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Effect of pesticides on the activity of soil enzymes (Urease) in Ground nut (*Arachis hypogaea L.)* soils.

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Abstract : Soil enzymes play a key role in energy transfer through the breakdown of soil organic matter and nutrient cycling, and therefore play an important role in agriculture. Therefore, this study was conducted to determine the effect of different concentrations of four selected pesticides (2 insecticides and 2 fungicides) on groundnut soil in black and red groundnut cultivated fields in Anantapuramu district, Andhra Pradesh, India. In a laboratory experiment, the effect of selected pesticides - dimethoate and thiodicarb, fungicides - fosety-Al and copper oxychloride on urease activity with different concentrations of 1.0-10 kg ha^{-1} was determined. Enzyme activity was determined in two parallel air-dried soil samples and incubated for 10, 20, 30 and 40days for soil enzyme i.e., urease was tested.Urease activity was significantly higher in insecticides compared to fungicides levels at 10 and 20 days of 5.0 kg ha^{-1} in black and red soil. In general, pesticides reduced the activity of soil enzymes, which decreased with increasing pesticide applications. Urease activity decreased dramatically as the incubation time increased to 30 and 40 days. The results of this study clearly show that field application rates do not affect enzyme activity during a longer incubation period (40 days). However, a higher concentration of fungicide (10 kg ha^{-1}) causes inhibition of enzymatic activity.

***IndexTerms* - Soil enzyme (Urease), Pesticides (dimethoate, thiodicarb,fosety-Al and copper oxychloride)**

I. Introduction

INTRODUCTION

Agriculture in many countries has been based in recent decades on monocultures, intensive use of synthetic fertilizers and pesticides, water and agricultural machinery[11]. Pesticides are chemical compounds used to control diseases and pests and to return nutrients to the soil [9]. Excessive use of synthetic fertilizers (containing nitrogen, phosphorus and potassium) and their use in combination with organic fertilizers has been shown to adversely affect native microbiota, soil productivity and texture, soil enzyme effects, human health and the environment [24] . Ananthapuram district is considered one of the most important ground nut growing districts in Andhra Pradesh with an average area, production and yield of 7.04 million ha, 3.65 million tons and 512 kg ha^{-1} respectively in the present millennium (2000-01 to 2018-19) [10].

The analysis of enzymatically controlled functional characteristics, such as carbon and nitrogen mineralization, could reveal how pesticides affect soil microbes. All biochemical changes in soil depend on or are connected to the existence of enzymes, therefore their activities are crucial [17]. Since Rotini (1935)[18] first described urease activity in soils, it has attracted a lot of attention since it is thought to play a key role in controlling the nitrogen supply to plants following urea fertilization [12]. Herbicides and fungicides appear to have little to no influence on urease activity, according to the majority of the research cited [19],[22],[23],[3],[20],[21],[5].
Since urea hydrolysis helps to maintain the availability of nitrogen to plants, it is generally advantageous when urease activity in soil is reduced as a result of pesticide treatment[1]. Contrarily, the fungicides validamycin and carbendazim increased urease activity, Approximately 70% and 13-21%, respectively[15], [23]. Except for carbendazim and validamycin, which tend to boost this enzyme activity, the addition of insecticides does not appear to impact or impede the urease activity. Due to the lack of research on this enzyme in recent years, it is challenging to determine a definite response of its enzymatic activity to pesticides.

2.Materials and Methods

2.1 Soils

Samples of a black clay soil and red sandy clay soils, collected from groundnut cultivated fields of Anantapur district, a semi-arid zone of Andhra Pradesh, India, to a depth of 12 cm, were air-dried and sieved through a 2-mm mesh screen before use[7]. Physico-chemical characteristics of the two soils were analyzed using standard methods and listed in Table 1.

2.2 Pesticides

In order to determine the influence of pesticides on microbial population and their activities, insecticides of thiodicarb, dimethoate and fungicides of fosetyl-Al, copper oxychloride were selected in the present study.

2.3 Enzymes Used In the Present Study

Urease activity: (E. C. 3.5.1.5)

To study the effect of selected 4 pesticides on urease, 5 g of dried black and red soils were taken separately in test tubes (12 x 125 mm) containing different concentrations of selected 4 pesticides 10, 25, 50, 75, and 100 µg g^{-1} soil 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 the water holding capacity (WHC), about 2 ml of deionized 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, 35 days. During incubation period certain amount of distilled water was added to maintain the WHC. Triplicate soil samples were withdrawn for the enzyme assay.

2.4 Assay of urease

Soil urease activity was based on the hydrolysis of urea. The assay of urease enzyme activity was determined using the method of [6] . At desired intervals, 1 ml of 3% urea (urease substrate) and 2 ml of 0.1 M phosphate buffer (pH 7.1) were added to one gram portion of soil samples and incubated for 30 minutes at 37°C in a water bath shaker. Then the tubes were placed in ice until ammonia was extracted with 10 ml of 2 M KCl and filtered through Whatman filter paper No.1. To 4 ml of the filtrate, 5 ml of phenol sodium nitroprusside solution and 3 ml of 0.03 M sodium hypochlorite solution were added. Mixture was shaken, and allowed for 30 minutes in dark, and the developed blue colour was measured at 630 nm in a Spectronic-20 D spectrophotometer.

3.Results and Discussion

3.1 Analysis of Physico chemical characteristics of soil samples:

Mineral matter of soil samples such as sand, slit and clay contents were analyzed by means of different sizes of sieves by following the method described by Alexander, 1977 [2]. The water holding capacity of soil samples was determined by adding distilled water up to the saturation point and then 60% water holding capacity of soil was calculated as described by Jhonson and Ulrich, 1960[8]. Soil pH was measured by mixing soil and water in the ratio of 1:1.25 using systronics digital pH meter with calomel glass electrode assembly. Organic carbon content in soil sample was estimated by the walkley Black method and organic matter was calculated by multiplying the values with 1.72 Jackson, 1971[7]. Electrical conductivity of soil samples after addition of 100 ml distilled water to 1 g soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method of micro-Kjeldhal method [7]. Content of inorganic ammonium-nitrogen in soil samples after extraction of 1M KCL by Nesslerization method [7]. Contents of nitrite-nitrogen by Barnes and Folkard, 1951 [25] and contents of nitrate-nitrogen by Brucine method [16] after extraction with water determined, respectively. Physico chemical properties of two soils samples were listed in the Table 1.

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

Properties	Black soil	Red soil
Sand (%)	80.2	63.6
Silt (%)	13.4	23.3
Clay (%)	6.4	13.1
pH[a]	8.0	7.5
Water holding capacity (ml g^{-1} soil)	0.45	0.33
Electrical conductivity (m.mhos)	264	228
Organic matter [b] (%)	1.85	0.054
NH_4^+- N (µg g^{-1} soil)[d]	8.42	6.69
NO_2^-- N (µg g^{-1} soil)[e]	0.56	0.41
NO_3^-- N (µg g^{-1} soil)[f]	0.92	0.81

a1:1.25 (Soil: water)

bWalkley-Black Method (Jackson, 1971)

cMicro-Kjeldhal Method (Jackson, 1971)

dNesslerization method (Jackson, 1971)

eDiazotization Method (Barnes and Folkard, 1951)

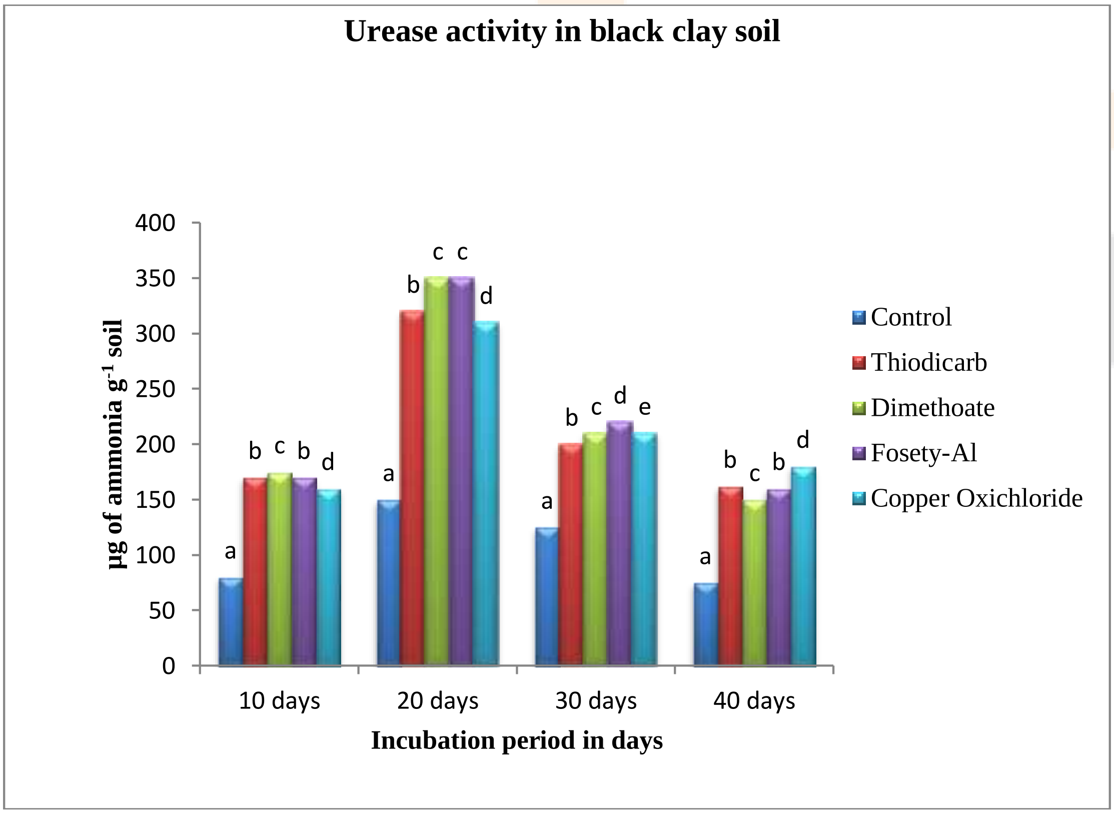
f Brucine Method (Ranney and Bartlett, 1972)

Table2. Influence of selected insecticides thiodicarb and dimethoate and fungicides of fosetyl-Al and copper oxychloride on activity of urease * in black soil after 10 days incubation

Pesticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate	Fosety-Al	Copper oxy chloride
0.0	80fe(100)	80e (100)	80e (100)	80d (100)
1.0	120c (150)	135d (169)	125d (156)	130d (162)
2.5	135b (169)	145c (181)	135c (169)	140c (175)
5.0	170a (212)	175a (219)	170a (212)	160a (200)
7.5	140b (175)	155b (194)	150b (187)	150b (187)
10.0	70d (87)	75e (94)	75e (94)	80d (100)

* Values are µg ($NO2^{-}$ + $NO3^{-}$) – N g^{-1} soil.
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 Duncan's multiple range (DMR) test.

FIGURE 1:



Influence of Thiodicarb, Dimethoate, Fosetyl-Al and Copper-oxychoride at 5.0 Kg ha^{-1} respectively on Urease activity* in black clay soil
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

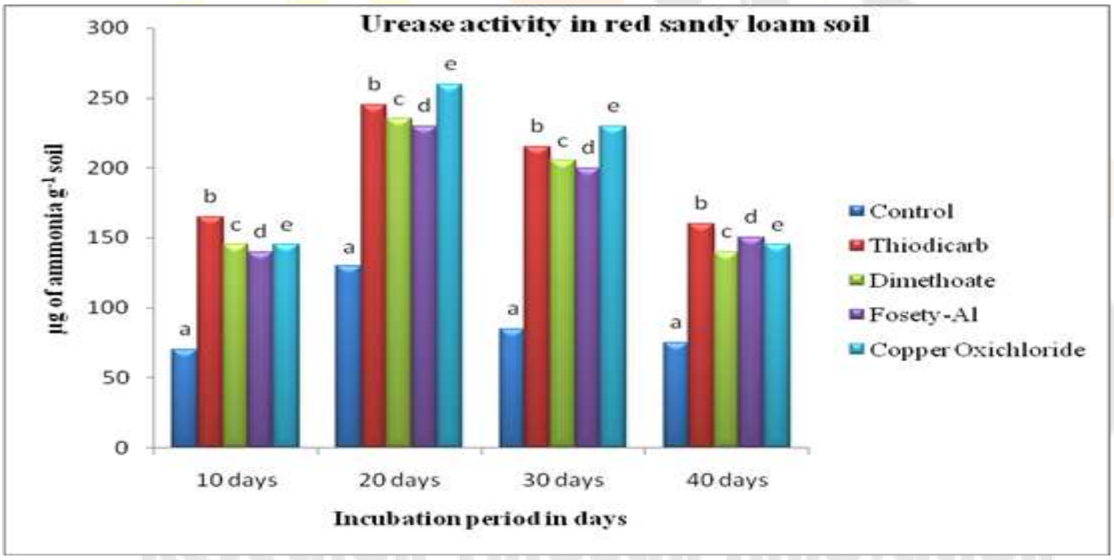
*Values plotted in figure are means of triplicates.

Table 3: Influence of selected insecticides thiodicarb and dimethoate and fungicides of fosetyl-Al and copper oxychloride on activity of urease * in red soil after 10 days incubation

Pesticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate	Fosety-Al	Copper oxy chloride
0.0	70e (100)	70d (100)	70c (100)	70e (100)
1.0	110d (157)	100c (143)	105b (150)	110d (157)
2.5	140b (200)	135b (193)	130a (185)	130b (186)
5.0	165a (136)	145a (207)	140a (200)	145a (207)
7.5	120c (171)	125b (178)	130a (185)	120c (171)
10.0	60e (86)	65d (93)	60c (86)	65e (93)

* Values are µg ($NO2^{-}$ + $NO3^{-}$) – N g^{-1} soil.
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 Duncan's multiple range (DMR) test.

Figure 2:



Influence of Thiodicarb, Dimethoate, Fosetyl-Al and Copper-oxychoride at 5.0 Kg ha^{-1} respectively on Urease activity* in red sandy loam soil
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.
*Values plotted in figure are means of triplicates.

3.2 Urease activity

The activity of urease, implicated in the hydrolysis of urea, was significantly enhanced, under the impact of pesticides (thiodicarb, dimethoate, fosetyl-Al and copper oxychloride) up to 5.0 kg ha^{-1} level in both soils, in comparison to controls. But at higher concentrations of 10 kg ha^{-1} were toxic to urease activity after 10 days incubation (Table 2). The activity of urease in terms of ammonia formed from urea was pronounced more in soil samples treated with 5.0 kg ha^{-1}.

3.3. Conclusion

The activity of urease was higher in black soil, received the thiodicarb, dimethoate, fosetyl-Al and copper oxychloride at 5.0 kg ha^{-1} incubated for 10 days. This increase in enzyme activity was continued up to 20 days of incubation and then there was turn down in enzyme activity, whereas the thiodicarb, dimethoate, fosetyl-Al and copper oxychloride have shown maximum enzyme activity at 5.0 kg ha^{-1} with 50 - 112, 69 - 119, 56 - 112, 62 - 100 % and 57 -136, 43 - 107, 50 - 100, 57 - 107 % whereas higher over control in black and red soils (Table 2). The enzyme activity was significantly decreased by a longer period of incubation up to 30 and 40 days in both soils (Fig. 1and 2).

3.4. Statistical Analysis:

The concentrations of the Urease enzyme were calculated on soil weight (over dried) basis. The insecticide treatments with untreated controls and the significant levels $P \leq 0.05$ 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 [14].

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