Influence of Selected Insecticides on Enzyme Activities in Groundnut (Arachis hypogaea L.) Soils

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ABSTRACT

The influence of acephate and imidacloprid on important soil enzyme activities, such as dehydrogenase and urease in two groundnut (Arachis hypogaea L.) soils, collected from Anantapur District of Andhra Pradesh, India, was studied under laboratory conditions. The activity of dehydrogenase, in terms of formazan formed from triphenyl tetrazolium chloride, was more pronounced in both soils treated with 2.5 kg ha⁻¹ of the acephate and imidacloprid. But higher concentrations (5.0, 7.5 and 10 kg ha⁻¹) were toxic to dehydrogenase activity. The activity of urease in terms of ammonia formed from hydrolysis of urea was higher in both soils, treated with acephate and imidacloprid at 5.0 kg ha⁻¹, but higher levels (7.5 and 10 kg ha⁻¹) were toxic or innocuous to urease activity.

Keywords: acephate, dehydrogenase, imidacloprid, soil enzymes, urease

INTRODUCTION

The economy of India is largely dependent on the quality and quantity of agricultural produce. Better harvest requires intensive cultivation, irrigation, fertilizers and more importantly pesticides to protect plants from pests and plant diseases. In India, about 15–20% of agricultural production is negatively influenced by pests (Bhalerao and Puranik 2007). Organophosphates, synthetic pyrethroids, carbamates, triazoles and organochlorine pesticides either singly or in combination are routinely used to control major pests which affect economically important crops like groundnut, cotton, and tomato (Megharaj et al. 1989; Rangaswamy and Venkateswarlu 1992; Vijay Gandi et al. 2007; Jayashree and Vasudevan 2007; Romeh et al. 2009). In modern agriculture, pesticides are used in large quantities for controlling not only pests and weeds but also improving the crop yield. A study of the effect of pesticides on soil microflora and their beneficial activities forms an important part of the pesticides risk assessment. However, intensive use of common pesticides can lead to toxicity to soils, which may inhibit several biochemical reactions. Due to a high degree of toxicity, some pesticides, particularly those persistent in soil, constitute a very important group of contaminants. When pesticides are applied to soils, they may interact with non-target soil microorganisms and exhibit chronic diverse effects on soil microflora (Omar and Abdel Sater 2001; Moorman 1989; Tu 1995; Pimentol and Levitan 1986; Sarfraz et al. 2009). Further, they affect ecological balance in terms of soil fertility (Aramendia et al. 2007; Swaminathan et al. 2009). Although these pesticides have been restrictively used or even banned in some countries for several years, their persistence and bioaccumulation can still be found in many soils and plants (Vinas et al. 2002). It is well known that a soil is an open but self regulating ecosystem with a large diversity of microbial populations (Kizilkaya et al. 2004). The living dynamic nature of living organisms is one of the important features of soil quality and often used as a bio indicator for soil health (Gianfreda et al. 2005; Sukul 2006).

Soil enzymes, that represent the major living organism activities, are involved in catalyzing various reactions necessary for organic matter metabolism, nutrient cycling, energy transfer, and crop productivity (Kizilkaya et al. 2004). Soil enzymes are potential indicators and act as biological catalysts of various important biochemical reactions to produce essential components besides playing an important role in soil fertility (Pascual et al. 2000; Garcia et al. 2000; Bending et al. 2006; Benedetti and Dilly 2006; Quian et al. 2009). The composition of the soil surroundings, insecticides, may be directly or indirectly influences the catalytic efficiency of soil enzymes (Bollag and Liu 1990; Min et al. 2001). Soil enzyme activities are used to assess the negative effects of pollutants such as pesticides, illicit drugs, petroleum hydrocarbons and heavy metals on soil ecosystem (Pandey and Singh 2006). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, only limited studies are available on influence of organochemicals on soil enzymes (Nannipieri and Landa 2000; Walker et al. 2001; Ramesh et al. 2003; Pessagno et al. 2008). Some of the microbial processes for assessing the effects of contaminants on soil health include dehydrogenase; an intracellular enzyme belonging to oxidoreductases present in all soil microorganisms used as a measure of total microbial activity in soil (Trevors 1984). Soil dehydrogenase is a specific kind of enzyme which plays significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Sebiono et al. 2011). The objective of the present study is to evaluate the effect of imidacloprid and acephate applied at normal field and high concentrations in laboratory. Urease and dehydrogenase activities are very important for soil quality (Wang et al. 2010). Hence dehydrogenase and urease activities were selected because of their significance in soils.
60% water holding capacity (WHC), about 2 ml of deionized distilled water. In order to maintain the same amount of distilled water was added to test tubes containing black soil and 1 ml into tubes containing red soil. Untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 7, 14, 28, and 35 days. During incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

**ASSAY OF DEHYDROPENTASE:** The method employed for the assay of dehydrogenase was developed by Casida et al. (1964). This method is based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.1 g of CaCO₃ and 1 ml of 0.18 mM aqueous solution of TTC and incubated for 24 h at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a Spectronic 20D spectrophotometer (Milton Roy Co.).

2. **UREASE ACTIVITY (E.C. 3.5.1.5)**

To study the effect of imidacloprid and acephate on urease, the soil samples were prepared according to the method described in the assay of dehydrogenase. Untreated soil samples were considered as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 10, 20, 30, and 40 days. During the incubation period, a certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the assay of urease by following the phenol hypochlorite method (Fawcett and Scott 1960). The influence of two insecticides at 2.5 and 5.0 kg ha⁻¹ on the rate of urease activity was also determined in two soil samples. After 10, 20, 30, and 40 days of incubation at room temperature (28 ± 4°C), triplicate soil samples of each treatment were withdrawn for the enzyme assay.

**ASSAY OF UREASE:** For the assay of soil urease, the soil samples were mixed with 4 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 1 ml of 1 M urea solution, and incubated for 30 min. After incubation, the enzymatic reaction was stopped by adding 10 ml of 2 M KCl at 4°C for 10 min. Suspensions were centrifuged at 5000 rpm for 5 min and the NH₄⁺ ions content in supernatant was determined by the phenol hypochlorite method (Fawcett and Scott 1960). Two ml of supernatant was mixed with 5 ml of phenol sodium nitroprusside and 5 ml of 0.02 M sodium hypochlorite and incubated for 30 min in the dark. The absorbance of the blue color formed was read at 630 nm in a Spectronic 20D Spectrophotometer (Milton Roy).**

**Statistical analysis**

The concentration of the dehydrogenase and urease was calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan’s multiple range test (DMRT) (Jaffer et al. 2010). All statistical analysis was performed at P ≤ 0.05 using SYSTAT statistical software package.

**RESULTS AND DISCUSSION**

**Dehydrogenase activity**

To determine the selective influence of the two pesticides (imidacloprid and acephate) on dehydrogenase activity, the soil samples were treated with different concentrations (1.0, 2.5, 5.0, 7.5, and 10 kg ha⁻¹) of the pesticides for 7 days and the treated samples were exposed to TTC, which is a water-soluble and its redox potential is about -0.08mV and functions as an electron acceptor for several dehydrogenases. Nearly all microorganisms reduce TTC into TPF. Dehydrogenase activity was enhanced in both the soil samples following the application of imidacloprid and acephate at the concentration of 2.5 and 1.0 kg ha⁻¹, whereas, higher levels (5.0 to 10.0 kg ha⁻¹) were either toxic or innocuous to the enzyme activity. The two soil samples (black and red soil) treated with imidacloprid and acephate at 10 and 25 µg g⁻¹ levels for 7 days and exposed to TTC for 24 h showed individual increments of 38-43, 30-48, 43-46 and 65-76% in dehydrogenase activity over the control. A
significant increase in the activity of dehydrogenase was noticed after the application of acephate at 2.5 kg ha\(^{-1}\) in red soil (Table 2) and also in the accumulation of formazan when the incubation period was raised to 21 days (Figs. 1, 2). Hence, the results in the present study clearly indicated that the activity of dehydrogenase in black soil was comparatively higher than in red soil. In contrast, Cycon et al. (2010) reported that dehydrogenase activity decreased in sandy loam soils in combination of mancozeb and dimethoate at higher concentrations. Similar observations were made by Monkiedje et al. (2002) with mfenoxam and metalaxyl and as well as azoxystrobins, tebuconazole and chlorothalonil by Bending et al. (2007). In the same manner dehydrogenase activity was decreased to 39.3% in unamended soils (Table 2) and by benomyl at 100 to 10000 μg g\(^{-1}\) soil. The extent of inhibition of dehydrogenase activity in peptone-amended soil to the controls. However, higher concentrations (7.5 and 10 kg ha\(^{-1}\)) were toxic to urease activity after 10 days' incubation whereas dehydrogenase activity was initially reduced by tefluthrin and unaffected by other pesticides in an organic soil after 2 weeks (Tu 1990). In some cases, dehydrogenase activity was unaffected by several pesticides (Chendrayan et al. 1980; Tu 1981).

The data presented in Table 2 reveals that significant inhibition of dehydrogenase activity occurred at higher concentrations (10 kg ha\(^{-1}\)) of imidacloprid and acephate in both black and red soils collected from groundnut-cultivated fields. Similarly, Gowda (1973) also reported the inhibition of dehydrogenase activity in peptone-amended soil by benomyl at 100 to 10000 μg g\(^{-1}\) soil. The extent of dehydrogenase activity of soil samples under the impact of selected insecticides at 2.5 kg ha\(^{-1}\) was also determined by incubating the insecticide-treated samples for 7, 14, 21, 28 and 35 days (Figs. 1, 2). In general, dehydrogenase activity was relatively lower in the soil maintained under non-flooded conditions as reported by Chendrayan et al. (1980). This is expected because dehydrogenase activity is significantly more pronounced in flooded soils, as most dehydrogenases are anaerobic in origin (Chendrayan et al. 1980). There was a progressive increase in the accumulation of formazan with increasing incubation period up to 21 days, which gradually decreased further. Hence, dehydrogenase activity was significantly enhanced with 2.5 kg ha\(^{-1}\) of the two insecticides up to 21 days of incubation. In fact, application of insecticides to soils led to an initial striking increase in dehydrogenase activity.

**Urease activity**

The activity of urease, implicated in the hydrolysis of urea, was significantly enhanced by the insecticides acephate and imidacloprid up to 5.0 kg ha\(^{-1}\), in both soils, in comparison to the controls. However, higher concentrations (7.5 and 10 kg ha\(^{-1}\)) were toxic to urease activity after 10 days' incubation (Table 3). The activity of urease in terms of am-

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**Table 2: Activity of dehydrogenase* under the impact of different concentrations of selected insecticides in soils (both black and red) for 24 h after 7 days.**

<table>
<thead>
<tr>
<th>Concentration of insecticides (Kg ha(^{-1}))</th>
<th>Imidacloprid</th>
<th>Acephate</th>
<th>Imidacloprid</th>
<th>Acephate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1850 ± 15.773 e</td>
<td>1850 ± 15.773 e</td>
<td>1020 ± 11.547 f</td>
<td>1020 ± 11.547 f</td>
</tr>
<tr>
<td>1.0</td>
<td>2560 ± 5.774 b</td>
<td>2405 ± 2.886 b</td>
<td>1460 ± 11.547 b</td>
<td>1660 ± 11.547 c</td>
</tr>
<tr>
<td>2.5</td>
<td>2650 ± 5.773 a</td>
<td>2749 ± 0.577 a</td>
<td>1490 ± 5.773 a</td>
<td>1800 ± 11.547 a</td>
</tr>
<tr>
<td>5.0</td>
<td>2215 ± 8.660 c</td>
<td>2000 ± 5.773 c</td>
<td>1440 ± 23.094 c</td>
<td>1730 ± 17.320 b</td>
</tr>
<tr>
<td>7.5</td>
<td>1950 ± 11.547 d</td>
<td>1670 ± 5.773 d</td>
<td>1300 ± 11.547 d</td>
<td>1600 ± 23.094 d</td>
</tr>
<tr>
<td>10.0</td>
<td>1450 ± 5.773 f</td>
<td>1020 ± 11.547 f</td>
<td>1220 ± 11.547 e</td>
<td>1580 ± 11.547 c</td>
</tr>
</tbody>
</table>

*μg formazan g\(^{-1}\) soil formed after 24 h incubation with triphenyl tetrazolium chloride (TTC). Means, in each time period, followed by the same letter are significantly different (P ≤ 0.05) from each other according to DMR test.
Imidacloprid and Acephate reduced urease activity in soils. According to Gianfreda et al. (1994), glyphosate enhanced urease activity in sandy loam soil (Tu 1980). In another study, urease activity decreased significantly after 30 to 60 days of incubation with the insecticide deltamethrin and the fungicide prothioconazole. In contrast, thiram at 10 ppm decreased urease activity in both sandy and organic soils after 7 days (Tu 1990). In another study, urease activity was not affected by the presence of glyphosate at 5.4 kg ha⁻¹ in soil (Davies and Greaves 1981). Pesticides, including organophosphorus insecticides, could disrupt urea hydrolysis in soils at higher doses ranging from 100-1000 ppm (Lethbridge et al. 1981). Fenamiphos at 18.6 kg ha⁻¹ reduced the activity of urease under field conditions but after 5 months’ activity was the same as in the control while no effect was observed under laboratory conditions (Ross et al. 1984; Ross and Speir 1985).

**CONCLUSION**

The results obtained in the present study clearly indicate that the insecticides imidacloprid and acephate profoundly enhanced the activities of both dehydrogenase and urease at recommended levels in agricultural system to control insect pests.

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