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Effect of Butachlor on Biochemical Process in Soil

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Authors' contributions

This work was carried out in collaboration between both authors. Author SC managed the literature searches, wrote the first draft of the manuscript and managed the analyses of the study. Author SP designed the study and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Butachlor is a very commonly used pre-emergence herbicide. In the present study, the effect of butachlor at three different application rates (0.5, 1.0 and 2.0 kg a.i. / ha) on the activities of some soil enzymes, microbial population and transformation and availability of C, N and P in an alluvial soil has been analyzed. The result showed that in general, the application of butachlor has significantly increased activities of most of the enzymes as well as the microbial biomass with greater retention, mineralization and availability of oxidizable C, N and P in soil. The stimulation in activities of enzymes dehydrogenase and phenol oxidase was found to be more pronounced when the herbicide was applied at half recommended field dose (hRFD). On the other hand, phosphatase, arylsulphatase and phenol oxidase were pronounced at double recommended field dose (dRFD). As compared to untreated control soil, the application of butachlor induced higher proliferation of total bacteria at recommended field dose (RFD), fungi at hRFD and actinomycetes at dRFD. Regarding the availability of plant nutrients, we also found that the greater retention of total N, exchangeable NH_4^+, and soluble NO_3^- was achieved by applying butachlor at RFD and that of organic C and available P by applying butachlor at dRFD. The correlation analysis showed

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significant positive correlation between hydrolase enzymes, phosphatase and arylsulphatase (r = 0.74), oxidoreductase enzymes, phenol oxidase and peroxidase (r = 0.706). The fungal population is related to total bacteria (r =0.73), actinomycetes (r = 0.50) and available P (r = 0.65) in soil. The results of the present investigation thus indicate that the application of butachlor significantly induces the growth and activities of microorganisms, resulting in greater retention, mineralization and availability of oxidizable C, N and P in soil but that the stimulation is depend on neither concentration of herbicide nor time of incubation.

Keywords: Pre-emergence; dRFD; hRFD.

1. INTRODUCTION

Herbicides are applied to control weeds at both the pre-emergence stage, via incorporation in soil and at the post emergence stage, via foliar application as well. In modern agriculture, the use of herbicides for combating unwanted weeds in crop fields has been increasing steadily. Since microorganisms are scavengers in soil and possess physiological variability, they degrade a variety of chemical substances including herbicides in soil to derive energy, C and nutrients for their growth and metabolism [1]. As a result microbial biomass increases [2], which in turn produces an enormous number of soil enzymes of different distinct chemical classes that are involved in the transformation of plant nutrients in soil [3]. Butachlor (N-butoxymethyl-2-chloro-2',6'-diethylacetanilide) is a selective choloroacetanilide herbicide that is used for the control of a variety of undesirable grasses and selective broad-leaved weeds in transplanted and direct seeded rice as well as in other crops. The dissipation [4,5], adsorption [6], mobility [7], photodegradation [8], bio-degradation [9] and bioavailability [10,11] of butachlor in soil have been well investigated. Soil microorganisms that are depleted because of single application of butachlor are rapidly restored due to repeated application of this herbicide. Butachlor had a notable inhibition to methane producing bacteria but no inhibition effect on hydrolytic, fermentative, hydrogen-producing and sulphate-reducing bacteria. In addition, Jena et al. [12] and Patnaik et al. [13] showed that butachlor at low concentration stimulate the activity of nitrogenase.

2. MATERIALS AND METHODS

An alluvial cultivable soil was collected from 0-15 cm depth from Mondouri Teaching Farm of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal After collection, the soil was air-dried at 30-35℃ in shade, ground and passed through a 2 mm sieve.

2.1 Chemical Analysis of Experimental Soil

2.1.1 Soil moisture

To measure the moisture content, about 50 g of soil sample was placed in an aluminium box and dried in an electric oven for over-night (at 105℃) to a constant weight. The percentage of moisture was calculated from the loss in weight of the soil and the result was expressed on an oven dry basis.

2.1.2 Soil pH

The pH of the soil was determined in soil-water (at 1: 2.5 ratio) suspension using Beckman's glass electrode with pH-meter.

2.1.3 Organic carbon

Organic carbon content of soil samples were estimated following Walkley and Black [14] wet digestion method.

2.1.4 Total nitrogen

Total nitrogen content of the soil samples were estimated following Kjeldahl's modified salicylic acid method [15].

2.1.5 Inorganic nitrogen

Soil samples were analyzed to determine the available inorganic mineral nitrogen (exchangeable NH_4^+ and soluble NO_3^-) content in 2N potassium chloride extract through distillation.

2.1.6 Exchangeable ammoniacal nitrogen

Exchangeable ammoniacal nitrogen was estimated from potassium chloride extract of the soil. Ten grams wet soil were placed in a 250 ml capacity conical flask and extracted with 100 ml of 2N KCl solution by shaking with the help of a mechanical shaker for 30 minutes. The soil particles were allowed to settle down and the contents were filtered through Whatman filter

paper (No. 1). In a distillation flask, 50 ml of the KCl extract was taken and the volume was made up to 450 ml with distilled water. The aliquot was distilled with powdered ignited magnesium oxide to collect approximately 250 ml distillate in 4% boric acid solution mixed with bromocresol green and methyl red indicator, which was then titrated against a standard H_2SO_4 solution at normal temperature.

2.1.7 Soluble nitrate nitrogen

After the estimation of soluble ammoniacall nitrogen, the flask was cooled and 0.5 g of Devarda's alloy was added. It was then distilled again and the distillate was collected in 4% boric acid solution mixed with bromocresol green and methyl red indicator, which was then titrated against a standard H_2SO_4 solution as stated above.

2.1.8 Available phosphorus

The available phosphorus content of the soil sample was determined using Olsen's extractant as outlined by Jackson et al. [15].

2.1.9 Total phosphorus

The content of total phosphorus in the soil samples was estimated following the method as described by Jackson et al. [15].

2.1.10 Pot culture experiment

The processed soil (1 kg each) was then put into polythene pots and used for the experiment. To study the effect of herbicides on soil microorganisms, soil enzymes activity and nutrient transformation in soil, a pot culture experiment was conducted under laboratory conditions with the herbicide butachlor applied to said soil in three doses viz. recommended field dose (RFD), half of the RFD (hRFD) and double the RFD (dRFD). The water content of the soil was adjusted to 60% of water holding capacity of the soil and it was maintained throughout the experiment by the periodic addition of distilled water. To avoid photo-degradation of the herbicide and evaporation loss of water from the soil, the pots were kept covered with black polythene sheet and were incubated in the dark at 30±1℃ for 80 days. All the treatments were replicated three times.

2.1.11 Soil sampling

Soil samples were collected at periodic intervals [0(1h), 15, 30, 45, 60 days] from the replicated pots of each treatment following the method as described by Das and Mukherjee [16]. The subsamples were immediately analyzed to determine microbial population, enzymatic activities and biochemical transformations.

2.1.12 Analysis of microbial population

Soil samples were analyzed to enumerate the colony forming units (CFU) of total bacteria, actinomycetes and fungi following serial dilution technique and pour plate method. The agar plates were incubated at 30±1℃ for 7 days and the CFU on the respective plates were counted.

Medium used for total bacteria, total actinomycetes and fungi were respectively,

- Thronton's agar medium was used to count the total bacteria in soil.
- Jensen's agar medium was used for counting the number of total actinomycetes population in soil.
- Martin's Rose Bengal medium was used for counting of fungi in soil.

2.2 Estimation of Activities Soil Enzymes

2.2.1 Arylsulphatase activity

Arylsulphatase activity was measured following the method of Tabatabai and Brenner [17] by adding acetate buffer (pH 5.8, 4ml), toluene (0.2 ml), and 25 mM p-nitrophenyl (PNP-S) sulphate (1 ml) to 1 g soil sample. The mixture was then incubated at 37℃ for 1 hour. Then 0.5 M $CaCl_2$ (1ml) and 0.5 M NaOH (4ml) were added. After shaking, the mixture was filtered through Whatman filter paper (No. 42) and p-nitrophenol (PNP) liberated into the filtrate was measured spectrophotometrically at 410 nm. The arylsulphatase activity was expressed in terms of pg PNP formed g^{-1} h^{-1}.

2.2.2 Phosphatase activity

The method was based on the determination of p-nitrophenol (PNP) released after the incubation of soil with p-nitrophenol phosphate (PNP-P) for 1 hour at 37℃ [18]. One gram soil was dispersed in a 50 ml volumetric flask, 4 ml distilled water and 1 ml 115 mM p-nitrophenol phosphate was added to it. It was then incubated for 1 hour at 37℃. After incubation, 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH were added to the flask. The contents of the flasks were mixed thoroughly and then filtered through Whatman filter paper No. 42. The amount of PNP released

from PNP-P was measured at 410 nm using a spectrophotometer. The phosphatase activity was expressed in terms of pg PNP formed g^{-1} h^{-1}.

2.2.3 Phenol oxidase and peroxidase activity

Phenol oxidase and peroxidase activity of soil were measured with slight modification. One gram soil was dispersed into a 30 ml centrifuge tube and treated with 5 ml (conc.) acetate buffer (pH 5.8). The mixture was vortexed in a Vortex cyclomixer for 5 minutes. For the determination of phenol peroxidase activity, 2 ml suspension was mixed with 2 ml (5 mM) catechol solution and 0.2 ml (0.3%) hydrogen peroxide which served as a test solution. The control solution was made from 2 ml soil suspension, 2 ml acetate buffer and 0.2 ml (0.3%) hydrogen peroxide.

For the determination of phenol oxidase activity, a reaction mixture was prepared by combining 2 ml soil suspension and 2 ml catechol (5 mM) solution. Accordingly, the control solution was made with buffer only without any catechol solution. In each case the tubes were incubated at room temperature for one hour and thereafter centrifuged at 10,000 rpm for 30 minutes. Finally, the absorbance was read at 460 nm in a spectrophotometer. The phenol oxidase and peroxidase activity was expressed in terms of catechol oxidized g^{-1} h^{-1}.

2.2.4 Dehydrogenase activity

Soil dehydrogenase activity was estimated by reducing 2, 3, 5- triphenyltetrazoliumchloride. Five gram soil sample was mixed with 50 mg $CaCO_3$ and 1 ml 3% (w/v) 2, 3, 5- triphenyltetrazolium chloride (TTC) and incubated for 24 hours at 37℃. Dehydrogenase enzymes convert TTC to 2, 3, 5- triphenylformazan (TPF). The TPF formed was extracted by adding 50 ml methanol in each solution, shaking for 10 minutes and keeping it in the dark for 30 minutes. This process of extraction was repeated three times. The extracts were filtered and absorption was measured at 485 nm with a spectrophotometer. The dehydrogenase activity was expressed in terms of pg of TPF formed g^{-1} h^{-1}.

2.3 Statistical Analysis

The results were evaluated by analysis of variance (ANOVA) and the statistical significance ($p < 0.05$) of difference between means within factors (herbicide and sampling period) was evaluated using Fisher's LSD method [19].

3. RESULTS AND DISCUSSION

The study was undertaken in order to examine the impact of butachlor, applied at three different concentrations viz. 0.5 Kg a.i./ha (hRFD), 1 Kg a.i/ha (RFD), and 2 Kg a.i./ha (dRFD) in soil, maintained at 60% of its maximum water holding capacity, along with untreated control, on the activities of some important soil enzymes (e.g., dehydrogenase, phosphatase, arylsulphatase, phenol oxidase and peroxidase), soil microbial population (bacteria, fungi and actinomycetes) and nutrient (carbon, total-N,NH_3-N, NO_3-N and available phosphorus) transformation in soil.

3.1 Effect of Butachlor on Soil Enzymes

The dehydrogenase activity in response to butachlor application at RFD and dRFD was decreased by 9.62% and 49.28% respectively, but at lower application rate (hRFD), the dehydrogenase activity was stimulated by 25.96% over control (Table 1). Two-way ANOVA showed significant ($p < 0.05$) difference in the dehydrogenase activity between different treatments. It has also been recorded that the highest stimulation of the dehydrogenase activity was observable after 30 days of application of butachlor at hRFD and RFD while butachlor at higher dose (dRFD) registered a higher DHA immediately (1 hr.) after application.

Phosphatase activity, in contrast to dehydrogenase activity, was stimulated significantly with increasing butachlor application rate. The percent stimulation of phosphatase activity were 1.79, 5.55 and 9.24 with application rates at hRFD, RFD and dRFD, respectively. Our results of increase in phosphatase activity in response to butachlor are in agreement with Shukla [20]. Moreover, butachlor, applied at hRFD and RFD exerted a harmful effect on activity after 60 days of incubation as compared to untreated control (Table 2).

The response of arylsulphatase activity to varying application rates of butachlor is similar to that of phosphatase activity. However, unlike phosphatase activity, a significant gradual increase in arylsulphatase activity, regardless of treatments concerned, was noticed throughout the experimental period. This holds true with untreated control and butachlor applied at hRFD. In case of RFD and dRFD, finally an inhibition of activity was recorded 60 days after application (Table 3).

Phenol oxidase activity was induced by butachlor application at hRFD and RFD but dRFD produced an inhibitory effect. Similar to the

changes in dehydrogenase activity of soil, the phenol oxidase activity, irrespective of treatments concerned, was also gradually decreased up to 30 days, of sampling followed by a progressive increase up to the end of the experiment (Table 4).

Table 1. Effect of butachlor on dehydrogenase activity (pg of TPF formed g $^{-1}$ hr^{-1})

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	8.11	7.58	10.91	9.55	5.45	8.32
Butachlor	0.5	10.21	8.25	14.13	10.71	9.1	10.48
	1.0	9.65	7.1	12.18	4.84	3.85	7.52
	2.0	8.78	4.29	2.64	2.51	2.88	4.22
	Mean	9.18	6.80	9.96	6.90	5.32	
LSD							
	Treatment		**Sampling days**			**Treatment x days**	
S. E. (m)	0.226		0.202			0.452	
CD(p<.05)	0.647		0.579			1.295	

Table 2. Effect of butachlor on phosphatase activity (pg PNP formed g"^{1} hr"^{1})

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	576.68	531.25	588.07	675.6	732.8	620.88
Butachlor	0.5	619.2	610.57	592.8	683.31	654.22	632.02
	1.0	629.4	607.35	681.6	685.8	672.6	655.35
	2.0	646.85	639.27	673.98	666.69	766.8	678.72
	Mean	618.03	597.11	634.11	677.85	706.61	
LSD							
	Treatment		**Sampling days**			**Treatment x days**	
S. E. (m)	0.065		0.058			0.130	
CD(p<.05)	0.187		0.167			0.373	

Table 3. Effect of Butachlor on Arylsulphatase activity (pg PNS formed g"^{1} hr"^{1})

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	661.8	688.8	700.8	722.4	726.6	700.08
Butachlor	0.5	667.2	691.2	697.8	706.2	772.2	706.92
	1.0	673.8	686.4	716.4	778.8	748.8	720.84
	2.0	690	705	713	775.6	768	730.32
	Mean	673.2	692.85	707	745.75	753.9	
LSD							
	Treatment		**Sampling days**			**Treatment x days**	
S. E. (m)	1.291		1.155			2.582	
CD(p<.05)	3.696		3.306			7.392	

Table 4. Effect of butachlor on phenol oxidase activity (pg catechol oxidized g^{-1} hr^{-1})

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	23.72	17.19	12.43	19.01	22.28	18.93
Butachlor	0.5	27.97	29.17	16.04	24.27	23.81	24.25
	1.0	25.6	19.29	15.33	22.31	19.73	20.45
	2.0	22.8	19.05	13.58	14.29	18.89	17.72
	Mean	25.02	21.18	14.35	19.97	21.18	
LSD							
	Treatment		**Sampling days**			**Treatment x days**	
S. E. (m)	1.291		1.155			2.582	
CD(p<.05)	3.696		3.306			7.392	

Contrary to phenol oxidase, peroxidase activity was stimulated by increasing rates of application. Maximum stimulation of activity occured with butachlor at dRFD (Table 5). It was also recorded that peroxidase activity, regardless of treatments, in contrast to oxidase activity, increased gradually up to 30 sampling days and decreased thereafter.

3.2 Effects of Butachlor on Soil Microbial Population

The effect of various treatments of butachlor on bacterial population in soil was compared. We found that bacterial population increased progressively with increasing concentration of applied butachlor. However, at higher application rate (dRFD) of butachlor, the population decreased below the value that was obtained from that with RFD. Two-way ANOVA showed that the effect of treatment on bacterial population was significant. Maximum stimulation was achieved by the treatment of this chemical at RFD followed by dRFD and hRFD. With respect to fungal population as affected by different treatments of this herbicide, maximum stimulation was achieved with dRFD (Table 7). Moreover, the stimulatory effect of butachlor applied at hRFD and RFD were similar. On the other hand, highest actinomycetes proliferation was registered with butachlor applied at hRFD while those with RFD and dRFD were similar (Table 8). In contrast, bacterial and fungal population of soil was affected by this herbicide whereas those of actinomycetes increased progressively up to 45 days of incubation and then decreased finally (Table 6).

3.3 Effects of Butachlor on Nutrient Transformation in Soil

The stimulation of microorganisms induced by the application of butachlor significantly increased the oxidizable C content of soil (Table 9). The effect was more pronounced when the herbicide was applied at dRFD followed by the treatment with hRFD. It was also observed that organic carbon content of soil was gradually increased up to 30 days of sampling and thereafter decreased followed by an increase.

The stimulation of microorganisms and their biochemical activities due to application of butachlor brought about greater mineralization of organic N, resulting in greater amounts of available N (Table 10) and subsequent loss of N from soil due to denitrification [21]. As compared

Table 5. Effect of butachlor on peroxidase activity (pg catechol oxidized g^{-1}) hr^{-1})

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	6.84	2.75	26.35	17.65	5.25	11.77
Butachlor	0.5	6.44	6.67	32.51	19.24	12.21	15.41
	1.0	12.88	22.37	16.95	23.23	6.91	16.47
	2.0	8.38	4.04	31.56	31.55	21.83	19.47
	Mean	8.63	8.96	26.84	22.92	11.55	
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	0.376		0.336		0.752		
CD(p<.05)	1.076		0.963		2.153		

Table 6. Effect of butachlor on bacteria (actual x 10^5)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	134.2	148.0	136.8	136.8	116.7	134.5
Butachlor	0.5	140.0	164.4	128.3	153.9	138.8	145.1
	1.0	137.5	172.0	137.1	174.1	129.8	150.1
	2.0	134.6	186.1	115.2	186.0	121.3	148.6
	Mean	136.6	167.6	129.3	162.7	126.6	
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	3.719		3.326		7.438		
CD(p<.05)	10.648		9.524		21.295		

Table 7. Effect of butachlor on fungi (actual x 10^4)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	18.0	17.1	16.2	17.8	16.6	17.1
Butachlor	0.5	17.8	21.3	19.0	20.2	20.8	19.8
	1.0	17.1	20.0	16.0	24.3	19.4	19.4
	2.0	17.1	27.0	17.4	21.5	20.5	20.7
	Mean	17.5	21.4	17.1	20.9	19.4	
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	1.084		0.969		2.168		
CD(p<.05)	3.103		2.775		6.206		

Table 8. Effect of butachlor on actinomycetes (actual x 10^5)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	136.0	138.0	142.2	134.6	140.3	138.2
Butachlor	0.5	139.8	193.3	193.7	199.2	176.7	180.5
	1.0	132.5	161.3	201.3	203.9	175.8	175.0
	2.0	136.1	170.5	185.2	211.0	175.6	175.6
	Mean	136.1	s165.8	180.6	187.2	167.1	
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	3.776		3.377		7.552		
CD(p<.05)	10.810		9.669		21.620		

Table 9. Effect of butachlor on organic-C (g/kg)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	8.43	8.35	8.21	8.00	8.72	8.34
Butachlor	0.5	8.58	9.53	8.92	8.28	8.26	8.71
	1.0	8.64	8.64	8.92	8.48	8.31	8.60
	2.0	8.49	8.87	9.85	8.28	8.55	8.81
	Mean	8.53	8.85	8.97	8.26	8.46	
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	0.055		0.049		0.110		
CD(p<.05)	0.157		0.140		0.314		

to untreated control soil, application of butachlor at higher concentrations retained higher amount of total N in soil more so when the herbicide was applied at RFD, followed by application of herbicide at dRFD. Moreover, application of butachlor at different concentrations significantly increased total N up to 30 days after application and decreased thereafter but finally increased. In addition, application of butachlor significantly augmented the availability of exchangeable NH4+ and soluble NO3- in soil (Tables 11 and 12). On an average, application of butachlor at RFD exerted the highest stimulation towards the availability of exchangeable NH4+ and soluble NO3-. The effect of sampling days at different treatments registered greater release of NH4+N and NO3-N after 15 and 60 days of sampling respectively.

The effect of different application rates of butachlor, regardless of sampling days, on changes in available P content of soil (Table 13) when considered, a progressive increase in available P was recorded with an increase in application rates of butachlor. It was also demonstrated that application of butachlor, exerted an alternate fall and rise in the release of available P in soil right from the beginning of the experiment.

Table 10. Effect of butachlor on total-N (g/kg)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	0.73	0.72	0.73	0.63	0.70	0.70
Butachlor	0.5	0.69	0.76	0.67	0.75	0.79	0.73
	1.0	0.73	0.78	0.76	0.75	0.77	0.76
	2.0	0.75	0.73	0.81	0.66	0.79	0.75
	Mean	0.72	0.75	0.75	0.70	0.76	0.74
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	0.006		0.005		0.012		
CD(p<.05)	0.017		0.015		0.034		

Table 11. Effect of butachlor on ammoniacal nitrogen (NH_3^- N) (mg/kg)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	36.3	40.4	45.2	47.9	45.3	43.0
Butachlor	0.5	38.2	78.4	53.7	56.0	45.6	54.4
	1.0	37.9	81.3	47.4	55.5	53.3	55.1
	2.0	36.8	72.8	47.4	57.5	46.4	52.2
	Mean	37.3	68.2	48.4	54.2	47.7	51.16
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	1.025		0.917		2.050		
CD(p<.05)	2.935		2.625		5.870		

Table 12. Effect of butachlor on nitrate nitrogen (NO_3^-N) (mg/kg)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	39.6	35.7	36.6	35.3	34.5	36.4
Butachlor	0.5	38.7	36.5	48.7	33.9	35.5	38.7
	1.0	39.4	41.2	45.8	38.3	51.0	43.1
	2.0	37.5	36.0	35.0	41.2	45.5	39.0
	Mean	38.8	37.3	41.5	37.2	41.7	39.3
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	0.766		0.685		1.532		
CD(p<.05)	2.193		1.962		4.387		

Table 13. Effect of butachlor on available P (mg/kg)

Treatment	Concentration (pg a.i. ha^{-1})	Sampling days					Mean
		0	15	30	45	60	
Control	0	2.44	3.23	2.43	2.55	2.19	2.57
Butachlor	0.5	2.66	3.38	2.18	2.98	2.76	2.79
	1.0	2.58	3.70	2.18	3.31	2.38	2.83
	2.0	2.75	3.36	2.08	3.80	2.95	2.99
	Mean	2.61	3.42	2.22	3.16	2.57	2.79
LSD							
	Treatment		**Sampling days**		**Treatment x days**		
S. E. (m)	0.065		0.058		0.130		
CD(p<.05)	0.187		0.167		0.373		

Table 14. Correlation data (r values) among different variables

	Phosphatase	Phenol Oxidase	Arylsulphatase	Peroxidase	Dehydrogenase	NH_3-N	NO_3-N	Total-N	Organic C	Available P	Bacteria	Actinomycetes	Fungi
Phosphatase	1												
Phenol Oxidase	-0.008	1											
Arylsulphatase	0.740**	-0.276	1										
Peroxidase	0.204	-0.706**	0.343	1									
Dehydrogenase	-0.434	0.158	-0.576**	-0.088	1								
NH_3-N	-0.012	0.008	0.108	0.045	-0.207	1							
NO_3-N	0.1	-0.248	0.17	0.129	0.027	0.039	1						
Total-N	0.185	0.064	0.062	-0.1	-0.287	0.155	0.05	1					
Organic C	0.018	0.001	-0.147	0.042	-0.144	0.314	0.023	0.393	1				
Available P	-0.128	0.145	0.135	-0.051	-0.355	0.609**	0.175	0.002	-0.16	1			
Bacteria	-0.214	0.073	0.069	-0.031	-0.194	0.688**	0.161	-0.133	-0.109	0.875**	1		
Actinomycetes	0.326	-0.209	0.530*	0.342	-0.179	0.499*	0.302	0.24	0.313	0.262	0.372	1	
Fungi	0.154	0.145	0.333	-0.121	-0.431	0.642**	0.035	0.107	0.042	0.651**	0.729**	0.501*	1

***. Correlation is significant at the 0.01 level (2-tailed)*

**. Correlation is significant at the 0.05 level (2-tailed)*

4. SUMMARY AND CONCLUSION

This experiment was conducted under laboratory conditions to examine the effects of butachlor at three application rates (0.5, 1.0 and 2.0 Kg a.i/ha), on the changes of growth and proliferation of some microorganisms (bacteria, fungi and actinomycetes) in relation to some enzyme activities (dehydrogenase, phosphatase, arylsulphatase, phenol oxidase and peroxidase) of soil along with the transformation and availability of C, N and P in an alluvial soil. In general, application of butachlor, significantly increased the microbial biomass and most of the enzyme activities. This was mainly due to greater retention, mineralization and availability of oxidizable C, N and P in soil. The stimulation of activities of dehydrogenase and phenol oxidase were more pronounced when the herbicide was applied at hRFD while that of phosphatase, arylsulphatase and phenol peroxidase at dRFD. As compared to untreated control soil, the application of butachlor registered higher proliferation of total bacteria at RFD, fungi at hRFD and actinomycetes at dRFD. Regarding the availability of plant nutrients, the greater retention of total N, exchangeable NH_4^+, and soluble NO_3^{--} was achieved by butachlor applied at RFD and that of organic C and available P by dRFD. However, the stimulation of soil enzyme activity, microbial population and the retention, mineralization and availability of oxidizable C, N and P were not consistent throughout the experimental period.

Soil is a heterogeneous system and is therefore difficult to generalize the influence of a foreign chemical on soil enzymes, microbial biomass and nutrient transformation with the help of a single soil and limited application rates. Therefore, a comprehensive approach involving varying types of soil and concentrations of chemical would be appropriate to understand the kinetic properties of soil enzymes in relation to microbial diversity and biomass carbon. This in turn would help in better understanding of the scenario of soil health and taking preventive measures for coping up with the reduction in quantity and quality of agricultural production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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