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RESEARCH PAPER

Assessment of soil micro flora in rice-wheat cropping system through continuous and rotational herbicide applications

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Abstract : A long term experiment was conducted at CCS Haryana Agricultural University, Regional Research Station, Uchani (Karnal), India. Assessment of fix and rotational herbicides with and without green manure on the microbial population were studied in wheat crop during 2017-18. The treatments included fix/continuous herbicide (Clodinafop) and rotational herbicide (Sulfosulfuron) to control weeds along with weed free and weedy checks. Rhizosphere samples of wheat were collected from different plots of fixed and rotational herbicides treated soil to assess the total bacterial count, phosphate solubilizing microorganisms, actinobacteria, fungi and free-living diazotrophs. Weed free and weedy check served as check (control) for total microbial populations in the soil. Initially, herbicidal application showed negative effect on the soil microbes but retain the number later on as compared to weedy check under both conditions. But the effects of the herbicides on the micro flora in soil could be nullified through green manure.

Key Words : Fix herbicides, Rotational herbicides, Green manure, Microbial flora, Rice-wheat

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INTRODUCTION

Weeds grow speedily and compete with the crops for nutrients, sunlight, space and/ or moisture during their life cycle. Herbicides form a principal component of weed management and are applied to control weeds in crops and cropping systems in many countries and India is not an exception. The use of these herbicides either continuously or rotationally may lead to many problems if used injudiciously. These herbicides directly or indirectly may reach the soil and get deposited in the top soil, the zone of maximum microbial activity (1) and may change the ecological niche of microbial flora and fauna influencing the soil structure and soil health profile (2). They directly or indirectly reach the soil either as drifts, runoff water or washed down through atmospheric precipitation (3). The prolonged intensive and indiscriminate use of agrochemicals adversely affects the soil biodiversity, agricultural sustainability, and food safety, bringing in long-term harmful effects on nutritional security, human and animal health. Most of the agrochemicals negatively affect soil microbial functions and biochemical processes. The alteration in diversity

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and composition of the beneficial microbial community can be unfavorable to plant growth and development either by reducing nutrient availability or by increasing disease incidence (4). These herbicides and their degrading products get accumulated in the soil and may upset the ecological equilibrium of soil micro flora and fauna by influencing the soil health (2). However, the indiscriminate use of pesticides leads to the degradation of soil's microbial status (5). In wheat, *Phalaris minor* (Canarygrass) alone affects wheat yield even to complete crop failure under serious situations of herbicide resistance. There are many effective herbicides for controlling *P. minor* in wheat like isoprutron is most effective and economical (6) upto evolution of new resistant biotypes in early 1990s and shift in weed flora with passage of time (7). To solve the above problems, new herbicides like clodinafop, fenoxaprop and, sulfosulfuron had been suggested (8). In rice-wheat system, clodinafop and sulfosulfuron had been used to control weeds in wheat in our experimental plots since 1999 at Regional Research Station Karnal, Haryana, India. In present study, our interest was to investigate the impact of fix herbicide (clodinafop) and rotational herbicide (sulfosulfuron) applied in wheat crop in rice-wheat system on soil micro flora under green manuring with *Sesbania aculeata*.

MATERIAL AND METHODS

A long-term field experiment was conducted at CCS Haryana Agricultural University, Regional Research Station, Karnal since 1999, in which continuous and rotational application of herbicides along with weed free and weedy checks were imposed under green manuring and without green manuring in rice-wheat cropping system (RWCS). During *Rabi* 2017-18, green manuring was done with *Sesbania aculeata* before rice crop. The herbicidal treatments were imposed on wheat field in RWCS with and without green manuring are given below:

– Fix or continuous herbicide treatment (Clodinafop 60g/ha) was given continuously since 1999 in long term herbicide trial plot.

– Rotational herbicide treatment (Sulfosulfuron 25g/ ha) as a component of herbicide in rotation between different herbicide. Rotation of three herbicides namely, clodinafop (60g/ha), fenoxaprop (120 g/ha), pinoxaden (50 g/ha) and sulfosulfuron (25g/ha) was followed over the years and during the *Rabi* 2017-18 sulfosulfuron was used as a rotational herbicide.

– Weed free (manual weeding).

– Weedy check (with weeds).

Soil sampling :

The soil samples were collected (0-15 cm depth) from fix and rotational herbicide treated plots, weed free and weedy checks from the long-term experimental field at RRS, Karnal, India to assess the total bacterial count, phosphate solubilizing microorganism (PSM), actinobacteria, fungi and free-living diazotrophs populations. The soil samples were collected randomly from four corners and one central site in each plot and bulked together. The soil samples from each treatment were collected at 30 and 60 days after treatment and replicated thrice. These samples were air-dried, mixed thoroughly and sieved using a mess (2.0 mm mesh size) to remove plant root debris and stones for further laboratory analysis. These soil samples were stored at 4°C in a refrigerator for further studies. The total microbial populations in soil were determined from untreated plots (weed free and weedy check) to compare with herbicide (fix or rotational) treated plot soils (13).

Total bacteria :

The sieved soil samples were used for enumeration of bacteria using soil extract agar medium. Serial dilution and pour plate method followed by standard plate count were used. After an appropriate incubation period of 2 days at 30±2°C, the colonies of microorganisms appearing on soil extract agar medium plates were counted and expressed in terms of colony forming units (cfu) per gram of soil on dry weight basis (9).

Free living diazotrophs :

The enumeration of microbial count was done on Jensen's N_2 free medium (10) for one week at 30±2 °C, the colonies of microorganisms appearing on Jensen's N_2 free medium plates were counted and expressed in terms of colony forming units (cfu) per g of soil on dry weight basis.

Phosphate solubilizers (PS) :

The sieved soil samples after appropriate dilution were used for enumeration of phosphate solubilizing microbial count using Pikovskaya's agar medium plates. Plates were then incubated at 30±2°C for 4-5 days and observed for the clearing zone around the colonies due

to the solubilization of inorganic phosphate by bacteria (11).

Actinobacteria :

Ken knight medium was used for enumeration of Actinobacteria count of diluted soil samples. The enumeration of Actinobacteria count was taken after an incubation period of 5-6 days at 30±2°C, the colonies of Actinobacteria appearing on Ken knight medium plates were counted and expressed in terms of colony forming units (cfu) per gram of soil.

Fungi :

For enumeration of fungi count of the sieved soil samples after appropriate serial dilution on Czapek's medium after an incubation period of 2-3 days at 30±2^{0}C. The colonies of fungi appearing on Capek's medium plates were counted and expressed in terms of colony forming units (cfu) per gram of soil on dry weight basis (12).

Statistical analysis :

Data generated on microbial count was subjected to analysis of variance by using OPSTAT software to compare the means of different exposure periods of herbicides with untreated checks. Critical difference at 5% level of significance was used to compare the data.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Bacterial population :

Total microbial population was observed more in green manure treated plots than without green manured plots. The reason might be the presence of more organic substances in green manuring. Initially at 30 days after treatment (DAT) of the herbicide spray, bacterial count was comparatively less in rotational herbicide than control, which recovered later on (Table 1 and 2). The population was 36.4 x 10^6cfu in fix herbicide, 24.9x 10^6 cfu in rotational herbicide and 36.0x 10^6 cfu/g of soil in weedy check under green manured treated plot at 30 DAT which was higher than without green manured plots where the population was 22.9 x 10^6 cfu in fix herbicide, 15.3x 10^6 cfu in rotational herbicide and 24.1x 10^6 cfu in weedy check. Later on at 60 DAT, the population increased under both conditions of green manuring. Similar results have been reported in the literature also (13) that there was initial suppression of micro flora and recovered later on. The toxicity appeared due to high initial concentration of herbicides in the soil. Later on, it decreased as microbes took part in degradation process. Similar results have been reported (14) that population

Table 1 : Effect of fix and rotational herbicides on total bacterial population in wheat crop in rice-wheat sequence with green manure

Treatments	Total bacterial population (x 10^6cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	31.7	36.4	39.7	24.4
Rotational herbicide (Sulfosulfuron)	19.9	24.9	37.7	21.9
Weed free	18.6	26.1	41.2	24.6
Weedy check	49.7	36.0	41.1	29.8
C.D. (P=0.05)		N/A		
C.V. (%)		27.4		

Table 2 : Effect of fix and rotational herbicides on total bacterial population in wheat crop in rice-wheat sequence without green manure

Treatments	Total bacterial population (x 10^6cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	21.8	22.9	25.7	20.2
Rotational herbicide (Sulfosulfuron)	13.2	15.3	18.7	17.9
Weed free	16.9	20.3	22.2	19.6
Weedy check	29.7	24.1	27.5	28.8
C.D. (P=0.05)		5.0		
C.V. (%)		15.5		

of microbes initially decreases but increases later on which could be attributed to decreased herbicide activity in soil due to microbial biodegradation and adsorption.

The toxic effects of herbicides were observed immediately when microbial population slightly suppressed in the soil after initial application. Total bacterial count was significantly higher in plots treated with fix herbicide as compared to rotational herbicide and weedy check under both green manure and without green manure. It indicated the safety of fix herbicide clodinafop to bacteria as compared to rotational herbicide sulfosulfuron. Further, in green manured conditions, more organic matters caused an increase in proliferation of soil biota as well as microbes become tolerant to clodinafop due to its continuous application and might have utilized it as a nutrient source.

Phosphate solubilizing microorganisms (PSM) :

Phosphate solubilizing microorganisms were more in green manure treated plots than without green manuring. The population was recovered at harvest almost equal in all treated as well as untreated plots in both green manured and without green manured conditions (Table 3 and 4). The population of PSM was less than the control in all treatments under both green manuring as well as in without green manuring. However, population of microbes increased with time in every treatment. Microorganisms, in general, degrade a variety of carbonaceous substances including the accumulated herbicides in soil to derive their energy and other nutrients for their cellular metabolism (15 and 16). As a result, amount of microbial biomass increases which favorably influences the transformation of plant nutrients in soil (17) . On the other hand, reports are also available on

Table 3 : Effect of fix and rotational herbicides on PSM in wheat crop in rice-wheat sequence with green manuring

Treatments	Phosphate solubilizing microbes (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	18.3	22.0	25.4	18.7
Rotational herbicide (Sulfosulfuron)	14.2	16.2	19.1	12.6
Weed free	19.1	22.0	29.9	19.2
Weedy check	21.2	22.0	29.9	23.1
C.D. (P=0.05)		N/A		
C.V. (%)		23.1		

Table 4 : Effect of fix and rotational herbicides on PSM in wheat crop in rice-wheat sequence without green manuring

Treatments	Phosphate solubilizing microbes (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	14.3	16.0	25.4	18.7
Rotational herbicide (Sulfosulfuron)	11.2	18.2	22.1	12.6
Weed free	9.1	14.0	19.9	19.2
Weedy check	18.2	22.0	29.9	23.1
C.D. (P=0.05)		N/A		
C.V. (%)		34.3		

Table 5 : Effect of fix and rotational herbicides on actinomycetes in wheat crop in rice-wheat sequence with green manuring

Treatments	Actinomycetes (x 10^3 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	13.7	17.6	18.7	8.8
Rotational herbicide (Sulfosulfuron)	10.3	17.3	19.4	7.7
Weed free	16.8	21.3	27.6	16.4
Weedy check	22.9	26.4	28.3	17.2
C.D. (P=0.05)		N/A		
C.V. (%)		34.3		

the adverse effect of herbicides on growth and activities of beneficial microorganisms in soil (18 and 19).

Actinobacteria and fungal count :

The actinobacteria and fungal population was increased at harvest compared to other dates of sampling. It was observed that there were no significant variation in actinobacterial and fungal populations (Table 5-8). At harvest, more number of fungal populations was observed in weedy check and weed free plot, when compared to herbicide sprayed plots (Table 7 and 8). Similarly, the populations of Actinobacteria and fungi increased, indicating that these microorganisms use herbicides as sources of biogenous elements (20).

The populations in herbicide treated plots were more or less similar to the control plots (un-weeded and weedy check) but overall observation show that herbicides have less effect on actinobacterial populations (Table 5 and 6). No inhibition was observed in both fix and rotational treatment with green manure and without green manure.The study suggests that the herbicide application at recommended dose to wheat crop in rice-wheat sequence caused transient impacts on microbial population growth. At normal field recommended rates, herbicides are considered to have no major or long-term effect on microbial populations (21-23). It has been reported that some microorganisms were able to degrade the herbicide, while some others were adversely affected depending on the application rates and the type of herbicide used (24- 25).

Free living diazotrophs :

The free living diazotrophs populations varied in both fix and rotational herbicide treatments with green manure

Table 6 : Effect of fix and rotational herbicides on actinomycetes in wheat crop in rice-wheat sequence without green manuring

Treatments	Actinomycetes (x 10^3 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	10.7	15.4	17.6	7.2
Rotational herbicide (Sulfosulfuron)	8.5	13.3	15.2	6.4
Weed free	11.8	19.3	23.5	14.2
Weedy check	17.9	26.4	28.3	17.2
C.D. (P=0.05)		N/A		
C.V. (%)		33.7		

Table 7: Effect of fix and rotational herbicides on total fungal population in wheat crop in rice-wheat sequence with green manuring

Treatments	Total fungal population (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	8.0	11.0	11.5	9.8
Rotational herbicide (Sulfosulfuron)	7.5	10.9	11.0	9.7
Weed free	14.8	23.8	25.3	18.6
Weedy check	20.2	24.1	28.4	19.1
C.D. (P=0.05)		5.9		
C.V. (%)		21.5		

Table 8: Effect of fix and rotational herbicides on total fungal population in wheat crop in rice-wheat sequence without green manuring

Treatments	Total fungal population (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix Herbicide (Clodinafop)	6.7	10.0	10.9	8.6
Rotational herbicide (Sulfosulfuron)	5.9	9.8	10.8	7.6
Weed free	12.5	21.7	23.1	18.2
Weedy check	17.6	24.4	28.5	19.7
C.D. (P=0.05)		5.2		
C.V. (%)		21.2		

Table 9 : Effect of fix and rotational herbicides on nitrogen fixing bacterial population inwheat crop in rice-wheat sequence with green manuring

Treatments	Nitrogen fixing bacterial population (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	25.6	34.5	35.3	21.6
Rotational herbicide (Sulfosulfuron)	21.4	32.0	33.1	20.4
Weed free	24.4	38.6	39.5	26.2
Weedy check	32.3	38.3	39.6	33.5
C.D. (P=0.05)		N/A		
C.V. (%)		25.1		

Table 10 : Effect of fix and rotational herbicides on nitrogen fixing bacterial population in wheat crop in rice wheat sequence without green manuring

Treatments	Nitrogen fixing bacterial population (x 10^4 cfu/g dry soil)			
	Before treatment	30 DAT	60 DAT	Harvest
Fix herbicide (Clodinafop)	22.9	31.4	32.8	18.1
Rotational herbicide (Sulfosulfuron)	18.5	26.0	27.7	16.6
Weed free	21.9	29.7	30.8	23.4
Weedy check	29.8	34.8	36.9	30.4
C.D. (P=0.05)		N/A		
C.V. (%)		22.2		

and without green manure (Table 9 and 10) on Jensen's N_2 free medium. The microbial population was inhibited only in rotation herbicide treatment which recovered later on. However, no significant results were observed in both treatments.

Conclusion :

Intense therapy of soil with pesticides can cause populations of beneficial soil microorganisms to turn down. Due to excessive use of chemical fertilizers and pesticides on the soil organisms there is affect similar to human overuse of antibiotics. Farm land biodiversity is a vital characteristic when assessing sustainability of agricultural practices and is of key international concern. Scientific figures point out agricultural intensification and pesticide use are among the main drivers of biodiversity loss. No portion of the population is entirely secluded against exposure to pesticides and the potentially severe health effects, though a unequal load is shouldered by the public of developing countries and by elevated threat groups in each country. From present study, it may be concluded that long term application of fix herbicide did not have significant effect on total soil microorganisms. It may also be concluded that the effects of the herbicides on the micro flora in soil could be nullified using the green manure. However, the impact differed depending upon the other experimental conditions, soil type, herbicide and its dose, and the sensitivity of the microbes. Ideally a pesticide must be lethal to the targeted pests, but not to non-target species, including man.

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