

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 9, September 2017

Effect of Insecticide Bifenthrin on Soil Enzyme Activity in Groundnut (*Arachis hypogaea* L.) Soils

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ABSTRACT : This study was undertaken to determine the effect of insecticide, bifenthrin on dehydrogenase activity in two groundnut (*Arachis hypogaea* L.) soils. The effect of the insecticide was assessed over a period of five weeks for dehydrogenase. Soil samples (red sandy loam and black clay soils) collected from groundnut (*Arachis hypogaea* L.) cultivated fields of Anantapuramu District of Andhra Pradesh, India were treated with insecticide at 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⁻¹) in the laboratory. The activity of dehydrogenase was significantly more treated with bifenthrin at levels of 5.0 kg ha⁻¹ in black and red soils respectively. But at higher concentrations of 7.5 and 10.0 kg ha⁻¹ respectively, bifenthrin were toxic to dehydrogenase activity. The activity of dehydrogenase was drastically decreased with increasing period of incubation up to 28 and 35 days.

KEYWORDS: bifenthrin, dehydrogenase, groundnut (*Arachis hypogaea* L.) soils.

I. INTRODUCTION

The use of pesticides has become an integral and economically essential part of modern agriculture. The sustainable agriculture involves optimizing agricultural resources and at the same time maintaining the quality of environment and sustaining natural resources. Pesticides are the substances intended for preventing, destroying, repelling any pest. Due to bulk handling or accidental release, they are accumulated in soil which leads to occasional entry into ecosystems that show lethal effects on living systems. The indiscriminate use of various pesticides to reduce the impact of pests on agricultural crops has increased over the years, especially in developing countries (Santhakumar and Balaji, 2000). The intensive use of these pesticides in agricultural soils, there may be an interaction with soil micro flora and their metabolic activities besides, controlling the different pest population (Baxter and Cummings, 2008). Groundnut (*Arachis hypogaea* L.) is an annual legume with 3-4 foliate leaves is an important contributor of vegetable oil in India. It occupies a prime place among oil seeds of our country. About 80% of the groundnut production is used for preparation of oil alone. Soil enzyme activity is a measure of soil metabolic activity and soil health. They play an important role in describing and making predictions about an ecosystem productions, quality and interactions among the subsystems both microbes and plants release enzymes. The soil microbial biomass is considered as active nutrient pool to plants and plays an important role in nutrient cycling and decomposition in ecosystem (De-Lorenzo *et al.*, 2001). Pesticides enable to achieve higher crop yields (Ray *et al.*, 2004, Pasquer *et al.*, 2005, Valenciano *et al.*, 2006), but at the same time negatively affect natural environment, including the soil environment, disturbing its homeostasis. Enzymes are secreted by floral and faunal organisms, but most often they are produced by microorganisms. Soil analysis includes the determination of the activity of intracellular enzymes, enzymes found on the cell surface and free enzymes. Their activity is related to the physical properties of the soil, organic matter content and the mechanism of action (Winding *et al.*, 2005). The physicochemical nature of the soil is important for persistence, metabolism and binding of pesticides in the soil (Burns, R.G 1982). Soil is a part of the terrestrial compartment, and supports all terrestrial life forms. Thus, without proper soil protection policies, numerous problems may arise, like reduction of soil

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fertility, erosion, groundwater contamination, insufficient water holding capacity and loss of biodiversity. The study of enzyme activity of soils may reflect the potential capacity of a soil to perform certain biological transformations which are important to soil fertility. Determination of dehydrogenase activity is one of the general criteria used to determine soil microbial activity. Dehydrogenase activity is an indicator of potential non-specific intracellular enzyme activity of the total microbial biomass.

II. MATERIALS AND METHODS

2.1 Soils used in the present study : Agricultural soil samples such as samples of black clay soil and red sandy loam soil, collected from groundnut cultivated fields of Anantapuramu District of Andhra Pradesh, India in a semi arid zone from the depth of 12 cm and mixed thoroughly to prepare a homogenate composite sample. Soil samples air dried at room temperature, were cleaned by removing plant material and other debris and passed through a 2 mm sieve and stored at 4°C prior to analysis.

2.2. Analysis of Physico- chemical characteristics of soil samples: 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). Water holding capacity of the soil samples were 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). pH of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using systronics digital pH meter with calomel glass electrode. 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). Electrical conductivity of soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method (Jackson, 1971). The inorganic ammonium nitrogen content in the soil samples after extraction of 1M KCl by Nesslerization method (Jackson, 1971) and contents of nitrite nitrogen (Barnes and Folkard, 1951), and the contents of nitrate-nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with distilled water were determined respectively. Physico-chemical characters of the two soil samples are listed in table 1.

2.3 Table1 Physico-chemical properties of the soils

Properties	Black soil	Red soil
Sand (%)	66.4	52.4
Silt (%)	23.6	26.9
Clay (%)	9.3	19.4
pH ^a	7.9	7.1
Water holding capacity (ml g ⁻¹ soil)	0.45	0.32
Organic matter (%) ^b	1.44	0.74
Total nitrogen (%) ^c	0.089	0.048
NH ₄ ⁺ - N (µg g ⁻¹ soil) ^d	8.57	7.02
NO ₂ ⁻ -N (µg g ⁻¹ soil) ^e	0.45	0.66
NO ₃ ⁻ -N (µg g ⁻¹ soil) ^f	0.92	0.74

^a1:1.25 = soil: water slurry

^b Walkley- Black method (Johnson and Ulrich, 1960)

^c Micro-Kjeldahl method (Johnson and Ulrich, 1960)

^d Nesslerization method (Johnson and ulrich, 1960)

^e Diazotization method (Ranney and Bartlett, 1972)

^f Brucine method (Barnes and Folkard, 1951)

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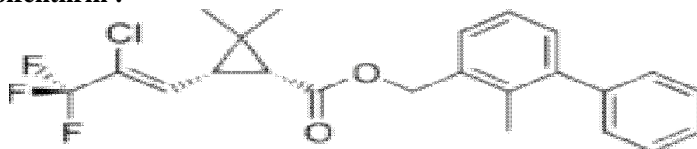
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2.4 INSECTICIDES USED IN THE PRESENT STUDY

Chemical structure of bifenthrin :



To determine the effect of selected insecticide on soil enzyme activities bifenthrin is selected for enzymatic activity. The literature reveals that the information regarding the effect of insecticide bifenthrin on microbial activities in groundnut soils is limited.

III. SOIL INCUBATION STUDIES

3.1 Dehydrogenase activity : To study the effect of bifenthrin on dehydrogenase, 5 g of air dried black clay soil and red sandy loam soil were taken separately in test tubes (12 × 125 mm) containing different concentrations of insecticide 10, 25, 50, 75, and 100 µg g⁻¹ soils which are equal to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha⁻¹ of field application rates. In order to maintain 60% water holding capacity (WHC), about 2 ml of deionized water was added to test tubes containing black clay 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 1, 2, 3, 4, and 5 weeks. During the incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

3.2 Assay of dehydrogenase: 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 solutions of TTC and incubated for 24 hours at 30°C. The triphenyl formazan (TPF) formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a spectrophotometer.

IV. STATISTICAL ANALYSIS

The concentration of dehydrogenase was calculated on the basis of soil weight (oven dried). Data were analysed 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.

V. RESULTS AND DISCUSSION

5.1 Dehydrogenase activity: Table 2: Activity of dehydrogenase* under the impact of different concentrations of selected insecticide in soils (both red and black) for 24 hrs after 7 days.

Concentration of insecticide (kg ha ⁻¹)	Black soil	Red soil
0.0	1659±5.664 d	1320±2.335d
1.0	2250±4.338c	1540±11.535c
2.5	2234±4.224b	2020±11.535b
5.0	2860±5.773a	2410±4.533a
7.5	1230±11.447e	1171±10.773e
10.0	1170±5.770f	1075±10.355f

Each column is mean ± S.E. for six concentration in each group; columns not sharing a common letter (a,b) differ significantly with each other (P ≤ 0.05) from each other according to DMR test

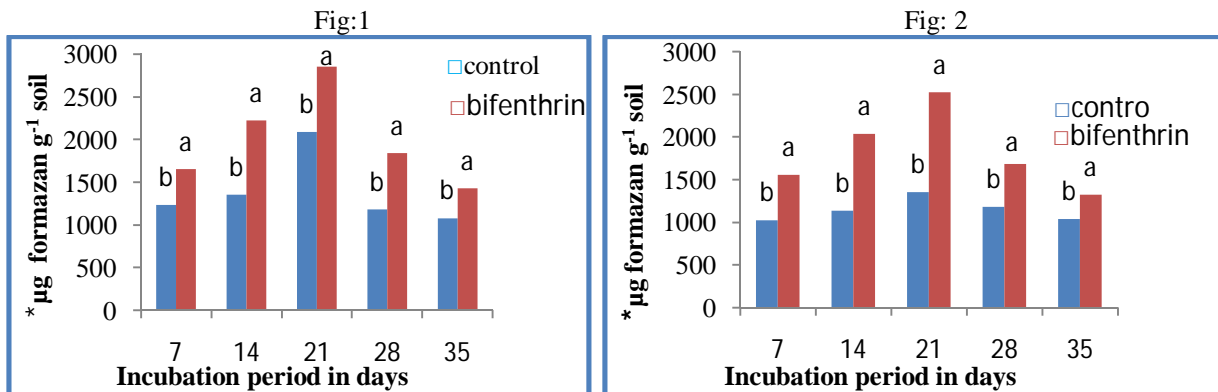


Fig.1 :Influence of selected insecticides at 5.0 kg ha⁻¹ on dehydrogenase activity in black clay soil after 24 hrs *µg formazan g⁻¹ soil formed after 24 hr incubation with triphenyl tetrazolium chloride (TTC). Means in each incubation period, followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan's multiple range test (DMRT).

Fig.2 :Influence of selected insecticides at 5.0 kg ha⁻¹ on dehydrogenase activity in red sandy loam soil after 24 hrs *µg formazan g⁻¹ soil formed after 24 hr incubation with triphenyl tetrazolium chloride (TTC). Means in each incubation period, followed by the same letter are not significantly different ($p \leq 0.05$) from each other according to Duncan's multiple range test (DMRT).

The data presented in the Table 2 reveals that significant inhibition of dehydrogenase activity occurred at higher concentrations (10 Kg ha⁻¹) of bifenthrin in both soils. The effect of pesticides generally decreased enzyme activity with the increase of incubation period. Increase in the concentration of pesticides up to 5 kg ha⁻¹ increase, higher concentration of pesticides decreased the rate of enzyme activities (7.5,10.0 kg ha⁻¹) determine the selective influence of the insecticide on dehydrogenase activity, the soil samples were treated with different concentrations 1.0, 2.5, 5.0,7.5,10 kg ha⁻¹ of the insecticide for 7 days and the treated samples were exposed to triphenyl tetrazolium chloride (TTC) which is a water soluble and its redox potential is about -0.08 mv and functional an electron acceptor for several dehydrogenases (Thalman 1968). However at higher levels (7.5,10.0 kg ha⁻¹) the insecticide were either toxic or innocuous to dehydrogenase activity following 7 days of incubation. At 5.0 kg ha⁻¹, the insecticide stimulated dehydrogenase activity up to 21 days which then gradually decreased. A significant increase in dehydrogenase activity up to 21 days was noticed.

Table 2 reveals that significant inhibition of dehydrogenase activity occurred at higher concentration (10 kg ha⁻¹) of bifenthrin in both black and red soils. In general dehydrogenase activity was relatively lower in the soil maintained under non flooded conditions as reported by (Chendrayan *et al.*,1980). The individual enzyme assays are useful as indicators of microbial activity. Similarly, Singh (2002) observed soil microbes and soil enzymes as an indicator of soil fertility in groundnut. These enzymes adsorbed on the organic and mineral soil particles are important for their activity in the rhizosphere soils (Ruggiero and Radogna, 1988). Taylor *et al.* (2002) described positive correlation between enzyme activities and organic matter content. Soil dehydrogenase activity is considered to be a valuable parameter for assessing the side effects of herbicide treatments on the soil microbial biomass (Stępniewska, Z., Wolińska, A. and Lipińska, R.: *Int. Agrophysics, et al* (2007). Dehydrogenases play a significant role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors (Zhang *et al.*, 2010). Many specific dehydrogenases transfer hydrogen to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Subhani *et al.*, 2001). Soil dehydrogenases are the major representatives of the Oxidoreductase

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enzymes class (Gu *et al.*, 2009). Among all enzymes in the soil environment, dehydrogenases are one of the most important, and are used as an indicator of overall soil microbial activity (Quilchano and Marañon, 2002; Gu *et al.*, 2009; and Salazar *et al.*, 2011), because they occur intracellularly in all living microbial cells (Moeskops *et al.*, 2010; Zhao *et al.*, 2010; Yuan and Yue, 2012). Moreover, they are tightly linked with microbial oxidoreduction processes (Moeskops *et al.*, 2010). Soil enzymatic activity plays an important role in catalyzing reactions indispensable in life processes of soil microorganisms, decomposition of organic residues, circulation of nutrients, as well as forming organic matter and soil structure. Presented and discussed above results are based on our studies. However, the discussion highlights the strong interactions between the soil environment, soil enzymes (dehydrogenases especially) and soil microorganisms.

VI. CONCLUSION

The results of the present study thus, clearly indicated that the insecticide, bifenthrin were profoundly enhanced the activity of dehydrogenase at 5.0 kg ha⁻¹. Based on the above results, it is concluded that the biological activities were not affected, by the insecticides applied at recommended levels in agricultural systems to control insect pests.

VII. ACKNOWLEDGMENT

The author (PR) are grateful to the University Grants Commission UGC-BSR, New Delhi, India, for financial assistance and at the same time we are very much thankful to the Department of Microbiology, Sri Krishna Devaraya University for providing all the necessary facilities in fulfilling our research.

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