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Soil microbial communities and enzymes as affected by herbicides of rice-wheat and soybean- wheat cropping system

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Abstract: A field study was conducted to study the long term impact of continuous use of herbicide on microbial activity in rice-wheat and soybean- wheat cropping system. In the present investigation, non herbicide treatments such as hand weeding and weedy check showed higher activity as compared with herbicide receiving treatments. In rice, among the two herbicides, application of butachlor had less adverse effect when compared to the application of anilophos on soil microorganisms. Actinomycetes population maintained stable after the application of herbicides. Among the different herbicide application practices, maximum dehydrogenase activity (27.7µg TPF/g soil/24hrs) and urease activity (44.5µg NH_4/g soil/24hrs) was observed in anilophos and butachlor treatment respectively. The treatment proceeding wheat crop did not influence the microbial and enzyme activities. In soybean, highest population of total bacteria (3.34×10^6cfu/g) and actinomycetes (2.47×10^3 cfu/g) were observed in one hand weeding treatment. The treatment proceeding wheat crop did not influence the basic microbial activities. However, it positively influenced dehydrogenase activity in all the three rabi season herbicides. This study clearly indicated that herbicide application had not significant effect on the soil microbial population and soil enzymes.

Keywords: Herbicides, Microorganisms, Rice, Soil enzymes, Soybean, Wheat

INTRODUCTION

Soil health with special reference to biological features maintaining the functions of both natural and managed ecosystems, is essential for sustainable agricultural fertility and productivity (Enriqueta-Arias *et al*., 2005). The worldwide application of pesticides guarantees production capabilities, but their heavy use, persistence and transfer cross-ecosystems and into trophic food webs all cause major environmental contaminations (Ackerman, 2007). Herbicides form the principal component of weed management in crops and cropping systems. The continuous use of herbicides may lead to many problems like residual toxicity, health hazards and mammalian toxicity. Many herbicides are directly applied to the soil and if applied by other methods eventually reach the soil either as runoff, drift or washed down through atmospheric precipitation (Das and Debnath, 2006). Herbicides and their degradation products generally get accumulated in the top soil to a depth of approximately 15 cm, the zone of maximum activity of soil flora and fauna, and may upset the equilibrium of soil microflora thereby influencing the future soil fertility and the general growth and development of crop plants (Schuster and Schroder, 1990). Generally herbicides are not harmful when applied at recommended rates (Govekar *et al*., 2014) but some herbicides may affect non target organisms including microorganisms (Latha and Gopal, 2010) such as bacterial population and fungal population (Kaur *et al*., 2014). These effects on non-target organisms may reduce the performance of important and critical soil functions such as organic matter decomposition, nitrogen fixation and phosphate solubilization which support the soil health, plant growth and in turn crop productivity. Some herbicide may even stimulate the growth and activities of the microflora (Lone *et al*., 2014). Most of the studies were focused on effects of single application of herbicides on soil microorganisms for a short period, which may not provide a realistic evaluation of such effects (Haney *et al*., 2000). Since, the present study was carried out to investigate the continuous herbicidal applications on soil microbial activity in rice - wheat and soybean- wheat cropping system.

MATERIALS AND METHODS

Field trial: A field study was conducted at Directorate of Weed Research (DWR), Jabalpur for two consecutive seasons (Kharif and rabi) during 2009-10 to study the long term impact of continuous use of herbicide on microbial activity in soil. Regular monitoring of soil microbial activity in long term herbicide trail will enable to find out the change in soil health. Considering these, long term herbicide trail consisting of butal-

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chlor1.5kg/ha, anilophos0.4kg/ha, 1hand weeding along with weedy check in rice as a main plot treatments and superimposed by isoproturon 1.0kg/ha, sulofsulfuron 25g/ha and clodinafop 60g/ha followed by 2,4,D 0.5kg/ha, 1hand weeding at 25 days after sowing along with weedy check in wheat as a subplot treatments were laid out in split plot design with three replication rice wheat cropping system. For soybean – wheat cropping system, treatments comprised of fenoxoprop 100g/ha PO, imazethapyr 100g/ha and 1 hand weeding at 30 Days after sowing DAS along with weedy check in soybean as main plot treatments and which were superimposed by isoproturon 1.0kg/ ha, sulfosulfuron 25g/ha and clodinofop 60g/ha followed by wheat as a subplot treatments were laid out in a split plot design with three replication.

Enumeration of microorganisms: The soil samples were collected from 0-15cm profile in all the plots at the time of harvest. The soils were soaked into 90 mL deionized water at the amount of 10 g, respectively. This mixed liquor was shaken for 10 min and kept still for 5 min. 1ml of the supernatant of the mixed liquor was diluted to proper dilution twice and inoculated in the diluted water at the constant temperature of 30ºC. All samples were performed in triplicate, and were used for enumeration microorganisms. The viable microbial counts were analyzed by the standard technique of serial dilution and pour plating. Enumeration of bacteria and fungi were carried out in soil extract agar medium (James, 1958) and Rose Bengal Agar medium (Parkinson *et al.,* 1971). The Kenknight's Agar medium (Wellingtonn and Toth, 1963) is used for enumeration of actinomycetes. After allowing for development of discrete microbial colonies during incubations under suitable conditions, the colonies were counted and the number of viable bacteria, fungi and actinomycetes [expressed as colony forming units (cfu)] per gram dry weight of soil was estimated by taking into account the soil dilutions.

Enzyme activities: Dehydrogenase activity was assayed by the method of Casida *et al*. (1964). Moist soil samples (4 g) were placed in 16 × 150 mm^2 test tubes to which was added 1 ml of 3% aqueous solution of 2,3,5-triphenyl tetrazolium chloride, 40 mg $CaCO_3$ and 2.5 ml distilled water. The contents of each tube were then mixed with a glass rod and incubated for 24 h at 37ºC. Triphenyl formazan (TPF) was extracted by transferring the soil with the aid of methanol from each tube to a funnel plugged with absorbent cotton and the colour intensity determined in a spectrophotometer at a wave length of 485 nm. The dehydrogenase activity was expressed as µg TPF formed /g soil /24hrs.

For urease activity, 10 gram soil was taken in a 100 ml volumetric flask and 1.5 ml of toluene was added, mixed well and incubated for 15 minutes. Then 10 ml of 10 per cent urea solution and 20 ml of citrate buffer were added, mixed thoroughly, stoppered and incubated for 3 hrs at 37° C. The filtrate was assayed according to Bremner and Mulvany (1978).

Statistical analysis: The data generated from the experiment which was laid out in split plot design and analyzed using SAS 9.1 software. Before analysis data was transformed using log transformation to make it normal (Panse and Sukhatme, 1976). Critical differences were worked out at 5% level of significance and presented.

RESULTS AND DISCUSSION

Microorganisms are a heterogeneous group of organisms whose enzymatic systems comprise 60-90% of the total metabolic activity of the soil (Lu *et al*., 2015). Population size, enzymatic activity and biodiversity of certain systematic and physiological groups of microorganisms may serve as bioindicators of changes taking place in the soil following herbicide application. Results of our studies have shown that generally, herbicides tended to reduce the total number of soil microorganisms. However the activity improved gradually. We also found that, in non herbicide treatments such as hand weeding and weedy check showed higher activity as compared with herbicide receiving treatments.

Rice- wheat cropping system: In rice crop, treatments such as hand weeding and weedy check, showed significantly higher population of microbes as compared with herbicide receiving treatments. Based on the statistical analysis, the microbial population was significantly influenced by herbicides spray. Among the two herbicides sprayed, application of butachlor had less adverse effect when compared to the application of anilophos on soil microorganisms (Table 1). The maximum bacteria, fungi population was observed in the weedy check treatment (3.90×10^6 cfu /g and 1.99×10^3 cfu /g) followed by one hand weeding treatment (2.95×10^6 cfu /g and 1.98×10^3 cfu /g) at harvesting stage (P=0.05). Among the treatments no significant reductions were observed in actinomycetes population. However, among the different herbicide application practices, maximum dehydrogenase activity (27.7µg TPF/g soil/24hrs) and urease activity (44.5µg NH_4/g soil/24hrs) was observed anilophos and butachlor treatment respectively (Table 1). In contrast to our findings, Hang *et al*. (2001) reported that the number of actinomycetes declined significantly after the application of butachlor, while that of bacteria and fungi increased. Author also recorded that, fungi were easily affected by butachlor compared to the bacteria. Based on our results, the treatment proceeding wheat crop did not significantly influence the microbial and enzyme activities.

In wheat (rabi) at harvesting stage, maximum population of total bacteria, fungi and actinomycetes were observed in weedy check treatment (2.84×10^6, 2.21×10^3 and 2.12×10^3cfu/g) (P=0.05). Higher amount of dehydrogenase (35.2µg TPF/g soil/24hrs) and urease (42.6µg NH_4/g soil/24hrs) activity were recorded in (P=0.05) weedy check treatment. However weedy check and one hand weeding recorded the statistically on par activity. Balasubramanian and Sankaran (2001) also found that initial suppression of

Table 1. Soil microbial activity as affected by herbicide in rice –wheat cropping system.

Treatments	Rice					Wheat				
	Soil microbial population (CFU/g soil)			Enzyme activity		Soil microbial population (CFU/g soil)			Enzyme activity	
	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	Dehydrogenase (µg TPF g^{-1} soil 24 h^{-1})	Urease (µg NH_4 g^{-1} soil 24 h^{-1})	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	Dehydrogenase (µg TPF g^{-1} soil 24 h^{-1})	Urease (µg NH_4 g^{-1} soil 24 h^{-1})
Herbicides (Kharif)										
Butachlor	2.40	1.31	2.13	23.5	44.5	2.54	1.74	1.90	21.4	41.4
Anilophos	2.35	1.23	1.94	27.7	43.2	2.49	1.84	2.04	23.2	39.3
1 Hand weeding	2.95	1.98	2.20	32.3	46.4	2.73	2.12	1.95	26.6	42.6
Weedy check	3.09	1.99	2.18	35.5	47.5	2.76	1.87	1.97	35.3	46.7
LSD(P=0.05)	0.058	0.048	0.055	4.1	5.2	0.053	0.053	0.056	3.9	5.1
Herbicides (Rabi)										
Clodinafop	2.47	1.52	2.02	29.4	41.4	2.51	1.70	1.82	27.3	39.4
Sulfosulfuron	2.53	1.44	2.03	32.8	39.4	2.47	1.71	1.91	31.1	36.4
Isoproturon	2.48	1.48	2.07	31.4	37.4	2.52	1.68	1.95	33.4	41.3
1hand weeding	3.03	1.84	2.21	33.5	43.4	2.83	2.16	2.06	34.1	42.1
Weedy check	2.96	1.86	2.22	38.6	42.6	2.84	2.21	2.12	35.2	42.6
LSD(P=0.05)	0.100	0.084	0.095	4.2	4.6	0.105	0.090	0.093	4.2	4.3

Table 2. Soil microbial activity as affected by herbicide in soybean –wheat cropping system .

Treatments	Soybean					Wheat				
	Soil microbial population (CFU/g soil)			Enzyme activity		Soil microbial population (CFU/g soil)			Enzyme activity	
	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	Dehydrogenase (µg TPF g^{-1} soil 24 h^{-1})	Urease (µg NH_4 g^{-1} soil 24 h^{-1})	Bacteria $\times 10^6$	Fungi $\times 10^3$	Actinomycetes $\times 10^3$	Dehydrogenase (µg TPF g^{-1} soil 24 h^{-1})	Urease (µg NH_4 g^{-1} soil 24 h^{-1})
Herbicides (Kharif)										
Fenoxoprop	2.52	2.90	1.85	24.4	43.1	2.87	2.87	2.09	26.1	41.4
Imazethapyr	2.85	2.86	1.86	21.5	39.1	2.75	2.87	1.97	22.3	42.5
1 Hand weeding	3.34	2.90	2.47	23.4	43.4	3.18	2.89	2.32	28.7	45.6
Weedy check	3.20	2.99	2.36	26.7	44.7	3.20	2.97	2.29	27.6	46.7
LSD(P=0.05)	0.060	0.059	0.055	3.6	5.5	0.063	0.059	0.054	3.5	5.2
Herbicides (Rabi)										
Clodinafop	2.90	2.89	2.11	31.6	36.7	2.83	2.79	2.05	32.6	33.4
Sulfosulfuron	2.94	2.90	2.05	32.7	37.6	2.89	2.84	1.95	34.7	31.2
Isoproturon	2.89	2.89	2.05	29.6	34.5	2.90	2.83	1.98	27.5	29.4
1Hand weeding	3.06	2.92	2.18	36.9	39.6	3.16	3.01	2.43	35.7	31.6
Weedy check	3.11	2.98	2.29	37.6	38.4	3.20	3.02	2.44	38.9	32.4
LSD(P=0.05)	0.102	0.103	0.095	4.3	4.4	0.101	0.103	0.095	4.7	4.5

soil microflora by the herbicide application in different soils. The toxic effects of herbicides normally appear immediately after the application when their concentration in the soil is highest. Later on, microorganisms take part in degradation process and herbicide concentration and its toxic effect decreases (Radivojevic *et al.,* 2004). Similarly, Chen *et al* (2009) recorded that dehydrogenase activity was significantly stimulated on application of herbicides. The herbicides did not affect the urease activity and the activity remained almost unchanged.

Soybean- wheat cropping system: In soybean, treatments such as hand weeding and weedy check showed statistically higher microbial activity as compared with herbicide receiving treatments. Among herbicide applied treatments, the highest population of total bacteria (3.34×10^6cfu/g) and actinomycetes (2.47×10^3 cfu/g) were observed in one hand weeding treatment (P=0.05) (Table 2). In the present study maximum fungi populations (2.99×10^3 cfu/g) dehydrogenase (26.7µg TPF/g soil/24hrs) and urease activity (44.7µg NH_4/g soil/24hrs) (P=0.05), were observed in weedy check plots (Table 2). The treatment proceeding wheat crop did not influence much the microbial activities. However, higher dehydrogenase activity recorded all the three herbicides such as sulfosulfuron, clodinafop and isoproturon respectively.

In rabi season, wheat the highest population of bacteria (3.20×10^6cfu/g) were found in the weedy check treatment followed by one hand weeding treatment (3.16×10^6cfu/g). Similarly, higher population of fungi (3.02×10^3 cfu/g) and actinomycetes (2.44×10^3 cfu/g) was recorded in weedy check followed by one hand weeding treatment (P=0.05). For enzyme activities, higher amount of dehydrogenase activity in 38.9(µg TPF/g soil/24hrs) and urease with 32.5(µg NH_4/g soil/24hrs) were found in weedy check treatment followed by one hand weeding treatment (P=0.05). Similarly, Hadizadeh (2010) found that the sulfosulfuron application rates didn't significant effects on the microbial population and enzymes.

Conclusion

Based on our results, it is apparent that legume intercropping highly supported microbial activity and further accelerated by organic matter incorporation when compared with rice- wheat cropping system. 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. Since we found that the herbicidal treatments at the level tested were not drastic enough to be considered deleterious to soil microbial and soil enzymes which are important to soil fertility. However, the effects were quite variable depending on the type of microbes investigated. This calls for in-depth analysis of specific microbial groups involved in key functions in the soil system.

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