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Effect of herbicides on fluorescent pseudomonads and spore forming bacilli

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The herbicides *viz.*, 2,4-DEE, butachlor, pretilachlor and pyrazosulfuron ethyl were evaluated at different concentrations of 1FR (Field rate), 2FR, 5FR, 10FR and 100FR for their effect on microbial population (Fluorescent pseudomonads, spore forming bacilli) and enzyme activities (β-glucosidase, protease, alkaline phosphatase and dehydrogenase) in laboratory microcosms. The result of the experiment revealed that butachlor, among the herbicides was more inhibitory to microbial population (7.85 to 34.20% reduction over control) and soil enzyme activities (5.03 to 19.11 % reduction over control) when compared to 2,4-DEE, pretilachlor and pyrazosulfuron ethyl. The different concentrations of herbicides also exerted significant influence on the population of bacilli. The highest population of bacilli was observed in control (5.799 log CFU g^{-1} soil) followed by treatments receiving 1FR (5.786 log CFU g^{-1} soil) and 2FR (5.781 log CFU g^{-1} soil). concentrations of herbicides. The lowest population was recorded with the 100 FR treatments (5.743 log CFU g^{-1} soil). Significant differences were also observed in the population of bacilli due to the interactive influence of herbicides and concentrations. The 1FR treatments of all herbicides recorded higher population comparable to the control, while butachlor at 100FR recorded the lowest population of 5.707 log CFU g^{-1} soil.

Key words : Fluorescent pseudomonads, Herbicides, Spore forming bacilli

INTRODUCTION

The present day agriculture depends upon high yielding varieties, inorganic fertilizers and pesticides to achieve the increased food production required to keep pace with the increasing population. The progressive modernization of irrigated rice cultivation in India, using the above technologies has led to tremendous increase in rice production, which has more than doubled over the last 35 years, mainly driven by 85% increase in productivity (Kumar, 2006).

Since the herbicides are used when the crop is either absent as pre-emergence or at its early stage of growth as post-emergence, a high proportion of the herbicide reaches the soil and accumulates in the microbiologically active top layer of 0-15 cm soil. Herbicides being biologically active compounds, an unintended consequence of the application of herbicides could influence the microbial ecological balance in the soil leading to significant changes in the populations of microorganisms and their activities and affecting the productivity of soils (Boldt and Jacobsen, 1998).

The increase in food production, till date, had come at the cost of the environment with both qualitative and quantitative degradation of land, water and bioresources (Sarkar and Ghosh, 2001). Hence, it has been advocated that, in the future, any increase in production should be obtained with practices that maintain or enhance the quality of the environment and that the environmental security should no longer be peripheral to the food and nutritional security. With this background, the present investigation was carried out to understand and predict the effect of herbicides *viz*., 2,4-D-2ethylhexyl ester (2,4-DEE), butachlor, pretilachlor and pyrazosulfuron ethyl on rice soil microorganisms and their activities, which could lead to their judicious use and thereby reduce their negative effects, if any on the environment.

MATERIALS AND METHODS

A laboratory incubation experiment was conducted using field soil obtained from wetlands of TNAU, Coimbatore, by devising microcosms to study the effect of different concentrations of herbicide formulations on cultivable microflora and potential enzyme activities.

Soil microcosms:

Soil obtained from wetlands of TNAU, Coimbatore was air dried and passed through 2 mm sieve. The soil in portions equivalent to 250 g oven dry weight was placed

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in 500 ml beakers, adjusted to the required level of moisture in flooded condition and pre incubated at 30 ± 1^{0}C for 3 days for conditioning. Appropriate quantities of the herbicide formulations were added to the soil to maintain concentrations of herbicides at control, FR (Field rate), 2FR (2 x Field rate), 5FR (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 ai. ha^{-1} for 2,4-DEE, 1.0 kg ai. ha^{-1} for butachlor, 0.30 kg ai. ha^{-1} for pretilachlor and 25g ai. ha^{-1} for pyrazosulfuron ethyl. Water was adjusted to the same levels in all the treatments including control. 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^{0}C in the dark for 30 days.

For sampling the soil at different intervals, overlying water was carefully removed and the surface soil was collected from a depth of 0-3cm using a spatula (Saeki and Toyata, 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 and enzyme activities. The population of fluorescent pseudomonads, spore forming bacilli were enumerated using serial dilution and plating technique (Parkinson *et al.,* 1971).

Statistical analyses :

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

The herbicides and the different concentrations caused significant differences in the fluorescent pseudomonad numbers as evidenced in Table 1. The results revealed that pyrazosulfuron ethyl (6.481 log CFU g^{-1} soil) and pretilachlor (6.471 log CFU g^{-1} soil) sustained higher population of pseudomonads compared to 2,4-DEE (6.447 log CFU g^{-1} soil) and butachlor (6.404 log CFU g^{-1} soil). Among the different concentrations, control and 1FR concentrations were found to sustain comparably higher populations (6.504 and 6.472 log CFU g^{-1} soil, respectively). The lowest population was observed at 100FR concentrations (6.376 log CFU g^{-1} soil). Significant interactive effect observed between herbicides and concentration revealed that control and the 1FR concentration of all herbicides recorded higher populations compared to 100FR concentrations. Butachlor at 100 FR concentrations was found to sustain the lowest population (6.247 log CFU g^{-1} soil). Non significant effects were observed for the effect of days, herbicide x days and days x concentration two way interactions and three way interaction on fluorescent pseudomonad population.

The population of spore forming bacilli was enumerated at different days after application of herbicides at different concentrations (Table 2). Significant differential response of bacilli population was observed towards herbicide, concentration and days of application. Significantly higher population was observed with pyrazosulfuron ethyl (5.784 log CFU g^{-1} soil) and pretilachlor (5.779 log CFU g^{-1} soil) than 2,4-DEE (5.770 log CFU g^{-1} soil) and butachlor (5.761 log CFU g^{-1} soil). The different concentrations of herbicides also exerted significant influence on the population of bacilli. The highest population of bacilli was observed in control (5.799 log CFU g^{-1} soil) followed by treatments receiving 1FR (5.786 log CFU g^{-1} soil) and 2FR (5.781 log CFU g^{-1} soil) concentrations of herbicides. The lowest population was recorded with the 100 FR treatments (5.743 log CFU g^{-1} soil). Significant differences were also observed in the population of bacilli due to the interactive influence of herbicides and concentrations. The 1FR treatments of all herbicides recorded higher population comparable to the control, while butachlor at 100FR recorded the lowest population of 5.707 log CFU g^{-1} soil.

Weeds are the major biological constraint in most rice growing areas of the world. Unlike the periodic outbreaks of insect pests and plant diseases, weeds are ever present and threatening. Problems associated with weeds in rice cultivation are mounting dramatically in South and Southeast Asia because of the reduced availability of affordable labour. The lack of suitable weed control alternatives has led to the increasing reliance on herbicides in many rice growing areas and their use is increasing as they are less expensive and convenient than manual labour, very effective and easy to use. In India, herbicide consumption is expected to increase in future as the use of herbicides has been expanding more rapidly than that of the other pesticides (Bhan and Mishra, 2001) used in pest and disease control.

Though many studies have been conducted on the effects of various sulfonyl urea herbicides on soil microorganisms, there are few studies available on the effects of pyrazosulfuron ethyl on soil microorganisms. Many studies have reported transient effects, similar to the effects of pyrazosulfuron ethyl in this study, on soil microflora by different sulfonyl urea herbicides. In this study, pyrazosulfuron ethyl had the least inhibitory effect on the microbial population when compared to the other herbicides. Aerobic bacteria, nitrogen fixing bacteria and

Table 1: Population of fluorescent pseudomonads as influenced by various herbicides under laboratory conditions

Herbicide concentration	Population of fluorescent pseudomonads (x 10^5 CFU g^{-1} dry soil)																
	Days after 2,4-DEE application				Days after butachlor application				Days after pretilachlor application				Days after pyrazosulfuron ethyl application				Mean
	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	
1FR	27.32	29.54	31.16	29.34	25.12	28.82	30.14	28.03	29.72	30.26	31.24	30.41	30.38	30.92	31.40	30.90	29.67
	(6.437)	(6.470)	(6.494)	(6.467)	(6.400)	(6.460)	(6.479)	(6.446)	(6.473)	(6.481)	(6.495)	(6.483)	(6.483)	(6.490)	(6.497)	(6.490)	(6.472)
2FR	27.30	29.12	30.45	28.96	24.84	27.98	29.64	27.49	29.16	29.98	30.84	29.99	30.03	30.26	31.00	30.43	29.22
	(6.436)	(6.464)	(6.484)	(6.461)	(6.395)	(6.447)	(6.472)	(6.438)	(6.465)	(6.477)	(6.489)	(6.477)	(6.478)	(6.481)	(6.491)	(6.483)	(6.465)
5FR	26.32	28.68	29.84	28.28	25.64	26.42	28.32	26.79	28.32	29.12	30.56	29.33	29.70	30.15	30.92	30.26	28.67
	(6.420)	(6.458)	(6.475)	(6.451)	(6.409)	(6.422)	(6.452)	(6.428)	(6.452)	(6.464)	(6.485)	(6.467)	(6.473)	(6.479)	(6.490)	(6.481)	(6.457)
10FR	25.86	27.42	27.98	27.09	23.00	22.84	22.62	22.82	27.65	28.63	29.42	28.57	29.53	29.62	30.45	29.87	27.09
	(6.413)	(6.438)	(6.447)	(6.433)	(6.362)	(6.359)	(6.354)	(6.358)	(6.442)	(6.457)	(6.469)	(6.456)	(6.470)	(6.472)	(6.484)	(6.475)	(6.431)
100FR	20.42	25.64	24.32	23.46	18.64	17.42	16.89	17.65	26.00	27.54	28.98	27.51	27.07	28.42	29.36	28.28	24.23
	(6.310)	(6.409)	(6.386)	(6.368)	(6.271)	(6.241)	(6.228)	(6.247)	(6.415)	(6.440)	(6.462)	(6.439)	(6.433)	(6.454)	(6.468)	(6.452)	(6.376)
Control	32.14	31.94	31.76	31.95	32.13	31.96	31.75	31.95	32.15	31.93	31.77	31.95	32.12	31.95	31.74	31.94	31.95
	(6.507)	(6.504)	(6.502)	(6.504)	(6.507)	(6.505)	(6.502)	(6.505)	(6.507)	(6.504)	(6.502)	(6.504)	(6.507)	(6.504)	(6.502)	(6.504)	(6.504)
Mean	26.56	28.72	29.25	28.18	24.90	25.91	26.56	25.79	28.83	29.58	30.47	29.63	29.81	30.22	30.81	30.28	28.47
	(6.421)	(6.457)	(6.465)	(6.447)	(6.391)	(6.406)	(6.415)	(6.404)	(6.459)	(6.471)	(6.484)	(6.471)	(6.474)	(6.480)	(6.489)	(6.481)	(6.451)

Factors	CD (P=0.05)	Factors	CD (P=0.05)	Factors	CD (P=0.05)
Herbicides (H)	0.029	HXC	0.072	HXCXD	NS
Concentration (C)	0.036	HXD	NS		
Days (D)	NS	DXC	NS		

Values in parenthesis are $\log_{10}$ transformed; Initial population of fluorescent pseudomonads before herbicide application: 32.68 x 10^5 CFU g^{-1} dry soil

NS-Non-significant

Table 2: Population of spore forming bacilli as influenced by various herbicides under laboratory conditions

Herbicide concentration	Population of spore forming bacilli (x 10^4 CFU g^{-1} dry soil)																
	Days after 2,4-DEE application				Days after butachlor application				Days after pretilachlor application				Days after pyrazosulfuron ethyl application				Mean
	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	7	15	30	Mean	
1FR	59.84	60.92	61.96	60.91	59.34	60.75	61.72	60.60	60.42	61.16	62.28	61.29	61.38	61.96	62.42	61.92	61.18
	(5.777)	(5.785)	(5.792)	(5.785)	(5.773)	(5.784)	(5.790)	(5.782)	(5.781)	(5.786)	(5.794)	(5.787)	(5.788)	(5.792)	(5.795)	(5.792)	(5.786)
2FR	58.54	60.28	61.42	60.08	58.02	60.15	61.00	59.72	59.36	60.82	61.84	60.67	60.24	61.04	62.00	61.09	60.39
	(5.767)	(5.780)	(5.788)	(5.778)	(5.764)	(5.779)	(5.785)	(5.776)	(5.773)	(5.784)	(5.791)	(5.783)	(5.780)	(5.786)	(5.792)	(5.786)	(5.781)
5FR	56.42	58.67	60.48	58.52	55.89	57.64	59.62	57.72	58.48	59.86	60.86	59.73	59.64	60.84	61.96	60.81	59.20
	(5.751)	(5.768)	(5.782)	(5.767)	(5.747)	(5.761)	(5.775)	(5.761)	(5.767)	(5.777)	(5.784)	(5.776)	(5.776)	(5.784)	(5.792)	(5.784)	(5.772)
10FR	54.64	56.26	58.26	56.39	54.26	55.65	56.28	55.40	57.32	59.36	60.16	58.95	58.76	60.02	60.82	59.87	57.65
	(5.738)	(5.750)	(5.765)	(5.751)	(5.734)	(5.745)	(5.750)	(5.743)	(5.758)	(5.773)	(5.779)	(5.770)	(5.769)	(5.778)	(5.784)	(5.777)	(5.760)
100FR	52.32	54.85	56.64	54.60	51.62	50.12	50.88	50.87	55.37	57.54	59.00	57.30	56.42	58.94	59.84	58.40	55.30
	(5.719)	(5.747)	(5.753)	(5.740)	(5.713)	(5.700)	(5.707)	(5.707)	(5.743)	(5.760)	(5.771)	(5.758)	(5.751)	(5.770)	(5.777)	(5.766)	(5.743)
Control	63.42	62.94	62.53	62.96	63.44	62.93	62.55	62.97	63.42	62.94	62.54	62.97	63.43	62.92	62.52	62.96	62.97
	(5.802)	(5.799)	(5.796)	(5.799)	(5.802)	(5.799)	(5.796)	(5.799)	(5.802)	(5.799)	(5.796)	(5.799)	(5.802)	(5.799)	(5.796)	(5.799)	(5.799)
Mean	57.53	58.99	60.22	58.91	57.10	57.87	58.68	57.88	59.06	60.28	61.11	60.15	59.98	60.95	61.59	60.84	59.45
	(5.759)	(5.772)	(5.779)	(5.770)	(5.756)	(5.761)	(5.767)	(5.761)	(5.771)	(5.780)	(5.786)	(5.779)	(5.778)	(5.785)	(5.789)	(5.784)	(5.774)

Factors	CD (0.05)	Factors	CD (0.05)	Factors	CD (0.05)
Herbicides (H)	0.008	HXC	0.019	HXCXD	NS
Concentration (C)	0.009	HXD	NS		
Days (D)	0.007	DXC	NS		

Values in parenthesis are log_{10} transformed; Initial population of spore forming bacilli before herbicide application: 63.87 x 10^4 CFU g^{-1} dry soil
NS – Non-significant

nitrifying bacteria were not decreased by the application of bensulfuron methyl a sulfonyl urea herbicide even at 10FR rates of application (Gigliotti *et al*., 1998). In this study it was observed that pyrazosulfuron ethyl at 1FR concentration after 30 days of incubation did not differ significantly from the control. Boldt and Jacopsen *et al*. (1998) showed that metsulfuron methyl, chlorsulfuron and thifensulfuron had a growth reducing effect on a number of strains of fluorescent pseudomonads at concentrations of 50 and 300 µg g^{-1} soil. In the current experiment, 1FR concentration of pryrazosulfuron ethyl transiently reduced the population of fluorescent pseudomonads, but the 100FR concentration at 0.125 µg g^{-1} soil was found to reduce the population of pseudomonads significantly after 30 days of incubation.

Conclusion:

Among the herbicides tested, the soil microbial population and enzyme activity inhibition followed a trend, butachlor > 2,4-DEE > pretilachlor > pyrazosulfuron ethyl.

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