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Soil Microbial Communities as Influenced by Intercropping and Herbicide Application in Autumn Sugarcane

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The effect of intercropping systems and herbicide application on soil microbial population was studied in Indo-gangetic plains at Ludhiana, India at two different sites during 2010-11 and 2011-12 respectively. The experiment consisted of 4 cropping systems {sole sugarcane, sugarcane + cabbage (1:1); sugarcane + peas (1:2) and sugarcane + garlic (1:3)} in the main plots and six weed control treatments {oxyfluorfen 0.176 kg & 0.234 kg ha^{-1} pre emergence, pendimethalin 0.562 kg & 0.75 kg ha^{-1} pre emergence, hand weeding and weedy check} in sub plots replicated thrice in a split plot design. The composite soil samples were collected at 0, 15 and 30 days after spray. There were 5.25 and 8.71% increase in population of bacteria with intercropping of peas as compared with sole sugarcane crop after a period of four weeks at site I and II respectively. Similarly, population of actinomycetes also increased under sugarcane and peas intercropping system. However fungal count did not vary under cropping systems. The highest microbial population was observed in unsprayed plots i.e. in hand weeding and weedy check as compared to those in herbicidal treatments. There was decrease in viable counts of bacteria, actinomycetes and fungi shortly after the spray and this effect was more pronounced with higher concentration of oxyfluorfen and pendimethalin at site I than site II. Thereafter, the microbial population recovered within 30 days to reach population not significantly different from the hand weeding and weedy check treatments. Therefore, intercropping in autumn sugarcane especially peas improves the soil microbiological environment while application of herbicide only temporarily suppress the microbes which bounce back within 3-4 weeks.

Key words: Intercropping, Oxyfluorfen, Pendimethalin, Soil microbes

Sugarcane is a major crop in tropical and subtropical regions of India, occupying about 5.03 million hectares. Autumn sugarcane is planted in wider rows (90 cm) and has a juvenile period of 110-120 days, conducive to conduct intercropping for the augmentation of productivity over space and time especially in subsistence farming situations[1]. Many crops can be successfully grown as intercrops in autumn sugarcane but vegetable crops having higher productivity, shorter maturity cycle, high in value provide greater income as compared with other crops[2,3,4]. Timely weed management is very important and conventional methods of weed control are not always feasible in intercropping systems. This has necessitated the

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use of herbicides; pendimethalin (N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine) and oxyfluorfen (2-chloro-±,±,±-trifluoro-*p*-tolyl 3-ethoxy-4-nitrophenyl ether) are the herbicides widely used in sugarcane and vegetable crops for effective weed control and increased the crop yields[5,6,7].

However, little is known about the effect of these herbicides applied in autumn sugarcane and vegetable based intercropping systems on soil microbial population. Studies showed that the extent of soil microbial diversity in agricultural soils is critical to the maintenance of soil health and quality. On the other hand, microbial diversity of soils is affected by crop management practices, and the count and composition of bacterial and fungal communities in soil can be interacted either directly by changing host plant physiology or indirectly by changing the patterns of root exudation[8]. Intercropping usually benefits from increased microbial number, and hence improved soil enzyme activity[9]. The addition of herbicides can cause qualitative and quantitative alterations in the soil microbial communities[10,11] that may affect the functional stability of the soil microflora and hence the soil health[8]. Some of the earlier workers reported that the application of herbicides at normal dose do not cause any change in total number of soil microorganisms whereas other workers reported that the herbicides may be stimulatory or inhibitory to specific groups of microbes[12,13]. The present study is an attempt in this direction to investigate how treatments of intercropping autumn sugarcane with different vegetables and application of herbicides would affect soil microbiological environment.

MATERIALS AND METHODS

Experimental details

The study was carried out during 2010-11 and 2011-12 in experimental fields located at Punjab Agricultural University, Ludhiana (247 m a.s.l., lat 30^0 56' N, long 75^0 52' E), India. The experiment was laid out in split plot design with cropping systems in main plots and weed control treatments in sub plots replicated thrice. The cropping systems were sole sugarcane, sugarcane + cabbage (1:1); sugarcane + peas (1:2) and sugarcane + garlic (1:3). The weed control treatments were oxyfluorfen 0.176 kg & 0.234 kg ha^{-1} pre emergence, pendimethalin 0.562 kg & 0.75 kg ha^{-1} pre emergence, hand weeding and weedy check. In both years, trials were conducted on separate experimental fields with different cropping history. The first experimental site was under maize – fallow rotation for the past three years while on the second experimental site, medicinal crops were grown under the poplar plantations from last five years. Hence both the sites varied in their soil physico-chemical properties reported in Table 1. The crops were sown on 22nd and 23rd October 2010 and 2011 respectively. Sugarcane (CoJ 85) was planted at 90 cm row spacing by using 50, 000 three budded setts ha^{-1} and one row of cabbage (4-5 weeks old seedlings transplanted), two rows of peas (55 kg seed ha^{-1}) and three rows of garlic (3500 kg cloves ha^{-1}) were accommodated in between the sugarcane rows. The herbicides were applied using flat fan nozzle by making solution in 750 L of water ha^{-1} on day following sowing in peas and garlic, whereas the transplanting of cabbage seedlings was done five days after spray of herbicides. The composite soil samples were taken at 0, 15 and 30 days after herbicides spray (DAS) from 0-15 cm soil depth and mixed so as to have a representative sample of the treatment and analysed for the effect on soil microbial populations. On the zero day, the herbicides were applied in the early morning and soil samples were collected in the evening, approximately 10 hours after spray.

Enumeration of microbial population in soil

The viable microbial counts were analyzed by using serial dilution and pour plating technique. Soil extract agar was used for count of total bacterial population. The population of actinomycetes was estimated on dextrose nitrate agar. The fungal population was cultured on Rose Bengal Agar[14]. The representative soil samples packed in sterilized polybags were opened under aseptic conditions and a part was drawn for serial dilution. Pre-sterilized standard glass petri-dishes were used for plating of diluted soil samples in triplicate and were incubated at $30+1^0$C in an inverted position for 5-7 days till the countable colonies of each type developed.

The respective colonies were counted by visual observations of their characteristics and growth pattern like fungi show mycelia cottony

growth on the agar surface with or without variously coloured spores; actinomycetes form white, dull white or grey coloured colonies of comparatively small size and with powdry appearance and bacteria form slimy wet or partially wet, minute pinhead to large spreading colonies on the agar surface. The microbial counts were expressed as colony forming units per gram (cfu g^{-1}) for which the colonies were counted. Mean of the three replicates was taken and divided by weight of the sample to calculate the count per gram soil. The data so obtained were multiplied by their respective dilution factors (10^6, 10^4 and 10^3 for bacteria, actinomycetes & fungi, respectively) to express the final count.

Statistical analysis

All data were subjected to ANOVA using statistical analysis software version 9.3 (SAS 9.3) to test for treatment effects and possible interactions. Normality, homogeneity of variance and interactions of treatments were tested. Where the ANOVA indicated that treatment effects were significant, means were separated at $P< 0.05$ with Duncan's multiple range tests. Means with same letter were non-significant at 5 per cent level of significance.

RESULTS AND DISCUSSION

The results of the research revealed an appreciable difference in soil microbial community in response to cropping systems and weed control treatments during the period of four weeks. The monitoring period is the most important part for the assessment of any herbicide effect 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 unusual with ecological consequences which may eventually be critical[15].

Effect of intercropping systems

The population of bacteria did not vary amongst cropping systems at zero day at both the sites during 2010-11 and 2011-12 (Table 2 & 3). After 15 DAS, significantly higher bacterial count was observed in the rhizosphere of peas (43.5×10^6 cfu g^{-1}) than garlic (40.1×10^6 cfu g^{-1}) which was at par with cabbage (42.6×10^6 cfu g^{-1}) intercropped with sugarcane and sole sugarcane (41.1×10^6 cfu g^{-1}). There was 5.25 and 8.71% increase in viable bacterial population of soil with intercropping of peas as compared to monocropped sugarcane after 30 DAS at site I and II, respectively. The population of actinomycetes did not differ significantly amongst the cropping systems on the same day at both the sites. The actinomycetes count increased at 15 and 30 DAS and was maximum in sugarcane intercropped with peas at site I and II, however it remained at par amongst rest three cropping systems after 30 DAS. Fungal count did not vary under sole cropping and intercropping of sugarcane during the period of four weeks at both the sites. Higher number of viable bacteria and actinomycetes in rhizosphere soil of peas intercropped with autumn sugarcane than rest of the cropping systems might be explained on the basis that since plant species differ in their biochemical composition, changes in plant diversity alter the quantity and quality of rhizodeposits and exudates, thereby control the composition and functioning of soil microbial communities[16,17]. Plants can modify their rhizosphere through nutrient, moisture and O_2 uptake from the rhizosphere as a result modify the microbial community[18]. Hence, the differential chemical reaction in the rhizopshere under different crops might be responsible for differences in microbial population under different cropping systems. Therefore, it was not striking to find the three intercropping systems and monocropping of sugarcane in this study had different effects. Further, there are many evidences that residues of different plant species had different decomposition rates[19,20].

Table 1. Soil characteristics of experimental fields

Characteristics	Site I	Site II
Sand (%)	78.3	80.0
Silt (%)	11.0	12.9
Clay (%)	10.7	7.1
pH	7.5	8.3
EC (ds m^{-1})	0.25	0.44
Organic carbon (%)	0.36	0.41
N(mg/kg)	108.3	122.1
P (mg/kg)	8.30	8.79
K (mg/kg)	66.9	95.1

Table 2. Soil microbial population (cfu g^{-1}) as influenced by cropping systems and weed control treatments at Site I (2010)

Treatments	Bacteria ($x10^6$)			Actinomycetes ($x10^4$) Days after spray			Fungi ($x10^3$)		
	0	15	30	0	15	30	0	15	30
Cropping systems									
Sugarcane sole	35.6 a	41.1 ba	41.9 b	36.3 a	40.0 d	34.4 b	25.4 b	33.0 a	33.2 a
Sugarcane + cabbage	33.3 a	42.6 ba	43.0 ba	37.1 a	42.3 b	34.6 ba	29.5 a	29.5 a	33.4 a
Sugarcane + peas	34.7 a	43.5 a	44.1 a	38.3 a	43.8 a	35.8 a	29.6 a	29.2 a	32.7 a
Sugarcane + garlic	33.3 a	40.1 b	42.8 ba	37.4 a	40.6 c	34.6 ba	29.8 a	29.5 a	33.4 a
SEm	0.92	1.24	0.72	0.70	1.03	0.14	1.72	0.32	0.28
F(p)	0.07	0.0006	0.02	0.33	<.0001	0.97	<.0001	0.34	0.82
Weed control treatments									
Oxyfluorfen 0.176 kg ha^{-1}	34.3 ba	42.4 a	43.0 a	37.6 ba	41.5 b	34.3 a	28.6 b	31.6 a	32.4 b
Oxyfluorfen 0.234 kg ha^{-1}	33.5 ba	39.4 b	42.6 a	37.4 ba	41.1 b	35.6 a	26.5 b	28.0 a	33.9 ba
Pendimethalin 0.562 kg ha^{-1}	32.5 b	42.9 a	42.4 a	36.6 b	42.2 a	34.4 a	27.3 b	31.9 a	34.0 ba
Pendimethalin 0.75 kg ha^{-1}	34.0 ba	38.4 b	42.9 a	36.5 b	40.3 c	35.3 a	26.5 b	31.1 a	30.0 c
Hand weeding	34.6 ba	44.1 a	43.8 a	35.8 b	42.4 a	33.4 a	30.8 a	30.9 a	33.8 ba
Weedy check	36.3 a	43.8 a	43.1 a	39.8 a	42.7 a	34.8 a	31.9 a	28.3 a	35.0 a
SEm	1.02	1.94	0.14	1.13	1.47	0.65	1.86	1.40	1.44
F(p)	0.09	<.0001	0.59	0.11	<.0001	0.43	<.0001	0.62	0.003
Interaction	0.70	0.09	0.28	0.06	<.0001	0.23	0.27	0.71	0.11

F(p) values of 0.05 or lesser means significant effect
Treatment means superscripted by different alphabets are statistically different

Table 3. Soil microbial population (cfu g^{-1}) as influenced by cropping systems and weed control treatments at Site II (2011)

Treatments	Bacteria ($x10^6$)			Actinomycetes ($x10^4$) Days after spray			Fungi ($x10^3$)		
	0	15	30	0	15	30	0	15	30
Cropping systems									
Sugarcane sole	30.2 a	40.7 b	41.3 c	25.3 b	29.3 b	30.6 b	24.5 a	22.3 a	25.3 a
Sugarcane + cabbage	29.6 a	39.4 d	43.1 b	26.8 ba	29.6 ba	30.8 b	22.8 a	22.6 a	24.6 a
Sugarcane + peas	29.6 a	41.8 a	44.9 a	29.6 a	33.8 a	35.0 a	24.9 a	24.4 a	25.8 a
Sugarcane + garlic	29.7 a	40.0 c	43.6 ba	26.8 ba	29.6 ba	30.8 b	22.8 a	22.6 a	25.7 a
SEm	0.21	1.04	1.51	1.44	1.73	1.75	0.89	0.80	0.45
F(p)	0.97	<.0001	<.0001	0.04	0.01	0.06	0.11	0.12	0.32
Weed control treatments									
Oxyfluorfen 0.176 kg ha^{-1}	28.4 b	39.6 c	43.1 a	25.9 a	29. 8 a	32.4 a	24.4 a	23.0 a	26.0 a
Oxyfluorfen 0.234 kg ha^{-1}	28.6 b	37.3 d	42.9 a	26.9 a	30.6 a	32.0 a	23.6 a	23.5 a	24.3 a
Pendimethalin 0.562 kg ha^{-1}	30.0 ba	42.8 a	43.0 a	26.6 a	30.9 a	29.8 a	23.1 a	23.3 a	24.8 a
Pendimethalin 0.75 kg ha^{-1}	27.8 b	38.0 d	43.0 a	27.6 a	29.8 a	30.3 a	23.0 a	23.5 a	25.4 a
Hand weeding	30.8 ba	41.8 b	43.8 a	26.5 a	30.0 a	33.9 a	23.9 a	22.3 a	26.0 a
Weedy check	33.1 a	43.4 a	43.5 a	29.4 a	32.4 a	32.4 a	24.6 a	22.3 a	25.6 a
SEm	1.62	2.07	0.34	1.00	0.81	1.24	0.53	0.47	0.57
F(p)	0.04	<.0001	0.32	0.431	0.641	0.47	0.74	0.77	0.29
Interaction	0.25	<.0001	.05	0.22	0.73	0.67	0.60	0.36	0.006

F(p) values of 0.05 or lesser means significant effect
Treatment means superscripted by different alphabets are statistically different

Effect of weed control treatments

Shortly after application of herbicides (0 day) significant differences in population of soil microorganisms (bacteria, actinomycetes, fungi) was noticed as compared to their population in unsprayed plots i.e. hand weeding and weedy check. Amongst the herbicides, the least bacterial count (32.5×10^6 cfu g^{-1}) was recorded shortly after the application of pendimethalin 0.75 kg ha^{-1}, which was at par with pendimethalin 0.562 and oxyfluorfen 0.176 & 0.234 kg ha^{-1}. The least counts of actinomycetes (35.8×10^4 cfu g^{-1}) and fungi (26.5×10^4 cfu g^{-1}) were observed with oxyfluorfen 0.234 kg ha^{-1} at zero day after spray during 2010-11. Both the herbicides viz. oxyfluorfen and pendimethalin at higher doses (0.234 & 0.75 kg ha^{-1} respectively) were more detrimental to soil microbes compared to their respective lower doses. Such inhibitory effect of herbicides used in study persisted upto 15 DAS on bacteria and actinomycetes. However, on the contrary, actinomycetes and fungal count did not differ significantly with herbicide application at 0, 15 and 30 DAS at site II.

The interaction effects of cropping systems and weed control treatments were significant only for population of actinomycetes at 15 DAS (site I) and bacteria at 15 & 30 DAS at site II (Fig. 1). Higher population of actinomycetes was recorded in sugarcane + peas intercropping system with all the weed control treatments except where pendimethalin 0.562 & 0.75 kg ha^{-1} was applied. At 15 DAS, bacterial population under unsprayed treatments in all the cropping systems were higher than the herbicides treatments, however, at 30 DAS, sugarcane and peas intercropping system recorded significantly higher bacterial count irrespective of the different weed control treatments compared to the rest three cropping systems.

Higher microbial populations observed in the unsprayed treatments might be due to the fact that healthy and conducive environment was present in soil for the survival and growth of micro organisms which change unfavorably in the herbicide treated plots. Pre emergence oxyfluorfen (0.234 kg ha^{-1} and pendimethalin (0.75 kg ha^{-1}) proved more detrimental to soil microbes than their respective lower doses. Application of pendimethalin (0.75 kg and 1.0 kg ha^{-1}) in mustard crop[21] and 1.0 kg ha^{-1} in cowpea[13] resulted in decrease in microbial count after one week. Some workers reported actinomycetes to be relatively resistant to herbicides and get affected at high concentration only[22,23]. The differential results obtained at site II (2011-12) showed that the effect of the herbicides on soil microbial populations depends on its concentration and soil physico-chemical properties. It has been noticed that soil properties like organic matter, soil texture, inorganic nutrients and pH affect soil microbial population and persistence of herbicides[24]. It has been

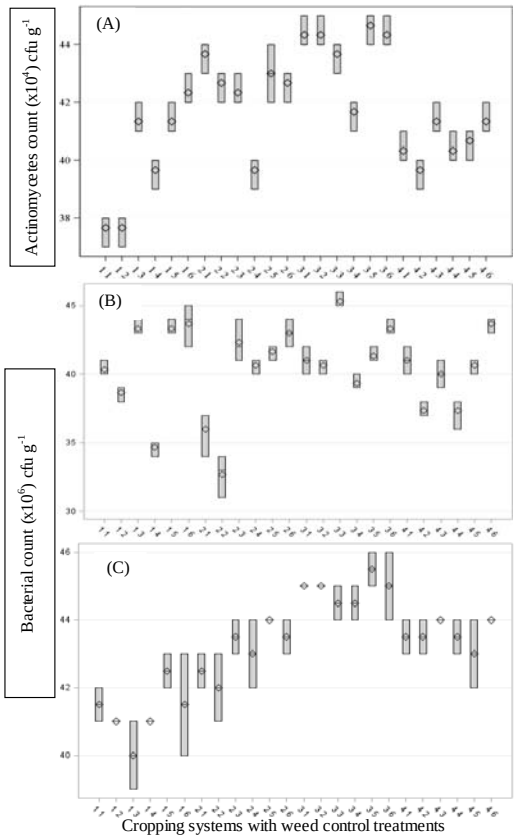

Fig. 1. Interaction effect of autumn sugarcane based cropping systems and weed control treatments on population of (A) Actinomycetes at 15 days during 2010 (B) & (C) Bacteria at 15 and 30 days during 2011 respectively.

On x-axis, 11-16 represents treatment combinations of sole sugarcane having 6 weed control treatments (WCT); 21-26 represents sugarcane + cabbage having 6 WCT; 31-36 represents sugarcane + peas having 6 WCT and 41-46 represents sugarcane + garlic having 6 WCT respectively

generally reported that combined factors such as inorganic nutrients, plant cover, root biomass, exudates and microclimatic environment of community affect herbicide degradation through their effect on diffusion, leaching and/or microbial growth and cellular metabolism[25]. Hence, the extent of change in microbial set up due to pesticide application depends on the chemical structure of the pesticide and the conditions where microbes live. However, regaining this set-up will be affected quickly by stopping pesticide application[26].

A close look at the data discussed above indicated a general rise in microbial count treated with herbicides reaching maximum around four weeks (Table 2 & 3) indicating that the microbial population started building up with the gradual degradation of herbicides to undo the inhibition of microbial growth. This could be due to the fact that the soil microflora is able to temporarily mineralize and use the degradation products of herbicides as carbon source for the growth of microbes and improves the soil health[27].

CONCLUSION

It could be concluded from the present study that the autumn sugarcane intercropped with peas (legume) increased the quantity of soil microbes than intercropping with cabbage, garlic and sole sugarcane. Pre emergence application of oxyfluorfen and pendimethalin at different doses was not detrimental to soil microbes (bacteria, actinomycetes and fungi). However, a temporary reduction on number of microbes was observed immediately after herbicide application and later the microbial population started to regain and there was increase in microbial count.

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