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Advances in Research on Noni Volatile Compounds

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Key words : Volatiles, Octanoic acid, decanoic acid, hexanoic acid

Abstract : In this review, the information about volatile composition in Noni (*Morinda citrifolia* L.) fruits and the methods used for their isolation are discussed. The results about volatile components of Noni at two ripening stages are also discussed. Ninety-six compounds have been identified, from which octanoic acid and hexanoic acid were found to be the major constituents. Due to noni maturation, octanoic acid, decanoic acid and (*E*)-2-nonenal decreased in their concentrations, while some esters with fruity odor, increased their contents. Two unsaturated esters, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased in their concentration from the ripe to over-ripe fruits. A specific part is dedicated to the recent studies about the changes of volatile compounds of Noni juice during the traditional process of fermentation/aging. Octanoic and hexanoic acids diminished in their concentrations; while esters of ethanol, 1-butanol and 1-hexanol, with their flavor fruity notes, increased. The concentration of esters of methanol and 3-methyl-3-buten-1-ol decreased during the fermentation/aging process, whereas major alcohols of Noni juice, 1-hexanol and 1-octanol, increased their concentration during the process. These chemical changes justify that fermented juice possesses a flavor less pungent than fresh juice due to reduction in hexanoic and octanoic acids contents, as well as a greater fruity note due to the increment in ester concentrations. The challenges in future will be: screening biodiversity by advanced techniques, identification and authentication of the starting materials and development of technological process for diminishing the pungent flavor of noni products.

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Introduction

Morinda citrifolia L. (Rubiaceae), commonly known as noni, is a plant typically found in the Hawaiian and Tahitian islands. It is believed to be one of the most important plants brought to Hawaii by the first Polynesians (Ross, 2001). Different parts of the tree, including the fruit, have been used traditionally as a folk remedy for many diseases like diabetes, hypertension, and cancer (Sang et al, 2002; Chan-Blanco *et al*, 2006; Potterat and Hamburger, 2007). The

consumption of the juice for improving health in many ways has shown a tremendous growth worldwide. The plant is a small evergreen tree with large bright green elliptical leaves. The fruit results from coalescence of the inferior ovaries of many closely packed flowers and its surface is smooth and has many polygonal sections. The immature fruit is green. As it matures the fruit becomes whiter in colour, and unless it is harvested at this stage, it simply falls to the ground. As it ripens, the fruit has a very pungent smell, similar to the odor of blue vein cheese and its taste is sour.

Although the fruits of noni have been used as a food, very few reports on their chemical composition are available (Wang, 1999; Ross, 2001; Kamiya, 2004; Chan-Blanco *et al*, 2006; Potterat and Hamburger, 2007), all of them related with the non-volatile components, and only two papers analyzed the volatile compounds (Farine *et al,* 1996; Lachenmeier *et al*, 2006).

Methods for the isolation of volatile compounds in fruits

The aroma of most fruits and fruit products consists of complicated mixtures, sometimes consisting of several hundred compounds. An analysis of odor – its identification and quantitative evaluation – can constitute a valuable source of information on the health quality of fruit, which includes both the sensory quality and the consumer's health safety.

The aroma compounds are mainly hydrophobic and instrumental analysis of volatiles must consider, as a first step, an isolation method suitable for separating these hydrophobic volatiles from the fruit matrix. The most frequently used methods for isolation and concentration of volatile compounds involve distillation, extraction, distillation-extraction, headspace techniques and the more recent sorptive techniques.

As no single isolation method yields an "accurate" picture of food aroma (Reineccius, 2006) isolation, analysis of aroma remains challenging. The isolation step may lead to artifacts, and the total volatile content in most cases is very difficult to relate to a sensory profile determined by a sensory panel. As no universal isolation method exists, it is essential to choose a method that yields an extract as representative as possible of the sensory properties of the food.

This part of the present review will focus on recent advances made with a sorptive extraction method: solid phase micro extraction (SPME) in noni fruits.

SPME, developed in the past decade, is a rapid, direct, inexpensive, and efficient technique for sampling different matrixes (Pawliszyn, 1997; 1999). SPME is a

multi-analyte extraction technique that requires no solvents; it minimizes artefact formation by heat and provides linear results over a wide range of analyte concentrations in a large variety of matrixes. In the field of food aroma analysis, the headspace SPME (HS-SPME) has proved to be an advanced and efficient tool for studies on aromas of food, including fruits and fruit products (Harmon, 2002; Marsili, 2002). In fact, SPME combined with gas chromatography-mass spectrometry (SPME/GC-MS) is a convenient technique for providing the aromatic fingerprint descriptions of each analyzed fruit. These are the reasons that the major part of papers about volatile compounds in this fruit included the use of SPME as isolation and concentration method.

Previous studies in Noni volatiles

The first paper related to the volatile compounds of noni was reported by Farine *et al*. (1996), who were interested in that ripe noni fruits are the only larval resources of *Drosophila sechellia*. In total, 51 volatile compounds were isolated and quantified by solvent extraction from the ripe fruit and analysis by GC-MS. The ripe fruit was characterized by a large amount of carboxylic acids, especially octanoic and hexanoic acids. The biological effects of the ripe fruit and its main acids were investigated with behavioural studies. Octanoic acid was responsible for the general toxicity of the fruit to most *Drosophila* species; *D. sechellia* was the only species which is resistant to this acid. Hexanoic acid had a unique effect, causing reversible coma but no mortality. Decanoic acid was inactive. A mixture of these three acids in proportions similar to those found in the fruit, mimics the effects of ripe noni fruits.

Many years later, Lachenmeier *et al*. (2006) reported the identification of 24 volatile constituents by HS-SPME-GC-MS using a PDMS fiber from commercial noni juices. In this paper, the authors were interested in the authentication of noni juices from different geographical origins. No quantitative data of volatile compounds was reported.

Volatile compounds in noni at two ripening stages

The volatile constituents of noni fruit were obtained by HS-SPME and analyzed by GC–MS using a fused silica capillary column (Pino *et al,* 2010). In total, ninety-six volatile compounds were identified (Table 1), sixty-seven of them reported for the first time in noni fruit, although they are often found in other fruits (Nijssen *et al,* 1996). The use of a more polar fiber in this study allows us the isolation of many polar compounds not previously found in the previous study using the non-polar PDMS fiber (Lachenmeier *et al*, 2006). The presence

of so many aliphatic esters in fruits of both maturity stages, mainly in the over-ripe ones, is interesting. This abundance of aliphatic esters has not been reported in previous studies (Farine *et al,* 1996; Lachenmeier *et al*, 2006). Alkyl esters of hexanoic and octanoic acids were the major ones in this family. All these esters have powerful fruity odor notes (Arctander, 1969).

In general, although terpenes are present in small quantities in both maturity stages, their contribution to the flavor of the fruit could be considerable, as in the case of limonene and linalool, which were found to possess intense citrus and flower-like odors, respectively (Arctander, 1969). Interestingly, three sulphur compounds were found for the first time in noni fruit, *e.g.* dimethyl disulfide, dimethyl trisulfide and 3-(methylthio)-1-propanol. No nitrogen-containing volatile compounds were found.

Major volatile compounds, at both maturity stages, were octanoic acid (@ 70% of total extract) and hexanoic acid (@ 8% of total extract), basically the same components as those previously reported (Farine *et al,* 1996), although in different proportions (58 and 19%, respectively). According to their odor notes (Arctander, 1969), both acids are responsible for the pronounced "rancid cheese" odor of noni fruit.

Although the majority of compounds remain without changes between the two maturation stages, the composition of some particular volatile compounds clearly differs in both maturation stages (Table 1). Some esters, with fruity odor notes, increased, while some compounds, mainly acids, decreased or even disappeared during ripening. The over-ripe noni fruit showed significantly higher amounts of methyl hexanoate, methyl octanoate, ethyl octanoate and methyl 4*E*-decenoate, while octanoic acid and decanoic acid concentrations significantly decreased. These changes probably indicate that esterification occurs during maturation, in a similar way as in other fruits (Wills *et al,* 1989). Only two unsaturated esters reported for the first time in this fruit, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased their concentration from the ripe to over-ripe fruits.

An unsaturated aldehyde related with lipid-degraded product, (*E*)-2-nonenal, decreased during fruit maturation. As reported for tomatoes (Gaillard *et al*, 1977), the activities of several enzymes seem to change during ripening of noni fruit, especially of those involved in the formation of this lipid-degraded product.

Volatile and nonvolatile acids of Noni fruit

The volatile acids of noni fruit were isolated by liquid-liquid extraction, derivatized as methyl esters and analyzed by GC-FID and GC-MS (Table 2) (Pino *et al*,

2009). In total, thirty-four acids were identified, many of them reported for the first time in noni. The qualitative profile of the identified volatile constituents is represented by linear aliphatic carboxylic acids produced by fatty acid biosynthesis or degradation; branched acids as 2-methylbutanoic acid, 3-methyl-2-butenoic acid and (*E*)-2-methyl-2-butenoic acid related with the synthesis of aliphatic amino acids; 2- and 3-hydroxyacids produced by the oxidation of a- and b-unsaturated long chain fatty acids; unsaturated aliphatic acids produced by the peroxydation of lipids; and some aromatic acids. One sulphur-containing acid was identified, 2-methylthioacetic acid, previously reported in other tropical fruits (Idstein *et al*, 1985).

The quantitative data in Table 2 show that the total amount of volatile acids per kilogram of edible fruit was 3.66 g. Major components were octanoic acid (3.06 g/kg) and hexanoic acid (0.33 g/kg). Both compounds were reported as the major volatile constituents in noni (FARINE et al, 1996).

Twenty-six nonvolatile acids were identified (Table 3), all of them reported for the fist time in noni, although they are widely distributed in nature. The qualitative profile of the identified compounds is represented by dicarboxylic acids and one tricarboxylic acid (citric acid) produced by the respiration process and some aromatic acids. Two fatty acids (linoleic acid and oleic acid) were also detected.

The quantitative data in Table 3 show that the total amount of nonvolatile acids per kilogram of edible fruit was 7.43 g. Major components were malic acid (3.28 g/kg), malonic acid (1.46 g/kg) and fumaric acid (1.03 g/kg).

Changes in volatile compounds during the fermentation/aging of Noni fruit by the ancient traditional process

There are two main methods employed to produce noni juice. In the traditional method, the fruit is collected when it is beginning to ripen and placed in a container for several weeks. At the end of this time a large percentage of the over-ripe fruit simply disappears into the juice; the residual fruits are mashed into a puree, and the juice is filtered to remove any remaining sediment. The dark brown juice is then ready for use. In another processing method, mature fruit (light amber colour) is pressed and the resulting juice becomes the finished product (Russell, 2000).

The composition of major volatile compounds of noni juice clearly differs during the fermentation/aging process (Table 4). These constituents represented more than 95% of the total composition. The behavior observed for these compounds was in dependence of its chemical structure: Major acids, octanoic

and hexanoic, diminished their concentration; while esters of ethanol, 1-butanol and 1-hexanol, with their flavor fruity notes, increased. Nevertheless, the concentration of esters of methanol and 3-methyl-3-buten-1-ol decreased during the fermentation/aging process. Therefore, this behavior indicates that in some cases occurred a selective esterification reaction during the process and in other there was a hydrolysis. On the other hand, major alcohols of noni fruit, 1-hexanol and 1-octanol, increased their concentration during the study.

The behavior as chemical group of these compounds (Fig. 1) indicated that the changes which take place during the fermentation/aging process were gradual. Acids and methyl esters gradually decreased their concentration, while alcohols and ethyl, butyl and hexyl esters increased during the process. It can be seen that at 60 days there occurred stability in the composition. These results give a scientific basement to the empirical approach of conserving the noni juice for two months before its extraction of the containers. These chemical changes also justify that final product possesses a flavor less pungent due to an important decrease of hexanoic and octanoic contents, as well as a greater fruity note due to the increment in ester concentrations.

Conclusions

A total of 96 volatile components of noni at two ripening stages, 67 of them for the first time, were isolated by headspace solid-phase microextraction and analyzed using GC-MS. Both maturation stages had several compounds in common. Octanoic acid (@ 70% of total extract) and hexanoic acid (@ 8% of total extract) were found to be the major constituents. Due to noni maturation, octanoic acid, decanoic acid and (*E*)-2-nonenal decreased their concentrations, while some esters (methyl hexanoate, methyl octanoate, ethyl octanoate and methyl (*E*)-4-decenoate), which their fruity odor notes, increased their contents. Two unsaturated esters reported for the first time in this fruit, 3-methyl-3-buten-1-yl hexanoate and 3-methyl-3-buten-1-yl octanoate, significantly decreased their concentration from the ripe to over-ripe fruits.

A combination of sampling methods with GC and GC-MS detection was used to analyze volatile and nonvolatile acids of noni fruit. Thirty-four volatile acids and twenty-six nonvolatile acids were identified. Octanoic acid and hexanoic acid were the major volatile acids, whereas malic acid, malonic acid and fumaric acid were the main nonvolatile acids.

The main changes of volatile compounds of noni juice during the traditional process of fermentation/aging were: major acids, octanoic and hexanoic, diminished their concentration; while esters of ethanol, 1-butanol and 1-hexanol,

with their flavour fruity notes, increased. The concentration of esters of methanol and 3-methyl-3-buten-1-ol decreased during the fermentation/aging process, whereas major alcohols of noni juice, 1-hexanol and 1-octanol, increased their concentration during the process. At 60 days of the processes occurred stability in the volatile composition of the noni juice.

The challenges in future will be: screening biodiversity by advanced techniques, identification and authentication of the starting materials, and development of technological process for diminishing the pungent flavor of noni products.

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Table 1. Volatile compounds in noni at two maturity stages (mean area %)

Compound	RI_{exp}	RI_{st}	Identification	Ripe	Over-ripe
Ethanol*	535	537	A	0.02a	0.02a
Acetic acid	602	600	A	0.01a	0.02a
1-Butanol	650	653	A	0.04a	0.04a
Pentanal*	701	706	A	0.01a	0.01a
Methyl butanoate*	728	729	A	0.01a	0.02a
3-Methyl-3-buten-1-ol	730	731	B	0.02a	0.03a
3-Methylbutan-1-ol*	741	741	A	0.02a	0.01a
2-Methylbutan-1-ol*	744	743	A	t	0.02
Dimethyl disulfide*	747	746	C	0.01	t
Ethyl isobutanoate*	750	751	A	0.01a	0.02a
2-Methylpropanoic acid	758	758	C	0.02a	0.01a
3-Methyl-2-buten-1-ol	772	774	C	0.03a	0.03a
Butanoic acid	793	790	A	0.03a	0.04a
Ethyl butanoate*	801	804	A	t	0.03
Butyl acetate*	809	811	A	t	t
3-Methylbutanoic acid*	837	836	A	0.20	-
Ethyl 2-methylbutanoate*	846	846	A	t	0.02
2-Methylbutanoic acid	862	860	A	t	0.16
1-Hexanol	870	871	A	0.29a	0.34a
3-Methyl-3-buten-1-yl acetate*	888	885	C	0.01	t
2-Heptanone	895	892	A	0.04a	0.04a
Propyl butanoate*	898	899	A	t	t
3-Methyl-2-hexanol*	906	909	C	0.01	0.01
Methyl hexanoate	930	927	A	0.37a	0.44b
a-Pinene*	938	939	A	0.01	t
Butyl isobutanoate*	950	949	A	0.01a	0.01a
Benzaldehyde	858	960	A	0.01a	0.01a
Dimethyl trisulfide*	969	970	C	0.01a	0.01a

Sabinene*	971	975	A	t	t
3-(Methylthio)-1-propanol*	980	980	C	0.01a	0.01a
Hexanoic acid	982	981	A	8.19a	8.16a
6-Methyl-5-hepten-2-one*	988	986	A	-	t
Myrcene*	993	991	A	0.03a	0.02a
Butyl butanoate*	996	995	A	0.03a	0.02a
Ethyl hexanoate	998	998	A	1.02a	1.07a
Octanal*	1000	999	A	t	t
Hexyl acetate*	1011	1009	A	t	0.11
3-Methyl-3-buten-1-yl isobutanoate*	1014	1013	C	0.03a	0.03a
Limonene	1030	1029	A	1.44a	1.89a
Benzyl alcohol	1033	1032	A	t	t
(*Z*)-b-Ocimene*	1034	1032	B	t	t
g-Terpinene*	1058	1060	A	-	t
Acetophenone*	1067	1065	A	-	t
1-Octanol	1069	1070	A	-a	0.01a
Terpinolene*	1088	1088	B	t	t
2-Nonanone*	1089	1090	A	0.04a	0.02a
Propyl hexanoate*	1094	1094	A	0.01a	0.01a
Ethyl heptanoate*	1097	1098	A	0.01	t
Linalool*	1099	1099	A	0.06a	0.09a
Nonanal*	1102	1101	A	0.03a	0.07a
Heptanoic acid	1109	1108	A	0.07a	0.04a
3-Methyl-3-buten-1-yl isopentanoate	1112	1113	C	0.03a	0.03a
2-Phenylethanol*	1117	1118	A	0.04a	0.05a
Methyl octanoate	1120	1120	A	4.47a	6.13b
2-Ethylhexanoic acid*	1122	1122	C	0.01	t
Hexyl isobutanoate*	1150	1152	B	0.03a	0.02a

Isobutyl hexanoate*	1153	1156	B	0.05a	0.04a
(*E*)-2-Nonenal*	1160	1161	A	0.28a	-b
Benzyl acetate*	1162	1162	A	0.01	t
Methyl 2-phenylacetate*	1177	1176	A	0.03a	0.02a
Octanoic acid	1179	1181	A	72.29a	70.47b
Butyl hexanoate*	1187	1188	A	0.75a	0.48a
a-Terpineol*	1190	1189	A	t	t
Methyl salicylate*	1193	1192	A	t	t
Ethyl octanoate	1199	1197	A	3.48a	4.58b
Decanal*	1201	1202	A	t	t
Methyl nonanoate*	1224	1227	A	t	t
3-Methyl-3-buten-1-yl hexanoate*	1241	1244	C	0.52a	0.33b
Ethyl 2-phenylacetate*	1246	1247	A	t	t
3-Methylbutyl hexanoate*	1255	1254	B	t	t
g-Octalactone*	1260	1261	A	t	t
Methyl (*E*)-4-decenoate*	1262	1263	C	-a	0.15b
(*E*)-2-Octenoic acid*	1269	1266	C	0.07	-
2-Phenylacetic acid*	1270	1268	A	t	-
Nonanoic acid	1271	1271	A	0.04a	0.03a
Methyl decanoate	1321	1326	A	0.71a	0.77a
Benzyl butanoate	1349	1347	A	t	t
Eugenol	1356	1359	A	0.08a	0.05a
Decanoic acid	1373	1371	A	0.24a	0.13b
Ethyl (*E*)-4-decenoate*	1380	1382	C	t	0.03
Propyl disulfide*	1388	1390	C	t	t
Hexyl hexanoate*	1393	1394	A	0.47a	0.45a
Butyl octanoate	1395	1396	A	0.76a	0.54b
Ethyl decanoate	1397	1397	A	0.09a	0.11a
Methyl 10-undecenoate*	1427	1429	C	0.06a	0.07a

3-Methylbutyl octanoate*	1453	1450	A	0.05a	0.05a
Pentyl octanoate*	1490	1491	A	t	t
3-Methyl-3-buten-1-yl octanoate*	1513	1518	C	3.20a	1.24b
Methyl dodecanoate*	1523	1526	A	t	t
Benzyl hexanoate*	1549	1547	A	0.02a	0.02a
Butyl decanoate*	1599	1590	A	t	t
Hexyl 2-phenylacetate*	1630	1631	B	t	t
2-Phenylethyl hexanoate*	1639	1642	A	0.01	t
Isopropyl tetradecanoate*	1835	1830	B	0.01a	0.03a
2-Phenyletyl octanoate*	1838	1842	B	t	t
Methyl hexadecanoate	1920	1922	A	t	t

t = lower than 0.01%, - not detected, RI_{exp} = experimental retention index on HP-5, RI_{st} = standard or literature retention index.

*reported for the first time in noni.

Values followed by the same letter are not significantly different at $p \leq 0.05$.

The reliability of the identification proposal is indicated by the following: A, mass spectrum and retention index agreed with standards; B, mass spectrum and retention index agreed with literature data; C, mass spectrum agreed with mass spectral database.

Table 2. Volatile acids of noni fruit (identified as methyl esters)

Compound	RI_{HP-5}	$RI_{HP-Innowax}$	mg/kg
Propanoic acid	646	896	t
Isobutanoic acid	685	927	15.7 ± 1.4
Butanoic acid	720	990	4.9 ± 0.3
2-Methylbutanoic acid	746	1007	6.7 ± 0.5
2-Hydroxybutanoic acid	835	1390	0.1 ± 0.01
3-Methyl-2-butenoic acid	844	-	19.7 ± 1.2
3-Hydroxybutanoic acid	859	1460	8.3 ± 0.6
(*E*)-2-Methyl-2-butenoic acid	871	1150	0.4 ± 0.1
2-Methylthioacetic acid	899	1380	9.6 ± 0.4
Hexanoic acid	927	1188	329.5 ± 12.5
(*E*)-2-Hexenoic acid	944	1217	0.5 ± 0.03
Heptanoic acid	1026	1272	20.7 ± 1.4
3-Hydroxyhexanoic acid	1048	1627	77.5 ± 4.0
Benzoic acid	1091	1596	8.6 ± 0.5
Octanoic acid	1127	1373	3057.9 ± 75.4
(*E*)-2-Octenoic acid	1149	1450	3.6 ± 0.2
Phenylacetic acid	1179	1747	4.8 ± 0.2
Nonanoic acid	1227	1472	6.2 ± 0.3
3-Hydroxyoctanoic acid	1250	1847	9.3 ± 0.3
(*E*)-4-Decenoic acid	1282	-	8.2 ± 0.3
Decadienoic acid	1293	1998	14.9 ± 1.1
(*Z*)-4-Decenoic acid	1310	1634	18.2 ± 1.2
Decanoic acid	1326	1584	19.7 ± 1.0
Anthranilic acid	1337	2181	t
(*E*)-Cinnamic acid	1379	2014	0.6 ± 0.02
10-Undecenoic acid	1402	1733	0.8 ± 0.02
Undecanoic acid	1426	1688	t
2-Hydroxydecanoic acid	1437	1790	3.0 ± 0.1
3-Hydroxydecanoic acid	1451	2038	6.4 ± 0.2
9-Hydroxydecanoic acid	1525	-	2.5 ± 0.05
Tetradecanoic acid	1724	1994	0.4 ± 0.01
Hexadecanoic acid	1923	2196	1.5 ± 0.05
Heptadecanoic acid	2022	2305	0.2 ± 0.01
Octadecanoic acid	2128	2412	0.6 ± 0.03

t = trace (< 0.1 mg/kg) - = no data

Table 3. Nonvolatile acids of noni fruit (identified as methyl esters)

Compound	RI_{HP-5}	$RI_{HP-Innowax}$	g/kg
Malonic acid	915	1479	1.46 ± 0.05
2,2-Dimethoxypropanoic acid	936	-	0.06 ± 0.003
2-Methylmalonic acid	969	-	0.01 ± 0.001
2-Furoic acid	980	1561	0.29 ± 0.02
Levulinic acid	981	1534	0.03 ± 0.001
2-Hydroxy-3-methylpentanoic acid	990	-	0.01 ± 0.001
Fumaric acid	1029	1529	1.03 ± 0.08
2-Methylsuccinic acid	1069	-	0.02 ± 0.001
Methylmaleic acid	1100	-	0.01 ± 0.001
Methylfumaric acid	1112	-	0.02 ± 0.001
2-Hydroxy-2-methylsuccinic acid	1122	-	0.01 ± 0.001
Malic acid	1138	-	3.28 ± 0.09
2-Methoxysuccinic acid	1156	-	0.04 ± 0.003
Pimelic	1167	1680	0.13 ± 0.01
Salicylic acid	1192	1744	0.04 ± 0.002
3-Methylglutaconic acid	1199	-	0.02 ± 0.001
Adipic acid	1243	1779	0.08 ± 0.003
Suberic acid	1446	1809	0.04 ± 0.002
Phthalic acid	1449	2203	0.30 ± 0.02
Citric acid	1460	2286	0.16 ± 0.01
4-Hydroxybenzoic acid	1465	-	0.12 ± 0.01
Vanillinic acid	1496	2559	0.09 ± 0.01
Azelic acid	1540	1908	0.11 ± 0.01
2-Ketoglutaric acid	1739	-	0.03 ± 0.001
Linoleic acid	2093	-	0.01 ± 0.001
Oleic acid	2103	2538	0.04 ± 0.002

- = no data

Table 4. Changes in major volatile compounds (mean peak area/internal standard peak area ratio) during fermentation/aging of noni juice

Compound	RI[a]	Time (days)								
		0	7	11	21	30	40	50	64	108
Methyl butanoate	729	0.01 a	0.01 a	0 b	0 b	0 b	0 b	0 b	0 b	0 b
Butanoic acid	790	0.03 a	0.02 a	0 b	0 b	0 b	0 b	0 b	0 b	0 b
Ethyl butanoate	804	0.03 a	0.11 b	0.14 b	0.19 c	0.23 cd	0.26 d	0.29 d	0.31 de	0.33 e
3-Methylbutanoic acid	836	0.20 a	0.15 b	0.12 b	0.08 c	0.06 c	0.03 cd	0.01 d	0.01 d	0.01 d
Ethyl 2-methylbutanoate	846	0.02 a	0.05 a	0.07 b	0.10 bc	0.12 c	0.14 c	0.16 cd	0.18 d	0.19 d
Ethyl 3-methylbutanoate	853	0.02 a	0.07 b	0.09 b	0.14 c	0.18 d	0.21 de	0.23 e	0.23 e	0.23 e
2-Methylbutanoic acid	860	0.02 a	0.01 a	0.01 a	0 b	0 b	0 b	0 b	0 b	0 b
1-Hexanol	871	0.30 a	1.12 b	1.42 c	1.84 d	2.25 e	2.61 f	2.95 g	2.98 g	3.00 g
Methyl hexanoate	927	0.35 a	0.23 b	0.20 bc	0.18 c	0.16 c	0.14 cd	0.13 d	0.12 d	0.11 d
Hexanoic acid	981	8.00 a	7.45 b	7.13 c	6.04 d	5.12 e	4.57 f	3.85 g	3.89 gh	3.92 gh
Ethyl hexanoate	998	1.09 a	6.01 b	7.02 c	8.63 d	9.76 e	11.05 f	11.96 g	12.00 g	12.03 g
Hexyl acetate	1009	0.01 a	0.34 b	0.45 c	0.65 d	0.79 e	0.94 f	1.00 f	1.02 f	1.00 f

3-Methyl-3-buten-1-yl isobutanoate	1013	0.03 a	0.02 ab	0.01 b	0.01 b	0.01 b	0.01 b	0.01 b	0.01 b	0.01 b
1-Octanol	1070	0.02 a	0.58 b	0.81 c	1.23 d	1.52 e	1.70 f	1.81 g	1.85 g	1.89 g
Methyl octanoate	1120	4.52 a	3.03 b	2.52 c	1.88 d	1.50 e	1.11 f	0.93 g	0.92 g	0.90 g
Octanoic acid	1181	72.00 a	60.81 b	55.63 c	49.80 d	45.12 e	40.10 f	35.30 g	35.33 g	35.36 g
Butyl hexanoate	1188	0.71 a	0.88 b	0.99 c	1.26 d	1.53 e	1.77 f	1.99 g	2.01 g	2.02 g
Ethyl octanoate	1197	3.40 a	10.08 b	12.61 c	17.31 d	20.21 e	23.92 f	26.06 g	26.09 g	26.12 g
3-Methyl-3-buten-1-yl hexanoate	1244	0.55 a	0.31 b	0.24 c	0.20 cd	0.18 d	0.15 d	0.13 de	0.12 e	0.13 e
Methyl decanoate	1326	0.68 a	0.44 b	0.37 c	0.29 d	0.23 e	0.18 f	0.15 f	0.15 f	0.14 f
Decanoic acid	1371	0.22 a	0.18 ab	0.16 b	0.11 c	0.07 cd	0.04 d	0.02 d	0.02 d	0.02 d
Hexyl hexanoate	1384	0.51 a	0.86 b	1.03 c	1.44 d	1.79 e	2.20 f	2.41 g	2.38 g	2.35 g
Butyl octanoate	1395	0.77 a	1.19 b	1.50 c	2.08 d	2.54 e	3.01 f	3.53 g	3.52 g	3.51 g
Ethyl decanoate	1397	0.10 a	0.42 b	0.59 c	0.83 d	1.02 e	1.14 f	1.20 g	1.21 g	1.22 g
3-Methyl-3-buten-1-yl octanoate	1518	3.15 a	2.37 b	2.01 c	1.41 d	0.89 e	0.40 f	0.10 g	0.12 g	0.14 g
3-Methyl-3-buten-1-yl decanoate	1718	0.03 a	0.02 a	0.01 a	0 b	0 b	0 b	0 b	0 b	0 b

a Retention index on AT - 5ms.

Different letters in the same row indicate significant difference at p 0.05.

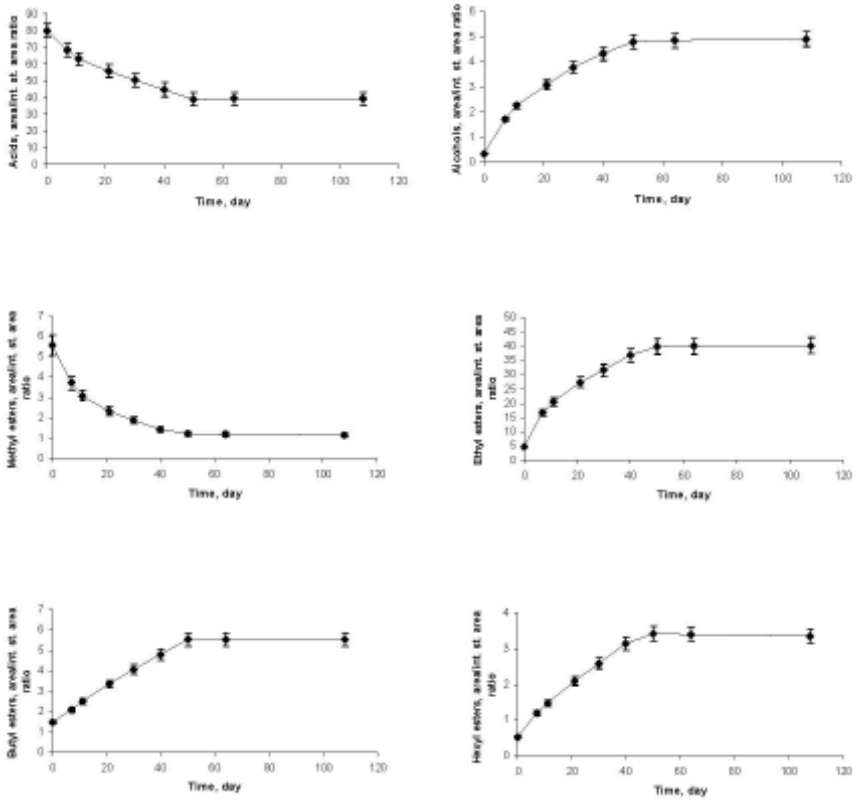

Fig. 1. Changes of major volatile compounds during fermentation/aging of noni juice.

Sustainable Management of biodiversity and Productivity of Medicinal Plants : Case Study of Noni

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Abstract : Currently, there is revival of interest globally in utilizing medicinal plants for human wellness. The global demand for herbal products is growing at the rate of 10-15% per annum. The world demand for herbal products is estimated to be currently US$ 62 billion, which is poised to grow many times by 2050. The 16 agro-climatic zones of South Asia harbour upto 50,000 plant species, of which at least 16,000 are believed to have medicinal properties. The emerging scenario of increasing the area under cultivation for direct access to communities and as raw material for commercial product preparation is very welcome. The World Health Organization (WHO) estimates that 4 billion people around the world use herbal medicines for their primary health care. In South East Asia alone Ayur veda, Unani, Siddha, Chinese, Amchi and Homeopathic traditional healing approaches make use of about 9000 plant species. Whatever available in nature in the forest and other lands are being indiscriminately cut/harvested and used. About 90% collection is from wild and 70% of plant collection involves destruction methods of harvest. Most of these plants are collected, sold and used by tribes for their livelihood. India alone has about 2500 species of medicinal plants and vast geographic area with high production potential. However, many of the very valuable medicinal plants are in the endangered position because not much care is taken to conserve these plants. One such plant is Noni, *Morinda citrifolia*, a plant along coastal belt and waste lands which has more than 150 plus nutraceuticals which are useful to mankind.

To ensure sustainable access to these plants of value in human wellness, we need to assemble, characterize and selectively conserve their biodiversity among and within species. To enhance their bio-mass output per unit area without detriment to quality, we should also identify high yielding varieties, cost-effective and eco-friendly crop production, post harvest and processing technologies.

Appropriate and affordable crop protection practices need to be developed for preventive and curative control of pest/disease, with emphasis on farm-level crop health monitoring to permit timely protection interventions. The scope for devel-

oping bio-pesticide formulations with longer shelf life and field stability, besides utilizing other bio-control agents having better adaptation to abiotic stresses should be assessed. There is scope to promote efficient and affordable organic practices/ inputs for enhanced crop productivity, on the basis of locally available bio-resources through holistic and on-farm experimentation.

The way forward for science-led research in crop improvement and management technology development through stake-holder participation and public-private partnership is discussed.

Introduction

"Wellness for All" continues to be a distant dream not only for India but also for the entire world. With the increase in life expectancy and the problems of over crowding, air and water pollution, degenerative disease stress, allergies, diabetes, rheumatic and arthritic conditions, neurological conditions, memory disorders are likely to grow. Currently, in India, about 85 per cent of women are anaemic on account of iron deficiency and 2.2 million children are afflicted with cretinism while another 6.5 million are mildly retarded. About 1.1 lakh women die every year of causes related to pregnancy and childbirth. This can affect the quality of life, productivity and the well-being of future generations. The health of women is especially important because if children are born to sick mothers, there will be problems in their later life.

Globally, there is revival of interest and awareness in utilizing medicinal plants for human wellness in various forms in the recent times. Health conscious people are more eager to go for organic and natural products to maintain their body and mind. A change in the status of merely collecting the existing medicinal plants in nature and in the natural forest has turned into cultivation of these crops for direct access to communities and as raw material for commercial product preparation which is a very welcome move. However, many of the very valuable medicinal plants are endangered because not much care has been taken to conserve these plants.

Bio Diversity

Flora of the world

Globally, 270,000 species of flora exists around the world of which 33,798 species are threatened with extinction. Though the current estimates put 13 percent of global flora are at risk of extinction (Table 1), the new study suggests that the number of plant species threatened with extinction may be more than three times higher than previously thought. According to a report published recently in the journal *Science,* between 22 and 47 percent of the world's plants are endangered.

Based on the study carried out in a diversity project in Ecuador, where they analyzed the status of nearly 4,000 plant species, it is estimated that a complete catalogue of the world's endangered plants could be assembled for around $100 per species annually, or with about $12 million for all of the world's biodiversity hotspots.

Table. 1 Status of Global and Indian Flora

Plant group of part of the world	No. of species of flora	No. of threatened species	Percentage of flora threatened	Remarks
Global flora	270,000	33,798	>13%	The figure of 33,789 includes 380 species classified as 'Extinct' in the wild, 371 species' Extinct' in the wild or 'Endangered' and 14,504 species as Rare.
Global tree	80,000-100,000	8753	9-11%	
Indian flora	15,900	1331	8%	

World wide it is estimated that around 21,000 species are of medicinal plants according to NTFP Partnership reports.

Out of 17,500 species of higher plants occurring in India, about 8,500 species are known to occur with ethno botanical properties. The 70% of medicinal plants are located in tropical areas and less than 30% are found in the temperate, alpine and higher attitude regions. . In Andaman and Nicobar Islands, there are 200 endemic and endangered species and one among them is Noni, *Morinda citrifolia,L.* (Gupta et al ., 2007).

Demand for Medicinal Plants

Indian per capita annual consumption of drugs valued at Rs.125 is the lowest in the world mainly because medicinal plants constitute the principal health care resources for the majority of population. The World Health Organisation (WHO) estimated that 80% of the population of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others, which are synthetic analogues built on prototype compounds, isolated from plants. Transition from synthetic drugs and microbially produced antibiotics to plant based drugs is rapidly gaining

acceptance. Global resurgence in the use of plant- based drugs is an opportunity for India to attain self- reliance and boost the export of herbal drugs.

The world market is growing by over 10% and at present it is US $ 64 billion. India is number two after China in market share but, the Indian share in export is only of about Rs. 446.3 crores, though India it self accounts for 16% of world market for it's internal needs . China and India are two great producers of medicinal plants having more than 40% of global biodiversity. China, besides meeting its domestic requirement, is earning US $ 5 billion per year from herbal trade. There is thus an enormous scope for India also to emerge as a major player in the global herbal product based medicines. However, this requires a grand strategic plan, which takes a holistic view of the entire situation to boost the export of Rs.10,000 crores by 2010 and minimising the import.

Plants as Sources of Medicines

Since ancient times, plants have been an exemplary source of medicine .About 2500 species are used in various classified systems of medicine like Ayurveda, Siddha , Unani etc. These systems of medicine used over 90% of their formulation from plant sources. The western systems of medicine too have a large component of drugs from plant origin.The forest areas have been the traditional source for plants and herbs.

At present, 90% collection of medicinal plants is from wild and 70% of plant collection involves destructive methods of harvesting. The current practices of harvesting are unsustainable and depleting the resources at faster rate. They pose a definite threat to the genetic stocks and the diversity of medicinal plants. In contrast, the demand for medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products, being non narcotic, having no side effect and sometime may be the only source of healthcare . India has about 45 000 plant species and among them, several thousands have been claimed to possess medicinal properties. Number of medicinal plants have been found useful in different inflammatory conditions (Srivatsava and Sahni,2000, and Rajput *et.al.,* 2004).

The Task Force, Government of India had identified a list of 25 plants which are having great demand and have been recommended for cultivation (Table.2).

Table.2.List of Medicinal Plants having great demand & Recommended for Cultivation

Sl. No.	Common Name	Botanical Name
1.	Amla	*Emblica officinales Gaertn*
2.	Ashwagandha	*Withania somnifera Dunal*
3.	Ashoka	*Saraca asoca (Ro xb.) DC*
4.	Atis	*Aconitum heterophyllum Wall*
5.	Baiberang	*Embelia ribes Burm. f.*
6.	Bael	*Aegle marmelos (L.) corr.*
7.	Brahmi	*Bacopa monnieri (L.) Pennell*
8.	Chandan	*Santalum album L.*
9.	Chirata	*Swertia chirata Buch. – Ham.*
10.	Goloe	*Tinospora cordifolia Willd*
11.	Guggal	*Commiphora wightii (Arn.) Bhandari*
12.	Rasaut	*Berberis aristata DC*
13.	Isabgol	*Plantago ovata Forsk*
14.	Jatamansi	*Nardostachys jatamansi* DC
15.	Kalmegh	*Andrographis paniculata* Nees
16.	Katki	*Picrorhiza kurroa* Royale ex Benth
17.	Kokum	*Garcinia indica* L.
18.	Kerth	*Saussurea lappa* C.B. clarke
19.	Mulethi	*Glycyrrhiza glabra* L.
20.	Pippali	*Piper longum* L.
21.	Madhuvashni	*Gymnema sylvestre* R. Br.
22.	Satavari	*Asparagus racemosus* Willd.
23.	Shankhapushpi	*Convolvulus pluricaulis* L.
24.	Safed musli	*Chlorophytum borivilianum*
25.	Senna	*Cassia angustifolia* Vahl.

Source : Task Force Report, GOI.

Morinda consists of 80 species present world wide and 12 species are found to exist in India. Noni , *Morinda citrifolia* L.

is one among them which can be considered as a nutritionally rich and under exploited fruit, with more than 150 nutriceuticals required for human health. It is also considered to be one of the important medicinal plants used for more than 2000 years .

Table 3.Plants and identified microbial bio active phytocompounds

Scientific name	Compound class	Compound	Activity (most relevant)
Allium sativum	Sulfaxide	Allicin	Broad spectrum
Anacardium pulsarilla	Polyphenols	Salicylic acids	*P. acnes*
Anemone pulsatilla	Lactone	Anemonins	Bacteria
Berberis vulgaries	Alkaloid	Berberine	Protozoa and bacteria
Camellia sinensis	Flavonoid	Catechin	Broad spectrum viruses
Carum carvi	-	Coumarins	Viruses, broad spectrum
Centella asiatica	Terpenoid	Asiatocoside	*Myobaerium leprae*
Cinchora sp.	Alkaloid	Quinine	*Plasmodium spp.*
Citrus sinensis	Terpenoid	-	Fungi
Croton cajucara	Essential oil	Linalool	*Leishmanio amazonenis*, fungi and bacteria
Erythroxylum coca	Alkaloid	Cocaine	Bacteria
Eucalypusglobulus sp.	Polyphenol	Tannin	Bacteria and viruses
Gloriosa superba	Alkaloid	Colchicina	Broad spectrum
Hydrastis canadensis	Alkaloid	Berberine	Bacteria, *Giargia duodenale*
Malus sylvestris	Flavonoid derivate	Phloretin	Broad spectrum
Matricaria chamonailla	Phenolic acid	Anthemic acid	*M. tuberculosis* and *S.typhimurium*
Melissa officinalis	Polyphenols	Tannins	Viruses

Millatia thonningii	Flavone	Alpinum-isoflavone	*Schistosoma* sp.
Ocimum basilicum	Essential oil	Terpenoids	Bacteria, *Salmonella sp.*
Olea auropaea	Aldehyde	Hexanal	Broad spectrum
Onobrychis viciifolia	Polyphenols	Tannins	Bacteria
Panax notoginseng	Saponins	-	Bacteria
Pimento dicica	Essential oil	Eugenol	Broad spectrum
Piper betd	Essential oil	Cathecol	Broad spectrum
Piper nigrum	Alkaloid	Piperine	Fungi, *Lactobacillus sp.*
Podocarpus nagi	Flavonol	Totarol	*P. acnes* and Grampositive bacteria
Rabdosia trichocarpa	Terpene	Trichorabdal	Helicobacter pylori
Rhamnus purshiana	Polyphenols	Tannins	Viruses, broad spectrum
Satureja montana	Terpenoid	Carvacrol	Broad spectrum
Vacinium spp.	Monosaccharide	Fructose	*Escheridia coli*
Vicia faba	Thionin	Fabatin	Bacteria
Vinca minor	Alkaloid	Reserpine	Broad spectrum
Curama longa	Terpenoids	Curcumin	Protozoa and bacteria
Aloysia tripphylla	Essential oil	Terpenoid	*Ascaris sp.*
Mentha piperita	Terpenoids	Menthol	Broad spectrum
Artemisia dracunlus	Polyphenols	Tannins	Helminthes and viruses

Source: Ahmed *et al*, 2007

Some commonly used poisonous drugs in the Indian system of medicine derived from plants are given in the Table below :

Table 4. Some commonly used poisonous drugs in Indian System of medicine

Plant name	Vernacular name	Part used	Common use	Adverse effect (in large doses)
Aborus precatorius L.	Indian liquoriceq	Seed	Diarrhea, dysentery, paralysis and skin diseases, antiseptic, uterine stimulant and anticancerouss,	Abrin causes edema and ecchymosi inflammation antifertility activity, antiestrogenic activity, abortifacient and oxytocic activity
Aconium casmanthum Stappex Holm	Aconite	Rhizome	Neuralgia, rheumatism, cardiac tomic and nerve poisons	Narcotic, powerful sedative, arrhythmia and hypertension
Gloriosa superba L.	Malanbar glory lily	Root	Anthelmintic, purgative, emetic, antipyretic, expectorant and toxic	Antifertility, vomiting, purging, gastrodynia and burning sensation
Croton tiglium L.	Croton	Seed	Abdominal disorders, constipation, helminthiasis, inflammation, leukoderma and dropsy	Depressor responses and neuromuscular blockade
Calotropis gigantean L.	Gigantic swallow wort	Latex and leaf	Paralysis, purgative and intermittent fevers	Violent purgative and gastrointestinal irritant

Cannabis sativa L.	Hernp	Leaf	Antidiarrhetic, intoxicating, stomachic and abdominal disorders	Neurotoxic, respiratory arrest, nausea tremors, insomnia, sexual impotence and gastrointestinal disturbance
Datura metel L.	Thorn apple	Seed and leaf	Antihelminthic and anticancerous	Insanity
Euphorbia neriifolia	Milk hedge	Latex	Insecticidal and cardiovascular	Emetic, irritant, apnea and pathological changes in liver, heart and kidney
Papaver somniferum L.	Poppy	Exudate	Diarrhea, dysentery, sedative, narcotic and internal hemorrhages	Highly narcotic
Semecarpus anacardium	Marking nut	Fruit	Antiseptic, cardiotoxic anticarcinomic liver tonic and uterine stimulants	Abortive
Nerium indicum mill	Oleander	Fruit and leaf	Antibacterial, ophthalmic and cardiotonices	Cardiac poison, paralysis and depress respiration, gastrointestinal, neurological and skin rashes
Strychnos nuxvomica L.	Snake wood	Seed	Appetizer, anthelmintic, purgative and stomachic	paralysis

Medicinal Plants Prohibited for Export from India

The Government of India has banned the export of under mentioned 29 plants, plant portions and their derivatives and extracts as such obtained from the wild, except the formulations* made there from.

1. *Cycas beddomei- Beddomes' cycad. (*
2. *Vanda coerulea -Blue Vanda ()*
3. *Saussurea costus*
4. *Paphiopedilium species- ladies slipper orchids ()*
5. *Nepenthes khasiana- Pitcher plant ()*
6. *Renathera imschootiana- Red Vanda ()*
7. *Rauvolfia serpentina (Sarpagandha)*
8. *Ceropegia species*
9. *Frerea indica (Shindal Mankundi)*
10. *Podophyllum hexandrum (emodi) (Indian Podophyllum)*
11. *Cyatheaceae species (Tree Ferns)*
12. *Cycadaceae species*
13. *Dioscorea deltoidea (Elephant's foot)*
14. *Euphorbia species (Euphorbias)*
15. *Orchidaceae species (Orchids)*
16. *Pterocarpus santalinus (Red Sanders)*
17. *Taxus wallichiana (Common Yew or Birmi leaves)*
18. *Aquilaria malaccensis (Agarwood)*
19. *Aconitum species*
20. *Coptis teeta*
21. *Coscinium fenestratum (Calumba wood)*
22. *Dactylorhiza hatagirea*
23. *Gentiana kurroo (Kuru, Kutki)*
24. *Gnetum species*
25. *Kamphergia galenga*
26. *Dactylorhiza hatagirea*
27. *Panax pseudoginseng*
28. *Picrorhiza kurrooa*
29. *Swertia chirata (Charayatah)*

Extending from Conservation to Cultivation

Generally, the past system has been that communities living in the neighborhood of forests and wild stands of medicinal plants have done well to selectively and

limitedly collect and use, with concern also to conserve the medicinal plants for future generations. Nevertheless, the emerging current scenario is of private and cooperative sectors seeking to scale up the product output from these plants for sale to end users in distant places, also as income generating enterprises benefitting the local communities as well. It is in this context that the medicinal plants like noni are being promoted for cultivation in newer areas which are endowed with favorable agro-ecological conditions. Thus ,there is indirect conservation of the crop, although some erosion might occur in the genetic diversity due to selection of genotypes for commercial productivity traits.

Of course, the consequences of moving these plants to new areas for commercial cultivation include their proneness to new pest/disease associations, besides needing the ecosystem services like pollination and organic matter decomposition by associated flora/fauna which are available in natural stands.

Noni – *Morinda citrifolia* .L

Indian Noni plant (*Morinda citrifolia L.*) belongs to the family *Rubiaceae.* It is commercially known as Noni, and also known as Indian Mulberry, Cheese fruit, Yellow fruit, Pain killer, Nono etc. It is a large shrub or a dwarf tree and native to South East Asia but has extensively spread throughout India and the Pacific Islands extending up to the Hawaiian Islands. It is known that Polynesians first took it from its homeland in Southern Asia. In Andaman and Nicobar Islands, *Morinda* is widely found growing in the coastal belts, rocky shores along fences, roadsides as well as in the wastelands of the islands. Two main varieties i.e., *Morinda citrifolia L.* var. *citrifolia* and *Morinda citrifolia L.* var. *bracteata* are available in plenty in A & N islands. They are locally known as Lorang, Burma phal, Pongee phal, Suraogi etc., by the tribals of Andaman and Nicobar Island (Singh, *et. al.,* 2005). Today, Noni grows in most regions of South Pacific, India, the Caribbeans, South America and the West Indies. Noni's broad proliferation gives testimony to its value to traditional cultures. Historically, it was known as the "queen" of all canoe plants. In Malaysia, it is known as Mengkudu. In South East Asia, it is known as Nhau. In the islands of the South Pacific particularly in Samoa and Tonga, the plant is known as Nonu. It is called, Noni in Raratonga and Tahiti, and Noni in the Marquesas Islands and Hawaii. In Australia, it is known as fromager, murier indien (French), Indian mulberry (English), nonu (Tahiti), nen, nin (Marshall islands, Chuuk) etc.. (Morton, 1992, Francis, 2003). The tropical humid climate is very much suitable for cultivation of *Morinda citrifolia L.* (Singh, *et. al.*, 2005).

Morinda tree attains a height of about 3-10 m. Plant forms have variation in fruit size, its morphology, odour of ripe fruit and number of fruits. Flower is white in colour, leaves are dark green, and fruits are greenish yellow, fleshy, fetid and soft

when ripe. Seeds are brown in color, conical to oblong in shape, has the ability to retain viability for a month if left in water. The wood is yellowish in color. The prime quality of the *Morinda* tree is its medicinal value and its nature to withstand any type of climatic conditions and environmental conditions and its competitiveness to grow on all types of soils like, loamy sand to very rocky soils. The species *citrifolia* is the best known to tolerate salty soils and salt spray. It is intermediate in shade tolerance and grows under the canopy of forests as well as in the open. *Morinda* grows naturally on the edges of mangroves, shorelines and on the landward side of beach and road sides as strand vegetation. The fruits may also be fed to pig livestock and poultry. *Morinda* is propagated either from seed or stem cuttings.

As the *Morinda* plants are quite susceptible to root knot nematode, the site selection should be done carefully in order to avoid those places. It should contain proper aerated soil with adequate drainage facilities and adequate light. Although it grows in varied agro climatic and soil conditions it does not grow well where winds are strong. The proper spacing for *Morinda* plants is 4 x 4 m. Plants of less than three years of age should be pruned after their first production of fruit. Pruning usually reduces the outbreak of pests and diseases. *Morinda* plants require only limited application of fertilizers. A fertilizer application of 10-20-20 kg NPK/ ha will be sufficient. *Morinda* plants need moderate irrigation but once they are established fully, they can withstand drought. Over watering can result in root knot nematode infestation and root rot (Nelson, 2005). *Morinda* fruits can be harvested when they change their colour from green to yellowish green. Usually these stages of fruits are suitable for shipping. For self use or local purpose, fruits can be harvested when ripe so that the juice can be squeezed easily from it. Fruits should be harvested 3 years after planting at any stage of development depending on the proposed processing method. Mostly producers prefer green fruits, where as, the processors prefer mature yellowish green fruit for processing. Noni fruits do not bruise or damage easily and need not be refrigerated.

Noni plants start flowering 8-10 months after planting. But it is suggested to remove all the flowers up to 1.5 to 2 years. This operation ensures better growth and bushy plant. Flowering and fruiting continues throughout the year. Commercial harvest starts from 20 to 24 months onwards. It yields 10 kg/plant after 24 months. It is reported that Noni plant is capable of giving yield up to 250 - 300 kg/tree under better cultivation condition after 7-8 years. Yield range may be 30 - 40 kg/plant in the initial stage. A well grown tree will produce an average of 90-100 kg / tree. It is reported that the productivity of the trees is up to 40-50 years. The harvesting can be done more than 6 to 7 times in a year

Traditional medicinal uses of Noni

\Leaves, flower and fruits are used in fever and as general tonic . Heated leaves are applied to chest to relieve cough and nausea .Juice of the leaves are used for lumbago, asthma.

The seeds are roasted and eaten and the young leaves are used as vegetables .Reddish purple dye is obtained from bark and roots and the roots give yellowish dye. Noni fruit juice is one of the food supplement in international market. Bark are used to treat eye problems, skin wounds and abscesses, gums and throat problems. Leaves, flower and fruits are used in fever and as general tonic. It is also used in respiratory ailments and constipation. Heated leaves are applied to chest to relieve cough and nausea The riped fruits are used in urinary problems while seed paste is used as shampoo.

Noni in the management of diseases

Noni juice has been scientifically confirmed to be remarkably safe, excellently efficient and highly effective in treating and also preventing a number of diseases. It is well documented that Noni is nontoxic to blood, blood elements and also to vital organs such as liver and kidney even at very high doses. Noni has a broad ranges of therapeutic effects such as analgesic, anti-inflammatory, antihypertensive, immune enhancing, anticancer, antibacterial, antiviral, antifungal, antituberculous, antiprotozoal, antioxidant, antistress and also sedative properties, Also Noni is effective in cough, nausea, colic, enlarged spleen, joint disorders such as gout and arthritis, senility, poor digestion, arthrosclerosis and drug addiction. These beneficial effects of Noni are strongly documented and well authenticated by valid scientific literature evidences. Also Noni has a strong cancer preventive effect. The various therapeutic benefits of Noni are due to the enriched phytoconstituents. The high therapeutic profile and safety potential of Noni has made it a popular health enhancer and food supplement world wide (Murugesh,2007).

R&D for Improved Crop Productivity : There are several areas of R&D that can make the cultivation/management of noni crop ,including harvest/post harvest handling, more efficient and cost-effective(Rethinam,2007).The following section illustrate some of the promising aspects of future R&D focus:

Focus on varietal productivity and quality attributes

The attributes among genotypes/land races that can maximize the fruit yield in noni should be identified so to use as baseline for further improvements through input manipulations.In the process of enhancing productivity care should be taken to keep up the quality of the produce as well.

Plant canopy development

While current fruit yield levels of noni in natural stands are around——kg per year per tree, there is need to adapt the canopy to be more compact and amenable to cultural operations including harvesting. Therefore, appropriate training and pruning strategies are to be worked out to maximize the yield, besides optimizing the crop density in cultivated fields. These practices will also have to be dovetailed to the needs of the locally promising land races/cultivars according to their natural growth habit.

Planting systems and companion cropping

The coastal soils in which noni grows well can be used for sole cropping with close or wide spaced planting arrangements. In addition, these could be considered for growing along with beneficial companion crops and/or intercrops. Depending upon their tolerance to root and light competition, the compatible crops can be grown so to diversify the income sources as well as permit polycultural options to the growers.

Way forward for R&D in Noni as a model crop

The areas of research for more productive and sustainable cultivation of noni among the smallholder and tribal communities include improvements in the genetic potential and agro-climatic adaptation of the land races/cultivars, more efficient systems of crop management including pruning, intercultivation, and organic manures besides eco-friendly crop protection practices which are also consumer-sensitive(Rethinam,2007).It is hoped that the valuable R&D contributions to this cause by the World Noni Research Foundation(WNRF) and the Centre for Organic Indian Noni (COIN) supported by the Indian Noni Cultivation Council(INCC) in promoting sustainable and profitable cultivation of noni for the cause of global wellness will be further strengthened and supported.

The close and constant involvement of the stakeholders starting with the local communities, extension/change agents, technical experts, private sector, and policy institutions is valuable to forge sustainable cultivation and conservation of this medicinal plant. There is particular opportunity for public -private partnership to play a key role in moving forward to lead to a balanced and fair play in utilizing the medicinal plants for the well being of the local communities as well as the linked industries.

Suggestions for the Future

1. Documentation of Medicinal Plants

The major source of indegenous medicines are minor forest produce, weeds as well as cultivated medicinal plants and some are known only to the traditional Vaidyas known to the family members and no documents may be available.So, there is need for documenting all these medicinal plants and their medicinal properties which will be of great use.

2. Conservation and Utilization of Medicinal Plants

Though Medicinal Board has been set up , it is still long way to promote setting up of adequate number of medicinal plants / herbal gardens in the country for conserving these medicinal plants ,most of them are endangered. This should form a part of indigenous medicinal research institutes .

3. Setting up of National Gene Banks, Conservatories nd Multilocation Resource Centres for Agro climatic Diversity

4. Community approach or Public Private Partnership in Setting up of Medicinal plants Gardens.

Medicinal Plants Conservation Areas are to be set up in different agro climatic areas in Public Private partner ship or Community mode for conserving as well as supplying nucleus Planting materials for multiplication.

5. Quality Planting Material Production

These medicinal plants have to be promoted to be grown in the farmers fields as cultivated crops by supplying adequate quantity of quality planting materials and providing technologies and also arrange for buy back system for getting fair market price.

6. Development of Improved Varieties and Technologies

Varieties and agro techniques for very important Medicinal plants are to be developed

7. Bioprospection and development of technologis

Product development, value addition and by product utilization and developing quality standards need to be looked into besides developing technologies for therapeutic, nutra ceutical, agrichemicals and health care products.

8. Validation of Medicinal Products through systematic Clinical tests for important diseases.

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Studies on spatial and temporal pattern of major pests and diseases to evolve standard sampling and monitoring protocols in a medicinal plant : Noni

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Keywords : Noni pests & diseases, visual scoring, on-farm-monitoring

Abstract : Noni (*Morinda citrifolia*) is among the important plants of medicinal value in India, presently being encouraged to be grown as farm plantations. Initial crop protection research in noni is therefore focused on evolving suitable sampling and visual severity scores as monitoring protocols for assessing the severity of the major pests and diseases.

The spatial and temporal patterns of pest /disease severity scores of the major pest/disease groups were studied at NRCN(COIN farm) ,TN, India during 2008-09.In the first study over 17 weeks, besides significant temporal variation in the scores, there was also spatial variation in the severity score between two blocks within the farm. In a further study among four blocks in the farm over three months, the occurrence of such spatio-temporal variations was confirmed, indicating the need for time-series as well as stratified sampling for such monitoring protocols. Significant overall correlation was also found in the scores for defoliating pests and leaf diseases with those of stem pest/ disease severity, besides for sucking pest with the leaf disease score. It appears that this system could be suitably adapted for routine on-farm noni pest/ disease monitoring.

In studies to standardize the sampling protocols for damage /infestation by the lacewing bug (*Dulinius conchatus*), while significant differences in the proportion of leaves with different visual rating scores were not found among the three noni ecotypes, they differed significantly for the number of lacewing bugs per leaf among leaves with particular severity score(3). Further, overall significant correlation was found between severity scores and numbers of lacewing bugs per leaf. Distinct differences in lacewing bug severity scores were also observed between the three canopy positions-top, middle and bottom.

To enable the assessment of relative efficacy of pest control options, the indicator leaf positions for sampling for lacewing bug infestation were identified as the top 3-5 leaves in seedling plants of noni .These studies have together laid the foundation for evolving appropriate protocols for the routine monitoring of pests and diseases, besides sampling to represent the overall pest infestation.

Introduction

Currently considerable interest is being shown for promoting farm level cultivation of noni (*Morinda citrifolia*), which is emerging as a promising source of nutraceuticals and so of immense use in wellness programs (Mathivanan *et al.,*2007Rethinam, 2008a,b; Rethinam and Sivaraman,2007Rethinam and Singh,2008;Singh et al.,2006; Surendran and Mathivanan,2006). Since shifting of these plants from natural stands to intensive mono cropping can render them more vulnerable to attack by pest and diseases (Nelson and Elevitch, 2006).

It is important to undertake concurrent R & D for evolving preventive and organic control practices. As such while the start up phase research on noni crop protection in India has focused on survey for insect pests and diseases in noni plantations (Jayakumar et al., 2008, 2009; Shanthakumar *et al* 2008; Ajanta Birah *et al*., 2009), concurrent initiatives have been taken up for assessing the relative severity of important sub groups of pest and diseases by visual assessments scores being developed for monitoring their relative incidence levels (Sithanantham *et al.,* 2009).The areas of focus in noni crop protection research should include bio-ecology studies on new pest associations, as in the case of the lacewing bug, which has earlier been only associated with another related species of Morinda (Mohanasundaram,1962), as already emphasized by Sithanantham(2007,2008). Since the variation in pest/ disease severity at spatial (intra farm and inter farm) level as well as the temporal pattern (seasonal variation) are the important dimensions to be covered in such routine pest monitoring systems(Jayakumar et al.2008,2009;Kannaiyan and Karthi,2008,2009;Sithanantham *et. al,*. 2008a), it was considered useful to focus the initial studies at NCRN on the spatio-temporal scenario of severity scores of the major pest/ disease sub groups on noni and the results are discussed here in, so to provide a basis for more holistic sampling system in visual scoring for monitoring at farm level.

Methodologies

Study1. Visual scoring for pest/disease severity- seasonal pattern (17 weeks; 3months)

1.1 At the NRCN, (COIN farm), weekly visual damage scores (1-5 scale) were recorded for our major pest/disease subgroups on 10 plants each in 2 blocks on a continuous spell of 17 weeks during April-August 2008.The main focus was to assess the temporal pattern of their severities and the data were analyzed by ANOVA.

1.2 Similar such seasonal study was also undertaken during a second spell of 3 months with sampling of five plants each in four blocks of the farm. The data were subjected to ANOVA.

Study2. Visual scoring- spatial pattern- (2 & 4 blocks in the farm)

2.1 The data on visual damage scoring to record the relative severities of the four subgroups of pests/diseases in the NRCN (COPIN farm) during this 17 week period was taken in 2 different blocks of the NRCN farm and the inter-block comparison was made with ANOVA.

2.2 Similar such spatial study was also undertaken during a second spell of 3 months with sampling of five plants each in four blocks of the farm. The data were subjected to ANOVA.

Study 3. Correlation studies of visual scores among 4 sub groups of pests/ diseases

The data on seasonal and spatial patterns of severity scores were also subject to correlation analysis to see if there was any association in the scores across the four sub-groups.

Study 4. Relative severity of lacewing bug damage/ infestation in three ecotypes

The three noni ecotypes growing at the farm were sampled for visual scores and to also assess the numbers of lacewing bugs per leaf. There were 10 plants per ecotype and the numbers of leaves in different visual score categories(score1-4) were counted. In addition the number of lacewing bugs per leaf in severity score 3 group were counted and compared for relative numbers per leaf. The data were analyzed by ANOVA.

Study 5. Plant canopy differences in lacewing bug damage severity.

The severity of lacewing bug damage was scored in three different canopy positions among two random branches across ten random plants. The canopy positions were compared by ANOVA.

Study 6. Selection of indicator leaf for assessing lacewing bug infestation

Noni seedlings of about 9-12 month age available in the farm (nursery block) with natural infestation of lacewing bugs were used in this study. Eighteen of them were randomly chosen and the numbers of nymphs and adults per leaf was counted in successive leaf positions from above. The data were subjected to ANOVA analysis.

Results and Discussion

1. Temporal pattern of severity scores

1.1 Seasonal pattern of damage score in 17 weeks

It was found that the visual score for 4 subgroups varied significantly over the 17 intervals studied, indicating that the temporal pattern offered substantial variations (Table 1).This result clarifies that the visual scoring system is adequately robust to capture time-series differences in severity of the four groups.

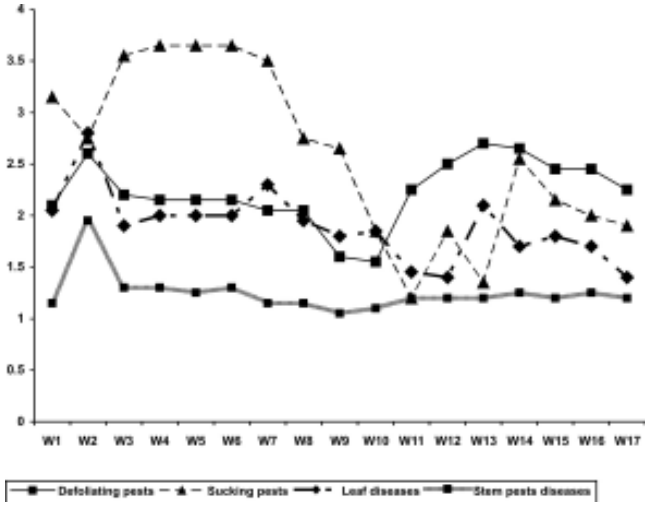
The peaks appeared to occur for defoliating pests in late July-early August, while for sucking pests it was late April to late May; for foliar diseases it appeared to be more intense during mid/late April and again in late, May compared to the other intervals; whereas for stem pests & diseases there was only one peak around Mid April only (Fig 1). These results have also provided an indication of the pattern of peaks in their severity across the common observation intervals.

Table 1 : Mean severity scores for five subgroups during 17 weeks: comparison for temporal pattern

Weeks	Defoliating pests	Sucking pests	Leaf diseases	Stem pests diseases
12.04.08	2.10 de	3.15 b	2.05 bcd	1.15 b
19.04.08	2.60 ab	2.75 c	2.80 a	1.95 a
26.04.08	2.20 cde	3.55 a	1.90 cde	1.30 b
03.05.08	2.15 cde	3.65 a	2.00 cd	1.30 b
10.05.08	2.15 cde	3.65 a	2.00 cd	1.25 b
17.05.08	2.15 cde	3.65 a	2.00 cd	1.30 b
24.05.08	2.05 e	3.50 a	2.30 b	1.15 b
31.05.08	2.05 e	2.75 c	1.95 cde	1.15 b
07.06.08	1.60 f	2.65 c	1.80 de	1.05 b
14.06.08	1.55 f	1.85 d	1.85 cde	1.10 b
21.06.08	2.25 bcde	1.20 e	1.45 fg	1.20 b
12.07.08	2.50 abc	1.85 d	1.40 g	1.20 b
19.07.08	2.70 a	1.35 e	2.10 bc	1.20 b
02.08.08	2.65 a	2.55 c	1.70 ef	1.25 b
09.08.08	2.45 abcd	2.15 d	1.80 de	1.20 b
16.08.08	2.45 abcd	2.00 d	1.70 ef	1.25 b
23.08.08	2.25 bcde	1.90 d	1.40 g	1.20 b
Significance	**	**	**	**

**- significant at p= 0.01

Fig 1: Overall pattern of severity scores for major subgroups of pests/ diseases.



The importance of seasonal pattern studies in understanding the build up and decline in pests/diseases as a group can provide a basis for preparedness ands well as for preventive strategies to limit their build up as visualized by Mathivanan(2007), Mathivanan and Sithanantham(2008),Sithanantham (2007)and Sithananthamet.al (2008a,b,2009).

1.2. Seasonal pattern of damage scores in 3 months (among 4 blocks) in farm

It was found that the months as units of observation did not appear to differ significantly, except in stem pest/disease scores (Table 2; Fig 2).Evidently, it would be useful to extend such study to more months so to provide a baseline for year round pattern of severity for each subgroup.

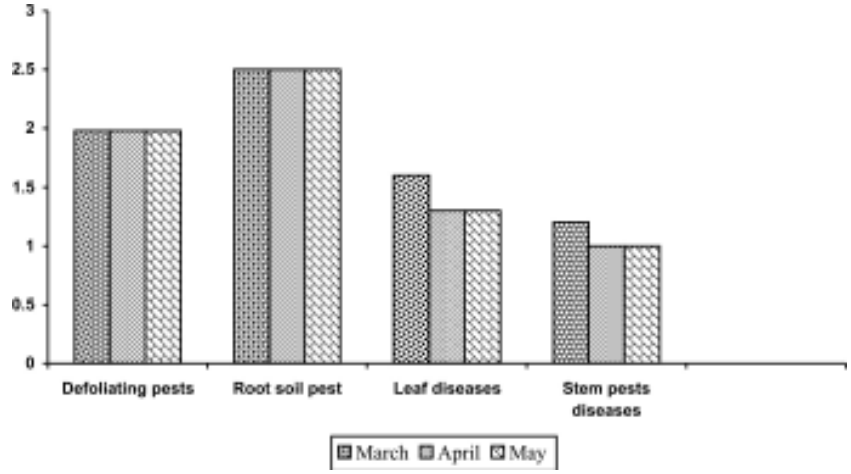
Table 2: ANOVA for comparison of 3 months for severity scores of four subgroups

Months	Defoliating pests	Root /soil-pest/disease	Leaf diseases	Stem-pests/ diseases
1	1.98	2.5	1.6	1.2[a]
2	1.98	2.5	1.3	1.0[b]
3	1.98	2.5	1.3	1.0[b]
Significance	NS	NS	NS	**

NS- Not significant ; **- significant at p= 0.01

The seasonal variation in severity scores even as monthly comparison was found to differ among the subgroups. These results tend to confirm the need for understanding the seasonal pattern of variations in severity scores, as means of quick and empirical tools for on-farm monitoring of the major pest/disease subgroups, leading to reliable prediction of their build up, as proposed by Sithanantham et al(2008).

Fig. 2 : Seasonal pattern of damage scores in 3 months for different pest/ disease groups.



2. Spatial pattern of pest/disease severity scores

2.1 Spatial patterns of damage scores in 2 blocks of the farm

The inter-block differences in severity scores between two blocks in the NRCN (COIN farm) during this period were found to be significant only for defoliating pests and not the other groups (Table 3). These results clarify that at least for this pest group is utility in continuing the spatial pattern assessments, by involving more blocks in the farm; this will provide a more holistic intra-farm scenario of the severity of these groups across the season. It is useful to extend these studies to include more blocks in the farm so to evolve a representative sampling strategy to capture the intra-farm variability in the pest/ disease severity scores. Udhaya lekha et al. (2008a) conducted trap position studies within this farm during 2007-08 and recognized the occurrence of similar intra-farm variability in catches of the adults of the defoliator insect pest-*Spodoptera* in Noni ecosystem.

Table 3 : Comparison of severity scores for two blocks in the NCRN farm, 2008

Blocks	Defoliating pests	Leaf suck pest	Leaf disease	Stem pest/ disease
IPM	2.33	2.64	1.85	1.26
DEMO	2.12	2.55	1.94	1.24
Significance	**	NS	NS	NS

NS- Not significant

**- significant at p= 0.01

2.2. Spatial pattern of damage score (4 blocks)

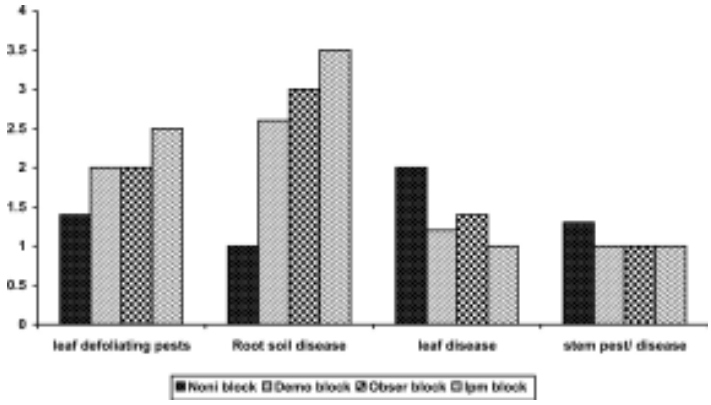
In the case of inter-block comparison (Table 4), there were significant differences between the blocks for each subgroup. Further, it was seen that the scores for root pest/ disease severity as well as defoliator damage levels appeared to be maximum in one block, with another block recording the highest mean scores for the other two subgroups The other two blocks seemed to record intermediate severity levels across the four groups. This is a clear case of spatial pattern of differences in severity at intra-farm level. Sithanantham *et. al.,* (2008) and Udhaya lekha *et. al.,* (2008) have indicated the importance of understanding intra-farm patterns in variability.

Table 4: ANOVA for severity scores in 4 blocks in the farm-4 pest/ disease groups.

Blocks	Defoliating pests	Root soil disease	leaf disease	stem pest/ disease
Noni block	1.4^{c}	1.0^{d}	2.0^{a}	1.3^{a}
Demo block	2.0^{b}	2.6^{c}	1.2^{bc}	1.0^{b}
Obser block	2.0^{b}	3.0^{b}	1.4^{b}	1.0^{b}
IPMm block	2.5^{a}	3.5^{a}	1.0^{c}	1.0^{b}
Significance	**	**	**	**

**- significant at p= 0.01

Fig. 3 Spatial pattern of damage scores in 4 blocks for different pest/ disease groups.



3. Correlation studies for severity scores of subgroups

3.1 Among data sets from 2 blocks for 17 weeks.

Correlation studies among the overall scores for the four subgroups showed significant association of defoliator damage with stem pest/diseases, besides that of sucking pests with foliar diseases and of these leaf diseases with stem pest/diseases (Table 5).Such studies on likely association can help building up suitable seasonal-cum-climatic prediction models for the target noni ecosystems, by linking these data with epidemiological and climatic parameters.

Table 5: Correlation matrix for major subgroups visual scoring means

		Defoliating pests	Sucking pests	Leaf diseases	Stem pests diseases
		V1	V2	V3	V4
IPM BLOCK	Defoliating pests	X	NS	*	**
	Sucking pests	NS	X	**	*
	Leaf diseases	*	**	X	**
	Stem pests diseases	**	*	**	X
DEMO	Defoliating pests	X	NS	NS	NS
BLOCK	Sucking pests	NS	X	**	NS
	Leaf diseases	NS	**	X	NS
	Stem pests diseases	NS	NS	NS	X
OVERALL	Defoliating pests	X	NS	NS	**
FARM	Sucking pests	NS	X	**	NS
	Leaf diseases	NS	**	X	**
	Stem pests diseases	**	NS	**	X

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

3.2.Correlation among data sets from 4 blocks for 3 months.

The extent of correlation between scores of subgroups was found to differ among individual block data, while the overall analysis brought forth some interesting trends in correlation among the subgroups (Table 6).For instance defoliating pests scores appeared to have significant positive correlation with sucking pest, foliar disease and root/soil pest/disease scores. (Table 6.5).The other subgroups also tented to show considerable correlation among them.

Never the less it is to be emphasized that these correlation studies have to be further extended to cover at least 2-3 years of continuous data collection in representative bench mark sites, and also linked to both phonological and climatic differences, so to lead to both action-based monitoring systems as well as to short term prediction leading to early warning systems development to assist the Noni farmers with advance information to undertake suitable preventive/curative interventions as indicated by Sithanantham et al(2009).As such these studies have provided useful indications and baseline characterization for planning the farm-level pest-disease monitoring systems on a sound basis, as also emphasized by Sithanantham et.al. (2008a).

Table 6.1-5. : Correlation among damage scores for individual blocks and overall.

Table 6.1 Noni block

	Defoliating Pest	Leaf suck pest	leaf disease	stem pest / disease	root/soil disease
defoliating pest	X	-	-	-	.[a]
leaf suck pest	-	X	-	-	.[a]
leaf disease	-	-	X	-	.[a]
stem pest/ disease	-	-		X	.[a]
root/soil disease	.[a]	.[a]	.[a]	.[a]	X

Table 6.2 Demo block

	Defoliating Pest	Leaf suck pest	leaf disease	stem pest / disease	root/soil disease
defoliating pest	X	.[a]	.[a]	.[a]	.[a]
leaf suck pest	.[a]	X	-	.[a]	-
leaf disease	.[a]	-	X	.[a]	-
stem pests/ disease	.[a]	.[a]	.[a]	X	.[a]
root/soil disease	.[a]	-	-	.[a]	X

Table 6.3 Observation block

	Defoliating Pest	Leaf suck pest	leaf disease	stem pest / disease	root/soil disease
Defoliating pest	X	.[a]	.[a]	.[a]	.[a]
Leaf suck pest	.[a]	X	-	.[a]	**
Leaf disease	.[a]	-	X	.[a]	-
Stem pests/ disease	.[a]	.[a]	.[a]	X	.[a]
Root/soil disease	.[a]	**	-	.[a]	X

Table 6.4: IPM block

	Defoliating Pest	Leaf suck pest	leaf disease	stem pest / disease	root/soil disease
Leaf defoliating pest	X	.[a]	.[a]	.[a]	**
Leaf suck pest	.[a]	X	.[a]	.[a]	.[a]
Leaf disease	.[a]	.[a]	X	.[a]	.[a]
Stem pests/ disease	.[a]	.[a]	.[a]	X	.[a]
Root/soil disease	**	.[a]	.[a]	.[a]	X

Table 6.5: Overall correlation (combined for 4 blocks)

	Defoliating Pest	Leaf suck pest	leaf disease	stem pest / disease	root/soil disease
Defoliating pest	X	**	**	-	**
Leaf suck pest	**	X	**	**	**
Leaf disease	**	**	X	**	**
Stem pests/ disease	-	**	**	X	**
Root/soil disease	**	**	**	**	X

a. Cannot be computed because at least one of the variables is constant.

*. Correlation is significant at the 0.05 level (2-tailed).

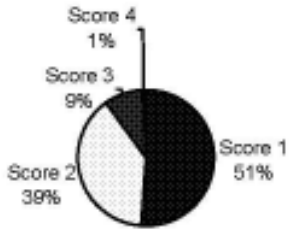
**. Correlation is significant at the 0.01 level (2-tailed).

4. Relative lacewing bug damage among three Noni ecotypes

The studies on relative proportion of different lacewing bug damage severity scores the sampled leaves in 3 Noni ecotypes showed that there was interesting trend of differential proportion of leaves showing the four different severity scores among them (Fig.4).Ecotype1 had more leaves with maximum severity score (4) than the other two ecotypes. Ecotype 3 had most leaves with light (score2) severity than the others.

Fig. 4 : Percentage of leaves with different severity scores in 3 ecotypes (4 scores)

Ecotype 1

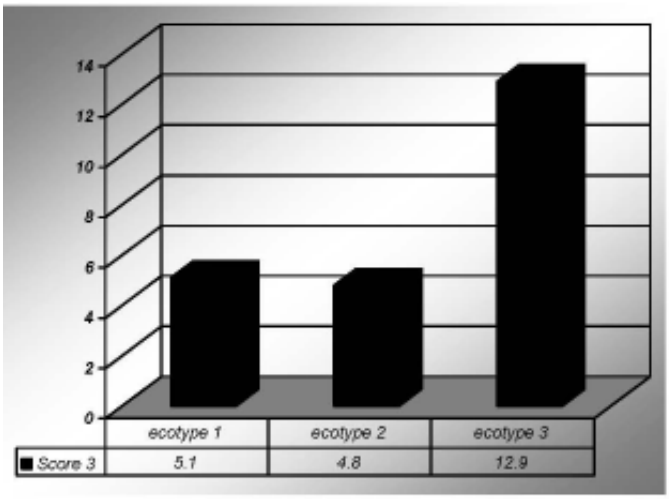


Ecotype 2

Ecotype 3

In the related assessment of pest numbers per leaf across the three ecotypes, it was found that ecotype 3 harbored significantly more numbers of lacewing bugs than the other two ecotypes (Fig.5).This study therefore brings out the infestation level as parameter complementary to damage score as the other parameter, so to better understand the nature of host plant resistance differences across the ecotypes.

Fig .5 Mean number of lacewing bugs per leaf (in score 3) among 3 Noni ecotypes

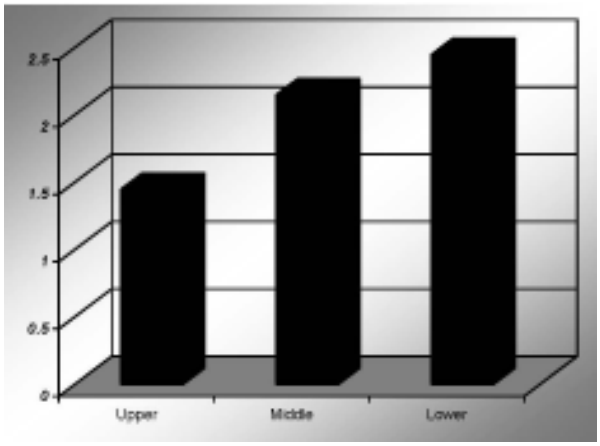


With such studies ,it is possible to identify genotypes of Noni with differential landing, survival and feeding potential of the lacewing bugs, besides distinguishing the three major mechanisms of host plant resistance- antixenosis, antibiosis and tolerance Mathivanan et al.(2008) have shown that fruit damage levels could differ among Noni ecotypes, while Sithanantham *et. al.,* (2008) mentioned the scope for such host plant factors in minimizing the crop losses due to pests, without extra investment in crop protection.

5. Relative lacewing incidence in three canopy positions

The sampling of leaf positions for assigning visual damage scores for the damage by the target pest, in the present case the lacewing bug, is required to cover different canopy positions. The differences in overall severity scores for the pest among the three canopy positions were found to be significant (Fig.6).There appeared to be a trend of decrease in severity with increase in the canopy level, the upper ones having the least severity, while the lower ones tended to have the maximum severity. Apparently the lower the canopy position, the more mature were the leaves and also the possibility of them having been associated longer with the feeding damage by the pest that those in the upper/outer canopy.

Fig. 6. Lacewing bug incidence in different canopy positions of grown up Noni plants



These results ,while confirming the need for sampling for lacewing bugs across the canopy levels, also suggest that stratified sampling to represent all three canopy levels may be appropriate for decision on timing of curative action, the lower canopy could well be adequate as sample for assessing the relative impact of different control options. These studies have not only brought out the basic information for pest/ monitoring as recommended by Sithanantham *et al*. (2008a), but also ensured the gathering of bio-ecological information on new pest associations as suggested by Sithanantham et al. (2008b).

6. Relative lacewing bug incidence in seven leaf positions

The total numbers of lacewing bugs (adults + nymphs) per leaf was found to differ significantly, so also the nymphs alone, but not the adults alone (Table 7).

Table 7. ANOVA for numbers of adults and nymphs in different leaf positions

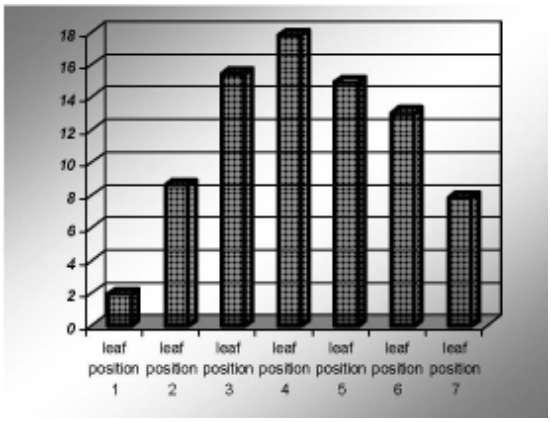
Source variable	f value	Significance	LSD(p=0.05)
Total numbers (adults+nymphs)	7.7598	**	5.55
Nymph number alone	11.2899	**	4.39
Adult number alone	1.2452 0	NS	-

NS=Not significant; **=significant at p=0.01

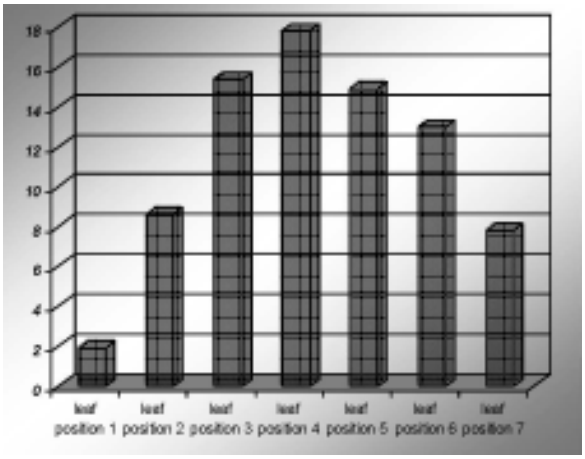
The total number of lacewings was the maximum at leaf position 4, but on par with positions 3 and 5 as well. (Fig.7).The leaf position 4 was also hosting the maximum numbers of nymphs and was on par with position 5 alone.

Fig.7 . Relative lacewing bug incidence in seven leaf positions

7.1. Total numbers (adults+nymphs) per leaf

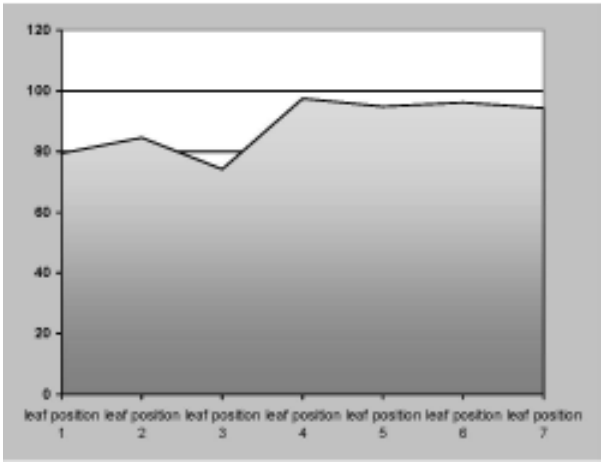


7.2.Number of nymphs alone per leaf



These results indicated that the leaf positions 4 and 5 may best represent the lacewing bug incidence, in host plant resistance and efficacy assessment of control options.

Fig. 8. Percentage of nymphs among total lacewing bugs in seven leaf positions



It is evident from these results that counting of nymphs would adequately represent the lacewing bug populations, which limits the error component as adults are often more mobile and so their relative abundance would be less dependable as an indicator of the pest build up. Further, as seen in Fig.8, the proportion of nymphs tends to be mostly above 80 percent of the total bug populations, which render their choice as realistic representatives of the overall pest numbers.

The biology studies on this pest by Dhanasekaran et al. (2007) have indicated that all the nymphal instars show linear morphometric and allometric growth pattern across the post-embryonic and adult stages. The present results add to the holisticity of our bioecology knowledge on this pest which is anew association on Noni although it has been reported as regular pest on a related species-*Morinda tinctoria (=pubescens*). (Jayakumar et al., 2008, 2009).

Conclusions and Way Forward

The present baseline studies on visual scores for monitoring the pests/ disease severity have shown that it is important to stratify the farm into adequate number of locations so to capture the spatial variations at intra farm level. In addition the need for periodical (preferably weekly) inspection to track time series changes in their severity as a basis for understanding the temporal pattern of changes in severity was also well brought out. Further the scope for understanding the possible interrelationship between the severity levels of the major subgroups through correlations over time and space has also been indicated.

Based on these results it should be possible to evolve suitable sampling strategy for capturing spatial variations in the target farms besides determining optimum intervals to track the temporal pattern. Ultimately we should evolve a farmer friendly visual

scoring system that catches such variability as well as alerts him of any impending build-up so to plan preventive interventions.

The other dimension of the present studies is to relate the sampling protocols for pests to their relative damage levels as well as their numbers per unit habitat. The scope for appropriate stratification for both between and within plant sampling for this newly associated pest-lacewing bug- is indeed a model for adopting in such new pest associations.

It is important that we further strengthen our research to widen and deepen our knowledge relating to pest monitoring and sampling for new pests in time and space, as these are of great value in evolving suitable protocols for the pest spectrum on this crop which is itself emerging as a new target for such crop protection research among such medicinally important plants in India.

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Exploitation of antibiotic producing plant growth promoting rhizobacteria and fungal antagonists for the management of foliar diseases of Noni

Keywords : Pseudomones fluorescens Bacillus subtilis, Alternaria alternate, colletotrichum gloesporioides.

Abstract : Noni is well known for its medicinal principles. Commercial cultivation of noni resulted in the outbreak of foliar diseases and fruit rot. Among all diseases, leaf blight caused by *Alternaria alternata* and anthracnose caused by *Colletotrichum gloeosporioides* play a vital role in reducing the yield. For the management of foliar diseases of noni, Plant Growth Promoting Rhizobacteria (PGPR) bestowed with antibiotic biosynthetic genes was explored for the management of the same. Twenty different isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* and the isolates of *Trichoderma viride* were screened *in vitro*. Among the different isolates of *Pseudomonas fluorescens* PF1, TDK1and MDU2 were effective in inhibiting the mycelial growth of *Alternaria alternata* and *Colletotrichum gloeosporioides in vitro*. Besides, the isolates TVI, TV2 and TV6 were effective in inhibiting the linear growth of both *A. alternata* and *C. gloeosporioides in vitro*.

Among the effective strains of *P. fluorescens* (PF1), the crude antibiotics of PF1 inhibit both the foliar pathogens under study. Genomic DNA of *P. fluorescens* (PF1) confirmed the presence of antibiotic biosynthetic genes namely phenazine and pyrrolnitrin. The isolate TDK1 confirmed presence of DAPG biosynthetic gene. HPLC analysis of the crude antibiotic confirmed presence of phenazine carboxamide and 2 hydroxy phenazine. NIST search through MASSPEC analysis also confirmed presence of phenazine. Analysis of the genomic DNA of *B. subtilis* isolate EPC 8 *confirmed* presence of the antibiotic gene iturin A (650bp), zwittermicin (950bp) and bacillomycin (400bp). The genomic DNA of EPC10 and EPC 11 showed presence of the antibiotic gene surfactin A of 300bp size. The isolates PF1, TDK1, EPC8 and TV1 were mutually compatible with each other.

The best performing isolates of *P. fluorescens* (PF1, TDK1), *B. subtilis* (EPC8, SVPR4) and *T. viride* (TV1) were formulated using vermicompost and neem cake as the career material. The shelf life of above isolates was maintained till 210 days after storage at room temperature. Studies on the effect of PGPR and *T. viride* under field conditions revealed that soil application of consortia formulated with *P. fluorescens* (PF1) + *B. subtilis* (EPC8) + *T. viride* (TV1) + *Azospirillum* using vermicompost and neem cake as pre monsoon application (August) and

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post monsoon application (January) at the rate of 5 kg/tree in the ratio of 8:1:1 of vermicompost, neemcake and biocontrol agents combined with foliar application of PF1+EPC8 at 45 days interval increased fruit yield and was effective in the management of *Alternaria* leaf blight and anthracnose of noni.

Introduction

Noni (*Morinda citrifolia* L.) is a small fruit-bearing, evergreen shrub, well known for its medicinal properties. The leaves, flowers, bark and roots on noni plants have medicinal properties (Dixon *et al*., 1999; Earle, 2001). The extracts of noni fruits can be used to cure bacterial, viral, parasitic and fungal infections by stimulating the immune system. It also prevents malignant tumors (Dixon *et al*., 1999; Earle, 2001). Noni belongs to family Rubiaceae and grown throughout the tropical countries. It is the native of Southeast Asia to Australia. The distribution of *M. citrifolia* is pan tropical. In India, it is cultivated in Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, Madhya Pradesh, Gujarat, Rajasthan and Andaman and Nicobar islands over 1850 acres. Recently, the farmers in Tamil Nadu, after realizing the commercial potential of noni, have entered into contract farming to cultivate the same. Intensive cultivation of noni has resulted in the outbreak of several diseases like leaf blight, anthracnose, black flag, fruit rot, stem blight, sooty mold, stem canker and algal leaf spot. These diseases result in considerable yield loss in terms of both quantity and quality of fruits. Foliar diseases caused by fungi can reduce leaf growth and fruit development of noni (Nelson, 2006). Hence, present study was carried out to device a suitable ecofriendly approach to manage the foliar diseases of Noni.

Materials and Methods

Isolation of the pathogens

The pathogens were isolated by tissue segment method (Rangaswami, 1958) on potato dextrose agar (PDA) medium. Sterilized leaf bits were placed in Petri plate containing potato dextrose agar (PDA) medium. The plates were incubated at 28 ± 2 °C for four days and observed for fungal growth. The fungus was purified by single spore isolation technique (Ricker and Ricker, 1936) and the purified isolates were maintained on PDA slants for further studies.

Identification of pathogens

The pathogens were identified up to species level based on their cultural and morphological characters. The pathogens associated with the diseased samples were confirmed from Fungus Identification Service, Mycology and Plant Pathology Group, Agharkar Research Institute, GG Agarkar Road, Pune, India which is an autonomous grant-in-aid institute under Department of Science and Technology, Government of

India and also from Indian Type Culture Collection and Identification, Culture Supply Services, Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India.

Molecular characterization of *A. alternata* and *C. gloeosporioides*

Detection of genus specific ITS region of *A. alternata* and *C. gloeosporioides* and sequencing

To confirm the isolates as *Alternaria* 18S rDNA specific primers ITS-F (5'GTCCTAACAAGGTTTCCGTA-3'; AJ297952) and ITS-R (5'-TTCTCC GCTTA TT G ATATGC-3'; AJ297953) were used to get 650 bp PCR amplicon of ITS region (Dong *et al*., 2002). To confirm isolates as *Colletotrichum* 18S rDNA or ITS region of C. *gloeosporioides* was amplified with Primers ITS5 (5' GGA AGT AAA AGT CGT AAC AAG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') to get 550 bp amplicon of ITS region and sequenced (Joon *et al*., 2008).

Isolation of PGPR from Rhizosphere soil

Sixteen antagonistic isolates of *B. subtilis* and twenty different isolates of *Pseudomonas fluorescens* were isolated from rhizosphere soil collected from various regions of Tamil Nadu by serial dilution technique and plated onto the Nutrient agar (NA) and King's B medium respectively. The isolates of *Bacillus* sp. and *Pseudomonas fluorescens* were identified according to the description given in Bergey's manual for Systematic Bacteriology.

Efficacy of bacterial antagonists against *A. alternata* and *C. gloeosporioides in vitro*

Nine mm mycelial discs of both pathogens were placed in the centre of Petri plate. Sterile Whatman No. 40 filter paper discs with six mm dia were placed one cm away from the edge at four sides centering surrounding the fungal discs. 25 µl of broth cultures of bacterial antagonists were dropped over the filter paper discs. Observations were taken after five days after inoculation for the presence of inhibition zone over the pathogens and nearer to the bacterial spot. Control was maintained with the sterile distilled water. The radial mycelial growth of the pathogens and per cent reduction over control was calculated by using the formula as follows

$$\text{Per cent inhibition over control} = \frac{C - T}{C} \times 100$$

Where, C- Mycelial growth of pathogen in control

T- Mycelial growth of pathogen in dual plate.

The effective isolates of *P. fluorescens* and *B. subtilis* against both the pathogens were used for further studies.

Efficacy of *Trichoderma spp* against *A. alternata* and *C. gloeosporioides in vitro*

Nine mm actively growing PDA culture discs of both *Trichoderma viride* and pathogens were placed on PDA medium 1.5 cm away from the edge of the Petri plates. On the opposite side of the medium in Petri dish, a nine mm culture disc of the fungal antagonist was placed. Three replications were maintained. PDA medium inoculated with the pathogen alone served as the control. The plates were incubated at room temperature (28 ± 2 °C). When the control plate showed full growth of the pathogen, the mycelial growth of the pathogen in plate containing fungal antagonist was measured. The results were expressed as per cent inhibition of mycelial growth over control.

Compatibility among effective bacterial isolates

PGPR isolates were tested for their compatibility by following the method described by Fukui *et al*. (1994). The compatibility was determined for *P. fluorescens* and *B. subtilis* isolate using nutrient agar medium. The bacterial isolates EPC8 and SVPR4 were streaked horizontally and isolates of *P. fluorescens*, Pf1 and TDK1 were streaked in such a way that Pf1, TDK1 were one cm apart and perpendicular to EPC8 and SVPR4. Compatibility was tested by overgrowth or inhibition of *P. fluorescens* and *B. subtilis* isolates.

Extraction of Antifungal proteins

The strains of antagonistic bacteria, *B. subtilis* and *P. fluorescens* were grown on nutrient and King's B broth respectively for 48 h at 25 °C. The broth is then centrifuged at 8000 rpm for 10 min. and culture filtrate was taken. Then 43 grams of ammonium sulphate were added to the culture filtrate and incubated for overnight. It was again centrifuged at 8000 rpm for 15 min. and pellets obtained were suspended in one ml phosphate buffer (pH 7.0). The cell extracts were centrifuged at 10,000 rpm for 10 min. at 4 °C and the supernatant was used for bioassay against pathogens.

Extraction of crude antibiotics produced by *B. subtilis* isolates

Isolates of *B. subtilis* were grown at 28 ± 2 °C in pigment production broth (PP) for five days. After incubation, they were centrifuged at 5000 rpm and the supernatant was adjusted to pH 2 with conc. HCl and it was extracted with an equal volume of benzene. The benzene layer was subjected to evaporation in water bath. After evaporation, the residues were resuspended in 0.1 N NaOH and tested for their efficacy.

Characterization of antibiotic biosynthetic genes of bacterial isolates

Detection of 2, 4-diacetylphloroglucinol (DAPG) and phenazine carboxylic acid (PCA) genes in fluorescent pseudomonads

PCR amplification was performed with 25 ìl reaction mixture which contained either approximately 20 ng of total DNA, 200 mM each dATP, dTTP, dGTP and dCTP (Genei), 20 pmol of each primer and 3 U of Ampli *Taq* DNA polymerase (Genei). The oligonucleotide primers Phl2a (GAGGACGTCGAAGACCACCA) and Phl2b (ACCGCAGCATCGTGTATGAG) developed from sequences within the biosynthetic loci for DAPG of *P. fluorescens* Pf1 were used for detection of DAPG, PCA2a (TTGCCAAGCCTCGCTCCAAC) and PCA2b (CCGCGTTGTTCCTCGTTCAT) developed from sequences within biosynthetic loci for PCA of *P. fluorescens* 2-79 were used to detect PCA (Raaijmakers *et al.*, 1997). Amplifications were performed with a thermal cycler (Eppendorf Master Cycler Gradient, Westbury, New York). The PCR program consisted of an initial denaturation at 94ºC for 2 min followed by 30 cycles of 94ºC for 60 s, 67ºC for 45 s and 72ºC for 60 s. Samples (9 ìl) of the PCR products were separated on a 1.2% agarose gel in TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3) containing 0.5 µg of ethidium bromide per ml at 75 V for 3 h. The amplified PCR products in agarose gel were visualized with a UV transilluminator and photographed using the gel documentation system (Alpha Innotech Corporation, San Leandro, California).

Detection of Iturin and Surfactin genes in *B. subtilis*

PCR amplification was performed with 25 ìl reaction mixture which contained either approximately 20 ng of total DNA, 200 mM each dATP, dTTP, dGTP and dCTP (Genei), 20 pmol of each primer and 3 U of Ampli *Taq* DNA polymerase (Genei).The oligonucleotide primers ITUD1F (GATGCGATCTCCTTGGATGT) and ITUD1R (ATCGTCATGTGCTGCTTGAG) developed for the iturin antibiotic gene and SUR3F (ACAGTATGGAGGCATGGTC) and SUR3R (TTCCGCCACTTTTTCAGTTT) developed for surfactin (Shiyi *et al.*, 2007). Amplifications were performed with a thermal cycler (Eppendorf Master Cycler Gradient, Westbury, New York). The PCR program consisted of an initial denaturation at 94 ºC for 3 min followed by 40 cycles of 94 ºC for 60 s, 60 ºC for 60 s (for iturin) and 57 ºC (for surfactin) and 72 ºC for 1 min. Samples (9 ìl) of the PCR products were separated on a 1.2 per cent agarose gel in TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8.3) containing 0.5 µg of ethidium bromide per ml at 75 V for 3 h. The amplified PCR products in agarose gel were visualized with a UV transilluminator and photographed using the gel documentation system (Alpha Innotech Corporation, San Leandro, California).

Sequencing of the Iturin and surfactin genes

Iturin and surfactin genes were amplified with the respective primers. Amplified

product was purified from each reaction mixture by agarose (1.2%, w/v) gel electrophoresis in TBE buffer containing 0.5 µg of ethidium bromide per ml. A small agarose slice containing the band of interest (observed under long-wavelength [312-nm] UV light) was excised from the gel and purified by using a QIA quick gel extraction kit (Qiagen, Inc., Chatsworth, California) according to the supplier's instructions. This purification was performed to remove primer dimers and other residues from the PCR amplification. PCR product was then subjected to sequencing. DNA sequencing was performed at Chromous Biotech Pvt. Ltd, Bangalore, India.

Sequence analysis of Iturin and Surfactin genes

The homology searches were performed using the BLAST program (Altschul *et al.*, 1990) though the internet server at the National Center for Biotechnology Information (National Institute of Health, Bethesda, USA). Sequences and accession numbers for compared genes were retrieved from the GenBank database. Sequence pair distances among related genes of the antibiotics were scored with the Clustal X (1.8) program and phylogenetic tree analysis was performed with the Treecon version 1.15. Newly obtained sequences were submitted in the GenBank database, New York, USA.

Development of formulations

a. Sterilization of carrier materials

Vermicompost was collected from the Department of Environmental Sciences, TNAU, Coimbatore and neem cake was purchased from market. Both were sterilized for three consecutive days in the autoclave at 120 psi for 30 min.

b.Preparation of bacterial and fungal suspension

A loopful of *P. fluorescens* and *B. subtilis* from 48 hours old culture were inoculated into the Kings B and nutrient broth and incubated in a rotary shaker at 150 rpm for 48 hours at room temperature (28 ± 2 °C). After 48 hours of incubation, the broth containing 9 x 10^8 cfu/ml was used for the preparation of vermicompost and neem cake based formulation. In case of *T. viride,* it was multiplied in the molasses yeast medium (30 g molasses, 5 g yeast and 1 l water) for five days.

c. Mixing bacterial and fungal suspension with carrier materials

After multiplication, the broth containing 8.5 x 10^7 cfu/ml was mixed with vermicompost and neem cake in the ratio 9:1:1. The mixture was shade dried for two days till moisture attained 20 per cent. The bioformulated product is packed in polypropylene bags and stored at room temperature.

d. Population dynamics of biocontrol agents in formulation

One gram of sample was derived from formulation and the populations (CFU) of *P. fluorescens*, *B. subtiliis* and *T. viride* were assessed at 15 days interval by serial dilution using Kings'B, Nutrient agar and *Trichoderma* selective medium, respectively.

e. Population dynamics of biocontrol agents in rhizosphere

One gram of rhizosphere sample was derived from formulation and the population (CFU) of *P. fluorescens*, *B. subtiliis* and *T. viride* were assessed at 15 days interval by serial dilution using Kings'B, Nutrient agar and *Trichoderma* selective medium, respectively.

Field Studies

Three field trials were conducted during October 2008 – March 2009 and June 2009 one at Badrapur and another at Hemmige in Karnataka and one at Hosur in Tamil Nadu to test the efficacy of the vermicompost and neem cake based bioformulations as well as liquid formulations against leaf blight disease. The trials were laid out in a randomized block design (RBD) with three replications by maintaining a plot size of 12 x 12 m^2 (eight plants / plot). The *var.* Andaman was used and the package of practices was followed as per the farmer practices and the treatments were given as described below.

T1 Soil application of vermicompost + neem cake + Pf1 in 8:1:1 ratio @ 5 Kg/ plant + foliar spray of Pf1 liquid formulation @ 0.2%

T2 Soil application of vermicompost + neem cake + EPC8 in 8:1:1 ratio @ 5 Kg/ plant + foliar spray of EPC8 liquid formulation @ 0.2%

T3 Soil application of vermicompost + neem cake + TV1 in 8:1:1 ratio @ 5 Kg/ plant + foliar spray of TV1 liquid formulation @ 0.2%

T4 Soil application of vermicompost + neem cake + (Pf1+EPC8) in 8:1:1 ratio @ 5 Kg/plant + foliar spray of Pf1 + EPC8 (1:1) liquid formulation @ 0.2%

T5 Soil application of vermicompost + neem cake + (EPC8+TV1) in 8:1:1 ratio @ 5 Kg / plant + foliar spray of EPC8 + TV1 (1:1) liquid formulation @ 0.2%

T6 Soil application of vermicompost + neem cake + (Pf1+TV1) in 8:1:1 ratio @ 5 Kg/plant + foliar spray of Pf1 + TV1 (1:1) liquid formulation @ 0.2%

T7 Soil application of vermicompost + neem cake + (Pf1+EPC8+TV1) in 8:1:1 ratio @ 5 Kg/plant + foliar spray of Pf1 + EPC8 + TV1 (1:1) liquid formulation @ 0.2%

T8 Foliar spray of Chlorothalonil @ 0.2%

T9 Control

Results and Discussion

Bioassay of crude antibiotics of *B. subtilis* by paper disc method against *A. alternata* and *C. gloeosporioides*

A. alternata

The crude antibiotics of *B. subtilis* isolates EPC8, SVPR4, SVPR2 and TMV2 were tested for their antifungal action against *A. alternata*. The crude antibiotic isolated from *B. subtilis* isolate EPC8 recorded the maximum per cent inhibition of mycelial growth (55.55 %) of pathogen at 100 ml concentration over untreated control, while other isolates of *B. subtilis* like SVPR4, SVPR2 and TMV2 showed 50.00, 42.22, 35.55 per cent inhibition over control with mycelial growth of 45.00 mm, 52.00 mm and 58.00 mm, respectively [Table 1]. The present studies agreed well with findings of Menaka (2006) who confirmed crude antibiotics of *B. subtilis* isolate BSM1 highly effective against *A. alternata*. Vasudeva and Chakravarthi (1954) reported that an antibiotic bulbiformin produced by *B. subtilis* inhibited the spore germination of *Alternaria spp*. by 63 per cent. Thus various studies clearly explained that the minimal inhibitory concentration of various antimicrobial metabolites vary in their antifungal action.

C. gloeosporioides

Crude antibiotic of SVPR4 significantly exerted the maximum inhibition of 57.77 per cent on the mycelial growth of the pathogen. This was followed by SVPR2, EPC8, and TMV2 recording 44.50 mm, 46.00 mm and 50.00 mm colony diameter which were 50.55, 48.88 and 44.44 per cent inhibition over control respectively [Table 1]. The results are in line with Mathiyazhagan *et al*. (2004) who reported that suppression of the mycelial growth of *C. cassicola* by *B. subtilis* isolate BSCBE4 and *P. fluorescens* isolate Pf1 is due to the production of antibiotics.

Table 1. *In vitro* bioassay of antibiotics of effective *B. subtilis* isolates against mycelial growth of *A. alternata* and *C. gloeosporioides*

Isolates	*A. alternata*		*C. gloeosporioides*	
	Mycelial growth (mm)*	% Inhibition over control	Mycelial growth (mm)*	% Inhibition over control
EPC8	40.00 [a]	55.55	46.00 [b]	48.88
SVPR4	45.00 [b]	50.00	38.00 [a]	57.77
TMV2	58.00 [c]	35.55	50.00 [d]	44.44
SVPR2	52.00 [d]	42.22	44.50 [c]	50.55
Control	90.00 [e]	-	90.00 [e]	-

* Mean of four replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

In vitro bioassay of antifungal proteins of effective *B. subtilis* isolates against mycelial growth of *A. alternata* and *C. gloeosporioides*

A. alternata

The cell proteins of *B. subtilis* isolates EPC8, SVPR4, SVPR2 and TMV2 were tested for their antifungal action against *A. alternata.* The cell protein extracts isolated from *B. subtilis* isolate EPC8 recorded a maximum of 66.50 per cent inhibition over untreated control. It was followed by SVPR4, TMV2 and SVPR2 showing 62.05, 46.14 and 28.35 per cent inhibition over control, with a mycelial growth of 34.15, 48.47 and 64.48 mm respectively. Control plate showed 90 mm mean mycelial growth of the pathogen [Table 2]. Similar results of efficacy of cell proteins in inhibiting mycelial growth of *P. aphanidermatum* were reported by Kavita (2004). This was further confirmed by Menaka (2006) that the cell proteins of *B. subtilis* isolates inhibited the mycelial growth of *A. alternata*.

C. gloeosporioides

SVPR4 significantly exerted the maximum inhibition of 61.27 per cent on the mycelial growth of the pathogen. This was followed by EPC8, SVPR2, and TMV2 recording 42.50 mm, 48.00 mm and 58.10 mm colony diameter which were 52.77, 46.66 and 35.44 per cent inhibition over control, respectively [Table 2]. Similar results were obtained by Nandinidevi (2008).

Table 2. *In vitro* bioassay of cell proteins of effective *B. subtilis* isolates against mycelial growth of *A. alternata* and *C. gloeosporioides*

Isolates	*A. alternata*		*C. gloeosporioides*	
	Mycelial growth (mm)*	% Inhibition over control	Mycelial growth (mm)*	% Inhibition over control
EPC8	30.15 [a]	66.50	42.50[b]	52.77
SVPR4	34.15 [b]	62.05	34.85 [a]	61.27
TMV2	48.47 [c]	46.14	58.10 [d]	35.44
SVPR2	64.48 [d]	28.35	48.00 [c]	46.66
Control	90.00 [e]	-	90.00 [e]	-

* Mean of four replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Compatibility studies

Compatibility among the effective bacterial antagonists

Several authors suggested the combination of introduced biocontrol agents have to be compatible to establish better and more consistent disease suppression (Baker, 1990). In our study, effective isolates of *P. fluorescens* (Pf1 and TDK1) and *B. subtilis* (EPC8 and SVPR4) were tested for their compatibility under *in vitro*. The results indicated that none of antagonistic bacterial isolates were inhibited by each other suggesting that the four biocontrol agents were compatible with each other [Table 3].Similarly; Lata (2009) reported the compatibility among the effective bioagents.

Table 3.Compatibility among the effective bacterial and fungal antagonists

	EPC8	SVPR4	TDK1	TV1
Pf1	+	+	+	+
TDK1	+	+	+	+
TV1	+	+	+	+

+ Compatible - Incompatible

Compatibility between the effective bacterial and fungal antagonists

Isolates of *P. fluorescens* (Pf1 and TDK1), *B. subtilis* (EPC8 and SVPR4) and fungal antagonist TV1 were tested for their compatibility under *in vitro*. The mycelial growth rate of *T. viride* isolate, TV1 in the presence of bacterial antagonist was compared with the control plate. Results indicated that the growth rate and mean mycelial diameter of the TV1 were not affected by presence of bacterial antagonists and in turn the bacterial growth was also not affected by presence of TV1 isolate, indicating compatibility between bacterial and fungal antagonists tested.

Detection of antibiotic biosynthetic genes in *Pseudomonas fluorescens* (Pf1) : Among the various isolates, isolate Pf 1 and TDK1 were effective both under *in vitro* and *in vivo*. Detection for presence of antibiotic biosynthetic genes revealed presence of phenazine and pyrrolnitrin in PF1. The gene DAPG was associated with TDK1 (Plate 1, 2, 3). The results are in confirmatory with the findings of Saveetha (2008).The biosynthetic genes present in the *Pseudomonas fluorescens* exhibit a wide spectrum of inhibitory action against the pathogens.

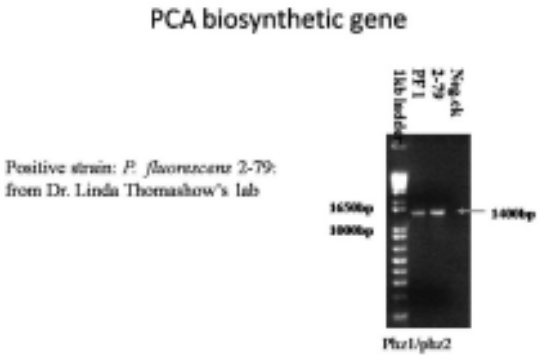

Plate 1: Presence of Phenazine carboxyclic acid gene in PF1

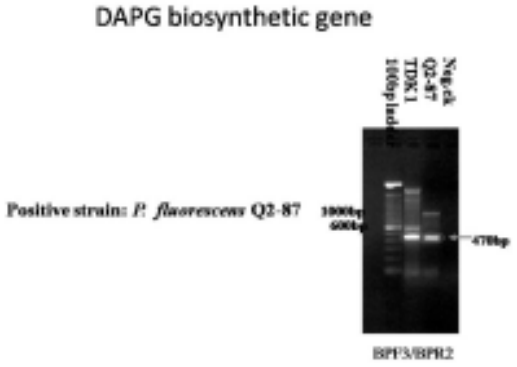

Plate 2: Presence of DAPG in TDK1

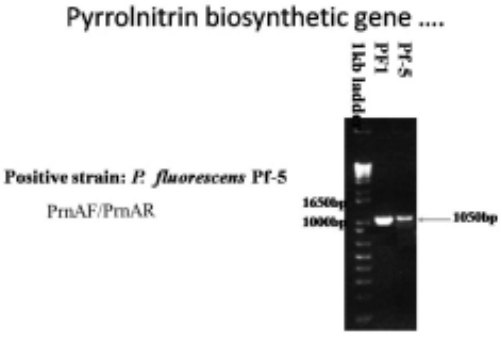

Plate 3: Presence of pyrrolnitrin in PF1

Detection of antibiotic biosynthetic genes in Bacillus subtilis (EPC8)

Among the various isolates, isolate EPC8 was effective both under in vitro and *in vivo*. Detection for the presence of antibiotic biosynthetic genes revealed presence of Iturin and surfactin in EPC8 (Plate 4 and 5). Similar results for the presence of antibiotic genes was reported earlier by Shiyi et al. (2003) and Sarangi *et al.* (2009). Antifungal nature of *Bacillus* isolates is attributed to presence of Iturin and surfactin genes.

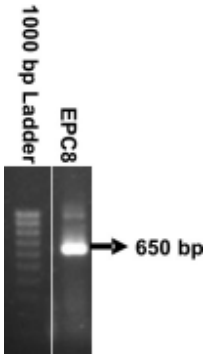

Plate 4: Presence of Iturin in EPC8

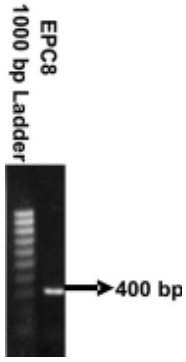

Plate 5: Presence of surfactin in EPC8

Population dynamics of effective bioagents on vermicompost and neem cake based bioformulations under *in vitro* condition

The study was conducted to know the multiplication rate of antagonistic bacteria and fungal antagonist on vermicompost and neem cake mixture. Besides, the study also aimed at standardizing the shelf life of the bioagents in the formulation comprising of vermicompost and neem cake mixture. Vermicompost, neem cake and bioagents were mixed in the ratio 8:1:1 and samples were drawn periodically and population density was recorded. The results clearly stated that *P. fluorescens* isolates (Pf1 and TDK1) started to multiply in the mixture and increased up to 120 days. After 120 days, the population started to decline. *B. subtilis* isolates (EPC8 and SVPR4) increased their population up to 150 days and maintained constant population up to 180 days and thereafter started to decline. On the other hand, the fungal antagonist *T. viride* increased population up to 150 days and reached to a load of $185x10^6$ cfu/g of the product and declined thereafter.

Hence, from the results, it can be concluded that the shelf life of *P. fluorescens*, *B. subtiliis* isolates and *T. viride* were maintained up to seven months after storage under room temperature [Table 4].

Table 4. Population dynamics of effective bioagents on vermicompost and neem cake mixture as carrier material

Antagonists	CFU x 107 at different days after storage*							
	0	30	60	90	120	150	180	210
Pf1	121.35	140.68	153.00	164.31	166.21	143.8	83.00	20.64
TDK1	110.56	138.30	148.34	153.80	158.80	138.40	76.40	14.50
EPC8	140.23	152.68	168.75	187.47	193.00	199.54	156.00	84.40
SVPR4	138.67	153.00	181.24	193.54	196.40	198.75	198.00	58.40
TV1	153.00	193.75	207.14	211.4	216.14	224.14	185.00	143.4

*Mean of three replications

Field experiments

Effect of different bioformulations on incidence of leaf blight and anthracnose diseases in noni under field conditions.

Leaf blight

Management of plant diseases by different biocontrol agents either suspension or through different formulations were reported by many workers (Kloepper and Schroth, 1981; Radjacommare *et al.,* 2002). In the present study, field trial on the management of noni leaf blight was initiated during 2009-2010 from August 2009 at Denkanikottai, Hosur. The treatment schedule comprising of the following treatments was given at 45 days interval. Soil application of vermicompost, neen cake and biocontrol agents were delivered to soil @ 5kg/tree in the ratio of 8:1:1. The soil application of vermicompost and neem cake was given only once. But, soil application of biocontrol agents was continued once in 45 days @ 500g/tree.

Among various treatments, soil application of VC+NC+PF1+ EPC8+TV1+AZO (8:1:1) combined with foliar application of PF1(5g/L)+ EPC8(5g/L) recorded 14.23PDI as against 56.35 PDI in control. It was followed by application of VC+NC+PF1+ EPC8+TV1 combined with FS of PF1+ EPC8 (17.58 PDI). Thus the experimental evidences prove that combined application of Pseudomonas, Bacillus through foliar spray combined with soil application of the same resulted in excellent control of the disease under field condition (Table 5). Foliar application with liquid formulation of P. fluorescens (Pf1) significantly reduced the incidence of leaf blight caused by Alternaria solani under glass house and field conditions (Manikandan, 2008). Similarly *P. fluorescens* strain Pf1 and TDK1 and B. subtilis were found effective against several plant pathogens (Vidhyasekaran and Muthamilan, 1995).

Table 5. Effect of different bioformulations on incidence of noni leaf blight incited by *Alternaria alternata* under field conditions – (2009-2010)

S. No.	Treatments	Spray I		Spray II		Spray III		Spray IV	
		PDI	PROC	PDI	PROC	PDI	PROC	PDI	PROC
1	VC+NC+PF1 combined with FS of PF1	25.25[f] (30.15)	57.80	24.87[f] (29.91)	57.39	22.96[f] (28.63)	60.50	20.97[f] (27.25)	62.79
2	VC+NC+BS (EPC8) combined with FS of BS (EPC8)	28.12[g] (32.02)	52.97	27.89[g] (31.88)	52.21	23.68[g] (29.12)	59.26	21.68[g] (27.75)	61.53
3	VC+NC+TV1 combined with FS of PF1	26.76[f] (31.15)	55.24	26.15[f] (30.76)	55.19	24.31[f] (29.54)	58.17	22.51[f] (28.32)	60.05
4	VC+NC+TV1 combined with FS of EPC8	27.16[f] (31.41)	54.57	25.95[f] (30.63)	55.53	23.81[f] (29.21)	59.03	23.00[f] (28.66)	59.18
5	VC+NC+PF1+ TV1 combined with FS of PF1+ EPC8	23.31[b] (28.87)	61.01	22.19[b] (28.10)	61.98	20.69[c] (27.06)	64.40	18.37[c] (25.38)	67.40
6	VC+NC+PF1+ EPC8+TV1 combined with FS of PF1+ EPC8	23.35[c] (28.90)	60.95	23.09[c] (28.72)	60.44	19.39[b] (26.13)	66.64	17.58[b] (24.79)	68.80
7	VC+NC+PF1+ EPC8+TV1+AZO combined with FS of PF1+ EPC8	21.24[a] (27.44)	64.48	20.21[a] (26.71)	65.37	16.54[a] (24.00)	71.54	14.23[a] (22.16)	74.75
8	Control	59.79[g] (50.64)	-	58.36[g] (49.81)	-	58.12[g] (49.67)	-	56.35[g] (48.65)	-
9	Farmers practice	38.56[d] (38.38)	35.51	32.54[d] (34.65)	44.59	29.28[d] (32.76)	49.62	28.36[d] (32.18)	49.67

PDI = Percent Disease Index, PROC= Percent reduction over control

* Mean of three replications
Value in the parenthesis are Arcsine transformed.

In a column mean followed by a common letter are not significantly different at the 5% level by DMRT.

Anthracnose

A field trial on the management of noni anthracnose with biocontrol agents like *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* was done through both soil and foliar application of the same either as individual organism or through combined application of the compatible strains. The trial was laid out at Denkannikottai, near Hosur. Among various treatments, soil application of VC+NC+PF1+ EPC8+TV1+AZO (8:1:1) combined with foliar application of PF1(5g/L)+ EPC8(5g/L) recorded 17.00 PDI as against 39.0 PDI in control. It was followed by the application of VC+NC+PF1+ EPC8+TV1 combined with FS of PF1+ EPC8 (20.00 PDI). Thus the experimental evidences prove that combined application of Pseudomonas, Bacillus through foliar spray combined with soil application of the same resulted in the excellent control of the disease under field condition (Table 6).

The results are in confirmatory with the findings of Sible George (2003) and Radjacommare (2002) who reported the combined application of bioproducts was effective against the diseases.

Table 6. Effect of different bioformulations on incidence of noni anthracnose under field conditions – (2009-2010)

S. No.	Treatments	Spray I		Spray II		Spray III		Spray IV	
		*PDI at 45 days after spraying	Percent reduction over control	PDI at 90days after spraying	Percent reduction over control	PDI at 135 days after spraying	Percent reduction over control	PDI at 180 days after spraying	Percent reduction over control
1	VC+NC+PF1 combined with FS of PF1	34.00^{c} (35.67)	19.62	36.00^{c} (36.87)	25.00	31.00^{c} (33.83)	24.39	24.00^{c} (29.33)	38.46
2	VC+NC+BS (EPC8) combined with FS of BS (EPC8)	31.26^{c} (34.00)	26.10	32.00^{c} (34.45)	33.34	28.00^{c} (31.95)	31.71	25.00^{c} (28.66)	41.03
3	VC+NC+TV1 combined with FS of PF1	34.00^{c} (35.67)	19.62	30.00^{c} (33.21)	37.50	30.00^{c} (33.21)	26.83	26.00^{c} (30.66)	33.34
4	VC+NC+TV1 combined with FS of EPC8	30.00^{c} (33.21)	29.08	28.00^{c} (31.95)	41.67	29.00^{c} (32.58)	29.27	24.00^{c} (29.33)	38.46
5	VC+NC+PF1+ TV1 combined with FS of PF1+ EPC8	30.00^{c} (33.21)	29.08	31.00^{c} (33.83)	35.42	28.00^{c} (31.95)	31.71	22.00^{c} (27.97)	43.59
6	VC+NC+PF1+ EPC8+TV1 combined with FS of PF1+ EPC8	28.00^{c} (31.95)	33.81	28.00^{c} (31.95)	41.67	25.00^{c} (30.00)	39.02	20.00^{c} (26.57)	48.72
7	VC+NC+PF1+ EPC8+TV1+AZO combined with FS of PF1+ EPC8	24.00^{c} (29.33)	43.26	27.00^{c} (31.31)	43.75	23.00^{c} (28.66)	43.90	17.00^{c} (24.35)	56.41
8	Control	42.30^{a} (40.57)	-	48.00^{a} (43.86)	-	41.00^{a} (39.82)	-	39.00^{a} (38.65)	-
9	Farmers practice	38.26^{b} (38.21)	9.55	44.00^{b} (41.56)	8.34	36.00^{b} (36.87)	12.20	32.00^{b} (34.45)	17.95

PDI = Percent Disease Index

* Mean of three replications

Values in the parenthesis are Arcsine transformed.

In a column mean followed by a common letter are not significantly different at the 5% level by DMRT.

Table 7. Population dynamics of biocontrol agents in the rhizosphere soil of Noni (2009-2010)

Treatments	0 day			45 days			90 days			135 days		
	CFU x 10^7		CFU x 10^6	CFU x 10^7		CFU x 10^6	CFU x 10^7		CFU x 10^6	CFU x 10^7		CFU x 10^6
	PF	BS	TV	PF	BS	TV	PF	BS	TV	PF	BS	TV
VC+NC+PF1 combined with FS of PF1	16	22	11	29	27	12	43	52	19	52	34	21
VC+NC+BS (EPCS) combined with FS of BS (EPCS)	17	19	6	26	45	13	31	49	17	34	62	17
VC+NC+TV1 combined with FS of PF1	16	16	9	26	31	24	32	34	19	52	40	37
VC+NC +PF1 +TV1 combined with FS of EPCS+PF1	14	18	11	24	29	16	24	27	12	30	31	23
VC+NC +PF1+EPCS+ TV1 combined with FS of EPCS+PF1	16	18	8	23	27	15	22	21	14	37	32	19
VC+NC+PF1+EPCS+TV1+AZO combined with FS of EPCS+PF1	12	21	7	27	27	19	21	24	17	34	31	14
CONTROL	13	16	7	17	18	11	17	19	9	28	20	13

Table 8. Population dynamics of biocontrol agents in the non rhizosphere soil of noni (2009-2010)

Treatments	0 day			45 days			90 days			135 days		
	CFU x 10^5		CFU x 10^4	CFU x 10^5		CFU x 10^4	CFU x 10^5		CFU x 10^4	CFU x 10^5		CFU x 10^4
	PF	BS	TV	PF	BS	TV	PF	BS	TV	PF	BS	TV
VC+NC+PF1 combined with FS of PF1	12	15	8	31	20	8	26	20	14	34	29	15
VC+NC+BS (EPCS) combined with FS of BS (EPCS)	12	19	4	20	40	12	24	30	13	23	31	13
VC+NC+TV1 combined with FS of PF1	14	11	12	22	15	24	21	18	21	31	24	19
VC+NC +PF1+TV1 combined with FS of EPCS+PF1	11	12	9	20	21	14	21	21	14	21	20	12
VC+NC +PF1+EPCS+ TV1 combined with FS of EPCS+PF1	13	14	11	21	23	12	20	14	9	20	17	14
VC+NC+PF1+EPCS+TV1+AZO combined with FS of EPCS+PF1	14	15	10	20	15	9	13	19	11	22	27	12
Control	10	12	4	11	12	4	13	12	6	17	14	11

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Isolation of Chemical Constituents from *Morinda tinctoria* bark and Evaluation of Anti-inflammatory activity

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Keywords : *Morinda tinctoria*; isolation; anti-inflammatory

Abstract : The ethanolic extract of *Morinda tinctoria* bark was used for the isolation of chemical constituents and evaluation of anti-inflammatory activity. To identify the anti-inflammatory component of this drug, an activity directed fractionation approach was adopted. The active fraction of the ethanolic extract of *Morinda tinctoria* bark was subjected to silica gel and column chromatography to yield a single compound and identified as anthraquinone glycosides. The anthraquinone glycoside 1-hydroxy-2-methyl-3-methoxy-9,10-anthraquinone and ethanolic extract were tested for anti-inflammatory effect by using carrageenan induced paw oedema served as acute models and formation granulation tissues by cotton pellets served as a chronic model in rats. The effect was compared with Indomethacin used as standard drug. Pretreatment with ethanolic extract of *Morinda tinctoria* bark (MTB) was administered orally 200, 400mg/kg(p.o) and isolated compound (5mg/kg) exhibited significant anti-inflammatory activity in acute and chronic models. These results indicate that ethanolic extract and 1-hydroxy-2-methyl-3-methoxy-9,10-anthraquinone contribute to the anti-inflammatory action of *Morinda tinctoria* bark.

Introduction

Morinda tinctoria belongs to family Rubiaceae, grows widly and is distributed throughout Southeast Asia. Commercially known as Nunaa, it is indigenous to tropical countries and is considered an important folk medicine. In the traditional system of medicine, leaves and root of *M. tinctoria* are used as anti-convulsant, astringent, deobstrent, and to relieve pain in the gout (Thirupathy kumaresan *et al.,* 2009). It was reported to have a broad range of therapeutic and nutritional values (Levand *et al.,* 1979). The major components identified in the Nunaa plant include octoaniacid, potassium, vitamin C, terpenoids, scopoletin, morindone, rubiatin and alizarin (Moorthy *et al.,* 1970, Singh *et al.,* 1976). The current study was undertaken to isolate the anthraquinone glycoside and get it examined by anti-inflammatory activity of ethanolic extract of *M. tinctoria* bark by carrageenan-induced paw odema and cotton pellet granuloma model.

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Materials and Methods

Instruments : Melting points were determined on a scientific model of melting point apparatus. A UV spectrum was recorded on a Double Beam UV-VIS Spectrophotometer and IR spectra on a Shimadzu 8900 FT-IR spectrometer. The NMR spectra (äppm, *j*, in Hz) were recorded in $CDCL_3$ using AM-500 spectrometer (500MHz) with tetra methyl silane (TMS) as an internal standard. A mass spectrum was recorded on QTOF micro mass UK electron spray Ionization mass spectrometer. Sample purity was checked by TLC (silica gel, precoated plates Merck, PF_{254} 10×5 cm, 0.25mm). The biochemical parameters of anti-inflammatory the rat paw odema were estimated by Plethysmometer.

Plant materials : The *M. tinctoria* stem bark (1.2kg) was collected in July 2009, from Kottapatty, Dindigul District, Tamil Nadu, India. The plant material was taxonomically identified by Dr.D.Stephen, Department of Botany, The American College, Madurai, Tamil Nadu, India. The bark of the plant materials was dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no 40 and stored in an airtight container for further use.

Preparation of extract: The dried powdered barks (1kg) were extracted with 80% ethanol (2.5 liter) in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semisolid mass was obtained (yield 14.2%). The ethanolic extract was concentrated to dryness in *vaccuo* at 35°C. Active constituents from the dried extract were separated by column chromatography with different solvent ratio.

Preliminary Chemical Test : The extract was subjected to preliminary screening for various active phytochemical constituents like alkaloids, carbohydrates, steroids, glycosides, protein, phenols, flavonoids and saponins.

Isolation : Dried barks (1kg dry wt) of *M. tinctoria* were extracted with 80% ethanol in Soxhlet apparatus after extraction solvent was distilled and concentrated to dryness in *vaccuo* at 35°C, with the concentrate (20g) was then partitioned between water and ethyl acetate with the aqueous layer was basified with ammonium hydroxide (pH 9) and extracted repeatedly with chloroform. The combined chloroform layer was concentrated to dryness in *vaccuo* with the concentrate (12g) separated by column chromatography in to various fractions of $CHCl_3$–MeOH(95:5, 90:10, 85:15) increasing polarity gradient elution. The $CHCl_3$–MeOH (85:15) fraction was concentrated to dryness in *vaccuo* at 35°C. The combined fractions were identified by TLC (10% Ethanolic KOH reagent) and recrystallized with methanol. These compounds were identified as anthraquinone glycoside respectively by chemical test (Borntrager's test) and spectral data.

Vehicle: The extract and isolated compound at different doses of 200, 400mg/kg and 5mg/kg suspended in aqueous Tween 80 solution (1%) and Indomethacin (10mg/kg Torrent, Bombay) in saline were used for the present study.

Animals and sample preparation : Adult Wister albino rats of either sex weighing between 120-150g were purchased from Experimental animal house Madurai, Tamil Nadu, India. Animals were housed at a temperature 25±2°C and relative humidity of 45-55% a 12:12 light: day cycle was followed. All animals were fed with standard pellet diet supplied by Amrut-rat mice feed, Mumbai. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment Government of India. The Animal Ethical Committee experimental protocol No. is UCP/IAEC/2009/050.

The test samples (*i.e.* EtOH extract and isolated compound) were first dissolved in 10% Tween 80 and diluted with 1% saline before being orally administered. The same volume of solvent was administered to control rats. Extracts were administered at 200, 400mg/kg once a day week and isolated compound also given by orally administered at 5mg/kg for the same period.

Acute Toxicity Studies: Acute toxicity study was performed for the extracts ascertain safe dose by acute oral toxic class method of organization of Economic Co-operation and Development as per 423 guidelines (OECD) 12. A single administration of starting dose of 2000mg/kg body weight/p.o of the MTB was administered to 3 female mice and observed for 3 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. When the experiment was repeated again with same dose level 2000mg/kg body weight/p.o of the MTB for 7 more days and observed for fourteen days, no change was observed from the experiments.

Anti-inflammatory activity

Carrageenan-Induced Paw Oedema: The rats were divided into 5 groups (n=3). The extract, isolated compound and standard drug used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 hours before the experiment. They were marked and numbered for identification. Paw oedema was induced by sub plantar injection into the rat right hind paw of 0.1ml sterile saline containing 1% carrageenan (control group). A group of rats were treated with MTB extract, isolated compound and standard drugs administered orally concomitantly with carrageenan injection. Control group of animals received the same volume of vehicle instead of the tested agents. The volume of the paw was measured by a Plethysmometer immediately after the injection as previously described. The increase in paw volume was taken as oedema volume. The percentage of inhibition of inflammation was calculated for comparison. The ratio of the anti-inflammatory effect of MTB extract and isolated compound was calculated by following equation anti-inflammatory activity (%) (1-D/C)× 100, where D represents the percentage difference in paw volume after extract and compound was administered to the rats, and C represents the percentage difference of volume in the control groups.

Cotton Pellet Granuloma Model : In cotton pellet, the animals were divided into five groups as described in the carrageenan induced paw oedema model. The animals were anaesthetized with Diethyl ether. The back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with Indomethacin, extract and isolated compound of *Morinda tinctoria* for 7 days. Then the pellets were dissected out and dried until the weight remains constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

Statistical Analysis : All the results were expressed as mean ± standard error (S.E.M). Data were analyzed using one-way ANOVA followed by Dunnett's *t*-test. $p<0.05$ was considered as statistically significant.

Results

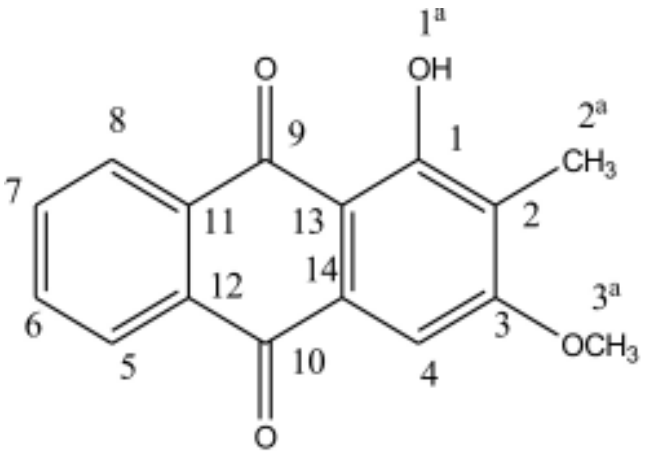
Preliminary Chemical Tests : The extract showed positive test for carbohydrates, glycosides, phenols and steroids.

Isolation of Anthraquinone Glycosides : The isolated compound a brownish yellow powder, is an anthraquinone glycosides (proved by Borntrager's chemical test), mp 238-240°C; UV ë$_{max}$ MeOH nm (log å): 248(2.1); 306(3.4); IR ë$_{max}$ (KBr) cm^{-1} 3424,3020,2928,1714,1594,1461,1276 and EI-MS *m/z* (rel.int.%) 268 (32,M^{+}), 236(14), 222(30) (calcd. For $C_{16}H_{12}O_4$). ^{1}H-NMR and ^{13}C-NMR see the Table-1.

Table 1. ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$, ^{1}H: 300 MHz; ^{13}C: 75MHz) spectral data of isolated compound.

C/H#	ä$_H$ (MHz)	ä$_C$	HMBC Correlations
1	-	163.5	-
2	-	124.0	C-2
3	-	161.8	-
4	7.33 (s)	120.8	C-3, C-14
5	7.26 (d)	128.1	C-6, C-11
6	7.08 (d)	119.8	-
7	7.12 (d)	120.0	C-6, C-8
8	7.28 (d)	127.9	C-7, C-12
9	-	176.8	-
10	-	182.6	-

11	-	136.1	-	
12	-	143.0	-	
13	-	139.3	-	
14	-	141.9	-	
1[a]	9.25 (s)	-	-	
2[a]	2.19 (s)	16.9		C-1
3[a]	3.51 (s)	60.3		C-3



1-hydroxy-2-methyl-3-methoxy-9,10-anthraquinone

Anti-inflammatory activity

Carrageenan-Induced Paw Oedema:The anti-inflammatory effect of the MTB extract and isolated compound and standard on the carrageenan-induced hind paw oedema mode are shown in Fig. 1. Standard drug Indomethacin (10mg/kg,p.o) produced a significant reduction in paw oedema volume (69.44%). Treatment with MTB extract, an isolated compound reduced the carrageenan-induced paw oedema volume in a dose dependant manner. MTB extract and isolated compound showed maximum ($p<0.01$) inhibition of 59.5%, and 47.2% at the dose of 400mg/kg and 5mg/kg after 4 h of treatment of carrageenan-induced paw oedema. Similarly, the inhibition was 55.5% ($p<0.01$) at the dose of 200mg/kg,p.o in pretreated rats.

Cotton Pellet Granuloma Model: The effect of MTB extract and isolated compound on cotton pellet induced granuloma in rats are shown Fig.2. In this, the mean weight of cotton pellets was determiner treatment with the MTB extract and isolated compound significantly decreased the granuloma weight 36.8%, 44.6% and 35.4% at the dose level of 200, 400mg/kg and 5mg/kg,p.o respectively. Similarly, 53.3% decrease was found in Indomethacin (10mg/kg,p.o) treated rats.

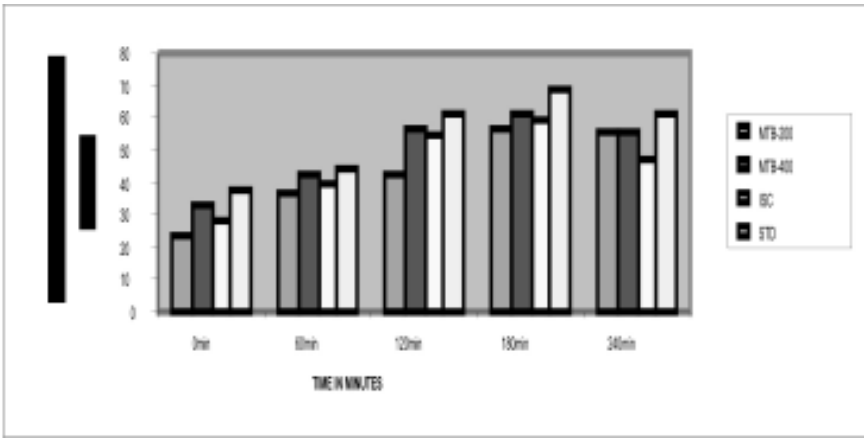


Fig. 1. Effect of ethanolic extract and isolated compound of *Morinda tinctoria* on Carrageenan induced paw oedema in rats.

Table 2. Effect of ethanolic extract and isolated compound of *Morinda tinctoria* on Carrageenan induced paw oedema in rats.

Groups	Dose	paw oedema (mm)			Inhibition (%)	
		0 hr	2 h	4 h	2 h	4h
Vehicle		2.2 ± 0.01	4.0 ± 0.02	3.6 ± 0.01	-	-
MTB Extract	200 mg/kg	1.6 ± 0.03	2.3 ± 0.03*	1.6 ± 0.01	42.5	55.55
MTB Extract	400 mg/kg	1.4 ± 0.02	1.7 ± 0.011	1.6 ± 0.05**	56.4	59.5
Indomethacin (STD)	10 mg/kg	1.2 ± 0.00	1.3 ± 0.01**	1.1 ± 0.01**	65	69.44
ISC-	5mg/kg	1.5± 0.01	1.8± 0.01*	1.9 ± 0.01**	42.6	47.2

n=3 per group, Values are mean ± SEM **p<0.01, compared with control.

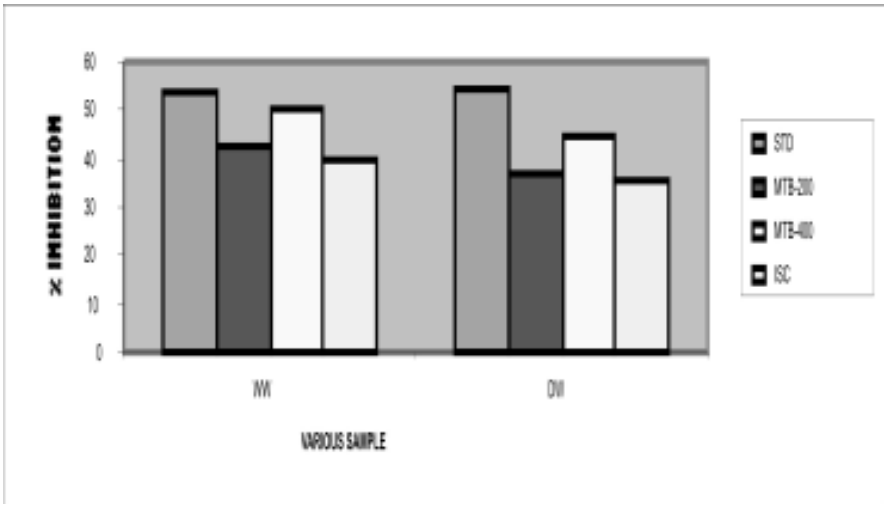


Fig.2. Effect of ethanolic extract and isolated compound of *Morinda tinctoria* on cotton pellet granuloma in rats

Table 3. Effect of ethanolic extract and isolated compound of *Morinda tinctoria* on cotton pellet granuloma in rats.

Group	Weight of Granuloma (mg)		(%) inhibition	
	Wet weight	Dry weight	For wet weight	For dry weight
Control	147 ± 3.22	49.33 ± 2.19	-	-
Indomethacin 10mg/kg	68.66 ± 3.18	23 ± 2.52	53.29	53.37
MTB Extract-200mg/kg	92.67 ± 2.33	37 ± 2.65	42.4	36.8
MTB Extract-400mg/kg	73 ± 4.16	27.67 ± 2.33	50.2	44.6
ISC 5mg/kg	91.33 ± 2.33	34.33 ± 0.88	39.8	35.4

n=3 per group, Values are mean ± SEM **p<0.01, compared with control.

Discussion

The shade dried stem bark of M. tinctoria (Rubiaceae) was extracted with 80% ethanol. Thus the phytochemical investigation shows presence of carbohydrates, steroids, phenolic compounds and anthraquinone glycosides in the extract. The residue of the ethanolic extract on column chromatography yielded the isolated compound. In column chromatography, a single compound was isolated in the elution of (CHCl3-85%: MeOH-15%). The isolated compound is a slight brownish yellow amorphous powder. The isolated compound gave positive test for anthraquinone glycosides.

1-hydroxy-2-methyl-3-methoxy-9,10-anthraquinone was obtained as an amorphous powder. Based on the molecular ion peak EI-MS at m/z 268 its molecular formula as C16H12O4 was suggested. The IR spectrum of 1 showed characteristic absorptions for hydroxyl (3424 cm-1) and ketone (1714 cm-1) groups (John, 2003). The UV spectrum showed absorption at nm (log e): 248(2.1); 306(3.4) suggestive of an anthraquinone skeleton (Pretsh et al., 2000). The 1H-NMR spectrum displayed a methoxy group by a signal at d ppm 3.51(3H, s, H-3a) which has connected carbon at d ppm 60.3(C-3a) in the HMQC plot and with a carbon at d 161.8 (C-3) in the HMBC spectrum. Presence of methyl group was evident from a signal at d (3H, s, H-2a) which had its connectivity with a carbon at d 16.9 (C-2a) in the HMQC and with carbons at d 124.0 (C-2), in the HMBC spectrum the signal at d (1H, s, H-1a) which had connectivity with a carbon at d 163.5 (C-1), in the HMQC signal at d 176.8 (C-9), d 182.6 (C-10). The assignments of 1H and 13C-NMR shifts are comparing well with the values of related partial structures reported in literature (Mulder-Krieger et al., 1982; Muzychkina et al., 1998; Bina et al., 2006). These data led to elucidate the structure of 1-hydroxy-2-methyl-3-methoxy-9,10-anthraquinone.

The anti-inflammatory effects of ethanolic extract and isolated compound of MTB in experimental animal models were studied. The potential of the MTB for its anti-inflammatory effect and short term toxicity was investigated. The effect of MTB ethanolic extract and isolated compound at the test dose of 200mg, 400mg/kg and 5mg/kg showed significant anti-inflammatory activity. Significant anti-inflammatory action was observed in carrageenan-induced oedema and also the chronic model.

It is well known that carrageenan induced paw oedema is characterized by biphasic events with involvement of different inflammatory mediators. In the first phase, (during the first 2 h after carrageenan injection), chemical mediators like histamine and serotonin play role while in second phase (2-4 h after carrageenan injection) Kinin and prostaglandins are involved (Sivaraman *et al*., 2010). Our results revealed that administration of extract and isolated anthraquinone compound inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. In cotton pellet induced grauloma, the extract and isolated anthraquinone fraction produced significant anti-inflammatory activity at the dose of 200mg/kg, 400mg/kg and 5mg/kg. The literature survey of plant reported presence of anthraquinone glycosides (Moorthy *et al.,* 1970) and phytochemical screening of ethanolic extract revealed that ethanolic extract of leaves of M. tinctoria contains various classes of phytoconstituents like alkaloids, sterols and anthraquinone glycosides. Several anthraquinone glycosides isolated from the medicinal plant were discovered to possess significant anti-inflammatory activity (Wang et al., 2002).

This study confirmed that ethanolic extract and isolated anthraquinone fraction from ethanolic extract of bark of Morinda tinctoria are responsible for its anti-inflammatory activity and the effects observed are attributing due to presence of anthraquinone glycosides in the plant. The toxicity studies of the plant suggest that it has reasonable safety margin justifying its wide application in various communities and lack of any reported side effects with traditional use of this plant.

In conclusion, the result of the study supports the traditional use of this plant in some painful inflammatory conditions. Further studies are currently in fact underway to characterize the active principles responsible for its anti-inflammatory activity.

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World Noni Research Foundation

With the mission of educating the people, the World Noni Research Foundation, a non-profit organisation dedicates itself to love and care for *Morinda citrifolia.,* through research and development. Learning from the wisdom of the simple people, WNRF aims at working with everyone to conserve and improve Noni towards sustainable human and ecological health. It will share the Noni's past glory, ethnobotany, history, science, benefits and its multiple uses with all. The INRF also serves as a facilitatory body for all Noni farmers, industries and consumers to establish a sustainable Noni economy network. The WNRF collectively represents the interests of all people in the Noni research and industry. It is an independent body and committed to exclusive Noni research and development. The WNRF website, journals and news letters are established to provide a non-biased forum for the researchers, consumers and industries to publicise their research findings and experiences with *Morinda* species.

WNRF believes that this synergistic effort of scientists and people of 'Noni Solidarity' would empower millions of ordinary masses to find their dignity and economic freedom, more naturally. This will lead to the realization of our vision "Healthy people, Healthy nation" in India and rest of the world.

Our Programmes Focus on :

- Conserving the *Morinda* species in India and rest of the world from its degradation.
- Organising "Noni Biodiversity Action Network" (NBAN) to save endangered (Red listed) *Morinda* species in the above regions.
- Developing Bioinformatics database on *Morinda* species existing in India and rest of the world and record all Indigenous Technical Knowledge about it.
- Supporting the research and development programmes on discovering the multiple potential of *Morinda* species in fields like pharmaceutical, nutraceutical, cosmetology, dye, agriculture, etc.
- Sharing the cutting edge action-programmes and research findings with researchers, farmers, consumers, food industry leaders, health - drug industry leaders, students and masses.
- Connecting the *Morinda* species researchers in India and rest of the world.
- Promoting the Indian Noni for health regenerative systems and processes through clinical studies & biotechnological research.
- Developing "Noni Villages" for Noni based socio-economic development of people at the grass-root level.
- Monitoring and encouraging quality *Morinda* products in the Market.
- Regenerating the glory of Indian Noni

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INSTRUCTIONS FOR AUTHORS

AIMS AND SCOPE

International Journal of Noni Research (IJNR) publishes original research and review articles on all aspects on Noni (*Morinda citrifolia* L.) and other species of *Morinda*. All submissions will be reviewed by the editorial board or by external references. The journal covers: diversity, cultivation, phytochemistry and clinical research, etc. related to Noni.

Three categories of paper will be considered for publication in IJNR.

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Authors are encouraged to submit their manuscripts via e-mail as attachment file to the E-mail ID : mail@worldnoni.com, palms02@hotmail.com and marisorna2008@gmail.com (A cover letter to be sent with the manuscript).

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3) Materials and Methods
4) Results
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Journal articles

Abbott, I.A. 1985. The geographic origin of the plants most commonly used for medicine by Hawaiians. Journal of Ethnopharmacology, 14: 213–22.

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Thesis / Dissertation

Surendiran, G. 2004. **Antimicrobial and Wound Healing Activity of Morinda tinctoria.** ***M. Sc. Thesis, University of Madras, Chennai, India.***

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