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Activity of soil urease as influenced by acetamiprid and carbofuran

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ABSTARCT

Soil enzymes play essential roles in catalyzing reactions necessary for nutrient cycling in the biosphere. They are also sensitive indicators of ecosystem stress, therefore their evaluation is very important in assessing soil health and quality. By keeping this point in view in the present study, we investigated the soil urease activity at low and higher doses (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}) and then incubated under laboratory conditions for different time intervals (10, 20, 30 and 40 d). Initially samples were analyzed for physicochemical characteristics, and then soil enzyme activities were determined after respective incubations. The enzyme activities were affected significantly ($P \leq 0.05$) when pesticide concentration was 10 kg per ha^{-1} soil. In contrast, insecticides application at low concentrations (1.0-2.5 kg ha^{-1}) caused stimulated effects. Especially, carbofuran was more effective than acetamiprid in the stimulation of enzyme activities at lower concentrations. Based on the results obtained in the present study, it is concluded that, although pesticide concentration had a somewhat inconsistent and erratic effects on activities of the soil enzymes, but it is true. Insecticides application at more than 7.5 kg ha^{-1} soils and prolonged incubations did have a negative impact on the investigated soil enzyme activities.

Key words: Ground nut (*Arachis hypogaea* L.), Soils, Urease enzyme

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INTRODUCTION

As a par of integarted pest management insecticides are predominantly used in modern agriculture, either simulataneously or in succession, to increase crop production but on the other hand they cause major environmental problems through out the world [1, 2]. With an increased pesticide use, concerns over public health and environment are rising. Since the 1950's, intensive farming methods have increased the range and effects of these agrochemicals. Once a pesticide is released into soil matrix, its effect will depend both on its characteristics and on those of the soil. Consequently, different pesticides may often exist together in the soil ecosystem at a given point of time. Considering the target and non-target effects of different pesticides in agriculture systems, organophosphorous and carbamate pesticides, first discovered in the 1930s, have been using continuously on a wide range of crops. Among them, carbofuran and acetamiprid are extensively used in the groundnut (*Arachis hypogaea* L.) and non-cereal cropping systems.

Acetamiprid, (E)- N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methyl- acetamidine, a new neonicotinoid insecticide, is being used for the control of Hemiptera, mainly aphids, Thyasnoptera and Lepidoptera on various

crops, especially vegetables, fruits, and tea [3]. Because of its special acting characteristics (systemic and contact insecticide), relatively low acute and chronic mammalian toxicity, acetamiprid is being more competitive than some conventional insecticides and has been considered as an important substitute to the organophosphate insecticides which have caused severe environmental pollution and pesticide resistance and now have been banned in many countries. Under the normal agricultural practice, the recommended dose of the acetamiprid is 25–75 g a.i. ha^{-1} in China and 0.3–0.6 Ib a.i. per acre in the USA.

Carbofuran (2,3-dihydro-2,2- dimethylbenzofuran-7-yl methyl carbamate) is a systemic non-ionic broad-spectrum carbamate insecticide/ nematicide, which is widely used in the Indian subcontinent to control nematodes in the soils. Considerable work has been done on the effects of carbofuran for the control of nematode population in the soils [4, 5], whereas the research work on the effect of carbofuran on the enzyme activities in soils is very scanty [6, 7]. The groundnut ranks seventh among the crops in terms of insecticide consumption in India [8]. Insecticides like acetamiprid and carbofuran are generally used against the Hemiptera, mainly aphids, Thyasnoptera, Lepidoptera, and nematodes in the groundnut fields. Thus, the studies are required to understand the effects of pesticide use on the different soil functions.

The biochemical processes driven by microorganisms are influenced by pesticides as a part of interaction between soil components and pesticides. Pesticides effects on soil microorganisms can be determined by the study of functional parameters such as carbon and nitrogen mineralization that are governed by enzymatic activities. Those activities play an important role because all biochemical transformations in soil depend on or are related to the presence of enzymes. They are indicators of biological equilibrium [9], fertility [10, 11] and changes in the biological status due to soil pollution [12, 13, 14, 15, 16]. Finally, the measurement of specific enzymatic activities may contribute to understand the metabolic processes involved in the biogeochemical cycles of nutrients.

Virtually, there is a lot of information available on the influence of insecticides on the urease activity in the soil. However, Mekapogu Madakka et al., [1] reported the effect of insecticides alone and in combination with pesticides on microbial diversty and urease activities in the soils. Gooty Jaffer Mohiddin et al. [2] with Acephate and imidacloprid on urease activity in soils revealed this enzyme but still need to know with the present insecticides. Hence, the present study was aimed at determining the influence of insecticides carbofuran and acetamiprid on the activity of urease in the two groundnut soils of Anantapur District, Andhra Pradesh, India.

MATERIALS AND METHODS

Urease Activity *(E.C. 3.5.1.5)*

Different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} levels) of the two insecticides were used to treat one gram portion of two soil samples in triplicates, to determine the urease activity after ten days of incubation at 28 ± 4° C. Triplicate soil samples were withdrawn for the assay of urease activity following the phenol hypochlorite method Fawcett and Scott [17] and also adopted by Gooty Jaffer Mohiddin et al. [18]. After 10, 20, 30 and 40 days of incubation at room temperature (28 ± 4°c), the influence of two insecticides, at 5.0 kg ha^{-1} level on the rate of urease activity was determined in two soil samples, in triplicates. Soil samples of each treatment withdrawn for the enzyme assay.

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 Fawcett and Scott [17] and adopted by Malkomes [19]. 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-20D spectrophotometer (Milton Roy). Rate of arylamidase, myrosinase, phosphatase, protease and urease enzyme activities were assayed at 10, 20, 30 and 40 days of soil incubation with the respective stimulatory concentrations of pesticides.

Statistical analysis

All the data were expressed on an air dry soil basis and were averages of three replicates. Data were analysed for

significant difference ($P \leq 0.05$) between pesticide-treated and untreated soils using Duncan's multiple range (DMR) test followed by Jaffer Mohiddin et al. [18].

RESULTS AND DISCUSSION

The activity of urease, implicated in the hydrolysis of urea, was significantly enhanced, under the impact of insecticides, carbofuran and acetamiprid up to 5.0 kg ha^{-1} level in both soils, in comparison to controls. But at higher concentrations of 7.5 and 10 kg ha^{-1} were toxic to urease activity after 10 days incubation (Tables 2 and 3). The activity of urease in terms of ammonia formed from urea was pronounced more in soil samples treated with 2.5 kg ha^{-1}. Where as the activity of urease was higher in black soil, received the acetamiprid than carbofuran at 2.5 kg ha^{-1} in both soils and carbofuran at 2.5 kg ha^{-1} in both soils, incubated for 20 days (Fig 1a, 1b). The enzyme activity was significantly decreased by longer period of incubation up to 30 and 40 days in both soils.

The experimental studies, conducted by Rangaswamy and Venkateswarlu [20], on addition of two organophosphates, monocrotophos and quinalphos at 2.5 kg ha^{-1} significantly increased urease activity at 10 day incubation in black and red soils. Pesticides have a complex effect on soil urease activity [21]. The activation effect was explained by three possible reasons, namely: (1) the result of direct activation of pesticides on enzymatic molecules; (2) the significant increase in cell wall permeability or cellular lysis, resulting in consequential increase in the accessibility of the substrate molecules to the intracellular enzymes [22]; or (3) a possible adsorption of the pesticides on inorganic and organic supports, and a competition between immobilized enzymes and pesticides, and subsequent release of free enzymatic molecules from matrices [23].

Urease activity was stimulated by the application of triazophos at 5 and 10 mg/kg to a clay loam soil, after two weeks incubation [24, 25, 26]. Thiophanate-methyl applied at 0.4 kg ha^{-1} level clay loam soil in combination with tridemorph and some herbicides resulted in stimulation of urease activity [27]. Omar and Abd-Alla [28] observed that urease activity was promoted on application of two fungicides pyrazofos (as Afugan) and propiconazole (as tilt) at 50 ppm. On the other hand, application of the formulated fungicides, captan, captan-folpet-folicidin and benomyl at recommended doses to an alluvial soil resulted in unchanged urease activity [24, 25, 26]. The fungicide, pyroxyfur applied to a sandy loam soil at 10 µg/gm of soil had no inhibitory effect on the activity of urease [29].

Further, Tu [30] reported that two fungicides, captafol and chlorothalonil also had no inhibitory effect on urease activity. In contrast, quintozene stimulated urease activity initially for four weeks before activity returned to a normal level in an alluvial soil at recommended field doses [27]. In a similar manner carbendazim at 0.2 kg ha^{-1}, enhanced urease level after three years when applied to several sandy loam soils in combination with a series of pesticides, and thereafter the activity remains unchanged. Similar results were obtained by folpet treatment at 1.0 mg/kg [31]. However, thiram in an organic soil applied at 5 and 10 mg/kg level inhibited urease activity at 10 mg/kg (more than 40%) but the activity remained normal within two weeks [25].

Depending upon the type of soil, few fungicides remained innocuous initially and then inhibited or stimulated urease activity. For instance, triazophos, captan, maneb and thiram at 5 and 10 mg/kg in a clay loam soil resulted in no inhibition of urease activity within seven days of incubation. When most of these pesticides were applied to an organic soil, urease activity was inhibited. On the other hand, maneb stimulated urease activity [24, 25, 26]. Similar results were also reported with captafol treated soils of different types, at 1.0 mg/kg soil [29]. In present study insecticides alone and combination with fungicides at higher concentrations of 7.5 and 10 kg ha^{-1} were toxic to urease activity after 10 days incubation. Similarly urease activity was inhibited by the application of quintozene at 10 and 100 ppm levels in black and red soils, incubated for 1 and 15 days [32]. According to Shukla and Mishra [33] urease activity was reduced in potato filed soil, by the application of benomyl (0.37 kg ha^{-1}), copper oxychloride (7.4 kg ha^{-1}) and dithane M-45 (2.0 kg ha^{-1}). Copperoxychloride and dithane M-45 are more effective than benomyl. Nagaraja et al. [34] found that inhibition of urease activity was dependent on concentration of the fungicide, in captan treated soil. The level of inhibition of urease was 500>100>10>0 ppm.

In the present study, the increase in activity of urease was more striking at 2.5 kg ha^{-1} and 5.0 kg ha^{-1} of the selected insecticides alone and in combination with fungicides. The results obtained in the present study indicated that the significant increase in urease activity was observed, when insecticides applied alone, and in combination with fungicides. There was no specific inhibitory or stimulatory effect was observed, when insecticides spiked in combination with fungicides, i.e, in both the cases nearly similar results were obtained. On this basis, insecticides

applied in combination with fungicides, at field application rates, to combat insects and pathogenic fungi at a time, doesn`t show any harmful effect on the urease activity of the soil in agricultural system.

Table 1: Physicochemical properties of the soils

Properties	Black clay soil	Red clay soil
Sand (%)	68.45	53.25
Silt (%)	21.45	27.12
Clay (%)	10.0	19.8
pH [a]	7.8	6.7
Water holding capacity (ml g^{-1} soil)	0.7	0.4
Electrical conductivity (m.mhos)	258	232
Organic matter [b] (%)	1.34	0.74
Total nitrogen [c] (%)	0.086	0.038
NH_4^+ - N (µg g^{-1} soil)[d]	6.96	6.01
NO_2^- - N (µg g^{-1} soil)[e]	0.58	0.42
NO_3^- - N (µg g^{-1} soil)[f]	0.94	0.73

Where,
a = 1:1.25 = Soil: Water slurry b = Walkley-Black Method [35],
c = Micro-Kjeldhal Method [35], d = Nesslerization method [35],
e = Diazotization Method [36], f = Brucine Method [37].

Table 2: Influence of insecticides on urease activity* in black soil after 10 days

Pesticide concentration (kg ha^{-1})	Acetamiprid	Carbofuran
0.0	64±1.154a (100)	64±1.154a (100)
1.0	75±5.773b (117)	70±0.577b (109)
2.5	104±2.886c (162)	102±11.547c (159)
5.0	93±1.732d (145)	85±0.577d (133)
7.5	82±8.66oe (128)	77±2.309e (120)
10.0	58±0.577f (91)	55±2.886f (86)

**µg ammonia g^{-1} soil formed after 30 minutes incubation at 37°C with urea.*
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.

Table 3: Influence of insecticides on urease activity* in red soil after 10 days

Pesticide concentration (kg ha^{-1})	Acetamiprid	Carbofuran
0.0	45±0.935a (100)	45±0.145a (100)
1.0	46±0.242a (102)	47±0.680a (104)
2.5	60±2.547b (133)	55±1.975c (122)
5.0	50±2.291c (111)	65±1.022b (144)
7.5	40±0.884d (95)	47±0.973d (104)
10.0	32±0.671e (71)	41±0.661e (91)

**µg ammonia g^{-1} soil formed after 30 minutes incubation at 37°C with urea.*
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.

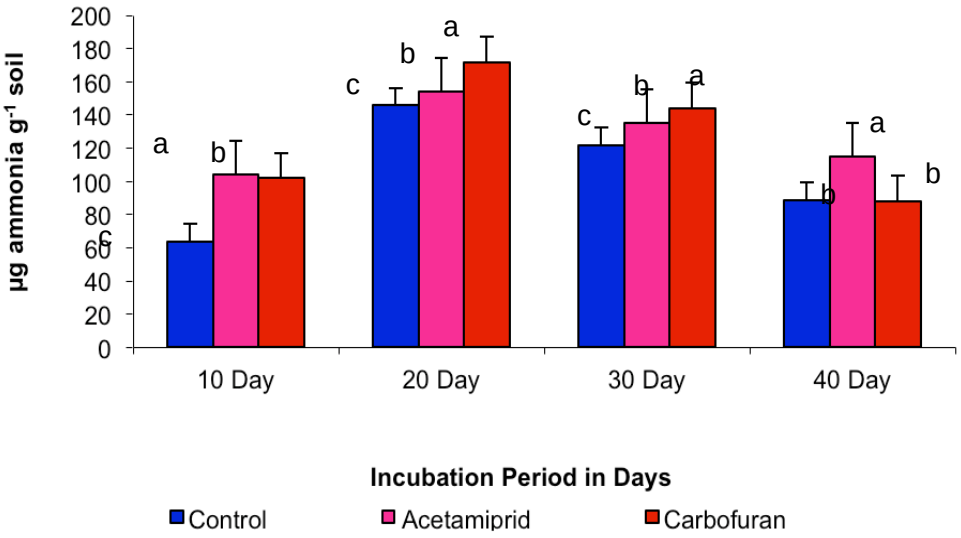

Figure. 1a: Influence of acetamiprid, carbofuran at 2.5 kg ha^{-1} flubendiamide and spinosad at 5.0 kg ha^{-1} respectively on urease activity* in black soil

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 three replicates*

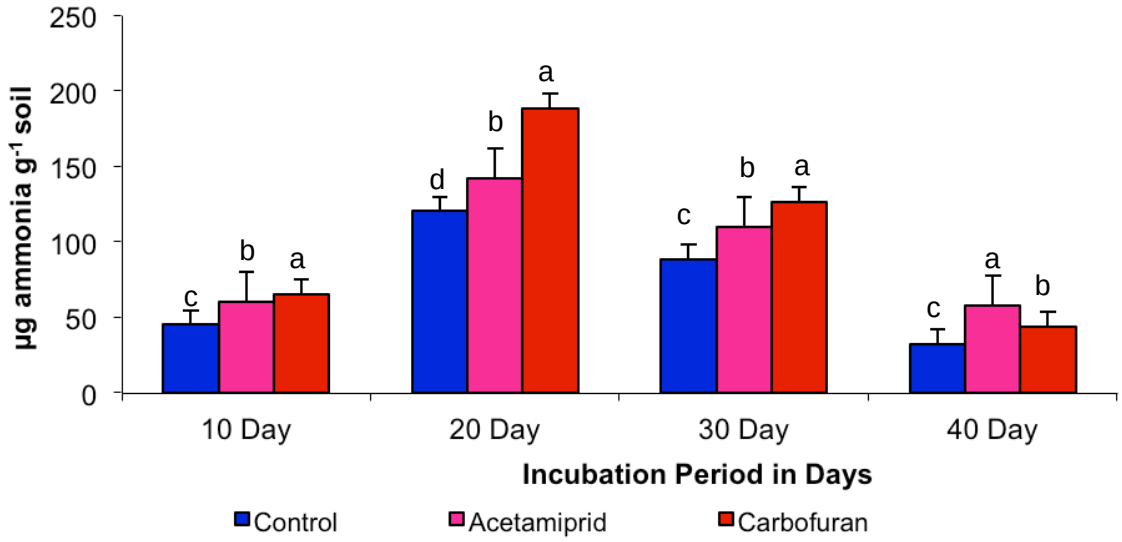

Figure. 1b: Influence of acetamiprid, carbofuran, flubendiamide and spinosad at 2.5 kg ha^{-1} on urease activity* in red soil

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 three replicates.

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