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The Study of Microbial Numbers Associated with Rhizosphere Soil of Spinacia Oleracea (L.) Treated with Fungicides During Kharif and Rabi Seasons

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Abstract

There are several field problems of crop plants 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 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 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 different ecological niches of *Spinacia oleracea* L. (Spinach) as test material. A comparative study of effectiveness of the biological fungicides- Neem cake. Neem oil and Turmeric and chemical fungicide Bavistin shows that while all the fungicides reduced the microbial populations over the season; Neem cake, Neem oil and Turmeric appears to be more effective than Bavistin in respect of reducing the Bacterial and Actinomycetes populations. Compared to the Control all the fungicides were ineffective in reducing the fungal populations. This also indicates biological fungicides - Neem oil, Neem cake and Turmeric are equally effective.

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

Introduction

There are several field problems of crop plants 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 aerial and subterranean 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 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 study of such associations are of special interest in the study of microbial ecology. The microbes associated with soil, rhizosphere and phylloplane were found to differ quantitatively and qualitatively. Several studies were carried out in the study of the rhizosphere microbiology on different plants by different workers. Higher number of fungal, bacterial and actinomycetes populations were recorded in rhizosphere and rhizoplane compared to the non-rhizosphere soil. This phenomenon was known as "Rhizosphere effect". 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.

The discovery of various isolating techniques in isolating diverse groups of microorganisms have revealed that diverse groups of microflora inhabit the soils and they are found in all habitats of the soil on this biosphere and they form an important soil biomass. Number of fungal, bacterial and actinomycetes species were reported to thrive well in the soil. Number of these microbes are very important as they are involved in recycling of organic waste, carbon nitrogen and phosphorous cycles, mineralization etc.,

The extensive studies on soil, rhizosphere and phylloplane microflora of different plants revealed diversified myco and microflora differing from plant to plant and region to region including some endemic species.

Relatively information of microbial ecology and effect of various chemicals on the qualitative and quantitative nature of microorganisms is meagre. Therefore, an attempt is made to understand the use of different biologically active fungicides and chemical fungicide on fungi. bacteria and actinomycetes populations in different ecological niches of *Spinacia oleracea* L. (Spinach) as test material. *Spinacia oleracea* L. is a greeny leafy vegetable and is commonly known as Indian Palak. These plants are succulent herbs with swollen nodes. This plant comes under Chenopodiaceae. The members of this family are cosmopolitan in distribution. Duration of the crop is 120-130 days. It flowers between 75-105 days after sowing. The plant is commonly used leafy vegetable rich in biotin, riboflavin and other micro and macro nutrients. It is widely grown by the farmers and also in kitchen gardens. The present study is aimed at:

1) Quantitative and qualitative estimations of mycoflora and qualitative estimation of bacteria and actinomycetes from rhizosphere

2) To understand the efficacy of applications of Neem cake, Neem oil, Turmeric as biological fungicides and Bavistin as Chemical fungicide on the population dynamics of microorganisms.

Materials and Methods

For the present study seeds of palak were sown in fifteen plots to study the efficacy of biological and chemical fungicides (eg: Neemcake, Neem oil. Turmeric and Bavistin) on the ecology and population dynamics of Fungi, Actinomycetes and Bacteria. The plants were raised in all fifteen plots of size 5' x 5' from these three plots for control, three plots each were used for biological fungicides (Neem cake, Neem oil and Turmeric) treatment and three plots for chemical fungicide (Bavistin). The plots were

treated with Neem cake, Neem oil, Turmeric and Bavistin from seedling stage to harvest at weekly intervals. Three plots each were sprayed with Neem cake, Neem oil and Turmeric @ 1 ml/lit and Bavistin was used as systemic and aerial fungicide 1gm/llit. Plant samples were collected at 20 days intervals treatment - wise from each of three plots and they were mixed thoroughly and immediately used for microbial and bio-chemical 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).

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

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.

Sampling of soil and rhizosphere soil. Sampling was regularly done for every 20 days. Soil samples were collected with a wedge sterilized with 70% alcohol from the top 5-6" of soil after scraping away an inch of surface soil into sterile containers. (Polythene covers). The rhizosphere soil samples were taken with the help of wedge by lifting up gently a block of soil with the plant intact. Then the root system along with sticky soil around was carefully removed and placed in fresh polythene bags. Soil and rhizosphere samples were quickly transported to the laboratory and the soil pH and moisture contents were determined immediately. Later, a small portion of the soil was taken from the same sample for the assessment of microflora.

Isolation of microflora : Dilution plate method: For quantitative estimation of fungi the dilution plate method of Waksman (1952) as described by Johnson and Curl (1972) was used, as it allowed qualitative and quantitative assessments. Five grams of sample was shaken by hand for 10 minutes to 20 minutes in 50ml sterile distilled water and successive dilutions were made as required 1:10,000, 1:20,000 and 1:30,000 dilutions were chosen for the quantitative estimation of fungi, actinomycetes and bacteria respectively. 1 ml of dilutant was transferred aseptically into sterile petridishes for each sample and the sterile medium was added. The suspensions were mixed well with the agar by rotating the plate in clockwise and anti-clockwise directions and then allowed to set.

RESULTS

MICROBIALNUMBERSANDPOPULATIONDYNAMICS

In order to study the efficacy of Neem cake, Neem oil, Turmeric as biological fungicides and effectiveness of chemical fungicide Bavistin on microorganisms a study was conducted using *Spinacia oleracea* L.(palak) as test plant. Neemoil and Turmeric was used as sprays and Neemcake and Bavistin were used as soil applications. The plants were treated with these fungicides at aninterval of 20 days and plants were analysed for bacteria and actinomycetes quantitatively and fungi both quantitatively and qualitatively.

a)MICROBIAL NUMBERSINRHIZOSPHERE

Table: 1 shows the number of fungi, bacteria and actinomycetes isolated from rhizosphere soilsduringKharifandRabiseasonsassociatedwith*Spinacia oleracea* L.Untreatedplants-Control.

The 'F' values for fungi, bacteria, actinomycetes are 4,5, 6.00 and 4.50 respectively for rhizospheresoilsduringKharif- I;3.7,4.30and5.60duringforRabi-I3.427,4.70and6.261forKharif-II;5.1,4.20and 5.20forRabiII.Thusthedifferencesaresignificant.In general the bacterial population were highest followed by actinomycetes and fungi in all theseasonsstudied.

Table:2showsmicrobialnumberspergrammaterialassociatedwiththerhizospheresoilsupporting*Spinacia oleracea* L.treated withNeemcake.

The 'F' values were 8.231, 3.407 and 10.157 respectively for fungi, bacteria, actinomycetes inrhizosphere soils during Kharif - I. The 'F values for fungi, bacteria, actinomycetes for Rabi - I are 4.9,5.667and15.575respectively.Thevaluesare significant.Themicrobialnumbersreducedalongwiththeplantageduringseasonsindicatingefficacyof Neem cakeinreducingthemicrobialpopulationsinthesoils.

Table:3showsthemicrobialnumberspergrammaterialassociatedwithrhizospheresoilssprayedwithNeemoil.

The'F'valuesforfungi,bacteriaandactinomycetesare5.1,8.619and3.550duringKharif-Iand3.7,51.561and3.754duringRabi-Irespectively.Thusindicatingsignificantdifferences.The data shows, there was gradual reduction of microbial numbers over the season indicating theeffectivenessofNeemoilonmicrobialnumbersinrhizospheresoilsduringtheentirecropseason.

Table: 4 shows microbial numbers per gram of rhizosphere soil associated with *Spinacia oleracea* L.sprayedwithTurmeric.

They 'F' values are 4.392, 6.165 and 5.3 respectively for fungi, bacteria and actinomycetes inrhizosphere soils during Kharif - 1. and 'F' values during Rabi - I are 6.632, 8.834 and5.40respectively.Thevaluesare significant.In general higher microbial numbers were observed in first 3 samples than in the last 3 samplesindicatingtheeffectivenessofTurmericspraysincontrollingrhizospheremicrobialpopulationlevelsof *Spinacia oleracea* L.overtheseason.

Table : 5 shows microbial numbers per gram of rhizosphere soil material associated with *Spinacia oleracea* L.treated withBavistin.

The 'F' values for fungi, bacteria and actinomycetes during Kharif-I are 3, 19.409 and 7.184respectively;The'F' valuesforfungi,bacteriaandactinomycetesduringRabi-Iare4.9,4.10and5.80respectively.The'F' valuesforfungi,bacteriaandactinomycetesduring Kharif-11are6.2,5.30and5.088 respectively; the 'F' values for fungi, bacteria and actinomycetes during Rabi-II are 5.7, 3.745and 5.690 respectively. The values are significant indicating significant differences between theseasonsandinbetweenthesamples formicrobialpopulations.

DISCUSSION

DYNAMICSOFMICROBIALNUMBERS

During both the seasons the fungal populations were higher during the first 3 samples compared tothelast3samplesindicatingthatthesoilssupportedhigherfungalpopulationsduringearlierpartofthe season than during the later part of the season. The trend was generally from high populationlevels to one of lower population levels during crop season. Such trend is also evident for bacteriaandactinomycetes duringboththeseasons.

It was observed that the populations of microorganisms reduced along with the season in thathigher population levels were observed in first three samples compared to the last three samples.ThisalsoindicateseffectivenessofBavistinshowing antimicrobialeffect overtheseason.

CONCLUSION

Acomparativestudyofeffectivenessofthebiologicalfungicides- Neemcake.NeemoilandTurmericand chemical fungicide Bavistin shows that while all the fungicides reduced the microbialpopulations over the season; Neem cake, Neem oil and Turmeric appears to be more effective thanBavistin in respect of reducing the Bacterial and Actinomycetes populations. Compared to theControl all the fungicides were ineffective in reducing the fungal populations. This also indicatesbiologicalfungicides-Neemoil, NeemcakeandTurmeric are equallyeffective.

TABLES

TABLE 1 : Microbial Numbers per gram material associated with Rhizosphere Soil of Spinacia oleracea.L. from untreated plants (Control)

S.No	Sampling Day	KHARIF (I)			RABI (I)			KHARIF (II)			RABI (II)		
		FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 3 X 3)	ACTI (10 ^ 3 X 2)
1	20 TH DAY	8.00	35.67	16.00	8.00	29.00	15.00	8.33	35.33	26.00	8.00	26.00	17.00
2	40 TH DAY	7.33	36.33	13.67	10.83	27.33	16.00	9.00	34.00	26.67	9.33	26.33	19.00
3	60 TH DAY	5.33	42.67	16.33	10.33	25.67	13.67	6.83	31.33	26.67	8.17	27.00	16.00
4	80 TH DAY	6.17	32.67	12.00	8.67	26.67	15.00	5.66	25.00	19.00	6.33	24.00	16.00
5	100 TH DAY	8.50	35.00	14.67	8.00	26.33	11.67	5.50	28.67	16.00	6.00	25.33	15.00
6	120 TH	4.67	33.0	14.0	6.83	27.3	12.3	6.17	27.3	17.0	6.17	21.3	13.0

	DAY		0	0		3	3		3	0		3	0
	AVERAGE	6.67	35.89	14.45	8.78	27.06	13.95	6.92	30.28	21.89	7.33	25.00	16.00
	CV % CD (0.5)	13.64 0.41	7.14 0.14	4.62 0.32	9.58 0.33	1.79 0.17	4.53 0.31	26.074 9	4.18 0.39	0.691 8.2	10.46 0.33	3.80 0.34	4.93 0.36

TABLE 2 : Microbial Numbers per gram material associated with Rhizosphere Soil of Spinacia oleracea.L. treated with Neem Cake

S.No	Sampling Day	KHARIF (I)			RABI (I)		
		FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)
1	20 TH DAY	14.00	21.00	18.00	8.50	19.67	11.67
2	40 TH DAY	15.16	23.33	16.00	7.17	29.33	14.67
3	60 TH DAY	9.83	26.33	17.67	7.00	27.67	17.33
4	80 TH DAY	5.00	16.00	11.00	5.50	16.00	17.33
5	100 TH DAY	4.67	14.00	11.00	8.00	15.00	9.00
6	120 TH DAY	7.50	12.67	8.33	9.00	10.00	5.00
	AVERAGE	9.36	18.89	13.67	7.53	19.61	12.50
	CV % CD (0.5)	20.6 0.730	13.5 1.055	8.4 0.559	10.41 0.33	14.5 1.146	9.5 0.600

TABLE 3 : Microbial Numbers per gram material associated with Rhizosphere Soil of Spinacia oleracea.L. sprayed with Neem Oil

S.No	Sampling Day	KHARIF (I)			RABI (I)		
		FUNGI (10 ^ 3)	BACTI 10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)
1	20 TH DAY	10.00	39.67	17.67	8.00	41.00	23.33
2	40 TH DAY	8.83	46.00	20.67	7.66	55.33	32.00
3	60 TH DAY	7.50	39.00	21.67	8.00	26.00	19.00
4	80 TH DAY	3.83	25.67	17.00	7.83	12.33	16.67
5	100 TH DAY	6.33	26.67	13.33	6.33	14.00	18.67
6	120 TH DAY	5.50	24.00	18.67	6.16	12.33	18.33
	AVERAGE	7.00	33.50	18.17	7.33	26.83	21.00
	CV % CD (0.5)	17.73 0.53	8.1 0.85	7.8 0.6	8.49 0.27	8.1 0.73	11.6 0.958

TABLE 4: Microbial Numbers per gram material associated with Rhizosphere Soil of Spinacia oleracea.L. sprayed with Turmeric

S.No	Sampling Day	KHARIF (II)			RABI (II)		
		FUNGI (10 ^ 3)	BACTI 10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)
1	20 TH DAY	13.67	26.67	14.00	7.67	81.67	16.33
2	40 TH DAY	12.00	22.67	12.33	7.83	35.00	12.00
3	60 TH DAY	5.67	29.33	11.33	3.67	58.33	16.00
4	80 TH DAY	7.33	19.00	10.33	2.67	31.00	10.67
5	100 TH DAY	5.00	17.33	9.67	3.50	50.00	10.00
6	120 TH DAY	5.00	15.67	10.00	4.16	33.67	13.67
	AVERAGE	8.11	21.78	11.28	4.92	48.28	13.11
	CV %	29.5	8.9	4.84	22.3	11.3	7.43
	CD (0.5)	0.941	0.746	0.29	0.564	1.407	0.48

TABLE 5: Microbial Numbers per gram material associated with Rhizosphere Soil of Spinacia oleracea.L. treated with Bavistin

S.No	Sampling Day	KHARIF (I)			RABI (I)			KHARIF (II)			RABI (II)		
		FUNGI (10^ 3)	BACTI. (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)	FUNGI (10 ^ 3)	BACTI (10 ^ 3 X 3)	ACTI (10 ^ 3 X 2)
1	20 TH DAY	10.33	25.00	20.67	8.50	17.33	18.00	11.25	18.67	16.00	7.00	32.00	29.00
2	40 TH DAY	8.33	33.67	22.33	6.50	15.33	16.00	10.33	21.67	16.67	6.50	30.33	25.33
3	60 TH DAY	7.33	29.00	15.67	7.83	19.00	17.00	8.17	25.67	17.67	8.17	38.00	29.00
4	80 TH DAY	6.67	16.00	10.33	6.00	14.00	12.00	6.50	19.33	10.33	6.50	29.00	17.00
5	100 TH DAY	7.66	14.00	11.33	7.67	12.33	15.00	9.50	13.67	11.67	7.00	30.00	16.00
6	120 TH DAY	7.00	13.00	10.00	5.17	15.00	13.33	7.67	17.67	10.33	5.50	14.00	14.00
	AVERAGE	7.89	21.78	15.06	6.95	15.50	15.22	8.90	19.45	13.78	6.78	28.89	21.72
	CV %	10.56	8.10.671	10.10.724	10.4	6.54	5.56	12.87	8.04	0.667 10.0	7.38	14.01.338	11.50.960
	CD (0.5)	0.34			0.32	0.47	0.39	0.46	0.64		0.22		

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