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EFFECT OF SELECTED PESTICIDES ON DEHYDROGENASE ACTIVITY IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) SOILS

Madhavi A[1], Srinivasulu M[1], Anuradha B[2], Rangaswamy V[1]*

1. Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu - 515003, Andhra Pradesh, India.
2. Viral Research and Diagnostic Laboratory (VRDL), Government Medical College, Anantapuramu - 515001, Andhra Pradesh, India.

ABSTRACT: Agrochemicals are used to improve economic production. Pesticides are the primary agricultural pollutants. Pesticides in the soil alter soil microbial diversity and soil enzymatic activity. In this study the impact of two insecticides, Oxydemeton Methyl, Emamectin Benzoate and two fungicides, Dithane Z-78, Benomyl on soil enzyme dehydrogenase activity in black clay soil and red sandy loam soil of groundnut (*Arachis hypogaea* L.) cultivated fields of Anantapuramu District of Andhra Pradesh, India was assessed. The soil samples were treated at different concentrations *i.e* 10, 25, 50, 75 and 100 ppm, which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) under laboratory conditions. The triphenylformazan (TPF) formed from tri-phenyl tetrazolium chloride (TTC) due to dehydrogenase activity was more pronounced at 2.5 kg ha^{-1}. Further increase in concentrations (5.0, 7.5 and 10.0 kg ha^{-1}) of pesticides declined the dehydrogenase activity gradually in both soils at 7 - day incubation period and also the enzyme activity declined in control and pesticide treated soils on prolonged period of incubation.

KEYWORDS: *Arachis hypogaea* L., dehydrogenase, pesticides, Oxydemetonmethyl and Benomyl.

Corresponding Author: Prof. Rangaswamy V* Ph.D.
Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu – 515003, Andhra Pradesh, India. Email address: rangamanjula@yahoo.com

1.INTRODUCTION

Soil is a living dynamic system containing many free enzymes, immobilized extracellular enzymes and enzymes within microbial cells. Microorganisms and enzymes play a key role in the functioning

of many ecosystems [1]. Currently, soils are becoming more polluted by pesticides molecules because of their wide use in agriculture practices. With an increased pesticide use, questions are rising on potential effects regarding public health and environment. According to [2], the ideal xenobiotic must be toxic to only target organisms, totally biodegradable and able to not leaving any intermediary compounds in the environment or being lixiviated to underground waters. Pesticides may influence more or less soil biological activities, which is the result of microbial and enzymatic transformations. Pesticides may be toxic to some important bacterial groups; other microorganisms are able to use some pesticides as energy and nutrient sources [3, 4]. In addition, some metabolites of pesticides may even be more adverse to microorganisms than the original compound [5]. Many of pesticides have shown adverse impacts on numbers and functions of diverse range of microorganisms. The reduction of bacterial and fungal biomass may effect soil respiratory activity, soil enzymes and microbial diversity as well as rates of carbon and nitrogen turn over [6, 7]. However, many factors such as soil structure and texture, pH, organic matter content, temperature and moisture influence pesticides effects on soil microorganisms [8, 9]. Microorganisms and enzymes, which are sensitive to stress and respond to contamination faster than other parameters, are potential indicators of soil quality [10-12]. [13] reported that soil enzymes respond quickly to changes in natural and anthropogenic factors that affect soil. According to [10] the most frequently analyzed soil enzymes are : dehydrogenases, catalase, phosphatase, amylase, cellulase, protease, urease and others. Soil dehydrogenase enzymatic activity (DHA) determination in soil was first initiated by [14]. 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 is used as a measure of total microbial activity in soil. Dehydrogenase occurs in all living microbial cells. 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. For the cultivation of groundnut (*Arachis hypogaea* L.) black and red soils are used predominantly in the Anantapuramu district of Andhra Pradesh, India. Black soil has highest organic matter content than red soil [15]. The aim of the present study is to analyze the influence of two selected insecticides, Oxydemeton Methyl, Emamectin Benzoate and two selected fungicides, Dithane Z-78 and Benomyl at normal field application rates and at high concentrations in the laboratory conditions.

2. MATERIALS AND METHODS

2.1 Soils:

Two soil samples of black clay soil and red sandy loam soil were collected from groundnut cultivated fields of Anantapuramu district of Andhra Pradesh, India from a depth of 12 cms near the rhizosphere region, air dried at room temperature and sieved through 2 mm sieve and stored at 4^0C prior to analysis.

2.2 Analysis of the 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 [16]. Water-holding capacity (WHC) of the soil samples was determined by adding distilled water upto the saturation and then 60% water holding capacity of soil was calculated as described by [17]. Soil pH was determined by using 1:1.25 soil to water ratio using Systronics Digital pH meter with calomel glass electrode assembly. Organic carbon content in soil samples was estimated by the Walkley and Black method and the organic matter was calculated by multiplying the values with 1.72 [18]. Electrical conductivity of soil samples after addition of 100 ml distilled water to 1g soil samples was measured by a conductivity bridge. The total nitrogen content in soil samples was determined by Micro-Kjehdal method. Inorganic ammonium-nitrogen content was estimated after extraction of soil samples with 1M KCl by the Nesslerization method and the contents of nitrite-nitrogen were determined by the method reported by [19], and the contents of nitrate-nitrogen by [20]. The physico-chemical characteristics of the two soil samples are listed in table 1.

2.3 Enzyme assay

2.3.1 Dehydrogenase activity:

The activity of dehydrogenase under the influence of two insecticides and two fungicides at different concentrations was determined in black clay soil and red sandy loam soil. Five grams of dried black and red soils were transferred into test tubes (12 x 125 mm) and treated with different concentrations of insecticides and fungicides to provide final concentrations of 10, 25, 50, 75 and 100 ug g^{-1} soil (equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} field application rates). The soil samples without pesticides treatment served as control. The water holding capacity (WHC) of soil was maintained at 60% by adding 2 ml of steam sterilized distilled water to the test tubes containing black soil and 1ml into the tubes containing red soil. All the treatments including controls were maintained in the laboratory at 28 ± 4^0C for 7, 14, 21, 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.

2.3.2 Assay of dehydrogenase:

The method employed for the assay of dehydrogenase was developed by [21-23]. This method is based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF). Each soil sample was treated with 0.1g of $CaCO_3$ and 1ml of 0.18 mM aqueous solution of TTC and incubated for 24 hrs at 30^0C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a U.V Visible Spectrophotometer (Thermo Scientific Evolution 201). Dehydrogenase activity was measured at 7, 14, 21, 28 and 35 days of incubation.

2.3.3 Statistical analysis:

The concentration of dehydrogenase 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) [24, 25]. The statistical analysis was performed at $P \leq 0.05$ using SYSTAT statistical software package.

3. RESULTS AND DISCUSSION

Red and black soils are the predominant in Anantapuramu district of Andhra Pradesh, India for cultivation of groundnut. The physico-chemical properties of soils were mainly depending on the nature of soil (Table 1).

Table No. 1: Physico-chemical properties of soils used in the present study

Properties	Black clay soil	Red sandy loam soil
Sand(%)	76.50	72.00
Silt (%)	18.00	25.00
Clay(%)	5.50	3.00
pH [a]	8.40	6.30
Water holding capacity (ml g^{-1} soil)	0. 48	0.34
Electrical conductivity (m.mhos)	266.00	246.00
Organic matter [b] (%)	0.94	0.80
Total nitrogen [c] (%)	0.05	0.03
NH_4^+ - N(μ g^{-1} soil)[d]	8.95	7.80
NO_2^- - N (μ g^{-1} soil)[e]	0.51	0.35
NO_3^- -N(μ g^{-1} soil)[f]	1.04	0.19

[a]1:1.25 (soil:water);

[b]Walkley-Black method

[c]Micro-Kjeldhal method

[d]Nesslerization method

[e]Diazotization method

[f] Brucine method

In black soils sand percentage is high (76%) when compared to red soils (72%). Both soils are alkaline in nature. Clay percentage is high in black soils (5%) than red soils (3%). The groundnut yield depends on infections caused by pests. To control pests, large quantities of pesticides are used to protect the crop which affects the soil conditions. Study on these soils helps to find the insecticides effect on enzyme activity. The biological activity is more in black soil than in red soil when insecticides were used at 2.5 kg ha^{-1}. Presence of enzymes depends on the type of the pesticide usage [26]. No information is available regarding the influence of Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl on soil enzyme activities. Soil enzyme activities are more

sensitive to the environment. They reflect the soil quality more quickly and directly [15]. When the Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl concentration was increased, the potential hazard to soil was increased at 7.5 to 10.0 kg ha^{-1}. Since enzyme activity is important indicator, and indicates the disturbance due to biotic or environmental stresses in the soil ecosystem. Our analysis revealed that dehydrogenase activity was significantly increased from 0.1 to 2.5 kg ha^{-1} whereas the activity was decreased at higher concentrations (2.5 to 10.0 kg ha^{-1}) of pesticides in both soils (Table 2). In black soils dehydrogenase activity is high at 2.5 kg ha^{-1} concentration for all pesticides and lowest at 10 kg ha^{-1}. The dehydrogenase activity was significantly enhanced at 2.5 kg ha^{-1} level in both soils treated with dithane Z-78 and showed individual increments of dehydrogenase activity ranging from a low increase in comparison to control (Table 2).

Table No. 2: Influence of different concentrations of selected insecticides and fungicides on dehydrogenase activity* in black soil after 7 days.

Pesticide Concentration (kg ha^1)	oxydemeton Methyl	Emamectin Benzoate	Dithane Z-78	Benomyl
0.0	250a (100)	250a (100)	250a (100)	250a (100)
1.0	275b (110)	280b (112)	285b (114)	275b (110)
2.5	380c (152)	375c (150)	380c (152)	360c (144)
5.0	325d (130)	315d (126)	320d (128)	325d (130)
7.5	300d (120)	295e (118)	300d (120)	305d (122)
10.0	210a (84)	200f (80)	250e (82)	205e (82)

*µg formazan g^{-1} soil formed after 24 hrs incubation with triphenyltetrazolium 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.

The stimulatory concentration (2.5 kg ha^{-1}) induces the highest dehydrogenase activity after 20, 30 and 40 days of incubation in black clay soils (Fig. 1 and Fig 2) with Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl when compared to control in black soil.

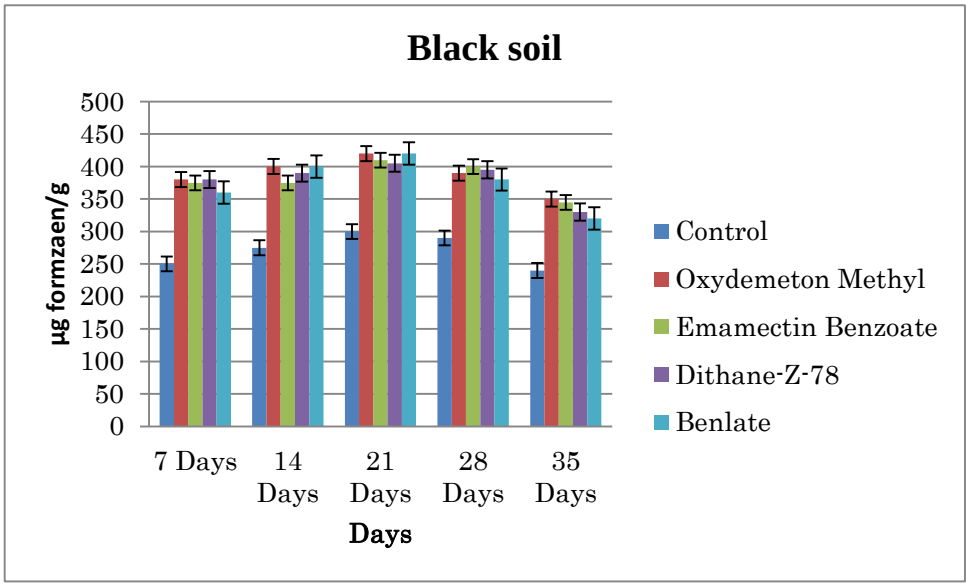

Figure 1. Influence of selected insecticides and fungicides at 2.5 kg ha^{-1} on dehydrogenase* activity in black soil.

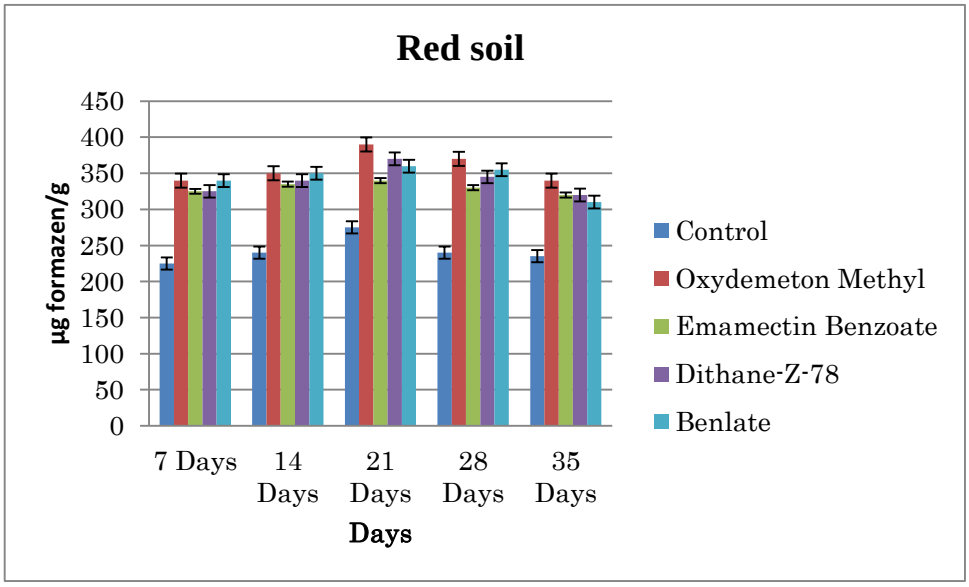

Figure 2. Influence of selected insecticides and fungicides at 2.5 kg ha^{-1} on dehydrogenase* activity in red soil.

In red clay soil a similar trend was followed by Benomyl, induces the highest dehydrogenase activity after 20, 30 and 40 days of incubation but spinosad showed a variable pattern at 30 and 40 days, the dehydrogenase activity remained same with control (Table 3).

Table No. 3: Influence of different concentrations of selected insecticides and fungicides on dehydrogenase activity* in red soil after 7 days

Pesticide concentration (kg ha^{-1})	Oxydemeton Methyl	Emamectin Benzoate	Dithane Z-78	Benomyl
0.0	225a (100)	225a (100)	225a (100)	225a (100)
1.0	250b (111)	240b (106)	230b (102)	260b (115)
2.5	340c (151)	325c (144)	325c (144)	340c (151)
5.0	220d (97)	250d (111)	285d (126)	280d (124)
7.5	210d (93)	200e (88)	205e (91)	220e (97)
10.0	175e (77)	180f (80)	180f (80)	195f (86)

*µg formazan g^{-1} soil formed after 24 hrs incubation with triphenyltetrazolium 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.

The relatively low activity of dehydrogenase might result from the toxic effect of Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl on soil microorganisms, which in turn produces dehydrogenase. The inhibition of dehydrogenase activity by Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl could be attributed to the properties of Oxydemeton Methyl, Emamectin Benzoate, Dithane Z-78 and Benomyl. Similar observations were made on the dehydrogenase activity [27, 28]. Some authors reported on other pesticide effects on the soils [29-31].

4. CONCLUSION

Dehydrogenase activity was significantly enhanced under the influence of pesticides at 2.5 or 5.0 kg ha^{-1} after 21 days of incubation in soils. Further increase in incubation period upto 35 days dehydrogenase activity is decreased. The results of the present study shows that the dehydrogenase enzyme activity gradually decreased by the application of pesticides above 2.5 or 5.0 kg ha^{-1} in both black and red soils.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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