Effect of pendimethalin and oxyfluorfen on soil enzyme activity

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ABSTRACT

A field experiment was conducted to study the effect of pendimethalin and oxyfluorfen on soil enzyme activities in raddish crop. There was significant difference observed in acid, alkaline phosphatase and dehydrogenase activities between the herbicide treatments and periods of study and their interaction. Acid, alkaline phosphatase and dehydrogenase activity was found to be increased from 0 days after application to 30 days after application (DAA) all the treatments and there was a decrease at the time of harvest. In the two seasons of study, phosphatases and dehydrogenase showed maximum activity at 30 DAA. The interaction effect (treatments and DAA) showed that the acid, alkaline phosphatase and dehydrogenase activity in the herbicide treated plots was significantly higher than the control among all the periods. Lower level of herbicide application increased enzyme activity as compared to higher dose of herbicide. Oxyfluorfen @ 0.15 kg a.i ha^{-1} showed inhibitory effect on enzyme activity where as urease levels in the herbicide treated plots were significantly lower than the control for all the growth stages.

In the modern day agriculture, a large number of agricultural chemicals are being used to control a wide variety of weeds, pests, diseases and parasites. A judicious and cautious use of these chemicals helps in sustaining the productivity at higher level while their indiscriminate use leads to serious ecological imbalances. The inhibition of soil enzymes by agrochemicals can be direct or indirect. Any action of the chemical altering the life functions of soil organisms could directly affect soil enzyme activity. An agrochemical may also modify the inter relationship between the particular group of organisms and this influences the amount and type of enzyme produced (Cerevelli *et al.*, 1978). Intensive use of herbicides without adequate knowledge on its effects on soil enzymes may have adverse impact on soil biochemical processes and cycling of nutrients. So, it is important to have knowledge of behavior of herbicides in the soil to avoid soil pollution and their side effects on soil micro organisms and succeeding crops. Though a lot of information is available concerning the influence of herbicides on soil micro flora and fauna, very little information is available concerning their effects on soil enzyme activities particularly those having a bearing on soil fertility i.e., urease, dehydrogenase, phosphatase etc. Hence, the study was conducted to understand the effects of preemergence herbicide pendimethalin on soil enzymes.Pendimethalin(N-(1-ethylpropyl)–3,4-dimethyl-2,6-dinitrobenzenamine) belongs to dinitroaniline group and oxyfluorfen belongs to diphenyl ether group used as pre-plant or pre emergence application for control of most of the annual grasses and broad leaved weeds in vegetables.

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MATERIALS AND METHODS

A field experiment was conducted during *rabi* and summer of 2006-07at College Farm, College of Agriculture, Rajendranagar. The soil was sandy clay loam in texture with pH 6.57, EC 0.16 dS m^{-1} and 0.53 % of organic carbon content. Radish was grown with six treatments and four replications in randomized block design. The crop was treated with the common dose of fertilizers. Treatment details involving two doses of Pendimethalin @ 0.5 and 0.75 kg a.i. ha^{-1}, oxyfluorfen @ 0.1 and 0.15 kg a.i. ha^{-1} applied at 2 days after sowing as pre emergence, hand weeding and un weeded check. Initial soil samples from surface layer (0-15 cm) were collected at 0,15,30, and 45 days after spraying of herbicides for the assay of enzyme activities like dehydrogenase, acid and alkaline phosphatase and urease.

Dehydrogenase enzyme activity of soils was determined by using the method given by Singh *et al.*(1980). Urease activity was assayed by quantifying the rate of release of NH^+_4 from the hydrolysis of urea as suggested by Rao (1989).The procedure of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977) was adopted for the assay of acid and alkaline phosphatases, respectively.

RESULTS AND DISCUSSION

Acid, alkaline phosphatase and dehydrogenase enzyme activity was influenced by herbicide treatments. The results indicated that significant differences existed between the herbicide treatments and periods of study and their interaction at 5 per cent level. Acid, alkaline phosphatase and dehydrogenase activity was found to increased in control from 0 DAA to 30 DAA in the all the

treatments showing a decrease there after at the time of harvest.

Perucci *et al.* (1990) showed that acid phosphomono esterase activity increased in herbicide treated soils during the first 10 days, but after 30 days, the activity decreased and no significant differences were observed after 60 days compared to the control. These findings may have been a result of the toxic effect of the herbicides on soil micro organisms. Some soil micro organisms were probably unable to survive the herbicidal treatments and consequently there was a temporary release of enzymes after cell diesis causing an immediate increase ion activity. A similar hypothesis has been put forth by Cerevelli *et al.* (1978). The subsequent decrease in phosphatase activity was probably related to proteolysis of non-stabilized extra celluar enzymes. These effects were generally more evident at the higher herbicide application rate. (Srinivas, 1993; Shukla,1997; Das *et al.*, 2003). The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978) as well as a direct measure of soil microbial activity (Garcia *et al.,* 1988). There has been increased activity of dehydrogenase at lower levels of herbicide as compared to control and at higher levels there was decreased activity.Which may be due to the availability of carbon source for the growth of microorganisms. These results were also in accordance with the Shukla (1997); Baruah and Mishra, (1986).

In current study there was always a stimulation of enzyme level at active growth of the crop plants. With both the herbicides and in the two seasons of study, acid and alkaline phosphatases showed maximum activity al 30 DAA. The acid phosphatase in general exhibited a two to three fold increased activity, while alkaline phosphatase showed three to fold increase at its peak when compared to zero DAS (control). The interaction effect showed that the acid, alkaline phosphatase and dehydrogenase level in the herbicide treated plots was significantly higher than the control among all the periods and also lower level of herbicide application increased enzyme activity as compared to higher dose of herbicide. Oxyfluorfen @ 0.15 kg a.i ha^{-1} showed inhibitory effect on enzyme activity. After first crop during summer, effect of herbicides on acid, alkaline phosphatase and dehydrogenase activity followed the same trend, but the enzyme activity was more as compared to first crop.

The urease activity increased from 0 DAA to 30 DAA in all the treatments. Thereafter the activity decreased at the time of harvest. From the interactions (treatment and DAA) it can be observed that the urease levels in the herbicide treated plots were significantly lower than the control for all the growth stages. The interaction effects at 15 DAA indicated that hand weeding and Un weeded check showed significantly higher urease activity than herbicide treated plots .

At 30 DAA, un-weeded check treatment showed maximum urease activity followed by hand weeding. In the herbicidal treatmental plots, inhibitory effect of urease was observed. At harvest, the urease activity was decreased as compared to 30 DAA. No significant difference was observed between the treatments. In case of the summer raddish crop, urease activity was more in control plots compared to herbicidal treatmental plots. But the activity of urease was found increased from 0 DAA to 30 DAA and at harvest it was decreased. The use of herbicides has become standard practice in modern day agriculture and these have influence on soil enzymes.

Phosphatases, urease and dehydrogenase were chosen for study because of their influence on transformation of N and P in soil and on microbial activity of soil. Letherberg and Burn (1976) reported time effect of phosphate pesticide on soils which are similar to the present study. They found that, in contrast to soil urease, jackbean urease was totally inactivated in a very short time by these chemicals. Thus, the soil enzymes are much better protected against the inhibitory action of these agrochemicals as compared to urease from plant and microbial sources. This can be best accounted for the stabilization of soil enzymes through their immobilization on soil colloids. They could show that part of the soil urease, which was extracellular was deactivated more quickly than the part which was an integral part of the soil humus complex. The inhibition of soil urease by herbicide can be due to competitive and non competitive.

The tapering of inhibitory effect of soil urease with time and the recovery during the later stages may be due to different reasons. i. The herbicides themselves are adsorbed increasingly irreversibly on the soil colloid with increase in time and this may result in decreased inhibition. ii. The herbicides on the microbial population may get stabilized after some time. iii. The partial degradation of these herbicides with time in the soil is also another factor for decrease in the inhibition. The recovery from inhibition at later stages may also be due to enzymes secreted by plant roots. Whatever be the mechanism of action, it is necessary that the actual affects be documented in the interest of improving agronomic practices.

Table1: Effect of pendimethalin and oxyfluorfen on activity of alkaline phosphatase (µg 4-nitrophenol g^{-1}soil h^{-1}) in radish

Treatments	Rabi, 2006-07				Mean	Summer, 2007				Mean
	Days after application					Days after application				
	0	15	30	45		0	15	30	45	
T$_1$- Pendimethalin @ 0.5 kg a.i.ha^{-1}	40.34	83.02	111.47	87.90	**80.68**	51.64	95.05	128.33	103.52	**94.63**
T$_2$- Pendimethalin @ 0.75 kg a.i.ha^{-1}	38.75	73.81	90.50	82.76	**71.45**	44.87	87.84	107.67	96.52	**84.22**
T$_3$-Oxyfluorfen @ 0.15 kg a.i.ha^{-1}	36.85	58.58	81.78	73.68	**62.72**	40.88	81.59	96.80	89.66	77.23
T$_4$- Oxyfluorfen @ 0.10 kg a.i.ha^{-1}	39.64	78.54	103.73	85.53	**76.86**	48.69	91.16	116.48	98.68	**88.75**
T$_5$-Farmers practice	33.79	52.45	85.03	76.58	**61.96**	36.89	70.84	84.59	72.56	**66.22**
T$_6$- control	34.78	56.20	89.65	78.45	**64.77**	38.98	75.44	87.78	68.88	**67.77**
Mean	37.36	67.10	93.69	80.81		43.66	83.65	103.61	88.30	
				SEm(±)	LSD(0.05)				SEm(±)	LSD(0.05)
Days				0.87	2.42				1.05	2.92
Treatments				1.07	2.97				1.82	3.57
Days × Treatments				2.14	6.46				3.65	7.77

Table 2: Effect of pendimethalin and oxyfluorfen on activity of acid phosphatase (µg 4-nitrophenol g^{-1} soil h^{-1}) in radish

Treatments	Rabi, 2006-07				Mean	Summer, 2007				Mean
	Days after application					Days after application				
	0	15	30	45		0	15	30	45	
T$_1$- Pendimethalin @ 0.5 kg a.i.ha^{-1}	22.85	61.28	95.48	58.26	**59.47**	42.51	87.76	110.69	93.67	**83.66**
T$_2$- Pendimethalin @ 0.75 kg a.i.ha^{-1}	20.64	52.21	87.50	56.31	**54.17**	37.32	73.47	96.65	86.71	**73.54**
T$_3$-Oxyfluorfen @ 0.15 kg a.i.ha^{-1}	18.75	30.79	56.56	54.91	**40.25**	35.94	58.51	83.76	61.40	**59.90**
T$_4$- Oxyfluorfen @ 0.10 kg a.i.ha^{-1}	21.35	58.06	92.38	56.93	**57.18**	40.87	81.35	109.83	90.82	**80.72**
T$_5$-Farmers practice	19.77	34.20	63.47	50.24	**41.92**	34.97	62.60	79.86	72.98	**62.60**
T$_6$- control	20.34	36.96	67.46	46.80	**42.89**	38.76	65.87	88.56	67.80	**65.25**
Mean	20.62	45.58	77.14	53.91		38.40	71.59	94.89	78.90	
				SEm(±)	LSD(0.05)				SEm(±)	LSD(0.05)
Days				0.73	2.03				0.93	2.57
Treatments				0.90	2.48				1.13	3.14
Days × Treatments				1.79	5.40				2.27	6.84

Table 3: Effect of pendimethalin and oxyfluorfen on dehydrogenase activity (μg TPF g^{-1} soil d^{-1}) in radish

Treatments	Rabi, 2006-07				Mean	Summer, 2007				Mean
	Days after application					Days after application				
	0	15	30	45		0	15	30	45	
T$_1$- Pendimethalin @ 0.5 kg a.i.ha^{-1}	178.24	542.85	760.62	420.75	**475.62**	362.24	632.44	862.45	603.24	**615.09**
T$_2$- Pendimethalin @ 0.75 kg a.i.ha^{-1}	182.56	380.95	580.44	382.25	**381.55**	350.33	520.36	721.36	530.65	**530.68**
T$_3$-Oxyfluorfen @ 0.15 kg a.i.ha^{-1}	178.65	241.33	460.62	360.81	**310.35**	360.82	462.25	651.74	481.72	**489.13**
T$_4$- Oxyfluorfen @ 0.10 kg a.i.ha^{-1}	181.45	460.52	642.33	401.65	**421.49**	360.45	582.24	782.24	562.95	**571.97**
T$_5$-Farmers practice	179.26	283.05	481.55	380.42	**331.07**	370.64	431.06	590.55	442.58	**458.71**
T$_6$- control	181.42	310.56	520.75	410.53	**355.82**	360.92	483.04	631.09	471.62	**486.67**
Mean	**180.26**	**369.88**	**574.39**	**392.74**		**360.90**	**518.57**	**706.57**	**515.46**	

					SEm(±)	LSD(0.05)			SEm(±)	LSD(0.05)
Days					8.0	22.0			8.0	21.0
Treatments					27.0	14.0			9.0	26.0
Days × Treatments					20.0	59.0			19.0	57.0

Table 4: Effect of pendimethalin and oxyfluorfen on urease activity (μg NH$^+_4$ released g^{-1} soil d^{-1}) in radish

Treatments	Rabi, 2006-07				Mean	Summer, 2007				Mean
	Days after application					Days after application				
	0	15	30	45		0	15	30	45	
T$_1$- Pendimethalin @ 0.5 kg a.i.ha^{-1}	3.81	5.38	8.07	6.81	**6.01**	4.42	6.83	9.32	8.13	**7.17**
T$_2$- Pendimethalin @ 0.75 kg a.i.ha^{-1}	3.45	4.26	7.54	6.64	**5.47**	3.93	5.96	8.71	7.05	**6.41**
T$_3$-Oxyfluorfen @ 0.15 kg a.i.ha^{-1}	3.39	3.97	6.83	5.74	**4.98**	3.70	5.83	8.26	6.85	**6.16**
T$_4$- Oxyfluorfen @ 0.10 kg a.i.ha^{-1}	3.64	5.23	7.83	6.82	**5.88**	4.27	6.65	8.98	7.75	**6.91**
T$_5$-Farmers practice	3.38	6.76	9.14	6.85	**6.53**	4.14	6.82	9.27	7.84	**7.02**
T$_6$- control	3.69	6.93	9.36	6.95	**6.73**	4.17	7.98	9.99	7.97	**7.53**
Mean	**3.56**	**5.42**	**8.12**	**6.36**		**4.10**	**6.68**	**9.09**	**7.06**	

					SEm(±)	LSD(0.05)			SEm(±)	LSD(0.05)
Days					0.17	0.48			0.16	0.43
Treatments					0.21	0.59			0.19	0.53
Days × Treatments					0.43	1.29			0.38	1.15

In current study there was always a stimulation of enzyme level at active growth of the crop plants. With both the herbicides and in the two seasons of study, acid and alkaline phosphatases showed maximum activity al 30 DAA. The acid phosphatase in general exhibited a two to three fold increased activity, while alkaline phosphatase showed three to fold increase at its peak when compared to zero DAS (control). Here again the problems are many because of difficulty in measuring microbial population/biomass. The overall result so far seems to be that at the most there is marginal decrease in biomass temporarily at usually high concentration of pesticide which later gets adjusted to equilibrium levels in time 2, 4 – D glyphosate and picloram which are post emergence herbicides caused enhancement of basal respiration only for a days following application, at concentration of 200 μg g^{-1}. Since, change in microbial variables occurred only at herbicide concentration much higher than what is normally applied in field, the side effects of these chemicals are probably of little ecological significance. A change in species composition of soil micro organisms may occur after pesticide application but elimination of a single species is very unlikely (Domsch, 1972). After initial disturbance, there is generally a tendency to restore the original level quickly, as there is rarely a total exposure of soil micro organisms to biologically active concentration of herbicide.

REFERENCES

Baruah, M. and Mishra, R. R. 1986. Effect of herbicide butachlor, 2, 4-D and oxyflourfen on enzyme activities and CO$_2$ evolution submerged paddy field soil. *Plant and Soil,* **96**:287-91.

Burns, R. G. 1978. Enzyme activity in soil – some theoretical and practical considerations. *In: Soil Enzymes* (Ed. Burns, R. G.), Academic Press Inc, New York, pp. 214-18.

Cerevelli, S., Nanniperi, P. and Sequi, P. 1978. Interaction between agrochemicals and soil enzymes. *In: Soil Enzymes* (Ed. Burns, R. G.), Academic Press Inc, New York, pp. 37-48.

Das, A. C., Debnath, A. and Mukharjee, D. 2003. Effect of the herbicides oxadiazon and oxyflurofen on phosphate solubilizing micro organiosn and their persistence in soil. *Chemosphere,* **53**:217-21.

Eivazi, F. and Tabatabai, M. A. 1977. Phosphatases in soils. *Soil Biol. Biochem.,* **9**:167-72.

Garcia, V. A., Proda, G. E., Sanchez, V. M., Byeno, F. D. and Garia, R. A. 1988. Adsorption of thiuron and dimethoate on soils. *Soil Sci. Soc. America J.,* **52**:1571-74.

Letherberg, G. and Burns, R. G. 1976. Inhibition of soil urease by organophosphorus insecticides *Soil Biol. Biochem.,* **8**: 99-02.

Perucci, P. 1990. Effect of the addition of municipal solid-waste compost on microbial biomass and enzyme activities in soil. *Biology and Fertility of Soils,* **10**: 221-26.

Rao, V. S. 1989. Distribution kinetics and some interactions of urease and phosphomonoesterases in soils. *Ph. D. Thesis,* Department of Soil Science and Agricultural Chemistry, Andhra Pradesh Agricultural University, Hyderabad. pp. 36-39.

Shukla, A. K. 1997. Effect of herbicides butachlor, fluchloralin, 2, 4- D and oxyfluorfen on microbial population and enzyme activities of rice field soils. *Indian J. Ecol.,* **24**: 189-92.

Singh, D., Chhonkar, P. K. and Pandey, R. N. 1980. Soil plant water analysis. *In: A Methods Manual.* Indian Agricultural Research Institute, New Delhi, pp:116.

Srinivas, D. 1993. Phosphomonoesterase activity in some soils of Andhra Pradesh: Distribution, Kinetics and variation due to plant cover, pesticide application and submergence. *Ph. D. Thesis,* Department of Soil Science and Agricultural Chemistry, Andhra Pradesh Agricultural University, Hyderabad, pp. 26-31.

Tabatabai, M. A. and Bremner, J. M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.,* **1**: 301-07.