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Effect of long-term application of herbicides on soil microbial demography in rice-wheat cropping sequence

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ABSTRACT

Increasing reliance of present day intensive agriculture on herbicide use has led to certain concerns about their eco-toxicological effects influencing various microbial populations and associated enzymatic activities, which may serve as indicators of soil quality. The effect of herbicides (cyhalofop butyl, isoproturon, butachlor and clodinafop) on soil microbial population of beneficial and other organisms was assessed over a period after 13 years in a *Rabi* and *Kharif* season. In the present study, herbicide application resulted in transient suppression of population of beneficial microorganisms including fungi. The microbial population regained its number by the time of harvesting of crops

Key words: Beneficial microorganisms, Fungi, Herbicides, Microbial population

Soil health with special reference to biological features maintaining functions of both natural and managed ecosystems, is essential for sustainable agricultural fertility and productivity (Enriqueta-Arias *et al*. 2005). In modern day agriculture, the use of herbicides for combating weeds in crop fields has been increasing steadily. Herbicides form a principal component of weed management in crops and cropping systems in many industrialized and developed countries. These are applied to improve crop yield and quality, and to maximize economic returns. Herbicides are bioactive, toxic substances if applied non-judiciously, which directly or indirectly inûuence soil productivity as well as agro-ecosystem quality such as residual toxicity, health hazards and mammalian toxicity.

During application of herbicides, a large portion of these chemicals accumulate in the top soil layer (0–15 cm), where most of the microbiological activities occur (Alexander 1978). As microorganisms are scavengers in soil and possess physiological variability, they degrade a great variety of chemical substances including the incorporated herbicides in soil (Das and Debnath 2006). Most of the studies till date have been focused on single application for a shorter period or in a single season which may sometimes provide a realistic evaluation of the effects of herbicides on soil microorganisms (Haney *et al.* 2000) but the knowledge about the effect of herbicides on soil enzymatic activities and microbial population over long-term applications has been limited. Therefore, the present study was designed to elucidate the effects of herbicides on soil microbial population after 13 years. This may help to provide a better understanding of the possible responses of soil microorganisms when exposed to different herbicides.

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MATERIALS AND METHODS

A long-term experiment on effect of continuous and rotational use of herbicides in transplanted rice-wheat rotation under All India Coordinated Research Project on Weed Control (AICRP-WC) was initiated in 1999 in Agronomy department, College of Agriculture, CSK Himachal Pradesh Krishi Vishvavidyalay, Palampur. Geographically, the AICRP-WC centre is situated at $32^{o}62$ N latitude and $76^{o}32$ E longitude at an elevation of 1290.8 meters above mean sea level. This area represents mid hill sub-humid climatic zone of Himachal Pradesh. The mean annual rainfall of about of about 2347.4 mm was received during the last five years at Palampur with the period of June to September being the wettest months of the year.

The soil of the site was clay-loam to silty-clay-loam in texture and taxonomically classified as Alfisols. The surface soil (0-15cm) had a pH range from 5.2 to 6.0 with a medium to high status of organic carbon. The soils were medium in available N and P and high in available potassium content. The studies on effect of herbicide application on soil micro flora and soil activity was assessed after 13 years of cropping in terms of microbial population.

Table 1. Treatment details of experimental long-term effect of continuous and rotational use of herbicides in rice-wheat rotation

Treatment Rice	Wheat
T1- Farmer's practice	Farmer's practice
T2- Butachlor 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (100% N through urea)	Isoproturon 1.0 kg + 2,4-D 0.75 kg/ha
T3- Butachlor 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (100% N through urea)	Clodinafop 75 g *fb* 2,4-D 0.75 kg/ha soproturon* 1.0 kg + 2,4-D 0.75kg/ha
T4- Butachlor 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (75% N through urea and 25% N through *Lantana*)	Isoproturon 1.0 kg + 2,4-D 0.75 kg/ha
T5- Butachlor 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (75% N through urea and 25% N through *Lantana*)	Clodinafop 75 g + 2,4-D 0.75 kg/ha soproturon* 1.0 kg + 2,4-D 0.75 kg/ha
T6- Cyhalofop-butyl 90 g *fb* 2,4-DEE 1.0 kg, butachlor* 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (100% N through urea)	Isoproturon 1.0 kg + 2,4-D 0.75 kg/ha
T7- Cyhalofop-butyl 90 g *fb* 2,4-DEE 1.0 kg/ha, butachlor* 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (100% N through urea)	Clodinafop 75 g *fb* 2,4-D 0.75 kg/ha Isoproturon* 1.0 kg + 2,4-D 0.75 kg/ha
T8- Cyhalofop-butyl 90 g *fb* 2,4-DEE 1.0 kg, butachlor* 1.5 kg *fb* 2,4-DEE 1.0 kg (75% N through urea + 25% N through *Lantana*	Isoproturon 1.0 kg + 2,4-D 0.75 kg/ha
T9- Cyhalofop-butyl 90 g *fb* 2,4-DEE 1.0 kg, butachlor* 1.5 kg *fb* 2,4-DEE 1.0 kg/ha (75% N through urea + 25% N through *Lantana*)	Clodinafop 75 g *fb* 2,4-D 0.75 kg/ha Isoproturon* 1.0 kg + 2,4-D 0.75 kg/ha

*Herbicides used in rotation

The experiment was initiated in the 1999 with nine treatments (Table 1) replicated three times in randomized block design. The rice and wheat crops grown in year 2012-13 were studied for herbicidal effects on soil microflora population.

Soil samples were collected (0-15 cm depth) from experimental field during wheat crop (2012-13) and rice crop (2013) to assess the total bacterial, fungi and free-living diazotroph populations using serial dilution technique (Agrawal and Hasija 1986). and pour-plate method. After appropriate incubation period, the colonies of microorganisms appearing on plates were counted following standard method (Pramer and Schmidt 1964). Nutrient agar medium (Johnson and Curl 1972) was used for total 2 bacterial count, Pikovskya's medium for phosphate solublising microorganism count, potato dextrose agar medium for fungi and Jensen's N free medium (Jensen 1951) was used for free living diazotrophs count (Wollum 1982).

The population count of microbes namely, bacteria, fungi, *Azotobacter* and phosphorus solubilising micro-organisms was analyzed to evaluate the effect of herbicides on their respective populations by plating soil dilutions. The counts were taken after an incubation period of 48 hours for bacteria, 48–72 hours for fungi, 96 hours for PSM, and one week for *Azotobacter*. The total count of the microorganisms was obtained by multiplying the number of cells per plate by the dilution factor, which was the reciprocal of the dilution.

RESULTS AND DISCUSSION

The effect of herbicides on soil microflora and other soil parameters in the long-term study on effect of continuous and rotational use of herbicides on shift in weed flora in transplanted rice-wheat rotation was analysed at different intervals from 2012-13 in rice-wheat crops. It was revealed that due to the impact of herbicide application, the population of microorganisms was not significantly influenced (Table 2). The herbicide application in wheat crop resulted in lower microbial population in herbicide treated treatments as compared to control. A population reduction of 55.0% of *Azotobacter* was recorded in clodinafop 75 g *fb* 2,4-D 0.75 kg/ha, isoproturon 1.0 kg + 2,4-D 0.75 kg treatment applied at 30 DAS. The population of Phosphate solublising microorganisms reduced to 62.7% on treatment of clodinafop 75 g + 2,4-D 0.75 kg isoproturon 1.0 kg + 2,4-D 0.75 kg/ha. Maximum bacterial reduction (52.1%) was observed with the application of clodinafop 75 g *fb* 2,4-D 0.75 kg isoproturon 1.0 kg + 2,4-D 0.75 kg/ha. Fungal population was not much influenced by application of herbicides and a maximum reduction (17.5%) was observed with the application of clodinafop 75 g/ha fb 2,4-D 0.75 kg, isoproturon* 1.0 kg + 2,4-D 0.75 kg/ha.

The population of total beneficial microorganism *i.e. Azotobacter* and phosphate solublising microorganism and bacterial population was also not influenced significantly in various treatments upon the application of continuous and rotational use of the herbicides in rice (Table 3). The population of

Table 2. Long term effect of continuous use of herbicides on soil microflora in wheat crop

Treatment	*Azotobacter* (×10^4 cfu/g dry soil wt.) *(142.46 ×10^4 cfu/g dry soil wt.)			Phosphate solublising microorganisms (×10^4 cfu/g dry soil wt.) *(87.28 ×10^4 cfu/g dry soil wt.)			Total bacterial population (×10^{6}cfu/g dry soil wt.) *(77.56 ×10^6 cfu/g dry soil wt.)			Total fungal population (×10^4 cfu/g dry soil wt.) *(66.77 ×10^4 cfu/g dry soil wt.)		
	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T1	101.0	122.6	128.6	47.2	57.0	64.5	43.3	55.2	62.2	58.8	59.6	62.0
T2	80.3	86.5	89.2	34.7	37.9	41.8	39.9	54.6	66.2	60.3	62.2	64.3
T3	66.3	85.6	91.4	38.9	40.3	48.2	40.4	53.1	58.5	55.0	58.6	60.7
T4	76.8	82.5	84.1	33.5	37.8	41.4	42.0	54.2	60.9	60.4	62.5	64.9
T5	65.1	79.1	82.1	32.5	38.5	40.5	42.1	50.1	57.3	56.2	58.3	60.6
T6	77.2	79.3	81.2	34.3	38.6	40.8	40.8	51.5	60.8	62.2	64.8	65.5
T7	67.8	79.0	86.8	36.1	41.4	44.3	37.1	47.8	52.2	59.3	63.5	65.3
T8	68.5	70.6	73.3	33.7	38.2	41.3	44.0	54.9	63.5	58.4	62.1	63.1
T9	64.1	77.6	82.9	32.9	36.6	39.3	39.9	50.3	51.9	59.1	60.7	62.3
LSD(P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Initial population of microorganisms before herbicide treatment

Table 3. Long term effect of continuous use of herbicides on soil microflora in rice crop

Treatment	*Azotobacter* (×10^4 cfu/g dsw.) *(94.56 ×10^4 cfu/g dsw.)			Phosphate solubilising microorganisms (×10^4 cfu/g dsw) *(84.27 ×10^{4}cfu/g dsw)			Total bacterial population (×10^6 cfu/g dsw.) (121.28 ×10^6 cfu/g dsw)			Total fungal population (×10^4 cfu/g dsw.) *(46.42 ×10^4 cfu/g dsw)		
	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T1	54.6	68.9	80.3	42.1	50.5	57.5	65.5	78.5	93.2	38.2	40.5	44.3
T2	45.1	54.5	66.5	35.8	39.5	43.9	52.2	61.8	70.4	35.5	35.0	37.3
T3	43.5	48.2	50.5	30.5	35.5	41.1	47.9	56.4	71.1	34.1	33.7	37.9
T4	45.9	55.7	60.1	22.6	25.9	29.8	55.6	68.4	84.1	34.0	37.1	39.9
T5	43.8	53.0	61.9	21.2	26.4	33.9	55.0	67.8	77.3	31.8	32.9	38.2
T6	47.9	55.1	60.2	25.3	31.4	37.1	51.6	58.5	70.6	32.7	34.6	35.9
T7	54.1	60.8	68.4	23.8	28.2	33.9	51.4	58.3	67.7	32.2	34.9	35.8
T8	48.5	55.2	60.2	24.9	29.6	34.5	56.8	69.1	77.3	32.4	33.7	36.0
T9	42.9	48.2	52.7	21.7	26.9	32.1	55.8	66.7	78.0	30.9	32.5	36.7
LSD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Initial population of microorganisms before herbicide treatment

Azotobacter reduced upto 54.6% after 30 DAS while the population of PSM reduced up to 74.24% and the population reduction of bacteria was observed to be up to 60.5%. Minimum reduction in fungal population was observed as 33.4%. Balasubramanian and Sankaran (2001) also reported initial suppression of soil microflora on herbicide application in different soils. The toxic effect of herbicides normally appear immediately after the application when their concentration in soil is highest. Later on, microorganisms take part in degradation process and herbicide concentration in soil and their toxic effects decrease (Radivojevic *et al*. 2004). The herbicides and their different concentrations affected fungal population but no significant differences were observed on population count at different days after herbicide application both in wheat and rice cropping seasons. No significant interaction effects were observed for the fungal population. The control plots showed highest population of fungi in both the crops. Deshmukh and Srikhande (1974) observed that 2,4-DEE at field application rate did not exert any effect on bacteria, fungi, actinomycetes 40 days after application. Similar effects were also observed in the herbicide treatments in both wheat and rice cropping system in the present experiment wherein the microbial population, 30 days after sowing and immediately after herbicide application, were numerically different compared to control treatment. As it was observed in the current study, herbicides in the soil showed temporary inhibition of *Azotobacter* and Phosphate solublising microorganism as well as bacterial population within the early period of application of herbicides followed by a recovery during the later period in rice and wheat. However, the population of fungi was not much influenced

It can be concluded from the present study that on herbicide application, a temporary suppression in population of beneficial microorganisms occur, but with the passage of time the population again recovers. The decline in inhibition at later stages can be attributed to decrease in herbicide activity in soil due to adsorption and microbial degradation (Saksena and Singh 1984). Microbial adaptation to these chemicals/herbicides or due to their degradation (Latha and Gopal 2010) may also be a possible reason. Besides, it can also be due to microbial multiplication on increased supply of nutrients.

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