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RESEARCH ARTICLE

IMPACT OF INSECTICIDES THIODICARB AND DIMETHOATE ON SOIL MICROBIAL ACTIVITIES (AMYLASE) IN TWO GROUNDNUT (*ARACHISHYPOGAEA*. L) SOILS

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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 inlaboratory conditions that the effect of two insecticides, ThiodicarbDimethyl N, N '- (thiobis ((methylimino) carbonyloxy)) bis (ethanimidothioate) and Dimethoate *(O, O*-dimethyl *S*-[2-(methylamino)-2-oxoethyl]) dithiophosphate) on enzyme activities, such as amylase and invertase in two soils collected from groundnut (*Arachishypogaea*. 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, Amylase activities 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 insectcides and decreased the rate of enzyme activity. However the stimulatory effect was continued up to 20 days of incubation in black and redsoils. 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 amylaseactivity in black and red soils.

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 (*Arachishypogaea* L.) cultivation, in the state (Anonymous, 2013), and itsproductivity 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; Romeh *et al*. 2009). In India, 15–20% of agricultural production is negatively influenced by pests (BhaleraoT. S. and PuranikP. R., 2007). An estimated annual loss of Rs. 150 crores in groundnut due to pests has been reported (Singh, V. 1980, 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 Ghewande, 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 0.99% not only troubles local metabolism or enzymatic activities (Pimentel, 1995; Topp, *et al*. 1997; Engelen *et al*. 1998; Carriger *et al*. 2006; 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; Gianfreda *et al*., 2005). The assessment of soil enzyme activities is simple, requires low labor costs compared to other biochemical

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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; 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 amylase 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 carbon 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 nitrite 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

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

Properties	Black clay soil	Red sandy loam soil
pH[a]	8.7	7.5
Electric conductivity (m.mhos)	272	220
Water holding capacity (ml g^{-1} soil)	0.45	0.33
Sand (%)	80.2	63.6
Silt(%)	19.8	23.3
Clay(%)	6.4	13.1
Organic matter[b(%)]	1.85	1.27
Total nitrogen[c](%)	0.087	0.054
NH_4^+- N (µg g^{-1} soil)[d]	8.42	7.74
NO_2^-- N (µg g^{-1} soil)[e]	0.56	0.41
NO_3^-- N (µg g^{-1} soil)[f]	0.92	0.81
[a]1:1.25 (Soil:Water)		

[b]Walkley-Black method (Jackson, 1971)
[c]Micro-Kjeldhal method (Jackson, 1971)
[d]Nesslerization method (Jackson, 1971)
[e]Diazotization method (Barnes and Folkard, 1951)
[f]Brucine method (Ranney and Bartler, 1972)

Insecticides and chemicals used in the 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.

Enzymes Used In the Present Study

Amylase Activity in Soils

Five gram portion of the soil samples were weighed and dispersed into sterile test tubes (25 x 150 mm). Stock solutions from selected insecticides were added at the rate of 10, 25, 50, 75 and 100 µg g-1 soil equivalent to field application rates of 1.0, 2.5, 5.0, 7.5 and 100 kg ha^{-1} respectively. Soil samples without insecticide treatment served as controls. Soil samples were mixed thoroughly for uniform distribution of insecticide added. Triplicates were maintained for each treatment at room temperature (28 ± 4°C) with 60% water holding capacity throughout the incubation period. After desired intervals of incubation, soil samples were extracted in distilled water for estimation of enzyme activities. Same method was used earlier to study pesticide-microbial interactions in (JayaMadhuri and Rangaswaamy. V 2006, Deborah,V and JayaMadhuri.R.2013).

Assay of Amylase

The method employed for the assay of amylase was developed by Cole, M.A., (1977) and followed by (Tu, C.M., 1981a &b]. The soil samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6ml of 0.2M of acetate phosphate buffer (pH 5.5) containing 2% starch was added to each of the testing samples and closed with cotton plugs. After 24 hours and 72 hours of incubation the testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Whatman No. 1 filter paper and the filtrate was assayed for amount of glucose in the supernatant was estimated

by Nelsonsomagyii method (Nelson N, 1944) in spectronic 20D spectrophotometer.

Statistical Analysis

The concentrations of the Amylase 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 (*Arachishypogaea* 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 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; Loganathan *et al*. 2002) and it has been observed that the responses of soil enzymes ondifferent 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 amylase activity was significantly increased in both soils by both pesticides from 1.0 to 5.0 kgha-^{1} whereas the activity was decreased at higher

Table 2 Influence of selected Insecticides on activity of Amylase* in black clay soil after 10 days in 24 hrs.

Insecticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate
0.0	198±2.23f -100	198± 2.23f -100
1.0	280±3.48d -141	294±1.17e -148
2.5	327±0.53c -165	475±2.27c -240
5.0	530±2.87a -268	670±2.30a -338
7.5	442±1.15b -223	515±2.42b -260
10.0	257±.1.73e -130	430±0.68d -217

*µg of glucose g^{-1} soil formed after 24 hours incubation with 2% starch.
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.

concentrations (7.5–10.0 kg ha^{-1}) of pesticides at 24 and 72hrs as shown in Table 2 and Table 3 respectivelywhen compared to control in black soils

Table 3 Influence of selected Insecticides on activity of Amylase* in black clay soil after 10 days in 72 hrs

Insecticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate
0.0	230±1.72f -100	230± 1.72e -100
1.0	304±1.15e -132	384±2.48c -167
2.5	465±1.53c -202	521±1.27b -227
5.0	672±5.77a -292	697±1.70a -303
7.5	542±2.15b -236	325±0.74d -141
10.0	357±.1.73d -155	192±1.98f -83

*µg of glucose g^{-1} soil formed after 72 hours incubation with 2% starch.
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.

Similarly amylase activity is increased by both pesticides from concentration of 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 and 72 hrs as shown in Table 4 and Table 5 respectively when compared to control in Red soils, with similar contrast results have been reported by many workers Amylase activity was enhanced by monocrotophos, quinalphos, cypermethrin and fenvalerate at levels ranging from 1 to 2.5 kg ha^{-1}, but was inhibited at concentrations of 5 and 10 kg ha^{-1} in groundnut soils (Rangaswamy and Venkateswarlu, 1992). Tu (1988) reported that malathion and permethrin at higher levels were stimulatory to amylase activity 3 days application, and several pesticides, including organophosphates at 5 and 10 mg kg^{-1} have been found to enhance amylase activity (Tu, 1982).

Table 4 Influence of selected Insecticides on activity of Amylase* in red sandy loam soil after 10 days in 24 hrs

Insecticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate
0.0	112±2.23f -100	112± 2.23f -100
1.0	194±3.48d -173	215±1.17e -192
2.5	222±0.53c -198	375±2.27c -335
5.0	432±2.87a -386	576±2.30a -514
7.5	352±1.15b -314	435±2.42b -388
10.0	157±.1.73e -140	334±0.68d -298

*µg of glucose g^{-1} soil formed after 24 hours incubation with 2% starch.
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.

Kennedy and Arathan, (2002) reported that utilization of carbofuran at 1 and 1.5 kg ha-1 fundamentally diminished the action of soil compounds, viz., alpha - amylase, beta - glucosidase, cellulase, urease and phosphatase up to 30 days after carbofuran application. In any case, use of carbofuran at the suggested level (0.5 kg ha-1) had no noteworthy impact upon the action of soil compounds, which are naturally huge as they assume a critical part not just in the dirt concoction and organic properties additionally influence the supplement accessibility to plants.

Table 5 Influence of selected Insecticides on activity of Amylase* in red sandy loam soil after 10 days in 72 hrs

Insecticide concentration (kg ha^{-1})	Thiodicarb	Dimethoate
0.0	150±1.12f -100	150± 1.12e -100
1.0	262±2.15e -175	294±2.48c -196
2.5	405±1.53c -270	471±1.17b -314
5.0	622±5.77a -415	647±1.70a -431
7.5	442±2.15b -295	325±0.74d -235
10.0	307±.1.73d -205	252±1.98f -168

*µg of glucose g^{-1} soil formed after 24 hours incubation with 2% starch.
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.

Rate of amylase action took after the same pattern of introductory incitement took after by restraint as reported by (Rangaswamy and Venkateswarlu, 1992 and Vijay Gundi *et al*. 2007).

CONCLUSION

Based on the results obtained from above, we are concluded that the observed stimulation or inhibition of these two enzymes at low or high concentrations of the insecticides could be attributed to number of populations of amylolytic organisms present in both soils, the amylase activity in both soils is profoundly increased in both pesticide concentrations 1.0 to 5.0 kg ha-^{1} at 24 hrs and 72 hrs, at higher contractions (7.5–10.0 kg ha-1) a suppressed activity in the amylase 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 amylase enzyme.

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