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Influence of the insecticides acetamiprid and carbofuran on arylamidase and myrosinase activities in the tropical black and red clay soils

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Abstract The objective of this study was to determine the effects of two insecticides, namely, acetamiprid and carbofuran on the enzymatic activities of arylamidase (as glucose formed from sinigrin) and myrosinase (as β-naphthylamine formed from L-leucine β-naphthylamide) in the black and red clay soils collected from a fallow groundnut (*Arachis hypogaea* L.) fields in the Anantapur District, Andhra Pradesh, India. The study was realized within the framework of the laboratory experiments in which the acetamiprid and carbofuran were applied to the soils at different doses (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}). Initially, the physicochechemical properties of the soil samples were analyzed. After 10 days of pesticide application, the soil samples were analyzed for the enzyme activities. Acetamiprid and carbofuran stimulated the arylamidase and myrosinase activities at lower concentrations after 10 days incubation. Striking stimulation in soil enzyme activities was noticed at 2.5 kg ha^{-1}, persists for 20 days in both the soils. Overall, higher concentrations (5.0–10.0 kg ha^{-1}) of acetamiprid and carbofuran were toxic or innocuous to the arylamidase and myrosinase activities. Nevertheless, the outcomes of the present study clearly indicate that the use of these insecticides (at field application rates) in the groundnut fields (black and red clay soils) stimulated the enzyme (arylamidase and myrosinase) activities.

Keywords Arylamidase · Myrosinase · Insecticides · Tropical soils

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Introduction

In the modern days, the advent of the nutrient responsive cultivars and the high yielding varieties leads to an intensive agriculture, which along with the mono cropping system leads to the outbreak of various pests and diseases. Thus, the use of agricultural chemicals becomes an imminent in the present-day agriculture. Long-term and intensive use of pesticides may contaminate agricultural land and adversely affect non-target organisms (Parr 1974; Hart and Brookes 1996; Fliessbach and Mader 2004; Srinivasulu et al. 2012). Thus, this makes concern in both the public and scientists all over the world. Most of the pesticides are organic or inorganic in nature, which has prolonged persistence in the environment. Despite the beneficial impacts of the pesticides in improving and stabilizing the

agricultural productivity of the control of obnoxious weeds, fungi, and insects, these allochthonous organic chemicals are known to contaminate the soil ecosystem. Hence, these contaminants pose a great threat to the different microbial processes, such as ammonification, nitrification, and phosphorus mineralization, which play an important role in recycling of the plant nutrients dependent on the balanced equilibrium existing among the various groups of microorganisms in the soil (Pimentel 1995; Johnsen et al. 2001; Sanchez et al. 2003). It is a well-known fact that only 1 % of the pesticide applied hit the target pest while the remaining 99 % of the pesticide drifts into the environment, subsequently contaminating the soil, water, and biota (Ranney and Bartlett 1972; Qui et al. 1981; Jaffer Mohiddin et al. 2013).

The parent compounds of the pesticides and their metabolites may affect the soil enzyme activities, indirectly by affecting the microbial populations in the soil (Turki and Dick 2003). Soil enzymes contain free enzymes, immobilized cellular enzymes, and enzymes within the microbial cells. These enzymes are derived from the microorganisms, plant roots, and soil animals. They play an important role in organic matter turnover and degradation of xenobiotics. Soil enzymes are, therefore, useful in describing and understanding the ecosystem quality and the interactions among the subsystem and to assess the effect of various inputs on the soil health. Many authors reported that the soil enzymes as indicators of the soil fertility are influenced by integrated ecological factors (Ramakrishnan et al. 2010; Srinivasulu and Rangaswamy 2013). The enzyme, arylamidase [α-aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2], catalyzes the hydrolysis of *N*-terminal amino acid of the peptides, amides, or arylamides. Because of the presence of such substrates in the soils, it is likely that this enzyme is associated with the N mineralization (Tu and Miles 1976; Acosta-Martinez and Tabatabai 2000; Srinivasulu and Rangaswamy 2013). Arylamidase is widely distributed in the tissues and body fluids of all animals (Tu and Miles 1976; Hiwada et al. 1980), plants, and microorganisms (Appel 1974). Arylamidase could be used to evaluate the soil resilience after pesticide disturbances (Tu and Miles 1976; Floch et al. 2011). However, little information is available on the effect of pesticides on activity of arylamidase in the groundnut-cultivated black and red clay soils. Among the majority of nutrients in the soil, N is a key element for many agricultural crops, but it is available only in trace quantities in the mineral form. Thus, arylamidase may play a key role in the initial reactions of the mineralization of amino acids in the soils; however, little information is available on the role of this enzyme in the soil nutrient cycling. Studies to understand the role of arylamidase in the soil N cycling and the factors (i.e., soil properties, trace elements, liming, tillage, crop residues, and management practices) that affect the activity of this enzyme will aid in the decision making and important for the fertility, productivity, and sustainability of soils.

On the other hand, myrosinase (thioglucosideglucohydrolase; EC 3.2.3.1) is an enzyme that hydrolyzes the glucosinolates to D-glucose and allelochemicals that have the biological potential to suppress the germination of weed seeds and some pathogens in the soil (Tu and Miles 1976; Angus et al. 1994; Brown and Morra 1997). Myrosinase is, thus, the fundamental factor for allelochemical expression derived from the glucosinolates and, hence, its study in the soil is of interest. This enzyme is noted in some microorganisms and released into the soils via root exudation and decomposition (Wilson and Tisdel 2001). Rask et al. (2000) reported that this enzyme is found in all glucosinolate-containing plants such as *Brassica* species and possibly in some bacteria and fungi. Products of glucosinolate hydrolysis include glucose, sulfate, and a number of active allelochemicals such as isothiocyanates, nitriles, thiocyanates, cyanides, and others depending on substrates and reaction conditions used (Gil and Macleod 1980). Only two reports are available on the effect of pesticides on the activity of myrosinase in soils.

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 (Singh and Strauss 1986). 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 (Skujins 1978; Di Sanzo 1981; El-Banhawy et al. 1998), whereas the research work on the effect of carbofuran on the enzyme activities in soils is very scanty (Megharaj et al. 1999; Kalam and Mukherjee 2001).

The groundnut ranks seventh among the crops in terms of insecticide consumption in India (Dudani and Sengupta 1992). 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. Virtually, there is no information available on the influence of these insecticides on the arylamidase and myrosinase activities in the soil. However, very recently, Srinivasulu and Rangaswamy (2013) reported the effect of insecticides alone and in combination with fungicides on arylamidase, dehydrogenase, and myrosinase activities in the soils. Hence, the present study was aimed at determining the influence of insecticides on the activities of arylamidase and myrosinase in the two groundnut soils of Anantapur District, Andhra Pradesh, India.

Materials and methods

Soils

The black and red clay soils with a known history of pesticides use (acetamiprid and carbofuran) were collected from the groundnut cultivated fields of Anantapur District, Andhra Pradesh, India, to a depth of 15 cm, air-dried at room temperature, and sieved through a 2-mm sieve before use. Physicochemical characteristics of the two soil samples were analyzed by standard methods and presented in Table 1.

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

[a] 1:1.25 = soil/water slurry

[b] Walkley-Black Method (Johnson and Ulrich 1960)

[c] Micro-Kjeldhal 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)

Pesticides

To determine the impact of insecticides on the soil enzyme activities, acetamiprid [a neonicotinoid (20 % SP)] and carbofuran [a carbamate (3 % CG)] were obtained from Daulatabad, Gurgaon-122001, Haryana, and Vantech Chemicals Ltd., Khazipally, Jinnaram Mandal, Medak District (A.P.), India.

Experimental design and soil incubation studies

Arylamidase activity (EC 3.4.11.2)

In order to determine the impact of pesticides on the arylamidase activity, acetamiprid and carbofuran were used in the following experimental design of the soil incubation studies. One gram portion of each soil was placed in 50-ml Erlenmeyer flasks and treated with different concentrations of the pesticides which were equivalent to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1}. Soil samples without insecticide treatments served as controls. Soil samples in flasks with and without insecticide treatments were incubated at the room temperature (28 ± 4 °C), and 60 % water holding capacity (WHC) was maintained throughout the incubation period. After

10 days of incubation, one gram portions of the soil samples in flasks were treated with 3 ml of 0.1 M THAM (Tris(hydroxymethyl)aminomethane) buffer (pH 8.0) and 1 ml of 8.0 mM L-leucine β-naphthylamide hydrochloride. These flasks were swirled for a few seconds to mix the contents and were stoppered and placed in a shaker cum incubator (37 °C) for 1 h. After incubation, the reaction was stopped by adding 6 ml of ethanol (95 %) to the each flask. The soil suspensions were immediately mixed and transferred into the centrifuge tubes and centrifuged for 1 min at 17,000×*g*. The supernatants were transferred into the test tubes separately to prevent any further hydrolysis of the substrate, and 1 ml aliquots of these supernatants were treated individually (in a second set of test tubes) with 1 ml of ethanol, 2 ml of acidified ethanol, and 2 ml of the *p*-dimethylaminocinnamaldehyde reagent. Solutions were mixed on a vortex mixer, after adding each of the reagents. The intensity of the resulting red azo compound was measured Spectrophotometrically at 540 nm (Hiwada et al. 1977).

Myrosinase activity in soils (EC 3.2.3.1)

One gram portion of each soil, in triplicates, was treated with selected pesticides at 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} concentrations. Soil samples in test tubes with and without insecticide treatments were incubated at room temperature (28 ± 4 °C). After 10 days of incubation, 0.2 ml of toluene, 2.3 ml of TES buffer (0.1 M, pH 7), and 0.5 ml of sinigrin prepared in 0.1 M TES (N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid) buffer (pH 7) to obtain a final concentration of 20 mM were added to each tube. The tubes were swirled for a few seconds to mix the contents. The tubes were stoppered and incubated at 37 °C. After 4 h, the contents were transferred into 50 ml plastic centrifuge tubes and the soil suspensions were centrifuged at 8000×*g* for 10 min and the supernatants were filtered through 0.45 μm MF-millipore membrane filter into the test tubes separately. During the centrifugation period, 2 ml of reagents from the diagnostic glucose kit was pipetted into a labeled test tubes and allowed to warm to assay temperature. One ml of the filtered supernatant was added to the labeled test tube containing 2 ml of reagents from the diagnostic kit and mixed by gentle inversion. The tubes were incubated at room temperature (28 ± 4 °C) for exactly 20 min and then immediately 25 μl of $AgNO_3$ (1 M) was added to stop the activities of all the enzymes. The absorbance of the pink color of the quinoneimine complex formed was measured with a Spectrophotometer adjusted to a wavelength of 505 nm (Wilson and Tisdel 2001).

Statistical analysis

All the data were expressed on an air dry soil basis and were averages of three replicates. Data were analyzed for significant difference ($P \leq 0.05$) between the pesticide-treated and untreated soils using Duncan's multiple range (DMR) test (Pal et al. 2005; Jaya Madhuri and Rangaswamy 2009; Jaffer Mohiddin et al. 2013).

Results and discussion

The black and red clay soils are predominantly used for the cultivation of groundnut (*A. hypogaea* L.) in the Anantapur District, Andhra Pradesh, India. The major constraints in the groundnut crop are the insects and fungus pests. Continuous and indiscriminate use of these pesticides causes a major risk of soil health (Jaffer Mohiddin et al. 2013). Hence, these soils were selected to study the effect of selected insecticides on the enzyme activities. In general, the organic matter content is high in the black soil. Therefore, the biological activity (nitrification and phosphatase activity) was also more pronounced in the black soil than in the red soil under the influence of insecticides alone and in combination with fungicides. Red soil indicates the presence of iron oxides, and the black color of the soil indicates that the soil has high organic matter content (Getenga and weil 2006; Srinivasulu et al. 2012).

Unfortunately, there is no information available on the influence of acetamiprid and carbofuran on arylamidase and myrosinase activities in the soils. However, as an important agent for the control of plant pathogens, acetamiprid and carbofuran often used at rates much greater than the recommended doses. Of course, when the acetamiprid and carbofuran concentration was increased, the potential hazard to soil would increase. Soil enzyme activities are more sensitive to the environment. They reflect the soil quality more quickly and directly (Jaffer Mohiddin et al. 2013). Since enzyme activity has been seen as a very sensitive index, any disturbance due to the biotic or environmental stresses in the soil ecosystem may affect the soil biological properties.

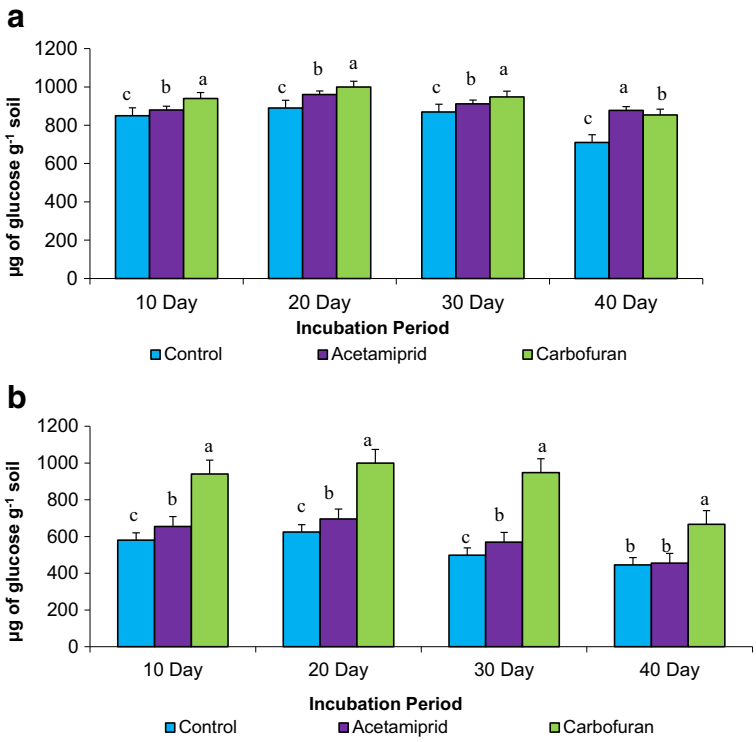

Fig. 1 Influence of carbofuran and acetamiprid respectively, on arylamidase activity in **a** black clay soil and **b** red clay soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figures are means of three replicates

Arylamidase activity

Our analysis revealed that the arylamidase activity was significantly increased as the insecticide concentrations were increased from 0.1 to 2.5 kg ha^{-1}, whereas the enzyme activity was adversely affected at higher concentrations of the insecticides. Furthermore, arylamidase activity increased in all pesticide-treated 10-day incubated soils up to 2.5 kg ha^{-1} than the controls. The enzyme activity was continued up to 20 days and then decreased gradually after 30 days of incubation (Fig. 1). Acetamiprid and carbofuran improved the arylamidase activity significantly in 10-day incubated soils. Acetamiprid at concentrations ranging from 1.0 to 5.0 kg ha^{-1} gradually increased the arylamidase activity and reached a maximum at the concentration of 5.0 kg ha^{-1} in the black and red clay soils. Application of acetamiprid above 5.0 kg ha^{-1} showed a negative effect on the arylamidase activity and exhibited a minimum activity at the 10.0 kg ha^{-1} (Tables 2 and 3). Similarly, Srinivasulu and Rangaswamy (2013) reported that the two insecticides, namely, monocrotophos and chlorpyrifos at a concentration of 2.5 kg ha^{-1} stimulated the activity of soil arylamidase. This stimulation was continued up to 20 days; whereas, the enzyme activity was greatly declined after 40 days. They also observed the drop in the arylamidase activity at higher concentrations of two insecticides. On the contrary, Floch et al. (2011) reported that the arylamidase activity, varied with the incubation time, but tended to return to its initial soil

Table 2 Influence of pesticides on the activity of arylamidase in black clay soil

Concentration of Inseticides (kg ha^{-1})	Black clay soil	
	Acetamiprid	Carbofuran
0.0	525 ± 2.886^{d} (100)	525 ± 2.886^{f} (100)
1.0	620 ± 1.154^{b} (118)	760 ± 4.041^{e} (145)
2.5	630 ± 3.464^{a} (120)	970 ± 2.309^{a} (185)
5.0	535 ± 1.154^{c} (102)	840 ± 1.154^{b} (160)
7.5	410 ± 5.773^{e} (78)	835 ± 1.732^{c} (159)
10.0	310 ± 2.886^{f} (59)	820 ± 1.154^{ad} (156)

Microgram of β-naphthylamine g^{-1} soil formed after 1 h incubation with L-leucine β-naphthylamide. 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 pesticides on the activity of arylamidase in red clay soil

Concentration of Inseticides (kg ha^{-1})	Red clay soil	
	Acetamiprid	Carbofuran
0.0	500 ± 4.082^{c} (100)	500 ± 2.886^{e} (100)
1.0	550 ± 0.577^{b} (110)	840 ± 1.732^{b} (168)
2.5	590 ± 5.773^{a} (118)	860 ± 4.041^{a} (172)
5.0	500 ± 0.577^{c} (100)	790 ± 2.309^{c} (158)
7.5	450 ± 3.464^{d} (90)	760 ± 6.027^{d} (152)
10.0	328 ± 1.154^{e} (65)	400 ± 3.464^{f} (80)

Microgram of β-naphthylamine g^{-1} soil formed after 1 h incubation with L-leucine β-naphthylamide. 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

level after a prolonged period (12 months) of incubation with the pesticides at 100 μg g^{-1} soil (10 kg ha^{-1}).

At the end of the 10-day incubation, 18 to 20 % increase in the arylamidase activity was observed in the black clay soil and 10 to 18 % increase in the red clay soil treated with acetamiprid when compared to the control (Tables 2 and 3). On the other hand, carbofuran in all the studied concentrations showed a marked increase in the arylamidase activity. However, in the red clay soil, the activity of this enzyme decreased gradually with the increased concentration of carbofuran and reached a minimum at 10.0 kg ha^{-1}. At the end of 10-day incubation, 45 to 85 % increase in the arylamidase activity was observed in the black clay soil and 68 to 72 % increase in the red clay soil treated with a carbofuran in comparison to control (Tables 2 and 3). Stimulated concentration (2.5 kg ha^{-1}) induced the highest arylamidase activity after 20, 30, and 40 days of incubation in the black clay soil with acetamiprid and carbofuran when compared to the control

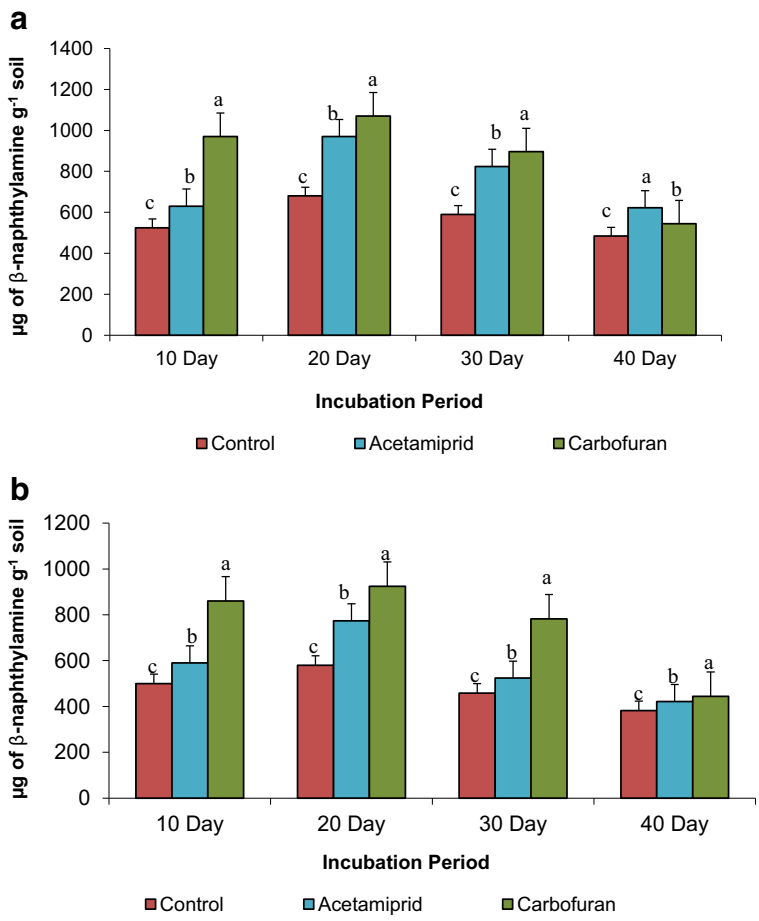

Fig. 2 Influence of carbofuran and acetamiprid respectively, on myrosinase activity in **a** black clay soil and **b** red clay soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figures are means of three replicates

(Fig. 1a),whereas in red clay soil, a similar trend was followed by acetamiprid, which induced the highest arylamidase activity after 20 days of incubation, and the trend was continued up to 40 days, but carbofuran showed a variable pattern in which the arylamidase activity remained the same with the control after 40 days (Fig. 1b). A relatively low activity of arylamidase might result from the toxic effect of the acetamiprid and carbofuran on the soil microorganisms; these in turn produce arylamidases. The inhibition of arylamidase activity by the acetamiprid and carbofuran could be attributed to the properties of the acetamiprid and carbofuran.

Myrosinase activity

Myrosinase activity increased in all pesticides treated soils up to 2.5 kg ha^{-1} than the controls at 10 days. The enzyme activity continued up to 20 days and then decreased gradually after 30 and 40 days of incubation (Fig. 2b). Acetamiprid improved the myrosinase activity significantly in the 10-day incubated soil samples. Similar types of results have been identified by Srinivasulu and Rangaswamy (2013) with monocrotophos, chlorpyrifos alone, and combination with mancozeb and carbendazim, respectively, at concentrations ranging from 1.0 to 5.0 kg ha^{-1} increased the myrosinase activity gradually and reached maximum at the concentration of 2.5 kg ha^{-1} in both soils. Above 5.0 kg ha^{-1}, acetamiprid showed minimum enzyme activity and exhibited negative effect at 10.0 kg ha^{-1} (Tables 4 and 5). In contrast, myrosinase activity was increased in all concentrations (1.0 to 10.0 kg ha^{-1}) in the red clay soil (Tables 4 and 5). At the end of the 10-day incubation, 1 to 6 % increase in myrosinase activity was observed in the black clay soil and 12 to 13 % increase in the red clay soil treated with the acetamiprid when compared to the control (Tables 4 and 5). After 10 days of incubation, 3 to 10 % increase in the myrosinase activity was observed in the black clay soil and 24 to 62 % increase in the red clay soil treated with the carbofuran in comparison to the control (Tables 4 and 5).

The results revealed that the myrosinase enzyme was rather sensitive to acetamiprid and carbofuran. Figure 2b showed the variation of myrosinase activity after acetamiprid and carbofuran applications. Although the enzyme activities of samples were lower than that of control, significant differences ($P \leq 0.05$) were found among the two studied enzyme activities between the treated soil samples and control (Tables 4 and 5). With the increase in incubation periods, the stimulation in the enzyme activities was also increased up to 20 days; further increase in the period of incubation, the enzyme activity was gradually decreased (Fig. 2b). Therefore, our results appeared to be consistent with previous reports, in which it was demonstrated that pesticides (at 2.5 to 5.0 kg ha^{-1}) stimulated myrosinase activity of soils (Srinivasulu and Rangaswamy 2013).

Pesticides normally have inhibition or stimulated functions on soil enzyme activities (Srinivasulu and Rangaswamy 2014). In our study, both insecticides induced the enzyme activities may be by stimulating the active site of the enzymes. The enzyme activities

Table 4 Influence of pesticides on the activity of myrosinase in black clay soil

Concentration of Inseticides (Kg ha^{-1})	Black clay soil	
	Acetamiprid	Carbofuran
0.0	850 ± 17.320^{b} (100)	850 ± 4.618^{b} (100)
1.0	860 ± 1.154^{d} (101)	880 ± 1.154^{d} (103)
2.5	900 ± 2.886^{a} (106)	940 ± 2.309^{a} (110)
5.0	830 ± 4.618^{c} (98)	816 ± 5.773^{c} (96)
7.5	700 ± 0.577^{e} (82)	630 ± 4.618^{e} (74)
10.0	695 ± 3.464^{f} (82)	600 ± 11.547^{f} (70)

Microgram of glucose g^{-1} soil formed after 4 h incubation with sinigrin. 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 5 Influence of pesticides on the activity of myrosinase in red clay soil

Concentration of Inseticides (kg ha^{-1})	Red clay soil	
	Acetamiprid	Carbofuran
0.0	580 ± 5.773^{c} (100)	580 ± 2.309^{e} (100)
1.0	650 ± 3.464^{b} (112)	720 ± 1.154^{c} (124)
2.5	655 ± 2.886^{a} (113)	940 ± 5.773^{a} (162)
5.0	450 ± 5.773^{d} (77)	875 ± 2.886^{b} (151)
7.5	400 ± 0.577^{e} (69)	630 ± 8.660^{d} (109)
10.0	280 ± 4.618^{f} (48)	490 ± 5.773^{f} (84)

Microgram of glucose g^{-1} soil formed after 4 h incubation with sinigrin. 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

especially arylamidase results are very high when compared to the previous reports; it is because of the factors affecting the enzyme activity especially toluene concentration, substrate concentration and other factors (Veronica Acosta-Martinez 2000). Earlier reports are similar with our studies; as reported by Tabatabai (1994), pesticides may directly interact with soil enzymes by binding with the active groups of the proteins and consequently affect their catalytic activities. Thus, in our study, stimulated or inhibitory effect of pesticides on some soil, enzyme activities could be partly explained by such direct interaction. Singh and Walker (2006) has identified a broad spectrum of microorganisms able to use organophosphorus compounds as sole source of carbon, nitrogen or phosphorus. These sources of available nutrients for microorganisms may consequently affect the biosynthesis mechanisms of enzymes by induction or repression phenomena.

Conclusions

The results of the present study clearly indicate that the application of insecticides, at field application rates (2.5 to 5.0 kg ha^{-1}), significantly enhanced the activities of arylamidase and myrosinase in the groundnut cultivated black and red clay soils. However, higher concentrations (7.5 and 10 kg ha^{-1}) of the two pesticides were either innocuous or toxic to the enzyme activities in soils. The most efficient pesticide for the enhancement of arylamidase and myrosinase activities in both soils was carbofuran. Overall, acetamiprid and carbofuran at the level of 1.0 to 2.5 kg ha^{-1} would not pose a threat to the soil enzymes. However, when the concentrations of acetamiprid and carbofuran were increased (5.0 to 10.0 kg ha^{-1}), the threat to soil, arylamidase, and myrosinase was increased.

No reports are available on the influence of these insecticides on the activities of arylamidase and myrosinase in the groundnut cultivating black and red clay soils. Hence, further investigation is needed to evaluate the influence of these insecticides on the enzyme activities in the agricultural soils which are important in cycling nutrients.

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Conflict of interest The authors declare that they have no competing interests.

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