptidase (ã-GT), bilirubin, creatinine (CRE) and blood urea nitrogen (BUN) were measured using diagnostic kits (Accurex, India) in a semi-automatic biochemical analyser (Star 21plus, India). Serum electrolytes sodium, potassium, chloride, total calcium and pH were estimated in a fully automated electrolyte analyzer (Cornley acculyte-5P, India).

B. Histopathology

Necropsy was done in all animals on day 29 except the satellite groups for which it was done on day 42. After blood collection, all the animals were euthanized for gross pathological examinations of all major internal organs. Organs such as the brain, eyes, spinal cord, lymph nodes lung (right and left), prostate, thyroid gland, heart, stomach, liver, kidney, adrenals, thymus, spleen, intestine, muscle, bone, ovary, uterus, testis and urinary bladder were collected from all the animals for histopathology.

The organs such as brain, heart, liver, spleen, kidneys, adrenals, thymus, and testis/ovaries were weighed and relative organ weights were calculated. However, it is planned to perform histopathological examination of the control and high dose group initially, if any histopathological findings were observed with the high dose group, the low and mid dose groups were to be studied. The organs were fixed at 10% neutral buffered formalin, trimmed and a 5ì thickness of tissue sections was stained with hematoxylin and eosin for histopathological Investigation.

3. Genotoxicity (Ames Test)

Members of the family rebiaceae, such a *Morinda citrifolia* L, are known to contain anthroquinones inside the roots. Anthroquinones could be isolated from cell suspension cultures of *Morinda citrifolia* (Leistner, 1975). However, in accordance with formerly published data (Zenk et al., 1975), these constituents could not be identified in noni fruits. Data from analyses submitted by the applicant paid particular attention to lucidin and rubiadan, because of the genotoxicity of these two anthroquinones. Neither compound was detected in the juice (detection limit determined for rubiadin: 10µg/kg). Genotoxicity studies of Original Noni *Garcinia gambogia* mix was investigated by Ames test.

In an in vitro bacterial reverse mutation test (plate incorporation and preincubation methods), mutagenic potentials of Noni juice *Garcinia gambogia* Mix were investigated (Aujoulat, 2003). Five strains of Salmonella typhimurium (TA98, TA100, TA102, TA1535 and TA1537) were used to investigate the effects of DNJ, both in the presence and absence of metabolic activation (±S9). In the plate incorporation method, the dose levels used were 12.5, 25, 50, 75 and 100 % V/V /plate. No statistically or biologically significant increases in the number of revertants were noted in any strain, either with or without metabolic activation. In the preincubation method, DNJ was used at dose levels of 12.5, 25, 50, 75 and 100 % /plate.

4. Allergenicity

The present study is to evaluate the safety and Allergenicity of Original Noni *Garcinia gambogia* Mix manufactured by Noni Biotech Private Limited, Kancheepuram. Allergenicity study of Original Noni *Garcinia gambogia* mix was investigated in BALB/c Mice model. Allergenicity study consist a total of 40 animals divided into eight groups containing 6 animals (3M+3F) per group for sensitization and 4 animals (2M+2F) per group for Passive Cutaneous Anaphylactic (PCA) reaction. The present study is to evaluate the safety and Allergenicity of Original Noni *Garcinia gambogia* Mix manufactured by Noni Biotech Private Limited, Kancheepuram. Allergenicity study of Original Noni *Garcinia gambogia* mix was investigated in BALB/c Mice model. Allergenicity study consist a total of 40 animals divided into eight groups containing 6 animals (3M+3F) per group for sensitization and 4 animals (2M+2F) per group for Passive Cutaneous Anaphylactic (PCA) reaction. Wheal and Flare reaction at the site of injection for allergenicity potential of test item.

Results and Discussion

The results of the acute and sub acute toxicity studies indicated no mortalities and there were no significant changes recorded in physical, physiological, haematological, biochemical and histopathological observations during the study period (data not shown). Under the experimental conditions and according to the criteria of the genotoxicity test (Ames assay) study plan, it was concluded that Noni juice-*Garcinia gambogia* Mix did not induce mutagenic effects in the bacterial reverse mutation test, either with or without metabolic activation. The results of the Ames assay showed that the Noni juice *Garcinia gambogia* Mix had no mutagenic effect to

any strain in this test. From the investigation of allergenicity studies indicated no allergenic reactions in the treated group and were comparable with negative control group animals. There were no significant changes observed in physical and physiological parameters under experimental conditions in BALB/c Mice model used for allergenicity study (data not shown).

Conclusion

Toxicity studies of Original Noni juice- *Garcinia gambogia* Mix, Batch no.45121 manufactured by M/s Noni Biotech Pvt. Ltd., Chennai, with label ingredients mentioned on bottle is *Morinda* Pulp Concentrate (6 fold concentrated *Morinda Pulp*), citric acid (INS330), sweetener (NS420), RO water, acidity regulator (INS330), food stabilizer (INS415), *Garcinia gambogia* and *Glycyrrhiza glabra*. Single dose toxicity in Swiss albino Mice and single and repeated dose (28 days) toxicity in Wistar Rats, Allergenicity study on Balb C Mice and Ames test, has been carried out at our laboratory as per Schedule Y of Drugs and Cosmetics (II Amendment) rules, 2005. The results of the single and repeated dose toxicity studies indicated no moralities and there were no significant changes recorded in physical, physiological, haematological, biochemical and histopathological observations during the study period. There were no allergenic reactions in allergenicity study, no mutagenic potential in Ames test under experimental conditions.

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References

Brett J. West, Shixin Deng and Jarakae Jensen. C. 2011. Nutrient and phytochemical analyses of processed noni puree. *Food Research International*, 44: 2295–2301.

Brett J. West, Leland D. White, Jarakae Jensen, C. and Afa K. Palu, A. 2009. Double-Blind Clinical Safety Study of Noni Fruit Juice. *Pacific Health Dialog*, 15(2): 21-31.

Charles, D. Boyd, Alika, M., Lawrance, J. M. and Katalin, C. 2006. Polynesian ethnobotanical: a critical role in drug discovery. *Pacific health dialog*, 5(2):337-340.

Freireich, E.J., Gehan, E.A., Rall, D.P., Schmidt, L.H. and Skipper, H.E. 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother Rep.,* 50(4):219–244.

Hirazumi, A., Furusawa, E., Chou, S. C. and Hokama, Y. 1994. Anticancer activity of *Morinda citrifolia* (noni) on intraperitoneally implanted Lewis lung carcinoma in syngeneic mice. *In: Proceedings of the Western Pharmacology Society, 37, pp: 145–146.*

Hirazumi, A., Furusawa, E., Chou, S. C. and Hokama, Y. 1996. Immunomodulation contributes to the anticancer activity of *Morinda citrifolia* (noni) fruit juice. *In: Proceedings of the Western Pharmacology Society, 39, pp: 7–9.*

Kaaber K. 2000. TAHITIAN NONI® Juice: active systemic anaphylaxis test in the guinea pig. Scantox Biologisk Laboratorium A/S, DK-426, Lille Skensved, Denmark.Guinea pig antigenicity study. TAHITIAN NONI® juice. 2000 Feb 29. Product Safety Labs (Eurofins Scientific, Inc). East Brunswick, New Jersey, USA.

Hirazumi, A. and Furusawa, E. 1999. An immunomodulatory polysaccharide-rich substance from the fruit juice of *Morinda citrifolia* (noni) with antitumour activity. *Phytotherapy Researches*, 13: 380–387.

Leistner, E. 1975. Isolation, identification and biosynthesis of anthraquinone in cell suspension culture. *Planta Medica* (Supl), 27:214-224.

McClatchey, W. 2002. From Polynesian healers to health food stores changing perspectives of *Morinda citrifolia* (Rubiaceae). *Integrated Cancer Therapy*, 1 :110-20.

Organization for Economic Cooperation and Development (OECD). 2002. Guidelines for the Testing of Chemicals/Section 4: Health Effects Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. Paris, France: Organization for Economic Cooperation and Development.

OECD Forum for the Future. 1998. Organisation for Economic Co-operation and Development: Advisory Unit on Multi-Disciplinary Issues. Expo 2000/OECD Forum for the Future: 21st Century Technologies: balancing economic, social and environmental goals: main issues and summary of the discussions of a Conference held on 7th and 8th December at Schloss Krickenbeck, Germany. Paris: OECD.

Organization for Economic Cooperation and Development (OECD). 2008. Guidelines for the Testing of Chemicals/Draft Updated Test Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents.

Organization for Economic Cooperation and Development (OECD). Guidelines for the Testing of Chemicals. Bacterial reverse mutation test Guideline, 471.

Organization for Economic Co-operation and Development (OECD). 2001. Guidelines for testing of chemicals, TG 423 (adopted – December, 2001) with minor modifications. The acute oral toxicity test was performed following the guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals, TG 423 (adopted – December, 2001) with minor modifications.

Pawlus, A.D. and Kinghorn, D. 2007. A review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement, *Morinda citrifolia* (Noni). *Journal of Pharmacy and Pharmacology,* 59 (12): 1587-1592.

Palu, A.K., Kim, A.H., West, B.J., Deng, S., Jensen, J. and White, L. 2008. The effects of *Morinda citrifolia* L. (Noni) on the immune system, its molecular mechanisms of action. *Journal of Ethnopharmacology*, 115 (3): 502-507.

Potterat, O. and Hamburger, M. 2007. *Morinda citrifolia* (Noni) fruit-phytochemistry, pharmacology, safety. *Planta Med*., 73(3):191-199.

Simla Basar, Johannes Westendorf. 2012. Mineral and trace element concentrations in *Morinda citrifolia* L. (Noni) leaf, fruit and fruit juice. *Food and Nutrition Sciences*, 3: 1176-1188.

Wang, M.Y., West, B.J., Jensen, C.J., Nowicki, D., Su, C., Palu, A.K. and Anderson, G. 2002 *Morinda citrifolia* (Noni) a literature review and recent advances in Noni research. *Acta Pharmacologica Sinica*, 23: 1127-1141.

West, B.J., Jensen, C.J., Westendrof, J. and White, L.D. 2006. A safety review of Noni fruit juice. *J. Food Science*, 71(8): 100-106.

Zenk, M.H., EL-Shangi., Schulte, U. 1975. Anthraquinone prodution by cell culture. *Planta Medica* (Supl), 27:97-101.

IV-OP-3

Phytochemical and biosafety analyses of Divine Noni® (*Morinda citrifolia* and *Garcinia cambogia* mix)

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Abstract

Morinda citrifolia L. popularly known as Noni is very popular in recent times due to its nutritional and medicinal properties. Noni products have been reported to show enormous health benefits in the treatment of various human diseases and disorders. The presence of more than 150 phytochemicals in different parts of Noni has been reported. Garcina cambogia extract is produced from the rind of the fruit which is being used in various herbal preparations mainly for its medicinal values. Similarly Glycyrrhiza glabra is used to treat a wide array of illnesses including lowering of cholesterol levels, healing respiratory tract disorders and boosting immunity levels. The commercial product of Divine Noni® (Morinda citrifolia and Garcinia cambogia Mix) is a perfect blend of M. citrifolia (Morinda Dried Fruit Extract), G. cambogia and G. glabra, whose nutritional values, health benefits and biosafety have been investigated. In addition, the presence of generic phyto-constituents (phytochemicals) in the formulated product of Divine Noni® has also been studied. Phytochemical analysis of Divine Noni® has revealed the presence of alkaloids, phenols, terpenoids and reducing sugar in high amount. Saponin was available in moderate amount and flavanoids, tannins and proteins were in trace. Interestingly, glycoside was completely absent in Divine Noni®. In addition, the presence of important phytocompound, iridoid in the Divine Noni® has also been recorded. The presence of iridoid in high quantity compared to other commercial noni products is quite interesting and important. Biosafety parameters of Divine Noni® such as acute

toxicity, sub acute toxicity, genotoxicity, allergenicity and cytotixicity have been studied using animal models and also human cell lines. Results of the biosafety studies have revealed that the Divine Noni® is safe for human consumption.

Keywords : Divine Noni®, *Morinda citrifolia*, *Garcinia combogia, Glycyrrhiza glabra,* phytochemicals, iridoid

V-OP-1

Therapeutic value of Divine Noni Gold in type 2 diabetic patients: Effect on insulin resistance, lipid profile and antioxidant status

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Abstract

Diabetes is a common metabolic disease of human being that has many adverse effects. The present study was carried out to examine the effect of Divine Noni gold, a commercial product from noni fruit juice on insulin resistance and glycemic indices in diabetes mellitus. This is a randomized, placebo-controlled trial in which 40 participants affected by diabetes were randomly assigned into Noni (NG) and placebo (PG) groups. The NG received 30 ml of Noni fruit juice whereas the PG received 30 ml placebo juice daily for 4 weeks. HbA1c, fasting blood sugar (FBS), fasting insulin, homeostasis model assessment insulin resistance index (HOMA-IR), ?-cell function (? %), insulin sensitivity (S %), AST (aspartate aminotransferase), ALT (alanine aminotransferase), triglycerides, total cholesterol, LDL, HDL, SOD, MDA and GSH were determined before and after the intervention. The results showed a substantial decrease in HbA1c, FBS, total cholesterol, LDL, triglyceride AST and ALT levels in Noni fruit juice treated patients compared with placebo group. In conclusion, Noni fruit juice treatment in type II diabetes patients for 4 weeks has a beneficial effect on improving the glycemic profile, as well as lipid and antioxidant profile.

Keywords : Noni fruit juice, placebo, hyperglycemia, insulin resistance, lipid profile

Introduction

Diabetes mellitus type 2 (T2DM) is one of the most widespread and fastest growing diseases in most of the countries (Gerstein *et al*., 2008). It is a clinical disorder which is diagnosed by

increase in blood sugar concentration and has been recognized for hundreds of years. This can be due to insufficiency of insulin secretion or insulin function or both. Patients with diabetes are anticipated to increase by more than 366 million in 2030, twice of the rate as compared to 2000 (Rathmann and Giani, 2004). Over 90% of diabetic patients are diagnosed with type 2 diabetes. The frequency of cardiovascular diseases is increased two to four fold in type 2 diabetic patients (Raza and Movahed, 2003). Type 2 *diabetes mellitus* is characterized clinically by hyperglycemia and insulin resistance. Hyperglycemia in type 2 diabetes results from an impaired insulin secretory response to glucose and decreased insulin effectiveness in stimulating glucose uptake by skeletal muscle and in restraining hepatic glucose production (Insulin resistance).

Insulin resistance has a key role in causing *diabetes mellitus* type 2 and the resultant cardiovascular disease is an important risk factor in diabetic patients (Minot-Gilchrist and Anderson, 2004). It is a condition in which body produces insulin but does not use it effectively. When people have insulin resistance, glucose builds up in the blood stream instead of being absorbed by the cells leading to T2DM. When this happens, the body puts out more insulin to stabilize blood glucose. Over time, this results in a condition called hyperinsulinemia which will cause other problems, including making it more difficult for the body to use stored fat for energy.

Many oral antidiabetic drugs are available for the treatment of metabolic syndrome and T2DM but none of them are well suited considering the side effects. Sulfonylurea and biguanides used for management of diabetes are expensive or have detrimental side effects or contraindication. Hence, there arises an urgent need to discover natural resources and study their potential on various identified targets in order to expand them as a new antidiabetic therapeutics for the management of *diabetes mellitus*.

Morinda citrifolia (Noni) is an evergreen small tree that grows in many tropical regions of the world. Noni fruit has a significant history of use as both food and medicine among pacific islanders and in Southeast Asia (West *et al*., 2006). Noni was highly valued for its variety of uses. The potential health benefits of fruit may be attributed to nutritionally important phytochemicals, such as flavonoids and lignans (Deng *et al*., 2007; Mohd Zin *et al*., 2006; Kamiya *et al*., 2004). Noni fruit juice is in high demand as an alternative medicine for different kinds of illness including diabetes and atherosclerosis. Noni juice has been found to exert an antioxidant effects in human athletes (Palu *et al*., 2008). Noni juice reportedly lowered serum cholesterol and triglycerides in

smokers (Wang *et al*., 2006). A potential antidiabetic and hepatoprotective property of fermented noni juice was proved (Nayak *et al*., 2011).

The present study was focused to find out the effect of Noni fruit juice on insulin resistance and glycemic indices in patients with type 2 diabetes. Since diabetes is known to induce the rise in blood sugar accompanied with marked increase in total cholesterol, triglycerides, LDL-C and reduction in HDL-C, the effect of Noni fruit juice on the levels of lipid parameters was studied. The hyperglycemic condition is known to increase free radical production as well as reduced antioxidant defense response leading to increased oxidative stress. Therefore, the antioxidant parameters such as, superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels were also estimated.

Materials and methods

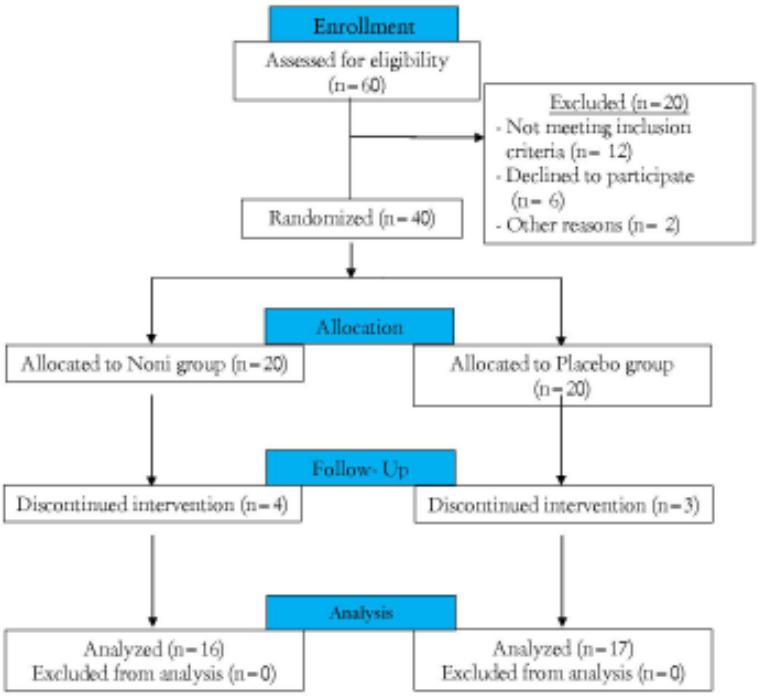

Fig.1 : Flow diagram of the studies conducted

Type of study, participants and sample size

This is a randomized, placebo-controlled trial with the participation of 40 patients with type 2 diabetes conducted at Thanjavur Medical College Hospital (TMCH), Thanjavur during June 2014.

Inclusion Criteria

- Subjects were male or female, aged between 20 and 58 years.
- Subjects with type 2 diabetes
- Subjects with poor glycemic control fasting blood levels of glucose greater than 126mg/dl and HbA_{1c} > 7.0% (preliminary screening).
- Subjects using common diabetes drug tablets every day
- Newly diagnosed patients

Exclusion Criteria

- Pregnant and lactating women
- Any known drug allergy
- Alcohol consumption
- Suffering from acute or chronic hepatitis
- Consumption of any other nutritional supplements
- History of serious adverse effects

Intervention

The patients were randomly categorized into 2 groups of Noni (NG) and Placebo (PG). NG group (20 patients) received Noni fruit juice (Divine Noni Gold) procured from World Noni Research Foundation (WNRF), Chennai and the PG group (20 patients) received a placebo juice as per the dosage form given below.

1. First 3 days - 5 ml twice daily, on an empty stomach (Morning and Evening).
2. Next 3 days - 10 ml twice daily, on an empty stomach (Morning and Evening)
3. Seventh day onwards – 15 ml twice daily, on an empty stomach (Morning and Evening).

The schedule was followed for 30 days. The subjects were advised to drink lot of water throughout the day. Follow-up of the patients was performed weekly by phone so as to control them for consumption of Divine Noni Gold, response to relevant questions and prevention of sample loss.

Biochemical Measurements

After 12 h fasting, a 5 ml sample of venous blood was taken from each patient by the laboratory technician at the beginning and the end of intervention. The centrifuge of sample was performed at the room temperature and at 3000 rpm for 10 min so as to separate serum. Fasting blood glucose level, fasting insulin, HbA1C, AST (aspartate aminotransferase), ALT (alanine aminotransferase), triglycerides, total cholesterol, LDL, HDL, SOD, MDA and GSH were determined with standard enzymatic kits using an auto analyzer. Homeostasis model assessment insulin resistance index (HOMA-IR), beta-cell function (â %) and insulin sensitivity (S %) were calculated using a software calculation, HOMA (HOMA calculator, version 2.2.2; Diabetes Trial Unit, University of Oxford, www.dtu.ox.ac.uk).

Ethical considerations

The objective and methods of the study were explained to the patients and the informed written consent was received from them. On the other hand, the clinical trial protocol was approved by the institutional ethics committee of the Thanjavur Medical College Hospital (TMCH). This study was also registered in Clinical Trial Registry of India (CTRI).

Results and Discussion

Demographics and measurements at Baseline

Out of 60 patients participated in the study, the following cases were excluded from the intervention: 12 patients not meeting inclusion criteria, 6 patients who had no tendency to continue and 2 for travel; the remaining 40 who continued to the end were investigated (Fig. 1). Further 7 patients were excluded from the intervention because of the protocol violation. The mean and standard deviation of age, sex, duration of T2DM and body mass index were presented in Table 1.

Table 1: Demographic data of the trial participants

Parameter	Noni group	Placebo group
Age (Years)	57.72±7.65	52.53±7.50
Sex (Male/Female)	4M/16F	6M/14F
Duration of T2DM	10.25±1.47	11.00±1.22
Body mass index (Kg/m^2)	28.36±4.14	28.78±5.51

Values were expressed as mean ± standard deviation.

Effect of Divine Noni Gold on glycemic indices and liver function test

The present study investigated the effect of Divine Noni Gold on the glucose profile in type 2 diabetic patients. Comparisons between glycemic indices and liver function tests in the studied groups before and after intervention Divine Noni Gold is presented in Table 2.

Table 2 : Comparisons between glycemic indices and liver function tests in the studied groups before and after intervention

Variables	Noni		Placebo	
	Baseline	End	Baseline	End
Fasting blood sugar (mg/dl)	191.59±27.27	172.77±10.42	187.45±16.25	183.78±25.95
HbA1C (%)	8.22±0.51	7.75±0.50	8.14±0.68	8.27±0.40
AST (IU/L)	28.53±5.64	26.25±3.56	30.10±5.64	31.54±6.43
ALT (IU/L)	23.21±6.89	21.50±5.85	31.52±11.01	32.49±8.96

The average fasting blood sugar level in the Noni group at the beginning of the study was 191.59±27.27 mg/dl which decreased to 172.77±10.42 mg/dl after 4 weeks of oral intake of Divine Noni Gold. The average fasting blood glucose level in the placebo group at the baseline of the study was 187.45±16.25 which was decreased slightly to 183.78±25.95 after the 4 weeks of placebo treatment. The results have shown that the Divine Noni Gold treatment notably decreased the fasting blood glucose and HbA1c in diabetic patients at the end of the study. In the present

study, fasting insulin levels in the Noni group were considerably lowered comparing with placebo group. The hypoglycemic effect in the test group may be due to the improved insulin sensitivity by the Noni fruit juice.

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin caused by the degeneration or hypo activity of the beta cells of the pancreas which resulted in the rise of extra cellular glucose level. Xeronine the major compound present in the Noni fruit juice plays a vital role in lowering blood sugar level. Xeronine combining with insulin helps more glucose combustion (oxidation) in peripheral tissues and also helps to enhance the cellular permeability to glucose leads to the reduction of extra cellular glucose level.

Hemoglobin A1c (HbA1c) is a minor component of hemoglobin to which glucose is bound. It is also referred as glycosylated hemoglobin. HbA1c levels are routinely measured in the monitoring of people with diabetes. HbA1c levels were depending on the blood glucose concentration. Levels of HbA1c are not influenced by daily fluctuations in the blood glucose concentration but reflect the average glucose levels over the prior six to eight weeks. Therefore, HbA1c is a useful indicator of how well the blood glucose level has been controlled in the recent past (over two to three months) and may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in people with diabetes. In the healthy patients, the HbA1c levels were found to be below 6%. The good control of diabetes would be achieved if the levels were maintained between 7-8%. In the present study, the HbA1c level at the beginning was 8.22±0.51 in the Noni group which was decreased to 7.75±0.50 after the 4 weeks intervention of the Noni juice. This result showed that the intervention of the Noni juice can be helpful in the good control of hyperglycemic condition. Whereas in the placebo group the levels of HbA1c was found to be 8.14±0.68 at the beginning and at the end of the study it was 8.27±0.40.

Treatment with Noni fruit juice for 4 weeks also decreased the blood levels of AST and ALT. AST and ALT activities are commonly measured to monitor liver damage. A mild or elevated activity of AST indicates liver injury or myocardial infarction (Feldman *et al*., 2000; Crook, 2006). In the present study, it was observed that the levels of the AST were decreased from 28.53±5.64 to 26.25±3.56 after the intervention. Likewise, the ALT enzyme activity was also found to be lowered from 23.21±6.89 to 21.50±5.85. As the result of the clinical biochemistry analysis, Noni fruit juice did not bring any damage to liver function of the patients. Whereas no such decreased activity was found in these enzyme levels in the placebo treated group.

Effect of Divine Noni Gold on insulin resistance indices

Insulin resistance is a condition in which the body produces insulin but does not use it effectively. When people have insulin resistance, glucose builds up in the blood instead of being absorbed by the cells, leading to type 2 diabetes or prediabetes. In insulin resistance, muscle, fat, and liver cells do not respond properly to insulin and thus cannot easily absorb glucose from the bloodstream. As a result, the body needs higher levels of insulin to help glucose enter cells. The beta cells in the pancreas try to keep up with this increased demand for insulin by producing more. As long as the beta cells are able to produce enough insulin to overcome the insulin resistance, blood glucose levels stay in the healthy range. Over time, insulin resistance can lead to type 2 diabetes and prediabetes because the beta cells fail to keep up with the body's increased need for insulin. Without enough insulin, excess glucose builds up in the bloodstream, leading to diabetes, prediabetes, and other serious health disorders.

For assessing insulin resistance, the present study focused on indices such as fasting insulin, HOMA-IR, S% and â% (Table 3). The results indicated that a notable decrease was found in fasting insulin and HOMA-IR in the patients who were taken Noni juice. An effective increase in the S% was also observed. â% had a decrease in the placebo group. No such changes were observed in the placebo group. Therefore, considering the results of these indices it may be concluded that Noni fruit juice is effective in glycemic control and in lowering insulin resistance.

Table 3 : Comparison of insulin resistance indices before and after the intervention in Noni and placebo groups

Variables	Noni		Placebo	
	Baseline	End	Baseline	End
Fasting insulin (UI/L)	12.25±0.41	4.65±0.85	11.87±0.32	11.03±0.53
HOMA-IR	1.91±0.13	0.7±0.12	1.92±0.28	2.2±0.51
Insulin sensitivity (%)	53.34±3.40	146.67±23.02	52.98±5.91	53.74±5.40
Beta-cell function (%)	31.99±6.07	48.67±2.57	36.24±7.95	32.58±6.98

Effect of Divine Noni Gold on antioxidant parameters

Hyperglycemia generates reactive oxygen species (ROS), which in turn cause damage to the cells in many ways. Damage to the cells ultimately results in development of secondary complications in diabetes (Jaganjac *et al*., 2013). Oxidative stress acts as a mediator of insulin resistance and its progression to glucose intolerance and installation of diabetes mellitus, subsequently favoring the appearance of atherosclerotic complications, and contributes to rise in many micro- and macrovascular complications. Hence there is a need to examine the antioxidant status of the patients.

Lipids are reported as one of the primary targets of ROS. Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes, such as malondialdehyde (MDA), that damage cell membranes. MDA had been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress. Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications. The increase in lipid peroxidation is also an indication of decline in defense mechanisms of enzymatic and nonenzymatic antioxidants. Increased MDA level in plasma, serum, and many others tissues have been reported in diabetic patients (Bandeira *et al*., 2012). In the present study, the MDA level was found to decreased (1.07±0.20) from the beginning level (1.93±0.40). The results showed that the intervention of Noni juice had reduced the lipid peroxidation and thus by prevents the cell damage.

Superoxide dismutase (SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion (O2) into hydrogen peroxide and molecular oxygen. SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water. In the present study, the activity of the SOD was found to be increased from 3.93±0.78 to 5.20±0.52 in Noni group (Table 4). Likewise, the level of GSH was found to be increased in the group administered with Noni. No changes observed in the placebo group. Hence, the Noni fruit juice has the protective role against histological damages. Intervention of Noni juice had improved the antioxidant status in diabetic patients.

Table 4 : Comparison of antioxidant parameters before and after the intervention in Noni and placebo groups

Variables	Noni		Placebo	
	Baseline	End	Baseline	End
SOD (IU/L)	3.93±0.78	5.20±0.52	3.68±0.67	3.85±0.94
GSH (IU/L)	1.85±0.52	2.37±0.73	1.80±0.41	1.74±0.51
MDA (IU/L)	1.93±0.40	1.07±0.20	1.76±0.54	1.85±0.43

Effect of Divine Noni Gold on lipid profile

Lipids play an important role in the pathogenesis of diabetes mellitus. Dyslipidemia is characterized by high plasma levels of total cholesterol, triglycerides with low plasma levels of HDL cholesterol. Hypertriglyceridaemia and hypercholesterolaemia are the major factors of diabetic state involved in the development of the atherosclerosis and coronary heart disease which are the secondary complications of diabetes.

Table 5 showed the level of serum lipids (TC, TG, HDL-C and LDL-C) in both the group. Comparing with the placebo group the levels of TC, TG and LDL-C were found to be decreased in Noni groups. Similarly, the notable increase in the HDL-C level was also observed in the test group. Thus, from the result, 4 week administration of Noni juice had positive effect on the lipid profiles in diabetic patients.

Table 5: Comparison of lipid profile before and after the intervention in Noni and placebo groups

Variables	Noni		Placebo	
	Baseline	End	Baseline	End
TC (mg/dl)	186.36±13.74	181.72±4.56	196.88±12.11	201.53±13.01
TG (mg/dl)	186.06±22.42	168.62±2.49	194.26±6.67	196.75±23.33
HDL-C (mg/dl)	33.97±2.29	35.67±2.52	34.31±2.35	35.68±5.53
LDL-C (mg/dl)	176.53±13.82	158.60±2.48	173.54±6.66	171.25±8.22

The results in this clinical trial study suggested that the daily consumption of Noni fruit juice for 4 weeks by patients with type 2 diabetes mellitus leads to lowering fasting blood sugar, HbA1c, fasting insulin, insulin resistance and increase of sensitivity to insulin. It also had a positive effect on lipid profile and also enhanced the antioxidant status of the patients. The present of the phytochemicals such as xeronine, scopoletin, iridoids and rutin may be responsible for the activity. Hence, Noni fruit juice could be used as a safe antihyperglycemic, antioxidant and antihyperlipidemic agent for type 2 diabetic patients.

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References

Bandeira, S. de M.G., Guedes, S., da Fonseca, L. J. S., Pires, A. S., Gelain, D. P., Moreira, J. C. 2012. Characterization of blood oxidative stress in type 2 diabetes mellitus patients: increase in lipid peroxidation and SOD activity, Oxidative Medicine and Cellular Longevity, vol. 2012, Article ID 819310, 13 pages.

Crook, M.A., 2006. Clinical Chemistry and Metabolic Medicine. 7th Edition. Hodder Arnold, London p.426.

Deng, S., Palu, A.K., West, B.J., Su, C.X., Zhou, B.N., Jensen, J.C. 2007. Lipoxygenase inhibitory constituents of the fruits of Noni (*Morinda citrifolia*) collected in Tahiti. *Journal of Natural Products*, 70 (5): 859-862.

Feldman, B.V., Zinkl, J.G., Schalm's. 2000. Veterinary Hematology, 5th ed. Philadelphia, Lea Febiger, p.1210-1218.

Gerstein, H.C., Miller, M.E., Byington, R.P., Goff, D.C., Bigger, J.T., Buse, J.B. 2008. Effects of intensive glucose lowering in type 2 diabetes. *New England Journal of Medicine,* 358(24): 2545-2559.

Jaganjac, M., Tirosh, O., Cohen, G., Sasson, S., Zarkovic, N. 2013. Reactive aldehydes-second messengers of free radicals in diabetes mellitus. *Free Radical Research*, 47(1): 39-48.

Kamiya, K., Tanaka, Y., Endang, H., Umar, M., Satake, T. 2004. Chemical constituents of *Morinda citrifolia* fruits inhibit copper-induced low-density lipoprotein oxidation. *Journal of Agricultural Food Chemistry*, 22; 52(19):5843-5848.

Minot-Gilchrist, D.V., Anderson, H.D. 2004. Insulin resistance-associated cardiovascular disease: potential benefits of conjugated linoleicacid. *American Journal of Clinical Nutrition,* 79(June (6 Suppl.)): 1159S-1163S.

Mohd Zin, Z., Hamid, A.A., Osman, A., and Saari, N. 2006. Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.). *Food Chemistry*, 94:169-178.

Nayak, B. S., Marshall, J. R., Isitor, G., and Adogwa, A. 2011. Hypoglycemic and hepatoprotective activity of fermented fruit juice of *Morinda citrifolia* (Noni) in diabetic rats. *Evidence-Based Complimentary and Alternative Medicine*, 2011:1-5.

Palu, A. K., Seifulla, R. D., and West, B. J. 2008. *Morinda citrifolia* L., (noni) improves athlete endurance: its mechanisms of action. *Journal of Medicinal Plant Research*, 2(7):154–158.

V-OP-2

In vivo effects of Divine Noni Gold on Cholinesterase activities and nitric oxide production in different brain areas of old F344 male rats

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Abstract

Background : Aging is a process characterized by the deterioration of physiological function. Morinda citrifolia (Noni; Indian mulberry) is known to possess anti-aging and memory enhancing properties. Morinda citrifolia has been widely used for treatment of a variety of diseases in Ayurvedic and folk medicine.

Purpose : The purpose of this study was to determine the effects of Divine Noni Gold (DNG) on cholinesterase activity and nitric oxide production in brain areas (Medial basal hypothalamus, Frontal cortex, Striatum and Hippocampus) of F344 rats. Methods: Oral administration of Divine Noni Gold (5%, 10% and 20%) was done twice a day for a period of 60 days (5 ml/kg body weight) in old (16-17 months) rats. Nitric oxide levels were analyzed using Greiss reagent system. Acetyl-, total- cholinesterases activities and butrylcholinesterase activity were measured in the medial basal hypothalamus and serum respectively.

Results : DNG increased nitric oxide level in hippocampus, frontal cortex and striatum, but it did not alter nitric oxide in medial basal hypothalamus. DNG (5%, 10% and 20%) significantly decreased butrylcholinesterase activity in serum and Acetyl-, total-cholinesterases activities in medial basal hypothalamus. Conclusion: Thus it can be concluded that Divine Noni Gold exerts differential effects on distinct brain areas, as shown by increased cholinergic and nitric oxide-mediated neuronal activity in the brain.

Keywords : *Morinda citrifolia*, medial basal hypothalamus, cholinesterase, aging, nitric oxide

Introduction

Aging is associated with a decline in immune and endocrine functions, and diminished responsiveness of central and peripheral catecholaminergic systems (Ader, 2001). With progressing age, there is a decline in the cholinergic neurotransmission, with concomitant loss of neuronal plasticity and cognitive functions of the brain which may result in neurodegenerative disorders. The cholinergic neurotransmission is regulated by the key enzymes – choline acetyltransferase (ChAT), which synthesizes the neurotransmitter, acetylcholine (ACh) and cholinesterases, which hydrolyses and terminates the activity of ACh. The cholinergic enzyme subtypes, including acetyl cholinesterase (AChE) and butyryl cholinesterase has been demonstrated to alter with advancing age (Sirviö *et al.*, 1991). Research has shown the role of acetylcholine (ACh) as a neurotransmitter in the behavioral, learning and memory (Blockland, 1996) and as an anti-inflammatory molecule. Moreover, inhibition of cholinesterases enhances the activity of cholinergic neurons in the central nervous system (CNS) and leads to suppression of inflammation (Geula *et al.*, 1995).

Nitric oxide (NO) is another neurotransmitter synthesized in CNS which is involved in memory and learning process. NO is synthesized in the brain in cognitive demand (Paul *et al.*, 2011). NO functions by stimulating soluble guanylyl cyclase to form the second messenger molecule, cyclic guanosine monophosphate (cGMP) in the target cells.

Morinda citrifolia (commonly known as 'Noni') fruit juice has been found to have immunomodulatory properties (Palu *et al.*, 2008). Neuroprotective effects of *Morinda citrifolia* is another important property that may be critical to the treatment of neurological disorders such as memory impairment, cognitive dysfunctions, neurodegenerative disorders, in the elderly especially, when the population of elderly is on the rise in several countries of the world (Li *et al.,* 2004).Although the beneficial effects of Noni have been reported in few studies (Palu *et al.*, 2008), the mechanism(s) of action(s) from the perspective of the neural functioning have not been investigated. Therefore, the present study was conducted to examine

the *in vivo* effect of the fruit juice, Divine Noni Gold, on the cholinesterase activities and nitric oxide production in various brain areas of old F344 rats.

Materials and Methods

Morinda citrifolia: *Morinda citrifolia* fruit juice (Divine Noni Gold®) was obtained from World Noni Research Foundation, Chennai, Tamil Nadu, India. Oral administration of Noni fruit juice (5%, 10% and 20%) was done twice a day for a period of 60 days (5ml/kg body weight) in old (16-17 months) F344 male rats.

Animals: Young (4-5 months), and old (16-17 months) F344 male rats were obtained from the National Institute of Nutrition at Hyderabad for the study. The animals were acclimatized to the animal house at SRM University for a period of one week. Divine Noni Gold (DNG) was obtained from World Noni Research Foundation, Chennai. Oral administration of Divine Noni Gold(5%, 10% and 20%) was done twice a day for a period of 60 days (5ml/kg body weight) in old (16-17 months) F344 male rats. Brain areas (Medial basal hypothalamus, Frontal cortex, Striatum and Hippocampus) were isolated from young and old F344 rats. Cholinesterase activities and nitric oxide production were analysed in homogenised tissue.

2.2. Cholinesterase activity

The Cholinesterase activity was measured using the Ellman's reagent, Dithionitrobenzoic acid (DTNB), with acetylthiocholine iodide as the substrate (Ellman *et al*, 1961). For the measurement of acetylcholinesterase activity, samples were diluted in 0.1 M phosphate buffer (pH 7.4) and were supplemented with the active component, DTNB (0.4 mM), and the specific butyrylcholinesterase inhibitor, ethopropazine hydrochloride (14 mM). The reaction mixture was incubated at room temperature for 20 minutes following which acetylthiocholine iodide (1 mM) was added and mixed well before reading in a spectrophotometer at 436 nm at a 60 second interval for 5 minutes. Results were expressed in terms of units/min/mg protein.

Butyrylcholinesterase and Total-cholinesterase activities were measured by diluting the samples in 0.1 M phosphate buffer (pH 7.4) and were supplemented with the active component, DTNB (0.8 mM). The reaction mixture was incubated at room temperature for 20 minutes, then

acetylthiocholine iodide (1 mM) was added, mixed well before reading in a spectrophotometer at 436 nm at a 60 second interval for 5 minutes. Results were expressed in terms of units/ min/mg protein.

2.3. Nitric oxide production

The total NO production was measured using the Griess reagent system (Fiddler, 1977) in the homogenized brain tissue samples. The samples were incubated with an equal volume of 0.1% N-1-napthylethylenediamine dihydrochloride in distilled water and 1% sulphanilamide in 5% phosphoric acid for 10 minutes. The purple color obtained was read at 520 nm in a spectrophotometer. Standard curve was obtained using serial dilutions of 0.1 M sodium nitrite in water. The results were expressed in terms of ¼g equivalents of sodium nitrite.

2.4. Statistical analysis

Data were analyzed using ANOVA by SPSS software package. Parameters that attained significance ($p<0.05$) with ANOVA were further analyzed by LSD post-hoc tests. All values are expressed as mean±S.E.M.

Results

Divine Noni Gold treatment Decreases Butrylcholinesterase activity in serum samples

Treatment with all doses (5%, 10% and 20%) of DNG significantly decreased butyryl cholinesterase activity in serum samples of old F344 rats compared to saline-treated group (Fig. 1).

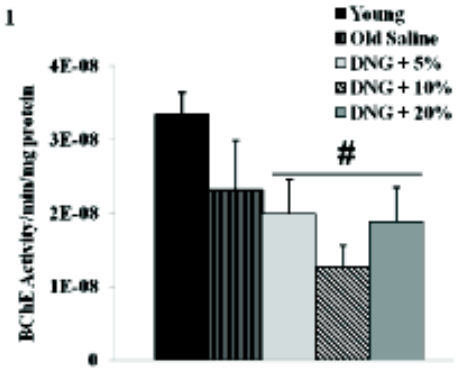

Fig. 1: Effects of Divine Noni Gold treatment on butyrylcholinesterase activity

Treatment with DNG decreased butyrylcholinesterase activity in serum samples of Old F344 rats compared to saline-treated group. *$p<0.05$compared to Young, #$p<0.05$ compared to age-matched control

Divine Noni Gold treatment Decreases Acetylcholinesterase (AChE) and Total Cholinesterase (TChE) activities in medial basal hypothalamus

DNG treatment (5% and 20% doses) decreased AChE activity in old rats; although 10% dose did not cause any significant difference in its activity compared to saline-treated group. TChE activity was found to be decreased with 10% and 20% DNG doses (Fig. 2).

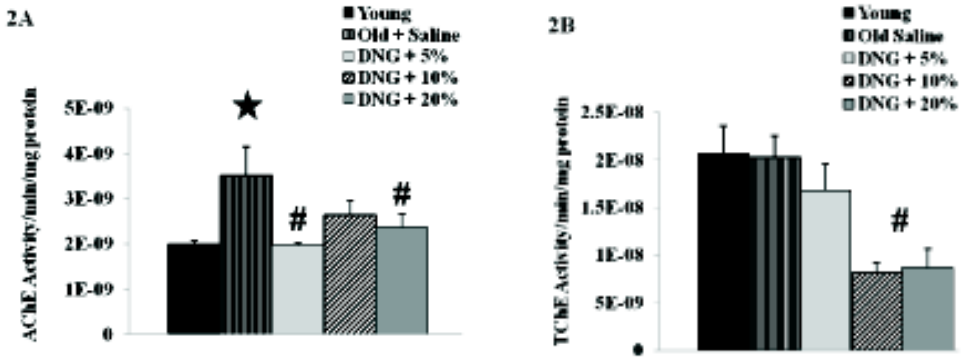

Fig. 2: Effects of Divine Noni Gold treatment on acetylcholinesterase and total cholinesterase activities in medial basal hypothalamus

DNG treatment decreased AChE and TChE activity in old rats (2A and 2B respectively). *$p<0.05$compared to Young, #$p<0.05$ compared to age-matched control

Divine Noni Gold treatment increases Nitric oxide production in Frontal cortex and Hippocampus

Treatment with of DNG significantly increased the NO production in frontal cortex (all doses) and hippocampus (10% and 20%) in old F344 rats. However in the striatum and medial basal hypothalamus there was no significant difference in NO production as compared to the saline-treated group (Fig. 3).

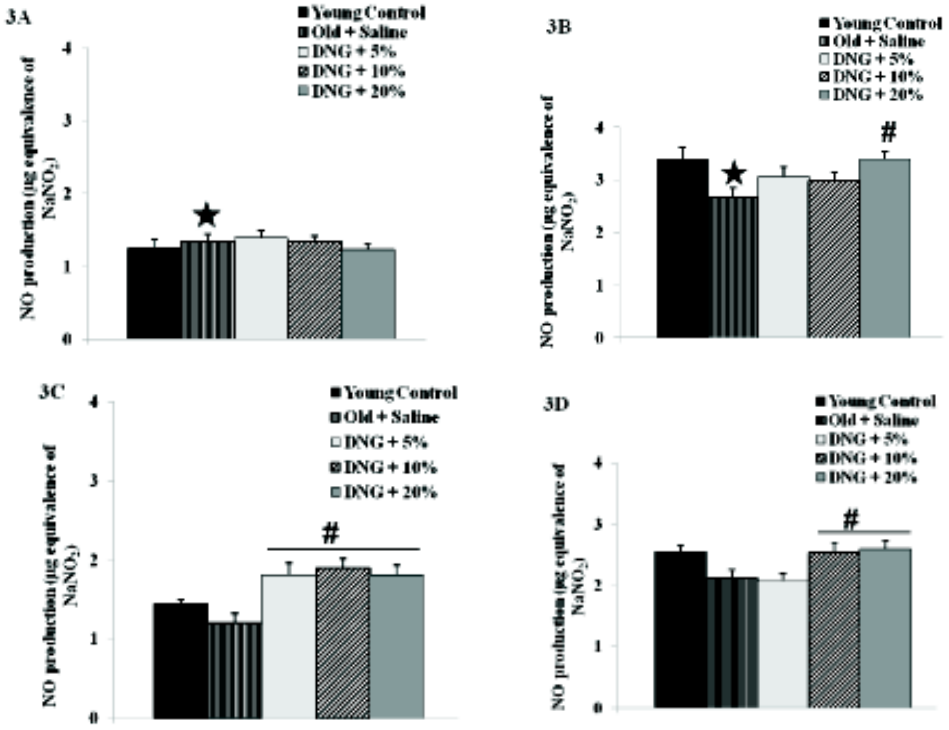

Fig. 3: Effects of Divine Noni Gold treatment on Nitric oxide production in different brain areas

There was an age related decline in nitric oxide production in Striatum (Fig. 3B). Treat ment with DNG significantly increased the NO production in striatum, frontal cortex and hippocampus (Fig. 3B, 3C and 3D). However in medial basal hypothalamus there was no significant difference in NO production (Fig. 3A) *$p<0.05$compared to Young, #$p<0.05$ compared to age-matched control.

Discussion

The present study was conducted to examine the effects of Divine Noni Gold on cholinesterase (ChAT) activities and nitric oxide production. The ACh-related cholinergic effects can be enhanced by increasing ChAT activity and lowering AChE activity. This study showed that DNG significantly decreases acetyl-, butyryl- and total cholinesterase activities which may contribute to enhanced cholinergic neurotransmission by increasing the levels of ACh. In the medial basal hypothalamus, 5% and 10% doses of DNG decreased the AChE activity, while TChE activity was found to be decreased by 10% and 20% doses. To further support our findings, it has also been shown that ethanolic extract of *M. citrifolia* and its fractions inhibit AChE activity in dose-dependent manner in scopolamine-induced memory impairment in mice (Pachauria *et al*., 2012). Another species, *Morinda officinalis* has been shown to possess protective effects against ²-amyloid-induced dementia by increasing the ACh levels and decreasing lipid peroxidation (Chen *et al*, 2013). Inulin-type oligosaccharides extracted from *M. officinalis* has also been demonstrated to show neuroprotective effects by suppressing the intracellular calcium overloading and simultaneously by enhancing nerve growth factor mRNA expression in corticosterone-lesioned PC12 cells (Li *et al.,* 204). These cellular effects may explain the prevention of neuronal damage and the reversal of neuronal deficits in rodents with cerebral ischemia by *M. citrifolia* fruit juice (Harada *et al*., 2009). The fruit juice may also facilitate protection from stress-induced impairment of cognitive functions by improving the blood vessel density in the hippocampal dentate gyrus (Muto *et al*., 2010). Although AChE is the predominant cholinesterase, the role of butyryl cholinesterase as compensatory and co-regulatory enzyme in ACh metabolism may be exemplified by the fact that it is more efficient under conditions of high brain activity when higher concentrations of ACh is released to render AChE becomes substrate-inhibited. It has been observed that there was a substantial decline in butyryl cholinesterase activity in serum of old rats with DNG treatment.

Nitric oxide as a neurotransmitter can influence the cerebral blood flow, local brain metabolism and has a role in morphogenesis and synaptic plasticity. DNG treatment increased NO production in frontal cortex, striatum and hippocampus; although in medial basal hypothalamus there was no change in its levels. Inhibition of NO synthesis has been shown to cause a decrease in acetylcholine (ACh) release and an impairment of retention of conditioned response in rats (Kopf *et al*., 2001). These findings clearly indicate that NO has a modulatory effect on

cholinergic activity and cognitive functions. In addition, comparative study carried out in young adult (3-4 month old) and old (13-17 month old) rats have shown that NO production decreases with declining levels of serum L-arginine and urinary excretion of nitrite by 30 to 50% in aged rats in comparison to young controls (Reckehoff *et al*., 1994). Taken together, it can be concluded that DNG exerts a differential effect on distinct brain areas to improve the neurotransmission and reduce the deteriorating effects of aging on CNS. Since aging is characterized by concurrent loss of neuroendocrine functions and immunosuppression resulting in the development of cancer, infectious, autoimmune and neurodegenerative diseases, the role of DNG warrants further studies so that it can be promoted as a functional food supplement for healthy aging process.

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References

Ader, R., Cohen, N. and Felten, D. L. 2001. Psychoneuroimmunology. 3rd ed. Academic Press New York.

Blockland, A. 1996. Acetylcholine: a neurotransmitter for learning and memory? *Brain Research Review,* 21:285–300.

Chen, D. L., Zhang, P., Lin, L., Zhang, H.M. and Liu, S.H. 2013. Protective effect of oligosaccharides from *Morinda officinalis* on beta-amyloid-induced dementia rats. *China Journal of Chinese Materia Medica*, 38:1306-1309.

Ellman, G.L., Courtney, K. D., Anders, V. Jr. and Feather-Stone, R.M. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7:88–95.

Fiddler, R. N. 1977. Collaborative study of modified AOAC method of analysis for nitrite in meat and meat products. *Journal-Association Off Analytical Chemists*, 60:594-599.

Geula, C. and Mesulam, M.M. 1995. Cholinesterase and the pathology of Alzheimer disease. *Alzheimer Disease and Associated Disorders*, 9:23–28.

Li, Y.F., Liu, Y.Q., Yang, M., Wang, H.L., Huang, W. C., Zhao, Y.M. and Luo, Z. P. 2004. The cytoprotective effect of inulin-type hexasaccharide extracted from *Morinda officinalis* on PC12cells against the lesion induced by corticosterone. *Life Science,* 75:1531-1538.

Harada, S., Hamabe, W., Kamiya, K., Satake, T., Yamamoto, J. and Tokuyama, S. 2009. Preventive effect of *Morinda citrifolia* fruit juice on neuronal damage induced by focal ischemia. *Biological and Pharmaceutical Bulletin*, 32:405-409.

Kopf, S.R., Benton, R. S., Kalfin, R., Giovannini, M.G. and Pepeu, G. 2001. NO synthesis inhibition decreases cortical ACH release and impairs retention of conditioned response. *Brain Research*, 894 : 141-144.

Muto, J., Hosung, L., Uwaya, A., Isami, F., Ohno, M. and Mikami, T. 2010. *Morinda citrifolia* fruit reduces stress-induced impairment of cognitive function accompanied by vasculature improvement in mice. *Physiology and Behavior*, 101:211-217.

Palu, A.K., Kim, A.H., West, B. J., Deng, S., Jensen, J. and White, L. 2008. The effects of *Morinda citrifolia* L. (Noni) on the immune system: its molecular mechanisms of action. *Journal of Ethnopharmacology*, 115(3): 502-506.

Paul, V. and Ekambaram, P. 2011. Involvement of nitric oxide in learning & memory processes. *The Indian Journal of Medical Research*, 133:471-478.

Pachauri, S. D., Tota, S., Khandelwal, K., Verma, P. R., Nath, C., Hanif, K., Shukla, R., Saxena, J. K. and Dwivedi, A. K. 2012. Protective effect of fruits of *Morinda citrifolia* L. on scopolamine induced memory impairment in mice: A behavioral, biochemical and cerebral blood flow study. Journal of *Ethnopharmacology*, 139:34-41.

Reckehoff, J. F., Kellum, J.A., Blanchard, E. J., Bacon, E. E., Wesley, A. J. and Kruckeberg, W. C. 1994. Changes in nitric oxide precursor, L-arginine and metabolites. Nitrite and nitrate with ageing. *Life Science*, 55:1895-1902.

Sirviö, J., Rakonczay, Z., Hartikainen, P., Kasa, P. and Riekkinen, P. J. 1991. The molecular forms of acetylcholinesterase in cerebrospinal fluid of normal subjects—effect of aging. *Journal of Neural Transmission*, General Section, 86:147-150.

V-OP-3

Divine Noni - A wonderful nutraceutical in preventing oxidative stress induced cataract formation

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Abstract

Oxidative stress and other toxic agents can induce apoptosis of lens epithelial cells that cause cataract formation in in vitro and in vivo conditions. The cataract patients may have deficient defence systems against oxidative stress and other agents responsible for cataract formation. This oxidative stress can trigger lens epithelial cell apoptosis, which may initiate cataract development. One of the benefits of Noni fruit juice is that it is rich in antioxidants. To investigate the involvement of 'Divine Noni' in protection against oxidative stress, chick lens epithelial cells were exposed to hydrogen peroxide (100 µM H2O2) over a time course of several hours, with and without pre-treatment of 'Divine Noni' and Noni fruit extract (Hexane). Cell Viability, during oxidative stress was determined by a method called MTT Assay method. Our results in the present investigation suggests that Noni juice and extract can preserve physiological functions of chick lens epithelial cells during oxidative stress, which suggests that 'Divine Noni' can be effectively used for cataract patients.

Keywords *:* oxidative stress, lens epithelial cells, cataract formation, apoptosis, MTT and 'Divine Noni'

Introduction

Cataract can be defined as opacity in the lens, is the main cause of blindness around the world (Baynes, 1991), and it can affects up to 80% of the human population over the age of 70, and seriously impair vision and quality of life (Chun-xiao Yang *et al*., 2013). Oxidative stress is the

main cause of Cataract, both the epidemiological and experimental studies have shown evidence that oxidative stress is a major mechanism in the initiation and progression of cataract (Beebe *et al*., 2010). Understanding the pathophysiology of cataract formation is important not only to advance the state of medical knowledge but also for public health purposes (Kadenbach *et al*., 2009; Bigsby *et al*., 1999). Cataract represents a large financial burden on health-care systems, and there remains a need to develop effective therapeutic agents for the prevention or treatment of cataract (Goswami *et al*., 2003; Gupta and Nandini, 1996). Scientists found that people at high risk of developing advanced stages of cataract formation, a leading cause of vision loss, lowered their risk by about 25 percent when treated with a high-dose combination of vitamin C, vitamin E, beta-carotene, and zinc. In the same high risk group which includes people with intermediate cataract formation in one eye but not the other eye the nutrients reduced the risk of vision loss caused by advanced cataract formation by about 19 percent (Spector *et al*., 1993; Singhal *et al*., 2012).

Morinda citrifolia L. is commonly known as Great *Morinda*, Indian mulberry, Beach mulberry and noni. The noni fruit has been used in tropical regions as both Food and folk medicine. The recent use of *M.citrifolia* as a dietary supplement has increased greatly and is reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anthelminthetic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects. Noni is rich with vitamin A, beta carotenoids, vitamin E, vitamin C, vitamin E, vitamin B complex, with all trace minerals like Ca, Mg, K, Zn, Molybdenum etc. Those all ingredients being present in one fruit; has made the Noni, a most powerful antioxidant (Mladenka *et al*., 2010; Marimuthu and Peter, 2011). Previously we have studied Cell Viability, intracellular reactive oxygen species, western blot analysis of various proteins involved during oxidative stress (by UV radiation and H_2O_2) and Flow cytometry analysis of cell survivability were determined.

Based on all these results, we hypothesized that Noni juice and extract would protect chicken lens epithelial cells from oxidative stress and hence would be beneficial in the treatment of cataract. To test this hypothesis, in vitro studies were carried out where chick lens epithelial cells exposed to H_2O_2. This study is designed to investigate the protective effect of Noni juice and extract against H_2O_2induced oxidative stress in chicken lens epithelial cells.

Materials and Methods

Lens Organ Culture: The lenses were isolated from chick heads brought from slaughter houses. The eyes were removed and the lenses were carefully dissected by a posterior approach. Lens Epithelial Cells (LECs) were separated from each of the dissected lenses by incubating in 1× Trypsin-EDTA solution for 1-2 minutes at 37°C. 0.5×10^{6} cells were cultured in a well of a 6-well culture plate containing 1.5 ml minimal essential medium (MEM199, M-3769; Sigma) containing 10% FBS for 6 days. Transparent lenses were selected for experimentation. All chick lens experiments (n=6) were performed in the MEM 199 containing 26 mM $NaHCO_3$ as buffer. The MEM 199 was prepared with ion-exchange double-distilled water, sterilized by filtration through 0.22-ìm filter with a pH adjusted to 7.4.

Preparation of Noni fruit extracts: Fresh Noni fruits were collected from Pullur NRTC, Krishna District of Andhra Pradesh. Fresh ripened fruits [peeled] were taken and cut into small pieces and 70 to 80gram was weighed and macerated and packed in muslin cloth and run the Soxhlet apparatus up to 8-10 runs with Hexane solution. This extract was filtered and concentrated under vacuum at 50°C.

Viability of cells assay: Cells were plated 24 hours before the initiation of the experiment, at a density of 5000 cells per well in 96-well plates. Cells were exposed to H_2O_2 (100ìM) from 0 to 12 hours. After exposure to H_2O_2 irradiation, cells were rinsed with 1×PBS (pH 7.4), and viability was assessed by MTT Assay method.

MTT Assay: Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. In this work the Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color.

Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance.

Procedure

96 well assay

1. Day one: The lens cells were transferred to T25 flask initially and trypsin EDTA was added and the trypsinized cells were removed and transferred to 15ml falcon tubes and centrifuged at 1200 rpm for 3 min.

- Supernatant was removed and resuspended the cells into 1.0ml within complete media (MEM+10%FBS).
- 100 µl cells were added into each well and incubated overnight at 37^{0}C in CO2 incubator.

2. Day two: Cells were incubated with 100µM H2O2 along with Devine Noni and Noni extract.

3. Day three: 20 µl of 5 mg/ml MTT was added to each well, one set of wells were kept as a control without adding noni juice and Noni extract.

- Plates were Incubated for 4 hours at 37^{0}C in CO2 incubator.
- Media was carefully removed after 4 hours of incubation, without disturbing the cells.
- Then 200 µl of MTT solvent (DMSO) was added.
- Covered with tinfoil and agitated the cells on orbital shaker for 15 min.
- Absorbance was taken at 590 nm with a reference filter of 620 nm.

Results

Results are presented below in figures and tables.

Fig. 1A: Eye balls separated from chick heads after that lens were carefully separated from the eye balls

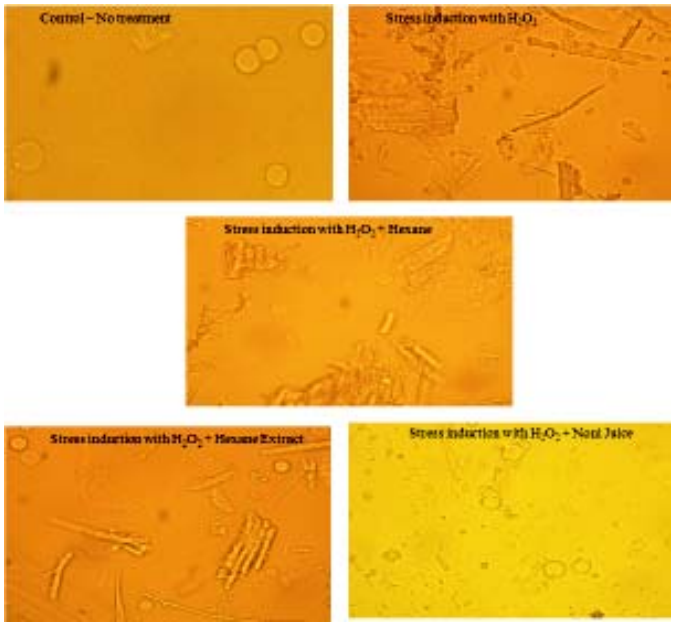

Fig.1B: Photographs of lens cells when cultured in presence of H2O2, hexane extract and Divine Noni.

Before MTT exposure

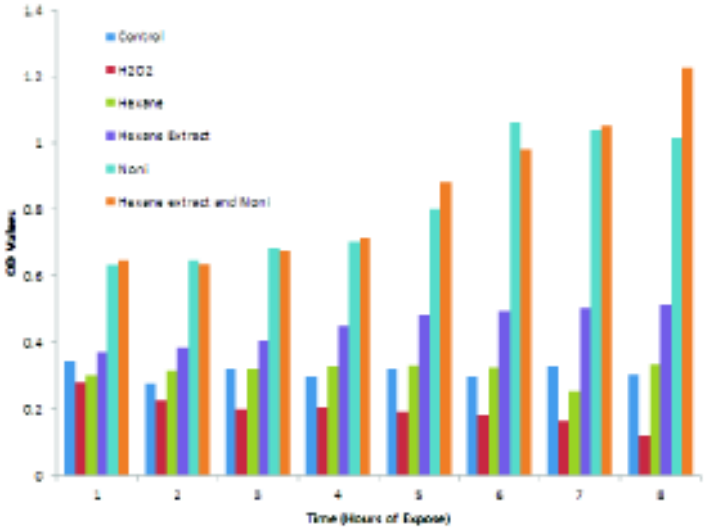

Fig. 2A: Time course (1-8hrs) of the effects of H2O2 on viability of Lens epithelial cells.

The control group was not treated with H2O2. Depicted mean ± SEM (n = 3) Data are expressed as a percentage of control group (non– H2O2- treated cells at each sampling time). The treatments are as follows: 1. Control; 2. H2O2 treated; 3. H2O2 along with Hexane; 4. H2O2 along with Hexane extract; 5. H2O2 and Noni juice; 6. H2O2 along with Noni juice and Noni extract.

After MTT exposure

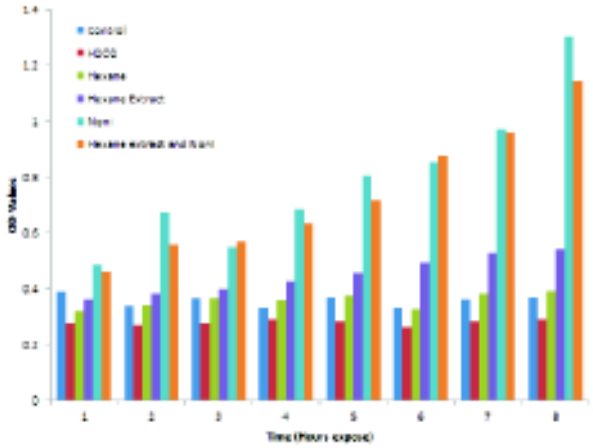

Fig. 2B: Time of the exposure (1-8hrs) with H2O2 (100µM) on viability of Lens epithelial cells.

The treatments are as follows: 1. Control; 2. H2O2 treated; 3. H2O2 along with Hexane; 4. H2O2 along with Hexane extract; 5. H2O2 and Noni juice; 6. H2O2 along with Noni juice and Noni extract.

The above experimental data revealed that effective concentration of Divine Noni along with Noni extract was found to be 100µM in preserving viability of lens epithelial cells and the effective time was found to be 7- 8 hours.

Discussion

The present study reveals that the cell death induced in chick lens epithelial cells by H2O2 is associated with production of intracellular ROS, decrease in cell viability and increase in late apoptotic cascade. 'Divine Noni' was effective in protecting LECs from death. An increase in cell viability and decrease in production ROS was observed with 100µM "Divine NONI". The above experimental data suggests that at pharmacological concentration of "Divine NONI", cellular morphology and viability are preserved. The reported protection from cataracts during the addition of 'Divine Noni' may be due to cumulative effect of all anti-oxidants against

oxidative stress toxicities in lens epithelial cells by decreasing in ROS production and increasing in cell viability.

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References

Baynes, J. W. 1991. Role of oxidative stress in development of complications in diabetes. *Diabetes*, 40: 405-412.

Beebe, D.C., Holekamp, N.M., Shui, Y.B. 2010. Oxidative damage and the prevention of age-related cataracts. *Ophthalmic Res,* 44:155-165.

Bigsby, R.M., Cardenas, H., Caperell- Grant, A., Grubbs, C.J. 1999. Protective effect of estrogen in rat model of age related cataracts. *Proc. Natl. Acad. Science USA*, 96: 9328 933.

Chun-xiao Yang, Hong Yan, Tian-bing Ding. 2013. Hydrogen saline prevents selenite induced cataract in rats. *Molecular Vision,* 19:1684-1693.

Goswami, S., Sheets, N.L., Zavadil, A., Chauhan, B.H. 2003. Spectrum a range of oxidative stress and response of human lens epithelial cell to H2O2 insult. *Invest opthalmol vis. Science*, 44:2084-2092.

Gupta, P. D. and Nandini, R. 1996. Cytoskeletal protein and cell shape. *Current Science*, 71:737-743.

Kadenbach, B., Ramzan, R., Vogt, S. 2009. Degenerative diseases, oxidative stress and cytochrome c oxidase function. *Trends Mol. Med.*, 15: 139-147.

Marimuthu, T. and Peter, P.I. 2011. World Noni Research Foundation- An overview, *World Noni Research Foundation, Chennai, India*, p.101.

Mladenka, P., Zatloukalova, L., Filipsky, T. and Hrdina, R. 2010. Cardiovascular effects of flavonoids are not caused only by direct antioxidant activity. *Free Radical Biol. Med.*, 49: 963-975.

Singhal, A.K., Naithani, V. Bangar, O.P. 2012. Medicinal plants with potential to treat Alzheimer and associated symptoms. *Int. J. Nutr. Pharmacol. Neurol Dis.*, 2:84 -91.

Spector, A., Guo-mang, W. Ren Rong, W., Garner, W.H. and Hans moll. 1993. The prevention of cataract caused by oxidative stress in cultured rat lenses. *Curr. Eye research*, 12:163-179.

V-OP-4

Exploitation of anti-inflammatory role of *Morinda citrifolia* L. in the skeletal muscle of intra-nigrally rotenone infused Parkinsonian rats

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder with the cardinal symptoms of bradykinesia, resting tremor, rigidity and postural instability that leads to abnormal movements and lack of activity which in turn causes muscular damage. Even though studies have been carried out to elucidate the causative factors that lead to muscular damage in PD, studies related to inflammatory events that occur in the skeletal muscle and a therapeutical approach to culminate the muscular damage has not been extensively studied. Hence, this study evaluated the impact of rotenone induced SNPc lesion on inflammatory mediators and the efficacy of Morinda citrifolia, a medicinal plant used by the Polynesians in safeguarding the myocytes. Assessment of inflammatory mediators such as NF?B and TNF? along with the analyses of mitochondrial function revealed that treatment with Morinda citrifolia fruit extract (MCE) significantly reverted the alterations in mitochondrial function by augmenting the enzymatic activities of Electron transport chain complexes and TCA cycle enzymes. MCE supplementation reduced the expression of inflammatory mediators NF?B and TNF? in the skeletal muscle and prevents the skeletal muscle damage induced by rotenone. Hence this preliminary study paves a way to show that the anti-inflammatory activity of MCE can be exploited to alleviate skeletal muscle damage induced by Parkinsonism.

Keywords : Parkinson's disease, skeletal muscle, oxidative stress, apoptosis, *Morinda citrifolia.*