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Pharmacological properties and clinical applications of *Morinda citrifolia* L.

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Abstract : *Morinda citrifolia,* also commonly referred to as Noni, has been widely used since ancient times in traditional medicine in South and Southeast Asia with various parts of the plant; roots, stems, barks, leaves, and fruits finding a variety of use in treatment of human ailments. In recent years, it has been increasingly used as a dietary supplement because its more than 200 phytochemical constituents possess multiple biological activities and thus, exert curative effects in a number of diseases such as inflammatory diseases, diabetes, cardiovascular diseases, infectious diseases, cancer, and female-specific problems such as menstrual difficulties, cognitive disorders, etc. Several *in vitro* and *in vivo* studies involving cell cultures and rodents, and a few human clinical studies have demonstrated that Noni's potential health benefits are a result of its antioxidant, immunostimulatory, anti-inflammatory, anti-tumorigenic and pro-apoptotic properties, and the direct modulation of intracellular signalling pathways by its phytochemicals. This review provides an update of findings on the pharmacological functions and clinical benefits of the phytochemicals at the cellular level in various diseases to emphasize the need for further experimental and clinical studies to explore the mechanism(s) of action(s) of already and yet to be determined phytochemicals of *M. citrifolia*. Although Noni is considered safe for consumption, it is essential to develop proper recommendations so that it may be safely used by various age groups of human beings as there is increased utilization of Noni as a dietary supplement.

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Introduction

Morinda citrifolia (Noni) is one among many herbal medicines used widely in Indian Ayurveda and Siddha medicine practice, and Polynesian folk-lore medicines for the past 2000 years (Singh *et al.*, 2011; Ramaswamy *et al.*, 2012; Whistler 1985). It is also called commonly referred to as Indian Mulberry, Ba Ji Tian, Nono or Nonu, cheese fruit, and Nhau in various medicinal practices that use it for treating various diseases because of its wide-ranging health benefits including therapy for cancer, arthritis, diabetes, hypertension, pain, infectious diseases, etc. (Whistler., 1991). *Morinda citrifolia,* belonging to the species Rubiaceae (coffee family), is

a small evergreen tree found in coastal regions characterized by a straight trunk, bright green and elliptical leaves, white tubular flowers, and ovoid greenish-yellow fruit. The mature Noni fruit has an unpalatable taste and odor but processing of the fruit has enabled it to be marketed as a nutraceutical for the treatment of various diseases (Wang *et al*., 2002; Potterate *et al*., 2007). Although all parts of the plant have been used in treatment of diseases, it is the leaves that are traditionally and widely used, followed by roots of the plant; but now the fruit juice is widely consumed as a supplement. Many secondary metabolites such as iridoid glycosides and triterpenoids (ursolic acid) in the fruit, and anthraquninones in roots have contributed to the beneficial therapeutic effects of Noni. Traditional use of Noni varies widely in Southeast Asia, South Pacific where it originated and Africa, Hawaiian Islands, and Caribbean where it has been brought for cultivation. Its root, stem, bark, leaves, and fruit is used externally as a poultice or applied directly to the body, or consumed orally as an infusion without or with fermentation. Topical application is meant for the treatment of sores, burns, cuts, and inflammation besides as a relief agent from headaches. Oral consumption of Noni has been used to treat diverse health problems that include improving immunity, regulation of female-specific problems such as menstruation, and cancer, helminthic, diabetes, mental diseases such as depression and drug addiction, heart diseases, obesity, and a wide variety of other ailments.

Phytochemicals in *Morinda citrifolia*

Noni is widely used as a dietary supplement by human beings all over the world because of increased publicity and marketing as a general cure for several acute and chronic diseases (Fairchild., 2004), as well as several scientific studies demonstrating its usefulness in the treatment of such diseases. Although the presence of xeronine and its precursor, proxeronine, have been reported to be in Noni (Heinicke., 1985), no peer-reviewed publications exist to support this data.

Analysis for phytochemical constituents of *M. citrifolia* by NMR spectroscopy and mass spectrometry (MS), and gas chromatography MS revealed the presence of more than 200 compounds (Potterate., 2007; Singh., 2012). These primarily consist of a number of anthraquinones (damnacanthal) and anthraquinone glycosides, fatty acids and their derivatives, iridoids and iridoid glycosides, lignans, neolignans, flavonol glycosides, phenylpropanoids, saccharides, triterpenoids and fatty acids. Several new compounds and those belonging to the glycoside and polysaccharide family have been identified that may have potential health benefits (Lv *et al*., 2011; Beh *et al*., 2012; Hu *et al*., 2012). The presence of carotenoids in the leaves, bark, and fruits, and especially its increased content in the leaves confers the ability to correct vitamin A deficiency (Aalbersberg *et al*., 1993). Noni fruits from Australia have been found to contain enough vitamin C and potassium to meet the daily requirements for humans (Peerzada *et al*., 1990). Vitamin E production was

enhanced in the callus of *M. elliptica* by modifying the medium conditions *in vitro* indicating that this vitamin may also contribute to the health benefits of Noni (Chong *et al*., 2004). The polysaccharide, mostly pectic polysaccharide content of the fruits from Vietnam was found to contain several monosaccharides and their products such as arabinose, galactose, galacturonic acid, and rhamnose (Bui *et al*., 2006).

Ten anatomical parts (bark, branches, flowers, leaves, pulp, immature and mature fruit, stem, heartwood and root) of Morinda citrifolia L. was taken for analysis of bioactive compounds using Reverse Phase – High Performance Liquid Chromatography (RP-HPLC). It revealed that polydatin and physcion were the major anthraquinones in plant parts, rhein and resveratrol in the roots, âcryptoxanthin, zeaxanthin, rutin, quercetin and myrecitin were the major carotenoids in noni parts, and gallic acid was the major phenolic in all the nine parts of noni except the immature fruit. Mature noni fruits had the maximum number of bioactive compounds suggesting that it can be used for various therapeutic purposes (Singh et al., 2012).

Pharmacological basis for the biological activities of *M. citrifolia*

Several studies have demonstrated antioxidant, analgesic and anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, cardioprotective, cognitive protective functions, etc. in both crude and pure constituents of Noni through *in vitro* and *in vivo* studies.

Antioxidant Activity of *M. citrifolia* has been one of the key activities responsible for several of Noni's therapeutic effects in humans (Wang *et al*., 2002). Recent studies have attempted to provide the phytochemical basis for the antioxidant properties of Noni. Oral administration of deacetylasperulosidic acid isolated from the Noni fruit has been demonstrated to reduce lipid peroxidation and enhance superoxide dismutase and catalase activity (Ma *et al*., 2013). The presence of vitamins C and E, and scopoletin and damnacanthal in Noni fruit have the capability to bind to ^{3}-D-crystallin, a lens protein with equal affinity, which may be useful in the prevention of cataract formation (Rentala *et al*., 2013). The antioxidant property of Noni fruit juice examined through HPLC-DAD (Diode Array Detector) and Electro Spray Ionization Mass Spectrometric detection (HPLC-ESI-MS) may be associated with phenolic compounds, iridoids, and ascorbic acid (Dussossoy *et al*., 2011). Spectroscopic analysis of Noni fruit revealed several compounds out of which a neolignan, americanin A, has been found to have potent antioxidant activity (Su *et al*., 2005).

Anti-inflammatory Activity of *M. citrifolia* is another important property of Noni that is critical to its therapeutic properties. The iridoid glycoside, monotropein, in *M .officinalis* root significantly promoted antinociceptive and anti-inflammatory action in mice with carrageenan-induced edema and inflammation that may be

facilitated through the inhibition of pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and nuclear factor-ºB (NF-ºB) activity (Choi *et al*., 2005; Shin *et al*., 2013). In addition, four saccharide fatty acid esters isolated from *M. citrifolia* fruit exhibited strong anti-inflammatory activity in mice (Akihisa *et al*., 2007). It is probable that these phytochemicals may be responsible for the inhibition of matrix metalloprotease-9 release from immune cells, thus causing analgesic and anti-inflammatory effects in mice and providing relief from debilitating pain and joint destruction found in arthritis (Basar *et al*., 2010). These effects of Noni may be also mediated through the direct inhibition of the key players in the inflammation process such as COX-1 and COX-2 activities and the production of nitric oxide (NO) and prostaglandins E2 (Dussossoy *et al*., 2011). Another compound that had potent anti-inflammatory activity was damnacanthal isolated from the Noni root and it mediated its action through its ability to bind weakly to H1 receptor (Okusada *et al*., 2011). Besides its effects at the receptor level, damnacanthal's mechanism of action include suppression of the NF-ºB activity leading to the inhibition of the expression of cytokines, COX-2, and iNOS and thus, can be potentially used for the treatment of diseases involving inflammation (Nualsanit *et al*., 2011). The leaves of Noni also exerted anti-inflammatory activity probably through selective inhibition of yet to be determined subsets of leukocytes (Serafini *et al*., 2011).

Anti-cancer Property of *M. citrifolia* may be mediated through its ability to enhance antioxidant properties and anti-tumor immunity. Damnacanthal treatment of K-rasts-NRK cells reversed the abnormal morphology and cytoskeletal structure without changing the amount and localization of Ras while its addition to epidermoid, breast, hepatocellular, cervical cancer cell lines suppressed their proliferation (Thai *et al*., 2010, Hiramatsu *et al*., 1993). Such anti-cancerous property of damnacanthal may be due to induction of caspase activity to promote apoptosis through the proapoptotic protein nonsteroidal anti-inflammatory activated gene-1 (NAG-1) that may enhance transcription factor CCAAT/enhancer binding protein ² (C/EBP²) (Nualsanit *et al*., 2012). Inhibition of HIF-1± protein and interfering with the intracellular signaling pathways involving PKB, ERK-1/2, JNK-1 and S6 in cancer cells or repressing IL-1², an inducer of HIF-1± by Noni may explain its anti-cancer functions besides its anti-angiogenic activity (Beh *et al*., 2012; Jang 2012; Hornick *et al*., 2003). *In vivo* administration of MMTV-neu transgenic mice with Tahitian Noni fruit juice resulted in significant reduction in tumor weight and volume and in longer tumor doubling times in mice (Clafshenkel *et al*., 2012). This was accompanied by significant inhibition of the growth of aggressive forms of cancer and also induced additional beneficial effects such as significant changes in mammary secondary ductule branching and lobuloalveolar development, serum progesterone levels, and estrous cycling. Phytochemical-rich Noni is able to influence the development and growth of cancer at the cellular and molecular level that can be exploited as a therapeutic option or as an adjunct therapy in the treatment of cancer.

Another crucial mechanism through which Noni can exert anti-tumor effects is by the augmentation of immunity that may improve immunosurveillance, enhance cytostatic and cytocidal activity of immune cells through immune molecules such as cytokines, or modulation of tumor antigen recognition. Polysaccharide-rich constituents in Noni were capable of stimulating the release of cytokines useful in the elimination of tumor cells such as tumor necrosis factor-alpha (TNF-±), interleukin-1² (IL-1²), IL-10, IL-12 p70, interferon-gamma (IFN-³) and nitric oxide (NO) while it had no effect on IL-2 and suppressed IL-4 release in mice with Lewis lung LLC peritoneal carcinoma (Hirazumi *et al.*, 1999). More importantly, it also improved survival time and combinatorial therapy with chemotherapeutic agents promoted the curative effects indicating a probable use of Noni as an adjunct therapy in cancer. Such anti-cancer immunity was shown to be mediated through enhancement of T helper (Th)-1 cytokines and suppression of Th2 cytokines involving macrophages, T cells, and natural killer (NK) cells (Furusawa *et al.*, 2003). In a significant finding, fermented Noni exudate was demonstrated to quickly improve innate immune system functions through the upregulation of NK cell activity followed by adaptive immune system and thereby, facilitating elimination of tumor cells in mice (Li *et al.*, 2008).

In addition, Noni was able to prevent carcinogen-induced DNA adduct formation in various organs and increase the expression of p53 and pro-apoptotic Bax while decreasing anti-apoptotic Bcl-2, Bcl-XL, and survivin that resulted in an increase in the activities of caspases-9 and -3 suggesting that Noni is capable of altering the cellular functional capacity to induce apoptosis (Wang *et al.*, 2001; Gupta *et al.*, 2013). Some of the glycosides in the Noni fruit juice suppressed 12-O-tetradecanoylphorbol-13-acetate (TPA)- and epidermal growth factor (EGF)-induced AP-1 activity that blocked the phosphorylation of c-Jun in mouse epidermal cell line indicating that such effects may also be possible in cancer (Liu *et al.*, 2001).

Out of a total of 44 peer-reviewed publications on the use of Noni in cancer, 19 directly dealt with cancer involving *in vitro* and *in vitro* studies in cell lines and animals, and human cancer (Brown., 2012). The studies in cell lines and animals demonstrate that Noni (1) stimulates immune system as outlined above, (2) exerts *in vitro* cytocidal (0-36%) effect depending on the type of cancer cells, and (3) moderately augments (25-45%) the survival rate in rodents. Further studies are warranted to focus on the isolation of active components and their mechanism(s) of action(s) in anti-cancer activity.

Anti-microbial Activity of *M. citrifolia* against bacteria, fungi, viruses, and parasites is one of the key functions that have been widely exploited as a traditional remedy in a number of countries. Several components of Noni exhibit pronounced anti-tubercular activity (Saludes *et al.*, 2002). Extracts of Noni leaves inhibited the growth of *M. tuberculosis* by 89% while the anti-TB drug, Rifampicin, had a growth-

inhibitory rate of 97%. Methanolic extract of *M. citrifolia* had potential antibacterial activities to both gram-positive *S. aureus* and Methicillin Resistant *S. aureus* (MRSA) indicating that the phytochemical constituents of Noni exert potent anti-bacterial activity against latest superbugs such as MRSA (Zaidan *et al*., 2005). Anthaquinones from *M. augustifolia* showed antimicrobial activity against *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Sarcinalutea, Candida albicans* and *Saccharomyces sake* (Xiang *et al*., 2008). *Helicobacter pylorus (H. pylori) in the stomach is* a common cause for stomach cancer, especially when the chronic inflammation caused by it is left untreated. The ethanol and ethyl acetate extracts promoted anti-adhesion of *H. pylori* to human gastric carcinoma AGS cells resulting in the down-regulation of CagA, IL-8, COX-2 and iNOS expression indicating Noni's anti-inflammatory properties in stomach (Huang *et al*., 2014). Presence of water-soluble components in *M. citrifolia* interfered with the morphological conversion of *C. albicans* and the germination of *Aspergillus nidulans* that provides an option for Noni as an anti-fungal therapy for candidiasis and aspergillosis (Banerjee *et al*., 2006; Jainkittivong *et al*., 2009).

Phytochemicals in *M. citrifolia* have been shown to exert strong antiviral activity against mild to severe pathogenic viruses. Powdered Noni fruit was able to provide protection against the cytopathic effect of HIV-1(IIIB) in lymphocyte cell lines and inhibited the *hepatitis C virus* (*HCV*) replication that may be due to damnacanthal and pheophorbide-a, a major catabolite of chlorophyll a (Ali *et al*., 2000; Selvam *et al*., 2009; Ratnoglik *et al*., 2014).

Most studies have focused on the use of *M. citrifolia* in inhibiting malarial parasites which is the major cause of mortality in the tropical countries. Although all the parts of *M. lucida* had significant effects on *Plasmodium berghei*, the stem bark extracts of the plant markedly inhibited the parasitaemia in mice because of the presence of digitolutein, rubiadin 1-methyl ether, and damnacanthal (Koumaglo *et al*., 1992; Obih *et al*., 1985). Extracts of *M. lucida* induced a dose-dependent inhibition of egg-hatching and larval migration of the parasitic nematode, *Trichostrongylus colubriformis* (Hounzangbe-Adote *et al*., 2005). The aqueous and ethanolic extracts of *M. citrifolia* fruits had a significant anti-helminthic activity against *Ascaridiagalli* in chickens suggesting its potential use in preventing infestation with roundworms which is a major cause of health problems in countries situated in tropical regions (Brito *et al*., 2009). In agreement with this notion, alcoholic extract of the leaves of *M. citrifolia* showed significant *in vitro* anti-helmintic activity against human *Ascaris lumbricoides* (Raj., 1975).

Anti-diabetic Activity of *M. citrifolia* is another important function that can be used to treat increasing incidence of diabetes and post-diabetic complications such as heart diseases and neuro- and retinopathy in India and abroad. Several of *Morinda sp.* alcoholic extracts decreased the fasting glucose levels, improved hepatic

and renal functions, and enhanced antioxidant activities in streptozotocin (STZ)-induced diabetic rodents possibly mediated through the bioactive molecules such as damnacanthol-3-O-beta-D-primeveroside and lucidin 3-O-beta-D-primeveroside(Soon *et al.*, 2002; Kamiya *et al.*, 2008). In addition, aqueous extracts of *M. lucida* significantly inhibited ± -amylase and ± –glucosidase activities further potentiating the anti-diabetic effects of Noni (Kazeem *et al.*, 2013). Similarly, the anti-diabetic effects of fermented *M. citrifolia* was demonstrated in STZ-induced diabetic rats where it reduced glycosylated hemoglobin (HbA1c) levels, enhanced insulin sensitivity, and significantly decreased serum triglycerides and low-density lipoprotein (LDL) cholesterol and such effects may have been due to the activation of peroxisome proliferator-activated receptor-(PPAR-) ^{3} and stimulated glucose uptake via stimulation of AMP-activated protein kinase (Lee *et al.*, 2012). Such hypoglycemic effects may been due to altering the intracellular signaling by the Noni molecules with the ultimate inhibition of hepatic FoxO1 mRNA expression and a concomitant increase in FoxO1 phosphorylation and nuclear expulsion of the proteins (Nerurkar *et al.*, 2012). It is important to elucidate whether the phytochemicals in Noni have insulin modulatory properties because fruit juice of *M. citrifolia* exerts synergistic functions with insulin in diabetic rodents that may have been due to certain compounds in Noni that can inhibit protein tyrosine phosphatase 1B resulting in insulinomimetic effects (Horsfall *et al.*, 2008; Nguyen *et al.*, 2013). Another complication in diabetic patients is poor healing of wounds because of high glucose levels and Noni hastened the process of wound healing in rats with STZ-induced diabetes via its ligand-binding to the Platelet-derived growth factor and A(2A) receptors (Palu *et al.*, 2010; Nayak *et al.*, 2007).

Cardioprotective Functions of *M. citrifolia* have been crucial to the treatment of cardiovascular problems that may or may not be related to diabetic complications. Noni caused vasodilatation by relaxation of vascular smooth muscles through endothelium-dependent involving nitric oxide-cGMP pathway and –independent pathways (Ettarh *et al.*, 2004). Such vasodilatory effects and spasmolytic effects of *M. citrifolia* may be mediated through the effects of phytochemicals that are capable of blocking voltage-gated calcium channels leading to the release of intracellular calcium and thus, exerting ameliorating effects in hypertension (Gilani *et al.*, 2010). Extracts of Noni have been shown to exert hypotensive effects, lower blood pressure in anesthetized dogs, and promote diuretic effects (Wang *et al.*, 2002). Dyslipidemia are disorders of lipoprotein metabolism that results in coronary heart disease and other cardiovascular problems directly or as an outcome of diabetes. It is characterized by a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles. The aqueous-ethanolic extracts of fruits, leaves, and roots of *M. citrifolia* had antidyslipidemic effect in high fat diet-fed rats through the inhibition of biosynthesis, absorption and secretion of lipids providing additional proof for its health benefit

in the treatment of heart problems (Mandukhail *et al.*, 2010). Few of the anthraquinones from the roots of *M. officinalis* enhanced adipocyte differentiation that may be a mechanism for weight loss therapy and prove to be beneficial in the treatment of diabetes and cardiovascular diseases (Liu *et al.*, 2012).

Neuroprotective effects of *M. citrifolia* is another important property that may be critical to the treatment of neuro-associated disorders such as memory impairment, cognitive dysfunctions, neurodegenerative disorders, etc in the elderly especially, when the population of elderly is on the rise in several countries of the world. Inulin-type oligosaccharides extracted from *M. officinalis* was shown to possess neuroprotective effects by suppressing the intracellular calcium overloading and simultaneously enhance nerve growth factor mRNA expression in corticosterone-lesioned PC12 cells (Li *et al.*, 2004). These cellular effects may explain the prevention of neuronal damage in rodents with cerebral ischemia by *M. citrifolia* fruit juice and the reversal of neuronal deficits (Harda *et al.*, 2009). It may also facilitate protection from stress-induced impairment of cognitive functions by improving the blood vessel density in the hippocampal dentate gyrus and attenuating the development of glucose intolerance (Muto *et al.*, 2010; Harda *et al.*, 2010).

Various extracts of Noni fruit juice markedly enhanced memory and cerebral blood flow and reduced oxidative stress and acetylcholinesterase activity in a scopolamine-induced amnesia mouse model suggesting that Noni not only improves cellular functions in the brain, but also reverses the behavioral deficits (Pachauri *et al.*, 2012). Further proof for this beneficial effect of Noni on improving memory deficits was observed in mice where Noni extract inhibited the STZ-induced memory dysfunction that was demonstrated to be due to increased levels of Brain-derived neurotrophic factor (BDNF), a key growth factor for the maintenance of neurons, acetylcholine, and ATP levels leading to a better cholinergic neurotransmission and antioxidative functions (Pachauri *et al.*, 2013). Similarly, administration of oligosaccharides of *M. officinalis* in rats with beta-amyloid-induced dementia enhanced the activities of antioxidant enzymes, acetylcholine, and Na+ /K+ -ATPase supporting the earlier studies where Noni improved brain energy metabolism and cholinergic neuronal activity (Chen *et al.*, 2013). The improvement in short- and long-term memory and exploratory behavior by Noni in dementia mouse model not only enhanced cholinergic neurotransmission but also inhibited monoamine oxidase-A activity that may have been responsible for the increase in the levels of serotonin and dopamine in the brain (Muralidharan *et al.*, 2010). These limited evidence for the improvement of neurobehavioral functions by Noni may be helpful in its use as a dietary supplement in the elderly population, people with cognitive dysfunctions, and patients with neurodegenerative disorders.

Clinical Applications of *Morinda citrifolia*

There are very limited studies pertaining to Noni's health benefits in human beings.

In one of the studies, it was used in women to determine whether it had anti-inflammatory and pain-reducing effects as reported in animals. Administration of Noni capsules to women with primary dysmenorrhea did not provide any health benefits such as attenuating bleeding or menstrual pain that may have been related to dosage, failure to monitor the compliance of Noni capsule consumption, sample size, etc. (Fletcher *et al*., 2013).

Drinking of Noni fruit juice by adult heavy smokers daily for a month significantly reduced cholesterol levels, triglycerides, and hs-CRP in association with a decrease in LDL and homocysteine, as well increases in HDL suggesting the beneficial of effects of Noni in preventing oxidative stress, dyslipidemia, and systemic inflammation (Wang *et al*., 2012). These beneficial biochemical effects may reduce the cancer risk in heavy cigarette smokers where drinking 1 to 4 oz of Noni juice daily for a month may block carcinogen-DNA binding or excising DNA adducts from genomic DNA (Wang *et al*., 2009). A gel containing the ethanol extract and juice pressed from the leaves was safe for topical use and may be useful in preventing UVB-induced skin injury, and therefore may be potentially considered for prophylactic and therapeutic use in skin cancer (West *et al*., 2009).

Controlled human trials documenting anti-diabetic effects of noni supplementation is not available but four population-based surveys have provided a link between noni consumption and the resulting increased glucose uptake (Nerurkar *et a*l., 2015). In another study, noni fruit juice consumption by Indian diabetic patients for a period of 6 months reduced fasting plasma glucose and LDL cholesterol levels while augmenting the HDL cholesterol level (Sathishkumar, 2007). Similar beneficial anti-diabetic effects of noni fruit juice was provided by another study where a month-long oral consumption of noni fruit juice resulted in substantial decrease in HbA1c, fasting blood sugar, total cholesterol, LDL, triglyceride aspartate aminotransferase and alanine aminotransferase levels (Ramesh *et al*., 2013). These effects may have been facilitated by bioavailability of scopoletin, iridoid glycosides, monotropein, and deacetylasperulosidic acid, or flavonoid quercetin (3,30,40,5,7-pentahydroxyflavone), and its glycoside, rutin (quercetin-3-*O*-b-rutinoside). However, proper randomized human trials examining the pharmacokinetics and bioavailability of these bioactive compounds have to be performed to establish the anti-diabetic activity of Noni in humans.

The following studies demonstrated some therapeutic effects in human beings:

(1) Aqueous extract of *M. citrifolia* enhanced the rate and the extent of ranitidine absorption by improving the gastrokinetic activity that may be due to its bioactive component, scopoletin, stimulating the 5-HT(4) receptor (Nima *et al*., 2012).

(2) Extracts of *M. citrifolia* stems and the morindicone and morinthone, bioactive molecules of Noni had potent anti-leishmanial activity against *L. major* that cause

cutaneous leishmaniasis in humans (Sattar *et al.*, 2012).

(3) Noni capsules containing dried fruit at a dose of 600 mg Noni extract significantly reduced the postoperative nausea and vomiting during the first 6 hours (Prapaitrakool *et al.*, 2010).

(4) Oral consumption of three or four capsules four times daily (6-8 g) in 51 advanced cancer patients was better in controlling fatigue, pain, and maintaining physical function and scopoletin may be one of the many bioactive molecules exerting such curative effects (Issell *et al.*, 2009).

Although the above studies may not strictly conform to the standard human clinical trials, the results from these disparate studies point to possible beneficial therapeutic effects in human beings and emphasize the need for a thorough systematic approach in establishing the usefulness of *M. citrifolia* in the treatment of diseases and cancer.

Conclusions and Future Directions

For several centuries, various parts of *M. citrifolia* have been used as a medicinal plant and it is currently used as a dietary supplement in a number of countries because of its anti-oxidant, anti-inflammatory, analgesic, anti-cancer, anti-diabetic, and anti-hypertensive properties. These findings are based on studies involving *in vitro* and *in vivo* cell cultures and animal models. The biological activity of Noni may be related to more than 200 phytochemicals that include anthraquinones, flavonoids, glycosides, iridoids, lignans, and triterpenoids but their individual contribution to the biological activity of Noni is yet to be fully elucidated. It is critical to develop techniques to identify and characterize yet to be isolated phytochemicals and also, examine the complex interactions between these phytochemicals as it is well known that biological effects are not mediated by a single component but through synergistic interactions among the various bioactive compounds.

Despite the wide-ranging benefits of Noni reported in literature, it is critical that some of the physiological and therapeutic effects of it are explored in detail in animal studies so that the mechanism(s) of action(s) are understood. It is important that pharmacokinetics of the various bioactive compounds are examined *in vivo* so that the contributions of their metabolites to the beneficial effects of noni can be fully ascertained. Such in-depth analysis will pave the way for development of targeted drugs to various ailments afflicting humans.

Although anecdotal and traditional knowledge about *M. citrifolia* is driving its use in humans as a dietary supplement, it is imperative that proper clinical studies are essential to examine the cellular, biochemical, and molecular mechanism of its action. It involves designing randomized double-blinded clinical studies with appropriate placebo controls in humans that will provide information about

bioavailability, safety, and benefits of long-term usage of noni and its bioactive components. This will aid in the development of Noni products with appropriate ingredients with reproducible quality useful not only for clinical trials but also marketing.

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Effect of Noni (*Morinda citrifolia* L.) on plaque induced gingivitis - A microbiological study

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Abstract : *Morinda citrifolia* L. (Noni) is a common traditional medicinal plant of the native people of the south pacific, which is used for the treatment of a broad variety of disease including gingivitis This scientific study was made to evaluate the effect of noni juice on plaque, gingivitis and colony forming units. Twenty samples with mild to moderate plaque and gingivitis were select of which. 10 used Noni fruit juice as mouth rinse for 28 days and the rest 10 continued with their normal oral hygiene measures. Their plaque and gingival scores and CFU counts were assessed before and after using Noni fruit juice. The results were positive as the noni fruit juice as mouth rinse resulted in decrease in plaque, gingival scores and number of bacteria in mouth. This paper explains about the dental benefits of Noni fruit juice on plaque, gingivitis and mouth residing bacteria.

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Introduction

Gingivitis and periodontitis are wide spread disease and are responsible for early loss of teeth. Gingivitis is usually caused by inadequate oral hygiene which has taken place over a certain period of time. Gingivitis is triggered by a plaque accumulation of the bacteria. Antibacterial mouth rinses like chlorhexidine are used for the treatment of gingivitis but they are accompanied with side effects like staining, allergy and lingering after taste. All these suggest for a natural remedy and nowadays usage of natural products have been increasingly popular. A daily treatment with Noni fruit juice is not much heard of and its effect on dental aspect lacks sufficient scientific research. The objective of the study was to find out the effectiveness of Noni fruit juice on plaque induced gingivitis. This is a type of study deals with clinical findings supported by microbiological evidence.

Morinda citrifolia L. (Noni) has been identified as edible plant by Merill (1943) in his technical manual-emerging food plants and poisonous plants of the Island of Pacific. It is a common traditional medicinal plant of the native people of the south pacific, which is used for the treatment of a broad variety of disease including gingivitis (Johnas *et al.,* 2013; Solomon, 1999). During the last 15 years, numerous

in vitro and in vivo investigations have been published about the biological activities, chemical constituents and preliminary phytochemicals of Noni (Lavand and Larson, 1979; Moorthy and Reddy, 1970; Simonsen ,1920). Sang *et al.* (2001) identified iridoid glycosides from the leaves of *Morinda citrifolia* L..

The research studies on Noni in dentistry are very few hence the future looks bright for this plant in curing dental diseases. Among many positive effects on health, current research results claim that Noni fruit juice is beneficial for fungal infections, bacterial infections, inflammations, immune enhancements and analgesic effects (Younos *et al.,* 1990). In the present study, we studied the influence of a daily treatment of teeth and gingiva with a fruit juice prepared from *Morinda citrifolia* L. (Noni). The reason for the effectiveness of noni juice against gingivitis could be either a reduction of the bacterial count and/or the reduction of inflammation associated with gingivitis/periodontitis. Noni juice contains scopoletinanthroquinones and terpenes which also decreases inflammation. Heinicke R. (1985) had reported the pharmacological activities of Noni. The study can promote large scale researches on noni and promote awareness among the people to use Noni fruit juice as a remedy for various health problems.

Materials and Methods

Twenty subjects are selected and divided into Group A (10 - control) and Group B (10 experimental) based on inclusion and exclusion criteria by random sampling. Group B participants were instructed to maintain proper oral hygiene measures along with rinsing the mouth using Noni fruit juice for duration of 4 weeks. The subjects were students of RVS dental college and hospital, Kannampalayam. And the study was conducted in department of periodontology.

The following materials were used for the study:

1. 0.85 % Na Cl
2. Sterile containers for collecting saliva sample
3. Mouth mirror
4. Periodontal probe
5. Explorer
6. Noni fruit juice
7. Containers and measuring jar

Statistical analysis used

Students paired 't' test and Wilcoxon signed rank test are used to compare plaque scores, gingival scores and CFU count before and after using noni juice.

The following criteria for inclusion and exclusion were used:

Inclusion criteria for groups (A & B)

1. Subjects in the age group of 18-21years
2. Subjects who have not used any mouth wash/rinse for past 6 months
3. Subjects having at least 20 natural teeth in the permanent dentition.
4. Subjects with mild to moderate plaque and gingivitis.
5. Subjects who were willing to use noni juice as instructed for 4weeks (group B only).

Exclusion criteria for groups A & B

1. History of antibiotic use in the past 3-4 weeks.
2. Subjects having allergy to noni juice.
3. Smokers – past and present.
4. Pregnant & lactating women.

Informed consent procedures

Informed consent was obtained from the subjects before starting the study.

Base line index scoring

The plaque index, gingival index and papilla bleeding index of all participants are recorded on day 0 and day 28 (after 4 weeks).

Plaque index

The plaque index by Silness.P & Loe .H (1967) is used to assess the thickness of plaque at the gingival area of the tooth on the index teeth.

Gingival index

Gingival index by Loe. H & Silness. P (1963) is used to assess the severity of gingivitis on the index teeth.

Sampling- Baseline CFU

On day 0, all the subjects are instructed to wash their mouth with the physiological saline (0.85% Nacl). This saline is collected in a sterile container and is serially diluted and plated in nutrient agar plates. The plates are incubated aerobically at 37degree for 24hours. After this incubation period, the number of colonies present in 1ml of saline is calculated.

Colony count is calculated by the formula:

Number of bacteria/ml = number of colonies / dilution × amount plated

Group A

Participants were instructed to continue their routine oral hygiene practice.

Group B

Procedure to practice noni mouth rinse

Patients in the noni group also instructed in methods to achieve proper oral hygiene, additionally they have to rinse the mouth with 30ml of noni juice plus 30ml water for 2min with subsequent swallowing once a day for a total duration of four weeks.

Subjects are instructed to take noni juice after brushing and in empty stomach.

They are also advised to take food half an hour after rinsing with noni juice.

Indices at day 28

- On day 28, the plaque index, gingival index of all the subjects are recorded as mentioned before.

CFU score at day 28

- After 28 days, the same procedure is followed for sample collection in both the groups and results are tabulated.

Results

The results of this study is presented and discussed below in tables 1 to 6 and discussed.

Table 1 : Comparison of plaque score before and after Noni treatment using paired t-test

Group		Mean	SSD	SEM	t-value	p-value
Group A	Before	0.8900	.27366	0.0865	1.3528	0.0106
	After	1.050	.25495	0.0806		
Group B	Before	0.9800	.27809	0.088	2.851	0.1929
	After	0.6200	.22998	0.081		

The mean plaque score of group B declined from 0.98to 0.62 after 28 days. However there is no significant reduction in group A.

Table 2 : Comparison of gingival score before and after noni treatment using paired t-test

Group		Mean	SD	SEM	t-value	p-value
Group A	Before	0.8750	.31114	0.0984	2.512	0.0218
	After	1.1900	.24585	0.0777		
Group B	Before	1.0350	.34323	0.1122	0.7554	0.4598
	After	0.8150	.33170	0.1216		

The mean gingival score of group B declined from 1.03 to 0.81 after 28 days. However there is no significant reduction in group A.

Table 3: Comparison of CFU score before and after noni treatment using paired t-test

Group		Mean	SD	SEM	t-value	p-value
Group A	Before	43.00	4.667	1.476	1.602	
	After	43.4	13.335	4.217		
Group B	Before	52.50	12.039	3.807	5.028	
	After	39.40	6.753	2.135		

The mean no of colonies in the case of group B declined from $52.5*10^3$ to $39.4*10^3$ after 28 days. However there is no significant reduction in group A.

Table 4 : Comparison of each parameters before and after Noni treatment using Wilcoxon signed rank test.

Group	Group A		Group B	
	Z	Asymp.sig (2-tailed)	Z	Asymp.sig (2-tailed)
Plaque index before with plaque index after	2.376	0.180	2.810	0.005
Gingival index before with gingival index after	2.809	0.016	2.318	0.005
No. of colonies before with no. of colonies after	1.602	0.182	1.387	0.127

Table 5: Correlation of parameters before Noni treatment

Group		Plaque Index	Gingival colonies	Number of
Plaque	Pearson correlation	1	0.414	0.395
	Sig.(2-tailed)			0.234
Gingival index	Pearson correlation	0.414	1	0.436
	Sig.(2-tailed)	0.234		.00
No. of colonies	Pearson correlation	10	10	1
	Sig.(2-tailed)	10		10
Plaque	Pearson correlation	1	0.660	0.742
	Sig.(2-tailed)		0.038	.00
Gingival index	Pearson correlation	0.660	1	0.645
	Sig.(2-tailed)	0.38		.002
No. of colonies	Pearson correlation	10	10	1
	Sig.(2-tailed)	10	10	

Table 6: Correlation of parameters after Noni treatment

Group		Plaque	Gingival Index	Number of colonies
Plaque	Pearson correlation	1	0.251	0.412
	Sig.(2-tailed)		0.485	
Gingival index	Pearson correlation	0.251	1	0.458
	Sig.(2-tailed)	0.485		.120
No. of colonies	Pearson correlation	10	10	1
	Sig.(2-tailed)	10	10	
Plaque	Pearson correlation	1	0.044	0.582
	Sig.(2-tailed)		0.903	
Gingival index	Pearson correlation	0.044	1	0.441
	Sig.(2-tailed)	0.903		.00
No. of colonies	Pearson correlation	10	10	1
	Sig.(2-tailed)	10	10	10

Discussion

Plants have always been among the coon sources of medicines, either processed as traditional preparation or used to extract active principles (Maiden, 1889) *Morinda citrifolia* L. has been used for more than 2000 years as traditional folk medicine for various diseases like headache, fever, arthritis, Gingivitis, diabetes, etc. (Mathivanan *et al.,* 2005; Wang *et al*., 1999; Brett, 2012). It is known to have dental benefits but sufficient scientific research has not been carried out on this field. Noni has anti-bacterial action and anti-inflammatory action. So noni extract has positive effects on plaque induced gingivitis (John Glang, 2013).

Plaque and gingival index were assessed for 20 subjects as clinical evidence. This was done to find the effect of Noni fruit juice on plaque induced gingivitis.

The plaque index scores at day 0 and day 28 for group A is taken. Based on results and statistical analysis, there is no significant reduction in plaque scores was seen in the case of group A. Mean scores indicate that there is an increase in plaque scores after 28 days in case of group A. This indicates that with time without any preventive measures there is increased accumulation of plaque. The mean plaque score of group B declined from 0.9800 to 0.6200 after 28 days indicating that there is significant reduction in plaque scores due to gargling of Noni fruit extract which supports our study.

The mean gingival score of group B declined from 1.035 to 0.8150 after 28 days indicating that there is significant reduction in gingival scores due to gargling of noni extract which supports our study. However no significant reduction was seen in case of group A. Mean scores indicate increase in gingival index after 28 days in case of group A. Thus gingivitis when left untreated can progress to periodontitis.

CFU count is taken to find out anti-bacterial effect of noni juice which provides the microbiological evidence. The mean number of colonies in case of group B declined from 52.50 to 39.40 after 28 days indicating that there is significant reduction in number of colonies due to gargling of noni extract. This indicates that Noni juice has anti-bacterial effect. However no significant reduction was seen in case of group A. Mean scores indicate increase in number of colonies after 28 days in case of group A.

Conclusion

As the results are positive it can be said that Noni fruit juice goggling causes reduction in plaque as well as gingivitis even it is practised for such a short time of 28 days. If practised daily it can develop as a healthy habit. From CFU count results it is evident that Noni has anti-bacterial effect as results show reduction in number of bacteria per ml after 28 days of n Noni fruit juice gargling. While the results on

control group is significant as it shows increase in plaque and gingival index scores. Also CFU count increases when sufficient oral hygiene measures are not taken in time. Both the plaque and gingival scores tally with CFU count which indicates that number of bacteria present in mouth is related to plaque and gingivitis. All these results indicate that Noni extract has significant effect on plaque and gingivitis. Hence Noni fruit juice has a array of therapeutic activities and has a promising note as a plaque control agent. From the results obtained it can be concluded that Noni fruit juice has significant effect on plaque and gingivitis.

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Etiology of dry and soft rot of Noni (*Morinda citrifolia*, L) fruits

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Abstract : Survey of Noni orchards at Tamil Nadu and Kerala revealed the presence of both dry and wet fruit rot. The pathogen associated with dry fruit rot of Noni was *Colletotrichum gloeosporioides* and *Alternaria alternata*. The wet or soft rot of Noni was caused by *Pantoea agglomerans*. The pathogen associated with Noni wet rot was confirmed for its identity through fatty acid methyl ester analysis, 16s rDNA analysis and through biochemical characterization. The primary spread ofNnoni dry fruit rot caused by *C. gloeosporioides* was due to the presence of dormant mycelium in the stems and barks and due to the fruiting body namely the acervuli. The primary spread by *A. alternata* is through infected crop leaf residues. The secondary spread of both the pathogen was through air borne conidia. Flowers on the fruits were also found to be infected by both *C. gloeosporioides* and *A. Alternata*. Aerodynamic studies revealed the association of conidia of both the dry rot pathogens in air. Presence of continuous inoculums in the Noni orchards favour the disease spread. Presence of scars on the fruit surface coupled with dew and increased humidity is highly favourable for the fruit rot epidemic. However, the wet fruit rot is severe only during the monsoon seasons.

Introduction

Noni (*Morinda citrifolia* L.) is known for its therapeutic use and grown throughout the tropical countries. The extracts ofNoni fruits stimulate the immune system and cure most of the diseases. Extensive cultivation of Noni has resulted in outbreak of diseases such as leaf blight, anthracnose, black flag, fruit rot, stem blight, sooty mould, stem canker and algal leaf spot. Fruit rot diseases leads to considerable yield reduction in terms of quantity and quality of fruits. Recent survey in the usual Noni growing areas of Tamil Nadu and Kerala revealed the outbreak of fruit rot diseases. However, perusal of literature revealed that the etiology behind the fruit rot disease was obscure. Hence, the present investigation was carried out to understand the host parasite relationship of fruit rot pathogens.

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Materials and Methods

Isolation of pathogens associated with dry and soft fruit rot

Pathogens associated with fruit rot were isolated from lesions of Noni flowers, fruits, and infected twigs through tissue segment method on potato dextrose agar (PDA) and nutrient agar for fungal and bacterial pathogens respectively. Plates were incubated at room temperature (28-30^0 C) and observed periodically. The growing edges of fungal hyphae developing from the infected tissues were then transferred aseptically to PDA slants and pure culture was stored at 4^0 C.

Identification of pathogens associated with dry fruit rot.

The pathogen was identified up to species level based on cultural and morphological characters. A loopful of fungal culture grown on PDA plates were taken on a glass slide and observed under image analyzer (CETI, with Capture pro software Medline Scientific Ltd, UK) under 40X magnifications for the presence of conidia and conidiophore. After confirming the spores, the culture was purified by single spore isolation technique and stored at 4 ¯C on PDA slants.

Identification of bacterial pathogen associated with soft /wet fruit rot.

Bacterial pathogen associated with soft rot of noni was isolated on NA medium, based on the cultural, morphological and biochemical characterization the pathogen was identified.

The identification was further confirmed through, FAME analysis and 16s DNA.

Morphological characteristics of bacterial pathogen

Morphological and cultural characters of bacterial pathogen was observed through microscope with the help of capture pro software.

Growth of bacterial pathogen in different media

The growth of bacterial pathogen was assessed in nutrient agar (NA) Luria bertani medium (LB) and Crystal Violet Pectate medium (CVP). An 18 hours old bacterial culture in a growing slant was transferred to 1 ml of sterile water and thoroughly shaked to get uniform suspension of bacterium. The concentration of bacterial cells was adjusted to 10^6 cells ml^{-1} using spectrophotometer. Twenty ml of the sterile agar medium in a test tube was inoculated with 100 µ1 of inoculum prepared as indicated above. Later it was shaked well and plated on to in nutrient agar medium Luria bertani and CVP medium in sterile Petri plates .The plates were incubated at (28 ± 2°C). The bacterial colonies were observed after 20 hours after inoculation.

Biochemical properties of the bacterial pathogen

Gram staining

Gram staining performed using 24 h old bacterial culture as described here under. The uniform suspension of the isolate prepared in sterilized distilled water was smeared on the cleaned glass slide and air dried. The smear was gently exposed to flame for two min and covered with crystal violet solution for 30 sec. Then the slide was gently washed with distilled water for a few min and covered with Lugols iodine solution for 30 seconds. The iodine solution was washed by using 95 per cent ethyl alcohol until no more color flows from the smear. The slide was again washed with distilled water, drained and safranin (counter-stain) was applied on the slide for 30 sec. The slide was then washed with distilled water, blotted using a country filter paper and air dried. The slide was then examined in the microscope under oil immersion (Aneja, 1993).

Biochemical characterization of bacterial pathogen was performed with gram negative bacteria identification kit supplied from Tulip diagnostics (P) Ltd, India. The identification kit comprises test such as 1.Lysine utilization test, 2.Ornithin decarboxylation test, 3.Phenyalanine deamination, 4.Urease detection test, 5. Nitrate reduction test, 6. H_2S production test, 7. Citrate utilization test 8. Glucose utilization test, 9. Adonitol utilization test, 10. Arabinose utilization test, 11. Lactose utilization test, 12. Sorbitol utilization test, 13. Oxidase detection test.

Kit containing 13 vials with differential medium were inoculated with 100 µl of 18-24 h old culture and incubated at 35-38° c for 24 h. Experiment was observed to examine color change after incubation so as to identify the pathogen. Pathogen was identified as *Pantoea agglomerans***.** In addition, reaction of bacterial pathogen also tested for KOH, Simmon citrate utilization and catalase test.

Fatty acid methyl ester of *P. agglomerans*

The bacterial fatty acid methyl derivatives were analyzed with Midi Sherlock®Microbial Identification System (Sherlock TSBA Library version 3.80; Microbial ID). Liquid culture (24h old) of bacteria was used to assess fatty acid. The fatty acids are extracted by saponification in dilute sodium hydroxide/methanol solution followed by derivatization with dilute hydrochloric acid/methanol solution to give the respective methyl esters (FAMEs). The FAMEs were then extracted from the aqueous phase by the organic solvent and the resulting extract was analyzed by Gas Chromatography (GC) the bacterial cells were killed by saponification. FAMEs are more volatile than their respective fatty acids and therefore more suitable to gas chromatography analysis. The Sherlock software automates all analytical operations and uses a sophisticated pattern recognition algorithm to match the unknown FAME profile to the stored library entries for identification.

Molecular characterization of *P. agglomerans* through amplification of 16s ribosomal RNA of *P. agglomerans*

Genomic DNA was isolated from the bacterial culture by using the bacterial Genomic DNA Isolation Kit (RKT09).The bacterial DNA were amplified with forward primer 16s forward primer: (5'AGAGTRTGATCMTYGCTWAC3) and16s reverse primer: (5'CGYTAMCTTWTTACGRCT-3')' .The 20 ìl mixture approximately contain 50 ng of total DNA, 5 mM each dNTPs, 20 pmol of each forward primer and reverse primer and 0.5 U of *Taq* DNA polymerase (Bangalore Genei, India). PCR amplification was performed in a thermocycler (Thermal Cylcer ABI2720, Chromous Biotech Pvt. Ltd, Bangalore, India).

Sequencing of bacterial 16s ribosomal RNA and sequence analysis of *Pantoea agglomerans*

Sequencing of amplicons were performed with ABI 3130 Genetic Analyzer (Chemistry: Big Dye Terminator version 3.1"Cycle sequencing kit. (Chromous Biotech Pvt. Ltd, Bangalore, India.). The biosynthetic gene homology searches were performed using the BLAST program though the internet server at the National Centre for Biotechnology Information, National Institutes of Health, and Bethesda, USA. Sequences and accession numbers for compared isolates were retrieved from the Gene Bank database. Sequence pair distances among related and different *Pantoea* spp isolates were scored with the CLUSTAL X (1.81) program and phylogenetic tree analysis was performed with Phylogenetic Tree Builder. Newly obtained sequences were submitted in the Gen Bank database, New York, USA.

Pathogenicity of dry fruit rot

The unripe maturedNoni fruits were used for pathogenicity.

The *C. gloeosporioides* and *A. alternata* were used for artificial inoculation on to unriped fruits at the rate of 5 x 10^5 conidia ml^{-1}. Conidia of *C. gloeosporioides* and *A. alternata* were harvested using 14 days old PDA culture by grinding it with phosphate buffer pH 7.0 in a sterile pestle and mortar. The fruits were washed with running tap water and surface sterilized with 0.1 per cent mercuric chloride and washed with sterile distilled water. The washed fruits were inoculated with conidial suspension after pin prick using sterile entomological pins.

The fruits inoculated with sterile distilled water served as control. The inoculated fruits were incubated in sterile poly propylene (PP) bags and sprayed with sterile water. To maintain humidity sterile cotton wools were moistened with sterile water and placed inside the PP bags. The bags were incubated at room temperature (28 ± 2ºC) for 72 hours. The infection was recorded periodically and the per cent infection of fruits was calculated. Three replications were maintained each replication consist of five fruits.

Pathogenicity of *C. gloeosporioides* on twigs

The healthy green twigs were collected from noni orchard. Twigs were sliced in to small bits (4-6 cm) and washed with running tap water, surface sterilized with 0.1 per cent mercuric chloride and washed with sterile distilled water. Later the surface sterilized twigs were inoculated with spore suspension of *C. gloeosporioides* at the rate of 5 x 10^5 conidia ml^{-1}. The twigs inoculated with sterile distilled water served as control. After inoculation the twigs were incubated in sterile poly propylene (PP) bags and sprayed with sterile water. To maintain humidity sterile cotton wools were moistened with sterile water and placed inside the PP bags. The bags were incubated at room temperature (28 ± 2ºC) for 72 hours.

Pathogenecity of C. *gloeosporioides* on flowers

Apparently healthy flowers of Noni were collected from noni orchard.

The collected noni flowers were surface sterilized with 0.1 per cent mercuric chloride for one minute and later serially washed thrice in sterile distilled water. Then the flowers were blot dried in sterile what man. No.1 filter paper. Ten sterilized flowers were placed at equal distance on the plates The conidia of *C. gloeosporioides* was harvested from 14 days old culture grown on PDA and suspended in phosphate buffer pH 7.0. The conidial suspension was adjusted to a load of 5 x 10^5 conidia ml^{-1}. The conidial suspension of *C. gloeosporioides* was sprayed to the flowers on plain agar in sterile Petri plate. Plates were incubated for three days at 28 ± 2 ºC. The flowers after the incubation were assessed for the colonization by *C. gloeosporioides* (Sergeeva *et al*., 2008).

Pathogenicity test *in vitro* for wet fruit rot

Moist chamber method

Pathogenecity was performed with healthy matured unriped fruit. Ten sterile tissue culture bottles (12.5X 6.00) were taken; inner side of bottle was lined with sterile moist filter paper. The surface sterilized fruits were kept inside the bottles placed with sterile moist cotton laid upto a height of 3 cm at the bottom. The healthy unripe fruits were inoculated with l00 µ1 of 18 h old bacterial pathogen after making four pinpricks randomly at the apical end of the fruit at the concentration of 10^6 cells ml^{-1}. Inoculated fruits were incubated at room temperature (28 ± 2 ºC) for 24 to 48hours. Controls were maintained by keeping sterile moist cotton at the apical end of fruit. The infection was recorded periodically and the per cent infection of fruits was calculated. Three replications were maintained each replication consist of 5 fruits.

Pathogenicity test in field

The bacterial pathogen was grown to log phase (48 h) in NA broth, and the cells were harvested by centrifugation at 4000 rpm for 10 min, washed in Phosphate

Buffer Saline (PBS) and resuspended in the same volume of PBS. The optical density was adjusted using a spectrophotometer to contain 10^6 cells ml^{-1}. Healthy unriped fruits were sprayed with bacterial pathogen at the rate of 1 ml by suspending it in 100 mg of carborundam powder to create mechanical injury to fruits using an aspirator. The inoculated fruits were then covered with polythene bags with 100 gauge thickness. The symptoms of the disease were observed at periodical intervals. A total of ten fruits were assessed for pathogenicity.

Histopathological studies of infected twigs and fruits

Histopatholgical studies were made by microtome sectioning of infected and uninfected twigs and fruits of noni followed by staining and microscopic observations. Infected fruit and twigs were examined for the presence of propagules of fungal pathogen with the help of image analyzer. At 10X x 40X magnification with CETI, capture pro software supplied by Medline Scientific Ltd, UK.

Mode of Spread

Source of infection

Twigs and fruits exhibiting brown lesion with pin head shaped acervuli were collected from noni orchard. Twigs and fruits were sliced in to small bits (4-6 cm) and washed with running tap water, surface sterilized with 0.1 per cent fmercuric chloride and washed with sterile distilled water. Later twigs and fruits were incubated at 5°C for 48 hours. After incubation the bits were examined under stereo zoom microscope for the presence of fungal growth.

Aerodynamic studies

Clean microscopic slides were evenly smeared with commercially available wax on one side of slide. Later the slides were tied with Noni tree in East, West, North and south directions at 30 cm, 60 cm, 90 cm, 120 cm, 150 cm, 180 cm and 210 cm height intervals from the ground level. After 24 hours the slides were examined under microscope for the presence of air borne conidia.

Results and Discussion

Survey for the incidence of fruit rot diseases

Survey was conducted in major Noni growing areas of Tamil Nadu and Kerala during September, 2010 .Survey revealed that the incidence of dry and wet fruit rot were found in surveyed area . Among five districts surveyed across two states, the high incidence of dry fruit rot was recorded in Coimbatore about 71.40 per cent followed by Aliyar Nagar of Coimabtore accounted 63.20 per cent. The lowest incidence of dry fruit rot (*Colletotrichum*) was observed in kodunallur, Ernakulum district of Kerala it ranged 24.80 per cent disease incidence. Among surveyed area Thrissur district was found with more incidence of *Alternarial* dry fruit rot about 45.00 per

cent followed by Coimbatore district it accounted 36.76 per cent .Survey across the districts of two states revealed that high incidence of wet fruit rot in Coimbatore (63.32 per cent) followed by Kodunallur it ranked 41.80 per cent incidence .The disease incidence of other place were detailed in Table 1. The careful observation of both the diseases revealed that there exists a marked difference in the symptom. Symptom was noticed on the flowers, fruits and twigs. As the fruits enlarge, the lesions also enlarge. It leads to splitting followed by drying and shrinkage of the fruits. Similarly, the infection of *C. acutatum* in olive tree caused blight of twigs, stem, inflorescence and later the infection spread to fruits also. The pathogen on the infected twigs remain as latent infection and get expressed only after fruit initiation and thereby causes infection to the fruit (Moral et al., 2009). Similarly, the results in the present study also explain the association of pathogen causing twig blight, stem blight, flower blight and fruit rot. Nakkeeran (*et al*., 2009) reported the occurrence of the infection of *C. gloeosporioides* and *A. alternata* on noni fruits. Similarly, the association of *A. alternata* and *C. gloeospoioides* was reported to cause leaf blight in Noni (Manjunath *et al*., 2010). The infection on the leaf and twigs might have spread to the fruits and caused dry fruit rot. Kaur *et al*. (2007) noticed flower infection as necrotic lesion lesions on unopened and opened flowers of citrus. The present study also confirmed the association of *C. gloeosporiodes* infection on flower and lead to drying of flowers followed by the fruit infection. Similarly, Masanto *et al.* (2009) reported the association of *C. gleosporioides* infected twigs and fruits of dragon fruit.

Table 1 : Distributions of Noni fruit rot diseases in major Noni growing areas of Tamil Nadu and Kerala

Place	District	% dry fruit rot *		% wet fruit rot
		Colletotrichum	Alternaria	
Coimbatore	Coimbatore	71.40 (57.68)a	36.76 (37.32)b	63.32 (52.72)a
Denkanikottai	Krishnagiri	63.20 (52.65)b	35.68 (36.67)c	37.98 (38.04)d
Tindivanam	Villupuram	45.64 (42.65)d	25.54 (30.35)e	24.30 (29.53)e
Aliyar nagar	Coimbatore	38.40 (38.29)e	30.87 (33.75)d	52.00 (46.14)b
Kodunallur	Ernakulam	24.80 (29.86)f	19.40 (26.13)f	41.80 (40.27)c
Viasa	Thrissur	54.63 (47.65)c	45.00 (42.33)a	26.24 (30.81)e

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT; Values in parentheses are arcsine transformed values

Symptoms of dry fruit rot caused by *Colletotrichum gloeosporioides*

Colletotrichum gloeosporioides infect all parts of noni plant irrespective of the stages of crop growth. The symptoms were observed on twigs, flowers and fruits. Symptoms of the infection caused by *C. gloeosporioides* on the flowers appear as dull brown lesions. The infected flowers dried within 48 h after infection. Examination of the flowers under stereo zoom microscope revealed the presence of brown necrotic spots on the corolla tube adhering on the noni fruit. The twigs infected by *C. gloeosporioides* were characterized by the presence of necrotic brown lesions with yellow halo. Later the lesions enlarge in size which was characterized with grey centre. On the grey centre number of minute, pin head shaped fructifications of the acervuli was observed .The necrotic lesions spread gradually towards fruits through peduncle and leads to the development of infection on flowers and fruits.

Symptoms of dry fruit rot occurring on the fruits incited by *C. gloeosporioides* are characterized by the presence of small circular reddish brown, slightly sunken necrotic spot on fruits. It later turns, into dark brown lesions, expands both upward and downward, coalesces, dry and shrinks. As fruits enlarge, the lesion also expands, leading to splitting and drying of fruits. Subsequently, small pinhead like acervuli appears on the infected tissues. Subsequently, the infected fruits shrinks, dries off and get mummified. After mummification the infected fruits were colonized by saprophytic moulds like *Fusarium* spp*, Aspergillus,* and *Penicillium*.

Symptoms of dry fruit rot caused by *A. alternata*

The dry fruit rot caused by *A. alternata* is characterized by the presence of black necrotic sunken spot of 2-3mm dia on the green unripe fruits. During favorable environment spot turns to dark black lesion and coalesced. Centre of the lesions are black in color with alternate concentric zonations. As the fruit expands it leads to splitting and drying of the fruits coupled with the saprophytic infection of other moulds.

Symptoms of soft rot caused by *Pantoea agglomerans* on fruits

The bacterial infection was associated with matured un-riped fruits. Symptoms were characterized by the presence of brown colored water soaked lesion on external surface of the fruit. Later with in 24 to 48 h after infection, the lesion spread to the entire fruit and the infected fruits emit a bad odour. The affected tissue becomes softened and rots subsequently. Later, the infection extends up to the peduncle and the fruits fall down after 24-48 h after infection.

Identification of dry rot pathogens

Colletotrichum gloeosporioides

The colony of the pathogen was white to pale grey on PDA medium. The pathogen produced hyaline septate, conidiophores bearing ovoid to cylindrical shape conidia, one celled, with one or two oil globules. The length and width of the conidia was 11.25 µm and 6.5 µm respectively.

Alternaria alternata

The colony colour on the PDA medium was dark brown. The colony was characterized by flat growth on PDA medium. It produced abundant, branched, septate, brown mycelia. Conidiophores were simple, olive-brown with septations. The conidia were smooth, brown to dark brown in colour, obclavate measuring 30.55 to 42.35µm x 11.73 to 17.30 mm, muriform or irregular with 0-5 cross septa.

Isolation and identification of pathogen associated with Soft / Wet fruit rot.

The pathogen associated with bacterial fruit rot was isolated on nutrient agar medium. Based on the morphological and biochemical characterization the pathogen was identified as *Pantoea agglomerans*. The identification was further confirmed through, FAME analysis and 16s rDNA.

Cultural and morphological characterization

The bacterial colony was yellow in colour. Colony morphology was mucoid with smooth margin on NA medium. . Bacterial cells were rod shaped. The bacteria showed negative reaction to Gram staining, indicating that it is a Gram negative bacterium.

Biochemical properties of bacterial pathogen

Series of biochemical test were performed with micro express kit for confirmation of bacterial pathogen. According to kit specification, the pathogenic bacteria posses 90% positive reaction for Citrus utilization, Nitrate reduction, Glucose utilization and Arabinose utilization, 75-89% for Phenylalanine Deamination, 26- 75% for Ornithine utilization and 25% for lactose utilization . Pathogenic bacteria exhibited negative reaction to lysine utilization, Urease detection, H_2S production, Adonital utilization, Sorbitol utilization and Oxidase detection (Table 3) Based on the micro express kit analysis, the pathogen was confirmed as *P. agglomerans.* In addition the bacterium also expressed positive reaction for KoH, Simmon citrate utilization and catalase test.

Table 3 : Biochemical characterization of P.agglomerans

S. No	Biochemical assay	Pantoea .spp
1	Lysine utilisation test	-
2	Ornithine decarboxylation	d
3	Phenylalanine deamination	(+)
4	Urease detection	-
5	Nitrate reductase test	+
6	H2S production	-
7	Citrate utilisation	+
8	Gucose utilisation	+
9	Adonitol utilisation	-
10	Arabinose utilisation	+
11	Lactose utilisation	(-)
12	Sorbitol utilisation	-
13	Oxidase detection	-

+ = positive (more than 90%); (+) = 76-89% positive; (-) = 11-25% positive; d= 26-75% Positive; - = negative (more than 90%)

Confirmation of *P. agglomerans* by FAME analysis

Identification of *P. agglomerans* was confirmed by Fatty acid methyl ester (FAME) analysis. Microbial identification system identified pathogenic bacteria as *P. agglomerans* with the similarity index (SI) of 0.536 with 0.3 separation of between the first and second choice. FAME analysis confirmed that the pathogen associated with soft rot of noni was *P. agglomerans.* This was in same line with the finding of Germida and Siciliano (2001) who identified wheat root colonizing bacterium *Enterobacter agglomerans* (*Pantoea agglomerans*) with the similarity index of 0.535. Coutinho *et al.* (2002) confirmed *Eucalyptus* leaf blight pathogen *P. ananatis* by fatty acid profiling.

Molecular confirmation *of P. agglomerans* through 16S rDNA analysis

The identity of soft rot bacterial pathogen was confirmed through PCR. The bacterial 16 rDNA was amplified with universal bacterial forward and reverse primers. Amplification of 16 rDNA revealed the presence of 1500bp amplicon. After amplification, the amplicon of 1500bp was purified from PCR product and sequenced. Partial sequences of *P. agglomerans* was submitted to NCBI, Gen bank, New York, USA, bearing accession no. JN036646. The sequence of the 1500bp of the amplicon is as follows.

16 S rDNA sequences analysis of pathogen

The 16S rDNA homology searches were performed using the BLAST program though the internet server at the National Center for Biotechnology Information, USA. The comparison of sequences of pathogens revealed 99 per cent homology with *P.agglomerans* (Table 4). The unweighted pair group method with arithmetic means (UPGMA) tree resulting rDNA sequences of pathogen had 99 percent similarity with sequences of *P. agglomerans* deposited in gen Bank. Genetic distance among the sequences from gen bank and sequence of 16S rDNA regions of pathogen is shown in Table 4. Based on the results, the pathogen was confirmed as *P. agglomerans.* The results are in agreement with the findings of Medrano and Bell (2007) where in they reported the amplification of 16 S r DNA region of *P. agglomerans* with16s -F and 16s -R specific primers that yielded 1.5 kb amplicon. Similarly Coutinho *et al.* (2002) differentiated *P. ananatis* from other species using 16 S rRNA primers. Krawczyk *et al.* (2010) identified *P. ananatis* associated with leaf spot disease of maize by using 16 s rDNA specific primers it yielded 1.5 kb amplicon.

Table 4 : Genetic similarity score between *P. agglomerans* and Gene Bank sequences

S. No.	Accession No.	Similarity score	Organism
1	HM130693	99.00	*Pantoea agglomerans* strain BJCP2
2	EU931561	99.00	*Pantoea agglomerans* strain ZFJ-6
3	JN036646*	99.00	*Pantoea agglomerans*
4	HQ443233	99.00	*Pantoea agglomerans* strain T2
5	HQ647265	99.00	*Pantoea agglomerans*
6	EF522820	98.00	*Pantoea* sp. 092305
7	GQ200831	99.00	*Pantoea dispersa* strain ND4
8	FJ560472	99.00	*Pantoea* sp. M3S5
9	JF958137	96.00	*Erwinia* sp. JSC-N3-112-2
10	HM130694	99.00	*Pantoea agglomerans* strain BJCP3

Pathogenicity

Pathogenicity of dry fruit rot by *C. gloeosporioides*

Koch postulates were proved for flower infection with *C. gloeosporioides* isolated from the infected flowers. The apparently healthy flowers sprayed after sterilization, with the conidial suspension of *C. gloeosporioides* (5 x 10^6 spore's ml^{-1}) resulted in the expression of brown necrotic spots on the corolla tube. It was followed by the colonization of *C. gloeosporioides* observed after 48h of incubation. Similarly, artificial inoculation of spore suspension of *C. gloeosporioides* (5 x 10^6 spores ml-

1) on twigs resulted with reproduction of brown lesions characterized with grey centre. The association of *C. gloeosporioides* with twig infection was confirmed by reisolation Inoculation of *C. gloeosporioides* on the fruits at the rate of 5 x 10^6 spores ml^{-1} after making pin prick injuries with sterile needles on to the green immature fruits developed necrotic brown, sunken spots after four days of inoculation. The spots later expand and coalesced. It resulted in the shrinkage and rotting of fruit. The pathogen was reisolated from the fruits and was confirmed as *C. gloeosporioides* based on their morphological and conidial characters.

Pathogenicity of dry fruit rot by *A. alternata*

Inoculation of *A. alternata* (5 x 10^6 spores' ml^{-1}) on to the healthy green immature fruits expressed small black necrotic spots after four days of inoculation. The spots later enlarged and were characterized by the presence of black concentric rings. Re isolation of pathogen from the infected tissue confirmed the association of *A. alternata*.

Pathogenicity of wet rot *in vitro*

Apparently healthy fruits were inoculated with the suspension of bacterial pathogen (10^6 cell m^{-1}) after pinprick. Later inoculated fruits were incubated in sterile bottles moistened with sterile absorbent cotton. Infected fruits expressed water soaked lesions at the point of inoculation after 24h of inoculation. The brown lesions extended both upward and downward leading to rotting of fruits coupled with the emission of a foul smell. The pathogen was reisolated and Koch's postulate was completed to confirm the pathogenicity.

Pathogenicity of wet rot in field

Soft-rot symptoms were observed on noni fruit after 24 - 48 h of inoculation of pathogen under field condition. The fruit without wound did not show any fruit rot symptoms. The bacteria caused soft rot disease and were reisolated from Noni fruit expressing the soft-rot symptoms. The Koch's postulates were also confirmed. However, the symptoms were not observed on uninoculated control.

Mode of Spread

Primary and secondary source of infection

Incubation of infected twigs and fruit bits in humid chamber at 5°C leads to germination of fructification. Microscopic observation of incubated twigs and fruit bits under stereo zoom microscope revealed the presence of dormant whitish mycelial growth and acervuli. Thin sections from incubated twigs revealed the presence of acervuli with black setae and capsule shaped conidia of *C. gloeosporioides*. Percent association of *C. gloeosporioides* on flowers, pericarp, twigs and fallen leaves were

32.5%, 28.0%, 62.0% and 23.0% respectively. Similarly, the per cent association of *A. alternata* on fallen leaves and fruit pericarp was 19.5% and 16.0% respectively (Table 2).

Table 2 : Per cent association of dry fruit rot pathogen of Noni

Source material	Per cent association of pathogens	
	C. gloeosporioides	A. alternata
Flowers	32.5	0.0
Pericarp	28.0	16.0
Twigs	62.0	0.0
Leaves fallen on to soil	23.0	19.5

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT; Values in parentheses are arcsine transformed values

Microscopic observation of slides after 24 h revealed the presence of the conidia of *C. gloeosporioides* and *A. alternata*. The number of conidia per microscopic field varied as per the height where the slides were tied on to the noni trees. Both the conidia were observed at a distance of 120 – 150 cm. The number of *Colletotrichum* conidia was 50 to 57 at 120 and 150 cm respectively. Similarly the maximum numbers of 52 to 54 conidia of *Alternaria* were observed at the height of 120-150 cm (Table 5). The deep seated dormant mycelium in the twigs and stem might colonize during the conducive period and lead to the production of acervuli during the death of the tissues. Hence, the dormant mycelium may serve as the primary source of inoculum to cause fruit rot of noni. Subsequently the conidia released from the fructifications may serve as the secondary source of inoculum for the further spread of the disease. Similar observation was noticed in olive fruits infected by anthracnose disease (Moral *et al*., 2009). Moreover, the aerodynamic studies on the dry fruit rot pathogens, indicated that the pathogens were air borne in nature.. This study suggested that the fungal spores of both pathogens were on middle part of the tree.

Table 5 : Trapping of air borne conidia of C. *gloeosporioides* and A. *Alternate* in Noni orchard

Direction	Different height													
	30 cm		60cm		90cm		120cm		150cm		180cm		210cm	
	C*	A*	C	A	C	A	C	A	C	A	C	A	C	A
East	10	7	23	9	33	24	50	33	57	35	26	11	13	8
West	13	6	24	14	30	25	54	34	52	38	23	16	11	6
North	12	5	22	10	34	22	53	35	54	37	25	13	14	7
South	11	8	21	11	31	21	51	36	55	32	27	14	12	9

Values in columns are mean of three replications

C*= values in respective column indicates number of *C. gleosporioides* conidia per microscopic field

A*= values in respective column indicates number of *A. alternata* conidia per microscopic field

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Disease management in medicinal crops

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Keywords : Medicinal plants, diseases, fungicides, biological control

Abstract : India is one of the twelve mega biodiversity hot spot regions of the world and one fifth of all the plants found in India are used for medicinal purpose. Medicinal plants are important for pharmacological research and drug development, not only when constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drug or as models for pharmacologically active compounds. The world market for plant derived chemicals *viz.*, pharmaceuticals, fragrances, flavours and colour ingredients exceed several billion dollars per year. Diseases can cause substantial losses in medicinal plants. Yield loss due to diseases is one of the important factors that limit successful production of all horticultural crops. Coleus (*Coleus forskohlii)* rose into prominence by virtue of its alkaloid, forskolin, a labdane diterpene present in the swollen primary roots (tubers). The productivity of Coleus has been hampered by its susceptibility to nematode, root rot and wilt diseases. The occurrence of major diseases of important medicinal plants and their management are discussed.

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Introduction

World Health Organization (2003) estimates that 80% of the world's population depends on traditional medicine for their health needs. In many developed countries, traditional herbal remedies are making a comeback as alternatives to modern medicine. Exports from India have increased from Rs. 460 crores in 1995 to 1200 crores in 2000 (Ghosh, 2000). The world of naturals is storming the globe with scientific rationale and trends that are fast emerging to support better health and life through plant and plant products. The demand for the products from the medicinal plants such as phytochemical, steroidal, biologically active compounds, alkaloids etc. is increasing in the national and international market.

According to the Hindu Survey of Indian Agriculture report, there was about US $ 60 billion sales of herbal medicines in the world and projected to increase at an average annual growth rate of 6.4 per cent reaching US $ trillion by the end of 2050 (Anon., 2007). India being the second largest exporter, next to China account for

about 13 per cent of global imports. To meet the demand, medicinal plants are being collected indiscriminately from forests resulting in dwindling supplies and endangering the survival of the species themselves.

Crop protection (pest and disease control) is a new area of research for medicinal plants. There is very little information available on the types of diseases on medicinal plants, and the effects of them on the plants. Yield loss due to diseases is one of the important factors that limit successful production of all horticultural crops. The success of controlling the diseases lies in correct identification of the pathogen and knowledge about their life cycles on a particular crop.

Coleus *(Coleus forskohlii)*

Coleus (*Coleus forskohlii* Briq.) syn. *Coleus barbatus* [(Andr.) Benth.] is an important medicinal plant belonging to the family Lamiaceae (Mint family). First found in India, it is now grown all over the world. *C. forskohlii* is a hardy perennial, having thrived for 3,000 years already. Mint family plants are also usually very strongly fragrant because they contain vast amounts of essential oils. The plant is fleshy and has a scent very similar to camphor. The plant has very high medicinal value. This compound is used for the treatment of glaucoma, congestive cardiovascular diseases, asthma, allergies, psoriasis, cancer metastases, obesity and weight loss (Shah *et al.,* 1980). This alkaloid has the unique property of activating all hormone-sensitive adenylate cyclase enzymes in biological systems (De souza & Shah, 1988). Forskolin content has been found to vary from 0.07 to 0.59% of dry tubers and just 1 g of forskolin costs $85, showing the importance of this crop (Gowda, 2000). In addition, forskolin is reported to have been used in the preparation of medicines preventing grey hair and restoring grey hair to its normal colour. Recently, with increase in the demand of forskolin, its cultivation has picked up in southern states with annual production of about 100 tonnes from 700 ha in India (Anon., 2004). Root juice is given to children suffering from constipation. Its foliage is employed in treating intestinal disorders and used as condiment since times immemorial.On account of increased exports, cultivation of Coleus is expanding in Tamil Nadu, Karnataka, Andhra Pradesh and the present area is about 5,000 acres. In Tamil Nadu, it is cultivated in Salem (Attur), Erode (Sathyamanagalam), Dindugal districts in about 2,000 acres under contract farming. Recently farmers have started to grow it as a crop because of its economic potential (Vishwakarma *et al.,* 1988).

Diseases

The crop is subjected to attack by many diseases *viz.*, stem blight caused by *Phytophthora nicotianae* var. *nicotianae*, wilt caused by *Sclerotium rolfsii*, leaf spot caused by *Botryodiplodia theobromae*, bacterial root rot caused by *Pseudomonas aeruginosa*, dry root rot caused by *Macrophomina phaseolina*, aerial blight caused by *Rhizoctonia solani*, collar rot complex caused by *Rhizoctonia*

bataticola, *Fusarium chlamydosporum*, *Sclerotium rolfsii* and root-knot nematode (*Meloidogyne* species). Among these, root-knot and wilt disease complex is occurring in severe form and cause heavy losses. Due to this disease complex the yield loss was ranged up to 50 to 60 per cent. Therefore, it is necessary to take effective control measures to save the crop from complete devastation.

Leaf spot

The lesions are initially brown and punctiform, becoming elliptic, subcircular to irregular and pale brown in colour. They are well delimited with a dark brown rim (up to 5 mm in diameter), distributed on the lamina, sometimes coalescing and leading to extensive necrosis and yellowing. A dematiaceous fungus (*Corynespora cassiicola*) was consistently found sporulating in the centre of the lesions.

Blight

It is common during monsoons or during period of high humidity. Symptoms include water soaked leaf spots that increased rapidly in size becoming light tan to brown and later necrotic. Severe infection results in defoliation and death of plants. *Rhizoctonia solani* has been reported to cause the leaf blight of *C. forskohlii*.

Root-rot/wilt

It is the major disease of *C. forskohlii* causing heavy losses (>50%) in south India. Root rot is a disease caused by a variety of fungi/bacterium species that love standing water. Disease shows various symptoms like yellowing and wilting of leaves, brown to black roots, oozing, putrefaction and decaying of roots and unhealthy plants. *Fusarium chlamydosporum* was found to be the causal agent of the disease. The symptoms include gradual yellowing, marginal necrosis and withering of leaves followed by loss in vigour and premature death. Such plants show discoloration of roots and complete decaying of tap and lateral root system. Such affected plants are finally killed due to severe root and collar rots. The infected tubers rot and emit bad odour.

Ralstonia solanacearum was reported to be causing vascular wilt of *C. barbatus*. The symptoms include initially brown later on becomes black roots due to decaying, oozing and putrefaction of roots. Boby and Bagyaraj (2003) reported that the fungus *Fusarium chlamydosporum* causes root rot of *C. forskohlii*. Kamalakannan *et al*. (2005) reported root rot disease (*Macrophomina phaseolina)* on *C. forskohlii.* The symptoms observed were yellowing and drooping of the leaves, blackening of the stem, rotting of the root, basal stem and bark peeling. The presence of black sclerotia was observed on the rotted portion. The mycelium was initially hyaline and later became grey in colour. Sclerotia were minute, black, round to oblong or irregular in shape with mycelial attachment.

Plant parasitic nematode fungal interactions

Senthamarai *et al.* (2006) observed nematode fungal disease complex involving *Meloidogyne incognita* and *M. phaseolina* on *C. forskohlii*. Patil *et al*. (2003) reported that the prevalence and severity of different diseases and nematode problems in north eastern Karnataka, on medicinal crops such as Coleus, Long pepper, Ashwagandha etc. and estimated the loss to the tune of 5-20 per cent due to the presence of root-knot, damping off, rhizome rot, seed rot, seedling blight and dieback diseases. Krishna Rao and Krishnappa (1994) observed that inoculation of nematode (*M. incognita*) along with fungus (*Fusarium oxysporum* f. sp. *ciceri)* at lower levels resulted in 19.88 per cent wilt incidence when compared to 6.66 per cent with fungus alone. However, maximum wilt incidence (66.70%) was observed when inoculated with 2 juveniles per g of soil and 25 g inoculum per 500 g soil of nematode and fungus respectively. Presence of root-knot nematode along with fungus adversely affected the root-knot disease.

Senthamarai *et al*. (2006) carried a glasshouse experiment to study the interaction of *M. incognita* and *M. pahseolina* on *C. forskohlii*. The nematode multiplication was adversely affected when fungus was inoculated prior to nematode. Simultaneous inoculation of nematode and fungus as well as nematode followed by fungus 15 days later, caused 100 per cent root-knot disease and significant reduction in plant growth compared to the inoculation of fungus alone or fungus inoculation prior to nematode. Significant reduction in number of branches, shoot length, root length, fresh shoot weight, dry shoot weight, dry root weight and tuber yield per plant was observed in simultaneous inoculation of *M. incognita* and *F. chlamydosporum* followed by *M. incognita* seven days prior to inoculation of *F. chlamydosporum* over untreated control.

Screening of popular Coleus genotypes for their reaction to *Meloidogyne incognita* and *Fusarium chlamydosporum*

In view of the long range programmes in management of complex diseases based on the use of resistant cultivars, the screening study helps in identifying promising cultivars having some degree of resistance/ tolerance to the nematode and/or fungus disease complex. Eleven genotypes were tested for their reaction to *M. incognita* and *F. chlamydosporum* together under glasshouse condition. Among 11 genotypes, yellow tubers showed maximum shoot and root length, whereas, minimum shoot and root length was noticed in Sunadolli local. Yellow tubers which showed least number of galls, root-knot index, nematode population, as compared to other genotypes. With respect to root rot index minimum root rot index was observed in genotype like Rabakavi local and yellow tubers (Kumar, 2008). Among 11 genotypes, yellow tuber and Rabakavi local genotypes showed resistant reaction, Orange tuber and Nimbanur local genotypes showed moderately resistant reaction. The study

indicated that the ability of nematode to induce wilt susceptibility was not uniformly consistent (Kumar, 2008).

Management of root-knot and wilt disease complex by organic amendments, plant products and bioagents

Amendments

Among the several methods of managing the plant diseases, soil amendments is one of the effective method. Amendments in the form of plant debris, green manures, farmyard manures, compost, oil cakes and fertilizers are known to improve crop productivity by improving nutrient status and soil tilth. Addition of amendments to soils might have increase microbial activities in soil to suppress diseases. Sivakumar and Marimuthu (1986) reported a significant reduction of 44.40 per cent over control in nematode population with neem cake applied at 100 kg per ha. Groundnut cake and neem cake along with carbofuran were used to manage *Meloidogyne incognita* (Reddy and Khan, 1991).

Goswami *et al.* (1993) indicated that organic amendments showed fluctuations in the mortality of *M. incognita* and among the organic amendments tested, mustard was the most nematoxic. Amendments of soil with decomposable organic matter is recognized as the most efficient method of changing soil and rhizosphere environment, thereby adversely affecting the life cycle of pathogens and enabling the plant to resist the attack of pathogens through better vigour or altered physiology. It was also reported that chemicals like ammonia and fatty acids liberated during the decomposition of neem cake could be one of the factors involved in nematode control. Apart from this, the neem cake itself contains formaldehyde (0.25%), which is another factor responsible for nematode control. Similarly, in the soil amended with saw dust, phenols are liberated which inhibit the growth and development of root-knot nematode (Sitaramaiah and Singh, 1978).

Effect of plant products on fungal wilt pathogens

Management of disease through fungicides alone leads to cause soil residual problem and health hazards, besides involving higher input cost. One of the recent approaches for plant disease management is exploitation of plant products. Mansoor (2006) reported neem leaf, seed powder, oil cake and two nematicides *viz.*, carbofuran and phorate alone and in combination reduced the root-knot development caused by *M. incognita*. Highest reductions in the nematode infections and corresponding improvement in plant growth was noted in pots treated with cake combined with carbofuran as well as increasing shoot length, shoot dry weight, root fresh weight, number of leaves of Japanese mint. Neem cake at two per cent reduced number of root galls and population densities of *M. incognita.*

Sahayarani (2003) reported wintergreen oil at a concentration of 0.05 per cent effectively inhibited the spore germination of powdery mildew pathogen of *Phyllanthus niruri*. In recent days, a special attention is being shown to the formulation of botanical fungicides since the formulated products are more effective than crude plant oils for its easy applicability and required in smaller quantity. Vanitha (2010) reported that 30 and 40 EC formulations of wintergreen oil, lemongrass oil and their combination significantly inhibited the mycelial growth and spore germination of *Alternaria chlamydospora* which causes leaf blight disease of *Solanum nigrum*. The compatibility of the formulations with biocontrol agents *viz., Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* showed that these three formulations were not inhibit the growth of biocontrol agents. The EC formulations tested for its storability at room temperature for different periods showed that they retained their antifungal effect up to 60 days.

The aqueous and 50% ethanol extract of *Annona squamosa, Azadircta indica, Eucalyptus* spp., *Ocimum sanctum, Lawsonia inermis, Allium schoenoprasum, Cinnamomum verum, Zingiber officinale, Piper nigrum, Calendula officinalis* species were found to have antifungal activity against *Fusarium chlamydosporum* causing root rot of *Coleus amboinicus* and *C. forskohlii* (Chathuri *et al.,* 2011).

Biocontrol agents

Chemical or physical methods against soil-borne diseases have limited efficacy or are too expensive. Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues which may lead to serious ecological problems. The literature on biological control of soil borne pathogens of medicinal plants is very limited. Fungi belonging to the genus *Trichoderma* are the most promising biocontrol agents against a range of plant pathogens under a variety of environmental conditions (Chet, 1987). Another important group of biocontrol agents is the pseudomonads. Even though *Pseudomonas fluorescens* is mainly considered as a PGPR, it can suppress wide range of pathogens including *Fusarium, Rhizoctonia* and *Pythium* (Nautiyal, 1997). Vesicular arbuscular mycorrhizal (VAM) fungi suppressing the activity of root pathogens is also a well documented phenomenon (Mohan and Verma, 1996).

A field study was undertaken to study the possibility of controlling the root rot disease of *C. forskohlii* using three biocontrol agents *viz*., *Glomus mosseae*, *Pseudomonas fluorescens, Trichoderma viride,* singly and in combination. Planting of coleus cutting was done in wilt sick soil. Inoculation with *T. viride* + *G. mosseae* gave the best result in controlling the disease. The same treatment also resulted in maximum growth, yield and root forskolin concentration of Coleus. Plants treated with *T. viride* + *G. mosseae* showed a disease severity index of 33.28% compared

to uninoculated control plants, which had a maximum disease severity index of 85.5%. The fungicide Emisan (0.2%) was not as effective as the biocontrol agents in controlling the pathogen (Boby and Bagyaraj, 2003).

Combination of NSKE 5 g per kg + *P. lilacinus* 10 g per kg + *T. viride* 10 g per kg + *P. fluorescens* 10 g per kg as soil application recorded lowest number of galls, root-knot index, root rot index, number of egg masses and nematode population. Tripathi and Singh (2006) reported combination of fungal biocontrol agents along with oil cakes is an ideal for controlling nematode population and increasing plant growth. Ramaprasad Shresti (2005) also reported application of *T. viride* + nemato for controlling wilt incidence, nematode population of root-knot nematode and number of galls on *C. forskohlii* under field conditions.

Effect of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on root rot and wilt, growth and yield of *Coleus forskohlii*

Biological control involving arbuscular mycorrhizal (AM) fungi (Mohan and Verma, 1996) and *P. fluorescens* (Nautiyal, 1997) is considered as a preferred disease management strategy because chemical control results in accumulation of harmful chemical residues leading to serious ecological and health problems. Management of root rot disease under low and high inoculum levels was assessed with four arbuscular mycorrhizal (AM) fungi and a strain of *P. fluorescens*. The AM fungus *Glomus fasciculatum* and *P. fluorescens* were the most effective that reduced the severity of root-rot and wilt of *C. forskohlii* by 56-65% and 61-66%, respectively, under lower and higher levels of pathogen *F. chlamydosporum*, *G. fasciculatum.* In plants treated with *P. fluorescens*, an increase of 97-223% and 97-172% in dry shoot and root weight, respectively, was observed. Increase in yields with both the biocontrol agents was accompanied by increase in P uptake (230-303%) and in K uptake (270-335%). The forskolin content of the roots was significantly increased (14-21%) by *G. fasciculatum*, *P. fluorescens* or *G. mosseae* under lower inoculum level of pathogen (Rakshapal Singh *et al.*, 2009).

AM fungi are known to increase the resistance of plants to soil-borne pathogens by modification of cell wall, production of antimicrobial compound and altered rhizosphere microflora (Sampangi and Bagyaraj, 1989). The disease suppressive effects of *P. fluorescens* are also well established and may result from production of antibiotics, siderophores, hydrocyanic acid, salicylic acid and competition for nutrients (Sharma, 2006).

Downy mildew disease

A new downy mildew disease that affects Coleus has recently been discovered and is causing alarm for both Coleus growers and researchers. The microscopic appearance of the coleus pathogen looks like *Peronospora lamii*, a familiar downy

mildew that affects some members of the mint family, such as Salvia. Downy mildews are not fungi; instead they are relatives of *Pythium* and *Phytophthora* and are more closely related to algae. They are favored by very moist, humid environments, such as those prevalent during plant propagation in the spring (Margery, 2006).

Symptoms

The symptoms include irregular, brown leaf spots, leaf twist and leaf drop. The leaf spotting can take the form of squamish patches bounded by larger veins. The fungus produces branched conidiophores that protrude through the stomata on the undersurface of leaves; ovoid conidia are produced on the pointed tips of the branches. At first, the spores are transparent and then turn grayish-brown as they mature, making the leaf undersurface appear somewhat 'dirty' to the naked eye. The sporulation can be sparse, but sometimes a thick, downy coating develops even on leaves that look perfectly normal. The disease can be spread when infected plants are moved or it gets on worker's hands or clothing. It also can be splashed or blown from one plant to another. The effective fungicides include Stature DM (dimethomorph), Chipco Aliette (fosetyl-Al), Alude (phosphorus acid), Compass (trifloxystrobin) and Heritage (azoxystrobin). For protection against downy mildews, mancozebs are effective and coppers are also helpful. Keeping stock plants after an outbreak of downy mildew will be a very risky proposition. Keeping seed-grown coleus separate from cuttings-grown coleus will be especially desirable. The importation of unrooted coleus cuttings from other countries is another possible avenue for introducing downy mildew, so offshore production sites also need to establish downy mildew management programs.

Noni (*Morinda citrifolia*) Noni is susceptible to attack by a wide range of pests and disease-causing pathogens. However, the damage depends upon the pest or pathogen and upon the environment.**Diseases** In damp, high-rainfall or flooded areas, noni is prone to certain plant diseases caused by fungi, leaf spot (*Colletotrichum* sp.) and stem, leaf and fruit blights (*Phytophthora* sp. and *Sclerotium rolfsii*). They can be minimized by sanitation (picking up or removing severely diseased leaves) or by periodic application of approved fungicides. Some foliar diseases caused by fungi (fungal leaf spots or black flag disease caused by the fungus-like *Phytophthora*) may significantly inhibit leaf growth and fruit development.**Black flag disease** Noni black flag was first discovered in 2000 in the Puna district near Opihikao on the island of Hawaii. The disease is caused by a *Phytophthora* sp. Foliar symptoms of noni black flag include: black leaf spots and leaf blight; brown to black stem blight; brown to black soft rot of fruits; fruit mummification; severe defoliation (hanging, diseased leaves are referred to "black flags"); blackened leaf veins; death of stems; plant death (Nelson, 2004). Roots and woody portion of the plant are not normally infected. Noni black flag is a major threat to noni farms in areas where the disease it occurs. Large yield losses and even

plant death are possible. Noni black flag is favored by frequent rains, high winds, warm weather and high relative humidity. Spores are dispersed by splashing rain and wind.

Control:

- Inspect noni plants regularly during and after periods of extended rainfall. Promptly prune, remove and destroy symptomatic foliage and fruits to reduce pathogen inoculum levels.
- Remove fallen or pruned branches, stems, leaves and fruits.
- Promote air circulation within the noni canopy to ensure rapid drying of leaves and fruits.
- Ensure good soil drainage.
- Control weeds around the noni plants.

The most common and severe problem for noni is root-knot disease caused by root-knot nematodes (*Meloidogyne* spp.). These soil-dwelling, root-parasitic roundworms are very destructive to noni and must be kept out of the nursery. The disease can cause farm failure. To keep nematodes out of nurseries, use soil-less media or only heat-treated soil for seedlings. Once established in a field, root-knot nematodes are virtually impossible to eradicate and can eventually result in plant death. Root-knot nematodes are best controlled by avoiding them i.e. by starting with nematode-free seedlings and by planting the seedlings in a nematode-free location. Avoid planting noni where it does not grow naturally and avoid fields where other crops have been planted. Rocky soils are best for noni cultivation. Proper use of irrigation, fertilizer, and composts can help in minimizing the damage caused by root-knot nematodes.

Glory Lily (*Gloriosa superba)*

Gloriosa superba L. (Liliaceae) is a striking tuberous climbing plant with brilliant wavy-edged yellow and red flowers. The name Gloriosa comes from the word gloriosus, which means handsome and superb from the word superba clearly alluding to the beautiful flowers which appear from November to March. Flame lilies are grown commercially for a chemical compound. They are also used by certain people to treat intestinal worms, bruises, infertility, skin problem and impotence. It is also said that sap from the leaf tip is used for pimples and skin eruptions.

Leaf blight – *Alternaria alternata*

Leaf blight disease was found on *Gloriosa* in West Bengal, India in 2004. Small brownish spots on leaves developed into concentric rings, which eventually darkened and coalesced to blight the entire leaf. The causal fungus was morphologically identified as *Alternaria alternata* (Fr.) Keissler. Symptoms appear in all stages. Initially, small, pale to brown, irregular or round spots appear on the leaves. Each

spot has a central necrotic lesion with concentric rings. In advanced stages, several spots coalesce together to from large blighted areas.

Management strategies

- Remove the infected plant debris from the field.
- Spray mancozeb (0.2%) or propiconazole (0.1%) or tebuconazole (0.1%) at the time of infection. Four to five sprays may be given at 15 days interval to check the leaf blight severity.

Root rot – *Macrophomina phaseolina*

Yellowing of leaves, discolouration and rotting of roots and dark brown lesions on the stem are the prominent symptoms of root rot disease. Rotting of tubers also occur. The presence of sclerotial bodies as small, black dot like structures, are seen on the stem portions. The pathogen survives in the soil for several years. The yield loss ranged up to 50 to 60 per cent due to this disease. The pathogen produces pycnidia which are black, flask shaped structures with an ostiole. Numerous pycnidiospores are released from the pycnidium.

Management

- Application of *Trichoderma viride* or *Pseudomonas fluorescens* at 2.5 kg/ha helps to prevent further multiplication of the pathogen.
- Soil drenching *Bacillus subtilis* (0.2%) can control the disease.

Tuber rot - *Sclerotium* spp.

It affects the underground tuber causing death of the plant. In the initial stages, infected tubers start becoming soft and the foliage exhibits yellow appearance. In advanced stages, the whole tuber gets infested giving an appearance of discoloured mass and the plant dies off.

Management

- Soil drenching with carbendazim (0.2%) can control the disease.
- Application of *Trichoderma viride* or *Pseudomonas fluorescens* at 2.5 kg/ha helps to prevent further multiplication of the pathogen.
- Provision of drainage facility, decreasing the frequency of irrigation can help in control of the disease.

Periwinkle (*Catharanthus roseus*)

Periwinkles are an extremely popular bedding flower because of their wide range of arresting colors and their heat- and drought-tolerance. However, these beautiful flowers are subject to a number of diseases that can turn gorgeous flower garden

into a wasteland if we do not properly prevent and control the issues. Careful planning and observation of periwinkle plants should enable us to keep them safe from these problematic infections.

Botrytis blight

Botrytis is a fungal infection that tends to manifest itself in small, black dots that cluster on the edges of the leaves of the periwinkle, then extend inward. The spores of the fungus splash onto the leaves during wet weather, then grow if the environment continues to be moist. This can be controlled by planting periwinkles in full sun and by removing any infected leaves as soon as we spot them.

Foliar blight

The disease was caused by *Phytophthora parasitica* and *Phytophthora nicotianae*. This infection creates dark brown streaks on the stem, then extends to leaves and, in some cases, flowers as well. Diseased leaves are gray-olive green to brownish. The rots progress from leaves to the stem and branches of the plant are killed. Yellow leaves and black leaf rots also occur near the stem. Movement of the fungus into the primary stem causes wilting of the entire plant.

Phytophthora nicotianae produces microscopic, hyaline (clear) spores called sporangia. These asexual spores are papillate (have a pointed tip), nearly spherical (round), and release swimming spores when water is available. They are attracted to host tissue or organic matter. If the environment is not wet enough, sporangia germinate by producing fungal threads or germ tubes. The fungus also produces a spherical spore with thickened walls. These are chlamydospores and do not release zoospores. They germinate by producing germ tubes. These spores are able to survive in soil for many months in the absence of the host. The fungus is favored by warmer temperatures (25 to 28°C) and moisture (Nagata and Aragaki, 1989).

Management

Use clean seeds and monitor seedlings for any sign of disease. Remove any diseased seedling immediately. Removal of diseased plants and plant parts reduces the amount of spores that spread disease. Fungicides are available that reduce disease levels. However, no chemical will eliminate this pathogen and the pathogen will always be present once it is introduced among susceptible plants. Mancozeb is a good contact fungicide and can reduce the number of leaf spots. Metalaxyl is an effective systemic fungicide that will protect leaves and new shoots.

Root and Stem Rot

This occurs when the roots and stems of periwinkles develop any of a variety of fungal infections. The stems turning black and the leaves are wilted and brown as if the plant is not getting any water. If there are plants with root and stem rot, remove

them from the bed immediately and treat the remaining plants with a fungicide to prevent the spread of the disease.

Leaf spot

Fungal leaf spot disease thrives on moist leaf surfaces and cause transparent, brown or black spots that disfigure periwinkle leaves. Flecks or black dots, the spore-bearing fruiting bodies surround some fungal spots. Often spots come together to form larger patches of dead tissue. Dig up and discard seriously infected plants together with the soil surrounding their roots. Spray at 10 day intervals with a flowable sulfur spray.

Canker and Dieback

Shoots blacken and die back to ground because of dieback disease. The tips of periwinkle vines turn dark brown or black, wilt and die back to the soil. The disease is most troublesome during rainy weather. Remove and destroy infected plants or plant parts. A spray of Bordeaux mixture may give some control, possibly reducing the spread of the disease to adjacent plants.

Conclusion

Protection of crop plants from disease causing agents has been the focal point of the scientists in dealing with plant pathogens. Various control strategies are adopted for the improvement of the productivity and quality of medicinal plants. But, before adopting any pest control strategies, assessment of population as well as estimation of the damage, are to be given due importance. In depth analysis and realization about the starting features of microbial ecology have impelled workers to replace the term 'control' with 'management'. No single method of management has given a lasting solution. Prohibitive costs of chemicals and their adverse ecological impacts of major compulsion invariably ask for diversions of research priorities from chemical method to other alternatives. The successive shifting of priorities from chemical to cultural and currently to integrated strategies demonstrates the elasticity of the scientific ideas. Integrated management systems constitute best management strategies and tactics for the disease complex at hand. There is hardly any comprehensive effort of highlight the significance of multi-pathogenic scenario vis-a-vis their integrated management.

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Management of dry and soft rot of Noni (*Morinda citrifolia* L.) fruits

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Abstract : Eighteen bacterial endophytes of *Bacillus subtilis* were screened for the management of Noni fruit rot under *in vitro* and *in vivo*. The studies reflected that, the *B. subtilis* isolates BS2 and BS8 having fengycin, surfactin, bacillomycin and iturin biosynthetic genes were effective against Noni fruit rot both under *in vitro* and *in vivo*. Studies on the extracellular metabolites of the isolates BS1, BS2 and BS8 through GSMS analysis confirmed the presence of as L-glutamic acid dimethy ester, Dodecanoic acid, Petadecanoic acid, 14 methyl ester and 1,2-benzenedicarboxylic acid, disooctyl ester, undecanoic acid,10-methyl ester, Hexadecanoic acid, Diglycerol, Tetradecanoic acid-12 methyester, Teradecanoic acid, 12-methyl-, methyl ester and 2-hexadeconal with antimicrobial properties.

Introduction

Extensive cultivation of Noni (*Morinda citrifolia* L.) has resulted in outbreak of dry and wet fruit rot. Fruit rot diseases leads to considerable yield diminish in terms of quantity and quality of fruits. Recent survey in the usual Noni growing areas of Tamil Nadu and Kerala revealed the outbreak of fruit rot diseases during monsoon seasons. Hence, the present investigation was carried out to understand the host parasite relationship of fruit rot pathogens and management of fruit rot with biocontrol agents.

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Materials and Methods

Isolation and characterization of *Bacillus* spp. against fruit rot pathogen
Apparently healthy matured Fruits and were cut into sections 2-3 cm long. The tissue was put in beaker, soaked in distilled water and drained. Fruit tissue was rinsed in 70% ethanol for 30 seconds and then sterilized with 0.1% HgCl2 for 3 minutes. The tissue was then washed ten times with sterile water (Gagne et al., 1987). Surface-disinfected tissue was aseptically macerated with homogenizers. Macerated tissue was diluted into 10^{-1} dilution by adding 9 volumes of sterile distilled water. Serial

dilution was made up to 10^{-6} dilution. 100µl from appropriate dilutions were spread plated on NA medium in sterile Petri plates. The bacillus species were tested for the presence of fengycin, surfactin, bacillomycin and iturin genes using the specific primers of the respective genes.

In vitro screening of antagonists

In vitro efficacy of bacterial antagonists against *C. gloeosporioides, A. alternata*

The antifungal activity of *Bacillus* isolates were tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. The radial growths of mycelia (mm) of antagonist and pathogen were measured and percent inhibition (PI) was calculated.

In *vitro* efficacy of *B. subtilis* against *P. agglomerans*

Antibacterial activity of the isolates of *B. subtilis* was assessed by pouring the nutrient agar medium seeded with 1 ml of *P. agglomerans* (10^8 cells ml^{-1}) in to sterile Petri plates. .Eighteen hours old *B. subtilis* cultures spotted at the rate of 5µl, one cm away from the periphery of Petri plates and incubated at room temperature at 28 ± 2 ÚC for 72 h. The area of inhibition zone was measured.

Bioassay of crude antibiotics against *Pantoea agglomerans* by agar diffusion method

NA medium was poured in sterile Petri plate and allowed to solidify. A 5mm diameter sterilized cork borer was placed inside the medium and wells were made in center of the Petri plate. The crude antibiotics extracted was poured at the rate of 100 m1. After drying of crude antibiotics 18 hrs old *P. agglomerans* was evenly streaked over NA medium. The inhibition zone was recorded after 24 hrs of incubation (28 ± 2 ÚC). Crude antibiotics of *B. subtilis* isolates which showed inhibition against both fungal and bacterial pathogens of noni were taken for GC-MS analysis to identify antimicrobial compounds responsible to arrest the growth of pathogens.

GC-MS analysis of crude antibiotics

Identification of the antibacterial metabolites was done by GC-MS analysis. The m/z peaks representing mass to charge ratio characteristic of the antimicrobial fractions were compared with those in the mass spectrum library of the corresponding Organic compounds.

Effects of *B.subtilis* against Noni fruit rot under field condition

Dry and wet fruit rot

Field experiment was conducted during 2010-2011 in Noni fields located at medicinal garden established in botanical garden at TNAU, Coimbatore. Field trail was aimed to assess the efficacy of liquid culture (48 h old) of *B.subtilis* in controlling the fungal and bacterial fruit rot of Noni. The trials were carried out in a randomized block design (RBD) with three replications. Treatment details of field spray are described below.

S. No	Treatments	Treatments Details*
1	T1	Foliar spray BS2 liquid formulation @0.1%
2	T2	Foliar spray BS2 liquid formulation @0.5%
3	T3	Foliar spray BS2 liquid formulation @1%
4	T4	Foliar spray BS8 liquid formulation @0.1%
5	T5	Foliar spray BS8 liquid formulation @0.5%
6	T6	Foliar spray BS8 liquid formulation @1.0%
7	T7	Foliar Spray with Difenaconazole@ 0.5%
8	T8	Untreated Control

As the disease outbreak was severe during monsoon season, pre monsoon spray was given during June 2010, followed by monthly spraying of antagonistic strains up to the month of October 210. Similarly untreated control was also maintained to assess the efficacy of *B. subtilis* under field conditions against fruit rot. *But 500 ppm of streptocycline was used as chemical check for the management of wet fruit rot.

Results and Discussion

Isolation of B. subtilis

Eighteen isolates of endophytic bacteria were isolated from various crops.

Detection of antibiotic biosynthetic genes of B. subtilis *isolates*

Surfactin

Biosynthetic gene specific primers SUR1F and SUR1R amplified a 441 bp of *surfactin* gene, for all 18 strains (BS1 to BS18) (Table 1). However, the sequence analysis confirmed the presence of *surf* genes in the isolates BS2, BS6 and BS15. The BLAST searches for amplified region had 97-100% homology with surf genes deposited in gen bank sequences like AF233756, AF534916, and AY040867. The bankit numbers for the strains BS2, BS6 and BS15 are 1445962, 1445855, 1451152 respectively.

Iturin

Iturin specific primers amplified with the fragment size 647bp of iturin gene. Among all strains of *B.subtilis* BS1, BS2, BS3, BS4, BS6, BS7, BS8, BS10, BS11, BS13, BS14 and BS18 strains were amplified for *iturin* gene (Table 1). Partial sequences of BS1,BS2, BS6, BS 8 *iturin* genes was sequenced and submitted to NCBI, Gen bank, New York, USA, bearing accession no. JF926689, JF926691, JF926690, and JF926692 respectively.

Table 1 : Molecular detection of antibiotics genes from the isolates of *Bacillus spp.*

S.No.	Isolates	Surfactin	Iturin	Bacillomycin	Fengycin
1	BS1	+	+	-	+
2	BS2	+	+	+	+
3	BS3	+	+	-	-
4	BS4	+	+	-	+
5	BS5	+	-	-	-
6	BS6	+	+	+	+
7	BS7	+	+	-	+
8	BS8	+	+	+	+
9	BS9	+	-	-	-
10	BS10	+	+	-	-
11	BS11	+	+	-	+
12	BS12	+	-	-	-
13	BS13	+	+	-	-
14	BS14	+	+	-	+
15	BS15	+	-	-	-
16	BS16	+	-	-	+
17	BS17	+	-	-	-
18	BS18	+	+	-	+

+ = Presence of biosynthetic gene; - = Absence of biosynthetic gene

Bacillomycin

Strains of *B.subtilis* BS2, BS6, and BS8 expressed bacillomycin gene amplified at 875 bp (Table 1).. Blast searches revealed that these sequences had 98-100 per cent similarity with gene bank sequences HM210890, HM234098, AY137375 .Sequences of bacillomycin gene were submitted with NCBI bearing the bankit no 1455644(BS2) and14553(BS7) respectively.

The genomic DNA of the strains BS1, BS2, BS4, BS6, BS7 BS8,BS11, BS14,BS16and BS18 were amplified at 964 bp .BLAST searches of this sequences showed 98 per cent similarity with AJ011849 (Table 1). Sequences of fengycin gene submitted with NCBI bear the bankit no.1455621(BS2) , 1441653 (BS3). Ongena (2008) reported cyclic lipopeptides of the surfactin, iturin and fengycin families from *Bacillus,* impart successful biocontrol activity by direct suppression of phytopathogens and reinforcing of the potential host plant through stimulating induced systemic resistance phenomenon. Among *Bacillus* isolates used in this studies BS2, BS6, and BS8 yielded positive for all antibiotic genes. However, the amplification of gene pertaining to zwittermycin with specific primers did not yield any amplicon, confirming absence of the gene in all the isolates used under study.

In vitro screening of antagonists against dry fruit rot pathogens

C. gloeosporioides

All 18 *Bacillus* isolates were screened for their antagonistic activity against *C .gloeosporioides.* Among various isolates of *Bacillus spp* screened; the isolate BS2 inhibited *C. gloeosporioides* to an extent of 56.67 percent over control. It was followed by BS8, BS1 and BS6 with percent inhibition of 54.44, 52.22, and 50.22 respectively. Isolates BS3, BS7 and BS17 were not found to differ significantly (Table 2). Similarly the PGPR was found effective against *C. gloeosporioides* both under *in vitro* and *in vivo* causing noni leaf blight (Nakkeeran *et al*., 2009; Manjunath *et al*., 2010).

A. alteranata

In vitro screening of *Bacillus* isolates against *A. alternata* revealed that the isolate BS2 inhibited *A. alternata* up to 61.11 per cent (Plate 19) .It was followed by the isolates BS8, BS1, and BS6 which inhibited *A. alternata* up to 59.11, 58.44 and 56.11 per cent respectively (Table 2). Similarly the PGPR was found effective against *A. alternata* both under *in vitro* and *in vivo* causing noni leaf blight (Nakkeeran *et al*., 2009; Manjunath *et al*., 2010).

Table 2 : In vitro antagonism of B. subtilis isolates against *C. gloeosporioides* and *A. alternata*

S. No.	Isolates	*C. gloeosporioides*		*A. alternata*	
		Mycelial growth of the pathogen (mm)*	Per cent inhibition over control	Mycelial growth of the pathogen (mm)*	Per cent inhibition over control
1	BS1	21.50	52.22 (46.27)c	18.70	58.44 (49.86)b
2	BS2	19.50	56.67 (48.83)a	17.50	61.11 (51.42)a
3	BS3	25.56	43.20 (41.09)d	23.68	47.38 (43.49)e
4	BS4	26.75	40.56 (39.55)e	23.70	47.33 (43.46)e
5	BS5	24.75	45.00 (42.12)d	21.50	52.22 (46.27)d
6	BS6	22.40	50.22 (45.12)c	19.75	56.11 (48.51)c
7	BS7	25.25	43.89 (41.48)d	22.00	51.11 (45.63)d
8	BS8	20.50	54.44 (47.54)b	18.40	59.11 (50.25)ab
9	BS9	33.50	25.56 (30.36)i	29.50	34.44 (35.93)h
10	BS10	31.75	29.44 (32.05)h	30.50	32.22 (34.58)h
11	BS11	34.75	22.78 (28.50)k	30.00	33.33 (35.26)h
12	BS12	33.75	25.00 (29.99)ij	31.50	30.00 (33.20)i
13	BS13	27.80	38.22 (38.18)f	24.80	44.89 (42.06)f
14	BS14	30.80	31.56 (34,17)g	27.80	38.22 (38.18)g
15	BS15	33.50	25.56 (30.36)i	29.5	34.44 (35.93)h
16	BS16	35.00	32.00 (34.44)g	28.00	37.78 (37.92)g
17	BS17	25.50	43.33 (41.16)d	23.60	47.56 (43.60)e
18	BS18	34.50	23.33 (28.88)jk	30.50	32.22 (34.58)h
19	Control	45.00	-	45.00	-

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT; Values in parentheses are arcsine transformed values

In vitro efficacy of *Bacillus spp.* against *P. agglomerans*

Antagonistic activity of *Bacillus* isolates against *P. agglomerans* was assessed through dual plate technique. Among the isolates of *Bacillus spp.* screened the isolate BS2 was found to record inhibition zone of 28mm. It was followed by the isolate BS8, which inhibited *P. agglomerans* to an extent of 25mm (Table 3).

Table 3: In vitro evaluation of Bacillus isolates against P. agglomerans

S. No	Isolates	Zone of inhibition* (mm)
1	BS1	24.00 (29.33) b
2	BS2	28.00 (31.94)a
3	BS3	20.0 0(26.56)d
4	BS4	18 .00(25.10)f
5	BS5	19.0 (25.84)e
6	BS6	22.00 (27.97 c
7	BS7	15.00 (22.78) j
8	BS8	25.00 (29.998)b
9	BS9	16.00 (23.57)hi
10	BS10	15.30(23.024)ij
11	BS11	15.80(23.41) hij
12	BS12	13.00(21.13) k
13	BS13	17.00 (24.34) g
14	BS14	16.50(23.96) gh
15	BS15	15.00(22.78) j
16	BS16	15.90(23.49) hij
17	BS17	18.50 (25.47) ef
18	BS18	15.50(23.18) ij
19	Control	0.00

*Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT; Values in parentheses are arcsine transformed values

Bioassay of crude antibiotics against *C. gloeosporioides* and *A. alternata*

Crude antibiotics of *Bacillus spp.* extracted from the effective isolates BS1, BS2, BS6 and BS8 were tested for their antifungal activity against *C. gloeosporioides* and *A. alternata* under *in vitro* through agar well diffusion techniques using 100 µl. The isolates BS2 and BS8 inhibited *C.goleosporioides* to an extent of 59.11% and 57.55% respectively. Bioassay of antibiotics against *A. alternata* revealed that isolates BS2 and BS8 had maximum inhibition accounting for 64.22 % and 62.44 % over control respectively (Table 4).

Table 4 : *In vitro* evaluation of antifungal and antibacterial activity of crude antibiotics from *Bacillus spp.*

	C. gleosporioides		*A. alternata*		*P. agglomerams*
	Mycelial growth* of the pathogen (mm)	Per cent Inhibition over control	Mycelial growth* of the pathogen (mm)	Per cent Inhibition over control	Zone of inhibition* (mm)
BS1	19.70 (26.34)^{d}	56.22	17.20 (24.50)^{c}	61.77	24.80 (29.86)^{b}
BS2	18.40 (25.39)^{a}	59.11	16.10 (23.65)^{a}	64.22	29.00 (32.58)^{a}
BS6	21.35 (27.51)^{c}	52.55	18.35 (25.36)^{d}	59.22	23.10 (29.87)^{c}
BS8	19.10 (25.91)^{b}	57.55	16.90 (24.27)^{b}	62.44	25.90 30.59)^{b}
Control	45.00 (42.12)^{e}	-	45.00 (42.12)^{e}	-	-

*Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT; Values in parentheses are arcsine transformed values

Bioassay of crude antibiotics against *Pantoea agglomerans* by agar diffusion method

Antibacterial activity of crude antibiotics was confirmed with agar diffusion method. Observation on control plates did not show any inhibition zone, where as plates amended with crude antibiotics 100 µl suppressed the growth of *P. agglomerans*. The crude antibiotics of the isolate BS2 recorded the maximum inhibition zone (29.00mm) followed by the isolate BS8 (Table 4). Several studies evidently explained that the antimicrobial compound synthesized from *B. subtilis* posses inhibitory activity against several phytopathogenic bacteria. These studies evidently revealed that inhibitory action of *Bacillus* isolates used in this study might be because of antimicrobial compound production.

Gas chromatography and mass spectrometry (GCMS)

Crude antibiotics were analyzed through GCMS to detect antimicrobial compound produced by different isolates of *Bacillus spp*. Result from three isolates BS1, BS2, and BS8 revealed the presence of antimicrobial compound belonging to fatty acid, lipopeptide, peptide, and aldehydes. NIST library search of the extracellular metabolite produced from Bacillus isolate BS2 revealed the presence of antimicrobial compound such as L-glutamic acid dimethy ester, Dodecanoic acid, Petadecanoic acid, 14 methyl ester and 1,2-benzenedicarboxylic acid, disooctyl ester. Similarly GCMS analysis for the presence of metabolites in the effective isolate BS8 revealed the presence of Undecanoic acid,10-methyl ester, Hexadecanoic acid, Diglycerol, and Tetradecanoic acid-12 methyester. Besides, compounds like Dodeconaic acid, Teradecanoic acid, 12-methyl-, methyl ester and 2-hexadeconal were detected with isolate BS1 (Table 5, 6, 7) . Shaligram and Singhal (2010) reported fatty ester group, tetradecanoic

acid was incorporated with cyclic structure of surfactin . The present result was supported by Melo 2009 who found antimicrobial activity of fatty acid methyl esters from *Bacillus* against several bacteria.

Table 5 : Antimicrobial compound from isolate BS2 identified through GCMS

Retention Time	Antimicrobial Compound	Group	Biological Activities
7.81	L-glutamic acid dimethy ester	Lipopetide	Glutamic acid is main polar compound of surfactin posses antimicrobial activity (Ongena et al. (2008)
11.41	Dodecanoic acid	Fatty acid	Antibacterial and antifungal activities (Ashwanikumar *et al.* 2011) Antimicrobial Compound (Seghal Kiran et al 2010)
15.29	Petadecanoic acid, 14 methyl ester	Fatty acid	Ghazala et al 2004 antifungal and antibacterial activity
21.51	1,2-benzenedicarboxylic acid, disooctyl ester	Fatty acid	Antimicrobial activity (jayaraman et al. 2010)

Table 6 : Antimicrobial compound from isolate BS8 identified through GCMS

Retention time	Antimicrobial compound	Group	Biological activity
10.8	Undecanoic acid, 10-methyl ester	Fatty acid group	Ghazala et al ., 2004 sauturated fatty acid from Tetraspora possessing antifungal and antibacterial activity against A. alternata, Rhizoctonis solani and Sclerotium rolfsii
15.32	Hexadecanoic acid	Fatty acid group	Ghazala et al 2004
5.20	Diglycerol	Fatty acid compound	Antibacterial activity Yamazaki et al. (2004)
13.96	Tetradecanoic acid-12 methyester	Fatty acid group	Ghazala et al., 2004

Table 7 : Antimicrobial compound from isolate BS1 identified through GCMS

Retention time	Antimicrobial compound	Group	Biological activity
11.59	Dodeconaic acid	Fatty acid	Antimicrobial compound (Seghal Kiran et al ., 2010) Antifunagal and Antibacterial activity (Robbel et al., 2010)
13.95	Tetradecanoic acid, 12-methyl-,methyl ester	Fatty acid	Ghazala et al., 2004 reported antifungal and antibacterial activity of tetradecanoic acid against fungal pathogens.
15.08	2-hexadeconal	Volatile compound	Antimicrobial compound from Naringi crenulata (Sarada et al., 2011)

Effect of *Bacillus* spp., on the incidence of fungal and bacterial fruit rot diseases under field conditions.

Dry fruit rot

Field experiment was laid out in the noni field located at Coimbatore. Different treatments were carried out with standard chemical check for the management of both fungal and bacterial fruit rot disease. Among the different treatments foliar application of liquid culture with 1% of BS2 was most effective under field condition. Minimum disease incidence of about 16.23 percent was recorded in plots sprayed at 1% of the *Bacillus* isolate (BS2). It was followed by the foliar application of BS8 which recorded 18.25 per cent fruit rot. But the disease incidence was maximum (43.80 per cent) in control plots. Noni trees sprayed with BS2 and BS8 recorded the fruit yield of 51.80 t/ha and 50.67 t/ha with cost benefit ration of 1:2.59 and 1: 2.53 respectively. Comparison of percent incidence recorded in chemical check resulted that the bio agents are quiet inferior in controlling fruit diseases (Table.8). Similarly the PGPR was found effective against *C. gloeosporioides* and *A. alternata* under *in vivo* causing noni leaf blight (Nakkeeran *et al*., 2009; Manjunath *et al*., 2010).

Table 8 : Effects of B.subtilis against noni dry fruit rot (*C. gloeosporioides* and *A. alternata*) under field condition

Treatments Details	Mean fruit rot (%)	Per cent reduction over control	Fruit yield per tree(kg/ha)	Fruit yield per (t/ha)	C:B ratio
BS2- Foliar spray @0.1%	30.23 (33.35)f	30.99 (33.82)f	95.23 [f]	38.09 [f]	1:1.90
BS2-Foliar spray @0.5%	23.38 (28.91)d	46.63 (43.06)d	103.63 [d]	41.45 [d]	1:2.07
BS2-Foliar spray @1.0 %	16.23 (23.75)b	62.94 (52.51)b	129.62 [a]	51.80 [a]	1:2.59
BS8 -Foliar spray @0.1%	31.05 (33.86)f	29.11 (32.65)f	92.22 [g]	36.87 [g]	1:1.84
BS8- Foliar spray @0.5%	26.46 (30.95)e	39.58 (38.98) e	99.81 [e]	39.93 [e]	1:2.00
BS8- Foliar spray @1.0%	18.25 (25.28)c	58.33 (49.79)c	126.67 [b]	50.67 [b]	1:2.53
Difenaconazole- Foliar Spray@ 0.5%	11.15 (19.50)a	74.54 (59.71) a	118.85 [c]	47.50 [c]	1:2.38
Control	43.80 (41.43)g	-	72.31 [h]	28.93 [h]	1:1.45

*Mean of three replications

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

Values in parentheses are arcsine transformed values.

Wet / soft rot

Foliar application of bio formulation had good impact in controlling bacterial fruit rot disease of Noni .Among treatments, foliar application with isolate BS2 at 1 % had controlled bacterial fruit rot to an extent of 65.53 percent over control with the fruit yield of 48.33 t/ha . It was followed by the foliar application of BS8, which reduced the incidence of fruit rot to an extent of 61.34% over control with fruit yield of 46.22 t/ha. However, among all the treatments, foliar application of antibiotic namely streptomycin sulphate @ 0.5% was effective than biocontrol agents (Table.9).

Table 9: Effect of *B. subtilis* against noni wet fruit rot *(P. agglomerans)* under field condition

Treatments Details	Mean fruit rot (%)	Per cent reduction over control	Fruit yield per tree(kg/ha)	Fruit yield per (t/ha)	C:B ratio
BS2- Foliar spray @0.1%	31.98 (35.04)^{f}	31.78 (34.31)^{f}	83.44^{f}	33.37^{f}	1:1.67
BS2-Foliar spray @0.5%	25.98 (30.64)^{d}	46.25 (42.84)^{d}	86.99^{e}	34.80^{e}	1:1.74
BS2-Foliar spray @1.0 %	16.66 (24.08)^{b}	65.54 (54.05)^{b}	120.83^{a}	48.33^{a}	1:2.42
BS8 -Foliar spray @0.1%	34.13 (35.74)^{f}	29.40 (32.83)^{f}	81.28^{g}	32.51^{g}	1:1.63
BS8- Foliar spray @0.5%	28.71 (32.40)^{e}	40.60 (39.58)^{e}	89.92^{d}	35.97^{d}	1:1.80
BS8- Foliar spray @1.0%	18.68 (25.61)^{c}	61.34 (51.55)^{c}	115.56^{b}	46.22^{b}	1:2.31
Streptomycin sulphate - Foliar Spray@ 0.5%	7.41 (15.80)^{a}	89.13 (70.83)^{a}	109.99^{c}	44.00^{c}	1:2.20
Control	48.35 (44.05)^{g}	-	54.98^{h}	21.99^{h}	1:1.10

*Mean of three replications

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

Values in parentheses are arcsine transformed values.

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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 referees. The journal covers diversity, cultivation, phytochemistry and clinical research, *etc*. related to Noni.

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Books or monographs

Lalithakumari, D. 2000. *Fungal Protoplast: A Biotechnological Tool*. Oxford & IBH Publishing Co., Pvt., Ltd., New Delhi. p184.

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