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Effect of butachlor on total microbial activity, Azotobacter and phosphate solubilizing fungal population

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ABSTRACT

Microbial activity and population dynamics of bacteria, fungi, actinomycetes, Azotobacter and phosphate solubilizing fungi (PSF) following application of butachlor was studied in a black cotton soil. Microbial population declined significantly due to butachlor treatment. Total population of fungi showed relatively more susceptibility than bacteria to butachlor. At recommended dose, the toxic effect of butachlor on total bacteria disappeared by 30 days after application (DAA), but the soil could not regain its lost population of Azotobacter, total fungi and PSF during the study period of 45 days. Total microbial activity in terms of CO_2 evolution from the soil sharply decreased by butachlor. Addition of glucose at 10 mg/kg soil nullified the adverse effect of butachlor on total microbial activity.

Key words : Herbicide, Soil respiration, Free living N fixing bacteria, P solubilizer

The effect of herbicide application on soil microbial environment is always of great concern. Depression in the bacterial growth due to application of different herbicides was reported by various workers (Shukla and Mishra 1997, Solomon 1999). However, the information in respect to the impact of herbicides on the specific group of soil microorganisms involved with the agriculturally important soil biochemical processes *viz.,* biological nitrogen fixation, P solubilization, *etc*. are very scanty. The common limiting nutrient for plant productivity is nitrogen, which has led to increased use of chemical N fertilizers inputs. However the economic and environmental costs of heavy use of chemical N fertilizers in agriculture are a matter of global concern. Biological nitrogen fixation offers an attractive and ecologically sound means of reducing external fertilizer input. Azotobacters are aerobic free living heterotrophic nitrogen fixing bacteria and are well adapted to neutral to alkaline soil conditions. They benefit the crop by excreting the fixed nitrogen as ammonium ion in the soil and also produce a number of growth promoting substances (Wani 1990). The benefits of Azotobacter inoculation on crop production in Indian soil have been well summarized by Narula and Yadav (1989). Yu *et al* (1993) reported about the adverse effect of butachlor on soil nitrogen fixing bacteria. Kole and Dey (1989a, 1989b) found that the application of fluchloralin, pendimethalin and oxadiazon had detrimental effect on proliferation of total, aerobic, non-symbiotic nitrogen fixing and P solubilizing bacteria in a Gangetic alluvial soil. P solubilizing fungi may play an important role in mitigating the P requirement of crops by using cheaper but scantly soluble P fertilizers. Beneficial influence of artificial inoculation with PSF has been observed for different crops under diverse agro-climatic conditions. Garetsen (1948) was the first to report the increased yield accompanied with the high phosphorus uptake in oat plants inoculated with phosphate solubilizing fungi. Hardly any report is available in respect to the impact of herbicides on this important group of fungi in Indian soils.

Butachlor is one of the widely used herbicides in India. The present investigation was carried out to evaluate the impact of butachlor on total microbial activity, Azotobacter and phosphate solubilizing fungal population in a black cotton soil.

MATERIALS AND METHODS

The surface soil (0-15cm) was collected from the DWSR farm, partially air-dried and ground to pass through 5 mm sieve. The soil belonged to sandy clay loam textural class with pH 6.84, EC 0.378 dS/m, clay 35.5% and organic C0.75%.

Enumeration of soil microbes

Soil was inoculated with commercially available biofertilizers containing Azotobacter and phosphate solubilizing fungi, and subsequently treated with butachlor at the rate of 0, 0.4, 0.8 and 1.6 mg/kg soil, mixed uniformly and transferred to plastic pots. Water was added to raise the moisture content to field capacity level and three replications of each treatment were incubated at room conditions. Soil sample was collected from the pots using a soil auger after 1, 3, 5, 10, 15, 20, 30 and 45 days of butachlor treatment and kept in refrigerator for the microbial analysis.

Microbial population in the soil sample was enumerated by following serial dilution technique (Agrawal and Hasija 1986). Nutrient agar medium, Martin's rose Bengal medium and Katznelson and Bose medium as described by Subba Rao (1988) were used for enumeration of total bacteria, total fungi and phosphate solubilizing fungi (PSF), respectively. In case of PSF 3 ml of 10% streptomycin sulphate solution was also added per litre of medium to prevent bacterial growth and only those fungal colonies around which a clear hollow zone was visible were counted. For enumeration of Azotobacter the medium as described by Brown *et al*. (1962) was used in the given study. The inoculated petriplates were inverted after solidification of the agar media and then incubated at 28°C for 1, 3, 5 and 10 days for enumeration of total bacteria, total fungi, Azotobacter and PSF, respectively.

Soil respiration

Soil respiration, as an indicator of total microbial activity, was measured by following the CO_2 entrapment technique. A 250 g amount of processed soil was taken in 1000 ml capacity conical flasks and water was added to the flask to raise the soil moisture at 50% of water holding capacity. The flasks were cotton plugged, kept under room condition for 7 days for stabilization of microbial activity and then butachlor was added at the rate of 0, 0.4, 0.8 and 1.6 mg/kg soil. Each treatment was replicated twice. A test tube containing 10 ml of 1N NaOH was placed in each flask, firmly corked the flasks to make them air tight and kept inside a BOD incubator at 28°C. A blank consisting of the flask without soil was similarly kept in the incubator. After the incubation for a desired period of time (1-16 h), test tubes were taken out of the flasks and a fresh set of test tubes containing 10 ml of 1N NaOH was again similarly placed inside the flasks and the sequence was continued up to 10 DAA. The contents of the test tubes were analyzed for the amount of CO_2 trapped in NaOH. A 5 ml amount of 2N $BaCl_2$ was added to the content of the test tube and then titrated to a phenolphthalein end point using 0.1N HCl. The amount of CO_2 evolved from soil during the exposure to the alkali was calculated by using following formula (Stotzky 1965).

$$\text{Milligram of C or } CO_2 = (B\text{-}V)\,N\,E$$

Where, B: volume of acid (ml) needed to titrate the blank, V: volume of acid (ml) needed to titrate the NaOH exposed to soil, N: normality of acid, E: equivalent weight (E=22 for CO_2).

In a separate set of flasks, glucose at the rate of 10 g/kg soil was added prior to butachlor treatment and subsequently soil respiration was measured similarly as mentioned above.

RESULTS AND DISCUSSION

Bacterial population

Total bacterial population in the control soil varied slightly from 25.4×10^5-28.2×10^5 cfu/g soil during the study period (Table 1). Contrary to the control, the bacterial population in the butachlor treated soils decreased sharply up to 5 DAA and thereafter showed an increasing trend. An increase in reproductive ability of bacteria with time after the initial phase of depression, resulting from toxic effect of herbicides, was earlier reported by various workers (Kole and Dey 1989b, Yu *et al*. 1993, Solomon 1999). There was no difference between control and butachlor 0.4 mg/kg soil treatments in terms of total bacterial population on 20 DAA. This indicated that the initial detrimental effect of butachlor applied at 0.4 mg/kg soil disappeared by 20 days. Similarly the soil regained its lost population of total bacteria by 30 DAA of butachlor at recommended level, i.e. 0.8 mg/kg soil treatment. However, the adverse impact on bacteria was still visible up to 45 DAA of 1.6 mg/kg soil butachlor treatment.

Table 1. Effect of butachlor on total bacterial population (in 10^5 cfu/g soil)

Butachlor (mg/kg soil)	Days after application							
	1	3	5	10	15	20	30	45
0	25.4	25.7	26.3	27.9	27.9	28.1	28.2	28.2
0.4	15.3	10.8	8.5	21.9	24.5	26.3	28.0	28.2
0.8	12.0	9.7	4.9	14.7	18.8	21.9	25.1	27.5
1.6	9.6	7.1	3.2	5.9	7.6	9.5	16.1	22.6
LSD (P=0.05)	2.6	2.4	2.9	2.9	2.3	2.2	3.1	3.0

cfu = Colony forming unit

Azotobacter population

Similar to total bacteria, Azotobacter population also declined in the butachlor treated soils (Table 2). Compared to the control, all the butachlor treatments showed significantly lower Azotobacter count throughout the period of study and the extent of decline in the population increased with increase in butachlor dose. Consequently, among the butachlor treatments the lowest Azotobacter population was recorded on each sampling day at its highest level of 1.6 mg/kg soil dose. Unlike total bacteria, Azotobacter could not regain its lost population during the period of study indicating relatively higher susceptibility of Azotobacter than the total bacterial population to butachlor. Piskorz (1998) reported decrease in Azotobacter population in soil due to glyphosate, fluazifop-P-butyl, haloxyfop, quizalofop-P-ethyl, sethoxydim, atrazine, cycloxydim and alloxydim application. Similar inhibitory effect of alachlor and atrazine on Azotobacter count was reported by Konstantinovic *et al*. (1999).

Fungal population

The fungal population in soil declined sharply after application of butachlor (Table 3). Unlike bacteria that showed lowest population on 5 DAA, fungal population decreased up to 10 days of butachlor application and thereafter started to regain their lost population. However, fungi could not regain their lost population even at 45 DAA in the butachlor treated soils. The result thus indicated that fungi were relatively more susceptible to butachlor than bacteria. Various workers (Shukla and Mishra 1997, Chauhan *et al*. 1994) have also reported earlier that, unlike bacterial population, fungus took more time to recover from the detrimental effect caused by applied herbicides.

PSF population

The population count of phosphate solubilizing fungi (PSF) in soil as recorded after 3 and 45 days of butachlor application is given in Table 4. Similar to total fungal count, PSF count also decreased with increasing level of butachlor and all the butachlor treatments showed significantly lower count as compared to control at 3 DAA. Similar trend was also noticed at 45 DAA with the exception that the PSF count at 0.4 mg/kg soil butachlor dose was statistically similar to that as recorded in control. It may be noted that, contrary to PSF count, the total fungal count at 0.4 mg/kg soil butachlor dose was significantly lower than the control.This indicated that the susceptibility of some of the PSF strain to butachlor could be relatively lesser than the overall susceptibility of the total fungal population. However, at 0.8 mg/kg soil dose, which is equivalent to the recommended dose of butachlor, similar to total fungal count the PSF count was significantly lower than the control at 45 DAA.

Table 2. Effect of butachlor on Azotobacter population (in 10^3 cfu/g soil)

Butachlor (mg/kg soil)	Days after application							
	1	3	5	10	15	20	30	45
0	20.5	21.0	20.4	21.0	20.7	20.6	19.9	20.5
0.4	13.3	10.6	11.2	12.6	13.8	14.7	17.5	13.3
0.8	11.5	10.2	7.0	9.3	10.1	11.9	16.0	11.5
1.6	9.7	8.1	5.6	6.8	7.8	8.9	13.5	9.7
LSD (P=0.05)	2.2	2.3	2.1	2.5	2.9	2.4	2.5	2.2

cfu = Colony forming unit

Table 3. Effect of butachlor on fungal population (in 10^3 cfu/g soil)

Butachlor (mg/kg soil)	Days after application							
	1	3	5	10	15	20	30	45
0	2.89	2.90	2.91	2.92	2.90	2.85	2.88	2.95
0.4	2.55	2.42	2.33	2.01	2.08	2.15	2.43	2.58
0.8	2.36	2.17	1.90	0.92	1.41	1.76	2.03	2.30
1.6	1.81	1.70	1.52	0.54	0.92	1.01	1.22	1.51
LSD (P=0.05)	0.21	0.32	0.29	0.23	0.31	0.35	0.28	0.27

cfu = Colony forming unit

Soil respiration

The cumulative amount of CO_2 evolved at different levels of butachlor application is presented (Fig.1). In absence of glucose the highest CO_2 evolution of 483.3 mg/flask was recorded in control and the amount decreased to 320.4, 185.8 and 182.1 mg/flask when the soil received butachlor application of 0.4, 0.8 and 1.6 mg/kg, respectively (Fig.1a). The data thus indicated detrimental effect of butachlor on total soil microbial activity. Significant decrease in the soil respiration, an important indicator of soil biological health, was also observed earlier following application of fluchloralin (Praharaj *et al*. 1997) and pendimethalin (Shetty and Magu 1997). Overlapping of the cumulative CO_2 evolu-tion curves indicated that there was no significant difference in total soil microbial activities between the 0.8 and 1.6 mg/kg soil butachlor treatments.

Table 4. Effect of butachlor on PSF population (in 10^3 cfu/g) 45th DAT

Butachlor (mg/kg soil)	3 DAA	45 DAA
0	1.29	1.40
0.4	1.02	1.33
0.8	0.70	1.21
1.6	0.43	1.05
LSD (P=0.05)	0.14	0.09

cfu = Colony forming unit

Irrespective of the butachlor treatment, the total amount of CO_2 evolution was much higher in presence of glucose than in absence of glucose indicating the occurrence of substrate-induced respiration (Fig.1). In presence of glucose, the cumulative amount of CO_2 evolved from the soil was 1968, 2032, 1888 and 1835 mg/flask at 0, 0.4, 0.8 and 1.6 mg/kg levels of butachlor application, respectively. As indicated by the Fig.1b, in presence of glucose there was no prominent effect of butachlor on total soil microbial activity during the incubation period.

The average rate of respiration at 0, 0.4, 0.8 and 1.6 mg/kg soil level of butachlor application was respectively 8.04, 5.36, 3.08 and 3.04 ìg CO_2 per hour per gm soil in absence of glucose and respectively 32.8, 33.9, 31.5 and 30.6 ìg CO_2 per hour per gm soil in presence of glucose. The observation thus showed that in terms of CO_2 evolution microbial activity was 4.1, 6.3, 10.2 and 10.5 times higher in presence of glucose than in absence of glucose at the corresponding level of butachlor. Compared to control, much higher increase in CO_2 evolution rate from the butachlor treated soil indicated that the addition of glucose to soil has not only increased the overall microbial activity but also substantially nullified the toxic effect of butachlor in terms of total soil microbial activity. As compared to control, at recommen-ded level of application, i.e. at 0.8 mg/kg level, butachlor decreased the average microbial activity during the incu-bation

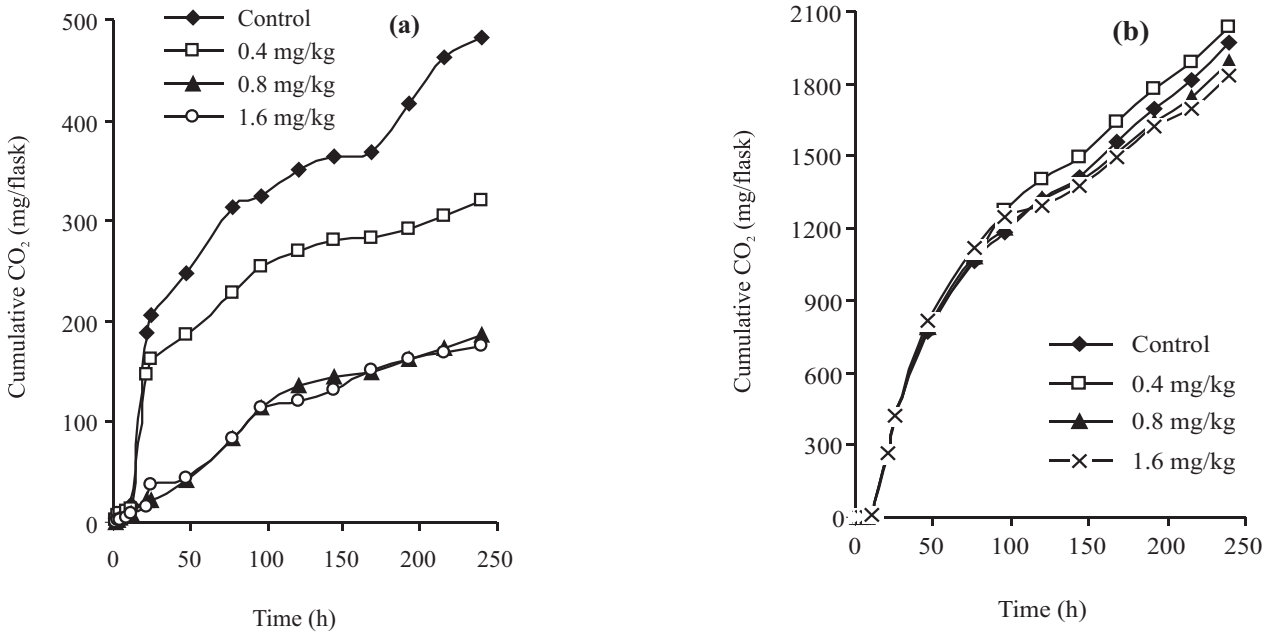

Fig. 1. The effect of butachlor on cumulative amount of CO_2 evolved with time from the soil receiving (a) 0 and (b) 2.5 g/flask of glucose addition

period by 62% in absence of glucose, whereas the corresponding value was only 4% in presence of glucose.

The finding thus indicated that butachlor is harmful to the given soil in terms of total microbial activity, however addition of easily degradable source of organic carbon to the soil prior to butachlor application could nullify this adverse impact.

REFERENCES

Agarwal GP and Hasija SK. 1986. *Microorganism in laboratory. A laboratory guide for Microbiology, Mycologyand Plant Pathology*. Print House, Lucknow, India : 137 p.

Brown ME, Burlingham SK and Jackson RM. 1962. Studies on Azotobacter species in soil. I. Comparison of media and techniques for counting Azotobacter in soil. *Plant and Soil* **17**: 309-319.

Garetsen FC. 1948. The influence of microorganisms on the phosphate uptake by the plant. *Plant and* Soil **1**: 51-58.

Kole SC and Dey BK. 1989a. Effect of aromatic amine herbicides on microbial population and phosphate solublizing power of the rizosphere soil of groundnut. *Indian Agriculturist* **33**: 1-8.

Kole SC and Dey BK. 1989b. Effect of aromatic amine herbicides on bacterial population and nitrogen fixing power of the rizosphere soils of groundnut. *Enviorment and Ecology* **7**: 850-853.

Konstantinovic B, Govedarica M, Jarak M and Milosevic N. 1999. Herbicide efficiency and their impact on microbiological activity in soil. In *Research progress in plant protection and plant nutrition*, AAM, Beijing, China Agriculture Press : 228-232.

Narula N and Yadav KS. 1989. *Biological Nitrogen Research in India*. Society for plant physiology and Biochemistry, New Delhi : 89-124.

Piskorz B. 1998. The effect of quackgrass (*Agropyron repens* L.) [*Elymus repens*] controlling herbicides on soil microorganisms. *Annals of Warsaw Agricultural University Agriculture* **32**: 59-64.

Praharaj AK, Saha N, Chakravarty A and Mukherjee D. 1997. Effect of Basalin on microbiological activity in soil. *Journal of Interacademicia* **1**: 43-47.

Shetty PK and Magu SP. 1997. Effect of pendimethalin on soil respiration and enzyme activities in the rizosphere of wheat. *Indian Journal of Environment and Toxicology* **7**: 39-41.

Shukla AK and Mishra RR. 1997. Influence of herbicides in microbial population and enzyme activity in potato (*Solanum tuberosum*) field soil. *Indian Journal of Agricultural Sciences* **67**: 610-611.

Solomon MG. 1999. The effect of atrazine application on some soil chemical and biological properties of acid sands of calabar. *Global Journal of Pure and Applied Sciences* **5**: 5-7.

Stotzky G. 1965. Microbial respiration. In : C.A. Black et al. (ed.) *Methods of soil analysis, Part 2*. Agronomy. American Society of Agronomy, Inc., Madison, Wisconsin : 1550-1572.

Subba Rao NS. 1988. *Biofertilizers in agriculture*, Second edition, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi 110 001 :189-202.

Wani SP. 1990. Inoculation with associative nitrogen fixing bacteria in cereal grain production improvement. *Indian Journal of Microbiology* **30**: 363-93.

Yu LQ, Zhang L, He SY and Zhu ZG. 1993. Limit action on microorganisms and effect on rice production of butachlor-fertilizer. *Chinese Journal of Rice Science* **7**: 55-57.