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Effect of Herbicides on Soil Microorganisms

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ABSTRACT

Herbicides being biologically active compounds, an unintended consequence of the application of herbicides is that it may lead to significant changes in the populations of microorganisms and their activities thereby influencing the microbial ecological balance in the soil and affecting the productivity of soils. The increasing reliance of rice cultivation on herbicides has led to concern about their ecotoxicological behaviour in the rice field environment. Hence, in this study, the herbicides viz., 2,4-DEE, butachlor, pretilachlor and pyrazosulfuron ethyl were evaluated at different concentrations of 1 FR (Field rate), 2 FR (two times field rate), 5 FR, 10 FR and 100 FR for their effect on total heterotrophic bacteria, fungi and actinomycetes in laboratory microcosms. The results of this experiment revealed that the application of herbicides reduced the population of all the bacteria counted during the study with butachlor showing highest reduction in the populations. This effect was stronger with increasing concentration of the herbicides employed. However, the populations at 1 FR (and also 2 FR for pyrazosulfuron ethyl) concentrations recovered within 30 days to reach populations not significantly different from the control treatments.

Key words : Bacteria, fungi, actinomycetes, 2, 4-DEE, butachlor, pretilachlor, pyrazosulfuron ethyl

INTRODUCTION

The herbicide consumption in India is expected to increase dramatically in future as the use of herbicides has been expanding more rapidly than that of the other pesticides (Bhan and Mishra, 2001). Herbicide usage, which was earlier confined to plantation crops, has now expanded to crops like wheat and rice which constitute about 42 and 30% of the total consumption of herbicides, respectively (Yadaraju and Mishra, 2002). The addition of herbicides can cause qualitative and quantitative alterations in the soil microbial populations and their enzyme activities (Min *et al*., 2002; Saeki and Toyota, 2004). Herbicide application may also kill species of bacteria, fungi and protozoa that combat disease causing microorganisms, thereby upsetting the balance of pathogens and beneficial organisms and allowing the opportunist, disease causing organisms to become a problem (Kalia and Gupta, 2004). In addition, change in the soil microflora has been listed as one of the possible causes of productivity decline in rice cropping systems (Reichardt *et al*., 1998). Thus there is a need to study the influence of herbicides on the microflora and their activities in flooded soils.

With this background, the present investigation was carried out with a view to understand the effect of herbicides viz., 2,4-D-2ethylhexyl ester (2,4-DEE), butachlor, pretilachlor and pyrazosulfuron ethyl on rice soil microorganisms for their judicious use.

MATERIALS AND METHODS

Soil Microcosms

Soil with no prior exposure to herbicides was obtained from wetlands of TNAU, Coimbatore. Air-dried and powdered soil (2 mm sieve) was placed in 500 ml beakers in portions equivalent to 250 g oven dry weight, and pre-incubated under flooded conditions at 30±1^{o}C for three days for conditioning. Appropriate quantities of the herbicide formulations were added to the soil to maintain concentrations of herbicides at control, FR (Field rate), 2 FR (2 x Field rate), 5 FR (5 x field rate), 10 FR (10 x field rate) and 100 FR. Soil without herbicide application was also maintained as control. The field rates of application for different herbicides were 0.75 kg/ha for 2,4-DEE, 1.0 kg/ha for butachlor, 0.30 kg/ha for pretilachlor and 25 g/ha for pyrazosulfuron ethyl. A 3 cm depth of overlying water was maintained in all the treatments. The treated soils were then covered with plastic sheets having small holes and incubated at 30±1^{o}C in the dark for 30 days. For sampling the soil at different intervals, the surface soil was collected from a depth of 0-3 cm (Saeki and Toyota, 2004). Samples were drawn at 0, 7, 15 and 30 days after application of herbicides and analysed for the effect of herbicides on soil microbial populations.

Count of Microbial Population in Herbicide Treated Soil

The population of total heterotrophic bacteria, fungi and actinomycetes was counted using serial dilution and plating technique. Nutrient agar medium (Allen, 1953) was used for count of total heterotrophic bacteria. The population of fungi was estimated on Martin's Rose Bengal agar medium with 1.25 g of streptomycin and 0.033 g of Rose Bengal in a litre of the medium (Martin, 1950). Actinomycetes population was counted using Kuster's agar (Kuster and Williams, 1964) medium. After allowing for development of discrete bacterial colonies during incubations under suitable conditions, the colonies were counted and the number of viable bacteria [expressed as colony forming units (CFU)] per gram dry weight of soil was estimated by taking into account the soil dilutions. All the data obtained from the above experiments were subjected to statistical analysis as per the method detailed by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Effect of Herbicides on Total Heterotrophic Bacteria, Fungi and Actinomycetes

The population of total heterotrophic bacteria, fungi and actinomycetes counted after application of herbicides is presented in Tables 1-3.

The population of bacteria was found to be significantly influenced by the type of herbicides, concentrations and the days after application of herbicides (Table 1). Among the herbicides, butachlor application significantly reduced the population of total heterotrophic bacteria (7.445 log CFU/g soil) compared to the herbicides pyrazosulfuron ethyl (7.546 log CFU/g soil) and pretilachlor (7.537 log CFU/g soil). The population was also found to be significantly influenced by the concentration of the herbicides used. The bacterial population with herbicides applied at 1 FR (7.533 log CFU/g soil) and 2 FR (7.521 log CFU/g soil) was significantly higher compared to 5 (7.504 log CFU/g soil), 10 (7.458 log CFU/g soil) and 100 (7.403 log CFU/g soil) times of the recommended rates. Significant differences were also observed in the bacterial population counted at different days after the application of herbicides. The bacterial population at 7 and 30 days after herbicide application was significantly higher (7.506 and 7.504 log CFU/g soil respectively) than the population at 15 days after herbicide application (7.484 log CFU/g soil). The herbicide x concentration interaction was significant for bacterial population, while the interactive effect of herbicide x days, days x concentration and the three-way interaction were non-significant.

The herbicides and their different concentrations affected the fungal population but no significant differences were observed in fungal population counted at different days after herbicide application (Table 2). Pyrazosulfuron ethyl (5.872 log CFU/g soil) and pretilachlor (5.864 log CFU/g soil) were found to support higher population than 2,4-DEE (5.846 log CFU/g soil) and butachlor (5.848 log CFU/g soil). Though the highest population was observed in control (4.907 log CFU/g soil), significantly higher population was also observed at concentrations of 1 FR, 2 FR and 5 FR (4.888, 4.886 and 4.883 log CFU/g soil, respectively) than 10 FR (4.871 log CFU/g soil) and 100 FR (4.863 log CFU/g soil). No significant interaction effect was observed for the fungal population.

The influence exerted by herbicides at different concentrations and at different days after application on the actinomycetes population is presented in Table 3. Among the herbicides, butachlor applied soils had significantly lower populations (4.015 log CFU/g soil) than pyrazosulfuron ethyl (4.162 log CFU/g soil), pretilachlor (4.148 log CFU/g soil) and 2,4-DEE (4.080 log CFU/g soil). The concentrations of herbicides significantly influenced actinomycetes population with the highest concentration (100 FR) recording the lowest population (3.914 log CFU/g soil). The highest population was recorded in the control treatment (4.235 log CFU/g soil) followed by that of 1 FR (4.159 log CFU/g soil) and 2 FR (4.144 log CFU/g soil). The population of actinomycetes at different days after herbicide application showed significant differences, with the highest population being recorded at 30 days (4.134 log CFU/g soil) and the lowest at seven days after herbicide application (4.063 log CFU/g soil). The interaction of herbicide x concentration and the herbicide x days was found to significantly affect the actinomycetes population. Higher population (4.185 log CFU/g soil) recorded with pyrazosulfuron ethyl at 1 FR concentration was

Table 1. Population of total heterotrophic bacteria as influenced by various herbicides under laboratory conditions

Herbicide conc.	Population of total heterotrophic bacteria (x 10^6 CFU/g dry soil)																Mean
	Days after 2,4-DEE application				Days after butachlor application				Days after pretilachlor application				Days after pyrazosulfuron ethyl application				
	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	
1 FR	33.62 (7.527)	31.00 (7.491)	35.92 (7.555)	33.51 (7.524)	31.62 (7.500)	29.63 (7.471)	34.99 (7.544)	31.32 (7.495)	34.42 (7.537)	35.94 (7.556)	36.92 (7.568)	35.76 (7.554)	35.24 (7.547)	36.42 (7.562)	37.00 (7.568)	36.22 (7.559)	34.20 (7.533)
2 FR	32.46 (7.511)	30.33 (7.482)	33.71 (7.528)	32.16 (7.507)	30.00 (7.477)	27.56 (7.440)	32.42 (7.511)	29.99 (7.476)	33.96 (7.531)	35.06 (7.545)	36.45 (7.562)	35.15 (7.546)	35.10 (7.545)	36.00 (7.556)	36.94 (7.568)	36.01 (7.556)	33.33 (7.521)
5 FR	30.28 (7.481)	28.00 (7.448)	31.66 (7.501)	29.98 (7.477)	29.36 (7.468)	26.00 (7.415)	30.00 (7.477)	28.45 (7.453)	32.64 (7.514)	34.01 (7.532)	35.84 (7.555)	34.16 (7.534)	34.60 (7.539)	35.62 (7.552)	36.53 (7.563)	35.58 (7.551)	32.04 (7.504)
10 FR	29.61 (7.471)	23.23 (7.366)	24.01 (7.380)	25.61 (7.406)	28.00 (7.447)	21.00 (7.322)	20.42 (7.310)	23.14 (7.360)	31.62 (7.500)	33.86 (7.530)	35.14 (7.546)	33.54 (7.525)	33.64 (7.527)	34.85 (7.542)	36.01 (7.556)	34.83 (7.542)	29.28 (7.458)
100 FR	26.24 (7.419)	20.33 (7.308)	19.00 (7.279)	21.85 (7.335)	23.60 (7.373)	19.00 (7.279)	18.25 (7.262)	20.28 (7.305)	28.16 (7.450)	30.42 (7.483)	32.45 (7.511)	30.34 (7.481)	29.33 (7.467)	30.12 (7.479)	33.46 (7.525)	30.97 (7.490)	25.86 (7.403)
Control	38.96 (7.591)	37.83 (7.578)	37.12 (7.570)	37.97 (7.580)	38.97 (7.591)	37.84 (7.578)	37.14 (7.570)	37.98 (7.580)	38.95 (7.591)	37.82 (7.578)	37.13 (7.570)	37.96 (7.580)	38.94 (7.590)	37.85 (7.578)	37.15 (7.570)	37.98 (7.579)	37.97 (7.580)
Mean	31.86 (7.500)	28.45 (7.446)	30.23 (7.469)	30.18 (7.471)	30.25 (7.476)	26.67 (7.415)	28.65 (7.443)	28.52 (7.445)	33.29 (7.521)	34.51 (7.537)	35.65 (7.552)	34.48 (7.537)	34.47 (7.536)	35.14 (7.545)	36.18 (7.558)	35.26 (7.546)	32.11 (7.500)

Factors	LSD (P=0.05)	Factors	LSD (P=0.05)	Factors	LSD (P=0.05)
Herbicides (H)	0.017	H x C	0.042	H x C x D	NS
Concentration (C)	0.028	H x D	0.030		
Days (D)	0.149	D x C	0.037		

Values in parentheses are log_{10} transformed. Initial population of bacteria before herbicide application : 39.40 x 10^6 CFU/g dry soil. NS–Not Significant.

Table 2. Population of fungi as influenced by various herbicides under laboratory conditions

Herbicide conc.	Population of fungi (x 10^4 CFU/g dry soil)																Mean
	Days after 2,4-DEE application				Days after butachlor application				Days after pretilachlor application				Days after pyrazosulfuron ethyl application				
	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	
1 FR	71.36	73.15	75.16	73.22	70.46	72.64	74.98	72.69	73.04	74.06	75.32	74.14	73.21	74.82	75.98	74.67	73.68
	(5.854)	(5.864)	(5.876)	(5.865)	(5.848)	(5.861)	(5.874)	(5.861)	(5.864)	(5.871)	(5.877)	(5.871)	(5.867)	(5.876)	(5.881)	(5.875)	(4.888)
2 FR	71.10	72.64	74.82	72.85	70.14	71.48	74.16	71.93	72.98	73.74	75.15	73.96	73.19	74.45	75.64	74.43	73.29
	(5.852)	(5.861)	(5.874)	(5.862)	(5.846)	(5.855)	(5.870)	(5.857)	(5.863)	(5.868)	(5.876)	(5.869)	(5.865)	(5.872)	(5.879)	(5.872)	(4.886)
5 FR	70.42	71.34	73.64	71.80	69.82	70.86	72.88	71.19	72.16	73.00	74.16	73.11	73.02	74.20	75.14	74.12	72.55
	(5.848)	(5.854)	(5.867)	(5.856)	(5.844)	(5.851)	(5.863)	(5.853)	(5.858)	(5.865)	(5.870)	(5.864)	(5.865)	(5.872)	(5.876)	(5.871)	(4.883)
10 FR	69.86	70.66	71.49	70.67	67.64	65.42	65.98	66.35	69.02	70.12	72.04	70.39	72.68	73.21	74.78	73.56	70.24
	(5.845)	(5.849)	(5.854)	(5.849)	(5.831)	(5.816)	(5.820)	(5.822)	(5.839)	(5.846)	(5.858)	(5.848)	(5.865)	(5.866)	(5.874)	(5.868)	(4.871)
100 FR	67.24	65.42	64.06	65.57	65.36	64.39	63.66	64.47	68.46	68.98	70.62	69.35	69.82	71.23	73.62	71.56	67.74
	(5.828)	(5.816)	(5.807)	(5.817)	(5.816)	(5.809)	(5.804)	(5.810)	(5.836)	(5.839)	(5.849)	(5.841)	(5.846)	(5.853)	(5.867)	(5.855)	(4.863)
Control	77.66	76.82	76.16	76.88	77.65	76.81	76.16	76.87	77.67	76.82	76.15	77.48	77.65	76.82	76.17	76.88	77.03
	(5.891)	(5.886)	(5.882)	(5.886)	(5.891)	(5.886)	(5.882)	(5.887)	(5.890)	(5.886)	(5.882)	(5.889)	(5.894)	(5.887)	(5.882)	(5.888)	(4.907)
Mean	71.27	71.67	72.56	71.83	70.18	70.27	71.30	70.58	72.22	73.09	73.91	73.07	73.26	74.12	75.22	74.20	72.42
	(5.853)	(5.855)	(5.860)	(5.856)	(5.846)	(5.846)	(5.853)	(5.848)	(5.858)	(5.864)	(5.869)	(5.864)	(5.867)	(5.871)	(5.877)	(5.872)	(4.883)

Factors	LSD (P=0.05)	Factors	LSD (P=0.05)	Factors	LSD (P=0.05)
Herbicides (H)	0.017	H x C	NS	H x C x D	NS
Concentration (C)	0.021	H x D	NS		
Days (D)	NS	D x C	NS		

Values in parentheses are $\log_{10}$ transformed. Initial population of fungi before herbicide application : 78.21 x 10^4 CFU/g dry soil. NS–Not Significant.

Table 3. Population of actinomycetes as influenced by various herbicides under laboratory conditions

Herbicide conc.	Population of actinomycetes (x 10^3 CFU/g dry soil)																Mean
	Days after 2,4-DEE application				Days after butachlor application				Days after pretilachlor application				Days after pyrazosulfuron ethyl application				
	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	
1 FR	12.01	14.46	15.94	14.14	11.26	13.86	15.63	13.58	13.42	15.00	16.36	14.93	14.00	15.62	16.42	15.35	14.50
	(4.080)	(4.160)	(4.203)	(4.148)	(4.052)	(4.142)	(4.195)	(4.130)	(4.129)	(4.177)	(4.214)	(4.173)	(4.146)	(4.194)	(4.215)	(4.185)	(4.159)
2 FR	11.98	13.90	15.06	13.65	10.58	12.98	14.92	12.83	13.01	14.96	15.92	14.63	13.66	15.04	16.16	14.95	14.01
	(4.079)	(4.143)	(4.178)	(4.133)	(4.025)	(4.113)	(4.175)	(4.104)	(4.115)	(4.176)	(4.202)	(4.164)	(4.135)	(4.177)	(4.208)	(4.173)	(4.144)
5 FR	10.32	12.22	14.36	12.30	9.32	11.65	14.00	11.66	12.64	14.15	15.18	13.99	13.00	14.56	15.22	14.26	13.05
	(4.014)	(4.087)	(4.157)	(4.086)	(3.970)	(4.066)	(4.148)	(4.061)	(4.103)	(4.152)	(4.181)	(4.145)	(4.114)	(4.163)	(4.182)	(4.153)	(4.111)
10 FR	9.86	10.53	13.28	11.22	7.65	7.42	8.54	7.87	11.12	13.04	14.64	12.93	11.33	13.94	15.02	13.43	11.36
	(3.994)	(4.023)	(4.123)	(4.047)	(3.884)	(3.869)	(3.933)	(3.895)	(4.047)	(4.116)	(4.166)	(4.110)	(4.054)	(4.144)	(4.176)	(4.125)	(4.044)
100 FR	7.54	6.21	6.54	6.76	5.42	4.32	4.16	4.63	9.62	11.62	13.61	11.62	10.62	13.00	14.56	12.73	8.94
	(3.878)	(3.793)	(3.816)	(3.829)	(3.734)	(3.635)	(3.620)	(3.663)	(3.985)	(4.066)	(4.134)	(4.062)	(4.026)	(4.114)	(4.163)	(4.101)	(3.914)
Control	17.89	17.02	16.64	17.18	17.88	17.02	16.65	17.18	17.90	17.03	16.63	17.19	17.89	17.01	16.64	17.18	17.18
	(4.253)	(4.231)	(4.221)	(4.235)	(4.252)	(4.229)	(4.222)	(4.234)	(4.254)	(4.232)	(4.221)	(4.236)	(4.252)	(4.230)	(4.221)	(4.234)	(4.235)
Mean	11.60	12.39	13.64	12.54	10.35	11.21	12.32	11.29	12.95	14.30	15.39	14.21	13.42	14.86	15.67	14.65	13.17
	(4.050)	(4.073)	(4.116)	(4.080)	(3.986)	(4.009)	(4.049)	(4.015)	(4.106)	(4.153)	(4.186)	(4.148)	(4.121)	(4.170)	(4.194)	(4.162)	(4.101)

Factors	LSD (P=0.05)	Factors	LSD (P=0.05)	Factors	LSD (P=0.05)
Herbicides (H)	0.023	H x C	0.054	H x C x D	NS
Concentration (C)	0.271	H x D	NS		
Days (D)	0.191	D x C	0.047		

Values in parentheses are $\log_{10}$ transformed. Initial population of actinomycetes before herbicide application : 18.30 x 10^3 CFU/g dry soil. NS–Not Significant.

comparable to the population recorded in control treatments, while the lowest population (3.663 log CFU/ g soil) was recorded in the treatment that received butachlor at 100 FR concentrations. The three-way interaction was found to be non-significant for actinomycetes population.

Deshmukh and Srikhande (1974) observed that 2,4-DEE at field application rate did not exert any effect on bacteria, fungi, actinomycetes after 40 days of application. Similar effect was also seen in the 1 FR treatment of 2, 4-DEE in the present experiment wherein the microbial population after 30 days of application was analogous to the control treatment. As was observed in the current study, butachlor at 5.5, 11 and 22 μg/g soil showed temporary inhibition of aerobic heterotrophic bacteria within the early period of eight days followed by a recovery during the later period in paddy soil (Min *et al*., 2002).

Pretilachlor at 0.45 kg/ha was not observed to appreciably affect the soil microbial communities (Murato *et al*., 2004). In the present study, it was observed that pretilachlor at 0.15 μg/g soil did not show differences in microbial population, when compared to control treatment after 30 days of incubation. Few studies are available on the effects of pyrazosulfuron ethyl on soil microorganisms, but studies with bensulfuron methyl–a sulfonyl urea herbicide by Gigliotti *et al*. (1998) have revealed transient effects on soil microflora, similar to the effects of pyrazosulfuron ethyl in this study.

The monitoring period is a most important part for the assessment of pesticide effects and a minimum of 30 days has been recommended for the recognition of persistent effects on soils. A delay of 30 days in the restitution of normality (recovery period) after herbicide application should be considered normal with ecological consequences being negligible, a delay of 60 days is not unusual, and the ecological consequences are tolerable and a delay of greater than 60 days is unusual with ecological consequences which may eventually be critical (Domsch *et al*., 1983).

Butachlor, among the herbicides was more inhibitory to microbial population (7.85 to 34.20% reduction over control) when compared to 2,4-DEE, pretilachlor and pyrazosulfuron ethyl. Among the various concentrations tested, the 100 FR treatment recorded a reduction of 11.57 to 47.96% over control treatment for soil microbial population.

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