Biol Fertil Soils (1996) 21:131–137
© Springer-Verlag 1996

ORIGINAL PAPER

A. Pandey · L.M.S. Palni

The rhizosphere effect of tea on soil microbes in a Himalayan monsoonal location

Received: 25 April 1994

Abstract Monthly investigations of the microbial population associated with tea soils, in terms of colony-forming units assessed by the plate-count method, were carried out at three different soil depths for a period of 12 months. Three groups of microbes, bacteria, actinomycetes, and fungi, were examined. Contrary to general observations, the rhizosphere : soil ratios were found to be consistently below 1 in samples taken from established tea bushes, indicating an overall negative rhizosphere effect. Interactions among certain microorganisms may also have contributed to this effect. Nevertheless, the rhizosphere of young tea plants and that of a number of other perennial plants, of different ages, growing in established tea fields, appeared to stimulate microbial growth. The negative effect of the rhizosphere of older tea bushes does not appear to be a common phenomenon that is related to the aging of plants in general, but seems to be unique and specific to tea plants.

Key words Rhizosphere · Non-rhizosphere · R : S ratio · Tea · Colony-forming unit · Actinomycetes · Bacteria · Fungi · *Camellia sinensis*

Introduction

The term rhizosphere was introduced by Hiltner (1904) to denote the region of the soil that is subject to the influence of plant roots. The "rhizosphere effect" is defined as the overall positive influence of interactions between plant roots and rhizoflora on the development of the plant (Starkey 1958; Katznelson 1965; Rovira 1965; Lynch 1987a; Bowen and Rovira 1989; Rovira 1991). Most studies on the rhizosphere effect have, however, been carried out on short-lived plants. Rovira (1965) cited the example of genge (*Astragalus sinicus* L.), a legume, for which rhizosphere : soil ratios between 100 and 400 have been reported. Values as high as 2000 are also on record (Fitter and Hay 1981). Rouatt and Katznelson (1961) studied rhizosphere : soil ratios for bacteria for different annual plants and found that the rhizosphere effect varied with the plant species, for example, ratios of 24.3, 5.5, 5.9, 3.3, 3.6, and 5.9 were recorded for red clover, flax, oats, maize, barley, and wheat, respectively.

Only a few reports have described the microflora associated with roots of perennial plants. Rangaswami and Vasantharajan (1962) carried out experiments using three species of six-year-old full-grown citrus trees during growing and non-growing period, and reported rhizosphere : soil ratios of 90–100, 5–6, and 3–4 for bacteria, actinomycetes and fungi, respectively, in the growing season; during the dormant season these ratios fell to 39–40, 2–3, and 4–6, respectively. Ivarson and Katznelson (1960) have also reported stimulation of the microbial population in the rhizosphere of yellow birch, with rhizosphere : soil ratios ranging from 8 to 10. Experiments have also been carried out under artificial conditions. For example, Baath et al. (1988) compared the growth of 10 bacterial isolates in the rhizoplane, rhizosphere, and the non-rhizosphere soil of rape seedlings grown in an otherwise sterile sand medium. They concluded that the introduced bacterial strains were dependent on the root for their growth, and that the degree of this dependence varied amongst the strains.

Tea (*Thea sinensis* = *Camellia sinensis*) is an economically important cash crop of India. A striking feature of the tea plant is that it is a small tree which is maintained as a shrub by continuous pruning cycles. Most Indian tea plantations are over 50 years old, and in this country root pruning is not practised. In the present study efforts were made to record basic information related to microbial dynamics in the tea rhizosphere. Isolations were performed

Anita Pandey (✉)[1] · Lok Man S. Palni
Biotechnology Division, C.S.I.R. Complex,
Palampur 176061, Himachal Pradesh, India

Present address:
[1] G.B. Pant Institute of Himalayan Environment
and Development, Paryavaran Bhawan, Kosi-Katarmal,
Almora 263643, Uttar Pradesh, India

at monthly intervals, from three different depths for 12 consecutive months.

Materials and methods

The present study was carried out in and around Palampur in Kangra district, Himachal Pradesh, India, during the period November 1990 to March 1992.

Climate

Tea plantations [mainly Chinery type, *Camellia sinensis* (L.) O. Kuntze] in the Kangra Valley cover an area of about 880 sq km and are located between 32.03° and 32.20°N and 76.37° and 76.80°E at 1000–1700 m above mean sea level in the mid-hills of the Himalaya. Data on rainfall, temperature, and evaporation for the study area, collected during the period November 1990 to October 1991, are given in Fig. 1.

Soil characteristics

Details of soil properties are given in Table 1. Soil organic matter was determined by the method of Walkley and Black (1934). The soils around Palampur have developed on glacial tills, granite, and gneiss, with a slope of 3–5%. The soil generally consists of 25% sand, 48% silt, and 25% clay. Thin discontinuous clay skins and Fe–Mn concretions appear at around 25 cm in depth, and thick continuous clay skins appear at around 50 cm in depth (Kanwar 1990).

Collection of soil samples

Soil samples were collected with the help of a "Carpentor" auger from three depths: 0–15, 15–30, and 30–45 cm. Only soil adhering very closely to tea roots was collected for rhizosphere studies. Non-rhizosphere soil samples were collected well away from tea bushes. Soil samples under young tea bushes were collected only from the upper most depth (0–15 cm). Unless stated otherwise, the samples were taken at monthly intervals for an entire year.

Soil samples (0–15 cm depth) under Assamica tea bushes [*C. assamica* (Mast) Kitam.] and under four other plant species were collected during two contrasting seasons, once in the dormant (November) and once in the acitve (April) growth period (Table 2). These included two wild plants (*Lantana camara* L. and *Pyrus pashia* Buch.-Ham.), in addition to *Grevillea robusta* A. Cunn. ex R. Br. and *Albizzia chinensis* (Osbeck) Merr., which are planted as shade trees in tea gardens. All cultivated tea bushes used in the present investigation (Chinery as well as Assamica) were raised from seed. These tea bushes were grown with a spacing of 1.4 m × 1.4 m, giving a planting density of 5100 bushes ha^{-1}. In established tea bushes the distance between the canopies of adjacent plants was negligible. Except for the Assamica bushes (Mansambal, Tea Estate, Palampur), all plants were growing in the Institute's Experimental Tea Garden at Banuri, Palampur.

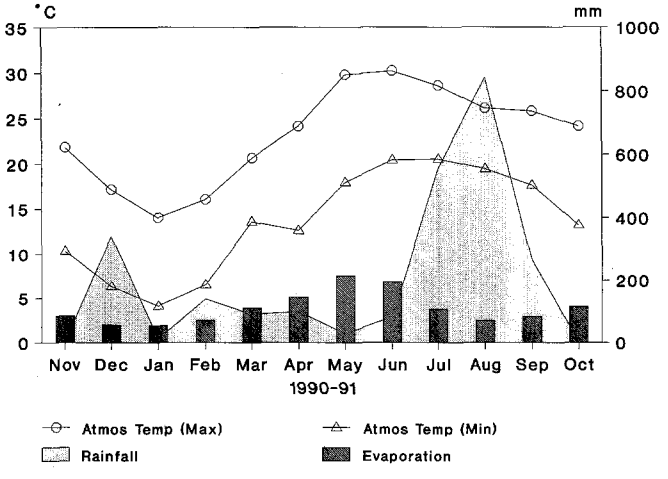

Fig. 1 Climate in the study area (*Atmos* atmosphere, *Temp* temperature, *Max* maximum, *Min* minimum)

Table 1 Soil characteristics of samples taken from the Institute's experimental tea garden at Banuri, Palampur. All values are based on triplicate samplings carried out at a monthly interval for 1 year (*Min.* minimum, *Max.* maximum, *R* rhizosphere, *S* non-rhizosphere soil)

Soil parameter		Soil depth (cm)					
		0–15		15–30		30–45	
		Min.	Max.	Min.	Max.	Min.	Max.
pH	R	5.2	5.8	5.2	5.9	5.3	5.9
	S	5.1	6.1	5.3	5.9	5.2	6.1
Moisture (%)	R	11.4	24.4	13.4	25.3	14.3	22.9
	S	13.1	23.1	14.6	20.8	13.7	21.9
Temperature (°C)	R	9.0	25.1	10.0	25.0	10.0	25.0
	S	9.0	27.0	9.5	25.0	9.5	26.0
Organic matter (% dry weight)	R	2.19	3.04	1.60	2.06	0.91	1.93
	S	1.67	2.36	1.29	2.09	0.80	1.77

Fertilizer use

Old tea bushes had been supplied with N:P:K at 90:90:90 kg ha^{-1} once a year in March. Young tea bushes, however, had been treated with an equal split dose of N:P:K, first in March and then in May, at the rate of 80:40:120 kg ha^{-1} per year.

Enumeration of microorganisms

To estimate the number of soil microflora, counts were calculated on the basis of serial 10-fold dilutions, in duplicate, using the pour-plate method using triplicate samples of 1 g soil, and an appropriate dilution (Johnson and Curl 1972); each value presented here is therefore an average of six individual counts. All Petri dishes (90 mm diameter) contained 25 ml medium, and the plates were incubated at 28–30 °C in the dark. Colony-forming units(CFU) were recorded after 1 week; the average number per gram oven-dry weight of soil was calculated as:

$$\text{CFU} = \frac{\text{Counts on the culture plate} \times \text{fresh weight of soil}}{\text{oven-dry weight of soil}}$$

The media (all from Hi Media, Bombay, India) used for these counts comprised (1) tryptone yeast extract for bacteria (5 g tryptone, 3 g yeast extract, 15 g agar, with volume made up to 1 litre with distilled water, pH 6.8); (2) caseinate-asparagine agar for actinomycetes (2 g sodium caseinate, 0.1 g asparagine, 4 g sodium propionate, 0.5 g dipotassium phosphate, 0.1 g magnesium sulphate, 0.001 g ferrous sulphate, 15 g agar, with volume made up to 1 litre with distilled water, pH 8.0); and (3) potato dextrose agar for fungi

Table 2 Comparison between the microbial population in the rhizosphere (0–15 cm soil depth) of established Chinery bushes (*Camellia sinensis*) with that of young tea, Assamica tea, and other plant species. Rhizosphere: soil microbial ratios are given in parentheses *CFU* colony-forming units. Significance: *letters* a–i represent *P* values of <0.001, <0.005, <0.010, <0.025, <0.050, <0.100, <0.200, <0.400, <0.500, respectively, versus >100-year-old established *C. sinensis*, and rhizosphere soil versus non-rhizosphere soil for values in parentheses

Plant species (age in years)	Bacteria (CFU $\times 10^4$ g^{-1})		Actinomycetes (CFU $\times 10^4$ g^{-1})		Fungi (CFU $\times 10^5$ g^{-1})	
	Dormant period	Active growth period	Dormant period	Active growth period	Dormant period	Active growth period
Wild plants						
Lantana camara	1856a	719a	264b	1108a	21a	30a
(?)	(5.6a)	(2.7a)	(1.4c)	(4.1a)	(1.4g)	(2.0e)
Pyrus pashia	1573a	542a	283a	529a	21a	20a
(?)	(4.7a)	(2.1a)	(1.5b)	(2.0a)	(1.4f)	(1.3e)
Shade trees						
Grevillea robusta	474a	394a	241a	321c	20a	30b
(7)	(2.0b)	(1.5b)	(1.4c)	(1.2d)	(1.4g)	(2.0d)
Albizzia chinensis	642a	563b	286a	643a	23a	27b
(3)	(2.7b)	(2.1b)	(1.6b)	(2.4a)	(1.6e)	(1.8d)
A. chinensis	480a	669a	233b	984a	16a	33a
(>50)	(2.0b)	(2.5a)	(1.3c)	(3.7a)	(1.1i)	(2.2d)
Tea						
Camellia sinensis	926a	728a	150c	358d	17a	21a
(4)	(3.8a)	(1.5b)	(1.0)	(1.6b)	(1.7f)	(5.0)
C. assamica	374b	79a	59d	167c	9b	11a
(>30)	(0.4b)	(0.1a)	(0.6d)	(0.5b)	(0.3d)	(0.8d)
C. sinensis	424b	262	119e	211d	9b	12e
(abandoned, >100)	(0.8d)	(0.9f)	(0.7d)	(0.8d)	0.6e)	(0.5f)
C. sinensis	84	251	99	223	7	5
(established, >100)	(0.3a)	(0.5a)	(0.6b)	(0.8d)	(0.5f)	(0.8g)

(potato infusion from 200 g potatoes, 20 g dextrose, 15 g agar with volume made up to 1 litre with distilled water, pH 5.6).

A numerical value for the rhizosphere: soil ratio (Katznelson 1946), representing microbial counts in the rhizosphere divided by the microbial counts in the non-rhizosphere soil, was used as an index of the rhizosphere effect on the microbial population.

The data were statistically analysed using Student's *t*-test (Snedecor and Cochran 1967).

In vitro demonstration of antimicrobial activity

The antimicrobial activity of the rhizosphere soil containing root exudates of established tea bushes was investigated by supplementing the medium with a soil extract. The five most abundant bacterial strains (nos. 2, 17, 21, 31, and 32) were selected as test organisms. A rhizosphere soil-water suspension was made at a 1:2 ratio. The soil suspension was shaken vigorously for 15 min and filtered through Whatman no. 1 filter paper. An isolated colony of the selected strain was suspended in 5 ml sterile water and a 10-fold serial dilution was made. The final dilution was plated using tryptone–yeast agar medium containing the rhizosphere soil extract. Plain tryptone–yeast agar medium was used in the control plates. All plates were incubated at 28 °C for 48 h and the counts were compared in control and treated Petri dishes.

Results

A clear suppression of the microbial population was found in the rhizosphere of established Chinery bushes; the most pronounced effect was observed on bacteria in the three groups of microorganisms studied. In general, the bacterial counts in control soils (non-rhizosphere) were significantly greater than those in the tea rhizosphere soil. This can be seen more clearly from the rhizosphere: soil ratios; values as low as 0.15 at 0–15 cm depth, 0.08 at 15–30 cm depth, and a still lower value of 0.04 at 30–45 cm depth were recorded (Fig. 2). Usually the bacterial population decreased with the increase in soil depth. However, during the peak winter months (January and February) the maximum bacterial population was recorded in the samples from the lowest depth, probably because that depth had cooled less. Although the bacterial counts increased in both the rhizosphere and the non-rhizosphere soil, the rhizosphere: soil ratios were still below 1 (Fig. 2). This increase in the bacterial population (30–45 cm depth) occurred with only two bacterial isolates.

Actinomycete counts were also suppressed, but to a lesser extent, in the tea rhizosphere. These counts appeared to be affected by seasonal fluctuations, too. Generally the actinomycete population declined during extreme winter months and also in the monsoon period. The optimal period for the proliferation of actinomycetes was from March to May, and also in September and October, when the temperature was moderate. For actinomycetes, also, the maximum counts were recorded at 0–15 cm depth. In the December samples, the rhizosphere: soil ratios were exceptionally high at 0–15 cm depth (Fig. 3). Fungi appeared to be the least suppressed group in the tea rhizosphere. Though the rhizosphere: soil ratios indicated a general trend of antifungal activity in the rhizosphere, the differences in counts between rhizosphere and non-rhizosphere soils were, by and large, not statistically significant, and the suppressive effect was relatively more pronounced at lower depths

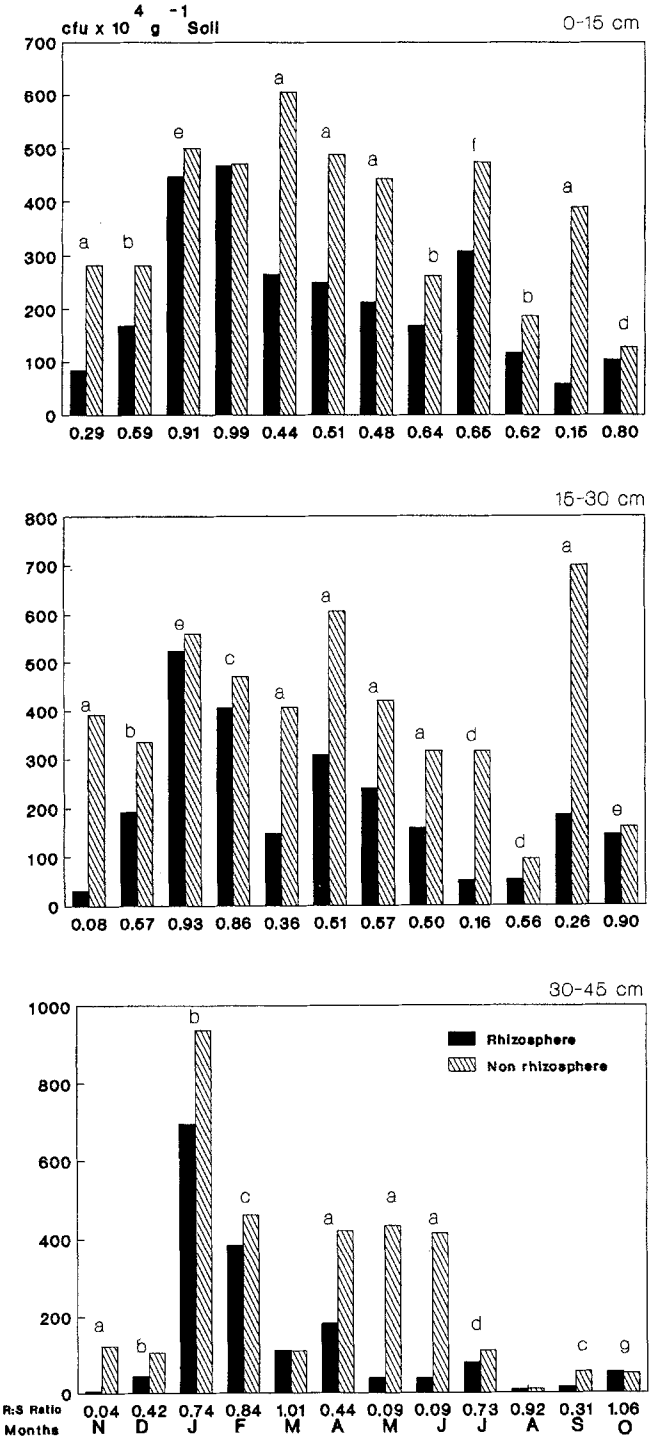

Fig. 2 Bacterial population in the rhizosphere of established Chinery tea bushes for the year 1990–1991 (*cfu* colony-forming units, *R*: *S* rhizosphere: soil ratio; for significance of *letters* see Table 2)

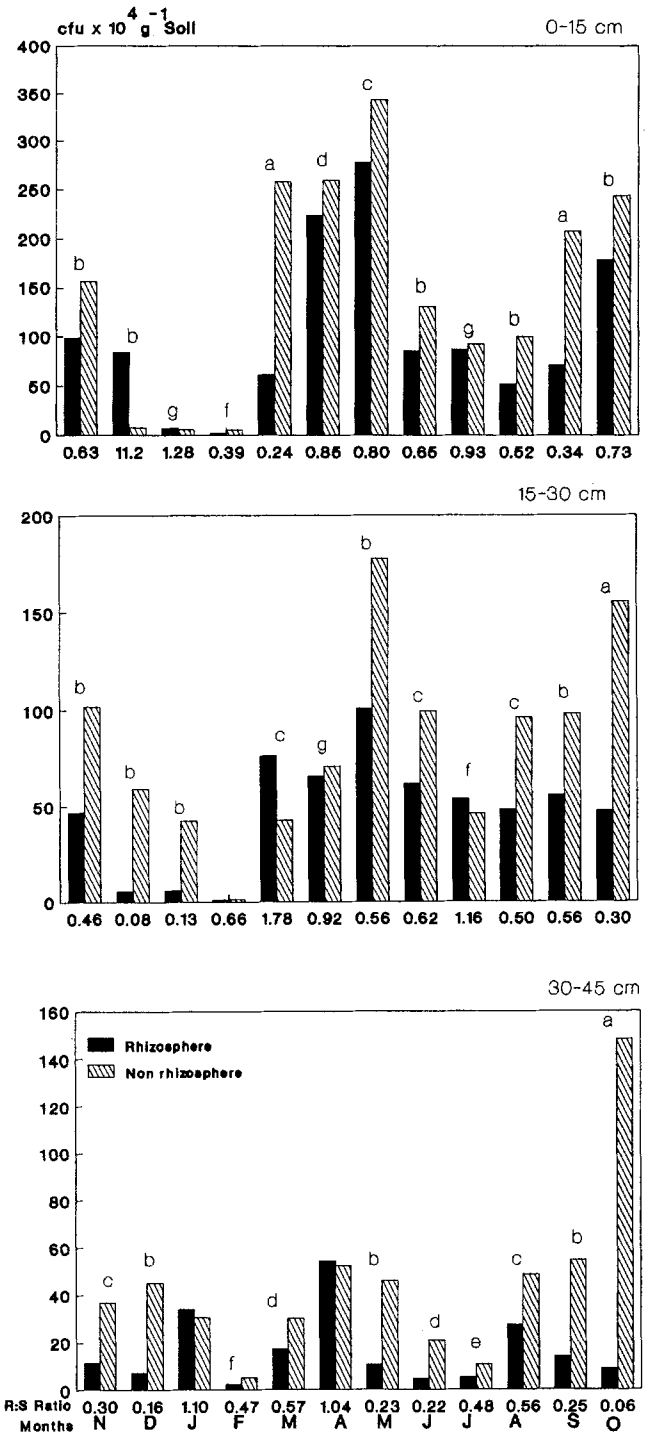

Fig. 3 Actinomycete population in the rhizosphere of established Chinery tea bushes for the year 1990–1991 (*cfu* colony-forming units, *R*: *S* rhizosphere: soil ratio; for significance of *letters* see Table 2)

(Fig. 4). The fungal population was highest during July, August, and September, indicating that the growth of fungi was favoured by higher moisture contents and moderate temperatures. As for bacteria and actinomycetes, the total fungal counts also decreased with increasing soil depth.

Microbial analysis of soil samples collected from established Assamica tea bushes also gave similar results (Table 2). In most of the Assamica samples rhizosphere: soil ratios were at par with the ratios for soil samples collected from Chinery tea bushes. Under Assamica tea bushes, also, bacteria were the most suppressed group,

resulting in rhizosphere: soil ratios of 0.1 in the active growth period and 0.4 in the dormant season. The ratios for actinomycetes and fungal counts were in the range 0.3–0.8 (Table 2).

The rhizosphere of abandoned tea bushes, also quite old, exhibited a similar inhibitory effect, but to a lesser extent. The rhizosphere: soil ratios for bacterial counts were calculated as 0.9 during the active growth season and 0.8 during the dormant season (Table 2). The young tea rhizosphere, however, provided a congenial environment for microbial growth; microbial counts were significantly higher in these samples, with rhizosphere: soil ratios as high as 5.3 for bacteria, 28.6 for actinomycetes, and 5.4 for fungi (Fig. 5). In addition, the rhizosphere of four other plant species (*Lantana camara, Pyrus pashia, Grevillea robusta*, and *Albizzia chinensis*) exhibited a promotive effect, in comparison to non-rhizosphere soil. These counts were also significantly higher than those recorded for the rhizosphere of established Chinery tea bushes (Table 2).

The antibacterial activity of tea root exudates was also observed by supplementing the rhizosphere soil extract in the medium. The number of colonies of strains 21 and 31 was reduced by more than 50% over the control (Table 3). The number of colonies of the other three bacterial strains was also reduced, but to a lesser extent. This preliminary experiment further indicated that at least some of the antimicrobial metabolites accumulate in the rhizosphere soil, and are water-soluble and heat-resistant.

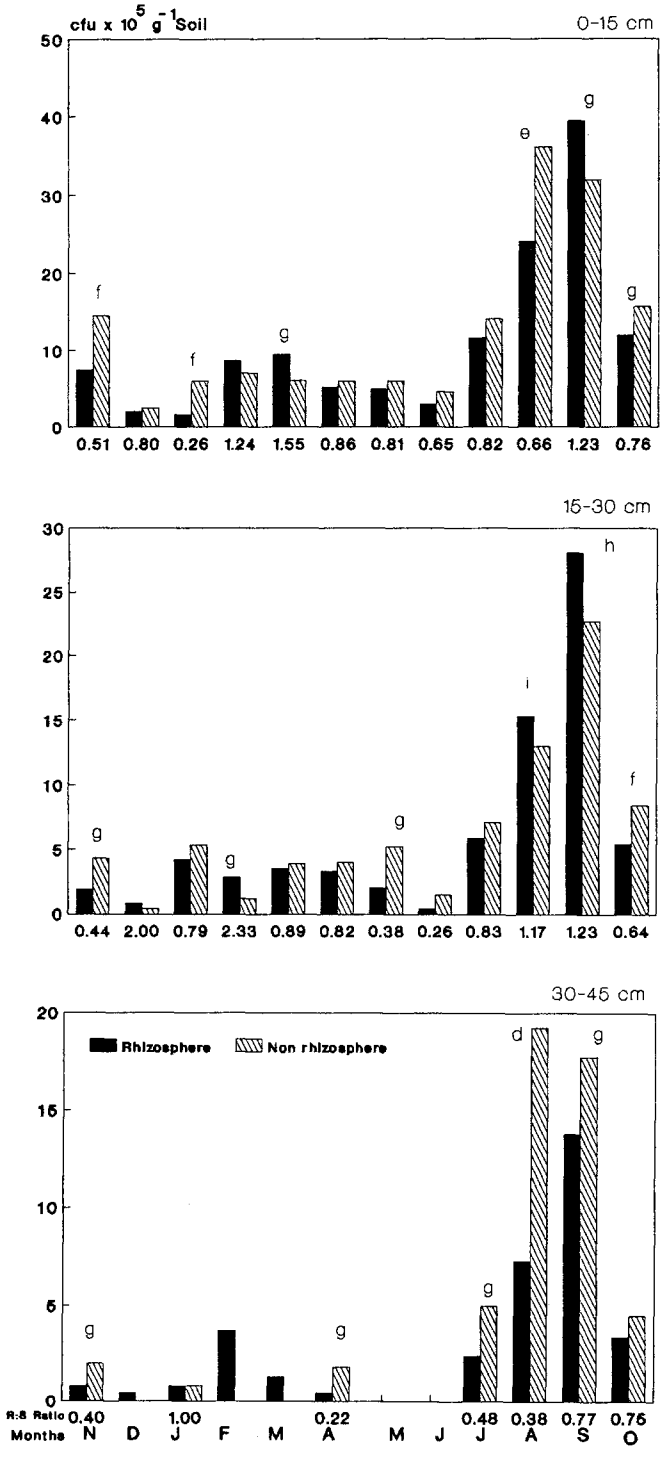

Fig. 4 Fungal population in the rhizosphere of established Chinery tea bushes for the year 1990–1991 (*cfu* colony-forming units, *R:S* rhizosphere: soil ratio; for significance of *letters* see Table 2)

Discussion

In general, the rhizosphere effect is expressed by greater microbial activity, and bacteria are the group most stimulated by the rhizosphere (Katznelson 1965). The present study, however, clearly indicates that the rhizosphere of established tea bushes may contain certain substances which are inhibitory to microbial activity. Among the three groups of microorganisms, bacteria appeared to be the most sensitive group. Actinomycetes and fungi were also affected by antimicrobial activity in the rhizosphere of established tea bushes, but to a lesser degree.

A number of possibilities may account for the suppressive effect of the tea rhizosphere (established bushes) on the microbial population. Tea roots, after attaining a certain age, may start secreting exudates that contain antimicrobial metabolites to which bacteria are the most susceptible group. There are a few reports on the secretion of inhibitory compounds by plant roots. A range of thio-

Table 3 Effect of rhizosphere soil on counts (colony-forming units $\times 10^4$ ml^{-1}) of five most abundant bacterial strains isolated from the rhizosphere of established tea bushes (*TY* tryptone-yeast; values are average of three readings)

Strain	Colony-forming units on		Reduction over control (%)
	TY agar + soil extract	TY agar (control)	
2	449	516	12.9
17	19	29	34.4
21	160	334	52.0
31	31	65	52.3
32	435	467	6.8

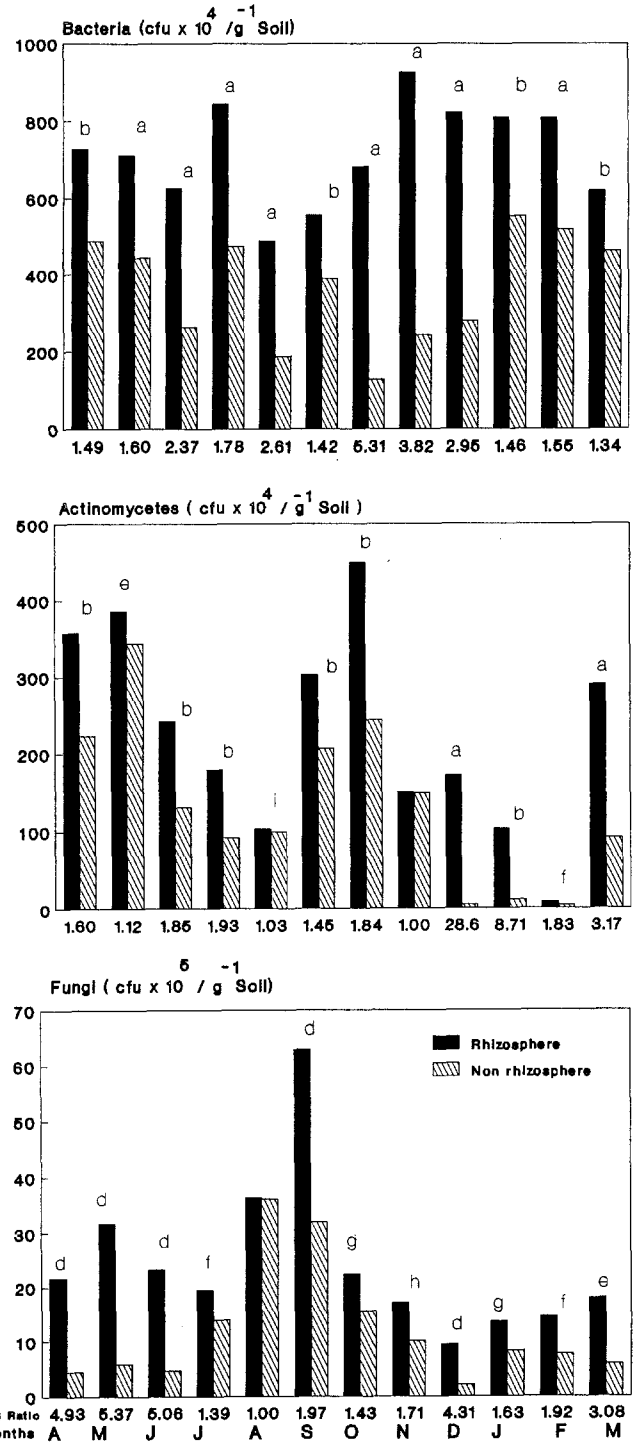

Fig. 5 Microbial population in the rhizosphere (0–15 cm depth) of young Chinery tea bushes for the year 1991–1992 (*cfu* colony-forming units, *R:S* rhizosphere:soil ratio; for significance of *letters* see Table 2)

phenes and benzofurans are secreted from *Tagetes patula* L. roots (Tang et al. 1987). A similar phenomenon is the two-to eightfold increase observed in terpene volatiles from roots of *Pinus sylvestris* L. upon becoming mycorrhizal (Krupa and Fries 1971). Root exudates are known to either stimulate or inhibit the growth of different species of microorganisms. For example, root exudates of *Crotalaria medicaginea* Lam. stimulated the growth of *Penicillium herquei*, *Aspergillus niger*, and *Alternaria humicola*, but significantly reduced the growth of *Trichoderma lignorum* (Sullia 1973). The inhibitory effect observed in the present study was more pronounced for cultivated tea bushes (Chinery as well as Assamica) in comparison to abandoned tea bushes. Cultivated tea bushes grow in close proximity to each other and the root exudates may accumulate in the rhizosphere from all sides, causing a marked inhibitory effect. Leaf litter may also contain antimicrobial substances which are slowly released upon decomposition. Abandoned tea bushes, however, are scattered, with a much reduced foliage and canopy. Possibly the root exudates are produced in lesser amounts from these bushes and fail to accumulate in the rhizosphere, hence resulting in slightly higher rhizosphere:soil values, although still well below 1.

In a few samplings, for example in the month of December, there were dramatic changes in actinomycete counts, perhaps because of sudden changes in environmental conditions. Whenever the sampling was done following a heavy rainfall the non-rhizosphere samples collected from 0–15 cm depth gave very low counts. Probably, in the absence of plant roots, actinomycetes are adversely affected by intense precipitation. In the rhizosphere, roots provide shelter to the microbial communities, resulting in apparently higher rhizosphere:soil values, compared with the normally low rhizosphere:soil values recorded for the established tea rhizosphere. Of the three groups of microbial communities studied, bacteria were dominant in the established tea rhizosphere. On the basis of data of 12 months and samplings at three depths, bacteria, actinomycetes, and fungi contributed 64.1, 15.8, and 20.1%, respectively, to the total microbial population in the rhizosphere. The bacterial contribution, as a percentage of total microbial counts, increased with increasing soil depth (51.4, 65.4, and 75.5% at 0–15, 15–30, and 30–45 cm, respectively) while the percentage contribution of actinomycetes and fungi was greatly reduced with increasing depth. A similar trend was also observed in non-rhizosphere soil samples taken from near the established tea garden, and in rhizosphere and non-rhizosphere soil samples collected from young tea bushes. In general, actinomycetes and fungi were more affected by environmental fluctations. For example during the extreme winter (January and February), when the soil temperature was below 5 °C, the actinomycete and fungal counts were extremely low while the bacterial population was at a maximum. However, the fungi were dominant during August and September, while the optimal period for actinomycetes was March to June.

Growth-inhibitory relationships or the antagonistic behaviour of microbial groups growing around established tea roots may also result in a reduced or smaller microbial population. This includes various categories of antagonism, such as competition, antibiosis, parasitism, and predation. These antagonistic activities in a suppres-

sive rhizosphere may maintain a low microbial population in the rhizosphere.

Also, the age of a plant should not be neglected as a factor influencing the microbial population. Counts in the rhizosphere of *Albizzia chinensis* were compared in young and established plants. Although some differences were seen, microbial suppression was not observed in the *Albizzia chinensis* rhizosphere.

Questions concerning the true number of microorganisms in soil and the best method of counting them remain unanswered. On the basis of the problems reported in the present study and elsewhere, the serial-dilution plate count method is suitable for determining the number of microorganisms in soil. If consistent methodologies are used, this technique can provide valuable information concerning relative microbial populations (Curl and Truelove 1986). The rhizosphere: soil ratio has been used to compare the rhizosphere populations of different plant species growing in different soils, and in plants of different ages, and of plants growing at different soil moisture contents (Rovira 1991).

The present results have been discussed and compared in terms of colony-forming units only and not on the basis of metabolic activity or the microbial biomass. During this study, a large number of bacteria, actinomycetes, and fungi were isolated and it was interesting that a few isolates were recorded in large numbers in the rhizosphere. These strains, through natural selection and continued exposure to antimicrobial metabolites, may have developed a kind of tolerance or resistance to the inhibitory components of root exudates. We are studying these strains further, as they may be closely associated with or specific to tea roots.

Every plant species provides an individual and specific site of microbial activity in the form of a rhizosphere. Similarly, every rhizosphere provides an opportunity for the isolation of microbial cultures that can be used for biotechnological applications. For example, natural rhizospheres are often inimical to pathogens because antagonists form part of the rhizosphere community (Lynch 1987b). During the present study period a large number of microorganisms were isolated and purified. These microbial isolates may be screened for possible use as biocontrol agents. Recent work has shown that inoculation of seedlings of forest tree species with coexistent rhizosphere bacteria promotes plant growth (Holl and Chanway 1992; Chanway and Holl 1993). The microbial community in an established tea rhizosphere should be more specific, owing to the prolonged length of time occupied by the plant species, and due to the competition among different types of microorganisms. Further experiments need to be carried out to study microbe–microbe interactions and plant–microbe interactions in this environment.

Acknowledgements Financial assistance was supplied by the Department of Biotechnology, Government of India, New Delhi.

References

Baath E, Olsson S, Tunlid A (1988) Growth of bacteria in the rhizoplane and the rhizosphere of rape seedlings. FEMS Microbiol Ecol 53:355–360

Bowen GD, Rovira AD (1989) The rhizosphere. In: Anderson JM, Ingram JSI (eds) Tropical soil biology and fertility. A handbook of methods. CAB International, Aberystwyth, pp 101–112

Chanway CP, Holl FB (1993) Ecotypic specificity of spruce emergence-stimulating *Pseudomonas putida*. Forest Sci 39:520–527

Curl EA, Truelove B (1986) The rhizosphere. Springer Verlag, Berlin Heidelberg New York

Fitter AH, Hay RKM (1981) Environmental physiology of plants. Academic Press, New York

Hiltner L (1904) Über neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie unter besonderer Berücksichtigung der Gründungung und Brache. Arb Dtsch Landwirtsch Ges 98:59–78

Holl FB, Chanway CP (1992) Rhizosphere colonization and seedling growth promotion of lodgepole pine by *Bacillus polymyxa*. Can J Microbiol 38:303–308

Ivarson KC, Katznelson H (1960) Studies on the rhizosphere microflora of yellow birch seedlings. Plant and Soil 12:30–40

Johnson LF, Curl EA (1972) Methods for research on the ecology of soil-borne plant pathogens. Burgess, Minneapolis

Kanwar BS (1990) Himachal Pradesh agricultural handbook. H. P. Vishva Vidyalays, Palampur

Katznelson H (1946) The rhizosphere effect of mangels on certain groups of micro-organisms. Soil Sci 62:343–354

Katznelson H (1965) Nature and importance of the rhizosphere. In: Baker KF, Synder WC (eds) Ecology of soil-borne plant pathogens. Univ Calif Press, Berkley, pp 187–209

Krupa S, Fries N (1971) Studies on the ectomycorrhizae of pine: 1. Production of volatile organic compounds. Can J Bot 49: 1425–1431

Lynch JM (1987a) Microbial interactions in the rhizosphere. Soil Microorg 30:33–41

Lynch JM (1987b) Biological control within microbial communities of the rhizosphere. In: Fletcher M, Gray TRG (eds) Ecology of microbial communities. Cambridge University Press, Cambridge, pp 55–82

Rangaswami G, Vasantharajan VN (1962) Studies on the rhizosphere microflora of citrus trees. 1. Quantitative incidence of microorganisms in relation to root and shoot growth. Can J Microbiol 8:473–477

Rouatt JW, Katznelson H (1961) A study of the rhizosphere soil of crop plants. J Appl Bacteriol 24:164–171

Rovira AD (1965) Interactions between plant roots and soil microorganisms. Annu Rev Microbiol 19:241–266

Rovira AD (1991) Rhizosphere research – 85 years of progress and frustration: In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Netherlands, pp 3–13

Snedecor GW, Cochran WG (1967) Statistical methods. Oxford and IBH Publishing Co, New Delhi

Starkey RL (1958) Inter-relations between microorganisms and plant roots in the rhizosphere. Bacteriol Rev 22:154–72

Sullia SB (1973) Effect of root exudates and extracts on rhizosphere fungi. Plant and Soil 39:197–200

Tang CS, Wat CK, Towers GHN (1987) Thiophenes and benzofurans in the undisturbed rhizosphere of *Tagetes patula* L. Plant and Soil 98:93–97

Walkley A, Black CA (1934) An examination of the Degtjareff method for determining soil organic matter and a proposed chromic acid titration method. Soil Sci 37:29–38