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Soil mycofloral responses following the exposure to 2, 4-D

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Abstract: *2, 4-Dichlorophenoxyacetic acid (2,4-D) is a post emergence herbicide. The tests were conducted to study the toxicity of 2,4-D using EC_{50} value in four dilutions. 2,4-D was applied at concentration of 25, 50, 75, 100, 200 and 300 mg l^{-1} in the potato Dextrose agar medium. The effect of this herbicide was evaluated as the colony forming unit (CFU). EC_{50} value for 10^{-3} dilution of soil was 138±5.944 mg l^{-1}. Soil physico-chemical parameters and mycofloral properties were also evaluated. Qualitatively 10 genera of fungi were observed in untreated soil, whereas 4 genera were found in 2, 4-D administered dose.*

Key words: *2, 4-D, EC_{50}, Mycofloral responses, Soil properties*
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Introduction

Microbial degradation of herbicides in soils is a function of three key variables**.** The ability of the microorganisms to degrade the pesticides, the quantity of these microorganisms in the soil, and the activity of the soil microbial enzyme system (Anderson, 1984). Herbicides when applied to the soil for the control of weed in crop fields have enormous effects on microbial activities. 2, 4-Dichlorophenoxyacetic acid (2, 4-D) is a member of phenoxy acid herbicides, which is used because of its effectiveness against certain weed species. Rosas and Storani (1987) reported that chemicals that interfere with the growth and activity of soil organisms might influence nutrient cycling, energy flow and other related fungal mediated processes. Frioni (1981), Schinner *et al.* (1983), Magu and Bhowmik (1984) and Sarnaik *et al.* (2006) studied the effects of phenoxy acid herbicide application on soil microorganisms and microbial activities in soil. Crecchio *et al.* (2001) investigated the impact of two herbicides on soil microorganisms at 70% moisture content of the field capacity and observed that some microorganisms can utilize herbicide as a carbon source. Nowak and Michalcewicz (1995) used LD_{50} values to evaluate the effect of herbicides on the soil mycoflora. Michael *et al.* (2005) observed toxicity (LC_{50}) of selected pesticides (benomyl, chlorothalonil, copper sulfate, dimethoate, glyphosate and aminomethylphosphonic acid) and solvents to the vesicular-arbuscular mycorrhizal (VAM) fungus, *Glomus intraradices*. Hasan and Abdel Satar (2000) studied the responses of soil fungi and sorghum with different concentrations of Linuron. Staddon *et al.* (2001) measured microbial activity of a vegetative buffer strip soil and studied the degradation and sorption of metchlor. Picton and Farenhorst (2004) studied the 2,4-D sorption and mineralization rates in five soils as influenced by soil characteristics and nutrient contents and observed that 2,4-D was weakly sorbed by soil. Herbicide sorption generally increased with increasing soil organic carbon content, but the extent of 2,4-D sorption per unit organic carbon varied among the soils due to differences in soil pH, clay content and organic matter quality. Herbicide mineralization rates were greater in soils that sorbed more 2,4-D per unit organic carbon and that had greater soil nitrogen contents.

The purpose of the present investigation was to assess the EC_{50} concentration of 2,4-D and its influence on mycoflora proportions of soil.

Materials and Methods

Soil and site: The soil used in experiment was sandy loam and it was collected from cultivated land near Kanya Gurukul Mahavidyalaya, Haridwar. Soil was not treated with any kind of herbicide. Soil was collected from the top 5 to 20 cm layers and sieved through a 0.5 mm screen. All the soil parameters were analyzed in the laboratory following Trivedy and Goel (1996).

Physico-chemical analysis: The soil was analyzed for some selected physico-chemical parameters. The soil temperature was measured using a mercury thermometer. Soil pH and electrical conductivity were measured in a 1:5 ratio of soil, water extract after shaking it for 30 min. Moisture content was measured on a dry weight basis. Alkalinity was measured as a capacity of soil extract (1:5 ratio) to neutralize a strong acid 0.1N HCl. Sulphate was determined in 1:5 soil extract by spectrophotometrically. Organic matter was analyzed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). Total nitrogen was determined by Kjeldahl (1883). Sodium and potassium were determined in ammonium acetate leachate (1N) following flame photometric method (Jackson, 1958). Cation exchange capacity was also determined as exchangeable sodium in ammonium acetate solution after repetitive washing with sodium acetate solution. Available phosphorus was extracted using 0.002 N H_2SO_4 and the concentration was determined by spectrophotometer.

Mycofloral analysis: For mycofloral analysis, serial dilution agar plating technique was adopted. Soil dilutions taken were 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. In this method potato dextrose agar medium was used for the culture of mycofloral proportions. Colony forming unit g^{-1} of soil was counted after 4-7 days.

For evaluating the EC_{50}, colony forming units ml^{-1} were counted after supplementing pre-sterilized PDA medium with known concentrations of 2,4-D, as 25, 50, 75, 100, 200 and 300 mg of an active ingredient/litre. 1ml of each soil dilution was pipetted out and spread over on a culture plate. The plates were then incubated at 28±2°C for 4-7 days and CFUs were counted. PDA was also supplemented with streptomycin, to suppress the bacterial activity. Mortality percentage of fungal cells were measured by using the formula

$$\% \text{ Mortality} = \frac{C_0 - C_l}{C_0} X100$$

Where

C_0 = Colonies formed ml^{-1} in control (Untreated sample)

C_l = Colonies formed ml^{-1} in pesticide treated sample.

EC_{50} dose of 2, 4-D was estimated following Probit method (Finney, 1971).

Results and Discussion

Physico-chemical and mycological parameters of soil: The mean values of physico-chemical parameters in present study are shown in Table 1 and mycological parameters are given in Tables 2 and 3. The micro-organisms are greatly affected by physical and chemical conditions of their environment. Soil is considered to be the most dynamic site of biological interactions. Microbial population and activity in soil can be regulated by soil's physico-chemical characters (Tiwari *et al*., 1987).

In the present study, temperature was 32±0.54°C. Temperature is one of the most important environmental factor affecting the growth and survival of organisms. Mishra (1965) observed that in summer months the fungal populations in soil decrease drastically, but it suddenly increases just after the onset of rain in June and July.

Alkalinity value during the study was observed as 0.808±0.043, meq/100 gm, which is comparatively low. Agarwal (1984) worked on mycoflora in usar soil and found that the soil with lower alkalinity contained large number of mycoflora and their number was decreased with progressive increases in alkalinity. Thus the present results are in accordance with the findings of Agarwal (1984). The pH of soil was slightly alkaline with value of 7.37±0.039. In the present study, soil procured from cultivated land was having only 1.3745±0.071% organic matter, 0.2861±0.006% total nitrogen, 0.2516±0.039% available phosphorus, 0.0474±0.005% potassium and 0.0059±0.006% sodium values that were not sufficient for the fungal growth and therefore sparse fungal colonies were observed (Table 2). Conductivity of the soil was 0.0459±0.003 mhoScm^{-1}, which is the manifestation of organic matter. Jha *et al.* (1991) observed a positive correlation between organic matter percent and fungal populations. High concentration of nutrients (N, P and K) is also due the presence of organic layer rich in mineral elements (Joshi *et al*., 1991).

Table - 1: Values of some selected physico-chemical characteristics of soil (Mean ±S.E. for ten observations each)

Parameters	Values
Temperature (°C)	32.00±0.547
Moisture (%)	3.00±0.519
pH	7.37±0.039
Conductivity (mhoS cm^{-1})	0.045±0.003
Alkalinity (meq/100 g)	0.808±0.043
Cation exchange capacity (meq / 100 g)	12.586±0.701
Organic matter %	1.3745±0.071
Total nitrogen %	0.2861±0.006
Sodium %	0.0059±0.006
Potassium %	0.0474±0.005
Sulphate %	0.0173±0.0021
Available phosphorus %	0.2516±0.039

In the present study, four dilutions *viz.*, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were taken of which the 10^{-3} dilution was most concentrated. In this dilution, *Mucor* sp was dominated, accounting 78.8% of total colony count. *Fusarium* sp, *Penicillium* sp and *Aspergillus* sp were also observed, contributing 7.31, 5.45 and 5.06% respectively, while *Verticillium* sp and *Cunnighmella* sp contributed only 1.16 and 0.134% respectively.

Table - 2: Percentage of different fungal species in control soil and with EC_{50} dose

Name of fungi	Control				EC_{50} dose			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Mucor sp	78.80	33.30	5.20	9.09	-	-	-	-
Aspergillus sp	5.06	14.34	40.16	30.30	84.52	55.55	-	33.33
Fusarium sp	7.31	12.07	12.98	4.16	1.96	-	-	-
Penicillium sp	5.45	22.42	20.68	14.84	12.50	11.11	-	-
Cunnighmella sp	0.31	-	-	-	-	-	-	-
Verticillium sp	1.16	3.41	3.47	4.16	-	-	-	-
Gliocladium sp	-	6.50	6.31	6.43	1.015	-	-	1.25
Alternaria sp	-	7.16	3.88	3.59	-	-	-	-
Helminthosporium sp	-	0.64	3.81	1.19	-	-	-	-
Curvularia sp	-	-	3.47	1.19	-	-	-	-

Table - 3: Total number of colony forming unit (CFU/mg) of fungal species of control soil and EC_{50} dose

Soil dilution	No. of colonies in control	No. of colonies in EC_{50} dose
10^{-3}	44.6±2.95	11.30±3.17
10^{-4}	8.40±1.13	1.66±1.00
10^{-5}	5.00±0.99	-
10^{-6}	3.40±1.00	0.33±0.003

In 10^{-4} dilution *Mucor* sp was also predominant contributing about 33.33%. *Penicillium* sp, *Aspergillus* sp, *Fusarium* sp, *Altrnaria* sp, *Gliocladium* sp, *Verticillium* sp and *Helminthosporium* sp contributed 22.42, 14.34, 12.07, 7.16, 6.50, 3.41 and 0.64% respectively.

In 10^{-5} dilution, *Aspergillus* sp was found dominant contributing about 40.16% followed by *Penicillium* sp (20.68%), *Fusarium* sp (12.98%), *Gliocladium* sp (6.31%), *Mucor* sp (5.20%), *Alternaria* sp (3.88%), *Helminthosporium* sp (3.81%). Percent of *Verticillium* sp and *Curvularia* sp were found to be least (3.47%).

In 10^{-6} dilution, *Aspergillus* sp was dominant contributing 30.30% followed by *Penicillium* sp (14.84%), *Mucor* sp (9.09%), *Gliocladium* sp (6.43%), *Fusarium* sp (4.16%) and *Verticillium* sp (4.16%) respectively. Delayed occurence of species of *Alternaria, Helminthosporium* and *Curvularia* contributing 3.59, 1.19 and 1.19% respectively was observed.

Effect of EC_{50} concentration of 2,4-D on soil mycoflora: In present study, the EC_{50} of 2,4-D was evaluated for 10-$^{-3}$ dilution. On the basis of estimated EC_{50} value mycofloral responses in the 10^{-4}, 10^{-5} and 10^{-6} were also observed. EC_{50} value for 10^{-3} dilution was 138.00±1.297 mg l^{-1} for the total colony-forming unit. Table 2 shows the fungal propagules (10^{-3} g^{-1} soil) in control soil and EC_{50} dose of 2, 4-D.

After application of EC_{50} dose to each dilution varied mycofloral responses were observed. 74.58, 80.15, 100 and 90.19% inhibition of mycofloral proportions were observed in 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions. In all three dilutions *Aspergillus* sp was dominant.

In 10^{-3} dilution, *Aspergillus* sp was dominant among the other dilutions. Whereas, *Penicillium* sp was 12.50%, *Fusarium* sp 1.96% and *Gliocladium* sp contributing 1.01% that was the least amount as compared to the 10^{-6} dilution.

In the 10^{-4} dilution, *Aspergillus* sp was also dominant contributing about 55.55%, in which only two species of *Aspergillus viz., A. tamari* (38.88%) and *A. ocharaceous* (16.66%) were present. Whereas, *Penicillium* sp contributing only 11.11%, that was least amount than 10^{-3} dilution.

In 10^{-5} dilution, there was no growth of any fungus. It showed 100% mortality after application of EC_{50} dose. In 10^{-6} dilution only one species of *Aspergillus i.e. A. candidus* (33.33%) and *Gliocladium* sp (1.25%) was present.

Adeleye *et al.* (2004) observed the effect of three herbicides namely, Agroxone, Atranex 50SC and 2,4-D amine on *Azotobacter vinelandii, Rhizobium phaseoli* and *Bacillus subtilis*. The results revealed that 2,4-D amine was the most toxic of the three herbicides studied and *Azotobacter vinelandii* was found to be most sensitive to the herbicide. There was a reduction in LC_{50} of herbicides with increased number of days. The percentage survival decreased with increased concentration of herbicides. Audus (1951) observed that phenoxy acid herbicides showed preliminary latent period before the soil become enriched with adopted microorganisms. He found that some microorganisms could grow on a substrate of 2,4-D as the sole carbon source, and its inoculation into new soil with 2,4-D resulted in an immediate breakdown of the herbicide. The similar effects were also observed in the present study. *Aspergillus* sp utilized 2, 4-D as a carbon source. Shaw and Burns (2004) observed enhanced biodegradation and mineralization in the rhizosphere of *Latifolium perenne* and *Trifolium paratense* for mineralization kinetics of 2,4-D. They observed that in non-planted soil there was significant reduction in lag phase and maximum mineralization rate was found for 25-60 days. Faulkner and Woodcock (1965) observed that *Aspergillus niger* first hydroxylate 2, 4-D in the 5th position on the phenyl ring. Clifford *et al.* (1964) also studied that phenoxy-acetic acid converted into a mixture of the 2, 3 and 4 hydroxy isomers. Faulkner and Woodcock (1961) observed that *Aspergillus niger* was capable of hydrolytic dechlorination. Abdel Fattah *et al.* (1983) investigated the toxic impact of two triazine herbicides on Egyptian soil fungi and found that it promoted the counts of total *Aspergillus* sp whereas the mycelium growth of *Alternaria alternata, Trichoderma viride, Myrothecium verrucaria, Cunninghamella echinulata, Gliocladium roseum* and *Penicillium verruculosum* was significantly suppressed**.** Omar (1998) identified thirteen fungal species isolated from pesticides treated soil and observed their ability to mineralize and degrade three organophosphate insecticides as a p source. All fungal species grew successfully on the culture media treated with the three used doses of insecticides (10, 50 and 100 ppm active ingredient) but the growth rate varied with the species. Out of thirteen fungal species, only some *Aspergillus* species *viz., A. niger, A. tamarii, A. terreus* and *Trichoderma harzianum* utilized pesticides as a nutrient source. In the present study *Fusarium* sp might be suppressed due to antagonistic effect of other fungal genera with 2,4-D. Suppression of *Fusarium udum* was observed with bavistain, dithane, difolatan, 2,4-D and machete by Rai and Upadhyaya (1983). They also observed that *Fusarium* was highly suppressed by antagonism from *Penicillium citrinum, A. niger, A. flavus, A. terrus* and *Trichoderma.* Zabaloy and Gomez (2005) studied the effect of five commonly used herbicides (2,4-D and glyphosate, dicamba, atrazine and metsulfuron-methyl) on the growth of rhizobial strains and observed that 2,4-D and glyphosate in solid medium inhibited and diminished the growth respectively in slow growing rhizobial strains. There was no strain which could use 2,4-D as sole C source.

The results of this study showed that the 2, 4-D has toxic effect on soil mycoflora. In contrast to the all-fungal suppression, only *Aspergillus* sp showed enhancement at higher concentration of herbicide, whereas, the *Mucor* sp was suppressed by higher concentration of 2, 4-D. Since *Aspergillus* sp utilized this herbicide as a carbon source, this fungus can be used as a bio agent for the remediation of pesticide.

References

Abdel Fattah, H.M., M.I.A. Abdel-Kader and S. Hamida: Selective effects of two triazine herbicides on Egyptian soil fungi. *Mycopathologia (Historical Archive)*, **82**, 143-151 (1983).

Adeleye, I.A., E. Okorodudu and O. Lawal: Effect of herbicides used in Nigeria on *Rhizobium phaseoli, Azotobacter vinelandii* and *Bacillus subtilis*. *J. Environ. Biol.,* **25**, 151-156 (2004).

Agarwal, S.C.: Microbial ecology of usar soils with special reference to fungal flora. *In*: Progress in microbial ecology (*Eds.*: K.G. Mukerjii, V.P. Agnihotori and R.P. Singh). Print House, Lucknow, India (1984).

Anderson, J.P.E.: Herbicide degradation in soil. *In:* Influence of microbial biomass. *Soil. Biol. Biochem.,***16**, 483-489 (1984)**.**

Audus, L.J.: The biological detoxification of 2,4-D in soils. Isolation of an effective organism. *Nature,* **166**, 356 (1951).

Clifford, D.R. and D. Woodcock: Metabolism of phenoxyacetic acid by *Aspergillus niger* van Tiegh. *Nature,* **203**, 763 (1964).

Crecchio, C., M. Curci, M., Pizzigallo, P. Ricciuti and P. Ruggiero: Molecular approaches to investigate herbicide-induced bacterial community changes in soil microcosms. *Biol. Fertil. Soils,* **33**, 460-466 (2001).

Faulkner, J.K. and D. Woodcock: Fungal detoxification. Pt.V. Metabolism of o- and p-chlorophenoxy acetic acids by *Aspergillus niger. J. Chem. Soc.,* **Part IV**, 5397-5400 (1961).

Faulkner, J.K. and D. Woodcock: Fungal detoxification. Pt.VII. Metabolism of 2,4-dichlorophenoxy acetic and 4 chloro-2 methylphenoxy acetic acids by *Aspergillus niger. J. Chem. Soc.,* **Part I**, 1187-1191 (1965).

Finney, D.J.: Probit Analysis. 3rd Edn. Cambridge Univ. Press, London and New York (1971).

Frioni, L.: Effect of atrazine, linuron and 2, 4-D amine on various biological properties of a soil sample. I - Field trial. *Rev. Argentina Microbiology,* **13**, 1-8 (1981).

Hasan, H.A.H. and M.A. Abdel Sater: Responses of mycoflora and sorghum to pre-planting soil incorporation with linuron herbicide. *Rostlinna – Vyroba,* **46**, 417-422 (2000).

Jackson, M.L.: Soil Chemical Analysis. Prentic-Hall Inc. Englewood cliffs, N.J. USA (1958).

Jha, D.K., G.D. Sharma and R.R. Mishra: Ecology of soil microflora and mycorrhizal symbionts in degraded forests at two altitudes. *Biol. Fertil. Soils,* **12**, 1- 7 (1991).

Joshi, S.R., M. Chauhan, G.D. Sharma and R.R. Mishra: Effect of deforestation on microbes, VAM fungi and their enzymatic activity in eastern Himalaya. Recwent res. *Ecol. Environ. Pollut.,* **6**, 141-152 (1991).

Kjeldahl, J.: A new method for the determination of nitrogen in organic matter. *J. Anal. Chem.,* **22**, 366 (1883).

Magu, S.P. and S. Bhowmik: Effect of 2 methyl-4-chlorophenocybutyric acid (MCPB) and disyston on legume-rhizobium symbiosis and rhizosphere microflora. *Zentralbl, Mikrobiol.,* **139**, 633-641 (1984).

Michael, W.T., R.E. James and W.G. Ronald: A new technique for determining the sublethal toxicity of pesticides to the vesicular-arbuscular mycorrhizal fungus, *Glomus intraradices*. *Environ. Toxicol. Chem.,* **17**, 1421-1428 (2005).

Mishra, R.R.: Seasonal distribution of fungi in four different grass consociations of Varansi. *Tropical Ecology,* **6**, 133-140 (1965).

Mishra, R.R.: Studies on ecological factors governing distribution of soil mycoflora. *Proc. Natn. Acad. Sci. India,* **36**, 205-222 (1966).

Nowak, A. and W. Michalcewicz: An attempt to use LD_{50} evaluates effects of herbicide on soil microflora. *Acta Microbiologica Polonica*, **44**, 277-283 (1995).

Omar, S.A.: Availability of phosphorus and sulfur of insecticide origin by fungi. *Biodegradation,* **9**, 327-336 (1998).

Picton, P. and A. Farenhorst: Factors influencing 2,4-D sorption and mineralization in soil. Part B-Pesticides food contaminants and agricultural wastes. *J. Environ. Sci. Hlth*., **39**, 367-379 (2004).

Rai, B. and R.S. Upadhyay**:** Competitive saprophytic colonization of pigeon-pea substrate by *Fusarium udum* in relation to environmental factors, chemical treatments and microbial antagonism. *Soil. Biol. Biochem.,* **15**, 187-191 (1983).

Rosas, S.M. and M.M.C. de Storani: The action of pesticides on microorganisms. I: Parathion. *Toxicity Assess.,* **2**, 293-303 (1987).

Sarnaik, S.S., P.P. kanekar, V.M. Raul, S.P. Taware, K.S. Chavan and B.J. Bhadbhade: Effect of application of different pesticides to soybean on the soil microflora. *J. Environ. Biol.,* **27**, 423-426 (2006).

Schinner, F., H. Bayer and M. Mitterer: The influence of herbicides on microbial activity in soil materials. *Bodenkultur,* **34**, 22-30 (1983).

Shaw, L. and R.G. Burns: Enhanced mineralization of ^{14}C 2,4-dichlorophenoxyacetic acid in soil from the rhizosphere of *Trifolium pretense*. *Appl. Environ. Microbiol.,* **70**, 4766-4774 (2004).

Staddon, W.J., M.A. Locke and R.M. Zoblatowicz**:** Microbiological characteristics of a vegetative buffer strip soil. *Am. J. Soil Sci. Soc.,* **65**, 1136-1142 (2001).

Tiwari, S.C., B.K. Tiwari and R.R. Mishra: The influence of moisture regimes on the population and activity of soil microorganisms. *Plant Soil,* **101**, 133-136 (1987).

Trivedy, R.K. and P.K. Goyal: Chemical and biological methods for water pollution studies. 3rd Edn., Environmental Publications or Aligarh, India (1996).

Walkey, A. and I.A. Black: An examination of the Degtjareff method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci.*, **37**, 29-38 (1934).

Zabaloy, M.C. and M.A. Gomez: Diversity of rhizobia isolated from an agricultural soil in Argentina based on carbon utilization and effects of herbicides on growth. *Biol. Fertil. Soils*, **42**, 83-88 (2005).