Journal of Environmental Biology
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January 2008, 29(1) 127-134 (2008)
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Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of *Coriandrum sativum*

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(Received: June 20, 2006 ; Revised received: December 11, 2006 ; Accepted: December 20, 2006)

Abstract: *The total heterotrophic bacteria, actinomycetes and fungus were enumerated from the rhizosphere and non – rhizosphere soil of 50 selected locally available medicinal plants in and around Bharathiar University. In all the plants, population of microorganism were higher in the rhizosphere soil than in the non rhizosphere soil. Among the microorganisms, bacterial population was higher in number followed by fungus and actinomycetes. Of the medicinal plants, the maximum rhizosphere effect was observed in Annona squamosa and the minimum effect was seen in Eclipta alba and Cassia auriculata. Among the bacteria the dominant species was Bacillus followed by Pseudomonas, Enterobacter, Corynebacterium, Micrococcus and Serratia. The Streptomyces species was found to be dominant followed by Deuteromycetes and Frankia among the actinomycetes. Among the fungal isolates Rhizopus was found to be higher in number followed by Aspergillus, Penicillium, Mucor and Fusarium. About 70.96% of the bacterial isolates were found to be nitrate reducers and 90.60% of the bacteria solubilised phosphate. The rhizosphere bacterial isolates were also capable of hydrolyzing starch, cellulose, casein, urea and gelatin. The isolates of bacteria, actinomycetes and fungus were also able to produce phytohormone Indole – 3 – acetic acid (IAA). The maximum IAA production was recorded by Fusarium sp (5.8 mg/l). The rhizosphere bacterial isolates showed resistance to 14 commercially used antibiotics. In an attempt to check the influence of these plant growth promoting microorganisms on the antimicrobial property of Coriandrum sativum against Escherichia coli MTCC - 443 and Aeromonas hydrophila MTCC - 646, the results observed was not encouraging since the inoculants did not influence the antibacterial property. However extensive and in depth study is required to find out the influence of rhizomicroorganisms on the antibacterial property of medicinal plants. The other results clearly indicated that the rhizosphere microorganisms could be exploited for its innumerable properties and active metabolites.*

Key words: *Rhizosphere effect, Pytohormone, Posphate solubilisation, Atibacterial property*
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Introduction

India has one of the richest plant medical cultures in the world. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plants as potential sources of medicinal substances. Soil microorganisms constitute world's largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystems. The rhizosphere, a narrow zone, adjacent to and influenced by, living plant roots (Kennedy, 1999), 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 bacterial taxa in the rhizosphere can be affected by several factors including plant species (Miller *et al*., 1989), soil type (Hoitink and Boehm, 1999), soil management practices (Rovira *et al*., 1990), microbial interactions (Hedges and Messens, 1990) and other environmental variables. The composition of bacterial community in the rhizosphere is important for the performances of the plant, as bacterial species can have beneficial, neutral or harmful relationships with the roots (Buchenauer, 1998; Atkinson and Watson, 2000; Sylvia and Chellami, 2001). Microorganisms have been intentionally introduced into soil and rhizosphere environments in attempts to enhance certain agriculturally beneficial activities such as improvement of aggregate stability (Lynch, 1981), suppression of plant pathogen (Maplestone and Campbell, 1989) and promotion of plant growth (Lambert and Joos, 1989). For several decades bacteria have been introduced into soil to improve plant growth (Cooper, 1959; Mishustin and Naumova, 1962; Brown, 1974; Kloepper *et al*., 1980; Schipper *et al*., 1995). To date many studies on the inoculation of plant growth promoting rhizobacteria have been focused on some economically important agricultural crops, and wild flora has not been considered as research target (Glick, 1995; Bashan, 1998). Hence the study was planned to find out the rhizobacterial association of medicinal plants and the effect of rhizobacterial inoculum on antibacterial activity of the selected medicinal plants. The objectives of the study are to enumerate, identify and characterize the microorganisms present in the rhizosphere and non-rhizosphere soil samples of selected medicinal plants, to find out the antibiotic resistance pattern of bacteria isolated from the rhizosphere soil, to screen indole-3-acetic acid producing microorganisms among the rhizosphere isolates and to study the influence of inoculated rhizosphere microorganisms on the antibacterial property of *Coriandrum sativum.*

Materials and Methods

Selection of medicinal plants: Fifty locally available medicinal plants in and around Bharathiar University were selected for the study. The plants chosen for the study and their medicinal properties are given in Table 1.

Sample collection: The rhizosphere samples were collected by gently uprooting the plants using sterile shovel. The plants were shaken to remove unwanted soil particles. The soil particles adhered to the roots were transferred to sterile polyethylene bags. Soil adjacent few centimeters away from the roots were considered as non-rhizosphere soil. The samples were carried aseptically to the laboratory and were processed with in 1-2 hours.

Enumeration of rhizosphere microflora: Rhizosphere microflora of the 50 selected medicinal plants was estimated by pour plate technique. Nutrient agar medium for bacteria, Actinomycetes isolation agar (Himedia) for actinomycetes and Sabourad's dextrose agar for fungi were used. Plates were incubated at 37°C. Bacteria were counted after 24 hr, actinomycetes and fungi were counted after 4 to 7 days of incubation. Representative colonies of bacteria, actinomycetes and fungus from each plate were picked and streaked on to the respective medium to obtain pure culture. The isolates were identified (Brenner *et al*., 2005; Alexopolous *et al*., 1995).

Rhizosphere effect (Subbarao, 2000): The quantitative rhizosphere effect of the plants was calculated using the formula:

$$R/S = \frac{\text{Number of microorganism per gram of rhizosphere soil}}{\text{Number of microorganism in a gram of non-rhizosphere soil}}$$

Identification of microorganism: The bacteria were isolated, purified and identified to various genera by morphological and biochemical tests (Brenner *et al.,* 2005). The fungal isolates were identified following the procedure given by Alexopoulos *et al.* (1995).

Enzyme hydrolysis: The bacterial isolates were checked for the production of various hydrolyzing enzymes like amylase, cellulase, urease and gelatinase (Harrigan and Mc Cance, 1972).

Phosphate solubilisation (Glodstein, 1986): Pikovskaya's agar plates were inoculated with bacterial cultures and incubated at 37°C for 48 hr. The bacterial colonies forming clear halos were considered as phosphate solubilisers.

Nitrate reduction (Eckerson,1924): Sterilized nitrate broth was inoculated with bacterial cultures and incubated at 37°C for 24 hr. After incubation, 0.5 ml of α naphthylamine and sulphanilic acid was added. Pinkish red colour indicated the production of the enzyme nitrate reductase by the bacterial isolates.

Production of IAA: Nutrient broth, glucose asparagines broth and Sabourad's dextrose broth supplemented with 5 mM L–tryptophan was inoculated with the isolates of bacteria, actinomycetes and fungus respectively. After incubation, few drops of Salkowski's reagent was added. A change of colour from pink to red indicated the production of IAA (Almonacid *et al.*, 1996).

Estimation of IAA production by fungus: Fungus cultures were inoculated into 100 ml of Sabouraud's dextrose broth supplemented with 0.5M tryptophan in a 250 ml conical flask. The flasks were incubated at room temperature for 7 days. After incubation the broth was filtered through filter paper to remove fungal mats. The filtrate was centrifuged to remove spores. One ml of the supernatant was mixed with 2 ml of Salkowski's reagent and left for colour development for 20 minutes. The red colour developed was estimated spectrophotometrically at 530 nm in a Hitachi UV–Vis spectrophotometer U3210. IAA standards (Himedia) were also prepared (1 to 10 ppm) and estimated simultaneously.

Intrinsic resistance to antibiotics: Resistance of bacteria to amoxycillin, bacitracin, chloramphenicol, erythromycin, gentamycin, kanamycin, methicillin, nalidixic acid, neomycin and rifampicin (30 mcg/disc); polymyxin-B (300 mcg/disc); cefazolin, tetracycline, trimethoprim, vancomycin (10 mcg/disc) was tested. The cultures were enriched in nutrient broth for six to eight hours. The enriched cultures were then swabbed over Muller Hinton agar (Himedia, Mumbai) plates using sterile cotton swabs. After 24 hr incubation period, the diameter of the inhibition zones was measured and compared with the chart of Kirby – Bauer sensitivity method modified in July 1969 (Scherring Corporation, USA and Bloomfield, New Jersy) and Performance Standards for Antimicrobial Disk Susceptibility Tests, NCCLS Jan, 2002 (HiMedia Laboratories) and classified as resistant, intermediate and sensitive.

Screening of antimicrobial property in medicinal plants: Among the three medicinal plants namely *Brassica juncea, Coriandrum sativum* and *Foenum graecum* whose whole plant crude extract initially screened for antimicrobial property with selected human pathogens namely *Escherichia coli* MTCC - 443*, Aeromonas hydrophila* MTCC - 646*, Vibrio cholerae* MTCC - 3249*, Staphylococcus aureus* MTCC - 737 and *Salmonella typhi* MTCC – 734, *Coriandrum sativum* alone exhibited antimicrobial property against *Escherichia coli* and *Aeromonas hydrophila*. Thus *Coriandrum sativum* was selected for further study.

Effect of rhizosphere microorganisms on the antimicrobial property of *Coriandrum sativum*: The bacterium (*Pseudomonas* sp - RB1), actinomycetes (*Streptomyces* sp – RA12) and fungus (*Fusarium* sp – RF4) were enriched in the broth. The cells of bacteria and spores of actinomycetes and fungus were harvested and fixed to 1OD. The inoculum containing 2 ml of bacteria + 1.5 ml of actinomycetes + 1.5 ml of fungus was added into different combinations (Table 2). Certified seeds of *Coriandrum sativum* (Rajendra hybrid Semence Pvt Ltd COR – 1338) were purchased for the study. The combinations were inoculated with 30 seeds per container and sprinkled with water regularly. The antimicrobial property of leaf, shoot and root was examined periodically on 15th, 30th, 45th, 60th, 75th and 90th day.

Results and Discussion

Total heterotrophic bacteria, actinomycetes and fungi enumerated from the rhizosphere and non – rhizosphere soil samples of 50 selected medicinal plants are given in Table 3.

The total heterotrophic bacteria in rhizosphere was found to be maximum of 280×10^4 CFU/g in *Acacia nilotica* and minimum of 18×10^4 CFU/g in *Cassia auriculata*. The maximum actinomycetes population

Table - 1: Medicinal plants chosen for the study and their properties

Medicinal plants	Common names	Medicinal property
Acacia nilotica	Prickly acacia, Babool Indian	Toothache, ulcer, rheumatism
Acalypha indica	Indian acalypha	Pneumonia, bronchitis, asthma
Aloe vera	Aloe	Anthelminthic, piles and rectal fissures
Alternanthera sessilis	Matsyakshi	Headache, to treat wound
Amaranthus viridis	Prickly amaranth	Snake bite
Andrographis paniculata	The creat	Gastric troubles
Annona squamosa	Custard apple	Purgative, headache
Azadirachta indica	Neem	Antiseptic, ulcer, small pox
Bauhinia pupurea	Bauhinia	Astringent, dysentery
Calotrophis gigantea	Madar	Scabies, fever
Cassia angustifolia	Indian senna	Laxative, purgative
Cassia auriculata	Cassia	Anthelminthic
Casuarina equistifolia	Beef wood tree, she oak	Dysentery, diarrhoea
Catharanthus roseus	Periwinkle	Astringent, diaphoretic
Chrysanthemum cinerarifolium	Chandramallika	Stomach ache
Citrullus colocynthis	Lemon	Leprosy, rheumatism
Coleus ambonicus	Braod leaf thyme	Headache, vomiting
Coriandrum sativum	Koriander	Diuretic, refrigerant
Cassia occidentalis	Negro coffee	Diuretic, purgative
Eclipta alba	False daisy	Snakebite and to check hair fall
Ervatamia corolaria	Crepe jasmine	Eyepain, toothache
Euphorbia heterophylla	Mexican fire plant	Reconditioning of stomach after delivery
Euphorbia thymifolia	Vomit weed	Astringent, skin disease, amenorrhegia
Gloriosa superba	Glory lily	Abortofacient, cancer, ulcer
Hibiscus rosa – sinensis	Shoe flower	Demulcent, refrigerant
Ixora cocinea	Ixora	Malaria
Jasminum sambac	Jasmine	To treat urine blockage
Lawsonia inermis	Henna, Mignonette	To keep body cool, jaundice
Lycopersicon esculentum	Tomato	Mouth ulcer
Mangifera indica	Mango	Diarrhoea, hemorrhage
Mentha arvensis	Pudina	Anorexia, gastric trouble
Momorsica dioica	Bitter gourd	Diabetes, antiseptic
Moringa pterygosperma	Drumstick	Hysteria, epilepsy
Murraya krenigii	Curry leaves	Astringent, dysentery
Musa paradisiaca	Banana	Anthelminthic, antiscorbutic
Nerium oleander	Nerium	Skin disease, scabies, asthma
Ocimum basilicum	Common basil	Diuretic, headache, neuralgia
Parthenium hysterophorus	Grays fever few	Cancer treatment
Phyllanthus acidis	Gooseberry	Cough
Phyllanthus amarus	Carry me seed	Jaundice, Chronic dysentery, scabies
Portulaca oleraceae	Puslane	Vermifuge, anthelmenthic
Punica granatum	Pomegranate	Diarrhoea
Rosa centifolia	Rose	Stomach ache
Santalum album	Sandal	Skin disease
Solanum nigrum	Black night shade	Bronchitis, cough
Tectona grandis	Teak	Dyspepsia, eye problems
Tephrosia purpurea	Wild Indigo	Diarrhoea
Trichoderma indicum	Lane grass	Cold, joint pains
Zizipus mauritiana	Indian plum	Dysentery, astringent

was 76 x 10^2 CFU/g in *Coleus amboinicus* and minimum was found to be 8 x 10^2 CFU/g in *Annona squamosa* and *Eclipta alba*. The maximum fungal population was 100 x 10^2 CFU/g in *Acacia nilotica* and minimum was recorded as 6 x 10^2 CFU/g in *Portulaca oleraceae.*

In the non – rhizosphere soil, the maximum heterotrophic bacteria was 221 x 10^4 CFU/g in *Coleus amboinicus* and minimum was 6 x 10^4 CFU/g in *Calotrophis gigantea*. The maximum actinomycetes population was found to be 48 x 10^2 CFU/g in *Euphorbia thymifolia* and minimum was 5 x 10^2 CFU/g in *Azadirachta indica* and *Eclipta alba*. The maximum fungal population was 66 x 10^2 CFU/g in *Coleus amboinicus* and minimum was found as 4 x 10^2 CFU/g in *Catharanthus roseus* and *Portulaca oleraceae.*

Table - 2: Different combinations of soil and vermicompost with rhizosphere microorganisms

S. No.	Combinations	Amount in grams
1	Red soil	750
2	Sterile soil	750
3	Vermicompost	750
4	Red soil + rhizosphere isolates	750 + 5 ml
5	Sterile soil + rhizosphere isolates	750 + 5 ml
6	Vermicompost + rhizosphere isolates	500 + 5 ml
7	Red soil + vermicompost	500 + 250
8	Sterile soil + vermicompost	500 + 250
9	Red soil + vermicompost + rhizosphere isolates	500 + 250 + 5 ml
10	Sterile soil + vermicompost + rhizosphere isolates	500 + 250 + 5 ml

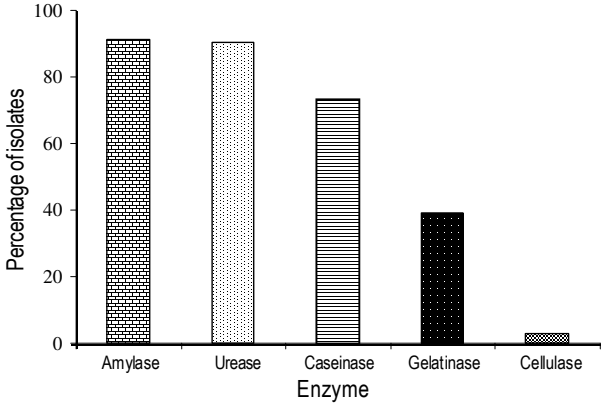

Fig. 1: Extracellullar enzyme producting rhizosphere bacteria

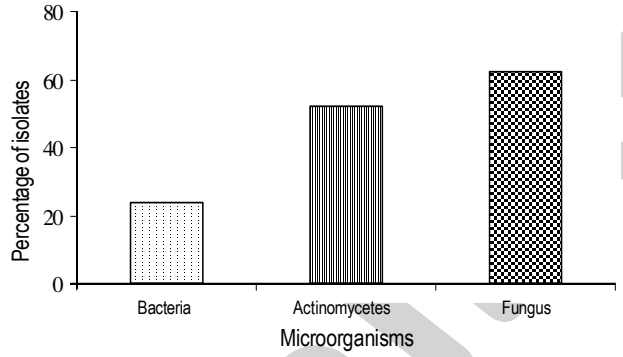

Fig. 2: IAA production by rhizosphere bacteria

The bacterial population was higher in the entire root zone of medicinal plants followed by fungal and actinomycetes population. Similarly the number of microorganisms was more in the rhizosphere soil than in the non – rhizosphere soil. The results can be related with the reports of Duine *et al.* (2005) who found the maximum rhizosphere population of 8.92 x 10^8 CFU /g and minimum rhizosphere count was 2.35 x 10^8 CFU/g of *Carex arenaria*. The maximum non – rhizosphere population was 6.61 x 10^6 CFU/g and the minimum bacterial number was 1.01 x 10^6 CFU/g. Wamberg *et al.* (2003) reported that the bacterial count in the rhizosphere was 7.45 x 10^7 CFU/g in *Pisum sativum.* The population recorded in our study was lesser when compared to earlier reports.

The actinomycetes population was more in the rhizosphere soil than in the non- rhizosphere soil in all the experimental plants. But Oza *et al.* (2002) reported that the population was remarkably high in the non-rhizosphere soil as compared to rhizosphere soil in the dominant plant species in the semiarid soil of Rajkot.

The total count of fungus in all selected medicinal plants of the present study was higher in the rhizosphere soil than in the non-rhizosphere soil. The results of the study are similar to that of Hissy *et al.* (1980), who studied the presence of more number of fungus in the rhizosphere soil than in the non- rhizosphere soil of five plants in Egypt.

The varying degree of population observed in the roots of the plants is due to the effect of the chemical composition of root exudate of the individual plants on the microorganisms. Many of the environmental factors such as temperature (Rovira, 1959), light (Hodge *et al.*, 1997) and atmospheric CO_2 concentration (Cheng and Johnson, 1998) are known to influence microbes in the rhizosphere. It is not yet known to what extent plants can select a constant rhizosphere community from highly contrasting reservoirs of bulk soil populations (Duine *et al.*, 2005).

Rhizosphere effect: The maximum rhizosphere effect was 11.19 x 10^4 in *Annona squamosa* and the minimum rhizosphere effect was recorded in *Eclipta alba* and *Cassia auriculata* as 1.06 x 10^4 (Table 3). The rhizosphere effect was higher in *Annona squamosa* and minimum in *Eclipta alba* and *Cassia auriculata*. The greater the rhizospheric effect the higher will be microorganisms number. Greater rhizosphere effect is seen with bacteria than the actinomycetes and fungi and only negligible changes are noted with regard to protozoa and algae (Subbarao, 2000). The rhizosphere effect greatly decreases as we move away from the root (Curl and Truelove, 1986). The varying types and quantities of rhizodeposits have been postulated to act as key factors influencing the density and diversity of the rhizospheric microorganisms (Grayston and Campbell, 1996).

Species distribution: The bacteria and fungus distribution in the rhizosphere soil are given in Table 4. 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*. In the rhizosphere soil, *Bacillus* population was found to be higher followed by *Pseudomonas*, *Enterobacter, Corynebacterium, Micrococcus* and *Serratia.* The rhizosphere accommodates a large number of saprophytic bacteria with stimulating, neutral or deleterious effects on plants (Berggren *et al.*, 2001). Among the actinomycetes isolates *Streptomyces* was found to be maximum followed by *Deutromycetes* and *Frankia* sp. In the soil 80% of actinomycetes population is *Streptomyces* (Subbarao, 2000). In fungus the highest genera recorded was *Rhizopus* followed by *Aspergillus, Penicillim, Mucor* and *Fusarium.*

Table - 3: Total heterotrophic bacteria, actinomycetes and fungus in the rhizosphere and non-rhizosphere soil of selected medicinal plants

	Medicinal plants	Bacteria (x 10^4 CFU/g)		Actinomycetes (x 10^2 CFU/g)		Fungus (x 102 CFU/g)		Rhizosphere effect x 10^4
		R	NR	R	NR	R	NR	
1	*Acacia nilotica*	280	150	60	47	100	42	1.87
2	*Acalypha indica*	60	49	11	8	26	14	1.22
3	*Aloe vera*	270	32	9	7	48	16	8.43
4	*Alternanthera sessilis*	54	22	34	8	57	25	2.45
5	*Amaranthus viridis*	47	42	37	30	36	31	1.11
6	*Andrographis paniculata*	112	96	16	12	33	16	1.16
7	*Annona squamosa*	235	21	8	7	16	12	11.19
8	*Azadirachta indica*	41	19	17	5	18	17	2.16
9	*Bauhinia purpurea*	86	28	13	7	23	20	3.07
10	*Calotrophis gigantea*	53	6	43	25	54	37	8.83
11	*Cassia angustifolia*	49	43	36	33	41	31	1.14
12	*Cassia auriculata*	18	17	16	12	31	26	1.06
13	*Casuarina equistifolia*	59	46	37	12	58	33	1.28
14	*Catharanthus roseus*	267	43	12	9	8	4	6.20
15	*Chrysanthemum cinararifolium*	75	49	48	27	25	16	1.53
16	*Citrullus colocynthis*	99	66	23	13	38	24	1.50
17	*Coleus amboinicus*	258	221	76	37	82	66	1.17
18	*Coriandrum sativum*	65	54	27	13	27	13	1.20
19	*Cassia occidentalis*	242	214	9	7	30	22	1.13
20	*Eclipta alba*	74	70	8	5	23	8	1.06
21	*Ervatamia coronaria*	66	51	11	7	28	19	1.29
22	*Euphorbia heterophylla*	60	36	12	9	32	27	1.67
23	*Euphorbia thymifolia*	100	59	47	48	55	27	1.69
24	*Gloriosa superba*	39	10	31	19	41	33	3.90
25	*Hibiscus rosa - sinensis*	294	158	33	29	72	33	1.35
26	*Ixora cocinea*	79	58	15	9	31	21	1.36
27	*Jasminum sambac*	66	55	18	10	26	19	1.20
28	*Lawsonia inermis*	61	46	26	17	26	11	1.33
29	*Limonia crenulata*	51	39	21	8	36	23	1.31
30	*Lycopersicon esculentum*	74	60	15	8	28	21	1.23
31	*Magnifera indica*	279	70	9	7	20	17	3.98
32	*Mentha arvensis*	95	58	16	13	22	12	1.64
33	*Momorsica diocica*	120	34	35	12	46	35	3.53
34	*Moringa pterygosperma*	36	25	13	5	50	39	1.44
35	*Murraya koenigii*	62	41	30	18	21	18	1.51
36	*Musa paradisiaca*	177	204	12	39	41	34	1.15
37	*Nerium oleander*	76	22	16	14	49	18	3.45
38	*Ocimum basilicum*	60	49	11	8	26	14	1.22
39	*Parthenium hysterophorus*	216	129	27	25	35	21	1.67
40	*Phyllanthus acidis*	44	15	22	23	43	40	2.93
41	*Phyllanthus amarus*	108	28	14	8	39	16	3.86
42	*Portulaca oleraceae*	107	59	19	10	6	4	1.81
43	*Punica granatum*	86	30	27	22	34	28	2.87
44	*Rosa centifolia*	66	25	12	5	35	21	2.64
45	*Santalum album*	46	14	13	7	23	20	3.29
46	*Solanum nigrum*	88	69	21	12	46	24	1.28
47	*Tectona grandis*	52	38	56	38	66	41	1.37
48	*Tephrosia purpurea*	46	21	15	12	48	29	2.19
49	*Trichoderma indicum*	70	54	18	9	39	30	1.30
50	*Zizipus mauritiana*	38	8	14	7	31	25	4.75

R = Rhizosphere soil, NR = Non-rhizosphere soil

Table - 4: Generic distribution of microorganisms in the rhizosphere soil of selected medicinal plants

S.no.	Bacteria	Percentage
1	*Bacillus*	80.7
2	*Pseudomonas*	9.6
3	*Enterobacter*	6.4
4	*Corynebacterium*	1.2
5	*Micrococcus*	1.0
6	*Serratia*	1.0
7	*Rhizopus*	43.8
8	*Aspergillus*	26.1
9	*Penicillium*	13.6
10	*Mucor*	9.5
11	*Fusarium*	6.8

Table - 5: Antibiotic resistance pattern of bacteria isolated from rhizosphere soil samples of selected medicinal plants

S. no.	Representative isolates	Pattern of resistance
1	*Pseudomonas* sp	B, Cz, C, E, K, M, Na, R, T, Tr, Va
2	*Bacillus* sp	B, Cz, E, M, P
3	*Bacillus* sp	Cz, E, P, R, Tr
4	*Bacillus* sp	Cz, C, Na, R, Tr
5	*Bacillus* sp	B, Cz, E, Na
6	*Bacillus* sp	……
7	Serratia sp	Cz, Na
8	*Bacillus* sp	K, P, Tr
9	*Bacillus* sp	Cz, Na
10	*Bacillus* sp	B, C, Na, P, R
11	*Bacillus* sp	B, Cz, M, Na, P, Tr
12	*Bacillus* sp	……
13	*Bacillus* sp	Cz, P
14	*Bacillus* sp	Cz, N
15	*Bacillus* sp	B, Na, P
16	*Bacillus* sp	A, B, Cz, M
17	*Bacillus* sp	Cz, Na, P, Tr
18	*Bacillus* sp	Cz, Tr
19	*Bacillus* sp	Cz, M, P, R, Tr
20	*Pseudomonas* sp	Am, B, Cz, E, M, P, R, Va
21	*Bacillus* sp	B, Cz, E, Na
22	*Bacillus* sp	Cz, Na, P, R,Tr
23	*Bacillus* sp	B, E, M, P, R
24	*Bacillus* sp	P
25	*Bacillus* sp	C, Na, Va
26	*E. Coli*	B, Cz, E, Na, P
27	*Bacillus* sp	Cz, R
28	*Bacillus* sp	B, E, R
29	*Bacillus* sp	……
30	*Bacillus* sp	B, Cz, E, Na, P, Tr
31	*Bacillus* sp	B, P, R,1

Selection of the isolates: A sub sample of 63 isolates all but one of which displayed strong tendencies for below mentioned traits was selected from the 310 isolates.

Hydrolytic enzymes: Starch hydrolyzing enzyme amylase was produced by 91.26% of the bacterial isolates, urease enzyme was produced by 90.32% of isolates, 73.6% of isolates exhibited caseinase enzyme activity, and 39% of isolated hydrolyzed gelatin and 3% of isolates produced cellulase (Fig. 1). All the bacterial isolates are capable of producing various hydrolytic enzymes like amylase, cellulase, gelatinase, urease and caseinase. A wide range of enzymes, which are of plant and microbial origin present in the rhizosphere, catalyze the breakdown of organic materials (Subbarao, 2000). The presence of these enzymes will help in breaking down the complex nutrients into simpler form and thus make available to plants. In turn the root exudates provide the substrate for the survival and growth of microorganisms in the rhizosphere.

Phosphate solubilisation and nitrate reduction: Among the bacterial isolates 70.96% of the isolates exhibited phosphate solubilizing capacity in Pikovskaya's agar medium and 90.3% of the isolates reduced nitrate to nitrite and ammonia. About 70% of the bacterial isolates were able to produce nitrate reductase, which converts nitrate to nitrite and ammonia. Nitrate reducing bacteria are common members of rhizosphere. Nijburg *et al.* (1997) enumerated nitrate reducing strains from the rhizosphere of *Glyceria maxima* that ranged from 3.2×10^6 to 3.3×10^8 CFU /g and they have also found that the total number of potential nitrate reducing strains in the rhizosphere significantly increased with NO_3^- addition.

Almost all the rhizosphere bacterial isolates were able to solubilize phosphate by producing phosphatase enzyme. Phosphorus is the second most limiting nutrient for plants. Phosphorus is an essential plant nutrient that is added to soil as soluble inorganic phosphates, a large proportion of which becomes insoluble and therefore unavailable to plants (Singh and Kapoor, 1994). Many species of bacteria are able to solubilise phosphates *in vitro* and most of them live in the plant rhizosphere. At present *Bacilli*, *Rhizobia* and *Pseudomonas* are most studied phosphate solubilisers (Rodriguez and Fraga, 1999) and most of them live in the plant rhizosphere. The results can be correlated with the work of Matsumoto *et al.* (2004) and Piex *et al.* (2005), who isolated the phosphate solubilising community from the rhizosphere of trees and grass.

IAA production: 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 (Fig. 2). The degree of IAA production by fungal isolates was estimated spectrophotometrically. Among them *Fusarium* species produced maximum 5.8 mg/l of IAA. The bacteria, actinomycetes and fungus isolated from the rhizosphere soil were able to produce IAA in the presence of tryptophan supplement. When compared to bacteria and actinomycetes, the IAA produced by fungus was higher in level and the maximum IAA produced was 5.8 mg/l by *Fusarium* sp. In addition to higher plants, numerous bacteria and fungi also have the ability to synthesize plant growth regulators such as indole – 3 – acetic acid and other indole related compounds (Furukawa *et al.*, 1996). Generally microorganisms isolated from the rhizosphere and rhizoplane of various crops have more potential of producing auxins than those from the root free soil (Arshad and Frakenberger, 1998).

The results can be related with the reports of Dey *et al*. (2004) who isolated the PGPR's that produced IAA like substances from the rhizosphere of *Arachis hypogaea*. The maximum production was 11.8 mg/l by one of the PGPR.

Antibiotic resistance pattern: Nearly 310 rhizomicroorganisms were isolated and identified (genera level) and 61 representative isolates were screened for the detailed study of enzyme hydrolysis, IAA production *etc.* Based on those criteria, a sub sample of 31 isolates showing strongest production was chosen for the antibiotic assay. Among them *Pseudomonas* sp exhibited maximum resistance pattern to 11 antibiotics and three of the *Bacillus* species were resistant to all the 15 antibiotics tested. Of the total 31 bacterial isolates checked for the antibiotic resistance pattern, about 61.51% of bacteria showed resistance to antibiotic cefazolin and all the isolates were sensitive to gentamycin (Table 5). *Pseudomonas fluorescens* exhibited a wide range of resistance to many antibiotics. Similarly many of the *Bacillus* species isolated from the rhizosphere also showed resistance to some of the antibiotics. The antibiotic resistance property is stored in extracellular DNA called plasmid. The antibiotic resistance property shown by the rhizosphere isolates indicate that the bacterial isolates got adapted to various commercially and regularly used antibiotics or similar allelochemicals produced by the plants. The source may be from the fertilizers added to soil, many of the animal wastes, municipal wastes.

Influence of rhizomicroorganisms and vermicompost amended soil on antimicrobial property of *Coriandrum sativum*

Antimicrobial property: *Coriander* seedlings were grown in various combinations and harvested. The antimicrobial property of the whole plant crude aqueous extract was analyzed on the 15th, 30th, 45th, 60th, 75th and 90th days against human pathogenic strain *Escherichia coli* MTCC - 443 and *Aeromonas hydrophila* MTCC - 646. In every stage the extracts produced 6 mm to 7 mm zone against *E. coli* and 7 mm to 8 mm zone against *A. hydrophila*. There was not much variation in zone formation with plants grown in different ratios and in different growth stages.

The growth of the *Coriandrum sativum* was medium in the ordinary soil while the growth was higher with vermicompost. The growth was less in sterile soil. This is because all the indigenous microorganisms present in the soil were killed during sterilization. In the ratios with the vermicompost addition, the growth of the plants was enhanced. The compost in general contains some suppressive microorganisms that inhibit the growth of the root pathogens. At the same time some useful microorganisms, can act as plant growth promoting rhizobacteria and thus increases plant growth.

In the study, the introduced rhizosphere isolates did not show any influence on the growth and antimicrobial property of *Coriandrum sativum*. The frequent failure of the performance of inoculants in the field was noticed, an observation that is difficult to explain because of poor understanding of the factors which influence survival, proliferation and dispersal of soil inoculants. When laboratory grown cultures are introduced to soil many bacterial species decline rapidly in numbers (Liang *et al.*, 1982). This may be due to predation, competition or nutrient limitation and varies according to the species of bacteria introduced, the soil type and numerous factors. To successfully use bacterial strains as inoculants that enter plant soil systems, the relationships between the inoculants and plant should be beneficial. Inspite of the deleterious effects of some microorganisms on plants, the beneficial effects are usually greater and the overall results are generally demonstrated by growth promotion and faster germination (Atlas and Bartha, 1998). Although there may be some artificial and trivial effects on plant growth promotion induced by the inoculation of some microorganisms, overall evidence showing significant plant growth effects induced by rhizosphere microorganisms are overwhelming (Gerhardson and Wright, 2002). The important mechanisms include direct phytohormonal action, plant disease suppression, enhancement of plant nutrient availability and the enhancement of other plant beneficial microorganisms (Gerhardson and Wright, 2002).

Thus the rhizosphere isolates from medicinal plants are able to solubilise phosphate, reduce nitrate, produce hydrolytic enzymes, phytohormone and have multiple antibiotic resistance capacity. These characteristics confirm that these rhizosphere microorganisms are plant growth promoting microbes. These isolates can be further used as bioinoculum and can be exploited for the synthesis of numerous metabolites, which can be used commercially. Further trials and indepth studies are required to check whether these PGPR's have influence over the enhancement of specific active principle of many other medicinal plants.

To unravel the complex microbial – faunal interactions in the rhizosphere, further research on rhizosphere processes requires a multidisciplinary approach and an interdisciplinary exchange of knowledge is essential. Although the study of rhizospheric bacteria is difficult due to high number of bacteria present in soil, characterization and identification of these bacteria are necessary for wide ecological studies of the plant rhizosphere.

Acknowledgments

The authors are very much thankful to Professor and Head, Department of Environmental Sciences, Bharathiar University, Coimbatore, India for providing necessary facilities to carry out this work.

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