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The Variance of Microbial Numbers Associated with Rhizoplane of *Spinacia Oleracea* (L.) Untreated and Treated with Different Biological and Chemical Fungicides During Kharif and Rabi Seasons

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Abstract

There are several field problems of crop and vegetable plants effecting yield potential which include pests and diseases. Most of the diseases in plants are caused by microorganisms such as fungi. bacteria and actinomycetes. The microbes are known to colonize diverse habitats and substrates including plantsThe colonisation of different microorganisms on the same leaf offers interesting study related interaction of microorganisms and host plant under constantly changing atmospheric conditions and the host plant. During their life cycle both the host and the pathogen are subjected to several biotic and abiotic factors. The information on microbial ecology of aerial parts and other ecological niches of vegetable crops will help in understanding quality product, production thresholds, pre and post-harvest pathology and bio-deterioration. Such studies are important in understanding pathogenicity and control of plant pathogens. An attempt is made to understand the use of different biologically active fungicides and chemical fungicide on fungi. bacteria and actinomycetes populations in rhizoplane of *Spinacia oleracea* L. (Spinach) as test material. There was positive effect of Neem cake in controlling fungi and actinomycetes, the bacterial populations were higher during Kharif season while fungi and actinomycetes populations were higher in Rabi season. Neem oil sprays were more effective in Rabi than in the Kharif. There was decrease in bacteria and actinomycetes populations when the plants were treated with Neem oil. Turmeric was effective in reducing the population levels of fungi and bacteria. On an average Bavistin was effective only on bacteria in reducing their population levels.

Key words: Spinacia oleracea, fungicides, Neem Cake, Rhizoplane, Neem Oil, Bavistin, Turmeric

Introduction

The aerial and subterranian portions of the plants are known to be congenial sites for the colonisation of the microbes. The microorganisms are known to interact with each other and with the host plant simultaneously, sometimes resulting in useful effects on the host plant and at other times causing disease conditions. The microbes are known to colonize diverse habitats and substrates including plants. The specialized ecological niches that are of importance to plants are soil, rhizosphere, rhizoplane and phylloplane. The association of the microbes with host plant depends on number of factors associated with the substrate, host plant interaction and microbe to microbe interaction. The roots are known to exert influence on the soil microorganisms as they release roots exudates which contain useful nutrients for the microbes. The microbes in turn exert influence on the plants in supplying useful micro and macro nutrients to the above ground community. The root exudates are not only known to influence the spore germination

but also inhibit microbial growth. Thus the quantitative and qualitative nature of the root exudates differ from plant to plant and variety to variety and in turn influence the microbial growth. A large number of these microbes are crucial to human welfare because they are involved in the recycling of organic waste, the carbon, nitrogen, and phosphorus cycles, mineralization, and other processes. They are also a source of organic acid and antibiotics, which are highly valuable.

An effort is made to comprehend the application of various chemical and biologically active fungicides. As test material, populations of actinomycetes and bacteria from rhizoplane of *Spinacia oleracea* L. (Spinach) were used. The present study aims to study

1) Quantitative and qualitative assessments of mycoflora and qualitative assessments of bacteria and actinomycetes from rhizoplane.

2) To comprehend the effects on the population dynamics of microorganisms in rhizoplane on the applications of neem cake, neem oil, and turmeric as biological fungicides and bavistin as chemical fungicide.

Literature review

Rhizoplane is an important ecological niche harbouring number of bacteria, actinomycetes and fungi. The term rhizoplane was proposed by Clark (1949) to refer to the immediate external surface of plant roots together with any closely adhering particles of soil or debris and microbial communities.

Cook and Lochhead (1959) isolated microorganism using root maceration technique Using serial root washings Harley and Waid (1955) successfully isolated rhizoplane microorganisms. Stover and Waite (1953) and Singh (1965) using root maceration technique isolated Fusaria and other root mycoflora. Patel (1926) isolated Agrobacterium and other bacteria from roots. Using boiling technique Sherwood (1958) isolated species of Aphanomyces from plant roots. Stover and Waite, (1953, 1954) recorded various species of Phytopthora. (Eckert and Tsao, 1962), Drechsler (1929) has been successful in isolating Pythium species from diseased roots of sugar cane and cotton.

Thirty bacterial isolates were obtained from the infected roots of carrot (Choi et al. 1989) and 14 isolated belonged to genus Pseudomonas and 16 to Ervinia. The isolates belonging to fluorescent Pseudomon as were identified. The diseases caused by the bacteria on the root are proposed to be called as ' Bacterial soft rot of carrot'. Three diseases of root of spinach were reported by Naiki (1984). They are Pythium causing 'damping off", Rhizoctonia causing 'root rot' and Fusarium causing 'wild' disease. Mycoflora of root surface in winter wheat and winter barley was reported by Grynderova and Hana (1990). They have found that genus Fusarium and Penicillium were dominant fungal flora. Nitrogen fixing bacteria were isolated (Gamo, 1991) from roots of rice, sugar cane, sorghum, maize, spinach, Chinese cabbage, soybeans, cucumber and from Brassica species. Azospirillum brasilense was isolated from spinach, cabbage and cucumber.

Methodology

The green leafy vegetable *Spinacia oleracea* L. belongs to Chenopodiaceae. These plants are herbs that are succulent and have swollen nodes.. The crop lasts for 120–130 days. After sowing, it takes 75–105 days for it to flower. The plant is utilised as a leafy vegetable that is high in macro and micronutrients such as riboflavin and biotin. Farmers cultivate it extensively, as well as in kitchen gardens. In order to examine the effects of chemical and biological fungicides (such as neemcake, neem oil, turmeric, and bavistin) on the ecology and population dynamics of fungi, actinomycetes, and bacteria, fifteen plots were seeded with palak seeds for this study.

Fifteen five-by-five-foot plots were used to raise the plants; three plots were utilised as controls, three plots each for biological fungicide treatments (Neem cake, Neem oil and turmeric), and three plots for chemical fungicide treatments (Bavistin). Weekly treatments of neem cake, neem oil, turmeric, and bavistin were applied to the plots from seedling stage till harvest. Neem cake, Neem oil, and turmeric were sprayed on three plots each at a rate of 1 ml/lit. Bavistin was employed as an aerial and systemic fungicide at a rate of 1 gm/lit. Plant samples from each of the three plots were taken at 20-day intervals according to treatment. After being well mixed, the samples were used right away for microbiological and biochemical analysis. Twenty plants from each treatments were collected and their agronomical characters were noted. Soil samples were also collected simultaneously and are subjected to microbial, physical and chemical analysis apart from recording soil pH. The general laboratory techniques used for this experiment were adopted as suggested by Booth (1971) and Hawksworth (1974).

Isolation and estimation of rhizoplane fungi: To isolate fungi from rhizoplane root pieces of 5mm length were. collected and were macerated and fungi were enumerated following by the technique of Stover and Waite (1953). The roots from which rhizosphere soil was collected were washed thoroughly with sterile water and dried between filter paper. Five grams of roots in 50ml of sterile distilled water were macerated in sterilized waring blender (Singh 1965) and serial dilutions were prepared from the blended material to get biochemical analysis for proteins, amino acids, carbohydrates, reducing sugars and phenols.

The following media were used

a) Potato Sucrose Agar (PSA) , Vegetable Agar Medium (VAM) for Fungi
b) Gluose Yeast Extract Agar Medium (GYE) for Actinomycetes
c) Beef Extract Peptone Agar Medium (BPA) for Bacteria

Microflora isolation using the dilution plate method: The dilution plate method of Waksman (1952), as reported by Johnson and Curl (1972), was employed for the quantitative estimate of fungi since it permitted both qualitative and quantitative evaluations. Five grams of the sample was manually shook in 50 millilitres of sterile distilled water for ten to twenty minutes, and additional dilutions were performed as needed. The dilutions of bacteria, actinomycetes, and fungus that were selected for quantitative measurement were 1:10,000, 1:20,000, and 1:30,000, respectively. For every sample, 1 ml of dilutant was aseptically put into sterile petridishes, and then the sterile medium was added. After thoroughly combining the suspensions with the agar, the plate was rotated in both clockwise and anticlockwise directions, and it was left to set.

Slide Preparation: Lactophenol and cotton blue in lactophenol were used as mounting and staining media for preparing semi-permanent slides which were sealed with D.P.X. mountant.

Microscopic observation: Meopta Research microscope with adequate high power has been used throughout the study. The fungi were identified and some fungal species were photographed using trinocular head. The data obtained was subjected to statistical analysis for drawing precise conclusions on various aspects as suggested by Snedecor and Cochran (1967).

Results

Microbial Numbers And Population Dynamics On Rhizoplane Of *Spinacia oleracea* L.

Table: 1 shows the number of fungi, bacteria, actinomycetes per gram material associated with rhizoplane supporting *Spinacia oleracea* L. among the untreated plants (Control) during Kharif andRabi seasons.
The 'F' values are 4.8, 4.483 and 5.90 respectively for fungi, bacteria and actinomycetes on rhizoplane during Kharif-1; 3.1, 4.052 and 4.20 during Rabi-1.3.5, 8.269 and4.30 during Kharif-II, 3.8, 28.495 and 19.488 for Rabi - II respectively. They are significant. The 'F' indicates the differences between the microbial numbers between different samples and between fungi, bacteria and actinomycetes and in between the seasons.

During Kharif-I the microbial numbers increased along with the crop season and plant age. It was found that the root surface of *Spinacia oleracea* L. harboured higher population levels of microorganisms during later part of the season than the earlier part of the season. This may be due to the exudation of nutrient materials by the plant usually resulting in rhizosphere effect. In general the highest population density was represented by bacteria followed by actinomycetes and fungi. It was also observed that the fungal populations were higher during Kharif season than during Rabi season. On the contrary the bacterial and actinomycetes populations were found to be higher during the Rabi season than during Kharif season.

A study was also made to understand the effect of biological fungicides like Neem cake, Neem oil and Turmeric and chemical fungicide Bavistin on the population levels of rhizoplane microflora of *Spinacia oleracea* L.

Table: 2 reflects the microbial numbers per gram material associated with rhizoplane of *Spinacia oleracea*e L. in the soils supplemented with Neem cake during Kharif and Rabi seasons.

The 'F' values for fungi, bacteria, and actinomycetes are 4.9, 7.170 and 4.20 respectively during Kharif ; 3.610, 11.753 and 3.606 during Rabi respectively. They are significant.

The fungi and actinomycetes populations showed progressive increase in population concentration along with the age of the plant. The data indicates that the fungal numbers were higher in rhizoplane during mature stages of the plant than at vegetative phases. However, the bacterial populations were reduced over the season and they were higher in numbers during vegetative phases compared to mature stages. In contrast to the Control the fungal and actinomycetes populations were relatively higher while bacteria populations were less than the Control indicating the positive effect of Neem cake on fungi and actinomycetes. It was also observed that the bacterial populations were higher during Kharif season while fungi and actinomycetes populations were higher in Rabi season.

Table: 3 shows the microbial numbers per gram material associated with rhizoplane of *Spinacia oleracea*e L sprayed with Neem oil during Kharif and Rabi seasons.

The 'F' values for fungi, bacteria and actinomycetes are 4.5, 13.723 and 15.959 during Kharif ; and 4.2, 8.717 and 21.574 during Rabi respectively. The 'F' values are significant.

In contrast to the untreated plants (Control) the microbial numbers decreased along with age of the plant indicating the effectiveness of Neem oil on rhizoplane microflora. In general the bacterial populations were higher than actinomycetes populations and the microbial numbers were higher in Kharif season than Rabi season indicating the Neem oil sprays were more effective in Rabi than in the Kharif season. On an average there was decrease in bacteria and actinomycetes populations when the plants were treated with Neem oil.

Table 4 shows the microbial numbers per gram material associated with rhizoplane of *Spinacia oleracea*e L. sprayed with Turmeric.

The 'F' values are 5.880, 8.505 and 6.343 respectively for fungi, bacteria and actinomycetes of rhizoplane during Kharif and 3.7, 8.760 and 4.10 during Rabi respectively. The values are significant.

The microbial populations reduced from high concentration during the vegetative phases of the plants to lower concentration during the flowering and matured stages in respect of fungi and bacteria. But the actinomycetes populations were found to be more during matured stages than during vegetative phases. This indicates the effectiveness of Turmeric in reducing the population levels of fungi and bacteria. In contrast to the Control the bacterial and actinomycetes numbers have been reduced. However, no significant differences in fungal populations between the Control and Turmeric treated plants were observed.

Table 5 shows the microbial numbers per gram material associated with rhizoplane of *Spinacia oleracea*e L. treated with Bavistin.

The 'F' values are 5.645, 37.093 and 20.201 respectively for fungi, bacteria and actinomycetes of rhizoplane during Kharif 5.004, 16.361 and 4.5 during Rabi. Thus indicating significant differences The fungal and bacterial populations decreased during flowering and matured stages of the plant compared to vegetative phases. On the contrary the actinomycetes populations were found to be higher in later part of the season than the earlier part of the season on the rhizoplane of *Spinacia oleracea*e L. However, actinomycetes populations increased over the crop season and is similar to Control and Bavistin appears to be ineffective in reducing the actinomycetes populations compared to Control. On an average Bavistin was effective only on bacteria in reducing their population levels.

Tables

TABLE 1: Microbial Numbers per gram material associated with Rhizoplane supporting *Spinacia oleracea* L. from untreated plants (Control)

S.No	Sampling Day	KHARIF			RABI		
		FUNGI (10^ 3)	BACTI (10^3X3)	ACTI (10^3X2)	FUNGI (10^3)	BACTI (10^3X3)	ACTI (10^3X2)
1	20 TH DAY	9.66	40.50	12.67	6.00	38.67	12.57
2	40 TH DAY	8.67	49.00	14.00	6.17	50.00	14.00
3	60 TH DAY	7.17	39.00	15.00	5.00	55.67	13.67
4	80 TH DAY	9.33	61.67	16.00	6.00	54.33	14.33
5	100 TH DAY	10.00	62.67	16.67	7.00	66.00	15.33
6	120 TH DAY	8.67	68.00	17.00	7.50	67.00	15.00
	AVERAGE	8.92	53.47	15.22	6.28	55.28	14.17
	CV %	5.83	9.6	3.94	9.17	8.8	3.07
	CD (0.5)	0.20	1.270	0.28	0.27	1.188	0.21

TABLE 2: Microbial Numbers per gram material associated with Rhizoplane supporting *Spinacia oleracea* L. treated with Neem cake

S.No	Sampling Day	KHARIF			RABI		
		FUNGI (10 ^ 3)	BACTI (10^3 X 3)	ACTI (10^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10^3X 3)	ACTI (10^3X 2)
1	20 TH DAY	9.67	33.67	13.33	4.50	33.33	13.67
2	40 TH DAY	7.67	46.00	11.67	5.00	48.33	13.00
3	60 TH DAY	8.00	47.00	13.67	10.00	37.67	14.00
4	80 TH DAY	8.33	33.00	12.23	12.00	30.00	17.00
5	100 TH DAY	8.33	32.33	14.00	11.00	17.67	18.00
6	120 TH DAY	10.67	24.33	14.50	16.83	16.00	23.00
	AVERAGE	8.78	36.06	13.23	9.89	30.50	16.45
	CV% CD (0.5)	7.16 0.24	8.2 0.884	3.62 0.24	26.2 0.886	10.7 1.055	10.1 0.741

TABLE 3: Microbial Numbers per gram material associated with Rhizoplane supporting *Spinacia oleracea* L. sprayed with Neem Oil

S.No	Sampling Day	KHARIF			RABI		
		FUNGI (10 ^ 3)	BACTI (10^3 X 3)	ACTI (10^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10^3X 3)	ACTI (10^3X 2)
1	20 TH DAY	12.00	58.00	10.67	12.67	26.33	12.67

2	40 TH DAY	12.67	48.00	17.00	9.67	23.33	21.33
3	60 TH DAY	11.33	37.33	12.00	8.00	22.33	12.67
4	80 TH DAY	10.17	37.00	13.00	6.00	16.67	8.00
5	100 TH DAY	7.17	29.67	12.00	7.00	10.67	7.00
6	120 TH DAY	8.84	27.00	6.67	5.50	7.67	4.00
	AVERAGE	10.36	39.50	11.89	8.14	17.83	10.95
	CV% CD (0.5)	14.87 0.56	6.9 0.780	6.4 0.397	12.70 0.39	13.5 1.013	10.6 0.614

TABLE 4: Microbial Numbers per gram material associated with Rhizoplane supporting *Spinacia oleracea* L. sprayed with Turmeric

S.No	Sampling Day	KHARIF			RABI		
		FUNGI (10 ^ 3)	BACTI (10^3X 3)	ACTI (10^3 X 2)	FUNGI (10 ^3)	BACTI (10^3X 3)	ACTI (10^3X 2)
1	20 TH DAY	11.33	26.33	10.33	8.50	35.00	16.67
2	40 TH DAY	15.17	27.00	14.00	9.00	40.00	13.67
3	60 TH DAY	8.00	24.00	14.00	14.67	50.00	19.00
4	80 TH DAY	8.17	17.67	10.00	8.17	26.67	20.00
5	100 TH DAY	4.17	18.00	26.00	7.84	28.00	21.00
6	120 TH DAY	3.50	10.33	30.00	6.00	18.00	25.00
	AVERAGE	8.39	20.56	17.39	9.03	32.95	19.22
	CV % CD (0.5)	31.4 0.984	10.2 0.826	16.1 1.191	18.61 0.62	10.3 1.064	8.98 0.71

TABLE 5: Microbial Numbers per gram material associated with Rhizoplane supporting *Spinacia oleracea* L. treated with Bavisitn

S.No	Sampling Day	KHARIF			RABI		
		FUNGI (10 ^ 3)	BACTI (10^3 X 3)	ACTI (10^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10^3X 3)	ACTI (10^3X 2)
1	20 TH DAY	13.67	38.67	8.00	16.00	41.67	14.67
2	40 TH DAY	13.00	36.33	6.33	17.00	42.67	15.00
3	60 TH DAY	10.00	42.67	5.33	14.84	30.33	17.67
4	80 TH DAY	9.00	29.00	20.33	10.67	24.00	22.00
5	100 TH DAY	6.67	20.00	13.67	8.17	17.33	18.00
6	120 TH DAY	4.50	10.33	11.33	8.67	14.67	19.00
	AVERAGE	9.47	29.50	10.83	12.56	28.45	17.72
	CV% CD (0.5)	21.7 0.767	6.8 0.654	9.9 0.574	24.3 1.000	14.3 1.277	6.17 0.47

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