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Effect of herbicides on soil microorganisms in direct-seeded rice

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ABSTRACT

The use of herbicides in direct-seeded rice may affect the biological equilibrium of the soil and thus influence the nutrient status, health and productivity of the soil. To study the effect of herbicides on soil microbial population of direct-seeded rice, field experiment was conducted at Punjab Agricultural University, Ludhiana (Punjab) during *Kharif* 2009 and 2010. Sixteen weed control treatments, *viz*. pendimethalin 0.75, butachlor 1.50, thiobencarb 1.50, anilofos 0.375, pretilachlor 0.75, oxadiargyl 0.09 and pyrazosulfuron-ethyl 0.015 kg/ha applied as pre-emergence and each followed by bispyribac 0.025 kg/ha at 30 days after sowing; two hand weedings and unweeded control were tested. The results revealed that viable microbial population was influenced to varying degrees with different weed control treatments during both the years. The herbicides, *viz*. pendimethalin, butachlor, thiobencarb, anilofos, pretilachlor, oxadiargyl and pyrazosulfuron-ethyl as pre-emergence and bispyribac as post-emergence were safe for soil microbial populations at recommended rate.

Key words: Actinobacteria, Bacteria, Direct-seeded rice, Fungi, Herbicides, Non-target organisms

To meet the global rice demand, it is estimated that about 114 million tonnes of additional milled rice need to be produced by 2035 which is equivalent to an overall increase of 26 per cent in the next 25 years (Kumar and Ladha 2011). To sustain present food self-sufficiency and to meet future food requirements, India has to increase its rice productivity by 3 per cent per annum but the possibility of expanding the area under rice in the near future is limited. There has, however, been stagnation in rice productivity in recent years and long-term experiments showed a declining trend in rice yield. Due to receding water table, rising costs of labour for transplanting of paddy and the adverse effects of puddling on soil health; direct-seeded rice (DSR) is gaining popularity. But, weeds are the main constraint for farmers practising direct- seeding so use of herbicides both pre- and post-emergence is required for good crop. 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 (Min *et al*. 2002, Saeki and Toyota 2004) and affecting the productivity of soils. When herbicides are applied in soil, they may exert certain side effects on non-target organisms. Therefore, there has been considerable interest on the influence of herbicides on the soil microflora and microbially mediated processes. The effects of these chemicals on certain variables are associated with microbial biomass and their activity (Wardle and Parkinson 1991). The increasing reliance of rice cultivation on herbicides has led to concern about their ecotoxicological behaviour in the rice field environment. Soil health and microbial diversity have become vital issues for the sustainable agriculture. Loss of microbial biodiversity can affect the functional stability of the soil microbial community and soil health. Generally, there are some negative effects of herbicides on the population level or composition of species. The impact of applied herbicides on the soil microbial populations were studied which included analysis of bacteria, actinobacteria and fungi counts. In Punjab, seven pre-emergence herbicides are being used in direct-seeded rice for chemical weed control, therefore, this work was carried out to estimate the counts of these microbes at different period of crop growth after their application.

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MATERIALS AND METHODS

The experiment was conducted at Students' Research Farm, Department of Agronomy, PAU, Ludhiana during *Kharif* season of 2009 and 2010. Ludhiana is situated in Trans-Gangetic Agro-Climatic zone, representing the Indo-Gangetic Alluvial plain at 30°56' N latitude, 75°52' E longitude and at an altitude of 247 m above mean sea level. The meteorological data recorded during rice growing season showed that the overall weather during 2009 remained good for the rice crop but it was not as conducive for rice during 2010. The average minimum temperature during the crop growing season of 2010 was higher by 1 °C than normal. The total rainfall of 818 and 627.6

mm were received during 2009 and 2010, respectively. The soil of the experimental site was loamy sand in texture with normal soil reaction and electrical conductivity, low in organic carbon and available N and medium in available P and K. The experiment comprised of 16 weed control treatments, *viz*. alone application of pendimethalin 0.75, butachlor 1.50, thiobencarb 1.50, anilofos 0.375, pretilachlor 0.75, oxadiargyl 0.09 and pyrazosulfuron- ethyl 0.015 kg/ha as pre-emergence and with sequential application of bispyribac 0.025 kg/ha at 30 DAS; two hand weeding and un-weeded control. The experiment was laid out in randomised complete block design with three replications. Rice variety '*PAU 201*' was seeded on 6th June 2009 and 4th June 2010 with tractor drawn conventional drill using primed seed after seed treatment with seed rate of 35 kg/ha in rows spaced at 20 cm. The recommended dose of fertilizers and plant protection measures for insect-pest and disease control were applied.

The composite soil samples were taken at 2 DAS (*i.e.* 0 days after spray of pre-emergence herbicides), 30 DAS (*i.e.* 0 days after spray of post-emergence herbicides and 30 days after spray of pre-emergence herbicides), 60 and 90 DAS and at harvest. Four samples of soil under each treatment were taken from 0-15 cm soil depth and mixed so as to have a representative sample of the treatment. The viable microbial counts were analyzed by the standard technique of serial dilution and pour plating. The bacterial population was estimated by growing on soil extract agar (Allen 1957) culture media composing 1.0 g glucose, 0.5 g KH_2SO_4, 0.1g KNO_3, 100 ml soil extract, 15 g agar, and 1000 ml distilled water with pH of 6.8-7.0. The population of actinobacteria was grown on dextrose nitrate agar (Küster and Williams 1964) culture media comprising 1 g dextrose, 0.1 g KH_2PO_4, 0.1 g $NaNO_3$, 0.1 g KCl, 15 g agar, and 1000 ml distilled water with pH of 7.0-7.2. The fungal population was cultured on rose bengal agar (Martin 1950) culture media having 10 g dextrose, 5 g peptone, 1 g K_2HPO_4, 0.05 g $MgSO_4.7H_20$, 0.033 g rose bengal, 15 g agar, and 1000 ml distilled water with pH of 5.5. After allowing for development of discrete bacterial colonies during incubations under suitable conditions, the colonies were counted and the number of viable bacteria, fungi and actinobacteria [expressed as colony forming units (cfu)] per gram dry weight of soil was estimated by taking into account the soil dilutions. Statistical significance of the treatment effects on different parameters was determined for the least significant difference (LSD) at 5% level of significance using analysis of variance for a randomised complete block design (Cochran and Cox 1957). Normality, homogeneity of variance, and interactions of treatments and years were tested. Interactions among years were significant; therefore, data were presented separately for each year.

RESULTS AND DISCUSSION

There was seasonal variation found in the microbial population observed at different periodical observation as most of the rainfall was received during the vegetative period. At 0 days after spray, statistically similar viable microbial counts were found in different pre-emergence herbicides and control (Table 1). At 30 days after spray, microbial count was not affected by different weed control treatments (Table 2). The herbicides and their degradation products generally take some time to accumulate in the soil and then affect the soil microflora. Actinobacteria were less affected as compared to bacteria and fungi. Actinobacteria are reported relatively resistant to herbicides and get affected at high concentration only as reported by Sondhia (2008). Raut *et al.* (1997) found that except for a slight initial suppressing effect for 0-3 days, butachlor 1.5 kg/ha stimulated the microbial activity of rice rhizosphere and increased significantly in 30 days.

The preceding trend followed when the observations on the microbial population was taken at 60 days after spray (Table 3). The microbial populations were not significantly affected by different weed control treatments. The microbial populations in the herbicide treated plots were more or less similar to the control plots (unweeded and hand weeding) thus indicating that herbicides have no detrimental effect on soil health at the applied doses. Roger (1995) also concluded that microbial activities were more sensitive to pesticides than population densities. Also, pesticide degradation in rice fields was favoured by high temperatures which usually stabilize in range favouring high microbial activity and further accelerated by organic matter incorporation. Chen *et al.* (2009) reported that microbial activity was suppressed shortly after butachlor application but was augmented after 37 days in both upper and lower soils. Min *et al.* (2001) reported that number of actinobacteria declined significantly after the application of butachlor at different concentrations ranging from 5.5 µg to 22.0 µg/g dried soil, while that of bacteria and fungi increased. Latha and Gopal (2010) reported that 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 field rate (FR) and also 2 FR for pyrazosulfuron-ethyl concentrations recovered within 30 days to reach populations not significantly different from the control treatments.

Table 1. Effect of weed control treatments on microbial population of soil at 2 DAS* in direct-seeded rice

Treatment	Dose (kg/ha)	Time of application	Viable counts in soil (cfu/g) 2009 Fungi ($\times10^3$)	2009 Actinobacteria ($\times10^4$)	2009 Bacteria ($\times10^6$)	2009 Soil moisture (%)	2010 Fungi ($\times10^3$)	2010 Actinobacteria ($\times10^4$)	2010 Bacteria ($\times10^6$)	2010 Soil moisture (%)
Pendimethalin	0.75	PE	23.2	16.1	20.7	14.3	17.9	19.0	16.9	12.9
Butachlor	1.50	PE	17.8	16.0	17.2	15.3	18.6	20.8	16.5	13.2
Thiobencarb	1.50	PE	22.1	16.9	18.2	18.2	18.0	21.7	14.3	15.2
Anilofos	0.375	PE	21.0	14.6	19.8	17.8	16.9	20.8	16.9	10.7
Pretilachlor	0.75	PE	24.0	14.0	20.2	12.9	18.8	19.3	15.6	11.1
Oxadiargyl	0.09	PE	17.1	14.7	17.5	14.5	18.3	20.7	14.7	12.2
Pyrazosulfuron-ethyl	0.015	PE	20.0	14.3	17.7	13.7	14.9	19.1	13.4	17.6
Unsprayed	-	-	15.5	13.9	15.8	16.1	17.2	19.7	16.6	18.8
LSD(P=0.05)			NS	NS	NS	-	NS	NS	NS	-

*2 DAS means 0 day after spray of pre-emergence herbicides, PE = Pre-emergence

Table 2. Effect of weed control treatments on microbial population of soil at 30 DAS* in direct-seeded rice

Treatment	Dose (kg/ha)	Time of application	Viable counts in soil (cfu/g) 2009 Fungi ($\times10^3$)	2009 Actinobacteria ($\times10^4$)	2009 Bacteria ($\times10^6$)	2009 Soil moisture (%)	2010 Fungi ($\times10^3$)	2010 Actinobacteria ($\times10^4$)	2010 Bacteria ($\times10^6$)	2010 Soil moisture (%)
Pendimethalin	0.75	PE	16.8	19.9	13.0	10.7	13.1	19.3	15.6	11.8
Pendimethalin *fb* bispyribac	0.75 *fb* 0.025	PE *fb*at 30 DAS	18.8	22.4	13.2	12.9	11.7	18.2	17.0	11.5
Butachlor	1.50	PE	16.8	21.8	11.2	13.4	16.1	19.0	12.8	11.7
Butachlor *fb* bispyribac	1.50 *fb* 0.025	PE*fb*at 30 DAS	19.9	21.1	14.8	12.4	14.5	21.3	12.7	11.5
Thiobencarb	1.50	PE	21.2	20.4	12.9	14.1	15.3	18.2	14.2	14.4
Thiobencarb *fb* bispyribac	1.50 *fb* 0.025	PE*fb* at 30 DAS	24.3	22.5	11.5	15.0	12.4	16.6	13.3	12.5
Anilofos	0.375	PE	20.2	18.3	12.4	13.4	11.3	18.0	13.6	12.8
Anilofos *fb* bispyribac	0.375 *fb* 0.025	*fb*at 30 DAS	19.0	20.8	14.6	16.1	15.9	21.0	13.3	15.8
Pretilachlor	0.75	PE	16.7	23.7	14.7	13.1	14.7	20.9	12.6	14.1
Pretilachlor *fb* bispyribac	0.75*fb* 0.025	PE *fb*at 30 DAS	16.2	22.0	12.9	14.9	12.6	18.7	14.6	9.5
Oxadiargyl	0.09	PE	21.8	19.4	13.4	11.6	14.4	17.0	15.7	12.9
Oxadiargyl*fb* bispyribac	0.09 *fb* 0.025	PE*fb*at 30 DAS	21.9	22.7	13.0	14.7	13.4	21.6	12.5	17.3
Pyrazosulfuron-ethyl	0.015	PE	22.8	18.7	14.1	14.9	12.8	19.3	15.0	13.7
Pyrazosulfuron-ethyl *fb* bispyribac	0.015 *fb* 0.025	PE*fb*at 30 DAS	15.6	19.0	11.9	14.8	12.0	18.1	14.6	11.1
Two hand weeding	-	25 and 45 DAS	17.0	18.3	10.9	12.6	14.3	18.2	14.0	11.4
Unweeded	-	-	18.1	11.7	13.7	13.5	14.5	17.8	13.0	13.0
LSD(P=0.05)			NS	NS	NS	-	NS	NS	NS	-

*30 DAS *i.e.* 0 day after spray of post-emergence herbicide

The counts of fungi and actinobacteria were significantly affected by different herbicides at 90 days after spray whereas that of bacteria remained unaffected (Table 4). Among different weed control treatments, there were significantly lower counts of fungi, actinobacteria and bacteria in the unsprayed and in two hand weeding. Significantly higher microbial populations in the herbicidal treatments at all stages of observation might be due to healthy and conducive environment for the microorganisms as compared to the control. No particular pattern of the microbial counts was observed among weed control treatments but the microbial counts were significantly lower in control plots. It may be concluded that there was increase in the biological properties of the soil in well aerated aerobic soil conditions found in direct seeded rice hence might be ascribed to the improvement in the nutrient status as well as physical conditions of the soil which resulted in better growth of the microorganisms. It could be further inferred that the microbial population started to regain after the weeds were also killed by the herbicides and got mixed in the soil during this period and these might have served to increase the nutrients. The degradation of herbicides may be serving as carbon source for growth of microbes.

Table 3. Effect of weed control treatments on microbial population of soil at 60 DAS in direct-seeded rice

Treatment	Dose (kg/ha)	Time of application	Viable counts in soil (cfu/g) 2009				2010			
			Fungi (×10^3)	Actinobacteria (×10^4)	Bacteria (×10^6)	Soil moisture (%)	Fungi (×10^3)	Actinobacteria (×10^4)	Bacteria (×10^6)	Soil moisture (%)
Pendimethalin	0.75	PE	19.8	36.9	21.9	9.4	14.0	12.6	14.1	15.6
Pendimethalin *fb* bispyribac	0.75 *fb* 0.025	PE *fb* at 30 DAS	19.6	32.9	22.7	9.7	12.2	13.0	13.4	18.2
Butachlor	1.50	PE	20.7	33.1	17.3	8.7	13.7	12.6	14.0	18.5
Butachlor *fb* bispyribac	1.50 *fb* 0.025	PE *fb* at 30 DAS	18.5	25.1	17.1	11.9	12.5	13.7	13.0	17.0
Thiobencarb	1.50	PE	22.4	34.3	19.0	10.7	13.0	11.8	13.7	17.9
Thiobencarb *fb* bispyribac	1.50 *fb* 0.025	PE *fb* at 30 DAS	20.5	33.1	21.2	10.7	10.1	11.6	13.7	16.8
Anilofos	0.375	PE	17.0	29.1	20.4	12.2	12.3	11.4	13.9	17.8
Anilofos *fb* bispyribac	0.375 *fb* 0.025	PE *fb* at 30 DAS	19.2	42.0	21.5	14.6	13.6	13.9	14.2	17.7
Pretilachlor	0.75	PE	21.6	30.2	16.9	30.0	12.8	11.5	13.3	18.9
Pretilachlor *fb* bispyribac	0.75 *fb* 0.025	*PE fb* at 30 DAS	20.9	30.4	16.2	10.6	12.3	11.0	13.8	17.5
Oxadiargyl	0.09	PE	21.7	36.1	18.3	14.8	10.6	11.5	14.4	17.4
Oxadiargyl *fb* bispyribac	0.09 *fb* 0.025	PE *fb* at 30 DAS	21.8	36.5	16.8	9.8	12.6	12.2	13.8	17.8
Pyrazosulfuron-ethyl	0.015	PE	18.3	30.8	16.9	10.8	11.6	11.6	15.3	18.7
Pyrazosulfuron-ethyl *fb* bispyribac	0.015 *fb* 0.025	PE *fb* at 30 DAS	17.9	28.5	19.9	11.2	11.4	10.7	13.3	19.7
Two hand weeding	-	25 and 45 DAS	19.1	32.6	19.7	11.6	11.5	10.9	15.0	18.2
Unweeded	-	-	17.8	30.2	16.2	12.6	11.5	9.7	13.4	16.1
LSD(P=0.05)			NS	NS	NS	-	NS	NS	NS	-

Table 4. Effect of weed control treatments on microbial population of soil at 90 DAS in direct-seeded rice

Treatment	Dose (kg/ha)	Time of application	Viable counts in soil (cfu/g) 2009				2010			
			Fungi (×10^3)	Actinobacteria (×10^4)	Bacteria (×10^6)	Soil moisture (%)	Fungi (×10^3)	Actinobacteria (×10^4)	Bacteria (×10^6)	Soil moisture (%)
Pendimethalin	0.75	PE	23.4	40.5	12.4	8.1	29.0	15.0	13.9	18.9
Pendimethalin *fb* bispyribac	0.75 *fb*0.025	PE *fb* at 30 DAS	23.8	38.2	12.7	11.9	26.1	14.3	14.8	17.2
Butachlor	1.50	PE	21.7	37.2	13.8	20.1	26.7	13.1	13.8	18.0
Butachlor *fb* bispyribac	1.50 *fb* 0.025	PE*fb*at 30 DAS	23.3	39.4	12.9	10.6	27.3	15.2	14.1	18.7
Thiobencarb	1.50	PE	22.8	35.3	13.6	13.7	27.4	13.3	13.3	17.4
Thiobencarb *fb* bispyribac	1.50 *fb* 0.025	PE*fb* at 30 DAS	23.5	33.5	9.8	12.6	28.8	13.7	13.4	17.7
Anilofos	0.375	PE	20.4	34.0	11.8	12.1	29.5	13.5	15.3	17.3
Anilofos *fb* bispyribac	0.375 *fb* 0.025	PE*fb* at 30 DAS	24.7	35.4	11.8	12.5	26.7	13.8	14.4	16.2
Pretilachlor	0.75	PE	21.4	39.6	12.4	16.9	27.3	15.6	11.1	17.6
Pretilachlor *fb* bispyribac	0.75*fb* 0.025	PE*fb* at 30 DAS	20.1	34.0	12.2	12.8	27.3	13.5	13.9	16.2
Oxadiargyl	0.09	PE	21.1	35.6	13.1	8.5	29.4	14.8	12.6	18.5
Oxadiargyl *fb* bispyribac	0.09 *fb* 0.025	PE*fb*at 30 DAS	23.6	35.9	13.3	13.5	29.1	13.2	13.1	14.3
Pyrazosulfuron ethyl	0.015	PE	19.7	33.6	12.8	14.4	27.8	14.6	11.1	15.2
Pyrazosulfuron ethyl *fb* bispyribac	0.015 *fb* 0.025	PE *fb*at 30 DAS	22.9	36.2	12.1	14.6	26.6	15.4	12.2	18.7
Two hand weedings	-	25 and 45 DAS	11.4	20.8	12.4	11.9	13.9	10.2	13.3	17.5
Unweeded	-	-	11.2	19.4	11.8	16.6	13.1	9.3	14.3	16.3
LSD(P=0.05)			5.1	7.7	NS	-	6.0	2.9	NS	-

The viable microbial counts were found to be statistically similar under the influence of different weed control treatments at harvest (Table 5). 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

Table 5. Effect of weed control treatments on microbial population of soil at harvest in direct-seeded rice

Treatment	Dose (kg/ha)	Time of application	Viable counts in soil (cfu/g)							
			2009				2010			
			Fungi (×10³)	Actinob-acteria (×10⁴)	Bacteria (×10⁶)	Soil moisture (%)	Fungi (×10³)	Actinob-acteria (×10⁴)	Bacteria (×10⁶)	Soil moisture (%)
Pendimethalin	0.75	PE	12.0	16.7	11.8	9.7	13.8	14.5	11.5	12.6
Pendimethalin *fb* bispyribac	0.75 *fb*0.025	PE *fb* at 30 DAS	11.6	14.7	12.9	13.5	13.1	11.5	12.1	9.8
Butachlor	1.50	PE	11.1	14.1	13.0	15.1	10.2	11.6	12.3	14.0
Butachlor *fb* bispyribac	1.50 *fb* 0.025	PE *fb* at 30 DAS	13.0	18.0	12.2	14.9	13.1	11.3	12.4	11.2
Thiobencarb	1.50	PE	10.6	16.2	12.7	12.7	10.5	10.9	13.2	9.4
Thiobencarb *fb* bispyribac	1.50 *fb* 0.025	Pefb at 30 DAS	11.3	15.9	11.5	12.1	11.2	12.1	12.1	7.9
Anilofos	0.375	PE	9.1	14.3	13.0	10.9	11.2	14.1	10.5	10.7
Anilofos *fb* bispyribac	0.375 *fb* 0.025	PE *fb* at 30 DAS	9.2	13.5	13.5	12.3	12.2	10.4	11.3	7.1
Pretilachlor	0.75	PE	11.2	13.8	11.2	13.4	12.7	10.5	11.6	12.2
Pretilachlor *fb* bispyribac	0.75 *fb* 0.025	PE *fb* at 30 DAS	9.9	11.7	11.9	11.7	11.4	10.9	12.4	10.2
Oxadiargyl	0.09	PE	12.1	12.2	11.2	10.1	12.2	11.0	13.1	7.9
Oxadiargyl *fb* bispyribac	0.09 *fb* 0.025	PE *fb* at 30 DAS	10.2	12.9	13.4	11.6	12.3	13.4	11.3	11.2
Pyrazosulfuron-ethyl	0.015	PE	14.5	13.9	11.6	9.1	11.8	12.3	11.5	10.9
Pyrazosulfuron-ethyl *fb* bispyribac	0.015 *fb* 0.025	PE *fb* at 30 DAS	11.2	10.2	11.2	13.3	12.5	10.3	12.3	10.8
Two hand weeding-	-	25 and 45 DAS	10.6	13.8	12.4	9.6	12.8	14.7	12.9	10.3
Unweeded	-	-	9.7	14.9	12.3	10.4	11.6	10.6	12.9	10.9
LSD(P=0.05)			NS	NS	NS	-	NS	NS	NS	-

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).

The microbial populations in the herbicide treated plots were more or less similar to the unsprayed control plots thus indicating that herbicides have no detrimental effect on soil health at the applied doses.

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