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Acid and alkaline phosphatase enzyme activity influenced by diuron

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Abstract
A field experiment was conducted at College Farm, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, during Kharif 2010 to study the effect of herbicides (diuron, pendimethalin, pyrithiobac sodium and quizalofop p butyl) on soil phosphatase activity in a clay loam soil. The effects of herbicides on soil enzyme activity showed that there was an increase in soil enzyme activity from 0 to flowering of the crop irrespective of the treatment of the soil. The activity of alkaline phosphatase remained the unaffected by the treatments; while the acid phosphatase activity was reduced under diuron at 1.0 kg ha^{-1} *fb* pyrithiobac sodium + quizalofop p ethyl PoE in both red and black soils.

Keywords: Diuron, polymulch, weed control, phosphatase

Introduction
Cotton being a long duration crop and widely spaced crop is prone to heavy weed problem. The lack of suitable weed control alternatives has led to increase in reliance on herbicides and their use is increasing as they are less expensive and convenient than manual labour, very effective and easy to use. Herbicides may affect non-target organisms including microorganisms (Latha and Gopal, 2010). These chemicals may exert an effect upon the enzymes of soil and biological activities of microorganisms in a variety of way as herbicides may have negative effects on the growth of rhizobia, nitrogen fixation, morphology etc. (Nweke *et al.*, 2007). Measurement of the activity of the soil microflora provides indexes of the biological state of the soils and hence the soil fertility. Assessment of the enzymes present in soils offers potential as an integrative index of the soil's biological status. The role of soil phosphatase is to remove the phosphate molecule from organic compounds such as phospholipids and nucleic acids. Once the phosphate is cleaved it becomes soluble and can be taken up by the cell. This is a very important activity because phosphate is often the limiting nutrient for microbial growth in soil. Diuron has a prolonged soil residual life (80-230 days) making it more suitable for cotton crop due its slow initial growth. However, in the research experiment conducted in PJTSAU, it was observed that diuron 80% WP applied at 1.0 kg ha^{-1} caused significant reduction in plant stand in black soils which shows the need for the re-evaluation of herbicide dose in black and red soils also. Thus environmental and soil concern have prompted the agricultural research to look for improved management strategies.

Materials and Methods
An experimental study was conducted at College farm, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana State during *kharif*, 2017. The experiment was conducted in red and black soil and laid out in a randomised block design with three replications. The treatments included three doses of diuron (0.5, 0.75 and 1.0 kg ha^{-1}), pendimethalin 38.7% CS at 677 g ha^{-1} as PE followed by sequential application of pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}, intercropping of cotton with green manure crop (sunhemp), mechanical weeding thrice at 20, 40 and 60 DAS (weed free), polymulch and unweeded control.
Mallika *Bt* was sown with a seed rate of 2.5 kg ha^{-1}. One-two seeds per hill were sown at a spacing of 75cm X 75 cm facilitating the use of power weeder in both directions in case of mechanical weeding. Diuron 80% W.P. at 0.5 kg ha^{-1}, diuron 80% W.P. at 0.75 kg ha^{-1}, diuron 80% WP 1.0 kg ha^{-1}, pendimethalin 38.7% CS 677 g ha^{-1} were sprayed on the third day, pyrithiobac sodium 10% EC 62.5 g ha^{-1}+ quizalofop p ethyl 5% EC 50 g ha^{-1} were sprayed at 2-3 leaf stage of the weeds. In the intercropping treatment the intercrop sunhemp was sown along with cotton. Polymulch was spread 8 DAS after emergence of the seedling. Mechanical weeding at 20, 40, 60 DAS was done with power weeder and an unweeded check was maintained.

The activity of phosphatase was evaluated on the day of spray, 3rd day of spray, 7th day of spray, 15th day of spray of the pre emergence and postemergence herbicide spray, at flowering and harvest. The soil was collected from top 5cm at each time.

Phosphatase activity

The procedure followed was of Tabatabai and Bremner (1969) [3] (Acid phosphatases) (Alkaline phosphatases). One gram of soil sample was taken in glass tube. To this 0.2ml of toluene followed by 4ml of MUB buffer pH 6.5 (for acid phosphatase), 4ml MUB buffer pH 11.0 (for alkaline phosphatase) and 1 ml of p- nitro phenyl phosphate (only for samples) were added. Glass tube was swirled for few seconds, stoppered and incubated for 1 hour at 37^0C. After incubation, 1ml of 0.5M $CaCl_2$ $2H_2O$ and 4ml of 0.5M NaOH was added, swirled and filtered. The intensity of yellow colour was measured with spectrophotometer at 420nm. Controls were run simultaneously following the same procedure except adding 1ml of p-nitro phenyl phosphate solution.

Results and Discussion

Acid Phosphotase Activity (µg PNP kg^{-1} soil day^{-1})

The activity was affected upto third day after PoE application in red soil and seventh day after PoE application in black soil. The activity of the acid phosphatase was increasing in treatmets till flowering and decreasing to harvest indicating the influence of the root mass of the crop on the microorgaanisms.

Red soil

On the day of PE spray (159-163) and third of PE spray (207-211), all the non herbicidal treatments were on par and recorded significantly highest urease activity. The highest dose of diuron at 1.0 kg ha^{-1} *fb* pyrithiobac sodium + quizalofop p ethyl PoE recorded significantly lowest urease activity at on the day of PE spray (95), 3rd day of PE spray (126), 7th day of PE spray (142), 15th day of PE spray (129), on the day of PoE spray (150) and 3rd day of PoE spray (174). The other dosages of diuron at 0.75 kg ha^{-1}, 0.5 kg ha^{-1} *fb* pyrithiobac sodium + quizalofop p ethyl PoE and Pendimethalin treatments also registered significantly lower activity of urease on the day of PE spray and 3rd day of PE spray when compared to the non herbicidal treatments and were comparable to each other but from the 7th day of PE spray were also comparable to non herbicidal treatments. There was no significant difference among the treatments at on the 7th day of PoE spray, 15th day of PoE spray, at flowering and at harvest.

Black soil

Similar to that of red soil, on the day of PE spray (172-175) and third of PE spray (208-214), all the non herbicidal treatments were on par and recorded significantly highest urease activity. The highest dose of diuron at 1.0 kg ha^{-1} *fb* pyrithiobac sodium + quizalofop p ethyl PoE recorded significantly lowest urease activity at on the day of PE spray (105), 3rd day of PE spray (142), 7th day of PE spray (154), 15th day of PE spray (179), on the day of PoE spray (192), 3rd day of PoE spray (250) and 7th day of PoE spray (282). The other dosages of diuron at 0.75 kg ha^{-1}, 0.5 kg ha^{-1} *fb* pyrithiobac sodium + quizalofop p ethyl PoE and Pendimethalin treatments also registered significantly lower activity of urease on the day of PE spray and 3rd day of PE spray when compared to the non herbicidal treatments and were comparable to each other but from the 7th day of PE spray were also comparable to non herbicidal treatments. There was no significant difference among the treatments at on the 7th day of PoE spray, 15th day of PoE spray, at flowering and at harvest.

Alkaline Phosphotase Activity (µg PNP kg^{-1} soil day^{-1})

There was no significant difference among the treatments observed in both red and black soils in case of alkaline phosphatase activity. The values of the alkaline phosphatase activity registered were comparatively greater than the acid soils as the soils are slightly alkaline in nature. Similar to that of the acid phosphotase the alkaline phosphatase activity was increasing from crop germination till flowering and later on decreasing to harvest indicating the influence of the root mass of the crop on the microorganisms.

The close perusal of the data indicates that the activity of the soil acid phosphotase was inhibited by the application of diuron and the inhibition increased progressively with increase in dosage of the chemical while the activity of alkaline dehydrogenase remained unaffected. However the activity of the acid phosphatase enzyme regained with the increase in incubation time. The inhibition of enzyme activity might be the direct effect of herbicide on phosphotase activity and also due to competitive and non-competitive inhibition. The increase in enzyme activity with time might be due to various reasons i.e., the herbicide effect on microbial population may get stabilized after some time and the herbicides themselves are adsorbed irreversibly on soil colloids with increase in time resulting in decreased inhibition. The partial degradation of the herbicide with time in soil may also be another factor for decrease in inhibition. The recovery from inhibition may also be due to enzyme secreted by plant themselves. The detracting effect of herbicides towards all microbes and enzyme activities decreased with time and this may also be due to microbial population and enzyme activities after initial inhibition due to microbial adaptation to these chemicals or due to their degradation. Similar results were reported by Lal *et al*, (2017) [2], Sireesha *et al*, (2012) [4], Kumari *et al*, (2018) [1] and Emurotu and Anyanwu (2016) [5].

Conclusion

The above study indicates that the activity of soil acid phosphatase enzyme was inhibitedby the application of diuron where the diminution of activity increased with the increase in dosage of the chemical. While the activity of the alkaline phosphatase remained unaffected.

Table 1: Soil alkaline phosphatase activity (μg Pnp kg^{-1} soil day^{-1}) as influenced by weed control options in red soil

Treatments	Preemergence				Postemergence				Flowering	Harvest
	on the day of spray	on the 3rd day	on the 7th day	on the 15th day	on the day of spray	on the 3rd day	on the 7th day	on the 15th day		
Diuron 80% WP 0.5 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	257	295	306	311	315	364	381	436	486	248
Diuron 80% WP 0.75 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	241	264	266	297	308	356	359	445	461	251
Diuron 80% WP 1.0 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	232	261	265	290	296	347	357	405	463	241
Pendimethalin 38.7% CS at 677 g ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	246	290	301	310	329	369	368	427	482	236
Cotton + sunhemp	251	290	312	322	328	373	390	453	494	252
Mechanical weeding at 20, 40, 60 DAS	245	295	310	317	323	366	383	446	493	249
Control (unweeded)	254	287	308	322	332	371	384	460	491	253
Polymulch of 0.25 mm thickness	249	293	307	322	332	367	390	458	489	248
SE(m)±	11.1	10.3	14.9	10.6	18.3	17.3	24.5	22.1	20.8	13.7
C.D. (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 2: Soil alkaline phosphotase activity (μg Pnp kg^{-1} soil day^{-1}) as influenced by weed control options in black soil

Treatments	Preemergence				Postemergence				Flowering	Harvest
	on the day of spray	on the 3rd day	on the 7th day	on the 15th day	on the day of spray	on the 3rd day	on the 7th day	on the 15th day		
Diuron 80% WP 0.5 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	273	284	295	311	315	364	381	436	486	248
Diuron 80% WP 0.75 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	241	250	264	297	308	356	359	445	463	251
Diuron 80% WP 1.0 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	240	246	261	290	296	347	357	405	461	241
Pendimethalin 38.7% CS at 677 g ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	266	270	287	310	323	369	368	427	482	236
Cotton + sunhemp	271	280	290	322	328	373	390	453	494	252
Mechanical weeding at 20, 40, 60 DAS	265	280	295	317	329	366	383	446	493	249
Control (unweeded)	274	278	290	322	332	371	384	460	491	253
Polymulch of 0.25 mm thickness	269	267	293	322	332	367	390	458	489	248
SE(m)±	11.2	14.8	10.3	10.6	18.3	17.3	24.5	22.1	20.8	13.7
C.D. (p=0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 3: Soil acid phosphatase activity (μg Pnp kg^{-1} soil day^{-1}) as influenced by weed control options in red soil

Treatments	Preemergence				Postemergence				Flowering	Harvest
	on the day of spray	on the 3rd day	on the 7th day	on the 15th day	on the day of spray	on the 3rd day	on the 7th day	on the 15th day		
Diuron 80% WP 0.5 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	137	169	182	197	207	216	274	300	348	193
Diuron 80% WP 0.75 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	126	148	187	184	201	214	271	291	357	190
Diuron 80% WP 1.0 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	95	126	142	129	150	174	267	271	348	200
Pendimethalin 38.7% CS at 677 g ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	135	182	181	207	210	217	276	301	356	196
Cotton + sunhemp	163	211	207	209	220	228	274	313	370	201
Mechanical weeding at 20, 40, 60 DAS	159	207	201	204	218	224	267	313	358	196
Control (unweeded)	161	207	205	213	219	228	276	306	352	193
Polymulch of 0.25 mm thickness	161	209	204	207	221	227	271	304	368	201
SE(m)±	5.4 0	6.50	7.52	6.95	7.87	8.19	12.64	13.4	11.2	8.4
C.D. (p=0.05)	16.41	19.80	22.64	20.86	23.84	24.18	NS	NS	NS	NS

Table 4: Soil acid phosphatase activity (µg Pnp kg^{-1} soil day^{-1}) as influenced by weed control options in black soil

Treatments	Preemergence				Postemergence				Flowering	Harvest
	on the day of spray	on the 3rd day	on the 7th day	on the 15th day	on the day of spray	on the 3rd day	on the 7th day	on the 15th day		
Diuron 80% WP 0.5 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	156	189	206	226	262	281	311	350	362	210
Diuron 80% WP 0.75 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	152	182	196	224	244	270	297	346	361	211
Diuron 80% WP 1.0 kg ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	105	142	154	179	192	250	282	344	343	219
Pendimethalin 38.7% CS at 677 g ha^{-1} *fb* pyrithiobac sodium 10% EC 62.5 g ha^{-1} + quizalofop p ethyl 5% EC 50 g ha^{-1}	155	193	202	229	248	281	311	353	368	213
Cotton + sunhemp	175	214	210	257	262	310	340	359	376	216
Mechanical weeding at 20, 40, 60 DAS	172	208	209	247	262	308	338	357	367	207
Control (unweeded)	173	210	208	253	263	307	337	356	358	204
Polymulch of 0.25 mm thickness	174	211	208	249	259	308	338	357	371	214
SE(m)±	4.0	6.51	6.12	7.32	8.59	9.51	10.52	6.4	10.4	9.2
C.D. (p=0.05)	12.2	19.85	18.54	22.40	26.20	28.90	32.10	NS	NS	NS

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