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Fungi associated with non-rhizosphere soil, rhizosphere soil and rhizoplane of *Vitex negundo* from Telangana

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ABSTRACT:

Fungal species associated with Non-Rhizosphere soil, Rhizosphere soil and Rhizoplane of *Vitex negundo* Linn. located in Bhadrachalam forest area of Telangana state have been studied employing standard methods and many agar media. Altogether 191 fungal species have been found associated with Non-Rhizosphere soil, Rhizosphere soil and Rhizoplane regions of *Vitex negundo* Rhizosphere soil has supported more fungal species. *Aspergillus* followed by *Penicillium*, *Fusarium* and *Curvularia* dominated all the three ecological niches. Around 22 fungal species are reported new additions to the fungi of Telangana state. Only qualitative data of fungi associated with *Vitex negundo* is presented.

Keywords: Fungi, dilution plate, soil plate, rhizosphere, rhizoplane, soil, *Vitex negundo*.

INTRODUCTION

Rhizosphere is a specialized ecological niche and it is a portion of the soil, which is adjacent to the root system of plant as influenced by the root exudates. Non-rhizosphere soil is the zone of the soil which is away from root system not influenced by the root exudates, rhizosphere is influenced by soil type, soil condition and host plant besides the stimulation effect or rhizosphere effect due to interaction of soil microbes and their ratio. The term rhizosphere was proposed by Hiltnar (1904). Rhizoplane is nothing but root surface zone harbouring many fungi and term was coined by Clark (1949). The fungi associated with non-rhizosphere, rhizosphere and rhizoplane were reported by number of workers (Abdel-Nasser *et al*., 2014; Anderson, 2004; Abdel-Hafez,1982; Jeffrey and Kaufman, 1996; Laurent Philippot *et al*., 2013; Mehrotra and Kakkar, 1972; Mukerji *et al*., 2006; Mwajita *et al*., 2013; Kapur and Mukerji, 2006; Shivanna and Vasanthakumari, 2011; Vasanthakumari and Shivanna, 2011). This kind of studies are interesting as the fungi amongst the microbes show many beneficial effects through metabolites like antibiotics, enzymes, plant growth regulators and others. Different types of microbes like fungi, bacteria, actinomycetes, algae and others interact with host plants. Though the research on non-rhizosphere and rhizosphere soil is more than 100 years still this ecological niche has become important from the point of interactions and beneficial effects. Though there are many studies on soil microbes and fungi of non-rhizosphere, rhizosphere soil including rhizoplane , there is a little or no studies on non-rhizosphere soil, rhizosphere soil and rhizoplane fungi of *Vitex nigumdo* Linn. , which is an important medicinal plant. Therefore data on fungal species associated with non-rhizosphere soil, rhizosphere and rhizoplane of *Vitex nigundo* has been presented in this paper.

MATERIALS AND METHODS

Vitex nigundo is a large shrub with three foliolate leaves**,** leaflets lanceolate, acuminate and flowers blue in terminal thyrasoid panicle, fruit is drupe and it belongs to *Verbenaceae* **(Figs. 1, 2)**. The chemical constituents are mostly flavones and iridoides. The leaves are used in aromatic tonic preparation as vermifuge, antiparasitic and anodyne. Fruit is used as a nervine tonic and vermifuge and flowers are used as cool astringent. The oil from leaf is used in rheumatism and also improves hair growth.

Vitex nigundo has been selected from Bhadrachalam forest localities of Telangana region. Non-rhizosphere, rhizosphere and rhizoplane samples were collected at monthly intervals for one year (2014-2015) under aseptic conditions. The collected soil samples are brought to the laboratory and subjected for the analysis of pH, moisture and fungi. The soils belong to sandyloam type with the pH of 7.8 and moisture percentage ranged between 20-30%, moisture percentage increases to 40% during rainy season.

Fig.1, 2- *Vitex nigundo*: Plant with flowering parts in sampling area.

The general laboratory techniques followed in this investigation were of Booth (1971) and Hawksworth (1974). The Potato Sucrose Agar (PSA), Tomato Agar Medium (TAM), Oat Meal Agar (OMA) and Vegetable Agar Medium (VAM) were used to isolate fungi from soil, rhizosphere soil and rhizoplane . Czepek-Dox Agar medium (CZA) was used for the identification of *Aspergilli* and *Penicillia* species. All the media are sterilized at 15 lbs pressure for 20 minutes.

Maintenance of cultures: Cultures were maintained on PSA slants and preserved in refrigerator. Sub culturing was done at 2-3 months interval. Fungi isolated from soil, rhizosphere soil and rhizoplane were maintained on PSA slants and stored at 4°c.

Slide preparation: Lactophenol and cotton blue in

Table-1. Fungal species isolated from different ecological niches of *Vitex nigundo* Linn.

S. No.	Name of the Species	NRS	RS	RP
1.	*Absidia* sp.	+	+	+
2.	*Acremoniella* sp.	-	+	-
3.	*Acremonium* sp.	+	+	-
4.	*Acrophialophora fusispora* (S.B.Saksena) Samson	+	+	-
5.	*Allescheriella* sp.	-	-	+
6.	*Alternaria alternate* (Fr.) Keissl	+	+	+
7.	*Aternaria atra* (Preuss) Woundenb & Crous	+	-	+
8.	*Aternaria fasciculata* (Cooke & Ellis) L.R. Jones & Grant	-	-	+
9.	*Aternaria humicola* Oudem	+	-	-
10.	*Aternaria longipes* (Elis & Everh.) E.W. Mason	-	-	+
11.	*Aternaria* sp.	+	+	+
12.	*Aternaria tenuissima* (Kunze) Wiltshire	-	+	+
13.	*Ascochyta* sp.	-	-	+
14.	**Aspergillus caespitosus* Raper & Thom	+	+	+
15.	*Aspergillus candidus* Link	+	+	+
16.	**Aspergillus chevalieri* Thom & Church	+	+	+
17.	*Aspergillus clavatus* Desm.	+	+	+
18.	*Aspergillus flavipes* (Bainier & RSartory) Thom & Church	-	-	+
19.	*Aspergillus flavus* Link	+	+	+
20.	**Aspergillus foetidus* Thom & Raper	-	+	+
21.	*Aspergillus fumigates* Fresenius	+	+	+
22.	**Aspergillus funiculosus* G. Sm.	+	+	+
23.	*Aspergillus humicola* Chaudhuri	+	+	+
24.	*Aspergillus luchuensis* Inui	+	+	+
25.	*Aspergillus nidulans* Eidam	+	+	+
26.	*Aspergillus niger* Tiegh	+	+	+
27.	*Aspergillus ochraceus* G.Wilhi	+	+	-
28.	**Aspergillus panamensis* Raper & Thom.	-	+	-
29.	*Aspergillus phoenicis* (Corda) Thom& Currie	-	+	-
30.	*Aspergillus restrictus* Raper & Thom.	+	+	+
31.	*Aspergillus sacchari* Chaudhuri & Sachar	-	+	-
32.	**Aspergillus sulphurous* Desm.	+	+	
33.	*Aspergillus sydowii* Bainer & Sartory	+	+	+
34.	*Aspergillus terreus* Thom	+	+	+
35.	*Aspergillus unguis* (Emile Weil & Gauden) Thom & Raper	+	+	+
36.	*Aspergillus ustus* Thom	+	+	+
37.	*Aspergillus versicolor* (Vuill) Tiraboschi	+	+	+
38.	*Aspergillus violaceo-fuscus* Gasperini	+	+	+
39.	*Aureobasidium pullulans* (de Bary & Lowental) G. Arnand	-	+	+
40.	*Bahupaathra* sp.	+	+	+
41.	*Botryodiplodia* sp.	+	+	-
42.	*Botryotrichum* sp.	+	-	-
43.	*Cephaliophora irregularis* Thaxt	+	-	-
44.	*Cercospora* sp.	-	+	+
45.	*Chaetomella raphigera* Swift	+	+	-
46.	*Chaetomium aureum* Chievers	+	+	+
47.	*Chaetomium globosum* Kunze	+	+	-
48.	*Chaetomium rufum* K.Ramakr	-	+	-
49.	*Chaetomium* sp.	+	+	+
50.	*Chaetomium spirale* Zopf.	+	+	+
51.	*Choanephora* sp.	+	-	-
52.	*Cladosporium cladosporioides* (Fresen) G.A. de Vries	+	+	+
53.	*Cladosporium herbarum* (Pers.) Link	+	+	+
54.	*Cladosporium oxysporum* Berk. M.A. Curtis	+	+	+
55.	*Cladosporium sphaerospermum* Penz	-	+	-
56.	*Cladosporium variabile* (Cooke) G.A. de Vries	+	-	+
57.	*Colletotrichum falcatum* Butler & Bisby	+	+	+
58.	*Cryptomela* sp.	+	+	+
59.	*Cunninghamella echinulata*	+	+	+
60.	*Curvularia brachyspora* Boedijn	+	-	-
61.	*Curvularia clavata* Jain	+	+	-
62.	*Curvularia eragraotidis* Itenn & Mayer	+	+	+
63.	*Curvularia lunata* (Walker)Boedijn	+	+	+
64.	*Curvularia lunata* var. *aeria* (Bat.J.A Lima & C.T. Vascont.) M.B.Ellis	-	-	+
65.	*Curvularia pallescens* Boedijn	+	+	+
66.	*Curvularia prasadii* R.L. Mathur & B.L Mathur	+	-	+
67.	*Curvularia* sp.	+	+	+
68.	*Dendryphiella vinosa* (Bark. & Curt) Reisinger	-	+	-
69.	*Dicyma* sp.	+	+	-
70.	*Doratomyces microspores* (Sacc.) F.J. Marten & G.Sm.	+	+	+
71.	*Drechslera australiensis* (Bugnicourt) Subr. & Jain	+	+	+
72.	*Drechslera bicolour* (Mitza.) Subr. & B.L. Jain	-	-	+
73.	*Drechslera dematioidea* (Bubak & Wrobl) Scharif	-	+	-
74.	*Drechslera halodes* (Drechsler) Subr. & Jain	-	-	+
75.	*Drechslera monoceras* (Drechsler) Subr. & Jain	+	-	-
76.	*Drechslera rostrata* (Drechsler) Richardson & Fraser	+	-	-
77.	*Drechslera* sp.	-	+	+
78.	*Exophiala* sp.	-	-	+
79.	*Fusarium avenaceum* (Fr.) Sacc.	+	-	-
80.	*Fusarium dimerum* Penzig	+	+	+
81.	*Fusarium equiseti* (Corda) Sacc.	+	-	
82.	*Fusarium moniliforme* J.Sheld		-	+
83.	*Fusarium nivale* (Fr.) Sarauer	+	+	+
84.	*Fusarium oxysporum* Schltdl	+	+	+
85.	*Fusarium poae* (Peck) Wollenw		+	+
86.	*Fusarium semitectum* Berk. & Ravenel	+	-	+
87.	*Fusarium solani* Sacc.	+	+	+
88.	*Fusarium* sp.	-	-	+
89.	*Gilmaniella humicola* G. L. Barron	-	-	+
90.	*Gliocladium* sp.	+	-	+
91.	*Graphium penicilloides* Corda	+	-	+
92.	*Graphium putredinis* (Corda) S. Hughes	+	-	-
93.	*Helminthosporium* sp.	-	+	-
94.	*Humicola* sp.	+	+	+
95.	*Humicola grisea* Traaen	+	+	+
96.	*Humicola nigrescens* Omvik	+	-	-
97.	*Hyalopus* sp.	+	+	+
98.	*Masoniella* sp.	-	-	+
99.	**Melanopsmma pomiformis* (Pres.ex. Fr.) Sacc	+	-	-
100.	*Memnoniella echinata* (Rivolta) Galloway	-	+	-
101.	*Monilia* sp.	+	-	-
102.	*Monodictys glauca* (Cooke & Harkn.) S.Hughes	-	+	-
103.	*Monosporium* sp.	-	+	-
104.	*Mucor haemiliensis* Wehmer	+	-	-
105.	*Mucor racemosum* Fresen	+	+	+
106.	**Mucor sphaerosperus* Hagem	-	-	+
107.	*Mucor variens* (H.Mart.) Fr.	+	+	+
108.	**Myrothecium brachysporum* Nicot.	-	+	-
109.	*Myrothecium gramineum* Lib.	+	+	+
110.	*Myrothecium verrucaria* Alb. Schwein.	-	-	+
111.	*Nakataea oryzae* (Catt.) J. Luo & N. Zhang	+	+	+
112.	*Neocosmospora vasinfecta* E F Sm.	-	-	+
113.	*Nigrospora oryzae* (Berk. & Broome) Petch.	+	+	+
114.	*Nigrospora sacchari* (Speg.) E.W. Mason	-	+	-
115.	Nodulisporium puniceum (Cooke & Ellis) Deighton	+	+	+
116.	*Paecilomyces fusisporus* S.B. Saksena	+	+	+
117.	*Paecilomyces griseola* Petch.	-	+	-
118.	*Paecilomyces humicola* Onions & G.L. Barron	+	+	+
119.	*Papularia spherospema* Corda	-	-	+
120.	*Penicillium* sp.	+		+
121.	*Penicillium chrysogenum* Thom.	+	+	+
122.	*Penicillium citreo-viride* Biourge	+	+	-
123.	*Penicillium decumbens* Thom.	+	+	+
124.	*Penicillium dierckxii* Biourge	-	-	+
125.	*Penicillium fructigenum* Takeuchi	+	+	
126.	*Penicillium funiculosum* Thom.	+	+	+
127.	*Penicillium herquei* Bainier & Sartory	+	+	+
128.	**Penicillium implicatum* Biourge	+	+	+
129.	*Penicillium miczynskii* K.M. Zaleski	-	-	+
130.	*Penicillium notatum* Westling	-	+	-
131.	*Penicillium oxalicum* Currie & Thom	+	+	-
132.	*Penicillium pallidum* G.Sm.	-	-	+
133.	*Penicillium purpurogenum* Flerov.	+	+	-
134.	*Penicillium restrictum* J.C. Gilman & E.V. Abbott.	-	+	+
135.	*Penicillium rubrum* Stoll	+	+	+
136.	**Penicillium spinulosum* Thum	-	+	-
137.	*Penicillium tardum* Thom	+	+	+
138.	*Penicillium variabile* Wehmer	+	+	+
139.	*Penicillium varians* G.Sm.	+	+	+
140.	**Periconia atropurpurea* (Berk. M.A. Curtis) M.A. Litv.	+	+	+
141.	*Periconia hispidula* (Pers.) E.W. Mason & M.B.Ellis	-	-	+
142.	*Pestalotiopsis mangiferae* (Henn.) Steyaert	-	-	+
143.	*Phoma ficuzzae* Brackel	+	+	+
144.	*Phoma nebulosa* (Pers.) Mont.	+	+	-
145.	*Phoma humicola* J.C. Gilman & E.V.Abbott	+	+	+
146.	*Phycomyces* sp.	+	+	+
147.	*Pithomyces atro-olivaceus* (Cooke & Harkn.) M.B.Ellis	-	+	+
148.	*Pithomyces flavus* Berk. & Broome	+	-	-
149.	*Pseudobotrytis* sp.	-	+	-
150.	**Rhinocladiella basitona* (de Hoog) Arzanlou & Crous	-	+	+
151.	**Rhinocladiella mansonii* (Castell.) Schol-Schwarz	+	+	+
152.	*Rhizoctonia bataticola* (Taubenth.) E.J. Butler	+	+	+
153.	*Rhizopus stolonifer* (Ehrenb.) Vuill	-	+	-
154.	*Rhizopus arrhizus* A. Fisch.	+	+	+
155.	*Scolecobasidium constrictum* E.V. Abbott.	+	+	-
156.	*Scolecobasidium humicola* G.L. Barron & L.V. Busch	+	+	+
157.	*Scolecobasidium* sp.	-	+	-
158.	*Scolecobasidium variabile* G.L. Barron & L.V. Busch	+	-	-
159.	*Scopulariopsis* sp.	-	+	+
160.	*Scopulariopsis brevicaulis* (Sacc.) Bainier	+	+	+
161.	**Scytalidium lignicola* Pesante	+	-	-
162.	*Sepedonium* sp.	+	+	-
163.	*Sphaeronaema* sp.	-	+	+
164.	*Spicaria divaricata* (Thom) J.C. Gilman & E.V. Abbott.	+	+	+
165.	**Spicaria elegans* (Corda) Harz.	+	+	+
166.	*Spicaria fumosorosea* (Wize) Vassiljevsky	+	-	+
167.	*Spicaria griseola* Sacc.	-	-	+
168.	*Spicaria helothis* Charles	+	-	-
169.	*Spicaria silvatica* Oudem.	+	+	+
170.	*Spicaria* sp.		+	-
171.	**Sporotrichum roseolum* Oudem. & Beij.	+	+	-
172.	*Stachybotrys chartarum* (Ehrenb.) S.Hughes	+	+	+
173.	**Stachybotrys pervispora* S.Hughes	+	+	+
174.	*Stachylidium bicolour* Link	+	+	+
175.	*Stemmaria* sp.	+	-	+
176.	*Syncephalastrum* sp.	+	-	-
177.	*Tetracoccosporium* sp.	-	+	-
178.	*Thamnidium* sp.	+	+	
179.	*Thielaviopsis paradoxa* (De Seynes) Hohn.	+	+	+
180.	*Torula allii* (Harz) Sacc.	+	+	+
181.	*Torula herbarum* (Pers.) Link	-	+	+
182.	*Torula* sp.	+	+	-
183.	**Trichocladium canadense* S. Hughes	-	+	-
184.	*Trichoderma koningi* Oudem.	+	+	+
185.	*Trichoderma viride* Pers.	+	+	+

Table 1 contd....

S. No.	Name of the Species	NRS	RS	RP
186.	**Trichurus spiralis* Hasselbr.	+	+	+
187.	*Tritirachium* sp.	-	-	+
188.	**Veronaea apiculata* (J.H. Mill., Giddens & A.A. Foster) F.B. Ellis	-	+	-
189.	**Wardomyces inflatus* (Marchal) Hennebert	-	+	+
190.	Yeast	+	+	+
191.	*Zygorhynchus moelleri* Vuill.	+	-	-
NRS= Non Rhizosphere Soil, RS=Rhizosphere Soil, RP= Rhizoplane, + = Present, - = Absent., *=Fungal species New to Telangana, India.				

lactophenol were used for mounting and staining to prepare semi-permanent slides which were sealed with D.P.X mountant.

Microscopic observation: Leitz Research microscope with adequate high power has been used throughout the study. The fungi were photographed using trinocular stereo research microscope.

The dilution plate technique, soil plate technique, root grinding and direct root pieces plating techniques were employed to study the fungi of root region in the present investigation.

Isolation and estimation of fungi from soil and rhizoplane

(a) Dilution plate method for isolation of fungi from soil: For quantitative estimation of fungi the dilution plate method of Waksman (1952) and as described by Johnson and Curl (1972) was employed. For qualitative and quantitative assessments five gram of soil sample was shaken by hand for 10 to 20 minutes in 50 ml sterile distilled water and successive dilutions were made as required. Petri dishes were incubated at room temperature 28+2°C for 3 days and observed for fungi. 1:10,000 dilution was chosen for the quantitative estimation of fungi. 1 ml of dilutant was transferred aseptically onto sterile Petri dishes for each sample and the sterile medium was added. The suspensions were mixed well with the agar by rotating the plate in clock-wise and anti-clockwise directions and then allowed to set. 0.01% streptopenicillin solution was added to avoid bacteria and others.

(b) Soil plate method for isolation of fungi from soil: This method was adopted from the direct inoculation technique of Warcup (1950) to study the ecological distribution of various species of fungi from soil. Micro samples of soil were transferred to Petri dishes with the help of micro spatula and were mixed directly with cooled Tamato agar medium (TAM) under aseptic conditions. The dishes were incubated at room temperature 28± 2ºC and fungal colonies were observed after 1 or 2 days.

(c) isolation of fungi from rhizoplane: To isolate fungi from rhizoplane root pieces of 5mm length after several washings with distilled sterile water were placed aseptically on agar plates containing Potato Sucrose Agar (PSA) (Harley and Waid, 1955). Root maceration technique (Stover and Waite, 1953) was used with slight modification to get a satisfactory number of fungal colonies. The roots from which rhizosphere soil was collected were washed thoroughly with sterile water and dried between filter paper. Five grams of roots in 50 ml of sterile distilled water was macerated in sterilized waring blender (Singh, 1965) and serial dilutions were prepared from the blended material to get a final dilution of 1/10,000 to islolate fungi. One ml of this dilutant was pippetted out into sterilized petridishes to which melted and cooled PSA medium was added. Plates were incubated for 5 to 7 days at room temperature 28± 2ºC. Individual colonies were picked up for further identification of fungi.

Identification of fungal species: Identification of the fungal isolates was made both on the dilution plates and soil plates. *Aspergillus* and *Penicillium* species were grown on Czepak's dox agar medium as recommended by Thom and Raper (1945) and were identified with the help of manuals written by Raper and Fennel (1965) and Raper and Thom (1949). Species of *Fusarium* are grown on potato sucrose agar medium and identified with the help of the manual written by Booth (1971, 1977). Remaining soil fungi grown on various media were identified with the help of the keys provided by Barron (1968), Barnett and Hunter (1972), Dix and John Webster (1995), Ellis (1971, 1976), Gilman (1957), Nagamani *et al.* (2006), Onions and Barron (1967), Rifai (1969), Seth (1970), Subramanian (1971) Thomas *et al.* (2013) and Tulloch (1972) in their respective manuals.

RESULTS AND DISCUSSION

Altogether 191 fungal species are found associated with non-rhizosphere, rhizosphere and rhizoplane of *Vitex nigundo.* Such a huge number of species have not been reported earlier in any of the research study conducted so far (**Table 1**). Around 130, 138 and 128 fungal species are found associated with non-rhizosphere soil, rhizosphere soil and rhizoplane of *Vitex nigundo,* respectively **(Table 2 & Fig. 3**). The highest number of fungal species association in rhizosphere may be because of specific root exudates present in *Vitex nigundo* (Mukerji *et al.* 2006) and other factors. Species representing *Aspergillus* followed by *Penicillium, Fusarium* and *Curvularia* dominated in Non-rhizosphere, rhizosphere soil and rhizoplane, respectively **(Table 3 & Figs. 4, 5, 6)**.

Table-2. Number of Fungal species in Non-rhizosphere, rhizosphere and rhizoplane.

S. No	Sampling Area	No. of Fungal species
1	NRS	130
2	RS	138
3	RP	128

NRS= Non Rhizosphere Soil RS=Rhizosphere Soil RP= Rhizoplane

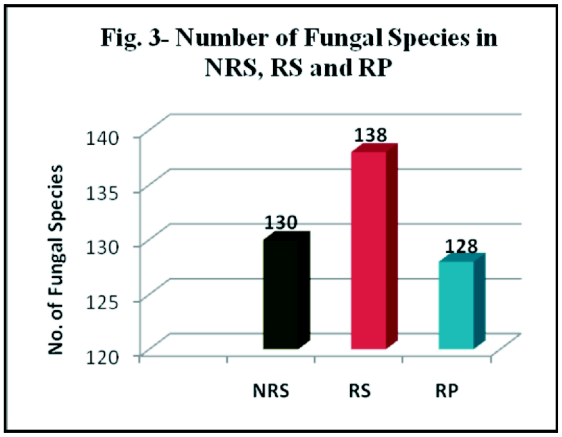
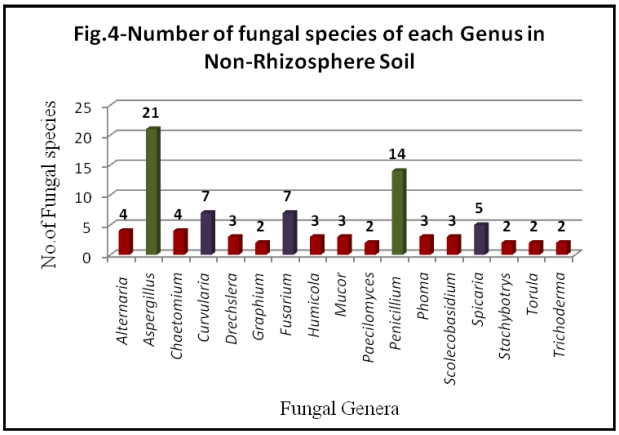
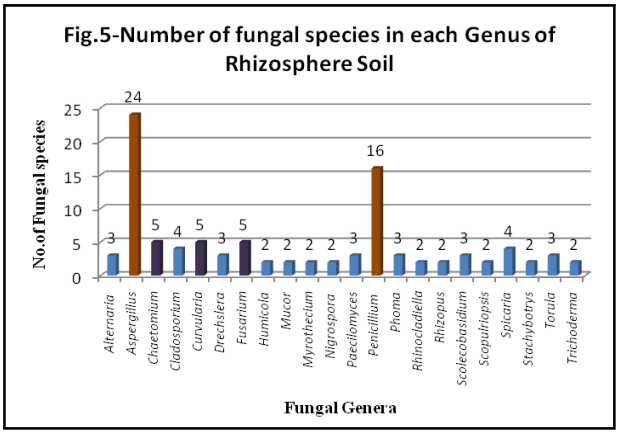


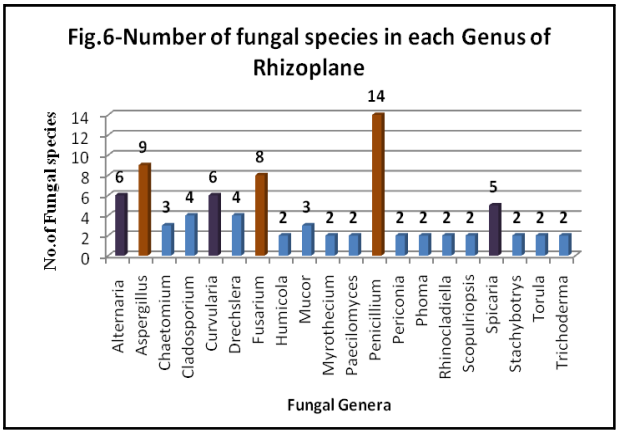
Table-3. Dominant genera associated with NRS, RS, RP of *Vitex nigundo*

S. No	Sample	Dominant Genera
1	NRS	*Aspergillus* (21) *Penicillium* (14) *Fusarium* (7) *Curvularia* (7)
2	RS	*Aspergillus* (24) *Penicillium* (16) *Fusarium* (5) *Curvularia* (5)
3	RP	*Penicillium* (14) *Aspergillus* (9) *Fusarium* (8) *Curvularia* (6)
NRS= Non Rhizosphere Soil RS=Rhizosphere Soil RP= Rhizoplane.		

Many species of *Fusarium*, one species of *Gleocladium*, three species of *Phoma* and *Rhizoctonia botaticum* the well known







root infecting and soil-borne fungi are found associated with rhizoplane. Species of *Aspergillus* and *Penicillium* are quite dominant in rhizoplane.

Altogether 22 fungal species are reported as new additions to Telangana state.Though such a huge number of fungal species are reported in the present investigation, but some fungal species have been reported earlier in non-rhizosphere, rhizosphere and rhizoplane of some other medicinal plants (Abdel-Hafez *et al.*, 2012; Abdel-Hafez, 1982; Adamović *et al.*, 2015; Anderson, 2004; Anna Muratova *et al*., 2003; Beula Rani *et al.*, 2016; Chandrashekar *et al.*, 2014; Curlevski *et al.*, 2010; Gabriele Berg and Kornelia Smalla, 2009; Jalander and Mamatha, 2015; Mwajita *et al.*, 2013; Mehrotra and Kakkar,1972; Odunfa and Oso, 1979; Patil and Morkhade, 2016; Puja and Dave, 2011; Ramesh *et al*., 2012; Rovira, 1991; Shivanna and Vasanthakumari, 2011 and Tamilarasi *et al.,* 2008).

CONCLUSION

In conclusion non-rhizosphere, rhizosphere and rhizoplane reports of *Vitex nigundo* have supported huge number of fungal species, which may be because of soil type, soil factors, root exudates, age of the plant and other related factors.

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