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Effect of Pretilachlor on Soil Enzyme Activities in Tropical Rice Soil

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Abstract Pretilachlor treatments, namely, recommended dose at 600 g a.i. ha^{-1} (RD), double the recommended dose at 1200 g a.i. ha^{-1} (2RD), ten times of the recommended dose at 6000 g a.i. ha^{-1} (10RD) along with control, were used to study the effects of pretilachlor on soil enzymes in tropical rice soil. Pretilachlor, at recommended dose completely dissipated 30 days after herbicide application. Twenty days after herbicide application, the dehydrogenase activity was inhibited up to 27%, 28% and 40% of initial values of RD, 2RD and 10RD treatments, respectively. Increase in fluorescein diacetate hydrolase activity was observed during the first 25 days post herbicide application up to 29%, 36% and 10% of initial values of RD, 2RD and 10RD treatments, respectively. β-Glucosidase activity in the experiment did not provide a specific trend. In general, urease and acid phosphatase activities were not influenced by pretilachlor application. There were significant differences in alkaline phosphatase activities among the treatments until 25 days after herbicide application. Hence, pretilachlor may cause short term transitory changes in soil enzyme parameters. However, it has negative impact on soil enzymes at very high dose.

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Soil enzymes are bio-indicators of soil management practices. They reflect microbial activities of soil. It is generally assumed that soil enzyme activities are much more sensitive towards soil degradation than chemical or physical parameters of soil (Dick 1994). Variations in enzyme activities can occur by external application of xenobiotics. Herbicide application can alter growth of microorganisms, thereby soil enzyme activities (Monkiedje et al. 2007). Besides the type of soil and agricultural practices, soil enzyme activities depend on the physico-chemical properties and quantity of the pesticide applied (Ramudu et al. 2011; Singh and Singh 2005; Ingram et al. 2005).

Soil enzyme activities, namely, fluorescein diacetate (FDA) hydrolase and β-glucosidase have critical role in C mineralization. Similarly, urease and phosphataes (acid and alkaline) play critical role in N and P cycle, respectively. Transformation of organic matter in soil is carried out by β-glucosidase, cellulase, and invertase enzymes to provide energy to microorganisms (Tabatabai 1994). FDA hydrolase is considered as an accurate expression of the total microbial activity (Das et al. 2011). Fluorescein diacetate (3′,6′-diacetylfluorescein) has been used to indicate indirectly the amounts of active fungi and bacteria present in soil. Dehydrogenases occur intracellularly in all living microbial cells and are an indicator of microbial activity (Wlodarczyk et al. 2002). Urease is a key component in the nitrogen cycle as it catalyzes the hydrolysis of urea into CO_2 and NH_3. Urease activity is found in a large number of soil bacteria and fungi (Sarathchandra et al. 1984). Phosphatases, exocellular

enzymes, are responsible for hydrolysis of organic P compounds to available inorganic P.

Despite selective mode of action of herbicides, non-target organisms in soil may also get affected. This can lead to reduction in microbial diversity and possible decrease in soil fertility (Johnsen et al. 2001). Pretilachlor [2-chloro-2′,6′-diethy1-*N*-(2-propoxyethyl) acetani1ide], a member of the acetanilide group of herbicides, is used for selective control of annual weeds in rice fields (Saha et al. 2012). Pretilachlor application reduced weed density and increased grain and straw yields in rice (Faruk et al. 2013; Rashid et al. 2012). Studies on effect of pretilachlor on soil enzymes are limited. Saha et al. (2012) reported enhanced dehydrogenase, β-glucosidase activity and decreased urease activity on the application of pretilachlor. Earlier, different researchers have reported increase, decrease or unchanged enzyme activity following various pesticide applications (Hussain et al. 2009). Earlier, we have reported recommended dose of pretilachlor did not record any significant changes in microbial properties of rice soil compared to control (Sahoo et al. 2016). The present investigation was carried out to determine any dose dependent negative effect of pretilachlor on soil enzyme activities in rice planted soil.

Materials and Methods

Analytical grade chemicals and solvents from Merck, India were used. Pretilachlor formulation (Craze® 50EC, Dhanuka Agritech Limited, Gurgaon, India) was purchased from local market. The persistence of pretilachlor in rice cultivated soil was studied during *Rabi* (winter) season of 2014–2015 in a pot experiment at National Rice Research Institute, Cuttack (20°27′10″N, 85°56′9″E; 24 m above mean sea level), in the eastern part of India. The soil was an Aeric Endoaquept (USDA, 1999) with sandy clay loam texture. The experiment was simulated as aerobic rice condition. The experiment was conducted with three treatments of pretilachlor, namely, recommended dose at 600 g a.i. ha^{-1} (RD), double the recommended dose at 1200 g a.i. ha^{-1} (2RD), ten times of the recommended dose at 6000 g a.i. ha^{-1} (10RD) along with untreated control, where no pesticide was applied. The 10RD dose was used to evaluate the potential hazards of pretilachlor on soil enzymes during undesirable events such as spillover effect, damage of devices, uncontrolled disposal and wash of spray equipments (Cycoń and Piotrowska-Seget 2015). Rice (var. Naveen, *Indica* type) seeds were sown in plastic pots (20×20 cm), each pot containing 4 kg of soil. Pretilachlor was applied as pre-emergence herbicide on second day of sowing to each pot as per the dose. Soil moisture was maintained at field capacity level. Recommended fertilizer application (N:P:K::100:50:50) was followed i.e. half dose of N and full dose of P and K were applied as basal at the time of filling up the pots. Remainder of the N was applied in two equal applications, once at tillering stage and another at panicle initiation stage. Each treatment was replicated thrice in a Completely Randomized Design. Each set of pots was removed at regular intervals [0 (before spray), after spray (2 h after spray), 1, 3, 5, 10, 15, 20, 25, 30, 45 days after pesticide spray] for quantification of pretilachlor residues from soil. Soil samples of 0 (before spray), 5, 10, 15, 20, 25, 30, 45 days were used to quantify different soil enzyme activities.

Pretilachlor was extracted from soil as per Sahoo et al. (2016). Soil sample (200 g) was collected from each treatment and 5 g of sub samples were transferred into 15 mL of Polytetrafluoroethylene centrifuge tubes. 7 mL of acetone was added into it. The tubes were shaken thoroughly on a horizontal shaker for 10 min. The contents were centrifuged at 3500 rpm for 10 min and the supernatant was transferred into conical flasks. Soils were re-extracted two more times using fresh 5 mL acetone each time. Acetone extracts were pooled and clean-up of the extracts were performed using PSA (25 mg) + Na_2SO_4 (100 mg). The contents were mixed in a vortex mixer followed by centrifugation at 3000 rpm for 10 min. Supernatants were filtered and evaporated to near dryness and 2 mL hexane was added for GC-ECD injection. Pretilachlor was analyzed in Agilent 6820 gas chromatograph (GLC) equipped with capillary column, HP-5 (30 m×0.25 mm×0.25 µm) and electron capture detector (ECD). The operating parameters of the instrument were: oven temperatures 100°C (1 min) → 15°C min^{-1} → 220 (2 min) → 10°C min^{-1} → 250°C (2 min), injection port at 200°C and detector at 300°C. Flow rate of nitrogen (carrier gas) was 30 mL min^{-1}, but through column it was 1 mL min^{-1} and injection was done in splitless mode. Under these operating conditions the retention time of pretilachlor found to be at 12.80 min with more that 80% recovery at different fortification levels. The coefficient of regression (r^2) for solvent matched calibration graph was >0.995 for pretilachlor. Matrix effect was <5% for different concentrations of pretilachlor in soil. The limit of detection (LOD) for pretilachlor was 0.01 µg g^{-1} of soil (signal:noise::3). The limit of quantitation (LOQ) for the method was 0.02 µg g^{-1} of soil (signal:noise::10).

Dehydrogenase activity (DHA) was determined by reduction of triphenyl tetrazolium chloride (TTC) (Casida et al. 1964). Soil samples were treated with $CaCO_3$ and TTC and incubated for 24 h at 37°C. The triphenyl formazan (TPF) was extracted from the reaction mixture with methanol and assayed at 485 nm. FDA hydrolase activity was measured by the potassium phosphate buffer method (pH 7.6) followed by extraction with chloroform/methanol (2:1 v/v) as described by Adam and Duncan (2001). β-Glucosidase activity was assayed by treating soil sample with toluene, modified universal buffer (pH 6.0) and

p-nitrophenyl-β-D-glucoside solution (Eivazi and Tabatabai 1977). After 1 h of incubation at 37°C, 0.5 M $CaCl_2$ and 0.1 M Tris (hydroxymethyl) amino methane buffer pH 12 was added. The suspension was filtered and the filtrate was measured at 420 nm. The amount of residual urea present in the soil suspension upon incubation was determined by non-buffer method of Zantua and Bremner (1977). Alkaline and acid phosphatase activities (Eivazi and Tabatabai 1977) were assayed by treating soil sample with toluene, Modified Universal Buffer (pH 6.5 for acid phosphatase and pH 11.5 for alkaline phosphatase) and *p*-nitrophenyl phosphate solution. After 1 h of incubation at 37°C, 0.5 M $CaCl_2$ and 0.5 M NaOH solution was added. The suspension was filtered, and the color intensities of filtrates were measured at 420 nm.

Data were analyzed following analysis of variance (SAS Software packages, SAS EG 4.3) and means of treatments were compared based on Tukey's minimum significant difference test (MSD) at 0.05 probability level.

Results and Discussion

The initial deposits of pretilachlor were 0.174, 0.968, 3.35 µg g^{-1} of soil for RD, 2RD and 10RD, respectively (Fig. 1). Pretilachlor residues declined to <0.02 µg g^{-1} within 30 days after herbicide application for RD treatment. Pretilachlor residues were found to be 0.023 and 0.533 µg g^{-1} soil at 45 days post herbicide application for 2RD and 10RD treatment, respectively. The dissipation of pretilachlor followed first order dissipation model. The half life values were 16.90, 17.76 and 36.47 days for RD, 2RD and 10RD treatments, respectively (Sahoo et al. 2016). Earlier in a field study, pretilachlor at 0.75 kg a.i. ha^{-1} dissipated to below detectable level (i.e. 0.005 µg g^{-1}) within 30 days after application (Dharumarajan et al. 2011). Dissipation of pretilachlor was observed in paddy field soil with half life of 7.52–9.58 days, respectively (Kaur et al. 2015). Acetanilide herbicides were degraded quickly by soil microbes (Sahid and Wei 1993). Dissipation of pretilachlor in this experiment may be due to the presence of soil microbes in combination with aqueous photolysis. Earlier author reported that photodecomposition hastened the degradation rate of pretilachlor (Kaur et al. 2015). Degradation of pretilachlor was enhanced under conditions of increasing soil temperature and moisture levels (Ismail and Handah 1999). In our earlier research, we reported soil pH, EC and organic carbon content of initial soil were 7.66%, 0.233% and 0.599%, respectively (Sahoo et al. 2016). pH, EC and organic carbon content varied from 6.39 to 6.69, 0.212–0.259 dS m^{-1} and 0.643%–0.414% among the treatments at 45 days after herbicide application.

Measurements of dehydrogenase activity represent immediate metabolic activities of soil microorganisms (Nannipieri et al. 2003). Dehydrogenase activities were inhibited by the application of pretilachlor during the experimental period (Fig. 2). Dehydrogenase activity in treated soil decreased over time compared to control. At 20 days after herbicide application, DHA was inhibited by 27%, 28% and 40% compared to initial values for RD, 2RD and 10RD treatments, respectively. Control soil had improved DHA compared to initial soil after 20 days of pretilachlor application. Dehydrogenase activity declined rapidly in

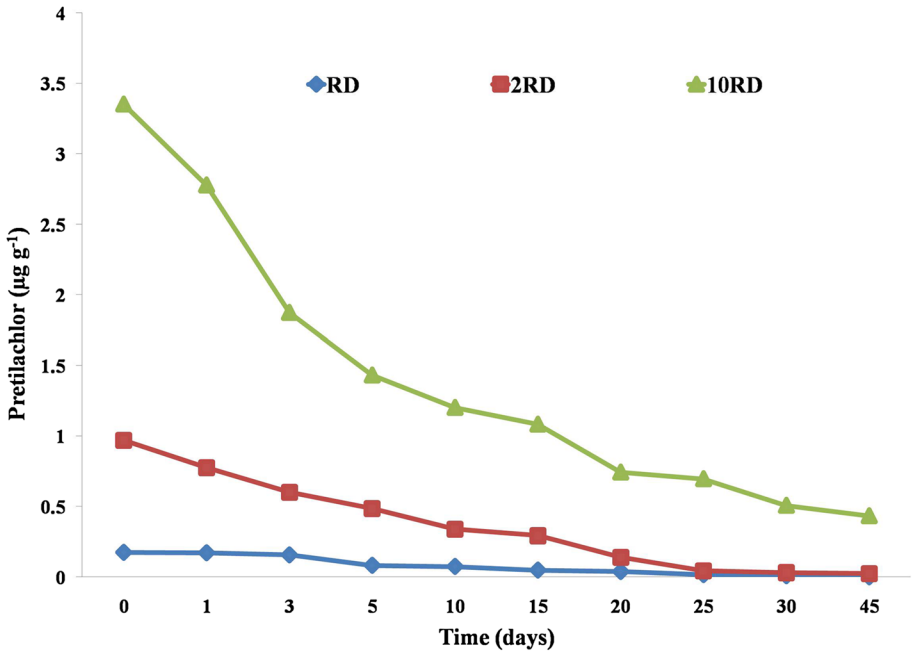

Fig. 1 Persistence of pretilachlor (µg g^{-1}) in tropical rice soil

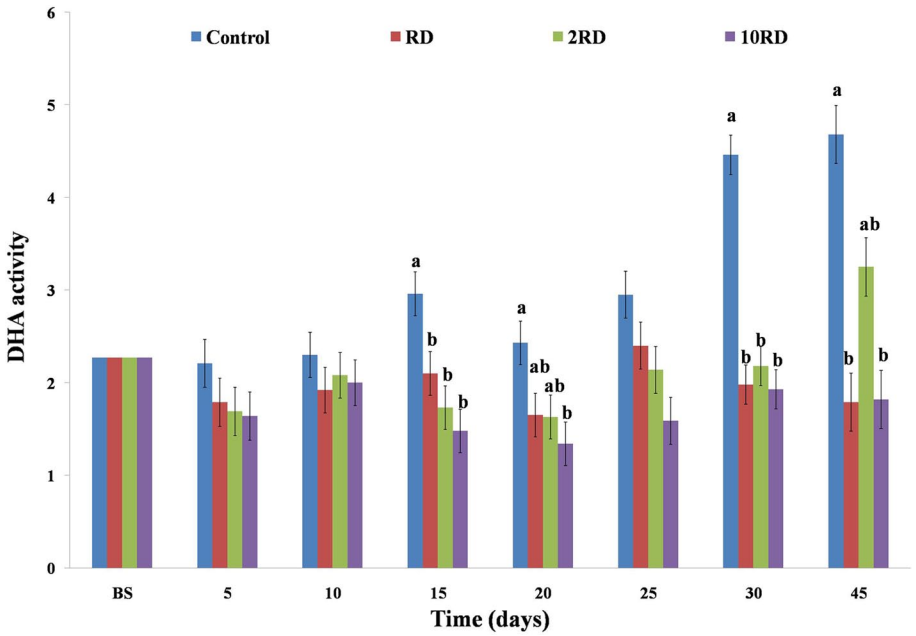

Fig. 2 Effect of pretilachlor on DHA (mg g^{-1} soil h^{-1}) (*Figures without letter* are nonsignificant at $p \leq 0.05$)

10RD treatment and was less compared to initial soil. Earlier reports are divided on stimulatory or negative effect of herbicides on soil enzyme activity. Saha et al. (2012) reported that pretilachlor stimulates the dehydrogenase activities. Butachlor enhanced the activity of dehydrogenase at increasing concentrations (Min et al. 2001). Contrary to them, higher concentration of acetochlor resulted in reduction in soil dehydrogenase activity (Cai et al. 2007). Similarly our results indicated that higher dose had more toxic effect on DHA and in turn the microbial activity of soil.

FDA activities were affected due to herbicide application for the RD treatment during the first 20 days post herbicide application (Fig. 3). At 10RD, FDA activities were restricted severely until 45 days after herbicide application. At 25 days after herbicide application, FDA activity for recommended dose was comparable with control soil. There was an increase of 29 %, 36 %, 10 % and 36 % FDA activity from initial values for RD, 2RD, 10RD and control treatments after 25 days of pretilachlor application, respectively. Compared to control, the FDA activity

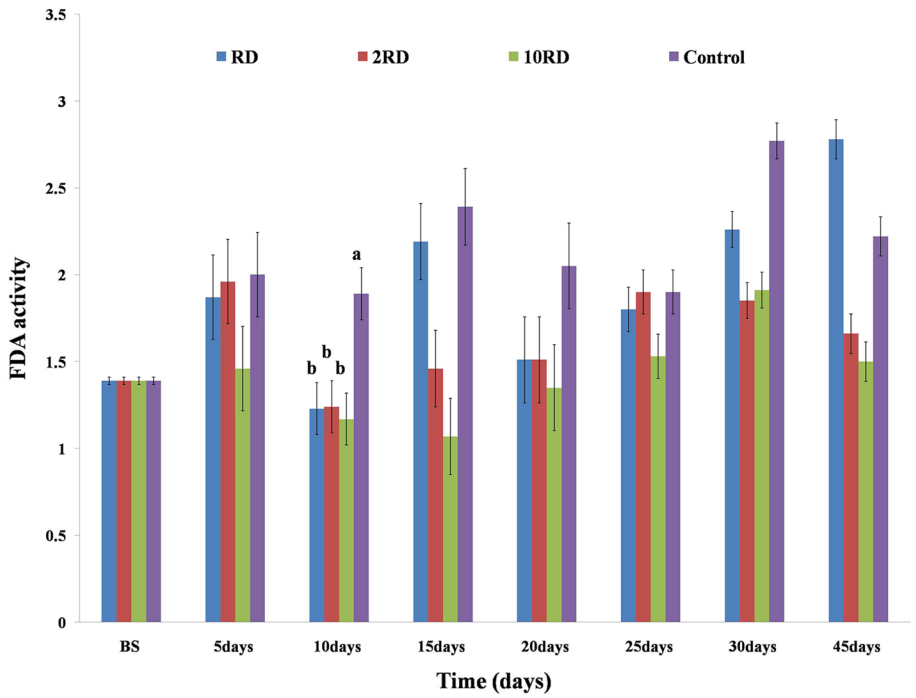

Fig. 3 Effect of pretilachlor on FDA (μg g^{-1} soil h^{-1}) (*Figures without letter* are nonsignificant at $p \leq 0.05$)

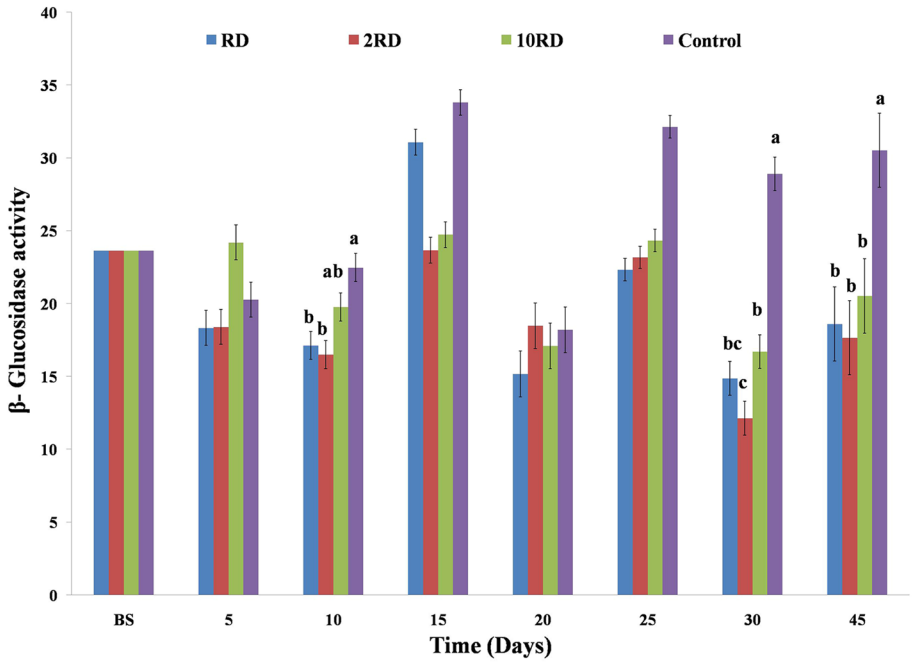

Fig. 4 Effect of pretilachlor on β-glucosidase activity (μg g^{-1} soil h^{-1}) (*Figures without letter* are non-significant at $p \leq 0.05$)

was −5 %, 0 % and −19.5 % for RD, 2RD and 10RD treatments, respectively during the first 25 days post herbicide application. Earlier it has been reported that FDA had been used to indicate indirectly the amounts of active fungi and bacteria present in soil (Schnurer and Rosswall 1982). The decrease in enzyme activity with increase in concentration is due to the inhibition of pretilachlor on microorganisms. This leads to less quantity of available enzymes in soil. Similar findings have been reported with profenofos (Kalam et al. 2004).

The hydrolysis products of β-glucosidase usually serve as energy sources for microorganisms in soil. β-Glucosidase activity in the experiment did not provide a specific trend (Fig. 4). There was non-significant difference among the treatments until 5 days after herbicide application. However, 10RD treated soil had maximum concentration of β-glucosidase activity. Higher values compared to control may be explained by increased availability of pesticide as carbon source material. Similar findings have been reported by Saha et al. (2012). β-Glucosidase activities was decreased

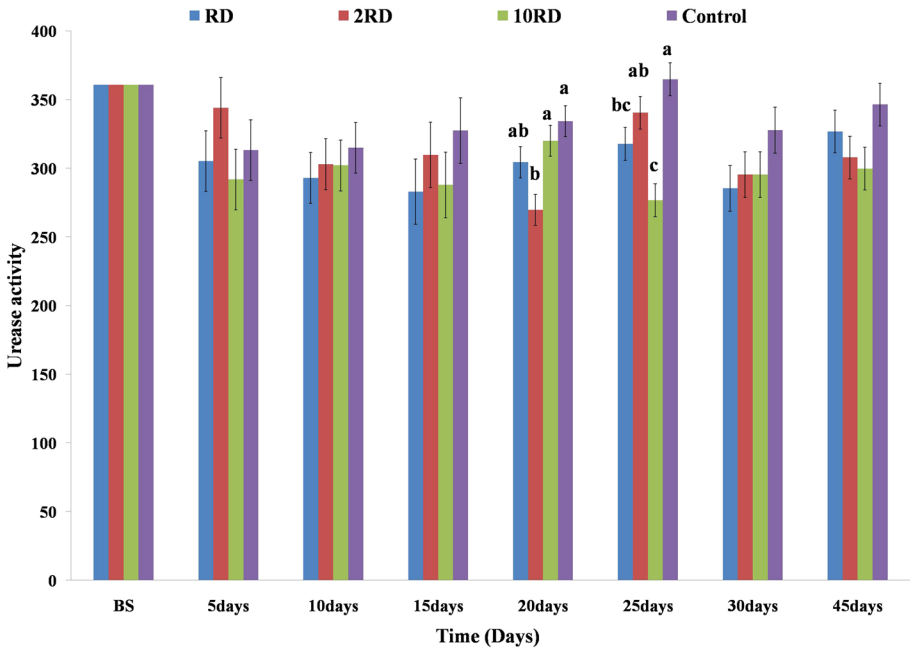

Fig. 5 Effect of pretilachlor on urease activity (μg g^{-1} soil h^{-1}) (*Figures without letter* are non-significant at $p \leq 0.05$)

Table 1 Effect of pretilachlor on acid phosphatase and alkaline phosphatase (μg g^{-1} soil h^{-1})

Treatment	BS		5 days		10 days		15 days		20 days		25 days		30 days		45 days	
	Ac P	Al P	Ac P	Al P	Ac P	Al P	Ac P	Al P	Ac P	Al P	Ac P	Al P	Ac P	Al P	Ac P	Al P
RD	19.42	7.32	24.27	4.67^{b}	20.14	8.22^{a}	27.3	7.01^{ab}	29.59	8.00^{b}	26.1	10.20^{a}	36.43^{b}	6.6	36.90^{ab}	9.51
2RD	19.42	7.32	23.28	4.19^{b}	26.24	5.72^{ab}	23.42	6.17^{b}	22.95	8.67^{ab}	29.96	10.05^{a}	36.92^{b}	7.91	38.56^{ab}	9.74
10RD	19.42	7.32	25.36	3.92^{b}	18.21	4.73^{b}	16.2	5.47^{b}	20.98	8.53^{ab}	20.41	7.87^{b}	28.16^{b}	7.53	30.34^{b}	10.23
Control	19.42	7.32	28.3	5.94^{a}	26.7	7.78^{a}	25.43	11.20^{a}	24.74	10.16^{a}	35.16	11.79^{a}	47.31^{a}	9.7	47.10^{a}	13.2
Tukey HSD at 5 %	NS	NS	NS	0.9	NS	2.69	NS	4.92	NS	1.89	NS	1.81	9.22	NS	10.37	NS

BS before spray, *Ac P* acid phosphatase, *Al P* alkaline phosphatase (means with same letter are not significantly different at $p \leq 0.05$)

compared to control 5 days after herbicide application. The reason could be inhibitory effect of pretilachlor on microorganisms. This led to less β-glucosidase activities in soil.

Urease is released from living and disintegrated microbial cells, and in the soil it can exist as an extracellular enzyme absorbed on clay particles or encapsulated in humic complexes (Marcote et al. 2001). Pretilachlor application inhibited urease activities. Though, non-significant interaction was observed among the treatments. Except for few cases urease activities was not influenced by pretilachlor application and its higher dose also did not change the dynamics of urease activities (Fig. 5). Before spray, the soil had 360.84 μg g^{-1} soil h^{-1} of urease activities, which changed over the time and reported to have 299.80–346.36 μg g^{-1} soil h^{-1} of urease activity. Different researchers have reported that urease activity following various pesticide applications may remain either unchanged, increase or decrease (Hussain et al. 2009). Ismail et al. (1998) reported a lower activity of urease due to the application of metsulfuron-methyl. Other herbicides such as chlorbromuron and S-ethyl dipropyl(thiocarbamate) have shown stimulatory effects on urease activity in sandy loam soil (Tu 1993).

No significant difference with respect to acid phosphatase was observed among the treatments until 25 days after herbicide application (Table 1). But 10RD treatment had least amount of acid phosphatase activities. Recommended dose treatment had positive effect on acid phosphates activities. Similar result was reported by Saha et al. (2012). They reported that application of pretilachlor improved acid phosphatase activities. In case of alkaline phosphatase activities, there were significant differences among the treatments until 25 days after pesticide application (Table 1). At par activities were recorded for control and RD treatments. Whereas, 10RD and 2RD treatments inhibited the production of alkaline phosphatase activities till 20–25 days after herbicide application. Phosphatase is an exocellular enzyme produced by many soil microorganisms and is responsible for the hydrolysis of organic P compounds to inorganic P. These enzymes are usually not free in solution but associated with soil constituents. Effect of pesticides on phosphatase is either stimulatory or inhibitory. Ismail et al. (1996) reported that metolachlor at 10 ppm and above caused a reduction in phosphatase activity over 28 days of study. Whereas, Perucci et al. (2000) found that field rate application of rimsulfuron and imazethapyr had a stronger effect on the acid phosphatase activity.

Effect of pesticides on soil enzymes varied not only on dose or characteristics of pesticides but also climatic factors, composition of soil, and organic matter content. The interactions between biotic and abiotic factors are very complex and many environmental factors may modify the reaction of microbial populations to pretilachlor. In general, higher rates of pretilachlor application had negative effects

on soil enzymes. But recommended dose did not have any negative effect on soil enzymes.

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