# RHIZOSPHERE AND RHIZOPLANE MICROFLORA OF COFFEE SEEDLINGS AS INFLUENCED BY COLLAR ROT AND BY SEED PRE-TREATMENT

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## ABSTRACT

**Rhizophere and rhizoplane microflora of coffee seedlings were studied by the dilution plate technique. The number of fungi, yeast and bacteria was higher in the rhizosphere than in the non-rhizosphere and rhizoplane. *Rhizoctonia solani*, the incitant of collar rot of coffee seedlings, altered the rhizosphere and rhizoplane microflora. The microflora of coffee seedlings obtained from seeds pre-treated with Carboxin and Quintozene markedly differed from that of seedlings with no seed treatment. Among the fungi isolated, *Aspergillus* spp. were the most predominant followed by *Fusarium* spp. *R. solani* was observed in the rhizosphere and rhizoplane of the untreated and collar rot-infected seedlings only. Seed treatment with fungicides decreased the fungal flora associated with plant roots.**

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**KEY WORDS:** Coffee. Rhizosphere. Rhizoplane. Microflora. Seed treatment. Collar rot.

## INTRODUCTION

Qualitative as well as quantitative differences between fungi of rhizosphere (immediate vicinity of plant roots) and non-rhizosphere soil have been reported (Katznelson and Richardson, 1943; Timonin, 1941). Sizova and Vasin (1961) found that the total number of fungi from rhizosphere soil of oak forests was higher than that in non-rhizosphere soil. Rangaswami and Vasantharajan (1962) quantitatively analyzed the rhizosphere population of *Citrus* species and found that fungi were 3 to 6 times more abundant in the rhizosphere than in the non-rhizosphere soil.

*Rhizoctonia solani*, a soil inhabitant that incites collar rot in coffee seedlings (Venkataramaiah, 1968), has been known to interact with other organisms in the soil. Since the work of Weindling and Fawcett

(1936), this interaction has become a favorite subject of research and speculation among many scientists.

The soil-borne nature of *R. solani* through sclerotia (Venkatasubbaiah and Safeeulla, 1983) and the speed with which the fungus spreads in the coffee nursery soil have prompted researchers to study the pattern of distribution of rhizosphere and rhizoplane microflora in the collar-rot infected plot under the influence of heavy soil inoculum pressure.

It is a common practice to treat coffee seeds with different fungicides to protect the seeds and seedlings from fungal infection (Venkatasubbaiah and Muthappa, 1981). Prasad and Rangaswami (1967) reported that seed treatments with sulfuric acid and captan influenced the rhizosphere microflora of cotton seedlings. Different factors such as soil pH and antagonistic microorganisms influencing the rhizosphere microflora for the benefit of plant growth have been indicated by Rangaswami and Bhagyaraj (1967).

The present study was undertaken to know the pattern of distribution of rhizosphere and rhizoplane microflora of coffee seedlings in nursery seed beds as influenced by collar rot disease and pre-treatment of seeds with fungicides.

## MATERIALS AND METHODS

Rhizosphere and rhizoplane microflora were isolated from samples collected from the following:

- Seedlings obtained from untreated coffee seeds (control).
- Seedlings obtained from coffee seeds treated with 0.05% Quintozene (Pentachloronitrobenzene).
- Seedlings obtained from coffee seeds treated with 0.05% Carboxin (Vitavax 75 WP).
- Collar rot-infected seelings.

Samples of rhizosphere soil were collected 40 days after sowing and serial dilutions were prepared (Johnson and Curl, 1972). Non-rhizosphere soil was obtained by removing and rejecting the first half-inch layer of top soil from the unseeded beds and then randomly taking approximately one-gram samples. These were treated similarly as the rhizosphere soil samples. Four replicate plates were prepared from the $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions.

For the isolation of rhizoplane microflora, roots in the dilution flasks containing rhizosphere soil were removed. Fifty-gram samples of these were immersed in other flasks containing 450 g of sterile distilled water and added with glass beads of 2-mm diameter. The flasks were reweighed and shaken for 20 min and serial dilutions were again made. Four replicate plates were likewise prepared from the $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions.

For detecting yeast and fungi, either malt extract agar (MEA) or potato dextrose agar (PDA) containing 50 ppm chloramphenicol and chlorotetracycline was used. PDA and MEA media plates were in-

cubated at 26°C for 10 days and colony counts were taken daily from the third day. Total plate count agar (TPCA - Difco composition) was used to get the total counts of bacterial colonies after 48 hours of incubation at 37°C. Fungal and bacterial colonies were isolated and maintained on PDA and nutrient agar slants, respectively, for subsequent identification.

## RESULTS AND DISCUSSION

The total number of fungi, yeast and bacteria (colony forming unit) in the rhizosphere and rhizoplane of coffee seedlings in the different treatments as recorded on MEA and PDA media are given in Tables 1 and 2, respectively. Seed treatment with fungicides considerably reduced the number of fungi and yeasts in both the rhizosphere and rhizoplane. Even the bacterial population was considerably reduced by fungicidal treatment. Non-rhizosphere samples showed lesser fungi ($12 \times 10^2$), bacteria ($242 \times 10^2$) and yeast ($2 \times 10^2$) than the rhizosphere and rhizoplane samples.

Tables 3 and 4 present the different species of fungi and yeast isolated from the rhizosphere and rhizoplane of the various treatments using PDA and MEA, respectively. Very few pigmented and non-pigmented yeasts were noted in both the rhizosphere and rhizoplane. Among the fungi, *Aspergillus* spp. were the most predominant followed by *Fusarium* spp. *R. solani*, the incitant of collar rot of coffee seedlings, was observed in the rhizosphere and rhizoplane of the control and collar rot-infected plots only. *Trichoderma harzianum*, the predominant antagonist of *R. solani* was not encountered in these plots. Gram-positive and gram-negative bacteria were also observed in both the rhizosphere and rhizoplane (Table 5).

Rhizosphere and rhizoplane microflora of control seeds and of fungicide-treated seeds also showed quantitative differences. As in other plants, greater fungal population was noted in the rhizosphere and rhizoplane of coffee seedlings than in non-rhizosphere soil. This may be attributed to the availability of more nutrients in the rhizosphere due to root excretions and sloughed-off root cells. The stimulation of rhizosphere and rhizoplane microflora by plant roots may also be due to possible excretion of inorganic substances (Lundegardh, 1945), amino nitrogen (Rovira, 1956) and accessory growth factors (Fries and Forsman, 1951) by plants.

Altered patterns of root exudation can promote or suppress the rhizosphere and rhizoplane microbial population depending on the type of stress. An increased cation leakage from the roots of downy mildew-affected sorghum plants had been observed by Balasubramanian (1974). The higher density of fungal flora in the rhizosphere and rhizoplane of collar rot-infected coffee seedlings may also be caused by the same phenomenon. Agnihothrudu (1961) found higher fungal populations in the rhizosphere of tea plants infected with charcoal stump rot. A

**Table 1.** Number of microflora in the rhizosphere and rhizoplane of untreated, treated and infected coffee seedlings as recorded on malt extract agar (MEA).

| Treatment | Number (cfu)[1] | | | | | |
|---|---|---|---|---|---|---|
| | Rhizosphere | | | Rhizoplane | | |
| | Fungi $x10^2/g$ | Yeast $x10^2/g$ | Bacteria $x10^2/g$ | Fungi $x10^2/g$ | Yeast $x10^2/g$ | Bacteria $x10^2/g$ |
| Untreated seeds | 137 | 8 | 1696 | 45 | 5 | 742 |
| Quintozene-treated seeds | 105 | 3 | 680 | 35 | 2 | 592 |
| Carboxin-treated seeds | 82 | 3 | 248 | 32 | 2 | 496 |
| Collar rot-infected seeds | 164 | 6 | 988 | 57 | 2 | 840 |

[1] cfu — colony forming unit

**Table 2.** Number of microflora in the rhizosphere and rhizoplane of untreated, treated and infected coffee seedlings as recorded on potato dextrose agar (PDA).

| Treatment | Number (cfu)[1] | | | |
|---|---|---|---|---|
| | Rhizosphere | | Rhizoplane | |
| | Fungi $x10^2/g$ | Yeast $x10^2/g$ | Fungi $x10^2/g$ | Yeast $x10^2/g$ |
| Untreated seeds | 61 | 12 | 38 | 5 |
| Quintozene-treated seeds | 36 | 4 | 12 | 1 |
| Carboxin-treated seeds | 24 | 8 | 11 | 3 |
| Collar rot-infected seeds | 62 | 5 | 39 | 4 |

[1] cfu - colony forming unit

**Table 3.** Percentage of different fungi and yeasts in the rhizosphere of untreated, treated and infected coffee seedlings as recorded on malt extract agar and potato dextrose agar.

| Isolated Microorganisms | Malt Extract Agar | | | | Potato Dextrose Agar | | | |
|---|---|---|---|---|---|---|---|---|
| | Untreated Seeds | Quintozene-Treated Seeds | Carboxin-Treated Seeds | Collar Rot-Infected Seeds | Untreated Seeds | Quintozene-Treated Seeds | Carboxin-Treated Seeds | Collar Rot-Infected Seeds |
| Fungi | | | | | | | | |
| *Aspergillus niger* | 69.2 | 78.9 | 76.2 | — | 47.0 | 71.2 | 75.6 | 2.2 |
| *A. candidus* | — | 7.8 | — | — | — | — | — | 16.8 |
| *A. flavus* | — | 5.2 | 6.7 | 33.6 | 6.8 | 4.5 | 2.3 | 1.3 |
| *A. ochraceous* | 24.0 | — | — | 3.3 | 10.5 | — | 2.3 | — |
| *A. ruber* | — | 1.0 | — | 5.0 | 3.9 | — | — | 4.2 |
| *Cladosporium* sp. | 2.0 | — | — | 4.5 | — | — | — | 4.5 |
| *Curvularia* spp. | — | — | — | — | 1.0 | 0.5 | — | — |
| *Fusarium oxysporum* | — | — | 2.1 | 23.6 | 15.6 | 8.4 | 13.5 | 18.4 |
| *F. solani* | — | — | 6.7 | 6.6 | 8.8 | 8.4 | — | 13.6 |
| *Helminthosporium* spp. | 1.2 | — | — | — | 1.4 | 1.5 | — | — |
| *Penicillium* spp. | — | 7.8 | 3.3 | 1.6 | — | — | — | — |
| *Rhizoctonia solani* | 1.2 | — | — | 17.6 | 1.5 | — | — | 35.4 |
| *Rhizopus* sp. | 2.6 | — | 6.7 | 3.3 | — | — | 2.6 | 4.5 |
| *Trichoderma harzianum* | — | — | — | — | — | 6.7 | 4.0 | — |
| Yeasts | | | | | | | | |
| Pigmented | 0.2 | — | — | — | 0.2 | — | — | — |
| Non-pigmented | — | — | — | 0.3 | — | — | — | 0.3 |

**Table 4.** Percentage of different fungi and yeasts in the rhizoplane of untreated, treated and infected coffee seedlings as recorded on malt extract agar and potato dextrose agar.

| Isolated Microorganisms | Malt Extract Agar | | | | Potato Dextrose Agar | | | |
|---|---|---|---|---|---|---|---|---|
| | Untreated Seeds | Quintozene-Treated Seeds | Carboxin-Treated Seeds | Collar Rot-Infected Seeds | Untreated Seeds | Quintozene-Treated Seeds | Carboxin-Treated Seeds | Collar Rot-Infected Seeds |
| Fungi | | | | | | | | |
| *Aspergillus niger* | 54.4 | 62.5 | 85.1 | 3.1 | 64.5 | 70.6 | 77.3 | 4.1 |
| *A. candidus* | 8.8 | 2.7 | 2.1 | 24.6 | 6.6 | 1.7 | 2.0 | 22.6 |
| *A. ochraceous* | — | — | — | 20.1 | — | 0.5 | — | 17.6 |
| *A. flavus* | — | 27.7 | — | 18.1 | 3.2 | 14.7 | — | 24.8 |
| *Fusarium oxysporum* | 5.5 | — | 2.5 | 13.0 | 6.4 | 4.0 | 4.0 | 13.8 |
| *F. solani* | 2.2 | 4.5 | — | 8.0 | 2.6 | 2.3 | 5.5 | 2.2 |
| *Penicillium* spp. | 15.5 | 5.5 | 8.5 | — | 14.2 | 2.7 | 6.6 | — |
| *Rhizoctonia solani* | 4.5 | — | — | 10.5 | 2.7 | — | — | 15.8 |
| *Rhizopus* sp. | 8.8 | — | — | 2.2 | 1.0 | — | — | 10.0 |
| *Trichoderma harzianum* | — | — | 2.2 | — | — | 2.5 | 4.2 | — |
| Yeasts | | | | | | | | |
| Pigmented | 0.2 | 0.1 | — | — | — | 0.1 | 0.1 | — |
| Non-pigmented | — | — | — | 0.1 | — | — | — | 0.2 |

**Table 5.** Percentage of bacteria in the rhizosphere and rhizoplane of untreated, treated and infected coffee seedlings as recorded on total plate count agar.

| Isolated Bacteria | Untreated Seeds | Quintozene-Treated Seeds | Carboxin-Treated Seeds | Collar Rot-Infected Seeds |
|---|---|---|---|---|
| Gram positive | | | | |
| Rhizosphere | 0.3 | — | — | 0.6 |
| Rhizoplane | 0.1 | — | — | 0.3 |
| Gram negative | | | | |
| Rhizosphere | 0.1 | — | — | 0.3 |
| Rhizoplane | 0.1 | — | — | 0.2 |

similar type of stimulation of fungal population in the rhizosphere was noted in Dolichos plants infected with Dolichos mosaic virus (Laxmikumari, 1964); in lodge pine seedlings with fusarial wilt and root rot pressure (Timonin, 1966); in pine seedlings with damping-off disease pressure (Hocking, 1968); in coffee plants with decline disease (Vishwanath et al., 1969); and in papaya plants with leaf reduction virus (Singh, 1972). On the contrary, Mishra and Kamal (1970) observed reduction in the rhizosphere fungal population of cotton, chili and tomato with viral infection. The higher number of rhizosphere and rhizoplane microflora obtained in the present investigation might be due to increased concentration of micronutrients leached into the rhizosphere soil of coffee seedlings colonized by the collar rot pathogen.

Low rhizosphere and rhizoplane microbial population density was found in seedlings from Carboxin- and Quintozene-treated seeds. Seedlings obtained from Carboxin-treated seeds showed the least mycoflora and bacterial populations. The observed reduction could be due to the elimination of seed mycoflora by the seed treatments. Pugashetty and Rangaswami (1969) reported varied changes in the rhizosphere microflora of cotton seedlings due to different pre-treatments given to the seed. The present findings support their results.

*R. solani* population substantially decreased in seedlings from seeds treated with both fungicides because Carboxin is systemic and Quintozene has been found to be effective against *R. solani* population in nursery seed beds when applied as seed dressing (Venkatasubbaiah and Muthappa, 1981).

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