Influence of Insecticides Thiodicarb and Dimethoate on Soil Microbial Activities (Phosphatase) in two Groundnut (Arachis hypogaea. L) soils.

A.Rekhapadmini, B. Anuradha and Rangaswamy. V*

Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India.

*Corresponding author
Rangaswamy. V
Professor, Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, India.
E-mail:rangamanjula@yahoo.com
Mobile No: +91 83338 32874

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Abstract
In Agricultural practices pesticides are used for crop production and to produce high yield. But indiscriminate and excessive use of pesticides in agriculture leads to environmental pollution and in soil it is not degrading. A pesticide disturbs the activities of soil enzymes and soil micro biota. So we investigated in laboratory conditions that the effect of two insecticides, Thiodicarb (Dimethyl N, N'-(thiobis ((methylimino) carbonyloxy)) bis (ethanimidothioate) and Dimethoate (O, O-dimethyl S-[2-(methylamino)-2-oxoethyl]) dithiophosphate) on enzyme activity, such as Phosphatase in two soils collected from groundnut (Arachis hypogaea. L) cultivated fields of Anantapuram district of Andhra Pradesh, India, by conducting experiments at different concentrations (10, 25, 50, 75, 100ppm) which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5,10.0 kg ha−1). In our present study we observed, Phosphatase activity were significantly enhanced at 2.5 and 5.0 kg ha−1 in black and red soils after 10 days of incubation. Furthermore increase in concentration of insecticides and decreased the rate of enzyme activity. However the stimulatory effect was continued up to 20 days of incubation in black and red soils. Whereas, the decline phase was started after 20 days and the minimum enzyme activities were noticed at the end of 40 days of incubation. But higher concentrations of insecticides at the level of 7.5 to 10.0 kg ha−1 were either toxic or innocuous to phosphatase activity in black and red soils.

Keywords:- Dimethoate, Thiodicarb, Groundnut (Arachis hypogaea. L) soils, SoilPhosphatase activity.

INTRODUCTION
India is one of the largest producers of oilseeds in the world and occupies an important position in the Indian agricultural economy (Kalamkar, S.S., 2006). Groundnut is called as the ‘King’ of oilseeds. It is one of the most important food and cash crops of our country (Madhusudhana, B., 2013). Anantapuramu, a semi-arid region of Andhra Pradesh, India although ranks first in area of groundnut (Arachis hypogaea. L.) cultivation, in the state (Anonymous, 2013) and its productivity is low fluctuating around 9 q/ha on average. More than 120 pests affect economically important crops like groundnut, cotton, and tomato (Rangaswamy and Venkateswarlu, 1992; Megharaj etal.1999; Jayashree and Vasudevan 2007; Vijay Gundi et al. 2007; and Romeh et al. 2009). In India, 15–20% of agricultural production is negatively influenced by pests (BhaleraoT. S. and Puranik P. R., 2007). An estimated annual loss of Rs. 150 crores in groundnut due to pests has been reported (Singh, V. 1980 and Amin, P.W., 1983). Pesticides are the important agrochemicals used for prevention of crops from pests. Their use has been largely increased in last few decades. (Sonia Sethi and Saksham Gupta, 2013).

Indiscriminate and excessive use of toxic synthetic pesticides damaged not only environment and agriculture but have also entered in to the food chain there by affecting all living beings. Indiscriminate use of synthetic pesticides in ground nut ecosystem lead to killing of useful organisms,
contamination in the food chain, pollution in air and water (NandagopalV. and Gbewande, M.P. 2004). When a pesticide is released deliberately or accidentally into the environment, about 0.1% is reaching the target organism, while the remaining 99.9% not only troubles local metabolism or enzymatic activities (Pimentel, 1995; Topp, et al. 1997; Engelen et al. 1998; Carriger et al. 2006; and Liu et al. 2008), but also disturbs soil ecosystem, and thus may affect human health by entering in the food chain, which has raised considerable public concern. Soil enzyme activity is believed to be sensitive to pollution and has been proposed as an index of soil degradation (Trasar-Cepeda et al., 2000; and Gianfreda et al., 2005). The assessment of soil enzyme activities is simple, requires low labor costs compared to other biochemical analysis (Ndiaye et al., 2000), and the results are correlated to other soil properties (Klose et al., 1999; Moore et al., 2000; Ndiaye et al., 2000; and Trasar-Cepeda et al., 2000). Further, it has been reported that any change in soil management and land use is reflected in the soil enzyme activities, and that they can anticipate changes in soil quality before they are detected by other soil analyses (Ndiaye et al., 2000).

The objective of this present study was to evaluate the effect of two insecticides on soil Phosphatase activity in two ground nut soils under normal field concentration and high concentrations because of their immense role in maintaining biodynamics of soil ecosystem and actively involved in soil Phosphorous cycle.

**MATERIALS AND METHODS**

**Soils used in the present study**

Black clay soil and Red sandy loam soil samples, with a known history of insecticide used were collected from fields of groundnut cultivated area of Anantapuramu District, Andhra Pradesh, India. The collected soil samples were chosen from a depth of 12 cm mixed, air-dried, and sieved through a 2-mm mesh prior to use. Two soil samples, a black clay soil and red sandy loam soil were used in the present study.

**Analysis of Physico-chemical properties of soils**

For soil sample characterization, selected physical and chemical properties were determined by using the well-established laboratory procedures. Potential for hydrogen ion (pH), of the soil samples was determined by mixing soil and water in the ratio of 1:1.25 using Systronic digital pH meter with calomel glass electrode assembly. The electrical conductivity of soil samples after addition of 100 ml distilled water to 1 g soil samples was measured by a conductivity bridge. Water-holding capacity (WHC) of the soil samples was determined by adding distilled water up to the saturation point and then 60 % water-holding capacity of the soil samples was calculated by Johnson and Ulrich (1960). Mineral matter of soil samples such as sand, silt and clay contents were analyzed with the use of different sizes of sieves by following the method of Alexander (1961). Organic carbon content in soil samples was estimated by Walkley Black method and the organic matter was calculated by multiplying the values with 1.72 (Jackson 1971). The total nitrogen content in soil samples was determined by the Micro-Kjeldhal method reported by Jackson (1971). The inorganic ammonium nitrogen content in soil samples after extraction of 1 M KCl by the Nesslerization method Jackson (1971) and the contents of nitrate nitrogen were determined by the method reported by Barnes and Folkard (1951), and the contents of nitrate-nitrogen by Brucine method Ranney and Bartlett (1972) after extraction with distilled water were determined. Physico- Chemical characters of the two soil samples are listed in Table 1

**Insecticides and chemicals used in the present study**

To determine the influence of selected insecticides on the groundnut soil, the commercial grades of Dimethoate-30 %EC from Rogorand Thiodicarb-75 %WP were obtained from Bayer’s Science and chemicals are from SRL Pvt. Ltd. India.

**Enzyme Used In the Present Study**

**Phosphatase activity (E.C. 3.1.1.1)**

The activity of phosphatase under the influence of the Insecticides at different concentrations was determined in black clay and red sandy loam soils. Two gram portions of soil samples were transferred into test tubes(12 × 125 mm) were treated with two Insecticides to provide final concentrations of 10, 25, 50, 75 and 100 g-isol (equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha-1 field application rates). The soil samples without Insecticides treatment served as control. All the treatments including controls were incubated in the laboratory at 28 ± 4 C by maintaining 60 % water-holding capacity. After 10 days of incubation period, triplicate soil samples were withdrawn for the assay of phosphatase (Tabatabai and Brenner 1969; and Srinivasulu et al. 2012). Similarly, the influence of the two insecticides at stimulatory concentration (5.0 kg ha-1) on the rate of phosphatase activity in two different soils was also determined in triplicate soil samples at 10, 20, 30 and 40 days of incubation.

**Assay of phosphatase**

For the assay of phosphatase activity, each soil sample was treated with 6 ml of 0.1 M maleate buffer (pH 6.5) and 2 ml of 0.03 M p-nitrophenyl phosphate and the tubes incubated at 37 °C for 30 min. After incubation, the tubes were placed on ice before the soil extracts were passed through Whatman No.1 filter paper. To suitable aliquots of the extract, 1 ml of 5 M CaCl2 and 4 ml of 0.05 M NaOH were added and the yellow color developed was read at405 nm in a Spectrophotometer (Milton Roy Company).

**Statistical Analysis**

The concentrations of the Alkaline Phosphatase enzyme were calculated on soil weight (over dried) basis. The insecticide treatments with untreated controls and the significant levels P≤0.05 between values of each sampling, each insecticide were performed using SYSTAT statistical software package to find the results of Duncan’s Multiple Range (DMR) test (Megharaj et al. 1999)

**RESULTS AND DISCUSSION**

The dark and red mud soils are overwhelmingly utilized for the development of groundnut (Arachis hypogaea L.) in the Anantapuramu local of Andhra Pradesh, India. The major limitations in the groundnut crop production are insects and fungi pests. Because of this reason pesticides are frequently used for crop protection. Continuous and indiscriminate use of these pesticides

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

<table>
<thead>
<tr>
<th>Properties</th>
<th>Black clay soil</th>
<th>Red sandy loam soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.7</td>
<td>7.5</td>
</tr>
<tr>
<td>Electric conductivity (m.mhos)</td>
<td>272</td>
<td>220</td>
</tr>
<tr>
<td>Water holding capacity (ml g⁻¹ soil)</td>
<td>0.45</td>
<td>0.33</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>77.2</td>
<td>63.6</td>
</tr>
<tr>
<td>Silt(%)</td>
<td>16.8</td>
<td>23.3</td>
</tr>
<tr>
<td>Clay(%)</td>
<td>6.0</td>
<td>13.1</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.85</td>
<td>1.27</td>
</tr>
<tr>
<td>Total nitrogen (%)</td>
<td>0.087</td>
<td>0.054</td>
</tr>
<tr>
<td>NH₄⁺- N (µg g⁻¹ soil)</td>
<td>8.42</td>
<td>7.74</td>
</tr>
<tr>
<td>NO₂⁻- N (µg g⁻¹ soil)</td>
<td>0.56</td>
<td>0.41</td>
</tr>
<tr>
<td>NO₃⁻- N (µg g⁻¹ soil)</td>
<td>0.92</td>
<td>0.81</td>
</tr>
</tbody>
</table>

¹:1.25 (Soil:Water)
²:Walkley-Black method (Jackson, 1971)
³:Micro-Kjeldhal method (Jackson, 1971)
⁴:Nesslerization method (Jackson, 1971)
⁵:Diazotization method (Barnes and Folkard, 1951)
⁶:Brucine method (Ranney and Bartler, 1972)

Table 2: Influence of selected Pesticides on activity of phosphatase* in Black clay soil after 10 days incubation.

<table>
<thead>
<tr>
<th>Pesticide concentration (kg ha⁻¹)</th>
<th>Thiodicarb</th>
<th>Dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>200±1.94f(100)</td>
<td>200±2.42f(100)</td>
</tr>
<tr>
<td>1.0</td>
<td>590±1.72d(295)</td>
<td>530±2.62d(265)</td>
</tr>
<tr>
<td>2.5</td>
<td>680±1.29c(340)</td>
<td>670±0.85c(335)</td>
</tr>
<tr>
<td>5.0</td>
<td>850±1.93a(405)</td>
<td>770±2.48a(385)</td>
</tr>
<tr>
<td>7.5</td>
<td>90±2.56b(45)</td>
<td>80±1.91b(40)</td>
</tr>
<tr>
<td>10.0</td>
<td>60±3.62e(30)</td>
<td>50±0.74e(25)</td>
</tr>
</tbody>
</table>

*µg of p-nitrophenol (PNP) g⁻¹ soil formed after 24 hours incubation with p-nitro phenyl phosphate (PNPP).

Figures, in parentheses indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan’s multiple range (DMR) test.

Table 3: Influence of selected Pesticides on activity of phosphatase* in red sandy loam soil after 10 days

<table>
<thead>
<tr>
<th>Pesticide concentration (kg h⁻¹)</th>
<th>Thiodicarb</th>
<th>Dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>120±1.94f(100)</td>
<td>160±2.42f(100)</td>
</tr>
<tr>
<td>1.0</td>
<td>390±1.72d(295)</td>
<td>230±2.62d(265)</td>
</tr>
<tr>
<td>2.5</td>
<td>480±1.29c(340)</td>
<td>570±0.85c(335)</td>
</tr>
<tr>
<td>5.0</td>
<td>710±1.93a(405)</td>
<td>670±2.48a(385)</td>
</tr>
<tr>
<td>7.5</td>
<td>70±2.56b(58.3)</td>
<td>80±1.91b(50)</td>
</tr>
<tr>
<td>10.0</td>
<td>40±3.62e(33.3)</td>
<td>40±0.74e(25)</td>
</tr>
</tbody>
</table>

*µg of p-nitrophenol (PNP) g⁻¹ soil formed after 24 hours incubation with p-nitro phenyl phosphate (PNPP).

Figures, in parentheses indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan’s multiple range (DMR) test.

causes a major risk of soil health. Hence, these soils were selected to study the effect of insecticides on enzyme activities. In general, the organic matter content is high in black soil it leads to pronounced more activity in black soil than in red soil under the influence of insecticides. There have been many reports of the effects of pesticides on soil enzyme activities (Anonymous 2011; and Loganathan et al. 2002) and it has been observed that the responses of soil enzymes on different pesticides are not the same. Soil enzyme activities are more sensitive to the environment. They reflect the soil quality more quickly and directly (Srinivasulu et al. 2012).

Since enzyme activity has been considered as a very sensitive indicator, any disturbance due to biotic or environmental stresses in the soil ecosystem may affect soil biological properties. Our analysis revealed that phosphatase activity was significantly increased in both soils by both pesticides from 1.0 to 5.0 kg ha⁻¹ whereas the activity was decreased at higher concentrations (7.5–10.0 kg ha⁻¹) of pesticides at 24 hrs as shown in Table 2 respectively when compared to control in black soils.
Phosphatases are a broad group of enzymes that are capable of catalysing hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Lawoski 1961). The phosphorus (P) cycle in soils plays a key role in the soil-plant environment because P is an essential nutrient for plant growth and development.

**Phosphatase activity**

From the above results, about 45–305 and 60–285 % increase in phosphatase activity over the control was noticed in the black soil with 20 days of incubation, whereas in the case of red sandy loam soil, the corresponding figures of the percentage enhancement by the two selected Insecticides were 33–405 and 25–385 % during the same period of incubation. In comparison, thiodicarb at 5.0 kg ha⁻¹ produced maximum stimulation in phosphatase activity in black clay soil than red sandy loam soil. At higher concentrations, i.e., 7.5 and 10.0 kg ha⁻¹, phosphatase activity was significantly inhibited by treating the selected soil samples with both the selected Insecticides. Among the two Insecticides treatments, thiodicarb produced a different stimulation over the control. In the present study, comparatively, black soil showed higher enzyme activity than red soil throughout the experiment. Phosphatase activity was significantly inhibited at higher concentrations, i.e., 7.5 and 10.0 kg ha⁻¹ in both the Insecticides treatments, gradually with the incubation periods, i.e., 20, 30 and 40 days (Rangaswamy and Venkateswarlu 1996). Therefore, in the present experiment, the maximum inhibition was recorded after 30 and 40 days of incubation periods. The enhancement in phosphatase activity over control was noticed in the black soil after 20 days of incubation. In the case of red sandy loam soil, the enhancement by the selected Insecticides was also obtained at 5.0 kg ha⁻¹ for 20 days of incubation and recorded in Table 2. The inhibitory effect was reduced upon further incubation, i.e., 30 and 40 days, due to the reduction in the concentration and degradation of the applied Insecticides (Fig. 1). Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms and thus in the cycling of it within the soil (Schneider et al. 2001). The activities of phosphatases were inhibited in sandy loam soil amended with captan during 94 days of incubation (Piotrowska et al. 2008). Phosphatase activity was slightly inhibited by the lower dosage of carbendazim, whereas higher dosage of carbendazim caused significant reduction in phosphatase activity 1 day after treatment (Yan et al. 2011). No significant inhibitory effect was observed on the activity of phosphatases by the repeated additions of chlorothalonil in soil (Yun Long et al. 2006).

As the incubation period increases, there was decreased average phosphatase activity within significant difference between various thiodicarb and dimethoate concentrations used in the present study. The decrease in the enzyme activity may be due to the decrease in the microbial population, destruction or inactivation of preexisting soil enzymes and the substrate limiting for enzyme induction. From the present study, the process of phosphorus solubilization was not distributed by the selected Insecticide treatment. Ahemad and Khan (2011) showed that P-solubilization was in a minor way affected at recommended doses, but majorly affected at higher doses of thiodicarb and dimethoate. This may be because the soil microbial population can solubilize the added insoluble phosphates and is enriched in the presence of higher concentrations of the selected Insecticides. In addition to that, the total population of microorganisms was decreased and resulted in the lesser utilization of the released phosphorus.

**CONCLUSION**

Based on the results obtained from above, we are concluded that the observed stimulation or inhibition of this enzyme at low or high concentrations of the insecticides could be attributed to number of populations of phospholytic organisms present in both soils, the
phosphatase activity in both soils is profoundly increased in both pesticide concentrations 1.0 to 5.0 kg ha\(^{-1}\) at 24 hrs, at higher contractions (7.5–10.0 kg ha\(^{-1}\)) a suppressed activity in the phosphatase enzyme with individual treatments of pesticides compared to control, the pesticides Thiodicarb and Dimethoate are as an important agents for the control of plant pathogens, Thiodicarb and Dimethoate is often not used at much higher than the recommended dosage in order to maintain soil health. A very few reports are available on the influence of insecticides on phosphatase enzyme.

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**References:**


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