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Effect of oxadiargyl on soil enzyme activity

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ABSTRACT

During both the seasons, application of FYM (10 t ha^{-1}) resulted in significantly higher level of activity of urease, acid and alkaline phosphatase and dehydrogenase enzymes in the soil growing spinach. Adoption of weed management options at different crop growth stages significantly influenced the activity of all these enzymes. While application of herbicide oxadiargyl at a lower dose of 0.75 kg a.i. ha^{-1} stimulated the activity of these enzymes. Comparable level of activity of these enzymes under the influence of different applied treatments at harvest stage of spinach crop signified progressive decrease in the influence of the applied herbicide in time. Changes in microbial activities occurred only at herbicide concentration much higher than what is normally applied in field. Thus, at the doses of herbicides applied in the field may not pose very serious threat to the soil ecological balance.

Key words: Acid, alkaline phosphatase, dehydrogenase, enzymes, oxadiargyl, urease

Application of herbicides is becoming a wide spread practice as weeds often pose a serious threat to crop yield. When herbicides are applied to soils, they generally disturb the natural eco-system through their effects on soil microbial and enzymatic activities (Cerevelli *et al.*, 1978). Application of herbicides is known to have side effects on soil enzymes. Soil enzymes play key biochemical functions in the overall processes in the soil system. They are important in catalyzing several important reactions necessary for the life process of microorganisms in soils. These enzymes are constantly being synthesized, accumulated, inactivated and decomposed in the soil, hence playing a role in agriculture and particularly in nutrient cycling (Tabatabai and Bremner, 1969). Intensive use of herbicides without adequate knowledge of its effects on soil enzymes may have adverse impact on soil biochemical processes and cycling of nutrients. Hence, a study was conducted to assess the effects of oxadiargyl (prominent herbicide widely using in paddy and vegetables) on soil enzymes *i.e.*, urease, phosphatase and dehydrogenase were chosen for study because of their influence on transformation of nitrogen and phosphorus and microbial activity in soil.

MATERIALS AND METHODS

A field experiment was conducted on an Alfisol at College Farm, College of Agriculture, Rajendranagar during the *kharif* and the *rabi* seasons, 2007-08. The soils had sandy loam texture with neutral soil reaction (pH-7.15) and non saline electrical conductivity (0.26 dSm^{-1}). It had low organic carbon content (0.47%), medium available nitrogen (267 kg ha^{-1}) and potassium (285 kg ha^{-1}) and low available phosphorus (0.18 kg ha^{-1}). The initial urease, dehydrogenase, acid phosphatase and alkaline phosphatase activities were24 µg NH_4^+released g^{-1} soil 2h^{-1}, 355 µg TPF g^{-1} day $^{-1}$, 40.5 and 62 µg of þ-nitrophenol g^{-1} soil h^{-1}, respectively. The experiment was laid out in a factorial randomized block design raising spinach as test crop. The treatment comprised of two levels of FYM *viz.*, 0 (S_1) and 10 t ha^{-1} (S_2) applied at subplots of the four main plot treatments *viz.*, (T_1) pre emergence application of oxadiargyl @ 0.75 kg ha^{-1}, (T_2) oxadiargyl @ 1.5 kg ha^{-1} applied two days after sowing, (T_3) hand weeding and (T_4) un weeded check. The treatment combination was replicated thrice. *Kharif* crop was sown on August 12th 2008 and harvested on 12th October, 2008, where as *rabi* crop was sown on 21.12.2006 and harvested on 20.02.2007. Recommended doses of fertilizers (25:25:50 kg NPK ha^{-1}) were applied uniformly to all the treatments in the form of urea, single super phosphate and muriate of potash as basal. Urea was applied as top dressing in 3 equal splits *i.e.*, 1/3rd after first picking, 1/3rd after second picking and 1/3rd after third picking of spinach crop. All the package of practices recommended for growing spinach were followed to ensure good crop growth and better yields. Representative surface soil sample were collected from the field before laying out the experiment for initial analysis. Immediately after irrigating the crop and after herbicide application treatment wise samples were collected at 15 days interval up to harvest.

The initial soil samples and the samples collected from different intervals were analysed by using standard procedures mentioned here. Soil reaction (*p*H) was determined in 1:2.5 soil :: water suspension using *p*H meter (Elico LI 610) (Jackson, 1967). Electrical conductivity of the soil was determined in 1:2.5 soil:: water extract by using conductivity bridge (Elico CM 180) (Jackson, 1967) and expressed as dS m^{-1}. Organic carbon in 0.5 mm sieve soil was determined by wet digestion method as suggested by Walkley and Black (1934) as described by Jackson (1967) and expressed as g kg^{-1}. Available nitrogen in the soil was determined by alkaline

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potassium permanganate method as described by Subbaiah and Asija (1956) and expressed as kg ha^{-1}. Available phosphorus was extracted from soil by Olsen's reagent. The blue colour was developed following ascorbic acid method of Watanabe and Olsen (1965) and the intensity of blue colour was determined at 420 nm using a UV-visible spectrophotometer (ECIL GS 570) and expressed as kg ha^{-1}. Available potassium was extracted from soil by using neutral normal ammonium acetate (Murh *et al.,* 1965) and was determined by using an Elico flame photometer (Elico CL 22 D) as described by Jackson (1967) and expressed as kg ha^{-1}. Enzymatic activities of soil *viz.*, urease activity was assayed by quantifying the rate of release of NH_4^+ from the hydrolysis of urea as described by Tabatabai (1977), dehydrogenase activity by quantifying the amount of TPF produced and expressed as gram sample per hour described by Cassida *et al.* (1964) and the phosphatase activity was assayed by quantifying the amount of p-nitrophenol released and expressed as ug of p-nitrophenol released g^{-1} sample hr^{-1} as described by Tabatabai and Bremner (1969).

RESULTS AND DISCUSSION

Urease activity

Soil urease catalyses the hydrolysis of urea to ammonical form which is subsequently oxidized to nitrate by nitrifying organisms of the soil. Changes in activity of urease enzyme measured in terms of the amount (µg) of NH_4^+ -N released g^{-1} soil 2 hr^{-1} at different growth stages of spinach during *kharif* and *rabi* seasons under the influence of different treatments were presented in table-1 and 2. Appraisal of the presented data revealed significant influence of crop growth stages; application of treatments and their interaction on activity of urease enzyme. An increasing trend in urease enzyme activity during vegetative growth stage reaching to its maximum at flowering (30 DAA) followed by decrease at the harvest stage (60 DAA) was observed. Similar results were also reported by Kumar *et al.* (2002). Application of FYM, though during the initial crop growth stage resulted in higher urease activity during both the seasons, its effect became non-significant as the crop growth advanced. The higher level of urease activity in organic manure (FYM) added plots might have been due to increased activity of organic carbon that acted as an energy source for proliferation of micro-organisms that produce diverse extra cellular enzymes while the lower level of this enzyme in unmanured plot might be due to the lack of this source (Kanchikerimatha and Singh, 2001). Weeding manually or by using herbicide also influenced urease enzyme activity in soil. The highest urease enzyme activity was observed when the herbicide oxadiargyl (T_1) was applied at lower dose (0.75 kg a.i. ha^{-1}) but by increasing its dose to 1.5 kg a.i. ha^{-1} (T_2) inhibited the activity of urease enzyme to its lowest level among different treatments during all the growth stages through both the seasons. The levels of urease activity under different weed management practices at harvest (60 DAA) were at par. This indicated that the inhibitory effect of higher dose of herbicide decreased with time which may be due to different reasons *viz.*, irreversible adsorption of herbicides on to soil colloids making them inactive; their partial degradation with time and or stabilization of microbial population in soil with time. Similar results were also reported by Nisha *et al.* (2006). The presented data suggested that the part of the extra cellular enzyme was deactivated more quickly than the part which was an integral part of soil humus complex. The higher urease activity in T_1 might be due to the increased availability of substrate organic carbon present in the herbicide resulting in proliferation of micro-organism (Nayak and Manjappa, 2010) while the inhibition of urease activity by herbicide may be competitive or non competitive (Letherberg and Burns, 1976).

Phosphatase activity

Phosphatases are a broad group of enzymes capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. In soil ecosystem, these enzymes are believed to play critical roles in P cycles, as evidence shows that they are correlated to P stress and plant growth. Phosphatase is an enzyme present in all microorganisms and increase in phosphatase activity is mainly due to increase in bacterial biomass. Temporal sequence in activity of this enzyme may be due to their differential production rates on account of physiological age of different groups of microorganisms present in the soil (Srinivas *et al.*, 2003).

Careful appraisal of the presented data (Table 3, 4, 5 and 6) revealed significant influence of crop growth stages, application of treatments and their interaction on the activity of both acid phosphatase and alkaline phosphatase enzymes in soil. An increasing trend from the day of application to 60DAA in the activity of both these enzymes was observed during both the seasons of study. The values were generally higher during *rabi* season compared to the *kharif* season. Application of FYM led to increased activity of both these enzymes during all the three stages of crop growth across both the seasons. Higher availability of substrate in FYM treated plots might have resulted in higher proliferation of micro-organism and thus higher phosphatase activity.

Weeding manually or by using herbicide also exerted significant role in the level of activity of both these enzymes during the first 30 days after their application across both the seasons. The highest acid phosphatase and alkaline phosphatase activities were observed when the herbicide oxadiargyl was applied at lower dose (T_1-0.75 kg a.i. ha-^{1}) but by increasing its dose to 1.5 kg a.i. ha^{-1} (T_2) inhibited the activity of both these enzymes.

Table 1: Effect of oxadiargyl on soil urease activity (µg NH^{+}_{4} released g^{-1} soil 2^{-1} h) in spinach during 2007-2008

Treatments Main/sub	*Kharif*						*Rabi*					
	0 DAA		30 DAA		60 DAA		0 DAA		30 DAA		60 DAA	
	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM
T_1: Oxadiargyl @ 1 kg a.i/ha	26.82	22.16	44.29	41.22	30.25	27.44	27.14	23.35	45.53	42.80	31.22	29.17
T_2: Oxadiargyl @ 2 kg a.i/ha	22.37	20.74	40.27	37.56	27.16	25.12	22.87	21.17	41.22	38.05	28.05	26.52
T_3: Hand weeding	24.15	21.35	42.22	39.22	28.72	26.75	25.06	22.08	43.55	40.10	29.15	27.45
T_4: un weeded check	23.26	20.10	41.85	38.22	28.05	26.17	24.12	20.85	43.05	40.22	29.18	26.35
Mean	**24.15**	**21.09**	**42.16**	**39.06**	**28.55**	**26.37**	**24.80**	**21.86**	**43.34**	**40.29**	**29.40**	**27.37**
LSD (0.05)												
M	**2.6**		**3.7**		**3.1**		**2.7**		**3.6**		**3.1**	
S	**2.2**		**3.4**		**2.8**		**2.2**		**3.02**		**2.8**	
M × S	**2.7**		**3.9**		**3.3**		**2.9**		**3.7**		**3.2**	

Table 2: Effect of oxadiargyl on soil acid phosphatase activity (µg of 4- nitrophenol g^{-1} soil h^{-1}) in spinach during 2007-2008

Treatments Main/sub	*Kharif*						*Rabi*					
	0 DAA		30 DAA		60 DAA		0 DAA		30 DAA		60 DAA	
	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM
T_1: Oxadiargyl @ 1 kg a.i/ha	49.22	42.85	68.25	61.28	74.56	68.35	51.43	44.65	70.54	64.75	81.16	74.05
T_2: Oxadiargyl @ 2 kg a.i/ha	43.05	37.80	59.50	52.21	72.50	64.58	43.55	38.25	62.45	55.36	75.36	68.42
T_3: Hand weeding	42.65	37.77	54.50	46.96	70.50	65.11	42.56	39.15	55.11	47.15	73.44	66.78
T_4: un weeded check	40.89	35.64	51.28	44.20	68.36	63.22	44.85	40.22	53.65	46.50	72.44	64.25
Mean	**43.95**	**38.52**	**58.38**	**51.16**	**71.48**	**65.32**	**45.60**	**40.57**	**60.44**	**53.44**	**75.60**	**68.38**
LSD (0.05)												
M	**3.9**		**5.2**		**6.8**		**4.1**		**5.3**		**6.8**	
S	**3.7**		**4.8**		**6.6**		**3.9**		**4.6**		**6.5**	
M × S	**4.2**		**5.5**		**7.2**		**4.4**		**5.8**		**6.9**	

Table 3: Effect of oxadiargyl on soil alkaline phosphatase activity (µg of 4- nitrophenol g^{-1} soil h^{-1}) in spinach during 2007-2008

Treatments Main/sub	*Kharif*						*Rabi*					
	0 DAA		30 DAA		60 DAA		0 DAA		30 DAA		60 DAA	
	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM
T_1: Oxadiargyl @ 1 kg a.i/ha	79.11	67.50	92.45	83.56	121.25	103.20	82.55	72.45	98.45	89.35	130.45	118.56
T_2: Oxadiargyl @ 2 kg a.i/ha	68.29	59.46	84.56	73.14	108.35	92.53	73.10	64.53	90.15	77.56	121.20	109.52
T_3: Hand weeding	66.25	55.26	85.36	75.35	98.52	88.40	69.45	62.43	92.10	81.25	113.46	102.35
T_4: un weeded check	65.16	56.16	82.45	73.40	101.35	87.35	68.45	63.15	91.44	79.58	109.25	98.55
Mean	**69.70**	**59.60**	**86.21**	**76.36**	**107.37**	**92.87**	**73.39**	**65.64**	**93.04**	**81.94**	**118.59**	**107.25**
LSD (0.05)												
M	**5.5**		**7.5**		**9.4**		**6.7**		**8.3**		**9.6**	
S	**5.3**		**7.2**		**8.9**		**6.3**		**7.9**		**9.2**	
M × S	**6.7**		**8.1**		**10.4**		**7.1**		**8.5**		**9.8**	

Table 4: Effect of Oxadiargyl on activity of dehydrogenase (µg TPF g^{-1} day^{-1}) in spinach grown soil during *kharif* and *rabi* 2007 -2008

Treatments Main/sub	*Kharif*						*Rabi*					
	0 DAA		30 DAA		60 DAA		0 DAA		30 DAA		60 DAA	
	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM	FYM	Without FYM
T_1: Oxadiargyl @ 1 kg a.i/ha	379.50	349.40	842.45	785.62	630.40	605.22	388.50	346.22	856.24	802.75	647.30	615.45
T_2: Oxadiargyl @ 2 kg a.i/ha	368.11	352.25	720.25	675.22	570.32	542.25	380.20	364.20	745.40	722.75	585.62	557.36
T_3: Hand weeding	368.10	346.22	685.25	650.78	531.12	522.75	374.36	349.40	733.80	693.46	578.69	550.46
T_4: un weeded check	356.25	334.62	675.22	640.74	553.04	528.30	360.22	334.62	726.84	678.26	567.45	546.78
Mean	**367.99**	**345.62**	**730.79**	**688.09**	**571.22**	**549.63**	**375.82**	**348.61**	**765.57**	**724.31**	**594.77**	**567.51**
LSD (0.05)												
M	**23.45**		**54.22**		**39.65**		**24.75**		**59.47**		**40.65**	
S	**20.40**		**50.43**		**34.76**		**22.10**		**55.78**		**38.45**	
M × S	**25.11.**		**56.85**		**40.64**		**26.78**		**61.27**		**43.65**	

The levels of activity of both the enzymes under different management practices at harvest (60 DAA) were at par. This indicated that the inhibitory effect of higher dose as well as the stimulatory effect at lower dose of herbicide decreased with time due to different reasons as discussed earlier. These observations are in agreement with those reported by Perucci *et al.* (1990).

Dehydrogenase activity

The dehydrogenase enzyme activity is commonly used as both, an indicator of biological activity in soils and as a direct measure of soil microbial activity. As could be observed from the data presented in table - 7 and 8, there was a consistent increase in dehydrogenase activity (µg TPF g^{-1} day^{-1}) from 0 days after application to 30 days after application and later decreased at harvest in both the seasons and in both the sub plots. The dehydrogenase activity was significantly higher in herbicide treated plots as compared to control. Dehydrogenase activity in soil was in order: oxadiargyl @ 0.75 kg ha^{-1} > oxadiargyl @ 1.5 kg ha^{-1} > hand weeding > un weeded check during the *kharif* season. Increased activity of dehydrogenase at lower levels of herbicide as compared to un weeded check was observed. This might be due to the availability of carbon source for the growth of microorganisms. These results were also in agreement with those observed by Baruah and Mishra, (1986). A change in species composition of soil micro organisms may occur after pesticide application but elimination of a single species is very unlikely. 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. Here again the problems are many because of difficulty in measuring microbial population/biomass.

The overall result so far points out to the marginal decrease in biomass temporarily at usually high concentration of pesticide which later gets adjusted to equilibrium levels in time. Since, change in microbial variables occurred only at herbicide concentration much higher than what is normally applied in field, the side effects of this chemical probably pose little ecological significance. The plant's root- soil- microbe interface is a totally different situation and has been recognized recently to be vital in plant nutrition. The rhizosphere abounds in microflora due to root exudates and exact role of pesticides in such low doses in affecting their activities still remains unclear.

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