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## Research Article

### IMPACT OF SELECTED INSECTICIDES AND FUNGICIDES ON PHOSPHATASE ACTIVITY IN GROUNDNUT (*ARACHIS HYPOGAEA L.*) SOILS

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#### ABSTRACT

Many xenobiotic compounds have long-term stability in soil, and their persistence results in significant impact on the soil ecosystem. A laboratory study was done to determine the impact of two selected insecticides, Oxydemeton Methyl (Organophosphorus), Emamectin Benzoate (Evermectin) and two selected fungicides Dithane-Z-78 (Dithiocarbamate), Benlate (Benzimidazole) on phosphatase enzyme activity in two different soil samples (Black clay soil and Red sandy loam soil) of Groundnut (*Arachis Hypogaea L.*) cultivated fields in Anantapuramu district of Andhra Pradesh, India. The soil samples were treated with insecticides and fungicides at five different concentrations i.e., 10, 25, 50, 75, 100 ppm, which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5, 10.0 kg ha<sup>-1</sup>). We observed that phosphatase enzyme activity significantly enhanced at 2.5 kg ha<sup>-1</sup> in both the soil samples after 10 days of incubation. After the prolonged incubation upto 30 and 40 days, there was a decline in phosphatase enzyme activity, However stimulation was observed with four pesticides incubated for 20 days at 2.0 kgha<sup>-1</sup>.

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#### INTRODUCTION

Pesticides have become an integral part of our modern life as a part of pest control strategies. Groundnut (*Arachis hypogaea L.*) is an important oil seed crop out of nine oil seeds crops grown in our county. The increased use of pesticides in agricultural soils cause soil contamination. Enzymes are the vital activators in life processes, like wise in the soil they are known to play a substantial role in maintaining soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system. Many of the pesticides used are persistent soil contaminants, whose impact may endure for decades and adversely affect soil conservation (Quazi *et al*, 2011; Defo *et al*, 2011; Ahemad and Khan, 2011a,b). Study of soil enzymes gives information about the release of nutrients in soil. Soil enzymes analysis helps to establish correlation with soil fertilization, microbial activity and biochemical cycling of various elements in soil.

The pesticides selected for the present study are Oxydemeton Methyl, Emamectin Benzoate, Dithane- Z-78 and Benlate. They influence soil phosphatase enzyme activity in Black and Red groundnut soils. The phosphatase enzyme activity by influence of these four chemicals was recommended by

Agriculture Department for control of pests on crops (Anonymous, 2016)

#### MATERIALS AND METHODS

##### Soils and pesticides

Black and Red soils are collected from Groundnut fields of Anantapuramu district to a depth of 12 cms, air dried and brought to the laboratory in sterile polythylene bags for soil experiments. Details of soil properties and pesticides used are mentioned in Table 1 and 2.

##### Soil incubation studies

The soil ecosystem stimulating non-flooding conditions consisting of 2g portions of soil samples were added to test tubes (10 ×150mm) and moisture to a water potential of 0.090 Mpa, in order to maintain 60% water holding capacity. The same model was used previously to elucidate the effect of pesticides on microbial activity (Jayamadhuri and Rangaswamy 2003 and Madakka *et.al* 2011)

##### Phosphatase activity

Two gram portions of each soil, in triplicates were treated with selected pesticides at five different concentrations i.e., 1.0, 2.5, 5.0, 7.5 and 10 kg ha<sup>-1</sup>. Soil samples without pesticide

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treatment served as control. Soil samples in test tubes with and without pesticide treatment were incubated at room temperature in the lab ( $28 \pm 4$  °C). After 10 days of incubation, soil extract was prepared in distilled water for assay of phosphatase as per Rangaswamy and Venkateshwarulu, 1996.

### Assay of Phosphatase

Soil samples were transferred to 100 ml Erlenmeyer flask and 0.2 ml of toluene, 6 ml of 0.1M maleate buffer (pH 6.5), and 2 ml of *p*-nitrophenyl phosphate disodium salt were added. The flask were swirled for a few seconds to mix the contents, stoppered and incubated at 37 °C for 30 min. The reaction was stopped by adding 1ml of 0.05 M CaCl<sub>2</sub> and 4 ml of 0.05M NaOH followed by swirling of the flask, for a few seconds and the soil suspension was filtered through a Whatmann No.1 filter paper. The liberated *P*-nitrophenol in the filtrate was determined at 410 nm in Thermo Scientific-Evolution 201 u.v/visible Spectrophotometer.

### Statistical Analysis

The concentration of phosphatase was calculated on soil weight (oven dried basis). The pesticide treatments were contrasted with untreated controls and the significant level P = 0.05 between values of each sampling and each pesticide were assessed using SYSTAT statistical software package to find the results of one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Jaffer *et al.*, 2010).

## RESULTS AND DISCUSSION

Phosphatases are a group of enzymes that catalyse the hydrolysis of both esters and anhydrides of phosphoric acid. The mineralization of organic phosphorous by the activity of phosphatase in soils makes one of the essential elements, phosphorous in soil for plant growth (Li *et al.*, 2004).

**Table 1** Physico-chemical properties of soils used in the present study

Properties	Black clay soil	Red sandy loam soil
Sand(%)	76.5	72.0
Silt (%)	18.0	25.0
Clay(%)	5.5	3.0
pH a	8.4	6.3
Water holding capacity (ml g <sup>-1</sup> soil)	0.48	0.34
Electrical conductivity (m.mhos)	266	246
Organic matter b(%)	0.94	0.801
Total nitrogen c (%)	0.05	0.034
NH <sub>4</sub> <sup>+</sup> - N(μ g <sup>-1</sup> soil) <sup>d</sup>	8.95	7.80
NO <sub>2</sub> <sup>-</sup> - N (μ g <sup>-1</sup> soil) <sup>e</sup>	0.51	0.35
NO <sub>3</sub> <sup>-</sup> -N(μ g <sup>-1</sup> soil) <sup>f</sup>	1.04	0.99

<sup>a</sup>1:1.25 (soil:water)

<sup>b</sup>Walkley-Black method (Jackson, 1971)

<sup>c</sup>Micro-Kjeldhal method (Jackson, 1971)

<sup>d</sup>Nesslerization method (Jackson, 1971)

<sup>e</sup>Diazotization method ( Barnes and Folkard, 1951)

<sup>f</sup>Brucine method (Ranney and Bartler, 1972)

Hence, phosphatase enzyme activity was measured under the influence of two insecticides Oxydemeton Methyl and Emamectin Benzoate, and two fungicides Dithane- Z-78 and Benlate. So the data obtained from these experiments are presented in the tables 2-5. Phosphatase activity increased in all individual tested pesticide- treated soils throughout the experiment. The stimulation in the activity of phosphatase

increased up to 2.5 kg ha<sup>-1</sup> and then turned down in both black and red soils after 10 days of incubation (tables 3 and 4).

**Table 2** Details of the pesticides used in the present study

Technical name	Commercial name	Chemical class	Commercial formulation	Sources
Oxydemeton Methyl	Metasystox	Organo phosphorous	25% EC*	UPL Ltd, Gujarat.
Emamectin Benzoate	EM-1	Evermectin	5% SG**	Dhanuka  Agritech Ltd, Gurgoan. Coromandal
Benlate	Benofit	Benzimidazole	50% WP***	International Ltd, Secunderabad. Indofil chemicals company, Maharashtra.
Dithane -Z-78	Zineb	Dithio carbamate	75% WP***	

\*Emulsifying concentration \*\* Soluble Granules \*\*\* Wettable powder

**Table 3** Effect of different concentrations of selected pesticides on activity of phosphatase \* in Black soil after 10 days

Concentration of pesticides (Kg ha <sup>-1</sup> )	Oxydemeton Methyl	Emamectin Benzoate	Dithane-Z-78	Benlate
0.0	97 ± 1.632 d	98 ± 2.209 d	97 ± 1.622 d	98 ± 1.13 d
1.0	110 ± 2.661 c	111 ± 1.421 b	112 ± 442 c	120 ± 3.333 b
2.5	140 ± 2.731 a	144 ± 1.123 a	155 ± 332 a	145 ± 2.886 a
5.0	120 ± 2.361 b	110 ± 4.343 b	125 ± 2.776 b	102 ± 1.154 c
7.5	99 ± 1.531 d	95 ± 2.123 d	99 ± 1.732 d	95 ± 2.866 d
10.0	88 ± 2.842 e	89 ± 2.886 c	90 ± 1.632 e	91 ± 1.732 d

\* μg P - nitrophenol (PNP)g<sup>-1</sup> soil formed after 3 hours incubation with P- nitrophenyl phosphate (PNPP)

Each column is mean ± S.E. for 6 concentrations in each group;

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

**Table 4** Effect of different concentrations of selected pesticides on activity of Phosphatase \* in red soil after 10 days

Concentration of insecticides (Kg ha <sup>-1</sup> )	Oxydemeton Methyl	Emamectin Benzoate	Dithane-Z-78	Benlate
0.0	60 ± 1.32 d	60 ± 1.32 e	60 ± 1.32 c	60 ± 1.32 d
1.0	76 ± 2.742 c	80 ± 4.322 c	77 ± 1.122 b	83 ± 1.521 c
2.5	105 ± 5.723 a	102 ± 5.321 a	99 ± 0.577 a	110 ± 0.521 a
5.0	82 ± 1.134 b	88 ± 1.154 b	80 ± 5.732 b	90 ± 8.214 b
7.5	70 ± 5.773 d	70 ± 3.881 b	70 ± 5.776 c	69 ± 0.532 d
10.0	55 ± 2.88 a	66 ± 3.841d	60 ± 5.341 c	66 ± 0.222 c

\* μg P - nitrophenol (PNP)g<sup>-1</sup> soil formed after 3 hours incubation with P- nitrophenyl phosphate (PNPP)

Each column is mean ± S.E. for 6 concentrations in each group;

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

As increase in the concentration of the pesticides, the stimulation of the enzyme activity was decreased after 2.5 kg ha<sup>-1</sup>. This enzyme activity continues up to 20 days of incubation and no declines afterwards (Table 5). Enhancement in phosphatase activity over control was recorded in Black and Red soils treated with Oxydemeton Methyl, Emamectin Benzoate, Dithane- Z-78 and Benlate at concentrations of 1.0 and 2.5 kg ha<sup>-1</sup> by the end of 10 days incubation, respectively (Table 3 and 4). The phosphatase activity was enhanced in both soils with and without pesticides upon further incubation for another 10 days (20- day interval). At 20-days interval, slight increment in the phosphatase activity in both soils with or without pesticides occurred in comparison to the activity of the respective increment at 10-day interval (Table 5).

**Table 5** Influence of selected Pesticides on phosphatase activity in black and red soils.

	Soil incubation, in days			
	10 days	20 days	30 days	40 days
<b>Black soil</b>				
Control	97 ± 1.632 a	120 ± 4.773 a	110 ± 4.341 a	80 ± 4.342 a
Oxydemeton Methyl (2.5 kg ha <sup>-1</sup> )	140 ± 2.73 b	170 ± 4.783 b	155 ± 2.781 b	130 ± 5.721 b
Emamectin Benzoate (2.5 kg ha <sup>-1</sup> )	144 ± 1.123 b	177 ± 1.321 c	140 ± 4.832 b	125 ± 5.771 b
Zineb (2.5 kg ha <sup>-1</sup> )	155 ± 3.32 f	175 ± 2.886 c	155 ± 2.866 c	110 ± 4.732 c
Dithane-Z-78 (2.5 kg ha <sup>-1</sup> )	145 ± 2.886 b	165 ± 2.834 d	152 ± 1.154 b	121 ± 3.421 b
<b>Red soil</b>				
Control	60 ± 1.32 a	89 ± 0.577 c	50 ± 5.773 d	42 ± 1.542 d
Oxydemeton Methyl (2.5 kg ha <sup>-1</sup> )	76 ± 2.72 b	120 ± 5.773 b	90 ± 5.772 c	70 ± 5.773 c
Emamectin Benzoate (2.5 kg ha <sup>-1</sup> )	105 ± 5.723 c	135 ± 2.866 a	120 ± 8.547 a	98 ± 7.154 a
Zineb (2.5 kg ha <sup>-1</sup> )	82 ± 1.134 d	125 ± 2.886 b	105 ± 2.886 b	72 ± 1.154 c
Dithane-Z-78 (2.5 kg ha <sup>-1</sup> )	70 ± 1.342 b	131 ± 0.577 a	100 ± 5.773 b	81 ± 0.577 b

\*µg P – nitrophenol (PNP)g<sup>-1</sup> soil formed after 3 hours incubation with P- nitrophenyl phosphate (PNPP)

Each column is mean ± S.E. for 6 concentrations in each group;

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

The present results indicating stimulation in phosphatase activity at 2.5 kg ha<sup>-1</sup> in both Black and Red soils (Table 3 and 4). After this concentration, increase in concentration of pesticides decreases the stimulation of enzyme activity. Phosphatase activity was improved in both the soils upon further incubation for another 10 days (20-day interval). Phosphatase activity declined in both pesticides- amended and control soils for further incubation for another 10 days (30- day interval). The release of *p*-nitrophenol from paranitrophenyl disodium ortho phosphate was significantly higher in Black clay soil when compared to Red soil (Table 5). Similarly, Mancozeb N<sub>10</sub> {10 times the normal application (60 kg ha<sup>-1</sup>)} brought about a 41% stimulation in activity after 14 days of incubation compared to control. But, after 28 days of incubation a 30% decrease in enzyme activity was recorded (Rasool and Zafar, 2010). In the pot study alkaline phosphatase activity was inhibited by swing at high doses and stimulated by unix (Jastrzebska and Kucharski, 2007). (Gopal et al, 2007) showed that 10 % of Azadirachtin granules at all doses exerted suppressive effect on phosphatase activity. Interestingly, (Quian et al, 2007) reported that the Validamycin stimulated its activity higher than that of control, only highest dose stimulated acid phosphatase activity by 29.7%. The Cadmium reduced the activity of phosphatase at early incubation time (1-7 days), while the reduction almost disappeared at the end of the incubation (Wang et al, 2007). When Cd (10 mg kg<sup>-1</sup>) was combined with butachlor (50 and 100 mg kg<sup>-1</sup>), the activity of phosphatase became lower than without combination at early incubation time, which indicated that the toxicity of Cd significantly increased (P < 0.05 or 0.001). However, when Cd (10 mg kg<sup>-1</sup>) was combined with butachlor (10 mg kg<sup>-1</sup>), the activity of phosphatase became higher than those without combination at the end of the incubation, which indicated that the toxicity of Cd decreased. (Piotrowska-Seget et al, 2008) noticed that the acid and alkaline phosphatase activities were

significantly reduced by soil treatment with captan. Obviously, phosphatase activity is instability in the beginning of 2 to 4 weeks incubation along with decomposing of mixed pesticides of deltamethrin and probinex in soil, and decline to last part of 12 weeks incubation (Rahmansyah et al, 2009). (Wang et al, 2009) showed that the phosphatase activity in copper concentrations of orchard soils significantly increased with increasing orchard ages ranging from 21.8 to 141 mg kg<sup>-1</sup>, and the CaCl<sub>2</sub>- extractable soil Cu concentrations varied from 0.00 to 4.26 mg kg<sup>-1</sup>. The soil mean C<sub>mic</sub> values varied from 43.6 to 116 mg kg<sup>-1</sup> in the orchard soils, and were lower than the value of the reference soil (144 mg kg<sup>-1</sup>). The ratio of soil C<sub>mic</sub> to total organic C (C<sub>org</sub>) increased from 8.1- to 18.3 mg C<sub>mic</sub> g<sup>-1</sup> C<sub>org</sub> with decreasing orchard ages, and was 26.1 mg C<sub>mic</sub> g<sup>-1</sup> C<sub>org</sub> for the reference soil. A significant correlation was observed between total or CaCl<sub>2</sub>- extractable soil Cu and soil C<sub>mic</sub> or C<sub>mic</sub>/C<sub>org</sub>, suggesting that the soil Cu was responsible for the significant reductions in C<sub>mic</sub> and C<sub>mic</sub>/C<sub>org</sub>. On the other hand (Cycon et al, 2010) reported that the acid and alkaline phosphatase was more sensitive to mancozeb + dimethomorph at 1500mg kg<sup>-1</sup> and its activity declined in both loamy soils and sandy loam soils. However, mancozeb, endosulfan and chlorpyrifos at 100 mg/ kg inhibited 50% activity of phosphatase (Sharma et al, 2010.) Similarly, (Suryakalyani et al, 2010) observed that acid phosphatase activity enhanced 1.8 times, respectively by the 14<sup>th</sup> day of incubation with 1ppm endosulfan.

## CONCLUSION

The application of the selected pesticides Oxydemeton Methyl, Emamectin Benzoate, Dithane- Z-78 and Benlate to soils increased the phosphatase enzyme activity upto 2.5 kg ha<sup>-1</sup> and decreased the enzyme activity when increased the pesticide concentration in both soils. Stimulation and pronounced activity of phosphatase by selective pesticides was noticed at 20 day period of incubation. Prolonged incubation upto 40 days of pesticide treated soils on the enzyme activity showed no effect. These results of the present study clearly indicate that these pesticides widely used in the cultivation of groundnut, at field application rates, enhance the activity of phosphatase enzyme in black and red soils of Anantapuramu District, Andhra Pradesh, India.

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