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# Qualitative and Quantitative Studies on Rhizosphere and Rhizoplane Microflora of *Trifolium alexandrinum* Linn.

S N SHUKLA* and R S DWIVEDI
*Department of Botany, Banaras Hindu University, Varanasi 221005*

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The R/S ratio depicted that quantitatively fungi, bacteria and actinomycetes per gram of dry soil were more in rhizosphere than in non-rhizosphere at all the stages of plant growth. The fungi, bacteria and actinomycetes per gram of dry soil were more in rhizosphere of secondary and tertiary roots as compared to primary ones. Qualitatively also, more of fungal species were encountered in the rhizosphere of primary, secondary and tertiary roots as compared to non-rhizosphere. Overall larger number of fungal species was isolated from the rhizosphere of primary root. Percentage occurrence of individual fungal species varied in rhizosphere and rhizoplane of primary, secondary and tertiary roots at all the stages of plant growth.

**Key Words: Rhizosphere, Rhizoplane, Microflora,** *Trifolium alexandrinum*

## Introduction

Antagonism between strains of rhizobia and other soil-inhabiting micro-organisms is known to cause defective nodulation in legumes (Chhonkar & Subba Rao 1966 and Shukla & Dwivedi 1979). However, the information about nature of microbial population around the root zone in legumes is inadequate. In the present study, experiments were conducted to investigate the quantitative and qualitative nature of microbial population in root region of *Trifolium alexandrinum* L. which is cultivated extensively as winter green fodder crop. Attempts have also been made to analyse the microbial population in rhizosphere and rhizoplane of primary, secondary and tertiary roots as well as non-rhizosphere soil for comparison.

## Materials and Methods

Isolation of microflora from rhizosphere (R) of primary, secondary and tertiary roots and from non-rhizosphere soil (S) was made at early, pre-flowering, flowering and fruiting stages of *Trifolium alexandrinum* plant raised in plots.

Plants were dug out by sterilized spatula with whole root system intact. The root system was gently tapped to remove the excess of soil particles. The primary, secondary and tertiary roots were separated from root system with a pair of sterilized scissors and collected separately in sterile 250 ml conical flasks containing 100 ml of sterilized distilled water. The flasks were shaken

*Present address*: Scientist S-1, Central Rice Research Institute, Cuttack 753006 (Orissa)

vigorously to get a well mixed suspension of rhizosphere soil and water. One ml soil suspension was inoculated from each flask into sterilized Petri dish and five replicates each were prepared for fungi, bacteria and actinomycetes. Fungi and bacteria were isolated on Martin's (1950) and Thornton' (1922) nutrient agar media respectively, while actinomycetes were isolated on agar medium of Kuster and William (1964). Sterilized and cooled (40°C) specific nutrient agar medium was poured into each Petri dish. The soil suspension was uniformly dispersed in the medium. The Petri dishes were incubated at 25°C±1, 30°±1 and 34°C±1 for fungi, actinomycetes and bacteria respectively. Roots were taken out of the flask after thorough washing and were used for rhizoplane study. Flasks containing the remaining soil suspension were placed in an oven at 105°C for 24 hr and then taken out of the oven, cooled and weighed. Weight of soil was recorded separately for each flask to determine fungi, bacteria and actinomycetes per gram of dry soil in rhizosphere.

The soil(s) samples were collected in sterilized conical flasks away from the root system at the corresponding stages of plant growth. The fungi, bacteria and actinomycetes were isolated using specific nutrient agar media as mentioned earlier by dilution plate technique (Warcup 1950).

Rhizoplane mycoflora were isloated employing the method of Harley and Waid (1955). Roots collected from the rhizosphere microflora were washed thoroughly 10 times with sterile distilled water. Each category of root was cut separately into small pieces of about 5 mm length with the help of a pair of sterile scissors after removing the adhered water drops by soaking in folds of sterile blotting paper. Five bits of each category of root were transplanted to each Petri dish containing sterilized nutrient agar medium (Martin 1950). Five replicates were prepared for each category of root. Petri dishes were incubated at 25°C±1 for a week.

Fungi growing in Petri dishes were observed after the incubation period of 7 days. The colonies of actinomycetes and bacteria were counted after their appearance on agar plates. The percentage occurrence of individual fungal species and the population of fungi, bacteria and actinomycetes per gram dry soil were computed as presented in tables 1 to 3.

## Results and Discussion

Percentage occurrence of individual fungal species varied in the rhizosphere and rhizoplane of primary, secondary and tertiary roots at all the stages of plant growth (tables 1 & 2). Overall, qualitatively greater number of fungal species was isolated from the rhizosphere of primary root. Members of Deuteromycetes were dominant with higher percentage occurrence. Certain fungi were common in the rhizosphere, non-rhizosphere and rhizoplane; while a few were confined to the certain category of roots. *Aspergillus flavus, A. nidulans, A. fumigatus, A. niger, A. terreus, A. luchuensis, Penicillium citrinum, P. javanicum, Fusarium poae, F. oxysporum, F. roseum, Alternaria tenuis, Acrophialophora fusispora, Paecilomyces varioti, Cladosporium herbarum, Curvularia lunata, Helminthosporium sativum, Papulaspora* sp., *Trichoderma lignorum, Verticillium terrestre*, white sterile mycelium and black sterile mycelium were dominant mycoflora on rhizosphere and rhizoplane of primary, secondary and tertiary roots at one or the other stages of plant growth (tables 1 & 2).

Quantitatively fungi, bacteria and actinomycetes per gram of dry soil were more in rhizosphere than in non-rhizosphere at all the stages of plant growth. Amongst the different categories of roots, the fungi, bacteria and actinomycetes per gram of dry

**Table 1** *Percentage occurrence of fungi in rhizosphere, non-rhizosphere and rhizosplane at early and pre-flowering stages of growth of T. alexandrinum*

| Isolates | Early stage | | | | | | | Pre-flowering stage | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rhizosphere | | | NR | Rhizoplane | | | Rhizosphere | | | NR | Rhizoplane | | |
| | PR | SR | TR | | PR | SR | TR | PR | SR | TR | | PR | SR | TR |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *Rhizopus stolonifer* | 2.04 | — | — | 3.26 | — | — | — | — | — | — | — | — | — | — |
| *R. oryzae* | — | — | — | — | — | 2.62 | — | — | — | — | 1.78 | — | 2.62 | — |
| *Mucor luteus* | — | 7.29 | — | 3.26 | 5.4 | — | — | 1.2 | — | 1.38 | — | — | — | — |
| *Cunninghamella echinulata* | — | 2.43 | — | — | — | — | — | 1.2 | — | — | 1.78 | — | — | — |
| *Syncephalastrum racemosum* | — | 2.43 | — | — | — | — | — | — | — | — | — | — | — | — |
| Sterile Phycomycete | — | — | — | — | — | — | — | 1.2 | — | — | — | — | — | — |
| *Aspergillus nidulans* | 6.14 | — | — | — | — | — | — | 1.2 | — | 5.52 | — | — | 7.86 | — |
| *Penicillium javanicum* | — | — | — | — | — | — | — | 1.2 | — | — | — | — | — | — |
| *Thielavia terricola* | — | — | — | — | — | — | — | — | 1.42 | — | — | — | — | — |
| *Chaetomium bostrychodes* | — | — | — | — | — | — | — | — | — | 2.76 | — | — | — | — |
| *Chaetoceratostoma longirostre* | — | 2.43 | — | — | — | — | — | — | 1.42 | — | — | — | — | — |
| *Cephalosporium roseogriseum* | — | — | — | — | — | — | — | — | — | 1.38 | — | — | 2.62 | — |
| *Trichoderma lignorum* | — | 7.29 | 3.12 | — | — | — | — | 2.4 | 1.42 | — | — | — | — | — |
| *Aspergillus clavatus* | — | — | — | — | — | — | — | — | — | 1.38 | — | — | — | — |
| *A. fumigatus* | 4.08 | — | — | — | — | — | — | — | 4.26 | 4.14 | — | 4.76 | — | — |
| *A. flavus* | 10.20 | 7.29 | 3.12 | — | 10.8 | 10.48 | 18.3 | 24.0 | 12.78 | 13.8 | 16.02 | 11.90 | 10.48 | — |
| *A. terreus* | 10.20 | 4.86 | 12.48 | 13.04 | — | 7.86 | 3.7 | 3.6 | 11.36 | — | 10.68 | — | 10.48 | 11.1 |
| *A. luchuensis* | 4.08 | — | — | 4.89 | 5.4 | 2.62 | — | 2.4 | 2.86 | — | — | 7.14 | 10.48 | 11.1 |
| *A. niger* | 4.08 | 14.58 | 12.48 | 14.67 | 18.9 | 20.96 | 22.2 | 12.0 | 11.36 | 16.56 | 17.8 | 11.90 | 15.72 | 14.8 |
| *A. sulphureus* | — | — | 3.12 | — | — | — | — | — | — | — | — | — | — | — |
| *A. candidus* | 4.08 | — | 3.12 | — | — | — | — | — | — | 4.14 | — | — | — | — |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Penicillium citrinum* | 14.21 | 2.43 | 24.96 | 21.19 | 16.2 | 20.96 | 25.9 | 12.0 | 6.1 | 11.04 | 11.06 | 16.28 | 13.10 | 14.8 |
| *P. chrysogenum* | 4.08 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *P. rubrum* | 2.04 | 2.43 | — | — | — | — | — | — | — | — | — | — | — | — |
| *Paecilomyces varioti* | 2.04 | 4.86 | — | — | — | — | — | — | — | — | — | — | — | — |
| *Acrophialophora fusispora* | — | — | 6.24 | 8.15 | — | — | — | — | — | 8.28 | 7.12 | — | — | 14.8 |
| *Torula allii* | — | — | — | 1.63 | — | — | — | — | — | — | — | — | — | — |
| *Humicola grisea* | — | — | — | 3.26 | — | — | — | 1.2 | — | — | — | — | — | — |
| *Cladosporium herbarum* | — | — | 3.12 | 3.26 | 8.1 | — | 3.7 | — | 4.28 | — | 10.68 | 4.76 | 2.62 | 11.1 |
| *Curvularia lunata* | — | — | — | — | — | — | — | — | 4.26 | — | — | — | 7.86 | — |
| *Helminthosporium sativum* | — | — | — | — | — | 2.62 | — | 4.80 | 4.26 | — | — | 9.52 | — | — |
| *Alternaria humicola* | — | 4.86 | 6.24 | — | — | 5.24 | — | — | — | — | — | 4.76 | — | — |
| *A. tenuis* | 12.18 | — | — | 6.52 | — | — | 7.4 | — | — | — | — | — | — | — |
| *Fusarium poae* | — | 4.86 | 9.36 | 3.26 | — | 18.72 | 7.4 | — | — | 9.66 | 7.12 | 16.66 | — | — |
| *F. roseum* | — | 2.43 | — | — | 8.1 | — | 3.7 | 4.8 | — | — | — | 2.38 | 10.48 | — |
| *F. oxysporum* | — | — | — | — | 13.5 | — | — | | | | | | | |
| *Myrothecium roridum* | 4.08 | 2.43 | 6.24 | — | — | — | — | — | 1.42 | 4.14 | — | — | — | — |
| *Papulaspora* sp. | — | — | — | — | — | — | — | — | — | — | 3.56 | — | — | — |
| White sterile mycelium | 12.18 | 2.43 | 6.24 | 6.52 | 6.4 | 7.86 | — | 4.8 | 11.36 | 11.04 | 5.34 | 3.17 | — | 7.4 |
| Dark sterile mycelium | 4.08 | — | — | 4.89 | 8.1 | 2.62 | — | 19.2 | 4.36 | 4.14 | 5.54 | — | 5.28 | — |
| Total No. of Species | 16 | 16 | 13 | 14 | 10 | 11 | 8 | 16 | 15 | 15 | 12 | 11 | 12 | 7 |

*PR*=Primary root; *SR*=Secondary root; *TR*=Tertiary root; *NR*=Non-rhizosphere

**Table 2** *Percentage occurrence of fungi in rhizosphere, non-rhizosphere and rhizoplane at flowering and fruiting stages of growth of* T. alexandrinum

| Isolates | Flowering stage | | | | | | | Fruiting stage | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rhizosphere | | | NR | Rhizoplane | | | Rhizosphere | | | NR | Rhizoplane | | |
| | PR | SR | TR | | PR | SR | TR | PR | SR | TR | | PR | SR | TR |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| *Rhizopus oryzae* | — | — | — | — | — | — | — | — | 3.32 | — | — | — | — | — |
| *Mucor luteus* | — | — | — | — | 8.48 | — | — | 2.82 | — | — | — | — | — | — |
| *Cunninghamella bertholletiae* | — | — | — | — | — | — | — | — | — | — | — | — | 5.70 | — |
| *C. echinulata* | — | 1.81 | — | — | — | — | — | — | — | — | — | — | — | — |
| *Aspergillus nidulans* | 3.52 | 5.43 | — | — | — | — | — | — | — | — | — | — | — | — |
| *Penicillium javanicum* | — | 18.10 | 26.10 | 8.45 | 12.72 | 15.19 | — | 9.8 | — | 6.78 | — | — | — | — |
| *Thielavia terricola* | 0.88 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *Chaetomium bostrychodes* | 0.88 | — | — | 1.69 | — | — | — | — | — | — | — | — | — | — |
| *Cephalosporium roseogriseum* | 1.76 | 1.81 | 2.76 | — | — | 8.68 | — | — | — | — | — | 7.89 | — | — |
| *Trichoderma lignorum* | — | 5.43 | — | — | 6.36 | 2.17 | — | — | 4.98 | — | — | — | — | 6.64 |
| *T. koningii* | — | — | — | 1.69 | — | — | — | — | — | — | — | — | — | — |
| *Aspergillus fumigatus* | — | — | — | — | — | — | — | — | 6.64 | 7.91 | 18.72 | — | — | — |
| *A. sydowi* | 2.64 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *A. flavus* | 5.28 | 18.10 | 2.76 | 3.38 | — | — | 6.44 | 9.8 | — | 7.91 | 8.32 | 10.52 | — | 9.66 |
| *A. terreus* | — | 3.62 | — | 1.69 | 6.36 | 8.68 | — | 7.0 | — | — | 8.32 | 7.89 | 2.85 | — |
| *A. luchuensis* | — | 1.81 | 1.88 | — | — | 4.34 | — | — | — | — | — | 5.26 | — | — |
| *A. niger* | 1.68 | — | 5.64 | 20.28 | 12.72 | 6.51 | 6.44 | 16.8 | 33.2 | 25.99 | 24.96 | 36.85 | 31.35 | 9.66 |
| *A. sulphureus* | 2.64 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| *Penicillium citrinum* | 39.66 | 36.20 | 41.36 | 28.50 | 10.60 | 17.36 | 16.10 | 15.5 | 23.24 | 16.96 | 16.64 | — | 14.25 | 16.10 |
| *P. rubrum* | 4.40 | — | — | — | — | — | — | — | 4.98 | — | — | — | — | — |
| *Verticillium terrestre* | — | — | 1.88 | — | 2.12 | — | — | — | — | — | — | 13.15 | — | 9.66 |
| *Paecilomyces varioti* | — | — | — | — | — | — | — | — | — | 6.78 | — | — | — | — |
| *Acrophialophora fusispora* | — | — | — | — | — | — | 6.44 | ∞.4 | 8.3 | — | — | — | — | — |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Humicola brevis* | — | — | - | 1.69 | — | — | — | — | — | — | — | — | — | — |
| *H. grisea* | — | — | — | — | — | — | — | — | — | — | 4.16 | — | — | — |
| *Cladosporium herbarum* | 5.28 | — | — | 10.14 | — | — | 9.66 | 5.8 | — | — | — | — | — | — |
| *Curvularia lunata* | — | — | — | 1.69 | — | 2.68 | 9.66 | — | — | — | — | — | — | — |
| *Helminthosporium sativum* | — | — | 1.88 | — | — | — | 6.44 | 1.4 | — | 4.52 | — | — | — | — |
| *Alternaria humicola* | — | — | — | — | — | 10.85 | — | — | — | 9.04 | — | — | — | — |
| *A. tenuis* | — | — | — | — | 2.12 | — | — | — | — | — | — | — | 5.7 | — |
| *Fusarium poae* | 7.92 | 3.62 | 9.4 | 13.52 | 14.84 | — | 19.32 | 9.6 | 6.64 | — | — | 2.63 | 19.95 | 45.08 |
| *F. roseum* | — | — | — | — | — | 11.36 | — | — | — | 5.65 | — | — | — | — |
| *F. udum* | — | — | — | — | — | — | — | — | — | — | — | 13.15 | — | — |
| *Myrothecium roridum* | — | — | — | — | — | — | — | — | 1.66 | — | 6.24 | — | — | — |
| *Sclerotium rolfsii* | — | — | — | — | — | — | — | 1.4 | — | — | — | — | — | — |
| *Papulaspora* sp. | — | — | — | — | — | — | 6.44 | — | — | 3.39 | — | — | — | — |
| *Rhizoctonia* sp. | — | — | — | — | — | 4.34 | 3.22 | — | — | — | — | 2.63 | — | — |
| White sterile mycelium | 2.64 | 3.62 | 2.76 | 8.45 | — | 4.34 | — | 1.2 | 6.64 | — | — | — | 8.55 | 3.22 |
| Black sterile mycelium | 3.52 | — | — | 1.60 | — | — | — | — | — | 4.52 | 8.32 | — | — | — |
| Total No. of species | 14 | 11 | 10 | 13 | 9 | 12 | 10 | 12 | 10 | 11 | 8 | 9 | 7 | 7 |

*PR* = Primary root; *SR* = Secondary root; TR = Tertiary root; NR = Non-rhizosphere

soil were higher in number in rhizosphere of secondary and tertiary roots as compared to primary ones. The maximum number of fungi was screened from the rhizosphere of secondary root at flowering stage while the minimum was on the primary root at early stage. The maximum number of bacteria and actinomycetes were isolated from the rhizosphere of tertiary root at early and pre-flowering stage respectively while the minimum were in the rhizosphere of primary root at flowering stage (table 3). The mode of colonization of fungi, bacteria and actinomycetes in the rhizosphere of primary, secondary and tertiary roots varied at different stages of plant growth. It has been presumed that the soluble organic compounds viz., carbohydrates, amino acids, vitamins and organic acids released from the roots of higher plants are wholly or partly responsible for the stimulation of microflora around the roots (Katznelson et al. 1955, Rovira 1956a, b,c, 1965, Schroth & Hildebrand 1964 and Bhuvaneswari & Subba Rao 1957). Besides

**Table 3** *Distribution of fungi, bacteria and actinomycetes per gram dry soil in rhizosphere and non-rhizosphere and R/S ratio at different stages of growth of* T. alexandrinum

| Stage of sampling | Isolate | Rhizosphere | | | Non-rhizosphere |
|---|---|---|---|---|---|
| | | Primary root | Secondary root | Tertiary root | |
| Early | No. of fungi (in thousand) | 12.432 | 14.520 | 13.230 | 12.200 |
| | R/S ratio | 1.019 | 1.190 | 1.090 | — |
| | No. of bacteria (in hundred thousand) | 8.763 | 14.690 | 15.790 | 5.990 |
| | R/S ratio | 1.462 | 2.451 | 2.630 | — |
| | No. of actinomycetes (in hundred thousand) | 1.530 | 2.057 | 3.079 | 1.610 |
| | R/S ratio | 0.950 | 1.277 | 1.910 | — |
| Pre-flowering | No. of fungi | 14.100 | 15.509 | 17.740 | 11.200 |
| | R/S ratio | 1.266 | 1.384 | 1.583 | — |
| | No. of bacteria | 6.482 | 8.065 | 9.060 | 5.100 |
| | R/S ratio | 1.270 | 1.580 | 1.770 | — |
| | No. of actinomycetes | 0.940 | 2.064 | 3.461 | 1.550 |
| | R/S ratio | 0.606 | 1.329 | 2.230 | — |
| Flowering | No. of fungi | 17.509 | 19.300 | 16.400 | 11.800 |
| | R/S ratio | 1.483 | 1.635 | 1.389 | — |
| | No. of bacteria | 6.755 | 10.793 | 11.272 | 7.916 |
| | R/S ratio | 0.853 | 1.363 | 1.423 | — |
| | No. of actinomycetes | 0.681 | 1.873 | 3.175 | 1.242 |
| | R/S ratio | 0.548 | 1.508 | 2.556 | — |
| Fruiting | No. of fungi | 13.260 | 16.380 | 14.240 | 9.600 |
| | R/S ratio | 1.381 | 1.706 | 1.483 | — |
| | No. of bacteria | 8.153 | 9.464 | 7.107 | 7.536 |
| | R/S ratio | 1.081 | 1.257 | 0.943 | — |
| | No. of actinomycetes | 1.130 | 1.972 | 1.052 | 1.042 |
| | R/S ratio | 1.084 | 1.892 | 1.005 | — |

amino acids and sugars, other ingredients such as vitamins, organic acids and certain other chemicals in root exudates of the test plant might also be responsible for variation in mode of colonization of microflora. The activity of bacteria and actinomycetes in the rhizosphere was more at early stage which showed that the 'factors' released from roots were more conducive for growth of bacteria and actinomycetes at initial stages of growth of leguminous plants.

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