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Effect of three selected fungicides on soil dehydrogenase, phosphatase, protease and urease enzyme activities in groundnut (Arachis hypogeae L.) fields

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Abstract

Soil enzyme activities namely dehydrogernase, phosphatase, protease and urease were studied by incubating fungicide treated (1,2.5, 5, 7.5 and 10.0 $kg.ha^{-1}$) and untreated groundnut soils at 7 (dehydrogenase) and 10 days. Except urease, all the three enzymes had stimulatory effect at $5kg.ha^{-1}$ in black and red soils than control. With increase in incubation time, enzyme activities also enhanced reaching maximum at 20 days.

Key Words: Interaction-Fungicides- Soil Enzymes- Groundnut Field

Introduction

Soil enzymes are a group of enzymes that play a substantial role in maintaining soil ecology.and chemical properties, fertility and soil health. They catalyze several vital reactions necessary for microbial processes in soil agriculture and described as biological fingerprints of soil.. They serve as sensitive indicators of ecological change (Das and Varma, 2011). Dehydrogenase is a soil enzyme that oxidizes soil organic matter and is commonly used asa biological indicator of microbial respiratory activity(Utobo and Tewari,2015). In soil, phosphatases have been the most studied enzymes contributing to P nutrition of plants and microbes. Like dehydrogenases, they can also be used as index of soil microbial activity (Makoi and Ndakidemi, 2008). Protease enzyme contribute to the breakdown of proteinaceous material present in soil to simpler nitrogen compounds available for plant nutrition (Nasreen et.al., 2012). Whereas urease is responsible for hydrolysis of urea fertilizers applied to the soil into ammonia and carbon dioxide. It may be degraded by soil proteolytic enzymes. The enzyme was very sensitive to toxic concentrations of xenobiotics (Yang et al., 2006).

Commercial fungicides namely dhanustin , Dithane M-45 and Contaf are widely used for control of fungal pests in agricultural crops like groundnut. These agrochemicals may affect local metabolism or soil enzyme activities besides controlling pest problem. They may have both negative and positive effects on function of soil enzymes (Liu et.al., 2008). So far little information is available on these fungicides behavior towards soil dehydrogenase, phosphatase, protease and urease.

Hence the present study is targeted to evaluate the response of the four enzymes with the selected fungicides in groundnut soils, major crop grown in Anantapur (Anonymous, 2010) . This

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information will be useful for analyzing environmental impact of the specific compounds on soil microbial and biochemical properties.

Materials and Methods

Soils and fungicides

Black and red soils were collected from groundnut fields of Anantapur District to a depth of 5 cm , air dried and brought to laboratory in sterile polythene bags for soil experiments. Details of soil properties and fungicides used is mentioned earlier (Jaya Madhuri and Rangaswamy, 2014).

Soil Incubation studies

Five grams of sieved soil sample was used for estimation of dehydrogenase activity. Whereas influence of the three fungicides on phosphatase and protease was determined in two gram portions of each soil. For urease, one gram of soil was incubated with the fungicides at 0,10,25, 50, 75 and 100ppm concentrations. These concentrations are equivalent to the field application rates of 0, 1, 2.5,5,7.5 and 10 $kg.ha^{-1}$. Triplicate soil samples were mixed thoroughly in order to maintain 60%water holding capacity throughout the experiment (Ramudu et.al., 2012).

Enzyme assays

Dehydrogenase assay was performed according to Casida et.al., (1964) and adopted by Srinivasulu and Rangaswamy, 2013 and 2014) as an index of microbial activity by reduction of 2,3,5 triphenyl tetrazolium chloride to formazen. Activity was measured at 485 nm using methanol as a blank.

The assay of phosphatase activity was based on that of Tabatabai and Bremner (1969) and adapted by Joanna Lemanowicz (2011) using p-nitrophenyl phosphate as substrate. Yellow coloured para nitro phenol formed due to the action of phosphatase was analysed spectrophotometrically at wavelength of 410 nm.

Protease activity was determined with the method outlined by Ladd and Butler (1972) and adopted by Subramanyam *et al.* (2011). Blue colour formed by amino acids release by action of protease on sodium caseinate was quantified by referring to absorbance values of tyrosine standard at 700nm

Activity of soil urease was expressed as amount of ammonia released from the substrate urea as per phenol hypochlorite method of Fawcett and Scott(1960) and adapted by Lakshmi Kalyani et.al., (2015).

Rate of dehydrogenase was estimated at 7, 14, 21 and 28 days of incubation at effective concentration only. Phosphatase, protease and urease rate of enzyme activities were noted at 10,20, 30 and 40 days incubation period.

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Statistical analysis

Data was statistically analysed using Duncan Multiple Range (DMR) test and used by Rahmansyah et.al., (2009). Insecticide treatments were contrasted with untreated controls and the significant difference ($P \leq 0.05$) between values of each sampling and insecticide was calculated on soil weight basis.

Results

Dehydrogenase

All the three fungicide treatments had a positive impact on dehydrogenase up to 5kg ha^{-1} in black and red soils (Tables 1,2). Maximum stimulation was observed at 5kg ha^{-1}, whereas for dithane and contaf at 2.5 $kg.ha^{-1}$. Especially in dhanustin treated black soil, stimulation was found even at 7.5 and 10 $kg.ha^{-1}$ and contaf only at 7.5 $kgha^{-1}$(Table 1). In red soil, dithane M-45 had also increase in enzyme activity at 7.5 $kg.ha^{-1}$(Table 2). Significant enhancement was noticed both in control and treated soils till 28 days of incubation, with a marked effect at 21 days (Tables 3, 4).

Table 1. Effect of different concentrations of selected fungicides on activity of dehydrogenase* in black soil after 7 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	47a (100)	47a (100)	47a (100)
1.0	80b (170)	91b (194)	71b (151)
2.5	152c (323)	148c (315)	120c (255)
5.0	201d (427)	82d (174)	104d (221)
7.5	115e (244)	43e (91)	68e (145)
10.0	73f (155)	24f (51)	35f (74)

***µg formazen g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC).**

Figures, in parentheses, indicate relative production percentages,

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Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Table 2. Effect of different concentrations of selected fungicides on activity of dehydrogenase* in red soil after 7 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	35a (100)	35a (100)	35a (100)
1.0	72b (205)	64b (183)	67b (191)
2.5	138c (394)	183c (522)	158c (451)
5.0	179d (511)	112d (320)	104d (297)
7.5	97e (277)	51e (145)	73e (208)
10.0	41f (117)	22f (63)	30f (85)

***µg formazen g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC).**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 3.. Effect of dhanustin at 5.0 kg ha^{-1} on activity of dehydrogenase* in black and red soils.

Treatment	Soil incubation, in days				
	7	**14**	**21**	**28**	**35**
			Black soil		
Control	47a	78a	214a	155a	108a
Dhanustin	201b	343b	392b	278b	143b
			Red soil		
Control	35a	62a	208a	124a	93a
Dhanustin	179b	254b	381b	242b	125b

***µg formazen g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC).**

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 4. Effect of dithane M-45 and contaf (both at 2.5 kg ha^{-1}) on activity of dehydrogenase* in black and red soils.

Treatment	Soil incubation, in days				
	7	**14**	**21**	**28**	**35**
			Black soil		
Control	47a	128a	301a	212a	108a
Dithane M-45	198b	394b	472b	330b	178b
Contaf	170b	382b	425c	305c	154c
			Red soil		
Control	35a	104a	284a	203a	133a
Dhanustin	183b	375b	4271b	320b	152b
Contaf	158c	358c	404c	285c	149b

***µg formazen g^{-1} soil formed after 24 hours incubation with triphenyl tetrazolium chloride (TTC).**

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Phosphatase

Similar to dehydrogenase enzyme, maximum activity was seen at 5 kgha^{-1} of fungicide treatments compared to control (Table 5) in black soil. With respect to red soil, similar trend was observed and innocuous effect at 7.5 kgha^{-1} concentration (Table 6). In both the soils, strong inhibition was seen at the highest concentration. Pronounced stimulation is with contaf than the two fungicides (Tables 5,6). Rate of enzyme activity had a significant increase at 20

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days of incubation. Activity declined slowly at 30 days incubation period and less than that of control at 40 days time interval (Table 7).

Table 5. Effect of different concentrations of fungicides on activity of phosphatase* in black soil after 10 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	102.10a (100)	102.10a (100)	102.10a (100)
1.0	114.30b (112)	107.00ab (105)	118.25b (115)
2.5	125.00c (122)	113.15b (110)	137.30c (134)
5.0	157.80d (154)	125.80c (123)	164.50d (161)
7.5	90.75e (89)	87.00d (85)	90.15e (88)
10.0	74.50f (73)	66.70fe (65)	78.90f (77)

***µg p-nitrophenol (PNP) g^{-1} soil formed after 3 hours incubation with p-nitrophenyl phosphate (PNPP).**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 6. Effect of different concentrations of fungicides on activity of phosphatase* in red soil after 10 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	93.00a (100)	93.00a (100)	93.00a (100)
1.0	108.30b (116)	104.75b (113)	113.50b (122)
2.5	118.80c (127)	113.20c (122)	121.75c (131)
5.0	127.50d (137)	122.10d (131)	130.80d (140)
7.5	90.00a (97)	85.00e (91)	91.00a (98)
10.0	67.50e (72)	63.50f (68)	70.25e (75)

***µg p-nitrophenol (PNP) g^{-1} soil formed after 3 hours incubation with p-nitrophenyl phosphate (PNPP).**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 7. Effect of dhanustin, dithane M-45 and contaf at 5.0 kg ha^{-1} on phosphatase activity[a] in black and red soils

Treatment	Soil incubation, in days			
	10	**20**	**30**	**40**
		Black soil		
Control	102.10a	151.25a	113.00a	77.10a
Dhanustin	157.80b	205.38b	184.30b	83.50b
Dithane M-45	125.80c	185.50c	146.20c	71.25a
Contaf	164.50b	211.00b	191.50b	89.85b
		Red soil		
Control	93.00a	135.00a	106.20a	68.70a
Dhanustin	127.50b	188.50b	157.75b	74.34b
Dithane M-45	122.10b	164.30c	120.00c	69.20a
Contaf	130.80b	192.25b	131.50d	76.50b

***µg p-nitrophenol (PNP) g^{-1} soil formed after 3 hours incubation with PNPP.**

Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.

Protease

When compared to control, all the treatments were increased at all concentrations tested. Unlike phosphatase, protease activity was maximum at 5 kgha^{-1} with dhanustin and contaf. But in dithane M-45, stimulation was more prominent at 2.5 kgha^{-1}in both soil samples(Tables 8,9).

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Similar trend was noticed in rate of enzyme activity at 20 days like phosphatase and urease (Tables 10, 11).

Table 8. Effect of different concentrations of selected fungicides on activity of protease* in black soil after 10 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	421a (100)	421a (100)	421a (100)
1.0	583b (138)	594b (141)	590b (140)
2.5	678c (161)	685c (162)	604c (143)
5.0	747d (177)	560d (133)	698d (166)
7.5	602e (143)	473e (112)	555e (132)
10.0	515f (122)	402f (95)	442f (105)

***µg tyrosine g^{-1} soil formed after 2 hours incubation at 30^0C with 1% casein.**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 9. Effect of different concentrations of fungicides on activity of protease* in red soil after 10 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	413a (100)	413a (100)	413a (100)
1.0	540b (130)	565b (137)	572b (138)
2.5	608c (147)	650cc (157)	647c (156)
5.0	694d (168)	602d (146)	691d (167)
7.5	525a (127)	550b (133)	535e (129)
10.0	451e (109)	424a (103)	427a (103)

***µg tyrosine g^{-1} soil formed after 2 hours incubation at 30^0C with 1% casein.**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 10. Effect of dhanustin, and contaf (both at 5.0 kg ha^{-1}) on activity of protease*in black and red soils

Treatment	Soil incubation, in days			
	10	**20**	**30**	**40**
		Black soil		
Control	421a	552a	394a	228a
Dhanustin	747b	821b	697b	471b
Cotaf	698c	814b	688b	465b
		Red soil		
Control	413a	538a	369a	202a
Dhanustin	694b	810b	681b	446b
Contaf	691b	803b	665b	434b

***µg tyrosine g^{-1} soil formed after 2 hours incubation at 30^{0}C with 1% casein**

Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.

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Table 11. Effect ofdithane M-45 at 2.5 kg ha^{-1} on activity of protease* in black and red soils

Treatment	Soil incubation, in days			
	10	**20**	**30**	**40**
		Black soil		
Control	421a	552a	364a	228a
Dithane M-45	685b	772b	617b	520b
		Red soil		
Control	413a	538a	345a	202a
Dithane M-45	650b	735b	598b	386b

***µg tyrosine g^{-1} soil formed after 2 hours incubation at 30^{0}C with 1% casein**

Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.

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Urease

Activity of urease under the impact of dhanustin, dithane M-45 and contaf up to 5 $kgha^{-1}$ was alleviated in comparison to control (Table 12,13). In the present study, striking stimulation of soil urease occurred at 2.5 $kg.ha^{-1}$ of the selected fungicides in the two soils. Similar to phosphatase, higher levels of 7.5 and 10.0 $kg.ha^{-1}$ of the fungicide treatment caused inhibition in enzyme activity at 10 days interval (Tables 12, 13,). Further it is evident that rate of urea hydrolysis was more rapid in the fungicide treated soils at stimulatory concentration for 20 days. It has declining tendency at later intervals(Table 14).

Table 12. Effect of different concentrations of selected fungicides on activity of urease* in black soil after 10 days

Fungicide Concentration (Kg ha^{-1})	Dhanustin	Dithane M-45	Contaf
0.0	25.38a (100)	25.38a (100)	25.38a (100)
1.0	33.63b (132)	32.88b (129)	34.45b (135)
2.5	40.75c (160)	41.75c (164)	42.13c (166)
5.0	27.88d (110)	28.50d (112)	30.75d (121)
7.5	20.25e (80)	19.63e (77)	22.48e (88)
10.0	14.00f (55)	12.28f (48)	15.83f (62)

***µg ammonia g^{-1} soil formed after 3 hours incubation at 37^0C with urea.**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 13. Effect of different concentrations of selected fungicides on activity of urease* in red soil after 10 days

Fungicide Concentration ($Kgha^{-1}$)	Dhanustin	Dithane M-45	Contaf
0.0	21.25a (100)	21.25a (100)	21.25a (100)
1.0	27.88b (131)	29.03b (136)	30.38b (143)
2.5	35.63c (167)	34.50c (162)	38.13c (179)
5.0	24.00d (113)	23.68d (111)	25.75d (121)
7.5	18.13e (85)	15.00e (70)	19.20e (90)
10.0	11.75f (55)	10.88f (51)	13.00f (61)

***µg ammonia g^{-1} soil formed after 3 hours incubation at 37^0C with urea.**

Figures, in parentheses, indicate relative production percentages,

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

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Table 14. Effect of dhanustin, dithane M-45 and contaf at 2.5 kg ha^{-1} on activity of urease [a] in black and red soils

Treatment	Soil incubation, in days			
	10	20	30	40
		Black soil		
Control	25.38a	33.50a	28.25a	20.10a
Dhanustin	40.75b	51.23b	44.38b	32.65b
Dithane M-45	41.75b	53.40b	46.20b	35.00b
Contaf	42.13b	55.25b	48.83b	38.75c
		Red soil		
Control	21.25a	28.75a	23.05a	15.83a
Dhanustin	35.63b	45.85b	38.45b	24.15b
Dithane M-45	34.50b	48.15b	41.38b	28.00b
Contaf	38.13b	50.25c	43.63b	32.65c

***µg ammonia g^{-1} soil formed after 3 hours incubation at 37^{0}C with urea.**

Means, in each column, followed by the same letter are not significantly different (P $\leq$ 0.05) from each other according to DMR test.

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Discussion

Better understanding of the role of dehydrogenase enzyme in environmental science will provide greater possibilities of using it as a diagnostic tool for ecosystem amelerioation as most areas of the world are often polluted by different compounds. Reports of stimulation in dehydrogenase on fungicide treatment in present study was observed by Singh and Kumar(2008); Sukul (2006) and Bending et.al., (2007). Singh and Kumar revealed that the insecticide acetamiprid increased dehydrogenase activity upto 22% after first treatment. According to Sukul (2006), metalaxyl fungicide application increased enzyme activity initially. Similar results were found by Bending et.al., (2007) with fungicides tebuconazole and chlorothalonil. However, the extent of increase varied significantly with different treatments, which may be ascribed to the use of pesticides as carbon and energy sources. Innocuous effect was noticed by earlier workers with pesticide treatment sometimes.

The present finding of inhibition at higher concentration of 10kg.ha^{-1} was indicated by few workers. As per the experimental findings of Menon et.al., (2005), two insecticides had strong inhibitory activity on the enzyme at 100ppm in semi arid fields of tropical India. With respect to application of fungicide chlorothalonil in combination with insecticide, similar action was noticed (Srinivasulu and Rangaswamy,2013). Strong inhibition of enzyme was found as a result of microbial response to soil amendment.

To date, there have been few studies examining the influence of pesticides management in the ecosystem on phosphatases activity in soil where major crops are grown. Understanding dynamics of enzyme activity in these systems is crucial for predicting nutrient uptake.

Phosphatases

Soil phosphatases because of their involvement in making phosphorus available to plants, need to be extensively studied in relation to pesticide use. Results obtained from the experiment show stimulation of phosphatase activity followed by fungicide treatment at field application rate of 5kg.ha.$^{-1}$ Rasool and Reshi (2010) declared that the fungicide mancozeb initiated stimulation under normal recommended dosage and ten times the normal dosage. In the same manner, phosphatase increased significantly in agricultural soil amended with the fungicide probineb at

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field application rate (Rahmansyah et.al., 2009). Enzyme activity was enhanced up to 7.5 kg.ha^{-1} than the controls in 10 days incubated soil samples (Srinivasulu et.al., 2012).

Contrarily, phosphatase activity in paddy cultivated soil showed a variable pattern in response to various concentrations of triazophos after 1st, 7th , 14th, 21st and 28thdays of incubation periods. Enzyme activity significantly decreased at all the concentrations of triazophos and there was much difference in the activity compared to untreated control (Lakshmi Kalyani and Suvarnalatha Devi, 2015). Coinciding with the above, activities of two phosphatases were also inhibited (22%) in a sandy loam soil incorporated with captan fungicide at all incubations reported by Piotrowska et.al., (2008). Decrease in enzyme activity may be ascribed to suppression of a sensitive fraction of soil biota.
In the present study , inhibition was observed in black soil but no significant effect in red soil at 7.5 kg.ha^{-1}. Whereas at 10kgha-1, similar negative effect was found in both soils. With the increase in incubation time, activity was more at 20 days incubation, following a declining trend later. Similar results were noticed by (Nasreen et.al., 2012) with the same fungicide mancozeb.

Protease

In accordance with the maximum increase in protease activity at 2.5 kg.ha^{-1} of dithane M-45 and 5 kgha^{-1} of dhanustin and contaf in black and red soils of groundnut, Mohiddin et.al., (2011) noticed that two insecticides acephate and imidacloprid at 25 and 50 ppm caused increment of 46-54% in the enzyme activity at 10 day interval respectively. Similar report was made by Ramudu et.al.,(2012) with combination of two fungicides. Stimulatory effect even at 7.5 kg.ha^{-1} was also indicated by the same authors. Contrary to the present result of innocuous nature of selected fungicides at the highest concentration of 10 kg.ha^{-1}, partial inhibition was found with mancozeb over control by Rasool and Reshi (2010).

Our experimental findings are also in agreement with the previous study carried by Srinivasulu et.al., 2011) who observed activity of protease higher in black soil up to 20 days of incubation followed by a sharp decline afterwards.

Urease

Unlike other enzymes, stimulation values obtained were low in both soils. Even at 5 kg.ha^{-1}, there was no significant increase in the activity. Yang e.tal.,(2006) showed that furadan enhanced urease activity in four soils. Rahmansyah et.al., (2009) also reported an increase in urease enzyme after two weeks incubation with fungicide probineb. Same findings were found by Maleeka Begum and Rajesh, 2015). But majority of workers stated inhibition of enzyme in fungicide amended soils, especially at higher concentration. According to Guo et.al., (2008), urease activity was inhibited at all concentrations with napropamide. Similarly, Cycon e.tal., (2010) noticed that activity declined with dimethomorph and mancozeb in loamy and sandy loam soils at higher concentration. Like wise, 20% decrease in activity was observed in chloropyrifos treated soil (Tejda et.al., 2010; Wang et.al., 2010).

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An increasing trend of enzyme activity upto 20 days followed by declining tendency was also revealed by earlier workers(Niemi et.al., 2009; Vavaoulidou 2009; Kalyani et.al., 2010; Bishnu et.al., 2012; Dutta etal., 2012; Riah et.al., 2014) with insecticide and fungicide amended soils.

Conclusions

Results show a strong positive influence on dehydrogenase, phosphatase, protease and urease in soils treated with 2.5 and 5 $kg.ha^{-1}$ dry soil and they were significantly higher than the control over the course of incubation which is increased maximum upto 20 days. However, a significant decrease in the three enzymes was observed in 30 and 40 days of incubation.

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