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Website: www.aarf.asiaEmail : editor@aarf.asia , editoraarf@gmail.com

MICROBIAL ASSOCIATIONS OF NEEM (*AZADIRACHTA INDICA* A. JUSS.)

K. Girish

Postgraduate Department of Microbiology, Maharani's Science College for Women, JLB Road, Mysuru – 570 005, India.

ABSTRACT

Azadirachta indica *A. Juss. (neem), native to India, is well known worldwide for its ethanopharmacological properties. Neem products have antibacterial, antifungal, insecticidal and other versatile biological activities. Neem plant provides a unique biological niche for the growth of microorganisms. Many microorganisms are found associated with this plant. These microbes may be pathogens causing disease on neem or endophytes and rhizosphere microorganisms having various bioactivities.*

Key Words*:* Neem, *Azadirachta indica*, pathogens, endophytes, rhizosphere microorganisms

INTRODUCTION

Azadirachta indica A. Juss. (Neem, Family: Meliaceae), is commonly called 'Indian Lilac' or 'Margosa'. From as early as the Vedic times neem has been aptly referred to as the '*Sarva Roga Nivarini*' in the Charaka Samhita. Neem is a plant with adaptability to diverse habitats. It has held the interest of phytochemists all over the world for its rich source of bioactive phytochemicals. The people of India have known the useful properties of neem since time immemorial and almost all parts of the plant are useful for the treatment of different ailments. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties (Girish and Shankara Bhat, 2008 a,b). Isolation of over 400 bioactive compounds has been

reported from various parts of neem. Neem is a natural source of insecticides, pesticides and agrochemicals (Bhramhachari, 2004).

The diversity of microbial life is enormous and the niches in which microbes live are truly amazing. One specialized and unique biological niche that supports the growth of microbes is the higher plants. Each plant supports a suite of microorganisms namely phytopathogens, endophytes and rhizopshere microorganisms. Neem tree is also associated with various microorganisms that exhibit different interactions, both harmful as well as beneficial.

PATHOGENIC MICROORGANISMS OF NEEM

In spite of its well-known antifungal, antibacterial and other versatile biological activities, neem is not free from microbial diseases. Many fungal and bacterial pathogens were reported on neem. The bacterial diseases reported on neem include leaf spot disease caused by *Pseudomonas azadirachtae* (Srivastava and Patel, 1969), *Xanthomonas azadirachtii* (Moniz and Raj, 1967; Chakravarthi and Gupta, 1975). The fungal diseases that have been reported on neem include pink disease caused by *Corticium salmonicolor* (Bakshi, 1976), twig canker and shot-hole incited by *Phoma jolyana* (Singh and Chauhan, 1984), leaf spot caused by *Pseudocercospora subsessilis* (Castellani and Mohamed, 1984), three foliage diseases *viz.*, two leaf spots, one leaf blight caused by *Colletotrichum capsici*, *Cercospora subsessilis*, and *Sclerotium rolfsii* respectively and one each of stem (stem rot by *S. rolfsii*) and root (wilt by *Fusarium solani*) were reported (Sankaran *et al*., 1986).

The other fungal diseases are leaf web-blight caused by *Rhizoctonia solani* resulting in premature defoliation (Sankaran *et al*., 1986; Mehrotra, 1989; Mehrotra, 1990), leaf spotting and blight incited by *Alternaria alternata* and *Colletotrichum gleosporioides* causing heavy premature defoliation and foliage damage (Mehrotra and Pandey, 1992). Root rot disease incited by *Ganoderma lucidum* (Tewari, 1992) and *Ganoderma applanatum* (Chakraborthy and Konger, 1995), damping-off caused by *Fusarium oxysporum* (Tewari, 1992), powdery-mildew caused by *Oidium azadirachtae* (Tewari, 1992), die-back disease incited by *Phomopsis azadirachtae* (Sateesh *et al*., 1997), collar-rot incited by *Fusarium semitectum* that causes sudden drooping of leaves and withering of tips (Uniyal, 1999), stem-rot caused by *Sclerotinia sclerotiorum* (Ghasolia and Shivpuri, 2004), *Ganoderma lucidum* causing root rots, cracking of the root and stem barks, heartwood rots, die-back of the branches, deformation of crown and eventually death

(Adedeji *et al*., 2014), and leaf blight, a moderate to severe foliar disease, causing severe defoliation incited by *Colletotrichum dematium* (Bhanumathi and Rai, 2007).

Die-back of neem is caused by *Phomopsis azadirachtae* Sateesh, Bhat and Devaki. The fungus affects leaves, twigs and inflorescence, irrespective of age, size and height of the tree. In severely affected trees it has resulted in almost always 100% loss of fruit production. The disease is spreading at an alarming rate and is found in all neem growing regions of India (Girish and Shankara Bhat, 2008b).

ENDOPHYTES FROM *AZADIRACHTA INDICA*

Endophytes are microbes that colonize the living internal tissues of plants without causing any immediate evident negative effects (Bacon and White, 2000; Janso and Carter, 2010). All vascular plants harbor endophytic organisms in all parts like leaves, stem, bark, fruit, and roots (Zhang *et al*., 2006). Endophytic fungi have been isolated from leaves, stems and roots of woody plants in the temperate regions and the tropics (Rodrigues and Petrini, 1997; Mahesh *et al*., 2005). They are potential producers of novel secondary metabolites with various bioactivities (Arnold *et al*., 2001; Strobel and Daisy, 2003). Isolation of a potent anticancer agent, taxol from *Pestalotiopsis microspora*, an endophyte of the yew tree and the phytohormone-producing fungus from rice plant, *Gibberella fujikuroi* suggests the potential of endophytes as a source of useful metabolites (Stierle *et al*., 1993; Strobel and Long, 1998).

Fungal endophytes have been observed within all healthy tissues of *A. indica* including mesophylls, palisade and spongy parenchyma and vascular tissue. The prominent fungal structures observed were the fungal mycelia and occasionally spores (Verma *et al*., 2012). The species richness and diversity of endophytic fungi are significantly affected by plant tissue. The aged tissues harbor more endophytes than the young (Rajagopal and Suryanarayanan, 2000; Verma *et al*., 2011).

Rajagopal and Suryanarayanan, (2000) recorded *Fusarium avenaceam* along with four sterile mycelia from the leaves of *A. indica*. The occurrence of these foliar endophytes was found to be influenced by environment, and type and chemistry of the host tissue. A total of 77 endophytic fungal isolates belonging to 15 genera were isolated from the inner bark of *A. indica*. The colonization frequency was 38.5%. The fungal composition included 71.4% of hyphomycetes, 18.2% of coelomycetes, 6.5% of ascomycetes and 3.9% of sterile mycelia (Table 1). *Trichoderma* spp., *Penicillium* spp. and *Pestalotiopsis* spp., were the most dominant

endophytes isolated in this study (Mahesh *et al*., 2005). Tejesvi *et al*., (2006) isolated endophytic fungal species *Fusarium, Pestalotiopsis, Myrothecium, Trichoderma, Verticillium and Chaetomium* from inner bark segments of ethno-pharmaceutically important tree species including *A. indica.* An endophytic fungus *Curvularia lunata* was isolated from neem leaf (Verma and Kharwar, 2006).

Table 1: Endophytic fungi isolated from inner bark of neem (*Azadirachta indica*)

	Endophytic fungi
Ascomycetes	
1a	*Chaetomium crispatum*
1b	*Chaetomium globosum*
Coelomycetes	
2	*Pestalotiopsis* spp.
3	*Phoma eupyrena*
4	*Phyllosticta* spp.
Hyphomycetes	
5	*Acremonium acremonium*
6a	*Aspergillus flavus*
6b	*Aspergillus niger*
6c	*Aspergillus oryzae*
7a	*Cladosporium acaciicola*
7b	*Cladosporium cladosporioides*
8	*Cochlonema verrucosum*
9	*Curvularia lunata*
10a	*Fusarium clamydosporum*
10b	*Fusarium moniliformae* var. *subglutinans*
10c	*Fusarium oxysporum*
10d	*Fusarium solani*
11	*Gliomastix* spp.
12	*Nigrospora oryzae*
13	*Penicillium* spp.
14	*Trichoderma* spp.
15	*Verticillium albo-atrum*
Sterile mycelia	

Source: Mahesh *et al*., 2005.

A total of 233 isolates of endophytic fungi representing 18 fungal taxa were obtained from segments of bark, stem, and leaves of *A. indica*. Hyphomycetes (62.2%) were the most prevalent followed by the Coelomycetes (27.4%) and Mycelia Sterilia (7.7%). The maximum species richness and frequency of colonization of endophytes was observed in leaf segments rather than stem and bark tissues. The dominant endophytic fungi isolated were *Phomopsis oblonga, Cladosporium cladosporioides, Pestalotiops*is sp., *Trichoderma* sp. and *Aspergillus* sp. Genera such as *Periconia, Stenella,* and *Drechslera* were also isolated (Verma *et al*., 2007). An endophytic fungus *Phomopsis* sp., was isolated from the stems of the neem plant (Wu *et al*., 2008). *Geotrichum* sp., was isolated from the leaves of the neem and *Chloridium* sp., was obtained from the root tissues of *A. indica* (Kharwar *et al*., 2009). *Nigrospora* sp. YB-141, an endophytic fungus was isolated from *A. indica* (Wu *et al*., 2009). 85 endophytic fungi of 10 genera were isolated from fresh *A. indica* leaves collected from Panchmarhi biosphere reserve. The endophytic fungi recovered belonged to hyphomycetes (68.2%), coelomycetes (19.99%), ascomycetes and sterile mycelium (5.88%) each. The most dominant endophytes observed were *Trichoderma* (21.17%), *Pestalotiopsis* spp. (16.47%) and *Penicillium* (15.29%) (Tenguria and Khan, 2011).

A total of 272 isolates of 29 filamentous fungal taxa were isolated from neem roots and fruits such as *Chaetomium crispatum, Chaetomium globosum* (Ascomycetes), *Cercinella mucoroides* (Zygomycetes), *Colletotrichum, Phyllosticta minima, Pestalotiopsis* (Coelomycetes), *Acremonium acutatum, Alternaria alternata, Alternaria dennsii, Alternaria chlamydospora, Alternaria longipes, Aspergillus niger, Aspergillus fumigatus, Aspergillus oryzae, Cladosporium cladosporioides, Cladosporium acaciicola, Curvularia lunata, Curvularia catenulata, Drechslera rostrata, Fusarium oxysporum, Ulocladium chlamydosporum, Chloridium virescens, Humicola grisea, Nigrospora oryzae, Scytalidium, Trichoderma viride, Penicillium cristata, Verticillium tenuissimum* (Hyphomycetes) and *Fusarium, Chaetomium globosum* (Mycelia sterilia). Species such as *Chaetomium, Chloridium, Scytalidium, Nigrospora* and *Verticillium* were exclusively isolated from the root samples, while *Humicola, Drechslera* and *Colletotrichum* spp. were obtained exclusively from fruits samples. Malt yeast extract agar (MYA), Mycological agar (MCA), Potato dextrose agar (PDA) and Nutrient agar (NA) media were used for the isolation of these endophytic fungi (Verma *et al*., 2011).

Xuan (2014) isolated a fungal strain from the fruit of *A. indica,* which was classified as a *Trichoderma* sp., by its morphological characteristics and ITS rDNA sequence analysis. An endophytic fungus identified as *Aspergillus* sp., was isolated from leaves of *A. indica* (Jain and Kumar, 2015). Four endophytic fungi *Chaetomium* sp., *Colletotrichum* sp., *Curvularia* sp. and *Trichoderma* sp., were isolated from the leaves of *A. indica* (Kumaresan *et al*., 2015). Patil *et al*., (2015) reported the isolation of *Cladosporium* sp., and *Curvularia* sp., from neem which showed greater production of extracellular amylase and cellulose enzymes. *Aspergillus flavus, Aspergillus* sp., *Alternaria* sp., *Colletotrichum truncatum, Trichoderma vridae, Cladosporium* sp., *Penicillum* sp., *Nigrospora* sp., *Fusarium* sp, and mycelia sterilia were isolated from leaf, stem and petiole samples of *A. indica* growing at five different locations (Taware and Rajurkar, 2015).

Verma *et al*., (2009) isolated 55 different isolates of actinomycetes from neem plant. They reported *Streptomyces* to be the dominant species followed by *Streptosporangium, Microbispora, Streptoverticillium, Saccharomonospora* sp., and *Nocardia*. A *Streptomyces* strain was isolated from the neem. The isolate was closely related to the type strain of *Streptomyces plicatus* sharing a 16S rRNA gene sequence similarity of 96% and this new strain was named as *Streptomyces* sp. mrinalini7 (Singh and Padmavathy, 2014). Seven novel endophytic bacterial species *viz*., *Bacillus amyloliquefaciens* (JNU-001), *Burkholderia denitrificans* (JNU-002), *Pseudomonas aeruginosa* (JNU-003), *Xanthomonas campestris* (JNU-004), *Azotobacter tropicalis* (JNU-005), *Acetobacter xylinum* (JNU-006) and *Azospirillum lipoferum* (JNU-007) were recovered from native neem varieties of Rajasthan state, India. Among these endophytic bacterial isolates obtained, *Bacillus amyloliquefaciens* (JNU-001) was dominant (Tiwari and Thakur, 2014). An actinomycetes strain was isolated from neem leaves and named as NEK5 (Vijayan *et al*., 2014). The endophytic *Streptomyces coelicolor* strain AZRA 37 was isolated from the surface sterilized root of neem plant (Kumar *et al*., 2016).

Bioprospecting of neem endophytes

Endophytes are a source of large number of bioactive secondary metabolites with unique structure including alkaloids, benzopyranones, flavonoids, phenolicacids, quinines, steroids, terpenoids, tetralones, xanthones and other (Tan and Zou, 2001). Such bioactive metabolites find wide range of applications such as agrochemical, antibiotics, immunosuppressant, autoparasitic, antioxidant and anticancer agents (Gunatilika, 2006). The bioactivite compounds found in the

host plant tissues might be due to the associated endophytes. Endophytic fungi (*Eupenicillium parvum*) synthesizes azadirachtin which is produced by host neem plant. This warrant to explore biochemical nature of endophytic fungi isolated from medicinal plants (Kusari *et al*., 2012). Several reports in the recent years show that the endophytic fungi from *A. indica* produce several bioactive compounds (Li *et al*., 2007; Wu *et al*., 2008; Kharwar *et al*., 2009; Wu *et al*., 2009; Verma *et al*., 2011). Nearly 32 compounds are reported from the endophytes of neem.

An isolate, *Drechslera* sp., produced 'pestasol' in liquid culture (Carroll, 1995). Endophytic fungal species isolated from inner bark segments of *A. indica* exhibited greater antifungal activity (Tejesvi *et al*., 2006). Tejesvi *et al*., (2007) screened *Pestalotiopsis* strains from *A. indica* for their antifungal activity and concluded *Pestalotiopsis* sp., can be good source of bioactive antifungal agents for better management of fungal pathogens. *Geotrichum* sp., was reported to produce two new chlorinated epimeric 1,3-oxazinane derivatives, that have significant activity against the nematodes *Bursaphelenchus xylophilus* and *Panagrellus redivivus* (Li *et al*., 2007). *Phomopsis* sp., produces some 10-membered lactones, these lactones have very promising activity against plant pathogens *Ophiostoma minus* and *Botrytis cinerea* with MIC values 31.25 and 62.50 µg/ml respectively (Wu *et al*., 2008). 'Javanicin' an antibacterial nephthaquinone was isolated and characterized from the endophytic *Chloridium* sp., obtained from root tissues of the *A. indica*. This highly functionalized nephthaquinone exhibited strong antibacterial activity against *Pseudomonas* spp., representing pathogens to both humans and plants (Kharwar *et al*., 2009). Antioxidant, antibacterial and antihypertensive activities were demonstrated by the extracts of *Pestalotiopsis* isolated from *A. indica* (Tejesvi *et al*., 2009). Two new solanapyrone analogues and three known compounds, solanapyrone, nigrosporalactone, and phomalactone were isolated from the fermentation culture of *Nigrospora* sp. YB-141, an endophytic fungus isolated from *A. indica*. The structures of the new compounds were elucidated on the basis of spectroscopic analysis. Most of the compounds exhibited no or only weak antifungal activities (Wu *et al*., 2009).

Kusari *et al*., (2012) reported the production of the natural insecticides, azadirachtin A and B, by an endophytic fungus *Eupenicillium parvum*, isolated from *A. indica*. *Saccharomonospora* sp., isolated from neem was used successfully to biosynthesize gold nanoparticles (Verma *et al*., 2013a). Anticestodal activity of the endophytic fungi *Nigrospora* sp., *Colletotrichum* sp., *Fusarium* sp., *Chaetomium* sp. and *Pestalotiopsis* sp. from neem plant was observed on protoscoleces of hydatid cysts of *Echinococcus granulosus*. An average anticestodal activity was

observed with different endophytic fungal strains, that is, *Nigrospora* (479±2.9), *Colletotrichum* (469±25.8), *Fusarium* (355±14.5), and *Chaetomium* (332±28.3) showing 64 to 70% protoscolicidal activity, except *Pestalotiopsis* sp. (581± 5.0), which showed promising scolicidal activity up to 97% mortality just within 30 min of incubation (Verma *et al*., 2013b). The crude extract of endophytic *Aspergillus* sp. isolated from *A. indica* exhibited antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and Gram negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas fluorescens* (MTCC 1748), antifungal activity against opportunistic fungal pathogens, *Candida albicans* (MTCC 227), *Candida glabrata* (MTCC 3814), and phytopathogenic fungi *Fusarium graminearum* (MTCC 2089) (Jain and Sharma, 2014).

Five new guaiane sesquiterpenes were isolated from the culture broth of the endophytic fungus *Xylaria* sp. YM 311647, obtained from *A. indica*. All guaiane sesquiterpenes showed moderate or weak antifungal activities in broth microdilution assay (Huang *et al*., 2015). Endophytic fungus *Aspergillus* sp., isolated from neem leaves showed antimicrobial activity against five Gram positive bacteria (*Streptococcus pyogenes, Streptococcus mutans, Staphylococcus aureus, Bacillus subtilis,* and *Bacillus megaterium*), two Gram negative bacteria (*Escherichia coli* and *Pseudomonas fluorescens*) and five fungal pathogens (*Candida tropicalis, Candida glabrata, Candida albicans, Alternaria solani* and *Fusarium graminearum*). Zone of growth inhibition against test pathogens ranged between 16-36 mm (Jain and Kumar, 2015). The methanolic extracts of endophytic fungi *Chaetomium* sp., *Colletotrichum* sp., *Curvularia* sp. and *Trichoderma* sp. showed promising antioxidant potential which was highest in *Chaetomium* sp., followed by *Curvularia* sp., *Colletotrichum* sp. and *Trichoderma* sp. The variation in the content of phenolic components and ascorbic acid levels reflected the antioxidant activity of the organisms studied (Kumaresan *et al*., 2015).

Actinomycetes isolated from neem plant were screened for their antibacterial and antifungal acti*vities. Streptomyces* had acute activity against *Pseudomonas fluorescens* and *Escherichia coli,* while an isolate of *Nocardia* sp. from leaves showed antagonism against *Bacillus sub*tilis. A few isolates of *Streptomyces, Nocardia* sp. and *Streptosporangium* sp. also showed significant antagonistic activity against root pathogens, including *Pythium* sp., and *Phytophthora* sp. (Verma *et al*., 2009). The *Streptomyces* sp. mrinalini7 isolate inoculated into model tomato plant significantly enhanced the biomass production of the plant and seed germination (Singh and Padmavathy, 2014). Actinomycetes strain NEK5 isolated from neem

leaves showed good antiphytofungal activity. The ethyl acetate extract of culture filtrate of NEK5 isolate inhibited the growth of *Fusarium* sp., *Pythium* sp., *Curvularia* sp. and *Cercospora* sp. (Vijayan *et al*., 2014). The *Streptomyces coelicolor* was treated with different concentrations of 5-azacytidine and evaluated for its antibacterial potential against five human pathogenic bacteria (*Aeromonas hydrophila* IMS/GN11, *Enterococcus faecalis* IMS/GN7, *Salmonella typhi* MTCC 3216, *Shigella flexneri* ATCC 12022 and *Staphylococcus aureus* ATCC 25923). The crude extract obtained from cultures treated with 25 μM concentration of 5-azacytidine, was found effective against all the five pathogenic bacteria tested (Kumar *et al*., 2016).

RHIZOSPHERE MICROORGANISMS OF NEEM

The rhizosphere is a narrow zone adjacent to and influenced by living plant roots (Kennedy, 1999). It is a site of high microbial activity in and around roots in soil (Sorenson, 1997). It harbors a great diversity of microorganisms affecting plant growth and health (Campbell and Greaves, 1990; Boehm *et al*., 1993). The diversity and composition of microbial taxa in the rhizosphere can be affected by several factors including plant species (Miller *et al*., 1989). The composition of microbial community in the rhizosphere is important for the performances of the plant, as microbial species can have beneficial, neutral or harmful relationships with the roots (Buchenauer, 1998; Atkinson and Watson, 2000; Sylvia and Chellami, 2001). Microorganisms in the rhizosphere are found to be more in population and having high metabolic rate rather than in non rhizosphere soil (Tamilarasi *et al*., 2008). There are many reports of neem rhizosphere microorganisms and their associated bioactivities.

Rhizosphere microflora of medicinal plants including *A. indica* was estimated. The total number of heterotrophic bacteria in the neem rhizosphere was 41 x 10^4 CFU/g, actinomycetes population was 17 x 10^2 CFU/g and fungal population was 18 x 10^2 CFU/g. The predominant bacterial species was *Bacillus* followed by *Pseudomonas, Enterobacter, Corynebacterium*, *Micrococcus* and *Serratia*. Among the fungus the most dominant species was *Rhizopus* followed by *Aspergillus, Penicillium, Mucor* and *Fusarium*. Among the actinomycetes, isolates of *Streptomyces* was found to be maximum followed by *Frankia* sp. The isolates of bacteria, actinomycetes and fungi were checked for IAA production. Among them 62.5% of fungal isolates produced IAA followed by 52.17% of actinomycetes and 23.7% of bacterial isolates (Tamilarasi *et al*., 2008).

Phosphate Solubilising Bacteria were isolated from rhizospheric soil of neem (Shankarrao, 2012). Neem rhizosphere soil can be the rich source for isolation of phosphate solubilizing microorganism, due to high P requirements to neem tree and other medicinal plants (Phavaphutanon *et al*., 1996), or due to long term association and interaction between neem root and microorganism found in the rhizosphere environments (Lucas Garcia *et al*., 2001). The bacterial P solubilization activity is due to secretion of organic acids such as oxalic, citric, formic, acetic, propionic, lactic, succinic and gluconic acid which chelate the cation bound to phosphate and convert it to soluble forms through their hydroxyl and carboxyl groups and production of acid /alkaline phosphatase enzyme (Chen *et al*., 2006).

The antagonistic activities of the PSB isolates from neem rhizosphere were determined against bacteria and phytopathogenic fungi. Isolates N-B (col-1), N-C (col-2) from neem rhizosphere showed potent antifungal activity against *Helminthosporium gramineum* and *Rhizopus oryzae*. Isolates N-B (col-1) also showed good antifungal activity against *Aspergillus niger* and *Ustilago maydis*. Comparitively, *R. oryzae*, *H. gramineum*, *A. niger* and *U. maydis* showed more sensitiveness to tested isolates than *Alternaria brassicicola*, *A. solani* and *Sclerotium rolfsii*. Both the isolates exhibited maximum antibacterial activity against *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Shankarrao, 2012). N-B (col-1) showed more than one PGPR trait such as phosphate solubilization, antifungal and antibacterial activity and phytohormone production. This isolate might promote plant growth directly, indirectly or synergistically in the soil environment.

Rhizosperic bacteria were isolated from neem and identified as *Sporosarcina*, *Streptococcus*, *Micrococcus luteus*, *Planococcus* and *Staphylococcus*. Intracellular secondary metabolites extraction was carried out using methanol and extracellular secondary metabolites were extracted by chloroform. These extracts exhibited significant antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Pandey and Singh, 2013). Bacterial strains named RHSAN-1 to 6 were isolated from neem rhizosphere of North 24 Parganas district of West Bengal (Biswas *et al*., 2016).

Phosphate-solubilizing rhizosphere fungus, *Talaromyces funiculosus* SLS8, was isolated from neem (*A. indica*) on saline soil. The fungus was tolerant to environmental stressors, salinity and agricultural systemic fungicides. Phosphate solubilization under different nutritional conditions was investigated by culturing *T. funiculosus* SLS8 in Pikovskaya liquid medium. The highest concentration of solubilised phosphate (187 mg P L^{-1}) was achieved after

5 days of incubation in the medium with glucose and ammonium sulphate (Kanse *et al*., 2015). The soil fungi have been reported to solubilize insoluble phosphates by secreting weak organic acids (Maliha *et al*., 2004; Chunqiao *et al*., 2009).

Both VAM and soil fungal diversity and frequency were studied in the neem rhizosphere from five ecogeographically different regions. Mycofloral diversity included *Aspergillus niger, A. flavus, A. nidulans, A. versicolor, A. fumigatus, Alternaria tenuis, A. alternata, Cladosporium, Cladosporium* sp., *Cephalospora* sp., *Candida albicans, Fusarium oxysporum, Pestalotia monorhinca, Paecilomyces, Monilia sitophila, Nigrospora oryzae* and *Rhizopus nigricans*. Saline-arid-parched soil exhibited three unique fungal species namely, *Monilia sitophila, Aspergillus versicolo*r and *Paecilomyces fusisporus*, whereas, the delta-wet region exhibited *Rhizopus nigricans* as its unique species. Overall, in the five regions studied, three VAM genera with nine species were observed, with *Glomus* being the predominant genus *viz., Glomus mosseae, Glomus microcarpum, Glomus macrocarpum, Glomus constrictum, Glomus fasciculatum, Glomus albida, Glomus multisubstance, Glomus deserticola, Gigaspora margarita, Acaulospora* species (Chary, 2011). This microbial diversity could be correlated with the azadirachtin-A content of the neem trees.

Field investigation was carried out to determine the arbuscular mycorrhizal fungi (AM) population and their diversity in neem-based agroforestry fields. *Glomus*, *Gigaspora* and *Sclerocystis* were the genera of AM present in the neem-based agroforestry system. Among the three genera, *Glomus* occurred most frequently with 15 species, three species were of *Gigaspora* and two were of *Sclerocystis*. *Glomus fasciculatum* was the predominant AM fungus infecting neem (Pande and Tarafdar, 2004). Arbuscular mycorrhizal (AM) fungi are recognized as an essential component of sustainable agricultural ecosystems (Jefferies, 1987; Barea, 1991).

Microorganisms are also intentionally introduced into the rhizosphere environments to enhance certain agriculturally beneficial activities mainly aiming at plant growth promotion (Tamilarasi *et al*., 2008). Inoculation of neem rhizosphere with AM fungi (Habte *et al*., 1993; Phavaphutanon *et al*., 1996) reduced fertilizer requirement in plant production. The effect of inoculation of neem with VA-mycorrhizal fungi (*Glomus fasciculatum*) and phosphorus solubilising bacteria was examined under nursery conditions to understand the compatibility between P solubilizing and P mobilising organisms in the neem rhizosphere. The results clearly indicated that combined inoculation markedly increased the plant growth of the neem seedlings

when compared to individual inoculants or uninoculated control, showing the synergistic effect (Kalavathi *et al*., 2000).

Neem seedlings were inoculated with arbuscular mycorrhizal (AM) fungi, *Glomus intraradices*, *Azospirillum brasilense*, and phosphate-solubilizing bacteria (PSB). Microbial inoculation resulted in greater plant height, increased mycorrhizal colonization, leaf area and number, root collar diameter, biomass, phosphorus, nitrogen and potassium content, and seedling quality. Microbial inoculation effects were greatest when seedlings were inoculated with a combination of microbes rather than individually. This clearly indicated that these microorganisms act synergistically (Muthukumar *et al*., 2001).

CONCLUSION

There are reports of endophytic and rhizosphere microorganisms of neem (*A. indica*). However they are limited. *A. indica* with appreciable medicinal values, the microbial population of the rhizosphere of this plant has not been studied so far in detail. Further studies are required in this direction as such studies might have enormous possibilities to provide an important data base regarding the microbial population of such novel ecosystem for exploration and evaluation of their bioprospective potentialities in future. The rhizosphere isolates of neem plant might have sufficient bioprospective potentiality in terms of suitable biofertilizer formulations for better crop production.

REFERENCES

1. Adedeji, G.A., Aiyeloja, A.A. and Omokhua, G.E. 2014. Occurrence and severity of *Ganoderma lucidum* (Fr.) P. Karst. on *Azadirachta indica* tree in University of Port Harcourt, Nigeria: implications for sustainable harvesting and replacement. Nat. Sci. 12(8): 123-128.
2. Arnold, A.E., Maynard, Z. and Gilbert, G.S. 2001. Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol. Res. 105: 1502–1507.
3. Atkinson, D. and Watson, C.A. 2000. The beneficial rhizosphere: A dynamic entity. Appl. Soil Ecol. 15: 99-104.
4. Bacon, C.W. and White, J.F. 2000. Microbial endophytes. Marcel Dekker Inc., New York. pp. 3-27.
5. Bakshi, B.K. 1976. Forest pathology: Principles and practice in forestry. Forest Research Institute, Dehra Dun, India.

6. Barea, J.M. 1991. Vesicular arbuscular mycorrhizae as modifiers of soil fertility. Adv. Soil Sci. 15: 1–40.
7. Bhanumathi, A. and Rai, R.V. 2007. Leaf blight of *Azadirachta indica* and its management *in vitro.* Afr. J. Agric. Res. 2(10): 538-543.
8. Biswas, K., Basu, J., Ghosh, A. and Giri, P. 2016. Study of rhizospheric bacterial population of Azadirachta indica (Neem) of North 24 Parganas district of West Bengal for bioprospective consideration. Int. J. Exp. Res. Rev. 6: 62-66.
9. Boehm, M., Madden, V. and Hoitink, H.A.J. 1993. Effect of organic matter decomposition level on bacterial species diversity and composition in relation to *Pythium* damping off severity. Appl. Environ. Microbiol. 59: 4171-4179.
10. Brahmachari, G. 2004. Neem - an omnipotent plant: a retrospection. Chembiochem. 5: 408-421.
11. Buchenauer, H. 1998. Biological control of soil borne diseases by rhizobacteria. J. Plant Dis. Protect. 105: 329-348.
12. Campbell, R. and Greaves, M.P. 1990. Anatomy and community structure of the rhizosphere. In: *The Rhizosphere* (Ed. Lynch, J.M.). Wiley, New York. pp. 11-34.
13. Carroll, G.C. 1995. Forest endophytes: pattern and process. Can. J. Bot. 73(Suppl. 1): S1316-S1324.
14. Castellani, E. and Mohamed, M.I. 1984. Diseases of *Azadirachta indica* in Somalia. 1 *Cercospora* disease. Riv. Agric. Subtrop. e Trop. 78: 733-739.
15. Chakraborthy, R. and Konger, G. 1995. Root-rot of neem (*Azadirachta indica*) caused by *Ganoderma applanatum*. Indian For. 121: 1081-1082.
16. Chakravarthi, B.P. and Gupta, D.K. 1975. A non- pigmented strain of *Xanthomonas azadirachtii* Moniz and Raj causing leaf spot of neem (*Azadirachta indica* A. Juss.). Curr. Sci. 44: 240-241.
17. Chary, P. 2011. A comprehensive study on characterization of elite neem chemotypes through mycofloral, tissue-cultural, ecomorphological and molecular analyses using azadirachtin-A as a biomarker. Physiol. Mol. Biol. Plants 17(1): 49–64.
18. Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil. Ecol. 34: 33–41.

19. Chunqiao, X., Ruan, C., Huan, H., Guanzhou, Q., Dianzuo, W. and Wenxue, Z. 2009. Isolation of phosphate-solubilizing fungi from phosphate mines and their effect on wheat seedling growth. Appl. Biochem. Biotechnol. 159: 330–342.
20. Ghasolia, R.P. and Shivpuri, A. 2004. Neem- A new host of *Sclerotinia sclerotiorum*. J. Mycol. Plant Pathol. 34: 200.
21. Girish, K. and Shankara Bhat, S. 2008a. Neem – A green treasure. EJBio. 4(3): 102-111.
22. Girish, K. and Shankara Bhat, S. 2008b. *Phomopsis azadirachtae* – the die-back of neem pathogen. EJBio. 4(3): 112-119.
23. Gunatilaka, A.A.L. 2006. Natural products from plant-associated microorganisms: Distribution, structural diversity, bioactivity and implication of their occurence. J. Nat. Prod. 69: 509-526.
24. Habte, M., Muruleedhara, B.N. and Ikawa, H. 1993. Response of neem (*Azadirachta indica*) to soil P concentration and mycorrhizal colonization. Arid Land Res. Manag. 7: 327–333.
25. Huang, R., Xie, X.S., Fang, X.W., Ma, K.X. and Wu, S.H. 2015. Five new guaiane sesquiterpenes from the endophytic fungus *Xylaria* sp. YM 311647 of *Azadirachta indica.* Chem. Biodivers. 12(8): 1281–1286.
26. Jain, P. and Kumar, T. 2015. Isolation of endophytic fungi from leaves of *Azadirachta indica* and preliminary screening for antimicrobial activity. World J. Pharm. Res. 4(1): 1882-1891.
27. Jain, P. and Sharma, P. 2014. Evaluation of antimicrobial activity of an endophytic *Aspergillus* sp. isolated from *Azadirachta indica* against plant and human pathogens. World J. Pharm. Res. 3(9): 808-814.
28. Janso, J.E. and Carter, G.T. 2010. Biosynthetic potential of phylogenetically unique endophytic actinomycetes from tropical plants. Appl. Environ. Microbiol. 76 (13): 4377–4386.
29. Jeffries, P. 1987. Use of mycorrhizae in agriculture. Crit. Rev. Biotechnol. 5: 319–357.
30. Kalavathi, B.P., Santhanakrishnan, P. and Divya, M.P. 2000. Effect of VA-mycorrhizal fungus and phosphorus solubilising bacterium in neem. Indian For. 126(1): 67-70.
31. Kanse, O.S., Whitelaw-Wecker, M., Kadam, T.A. and Bhosale, H.J. 2015. Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the neem rhizosphere. Ann. Microbiol. 65(1): 85–93.

32. Kennedy, A.C. 1999. The rhizosphere and spermosphere. In: *Principles and Applications of Soil Microbiology* (Eds. Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G. and Zuberer, D.A). Prentice Hall, New Jersey.
33. Kharwar, R.N., Verma, V.C., Kumar, A., Gond, S.K., Harper, J.K., Hess, W.M., Lobkovosky, E., Ma, C., Ren, Y. and Strobel, G.A. 2009. Javanicin, an antibacterial nephthaquinone from an endophytic fungus of neem, *Chloridium* sp. Curr. Microbiol. 58(3): 233–238.
34. Kumar, J., Sharma, V.K., Singh, D.K., Mishra, A., Gond, S.K., Verma, S.K., Kumar, A. and Kharwar, R.N. 2016. Epigenetic activation of antibacterial property of an endophytic *Streptomyces coelicolor* strain AZRA 37 and identification of the induced protein using MALDI TOF MS/MS. PLoS ONE 11(2): e0147876. doi:10.1371/journal.pone.0147876
35. Kumaresan, S., Karthi, V., Senthilkumar, V., Balakumar, B.S. and Stephen, A. 2015. Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of *Azadirachta indica* A. Juss (Neem) from Chennai, India. J. Acad. Ind. Res. 3(8): 355-361.
36. Kusari, S., Verma, V.C., Lamshoeft, M. and Spiteller, M. 2012. An endophytic fungus from *Azadirachta indica* A. Juss. that produces azadirachtin. World J. Microbiol. Biotechnol. 28(3): 1287-1294.
37. Li, G.H., Yu, Z.F., Li, X., Wang, X.B., Zheng, L.J. and Zhang, K.Q. 2007. Nematicidal metabolites produced by the endophytic fungus *Geotrichum* sp. AL4. Chem. Biodivers. 4: 1520–1524.
38. Lucas Garcia, J.A., Probanza, A., Ramos, B. and Gutierrez Manero, F.J. 2001. Genetic variability of rhizobacteria from wild populations of four *Lupinus* species based on PCR-RAPDs. J. Plant Nutr. Soil Sci. 164: 1–7.
39. Mahesh, B., Tejesvi, M.V., Nalini, M.S., Prakash, H.S., Kini, K.R., Subbiah, V. and Shetty, H.S. 2005. Endophytic mycoflora of inner bark of *Azadirachta indica* A. Juss. Curr. Sci. 88(2): 218-219.
40. Maliha, R., Samina, K., Najma, A., Sadia, A. and Farooq, L. 2004. Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms under in vitro conditions. Pak. J. Biol. Sci. 7: 187–196.

41. Mehrotra, M.D. and Pandey, P.C. 1992. Some important nursery diseases of *Azadirachta indica* and their control. In: *Monograph of Neem* (Azadirachta indica A. Juss), (Ed. Tewari, D.N.). International Book Distributors, Dehra Dun, India.
42. Mehrotra, M.D. 1989. Leaf blight of some hardwood species in Assam and Meghalaya and its control in the nursery. Indian For. 115: 378-384.
43. Mehrotra, M.D. 1990. *Rhizoctonia solani*, a potentially dangerous pathogen of Khasi pine and hardwoods in forest nurseries in India. Eur. J. For. Pathol. 20: 329-338.
44. Miller, H.J., Henken, G. and VanVeen, J.A. 1989. Variation and composition of bacterial populations in the rhizosphere of maize, wheat and grass cultivars. Can. J. Microbiol. 35(6): 656-660.
45. Moniz, L. and Raj, H.H. 1967. *Xanthomonas azadirachtii* sp. nov. causing leaf spot disease in *Azadirachta indica* A. Juss. Indian J. Microbiol. 7: 159-160.
46. Muthukumar, T., Udaiyan, K. and Rajeshkannan, V. 2001. Response of neem (*Azadirachta indica* A. Juss) to indigenous arbuscular mycorrhizal fungi, phosphate-solubilizing and asymbiotic nitrogen-fixing bacteria under tropical nursery conditions. Biol. Fertil. Soils. 34: 417–426.
47. Pande, M. and Tarafdar, J.C. 2004. Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. Appl. Soil Ecol. 26: 233–241.
48. Pandey, A. and Singh, A. 2013. A comparative study on secondary metabolites producing microbes isolated from rhizospheric and non-rhizospheric region of *Azadirachta Indica* and *Oscimum Tenuiflorum*. Int. J. Pharm. Res. Allied. Sci. 2: 36-48.
49. Patil, M.G., Pagare, J., Patil, S.N. and Sidhu, A.K. 2015. Extracellular enzymatic activities of endophytic fungi isolated from various medicinal plants. Int. J. Curr. Microbiol. App. Sci. 4(3): 1035-1042.
50. Phavaphutanon, L., Davies, F.T.Jr. and Duray, S.A. 1996. Growth, root alteration, and nutrient uptake of neem tree (*Azadirachta indica* A. Juss) seedlings in response to vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition. Int. Tree Crops J. 9: 59-67.
51. Rajagopal, R. and Suryanarayanan, T.S. 2000. Isolation of endophytic fungi from leaves of neem (*Azadirachta indica*). Curr. Sci. 78: 1375–1378.
52. Rodrigues, K.F. and Petrini, O. 1997. Biodiversity of endophytic fungi in tropical regions. In: *Biodiversity of Tropical Micro Fungi* (Ed. Hyde, K.D.). Hong Kong University Press, Hong Kong. pp: 57-69.

53. Sankaran, K.V., Balasundaran, M. and Sharma, J.K. 1986. Seedling diseases of *Azadirachta indica* in Kerala, India. Eur. J. For. Pathol. 16: 324-328.
54. Sateesh, M.K., Shankara Bhat, S. and Devaki, N.S. 1997. *Phomopsis azadirachtae* sp. nov. from India. Mycotaxon. 65: 517-520.
55. Shankarrao, O. 2012. Isolation and characterization of phosphate solubilising bacteria from rhizospheric soil samples. Online Int, Interdiscip, Res, J. 2(4): 28-39.
56. Singh, I. and Chauhan. 1984. *Phoma jolyana*, a new pathogen on neem (*Azadirachta indica*). Indian For. 110: 1058-1060.
57. Singh, M.J. and Padmavathy, S. 2014. Isolation, screening and characterization of endophytic PGPR actinomycetes present commonly in neem and tulsi leaves -in vitro study (tomato). Int. J. Recent. Sci. Res. 5(3): 574-579.
58. Sorensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. In: *Modern soil Microbiology* (Eds. Van Elas, J.D., Trevors, J.T. and Wellington, E.M.H.). Marcel Dekker, New York. pp. 21-45.
59. Srivastava, S.K. and Patel, P.N. 1969. Epidemiology of bacterial leaf spot, blight and shot-hole disease of neem in Rajasthan. Indian Phytopathol. 22: 237-244.
60. Stierle, A., Strobel, G. and Stierle, D. 1993. Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260*:* 214–216.
61. Strobel, G. and Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67: 491-502.
62. Strobel, G.A. and Long, D.M. 1998. Endophytic microbes embody pharmaceutical potential. ASM News. 64*:* 263–268.
63. Sylvia, D.M. and Chellemi, D.O. 2001. Interactions among root-inhabiting fungi and their implications for biological control of root pathogens. Adv. Agron. 73: 1-33.
64. Tamilarasi, S., Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy. 2008. Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum.* J. Environ. Biol. 29(1): 127-134.
65. Tan, R.X. and Zou, W.X. 2001. Endophytes: A rich source of functional metabolites. Nat. Prod. Rep. 18: 448-459.

66. Taware, A.S. and Rajurkar, S.K. 2015. Diversity assessment of endophytic fungi from *Azadirachta indica* A. Juss. from various regions of Aurangabad, Maharashtra (India). Int. J. Innov. Sci. Eng. Technol. 2(7): 96-107.
67. Tejesvi, M.V., Kini, K.R., Prakash, H.S., Subbiah, V. and Shetty, H.S. 2007. Genetic diversity and antifungal activity of species of *Pestalotiopsis* isolated as endophytes from medicinal plants. Fungal Divers. 24: 37-54.
68. Tejesvi, M.V., Mahesh, B., Nalini, M.S., Prakash, H.S., Kini, K.R., Subbiah, V. and Shetty, H.S. 2006. Fungal endophyte assemblages from ethnopharmaceutically important medicinal trees. Can. J. Microbiol. 52: 427-435.
69. Tejesvi, M.V., Tamhankar, S.A., Kini, K.R., Rao, V.S. and Prakash, H.S. 2009. Phylogenetic analysis of endophytic *Pestalotiopsis* species from ethnopharmaceutically important medicinal trees. Fungal Divers. 38: 167-183.
70. Tenguria, R.K. and Khan, F.N. 2011. Distribution of edophytic fungi in leaves of *Azadirachta indica* A. Juss. (Neem) of Panchmarhi biosphere reserve. Curr. Bot. 2(2): 27-29.
71. Tewari, D.N. 1992. Monograph of neem (*Azadirachta indica* A. Juss.). International Book Distributors, Dehra Dun, India.
72. Tiwari, K. and Thakur, H.K. 2014. Diversity and molecular characterization of dominant *Bacillus amyloliquefaciens* (JNU-001) endophytic bacterial strains isolated from native neem varieties of Sanganer region of Rajasthan. J. Biodivers. Biopros. Dev. 1: 115.doi:10.4172/2376-0214.1000115
73. Uniyal, K. 1999. Collar-rot in *Azadirachta indica* and its control. Indian For. 125: 513-516.
74. Verma, V.C. and Kharwar, R.N. 2006. Efficacy of neem leaf extract against its own fungal endophyte *Curvularia lunata*. J. Agric. Technol. 2(2): 329-335.
75. Verma, V.C., Anand, S. and Ulrichs, C. 2013a. Biogenic gold nanotriangles from *Saccharomonospora* sp., an endophytic actinomycetes of *Azadirachta indica* A. Juss. Int. Nano Lett. 3: 21–28.
76. Verma, V.C., Gangwar, M., Yashpal, M. and Nath, G. 2013b. Anticestodal activity of endophytic *Pestalotiopsis* sp. on protoscoleces of hydatid cyst *Echinococcus granulosus*. BioMed. Res. Int. 308515: http://dx.doi.org/10.1155/2013/308515
77. Verma, V.C., Gond, S.K., Kumar, A., Kharwar, R.N., Boulanger, L.A. and Strobel, G.A. 2011. Endophytic fungal flora from roots and fruits of an indian neem plant *Azadirachta*

indica A. Juss., and impact of culture media on their isolation. Indian J. Microbiol. 51(4): 469–476.

78. Verma, V.C., Gond, S.K., Kumar, A., Kharwar, R.N. and Strobel, G.A. 2007. The endophytic mycoflora of bark, leaf and stem tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). Microb. Ecol. 54: 119–125.
79. Verma, V.C, Gond, S.K., Kumar, A., Mishra, A., Kharwar, R.N. and Gange, A.C. 2009. Endophytic actinomycetes from *Azadirachta indica* A. Juss.: Isolation, diversity, and anti-microbial activity. Microb. Ecol. 57: 749–756.
80. Verma, V.C., Singh, S.K. and Kharwar, R.N. 2012. Histological investigation of fungal endophytes in healthy tissues of *Azadirachta indica* A. Juss. Kasetsart J. (Nat. Sci.) 46: 229-237.
81. Vijayan, V.M., Radhakrishnan, M. and Balagurunathan, R. 2014. Bioprospecting of endophytic actinomycetes for antiphytofungal activity. Int. J. Chem. Tech. Res. 6(5): 2689-2694.
82. Wu, S.H., Chen, Y.W., Shao, S.C., Wang, L.D., Li, Z.Y., Yang, L.Y., Li, S.L. and Huang, R. 2008. Ten-membered lactones from *Phomopsis* sp., an endophytic fungus of *Azadirachta indica*. J. Nat. Prod. 71(4): 731–734.
83. Wu, S.H., Chen, Y.W., Shao, S.C., Wang, L.D., Yu, Y., Li, Z.Y., Yang, L.Y., Li, S.L. and Huang, R. 2009. Two new solanapyrone analogues from the endophytic fungus *Nigrospora* sp. YB-141 of *Azadirachta indica*. Chem. Biodivers. 6: 79–85.
84. Xuan, Q.C., Huang, R., Miao, C.P., Chen, Y.W., Zhai, Y.Z., Song, F., Wang, T. and Wu, S.H. 2014. Secondary metabolites of endophytic fungus *Trichoderma sp.* ym 311505 of *Azadirachta indica*. Chem. Nat. Comp. 50: 139-141.
85. Zhang, L.R., Wanf, J., Son, N., Zhang, S., Hu, J. and Kuai, J. 2006. Exploring novel bioactive compounds from marine microbes. Curr. Opin. Microbiol. 8: 276-281.