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Original Research Article

Effect of Different Herbicides on Soil Microbial Population Dynamics in Rabi Maize (*Zea mays* L.)

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A B S T R A C T

A field experiment was conducted during *rabi* season 2011-12 and 2012-13 at research farm of Bihar Agricultural University, Sabour, Bhagalpur to study the effect of different weed control practices on soil microbial population dynamics at various time intervals of winter maize. In this experiment, total nine treatments comprised of acetochlor 90% EC at 1.25 l ha^{-1}, 1.875 l ha^{-1}, 2.5 l ha^{-1}, 3.125 l ha^{-1} and 5.0 l ha^{-1}, atrazine 50% WP at 2.0 kg ha^{-1} and 2, 4-Diethyl ester 38% EC at 1.315 l ha^{-1}, weed free and weedy check laid out in randomized block design replicated thrice. Results indicated that the herbicide treatments of acetochlor 90% EC, atrazine 50% WP and 2, 4-D Ethyl Ester 38% EC though did not vary significantly in microbial population of bacteria, fungi and actinomycetes initially, but after herbicide application, they differed significantly for a short period of time. Beyond 40 days after application of herbicides and up to harvest, the population increased considerably in each case justifying no long term adverse effect of herbicides on the beneficial soil micro fauna and flora. The bacterial population was adversely affected, followed by fungi and actinomycetes in descending order. But at the time of harvest of the crop, the microbial population attained with all the treatments, the level equal to that of initial level or even more than original level in some treatments. The trend was similar in bacteria, fungi and actinomycetes. It is clear that the effect of herbicides on soil microbes is only temporary. The adverse effects of herbicides were gradually reduced with passage of time and practically, there was no adverse effect of acetochlor, 2, 4-Diethyl ester and atrazine herbicides on soil microbial activities in terms of fungi, bacteria and actinomycetes population after harvest of maize.

Keywords

Acetochlor, Herbicide, Microbial population and Winter Maize

Introduction

Maize (*Zea mays* L.) is one of the most important cereals in the world agricultural economy both as food and fodder and is regarded as 'queen of cereals'. Weeds are harmful or obnoxious and troublesome for proper growth and development of winter maize. They have strong competition with crop for sun light, water, nutrients, space and other growth factors. Their eradication through use of manual labour not only costly and time taking but also there is not easy availability of labour at the time of requirement. Therefore, use of suitable herbicides would be better substitute from economic point of view. Ramesh and Nadanassababady (2005) found that

significant differences in population of soil bacteria, fungi and actinomycetes were noticed shortly after application of herbicides i.e. 5 days after sowing as compared to their population before herbicide application which was in conformity with the results of Jing *et al.*, (2010).

Herbicides applied in crop fields for weed control are reported to have affected the soil microorganisms living in soil and also in the rhizosphere of crops and weeds. There was a temporary setback in microbial population due to application of herbicides and microbes adopted themselves to the new substrate to grow normally 25 days after herbicide application. The population of soil heterotrophs was affected with herbicide application and these adverse effects reduced gradually with the passage of time, up to 20 days of application of pre-emergence herbicides, there was a decrease in bacterial, fungal and actinomycetes population and after 30 days, the microbes multiplied to their original number. Use of herbicides in agriculture system may usually disturb and alter the biological equilibrium in soil (Grossbard, 1976). Sandor (2006) reported that herbicides decreased the number of total viable bacteria and microscopic fungi. The population of nitrifying bacteria and cellulose degrading bacteria increased significantly.

Field doses of herbicides are often safe for soil microbes but their response to herbicide application cannot be predicted for all environments. This is because of the herbicide-microbe interaction depends not only on molecular configuration of herbicide, but also on many soil and climatic factors like temperature, soil moisture and acidity. Keeping in view, the present investigation was carried out to find out the effect of chemical herbicides on soil microorganisms population to be studied thoroughly for effective use in soil for better adoption of weed control measures to combat the menace of weeds in winter maize.

Materials and Methods

The field experiment was conducted at Bihar Agricultural College Research Farm of Bihar Agricultural University, Sabour in 2011-2012 and 2012-13. Maize used in the experiment was laid out in randomized block design with three replications consisting of nine treatments *viz*., acetochlor 90% EC at 1.25 l ha^{-1}, 1.875 l ha^{-1}, 2.5 l ha^{-1}, 3.125 l ha^{-1} and 5.0 l ha^{-1}, atrazine at 2.0 kg ha^{-1} and 2, 4-Diethyl ester at 1.315 l ha^{-1}, weed free and weedy check in each replication. Herbicidal treatments were sprayed after sowing of seed with hand knapsack sprayer. Maize crop was fertilized with 120 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1}, respectively. One third of nitrogen and full dose of phosphorus through di-ammonium phosphate and potash through muriate of potash at basal into the soil during final ploughing. Recommended dose of nitrogen through urea was applied in three split equal doses at basal, knee high and tasseling stage in maize rows. Excluding the weed management practice, all the recommended improved package of practices of winter maize was followed in this experiment including the general plant protection measures. Prior to sowing, the seeds were treated with carbendazim @ 2 g kg^{-1} seed followed by chlorpyriphos @ 8 ml kg^{-1} seed. The treated seeds were kept under shade for overnight before sowing in the field.

The methods employed for analyzing the microbial properties of the experimental soil before treatment and at harvest were performed. Soil samples from the

experimental plots were collected from the space between the rows at a depth up to 0-15 cm on different dates *viz*., initial (pre-treatment), 10, 20, 30, 40, 50, 60 day after application (DAA) of herbicide and at harvest stage of the crop. The soil samples from different replicates for the same weed control treatment were pooled together and then composite soil samples of each herbicidal treatment were taken for microbial analysis by using dilution plate technique following standard methods. Soil dilutions were prepared in sterile distilled water by constant shaking and plating was done separately in replicates in specific media like for bacteria- Thornton's agar medium, 1922 at 10^{-6} dilutions, for fungi- Martin'rose bengal streptomycin agar medium, 1950 at 10^{-4} dilutions and for actinomycetes- Jensen's agar medium, 1930 at 10^{-5} dilutions. The plates were incubated at 28 ± 1^0C for maximum duration of 7 days in BoD incubator and observations in terms of counting of number of colonies per plate were made.

Results and Discussion

Soil microbial properties

The impact of different herbicides including acetochlor 90% EC on soil micro-flora *viz*., fungi, total bacteria, and actinomycetes (Table 1, 2 and 3) as recorded at different time of observations (Initial, 10, 20, 30, 40, 50, 60 days after application and at harvest) are discussed below:

Soil fungi counts (CFU x 10^4 g^{-1} oven dry soil)

Data on the population of fungi in soil at different time intervals of the crop is presented in Table 1. There was no significant difference in the initial population of fungi (*Trichoderma viride* and *Trichoderma harzianum*) in different plots (Table 1). However, all the tested herbicides acetochlor

90% EC and standard herbicides (atrazine 50% WP and 2, 4-D Ethyl Ester 38% EC) treatments showed significant adverse effect on the population of fungi in rhizosphere soil of winter maize up to 30 days after application of herbicides exhibiting soil fungi population in lower range with increasing acetochlor 90% EC herbicide doses. Reduction in their population occurred in all herbicide treated plots up to that period over the weed free and weedy check. Thereafter, fungi population was gradually increasing at subsequent time intervals and at harvest. Their population increased considerably in herbicidal treated plots though the weed free (T_8) and weedy check (T_7) exhibited higher value of fungi population than other herbicide treated plots. Initially the fungi population in weed check plot (T_7) was lower over weed free (T_8), however, it remain continued in superior value over weed free for rest of time intervals and harvest. But at harvest, overall the data showed increase in population was higher than the initial population of the fungi in all the treatments at 40 DAA, 50 DAA, 60 DAA and at harvest (Table 1).

Li *et al.,* (2005) revealed that acetochlor at high concentrations of 150 and 250 mg kg^{-1} had an acute and chronic toxicity on both soil fungal population and total fungal biomass, but at a low concentration of 50 mg kg^{-1} had stimulating effect that was stronger with total fungal biomass than with soil fungal population. Cal *et al.,* (1993) reported that atrazine and alachlor herbicides decreased fungal populations without altering bacterial population. Konstantinovic *et al.,* (1999) reported that alachlor/atrazine herbicides reduces the

population of bacteria and increases the population of fungi and actinomycetes. Alachlor was generally more inhibiting towards the microbes as compared to atrazine. Increased herbicide doses increased the inhibitory effect, which was best exhibited at the beginning of the growing period.

Soil bacteria counts (CFU x 10^6 g^{-1} oven dry soil)

Data on the population of bacteria in soil at different intervals of growth stages of the crop is presented in Table 2. There was no significant difference in the initial population of total bacteria (*Pseudomonas fluorescence*, *Rhizobium* and *Azotobacter*) in different plots (Table 2). However, all the tested herbicides acetochlor 90% EC and standard herbicides (atrazine 50% WP and 2, 4-D Ethyl Ester 38% EC) showed significant influence on the population of total bacteria in rhizosphere soil of winter maize up to 30 days after application of herbicides showing soil bacterial population in lower values with increasing acetochlor 90% EC herbicide doses. Reduction in their population occurred in all the herbicide treated plots up to that period over the weed free and weedy check. Thereafter, bacteria population was gradually increasing at subsequent time intervals *viz.* 40 DAA, 50 DAA, 60 DAA and at harvest, their population increased considerably in herbicidal treated plots though the weed free and weedy check showed higher bacteria population than other herbicide treated plots. Initially the bacteria population in weed check plot (T_7) was lower over weed free (T_8), however, it remain continued superior value for rest of the time intervals and harvest. But at harvest, the data showed that their population increased considerably in herbicide treated plots and higher than the initial population of bacteria (Table 2).

Olabode *et al.*, (2010) reported that response of soil microorganisms to atrazine doses varied depending upon herbicides. Bacterial population was inversely related to atrazine doses with the highest value for control (290 x 10^6 cfu g^{-1} soil) and the least in 100% recommended dose (29 x 10^6 cfu g^{-1} soil). Fungi and actinomycetes, however, showed a reverse trend. Jing *et al.*, (2010) reported that an experiment was conducted to determine acetochlor effect on soil microbial activity and indicated that number of bacteria and actinomycetes increased two weeks after applying acetochlor, then inhibited by acetochlor @ 30 mg kg^{-1} at 30^{th} day. After that, actinomycetes population recovered while bacteria population was still inhibited till 60^{th} day. Fungi population decreased at 14^{th} day after applying acteochlor, but then recovered at 30^{th} day and was stimulated at 60^{th} day.

Soil actinomycetes counts (CFU x 10^5 g^{-1} oven dry soil)

Data on the population of actinomycetes in soil at different intervals of growth stages of crop is presented in Table 3. All the herbicide treatments showed significant influence on the population of actinomycetes in rhizospheric soil of winter maize indicating in lower range with increasing herbicide doses. Like the bacteria and fungi, no significant difference in initial population of actinomycetes in different plots was observed (Table 3).

However, significant differences were recorded between herbicide treated plots, weed free and weedy check plot up to 30 days after application of herbicide. Thereafter, at harvest the population of actinomycetes increased to a considerable level (Table 3). Soil actinomycetes population was found in lower values with increasing acetochlor 90% EC doses.

Table.1 Effect of different treatments on population of fungi in soil (Pooled mean of two years)

Treatment	Fungi population (CFU x 10^4 g^{-1} soil)							
	Initial	10 DAA	20 DAA	30 DAA	40 DAA	50 DAA	60 DAA	Harvest
T_1- Acetochlor 90% EC @ 1.25 l ha^{-1}	53.51	32.50	36.98	41.27	52.32	59.46	64.75	94.00
T_2- Acetochlor 90% EC @ 1.875 l ha^{-1}	52.34	29.52	33.46	39.67	50.31	58.57	62.77	88.50
T_3- Acetochlor 90% EC @ 2.5 l ha^{-1}	54.01	27.97	32.23	36.57	47.55	57.96	61.13	87.46
T_4- Acetochlor 90% EC @ 3.125 l ha^{-1}	53.57	26.32	28.03	32.58	46.22	54.45	59.69	86.06
T_5- Atrazine 50% WP @ 2.0 kg ha^{-1}	53.59	26.01	27.61	33.80	47.14	56.08	60.63	88.03
T_6-2,4-D Ethyl Ester 38% EC @ 2.65 l ha^{-1}	50.49	34.66	39.25	43.59	53.96	62.16	67.94	95.43
T_7- Weedy check	52.93	53.07	56.67	61.54	68.72	78.80	85.80	116.39
T_8- Weed free	54.11	47.81	50.47	56.63	61.75	73.90	80.92	111.36
T_9- Acetochlor 90% EC @ 5.0 l ha^{-1}	54.17	24.43	25.98	29.88	44.53	51.75	55.69	83.59
CD (P=0.05)	NS	0.93	1.44	1.78	1.85	2.09	2.17	4.03

DAA- Days after application NS- Non significant

Table.2 Effect of different treatments on population of total bacteria in soil (Pooled mean of two years)

Treatment	Total bacteria population (CFU x 10^6 g^{-1} soil)							
	Initial	10 DAA	20 DAA	30 DAA	40 DAA	50 DAA	60 DAA	Harvest
T_1- Acetochlor 90% EC @ 1.25 l ha^{-1}	35.23	24.63	30.35	36.62	44.12	51.78	64.84	101.73
T_2- Acetochlor 90% EC @ 1.875 l ha^{-1}	34.73	19.74	26.39	32.08	38.30	48.33	60.92	96.55
T_3- Acetochlor 90% EC @ 2.5 l ha^{-1}	32.91	19.29	24.62	29.29	36.79	46.60	59.34	93.80
T_4- Acetochlor 90% EC @ 3.125 l ha^{-1}	34.89	17.53	22.05	28.57	36.01	45.09	57.07	86.38
T_5- Atrazine 50% WP @ 2.0 kg ha^{-1}	33.24	17.91	23.55	28.55	36.53	45.87	56.70	91.37
T_6-2,4-D Ethyl Ester 38% EC @ 2.65 l ha^{-1}	35.25	24.09	29.70	35.18	41.59	49.10	62.77	97.53
T_7- Weedy check	35.50	37.51	41.44	47.88	58.76	69.09	77.50	116.96
T_8- Weed free	36.87	34.95	38.94	43.00	51.69	59.36	68.70	112.89
T_9- Acetochlor 90% EC @ 5.0 l ha^{-1}	33.59	17.26	21.93	27.68	35.89	43.51	53.32	85.02
CD (P=0.05)	NS	0.89	1.43	1.70	1.80	2.01	2.18	3.72

DAA- Days after application NS- Non significant

Table.3 Effect of different treatments on population of actinomycetes in soil (Pooled mean of two years)

Treatment	Actinomycetes population (CFU x 10^5 g^{-1} soil)							
	Initial	10 DAA	20 DAA	30 DAA	40 DAA	50 DAA	60 DAA	Harvest
T_1- Acetochlor 90% EC @ 1.25 l ha^{-1}	41.74	34.60	38.19	42.37	50.97	56.13	58.99	94.24
T_2- Acetochlor 90% EC @ 1.875 l ha^{-1}	41.27	30.26	34.70	40.50	46.61	50.14	54.03	91.06
T_3- Acetochlor 90% EC @ 2.5 l ha^{-1}	41.90	29.23	32.89	38.71	44.62	48.53	51.21	89.30
T_4- Acetochlor 90% EC @ 3.125 l ha^{-1}	41.70	28.61	29.99	37.23	42.11	45.25	49.60	82.59
T_5- Atrazine 50% WP @ 2.0 kg ha^{-1}	42.27	27.84	31.42	37.62	48.36	52.30	54.80	79.50
T_6-2,4-D Ethyl Ester 38% EC @ 2.65 l ha^{-1}	42.42	32.79	35.55	39.84	49.39	53.10	56.59	82.30
T_7- Weedy check	42.72	47.12	54.73	56.76	67.69	73.92	78.73	101.16
T_8- Weed free	43.77	44.20	48.76	52.19	61.84	69.13	72.61	107.99
T_9- Acetochlor 90% EC @ 5.0 l ha^{-1}	41.71	26.15	30.16	35.47	40.90	44.07	46.76	85.33
CD (P=0.05)	NS	0.55	0.22	0.31	1.54	1.47	2.14	4.18

DAA- Days after application NS- Non significant

Kulashrestha *et al.,* (1975) stated that atrazine @ 0.5, 1.0 and 2.0 kg ha^{-1} did not leave any herbicide residues. He also reported that atrazine @ 1.5 kg ha^{-1} as pre-emergence and atrazine @ 1.5 kg ha^{-1} as broadcast persisted up to 47 days in silty clay loam soil. Saikia *et al.,* (2000) reported that atrazine content showed initial slow rate of loss of atrazine in sandy loam soil up to 20 days and thereafter faster dissipation and at maize harvest (90 days), no detectable residue was found. They also reported that atrazine right from 1.0 to 2.0 kg a.i ha^{-1} in maize did not leave any detectable residues. Saikia and Pandey (2001) stated that atrazine was biologically active in sandy loam soil up to 90 days and its biological activity was well pronounced at 2.0 kg a.i ha^{-1}. Ayansina and Oso (2006) concluded that higher concentrations of atrazine herbicide resulted in much lower microbial counts as compared to soils treated with recommended herbicide dose. Herbicides also exhibited the elimination of some microbial species.

Das *et al.,* (2002) noted that alachlor and atrazine herbicides were not always inhibitory in action and stimulatory effect on important soil enzyme activity as well as microbial population was observed.

Reddy *et al.,* (2012) observed that atrazine at 0.25 to 0.5 kg ha^{-1} as pre-emergence and pendimethalin @ 0.5 kg a.i ha^{-1} as pre-emergence in maize did not leave any significant residues. Nikolova and Baeva (2004) indicated acetochlor decreased the growth of soil microorganisms.

Zhen *et al.,* (2013) reported that the dissipation half-life of acetochlor were 2.8 and 3.4 days in the rhizosphere and bulk soil, respectively. As compared to the bulk soil, microbial communities in the rhizospheric soil were inclined to be affected by acetochlor. Bacterial growth was most likely increased, however, fungal growth was prone to be inhibited. Soil microbial community was significantly affected by acetochlor at its early 15 days stage. Residual acetochlor did not confer long term impairment on viable bacterial counts in the rhizosphere and bulk soil.

In all the three cases (total bacteria, fungi and actinomycetes), the herbicide treatments though did not vary significantly in the plots of tested herbicide acetochlor 90% EC and the standard herbicides (atrazine 50% WP and 2, 4-D Ethyl Ester 38% EC) application initially, but after herbicide application, they differed significantly for a short period of time. Beyond 40 DAA and up to harvest, the population increased considerably in each case justifying no long term adverse effect of tested herbicides on the beneficial soil micro fauna and flora. The bacterial population was adversely affected, followed by fungi and actinomycetes in descending order. The adverse effects of herbicides were gradually reduced with passage of time and practically, there was no adverse effect of acetochlor, 2, 4-Diethyl ester and atrazine herbicides on soil microbial population as a whole.

Shortly after application of herbicides (5 days after sowing) significant differences in population of soil microorganisms (bacteria, fungi, actinomycetes) was noticed as compared to their population before herbicide application which was in conformity with the results of Jing *et al.,* (2010). Such inhibitory effect of herbicides used in the study persisted upto 45 days after sowing of the crop with respect to either pre-emergence single herbicide or herbicide mixture spray. However, under sequential application of pre-emergence herbicide on 3 days after sowing followed by post-emergence spray on 20 days after sowing,

the effect of herbicides on soil microorganisms population extended beyond 20 days after sowing of the crop. But at the time of harvest of the crop, the microbial population with all the treatments attained, the level equal to that of initial level or even more than original level of population in some treatments. The trend was similar in bacteria, fungi and actinomycetes. It is clear that the effect of herbicides on soil microbes is only temporary.

It might be concluded that there was no ill impact of herbicidal treatments on the soil microbial activities in terms of fungi, bacteria and actinomycetes population after harvest of maize.

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