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STUDIES ON ROOT REGION MICROFLORA OF HEALTHY AND WILTED LINSEED PLANTS IN RELATION TO SOIL POLLUTANTS

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Qualitative and quantitative differences were observed in microbial population of root region of healthy and wilted linseed plants (*Linum usitatissimum* L.) as well as in polluted and unpolluted soils. The number of fungi was higher in unpolluted soil than in soil polluted with ammonium hydroxide, raw sewage and cement dust. However, greater number of actinomycetes and bacteria were recorded in ammonium hydroxide and raw sewage polluted soils respectively. *Fusarium oxysporum* f. sp. lini was frequently present in high population on the rhizoplane of wilted plants throughout the study period.

A marked difference in succession of fungi in rhizosphere and rhizoplane region of excised healthy and wilted host roots was seen in early stages. The microbial population decreased on decaying roots of healthy and wilted host in all the soil samples as senescence and decomposition advanced.

Key Words : Linseed plants, soil pollutants, microflora.

Linseed (*Linum usitatissimum* L.) is an important oil seed and fibre yielding crop which suffers greatly from wilt disease caused by *Fusarium oxysporum* f. sp. *lini* (Bolley, Snyder and Hansen). The host roots and pathogenic and non-pathogenic organisms exist in soil in close proximity to each other. Thus, the ecology of microbes and the survival and pathogenecity of the soil-borne pathogen depend on soil characteristics and soil conditions. Soils, polluted with different pollutants, are known to exert diverse effect on qualitative and quantitative nature of mycoflora (Rai and Upadhyay, 1988). Role of ammonia as a volatile mycostatic factor on soil microbes was observed by Ko and Hora (1974). Sludge compost may be a useful soil amendment to decrease the severity of certain important diseases (Lewis *et al.*, 1981) Inhibition of the microbial population due to toxic effluent in sewage was reported by Downing (1971). Pesticides, industrial effluents and wastes, raw sewage and ferti-lizers are excessively applied in agricultural fields bringing change in soil properties (Ayanaba, 1981: Kayosato, 1983; El-Naim *et al.*, 1986) which greatly affect microbial population and host pathogenesis. Such changes either aggrevate or suppress root diseases. A little work has been done on direct or indirect influence of soil pollutants on development of diseases. In view of this a detailed investigation on root region microflora of healthy and wilted linseed plant, in relation to soil pollutants, was undertaken.

MATERIALS AND METHODS

The polluted soil samples were collected in sacks from various localities. Ammonium hydroxide polluted soil was collected from Sahupuri, around Hari Fertilizers Industry, raw sewage polluted soil from Dinapur sewage farm, cement dust polluted soil from Chunar around cement factory and unpolluted control soil from Botanical Garden, Banaras Hindu University (B.H.U.). All these localities are situated in close vicinity of B.H.U.

The collected soil samples were put in earthenware pots (23cm x 36cm). The seeds of local linseed variety were sown in each pot and all the pots were then kept in Botanical Garden in open and watered regularly as and when required. Thinning was done after 30 days leaving 18 plants per pot. Soil samples were collected at different stages of plant growth and also after excision of the host for physico-chemical analyses as well as for isolation of microflora.

Physico-chemical analyses of soil samples : The moisture content was determined by drying the soil samples at constant weight in an electric oven at 105°C for 48 h. pH was measured by digital pH meter using a glass electrode. The total organic carbon was estimated by rapid titration method as described by Piper (1944).

Isolation of rhizosphere, non-rhizosphere and rhizoplane microflora of healthy and wilted linseed

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Table 1: Numerically important non-rhizosphere, rhizosphere and rhizoplane mycoflora of healthy and wilted linseed plants in unpolluted and polluted soil samples.

Name of Fungi	AHP					RWS					CD					UNP				
	NR*	HR*	WR*	HRP+	WRP+	NR*	HR*	WR*	HRP+	WRP+	NR*	HR*	WR*	HRP+	WRP+	NR*	HR*	WR*	HRP+	WRP+
Acrophialophora fusispora	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
Alternaria alternata	-	-	-	9	-	-	-	-	3	12	-	-	-	-	3	-	-	-	8	2
A. tenuissima	1	1	1	5	2	1	3	-	-	-	-	-	1	4	-	-	1	-	1	-
Aspergillus candidus	-	-	-	-	-	-	-	-	-	1	-	-	3	-	-	-	1	6	-	
A. flavus	2	7	3	12	12	10	4	2	15	9	20	8	7	12	6	10	7	8	13	
A. flavipes	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	1	-	-	
A. fumigatus	14	10	4	-	8	21	18	2	9	7	15	6	11	8	-	25	6	1	-	-
A. nidulans	5	2	-	-	-	3	1	-	-	-	-	-	-	-	-	2	3	2	-	5
A. niger	11	7	2	22	13	8	7	5	15	8	3	3	2	17	4	13	4	4	16	4
A. sulphureus	-	-	-	-	-	-	1	7	-	3	-	-	-	-	-	1	-	1	2	-
A. sydowi	6	-	-	-	2	2	-	-	-	-	-	-	-	-	1	2	-	-	-	
A. terreus	2	2	1	10	11	-	3	-	-7	1	5	3	14	7	3	-	-	6	8	
Black sterile mycelium	1	-	1	7	-	3	2	2	3	2	-	-	1	-	-	2	-	-	-	4
Cladosporium cladosporioides	9	9	6	6	7	15	1	7	8	11	9	4	4	9	10	11	6	2	7	15
C. herbarum	-	-	-	-	-	2	2	3	-	1	2	-	3	-	-	-	-			
Cunninghamella achinulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13	9
Curvularia lunata	1	-	-	-	4	2	1	1	-	4	-	-	-	-	-	-	2	-	5	-
Drechslera australiensis	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	1	1	1	5	-	-	-	-	2	-	1	-	-	7	-	1	1	-	-	-
F. oxysporum f.sp. *lini*	18	38	74	59	83	18	35	72	67	96	8	72	59	82	11	44	75	73	92	
F. poae	-	-	-	5	2	1	3	1	-	-	1	-	-	7	-	1	3	-	7	3
F. semitectum	-	-	-	-	-	-	-	-	-	-	-	-	-	2	4	-	-	-	-	-
Grey sterile mycelium	-	-	-	-	-	-	-	-	4	6	-	-	-	-	-	-	-	-	-	-
Helminthosporium sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-
Paecilomyces fusispora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		
Papulaspora sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-
Penicillium chrysogenum	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	1	1	-	-	-
P. citrinum	5	5	-	-	-	1	4	1	4	-	17	3	-	-	4	3	1	-	-	-
P. decumbense	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. frequentans	5	4	1	1	3	-	5	6	6	-	-	5	1	4	8	8	1	-	-	-
P. granulatum	1	1	-	-	-	2	-	-	-	3	-	-	-	-	-	1	1	1	3	5
P. rubrum	-	-	-	-	-	-	-	-	-	-	-	1	-	3	3	-	-	-	5	2
P. rugulosum	-	7	1	5	1	5	3	-	-	5	6	1	3	-	15	2	3	1	1	-
P. javanicum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	-	-	-
Rhizopus nigricans	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	1	1	-	-	-
Rhizoctonia solani	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-
Scopulariopsis brumptii	-	1	1	-	-	1	2	1	1	-	-	1	-	2	3	-	-	-	-	-
Torula sp.	-	-	-	-	-	-	-	-	-	-	1	1	-	2	2	-	-	-	-	-
Trichoderma harzianum	-	-	-	-	-	-	-	-	-	-	1	2	2	-	-	1	1	-	7	-
T. viride	6	2	6	1	2	-	2	-	-	-	-	3	-	-	-	-	-	-	-	-
White sterile mycelium	3	8	-	12	9	1	4	3	3	7	-	1	7	4	3	3	2	-	5	
Yellow sterile mycelium	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3	-	-	-	-	-
Total No. of species	25	20	13	17	17	23	26	14	17	15	20	23	17	18	16	27	24	15	20	15

Average number of microbes g^{-1} dry soil

Before excision:

Soil samples	Fungi			Actinomycetes			Bacteria		
	NR	HR	WR	NR	HR	WR	NR	HR	WR
AHP	631	6162	6992	108	1408	2852	57	624	466
RWS	652	6471	8721	61	659	529	133	1647	1204
CD	604	6151	6478	86	1020	816	86	580	652
UNP	636	6440	8807	86	995	595	65	671	550
After excision:									
AHP	399	4863	6296	64	926	627	37	527	366
RWS	418	5491	7054	36	555	431	83	1262	850
CD	521	4860	5216	52	630	508	55	598	474
UNP	380	3817	7497	53	867	565	25	586	421

* - Per cent occurrence
+ - `` ''frequency
NR, Non-rhizosphere; HR, Healthy host's rhizosphere; WR, wilted host's rhizosphere; Hrp, Healthy host's rhizoplane; WRP, Wilted host's rhizoplane. AHP, Ammonium hydroxide polluted soil; RWS, Raw sewage polluted soil; CD, Cement dust polluted soil; UNP, Unpolluted soil.

plants : The microflora was isolated by soil dilution plate technique followed by Upadhyay and Rai (1982). The non-rhizosphere soil samples and the roots of linseed plants were collected from pots containing different polluted and unpolluted soil samples, as well as from the experimental field. The replicate Petri dishes for each sample and dilution were inoculated with 1 ml aliquot of the rhizosphere and non-rhizosphere soil suspension, separately for fungi, actinomycetes and bacteria. Separate sterilized pipettes were used for each dilution. The inoculated Petri dishes were incubated for each dilution. The

inoculated Petri dishes were incubated for a week at 25±1°C, 30±1°C and 37±1°C, for isolation of fungi, actinomycetes and bacteria, respectively and the colonies appeared in plates were examined and identified in case of fungi. In case of bacteria and actinomycetes, only the total number of colonies were counted.

The rhizoplane mycoflora was isolated by the method described by Harley and Waid (1955) and followed by Upadhyay and Rai (1982). Three replicate Petri-dishes each containing 20 ml Martin's agar medium in each plate were inoculated with five root bits. The plates were incubated at 23±1°C for a week and the fungi appeared were identified and recorded.

RESULTS AND DISCUSSION

The fungi per g^{-1} dry soil was found to be more in rhizosphere of healthy linseed plants as compared the rhizosphere of the wilted plants and non-rhizosphere of unpolluted and polluted soil samples. More number of fungal species was recorded in un-polluted control soil followed by raw sewage, ammonium hydroxide, and cement dust polluted soil (Table 1). The greater number of fungi in unpolluted soil, as compared to the polluted soil, may be due to presence of toxic substances in the latter. Amongst the polluted soil samples sewage soil harboured higher number of fungal species as compared to the others. Abdel Mallek *el al.* (1988) have reported that the fungal count is significantly raised due to soil treatment with low dose of sludge. Lesser number of fungi recorded in ammonium hydroxide polluted soil reflects the fungicidal nature for ammonia which has already been demonstrated for various fungi by a few workers (Gilpatrik, 1969 and Tsao and Oster, 1981). The increase in pH due to buffering capacity could have reduced microbial activity in cement dust polluted soil. The number of fungi in g^{-1} dry soil was higher in unpolluted garden soil followed by raw sewage, ammonium hydroxide and cement dust polluted soil (Table 1). The possible reason for the decrease in all the polluted soil samples may be due to influence of the pollutants on pH, moisture and aeration of soil. Physico-chemical status of soil determines the fungal population in the proximity of roots during development of the plants (Singh, 1977). The pH was found to be highest in cement dust polluted soil. The increase in pH due to the buffering capacity could reduce the microbial population in cement dust. The increase in pH could be due to accumulation of calcium and aluminium hydroxide formed during hydration of cement dust (Pajenkemp, 1961; Ozaja, 1962). The decrease in fungal population in soil varies with different moisture regimes. The per cent moisture was found to be more in ammonium hydroxide polluted soil followed by cement dust polluted soil, unpolluted garden soil and raw sewage polluted soil. The difference in moisture content attributed to the difference in soil texture. The physical characteristics of the soil are equally important as biological and chemical characteristic for pollutant attenuation (Fuller *et al.*, 1983).

The dominant species recorded from all the samples were *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, Black sterile mycelium, *Cladosporium cladosporiodes*, *Fusarium oxysporum* f. sp. *lini*, *Penicillium citrinum*, *P. frequentans*, *P. rugulosum* and white sterile mycleium (Table 1). When soil physical properties are altered by the pollutants, the organisms suited to the changed condition survive whereas, the susceptible ones disappear or become restricted. The presence of high amount of toxic substances indicated that complets destruction of the microflora is rarely possible.

Aspergillus flavus, *A. fumigatus*, *A. niger*, *Cladosporium cladosporiodes*, *Fusarium oxysporum* f. sp. *lini* were isolated as dominant species from all the soil samples of rhizosphere of healthy and wilted plants (Table, 1) Restriction of some fungi to various habitats in rhizosphere of healthy and wilted hosts was recorded as under : *Aspergillus terreus*, *Penicillum frequentans*, *P. rugulosum*, *Scopulariopsis* sp. and *Trichoderma viride* in ammonium hydroxide polluted soil, but only *P. citrinum* in rhizosphere of healthy plants : *Curvularia lunata*, *P. citrinum* *P. rugulosum* and *Scopulariopsis* sp. in raw sewage polluted soil : *Penicillium frequentans* in cement dust polltued soil and *Penicillium granulatum* and *P. rugulosum* in unpolluted sample (Table 1). *Fusarium oxysporum* f. sp. *lini* was always recorded in the rhizosphere region of wilted plants due to which possibly population of other fungi got suppressed.

A marked difference in the succession of fungi in rhizosphere and rhizoplane of excised healthy and wilted linseed roots was observed in all the soil samples in the early stages. Upadhyay and Rai (1982) have also reported difference in succession of fungi in the rhizosphere and rhizoplane regions of excised healthy and wilted pigeon pea roots. *Aspergillus nidulans*, *Penicillium granulatum* and *P. rugulosum*

were recorded as the primary colonizers on the decying roots in unpolluted soil samples; *Alternaria tenuissima, Cephalosporium roseo-griseum, Caldosporium herbarum* and *Curvularia lunata* in raw sewage; *Aspergillus nidulans, Penicillium citrinum, P. freqentans* and *P. rugulosum* in ammonium hydroxide and *A. terreus, F. semitetum, P. chrysogenum* and *Torula* sp. in cement dust polluted soil. The primary colonizers were followed by *Curvularia lunata, Scopulariopsis brumptii* and *Trichocderma harzianum* in unpolluted soil : *Cephalosporium roseogriseum, Macrophomina* sp., *Mortierella subtilissima* and *Pythium* sp. in raw sewage polluted soil: *Cunninghamella echinulata, Phoma* sp. and *Spicaria simplicissima* in ammonium hydroxide polluted soil and *Curvularia lunata, P. chrysogenum, scopulariopsis brumpti* and *Trichoderma harzianum* in cement dust polluted soil. *Aspergillus flavus, A. fumigatus, C. cladosporioides* and *F. oxysporum* f. sp. *lini* were recorded as the dominant species on decaying roots at all the stages of colonization in all the soil samples. Waid (1974), while reviewing the coloization and succession of fungi on decaying roots, pointed out that dead roots can act as reservoir of infection for soil-borne plant pathogens. The effect of concentration of organic chemicals on their biodegradation by natural microbial communities were observed by Boethling and Alexander (1979). Metal laden sewage sludge affected soil microbial population for nutrient cycling (Larry and George, 1982). Plant residues are major source of energy and nutrient for use by heterotrophic microorganisms in soil and affect biological, chemical and physical properties of the soil.

The population of *F. oxysporum* f. sp. *lini* was recorded higher in rhizosphere and rhizoplane of wilted host. The large population confined to the rhizosphere of wilted plants than that of the healthy plants may be due to the dominance of *F. oxysporum* f. sp. *lini* in the former because after wilting the pathogen enters into a saprophytic phase forming large number of conidia. Waid (1974) pointed out that dead roots can act as reservoir of infection for soil-borne pathogens. The population of *F. oxysporum* f. sp.*lini* was found to be more in unpolluted control soil compared to the polluted samples. This might be partly due to change in texture of the soil and partly due to presence of some inhibitory substances in the polluted soil samples.

The bacteria g^{-1} dry soil was found to be more in the rhizosphere of healthy and wilted plants than in non-rhizosphere in case of raw sewage polluted soil (Table 1). The highest number of bacterial colonies were recorded from the rhizosphere of healthy plants compared to the wilted ones from raw sewage polluted soil. Bacteriological characteristics of soil and water was reported by Fayes *et al.*, (1988). The increase in bacterial population in raw sewage irrigated soil as compared to other soil samples may be due to presence of more organic matter and humus in the former. The actinomycetes g^{-1} dry soil was recorded highest in case of ammonium hydroxide polluted soil (Table 1). The reason for lesser number of bacterial population in this soil sample may be due to large number of actinomycetes which could have suppressed them.

A significant variation ($p = 0.01/0.05$) in the population of fungi, bacteria and actinomycetes was found in relation to the association i.e. non-rhizosphere and rhizosphere of healthy and wilted plants.

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