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ORIGINAL PAPER

Influence of insecticides alone and in combination with fungicides on enzyme activities in soils

M. Srinivasulu · V. Rangaswamy

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Abstract Two insecticides, monocrotophos and chlorpyrifos alone and in combination with two fungicides, mancozeb and carbendazim, respectively, were assessed for their effects on the activities of arylamidase (as glucose formed from sinigrin), dehydrogenase (in terms of triphenyl formazan formed from triphenyl tetrazolium chloride) and myrosinase (as β-naphthylamine formed from L-leucine β-naphthylamide) in vertisol and laterite soils collected from a fallow groundnut (*Arachis hypogaea* L.) field. The influence of selected pesticides, alone and in combination on enzyme activities was concentration dependent; the activities increased with increasing concentration of the pesticides up to 2.5 kg ha^{-1}, whereas application of monocrotophos alone showed maximum enzyme activities up to 5.0 kg ha^{-1}, in both soils. However, higher concentrations (7.5 and 10 kg ha^{-1}) of the pesticides were either innocuous or toxic to the enzyme activities. The significant stimulation in the activities of arylamidase, dehydrogenase and myrosinase, was associated with 2.5 kg ha^{-1}. The maximum stimulation in arylamidase and myrosinase activity was observed at 20-day incubation, and the enzyme activities decreased gradually at 30 and 40 days of incubation. Significant increase in dehydrogenase activity was observed at 21-day incubation, and the enzyme activity decreased gradually at 28 and 35 days of incubation in both vertisol and laterite soils. The results of the present study thus, clearly, indicate that application of the insecticides alone or in combination with fungicides, in cultivation of groundnut, at field application rates improved the activities of arylamidase, dehydrogenase and myrosinase in soils.

Keywords Arylamidase · Dehydrogenase · Groundnut · Myrosinase

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Introduction

The major constraints in groundnut (*Arachis hypogaea* L.) production are insects and pathogenic fungi. Hence, insecticides and fungicides are frequently used for crop protection. Continuous and indiscriminate use of these pesticides causes a major risk of soil health (Srinivasulu et al. 2012). Groundnut, the most important oil seed crop of India, is attacked by several insect pests resulting in a marked reduction in yield (Ayyanna et al. 1982; Patil and Shekarappa 2002). The entry of insecticides in soils due to agricultural practices may disturb the delicate balance of microflora thereby affecting recycling of nutrients and soil fertility (Alexander 1977). Soil enzymes are indicator of biological equilibrium, fertility, quality and changes in the biological status of soil due to pollution (Kucharski et al. 2000; Antonious 2003). Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Liu et al. 2008). Negative impact of pesticides on soil enzymes like hydrolases and dehydrogenase activities has been widely reported in the literature (Tu 1992; Ismail et al. 1998; Menon et al. 2005). Hence, the present investigation on influence of insecticide and fungicide combinations on soil enzyme activities could be useful in minimizing the toxicity of pesticides on enzymes and improving their activities in groundnut soils. There is also evidence that soil enzymatic activities are increased by some pesticides (Megharaj et al. 1999a).

The enzyme, arylamidase [α-aminoacyl-peptide hydrolase (microsomal) EC 3.4.11.2] catalyzes the hydrolysis of a N-terminal amino acid from peptides, amides or arylamides. Because of the presence of such substrates in soils, it is likely that this enzyme is involved in N mineralization (Acosta-Martinez and Tabatabai 2000). Arylamidase is widely distributed in the tissues and body fluids of all animals (Hiwada et al. 1980), plants and microorganisms (Appel 1974). Arylamidase could be used to evaluate soil resilience after pesticide disturbances (Floch et al. 2011). However, not much information is available on the effect of pesticides on the activity of arylamidase in soils.

Dehydrogenase (EC 1.1.1.1) is a catalyst of aerobic respiration, and it could be used as an index for the total oxidative activities of the cell. Therefore, dehydrogenase activity in soil could be used as measurement for overall microbial activity (Chendrayan et al. 1980; Pandey and Singh 2006). Mayanglambam et al. (2005) studied effect of the organophosphate insecticide, quinalphos, on dehydrogenase activity after 15 days of incubation in soil. Dehydrogenase activity was recovered after 90 days of treatment which may be due to adoption of soil microorganisms to counter the effect of chemical stress in hostile conditions.

Myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1) is an enzyme that hydrolyzes glucosinolates to D-glucose and allelochemicals that have biological potential to suppress germination of weed seeds and some pathogens in soil (Angus et al. 1994; Brown and Morra 1997). Myrosinase is, thus, the key factor for allelochemical expression derived from glucosinolates and hence its study in soil is of interest. This enzyme is found in some microorganisms and released into soils via root exudation and decomposition (Turki and Dick 2003). Nevertheless, no reports are available on the effect of pesticides on the activity of myrosinase in soils.

Monocrotophos [dimethyl-(E)-1, 2-methyl carbamoyl vinyl phosphate] is an organophosphorus insecticide and acaricide. It was first produced by Ciba AG and Shell Development Co. in 1965, and has been widely used to control a variety of insects on crops, such as cotton, sugarcane, peanuts and tobacco because of its low cost and effectiveness (Sha 1999). Chlorpyrifos (0,0-diethyl-0-3,5,6-trichloro-2-pyridinol phosphorothioate), a broad spectrum organophosphorus insecticide, is most widely used for the control of white grubs in groundnut crop. Mancozeb [[(1,2-ethanediylbis-[carbamidodithioato]] (2-)] manganese, mixture with [[1,2-etha-nediylbis-[carbamodithioato]]-(2-)] zinc, is a fungicide of the carbamate pesticide family. In addition to other pesticides, it is extensively used for control of early leaf spot in groundnut. Further carbendazim (methyl-[2-^{14}C] benzimidazol-2-yl-carbamate) is also one of the mostly used members among the benzimidazole family of fungicides and is mainly used to control late leaf spot and rusts in groundnut crop.

Virtually not much information is available on the influence of insecticide and fungicide combinations on arylamidase, dehydrogenase and myrosinase activities in soil. However, very recently, the effect of insecticide and fungicide combinations on nitrification and phosphatase activities in soil was reported (Srinivasulu et al. 2012). The present study was carried out to determine the influence of insecticides alone and in combination with fungicides on the activities of arylamidase, dehydrogenase and myrosinase in two groundnut soils of Anantapur district in Andhra Pradesh, India from August 10, 2009 to February 12, 2010.

Materials and methods

Soils

A vertisol soil (pH 8.6; organic matter 1.48 %; total nitrogen 0.091 %; EC 265 (m.mhos); sand 68.3 %; silt 22.7 %; clay 19.0 %) and a laterite soil (pH 8.0; organic matter content 0.76 %; total nitrogen 0.052 %; EC 247 (m.mhos); sand 53.3 %; silt 27.1 %; clay 19.6 %) were collected from groundnut cultivated fields of Anantapur district of Andhra Pradesh, India, to a depth of 12 cm, air-dried and sieved through a 2-mm sieve before use.

Pesticides

To determine the impact of pesticides on arylamidase, dehydrogenase and myrosinase activity, monocrotophos (36 % EC), and chlorpyrifos (20 % EC) alone and in combination with mancozeb (75 % WP) and carbendazim (50 % WP), respectively, were selected in the present study. For the incubation studies, commercial formulations of tested pesticides dissolved in distilled water were used.

Experimental design and soil incubation

Arylamidase activity in soils

To investigate the effects of monocrotophos and chlorpyrifos, singly (10, 25, 50, 75 and 100 μg g^{-1} soil) and in combination (i.e. monocrotophos + mancozeb and chlorpyrifos + carbendazim) at the same concentration (5 + 5, 12.5 + 12.5, 25 + 25, 37.5 + 37.5 and 50 + 50 μg g^{-1} soil) on the activity of arylamidase, 1 g portions of each soil was placed in 50 ml Erlenmeyer flask and treated with different concentrations of pesticides which are equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. Soil samples without insecticide served as controls. Soil samples in flasks with and without insecticide treatment were incubated at room

temperature (28 ± 4 °C) and maintained at 60 % water holding capacity (WHC) throughout the incubation period. After 10 days of incubation, 1 g portions of soil samples in 50 ml Erlenmayer flasks were treated with 3 ml of 0.1 M THAM buffer (pH 8.0) and 1 ml of 8.0 mM L-leucine β-naphthylamide hydrochloride. The flasks, swirled for a few seconds to mix the contents, were stoppered and placed on a shaker cum incubator (37 °C) for 1 h. After incubation, the reaction was stopped by adding 6 ml of ethanol (95 %) to each flask. The soil suspension was immediately mixed and transferred into centrifuge tubes and centrifuged for 1 min at 17,000×*g*. The supernatant was transferred into test tubes to prevent any further hydrolysis of the substrate, and 1 ml aliquot of this supernatant was treated (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. The solution was mixed on a vortex mixer, after adding each one of the reagents. The intensity of the resulting red azo compound was measured at 540 nm in a Spectrophotometer (Hiwada et al. 1977).

Dehydrogenase activity in soils

Five gram portions of each soil, in triplicates, were treated with the selected pesticides at 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} concentrations. Soil samples without insecticide treatment served as controls. Soil samples in test tubes with and without insecticide treatment were incubated at room temperature (28 ± 4°C). After 7 days of incubation, the soil samples were withdrawn and the activity of dehydrogenase was quantified by the estimation of triphenyl formazan released from triphenyl tetrazolium chloride (Rangaswamy et al. 1994; Pandey and Singh 2006). Soil samples (5 g) were treated with 0.1 g $CaCO_3$ and 1 ml of 0.18 M aqueous triphenyl tetrazolium chloride, and incubated for 24 h at 37 °C. Then, the reaction mixture was treated with methanol for extraction of triphenyl formazan formed which was read at 485 nm in a Spectronic-20 D spectrophotometer. Further, the rate of dehydrogenase activity was measured after 14, 21, 28 and 35 days of incubation with the stimulatory concentration (2.5 or 5.0 kg ha^{-1}) of the selected pesticides in both soils.

Myrosinase activity in soils

One gram portions of each soil, in triplicates, were treated with the selected pesticides at 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} concentrations. Soil samples without insecticide treatment served as control. Soil samples in test tubes with and without insecticide treatment 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 buffer (pH 7) to obtain a final concentration of 20 mM were added to each tube. The tubes were swirled for 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 suspension was centrifuged at 8000×*g* for 10 min and the supernatant was filtered through a 0.45-μm MF-millipore membrane filter into a test tube. During the centrifugation period, 2 ml of reagents from the diagnostic glucose kit was pipetted into a labeled test tube and allowed to warm to assay temperature. One milliliter 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 activity of all the enzymes. The absorbance of the pink colour of the quinoneimine complex formed was measured with a Spectrophotometer adjusted to a wavelength of 505 nm (Turki and Dick 2003).

Statistical analysis

All data, expressed on an air dry soil basis, were averages of three replicates. Data were analysed for significant differences ($P \leq 0.05$) between pesticide treated and untreated soils using Duncan's multiple range (DMR) test (Megharaj et al. 1999b; Jayamadhuri and Rangaswamy 2009).

Results and discussion

The vertisol and laterite soils are mainly used for the cultivation of groundnut (*Arachis hypogaea* L.) in the Anantapur district of Andhra Pradesh, India**.** For this reason, insecticides and fungicides are applied simultaneously or one after another for crop protection and this type of pesticide application leads to a major risk in the soil quality. Therefore, these soils were selected to study the effect of insecticides alone and in combination with fungicides on the arylamidase, dehydrogenase and myrosinase activities in soils. In the present study, the enzyme activities were enhanced more in vertisol soil than in laterite soil, under the influence of insecticides alone and in combination with fugicides, due to the presence of high organic content in black soil. Black colour in (vertisol) soil indicates that the soil has high organic matter content and red (laterite) soil indicates the presence of iron oxides (Getenga and Weil 2006).

Arylamidase activity

Arylamidase activity increased in all individual and binary mixtures of pesticide treated and 10-day incubated soils up

to 2.5 or 5.0 kg ha^{-1} than the controls. The enzyme activity continued up to 20 days and then decreased gradually after 30 and 40 days of incubation (Fig. 1). In contrast, Floch et al. (2011) reported that arylamidase activity, varied with incubation time, but tended to return to its initial soil background level after prolonged period (12 months) of incubation with pesticides at 100 μg g^{-1} soil (10 kg ha^{-1}). Monocrotophos either singly or in combination improved the arylamidase activity significantly in 10-day incubated soils. Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} increased the arylamidase activity gradually and reached maximum at the concentration of 5.0 kg ha^{-1} in both soils. Application of monocrotophos above 5.0 kg ha^{-1} showed negative effect on arylamidase activity and exhibited minimum activity at 10.0 kg ha^{-1} (Tables 1, 2). At the end of 10-day incubation, 36–76 % increase in arylamidase activity was observed in vertisol soil and 14–82 % increase in laterite soil treated with monocrotophos when compared with control (Tables 1, 2). Chlorpyrifos at the concentrations of 1.0 and 2.5 kg ha^{-1} showed marked increase in arylamidase activity and above this concentration the activity decreased gradually and reached minimum at 10.0 kg ha^{-1} in both soils. At the end of 10-day incubation, 6–34 % increase in arylamidase activity was observed in vertisol soil and 2–48 % increase in laterite soil treated with chlorpyrifos in comparison to control (Tables 1, 2). The combination of monocrotophos with mancozeb and chlorpyrifos with carbendazim increased arylamidase activity at 1.0 and 2.5 kg ha^{-1} of each pesticide in both soils. However, amendment of the soils with higher concentrations of pesticides (7.5–10 kg ha^{-1}) resulted in minimum enzyme activity (Tables 1, 2). In contrast, 2,4-D ((2,4-dichlorophenoxy) acetic acid) did not affect the activity level of

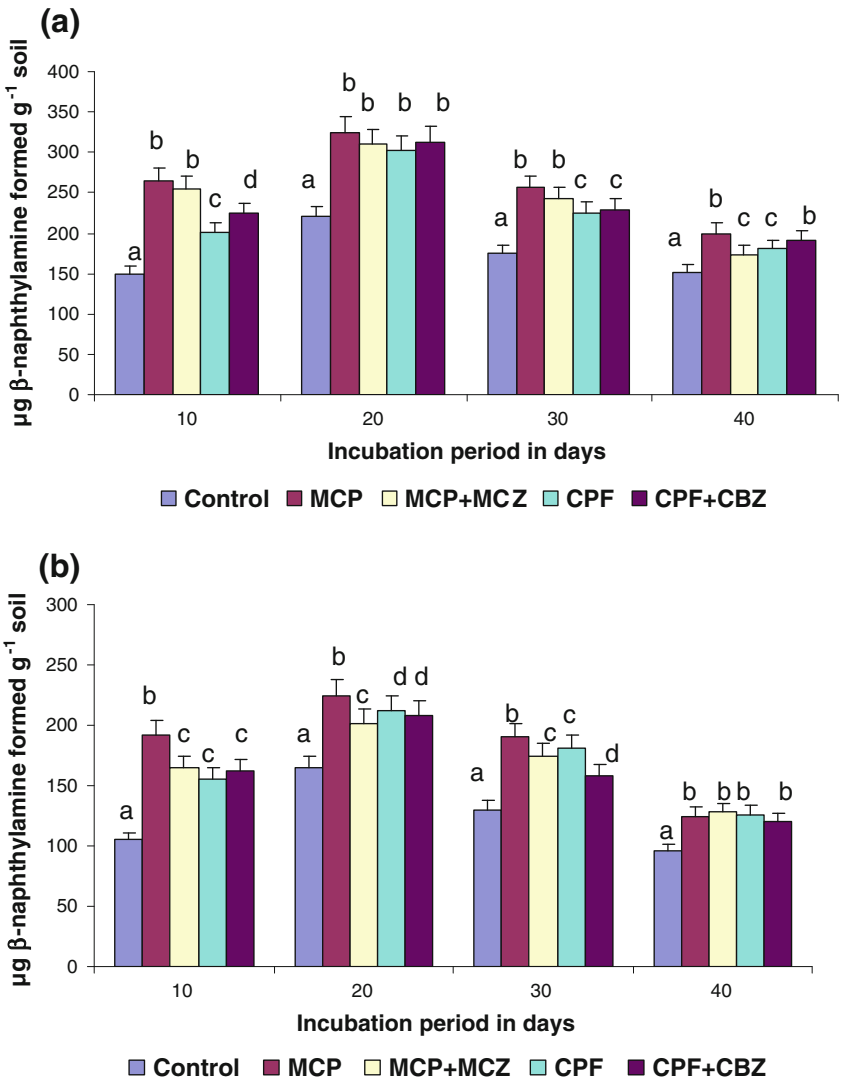

Fig. 1 Influence of monocrotophos (at 5.0 kg ha^{-1}), chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, on arylamidase activity in **a** vertisol soil and **b** laterite soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figures are mean ± standard errors of three replicates

MCP = Monocrotophos, **MCZ** = Mancozeb, **CPF** = Chlorpyrifos, **CBZ** = Carbendazim

Table 1 Influence of selected pesticides alone and in combination on the activity of arylamidase in vertisol soil after 10 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	150^{a} (100)	150^{a} (100)	150^{a} (100)	150^{a} (100)
1.0	205^{b} (136)	210^{b} (140)	160^{a} (106)	180^{b} (120)
2.5	220^{c} (146)	255^{c} (170)	201^{b} (134)	224^{c} (149)
5.0	265^{d} (176)	225^{b} (150)	152^{a} (101)	160^{a} (106)
7.5	200^{b} (133)	200^{c} (133)	145^{a} (96)	146^{a} (97)
10.0	140^{a} (93)	145^{a} (96)	110^{c} (73)	101^{d} (67)

μg 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. Values represented in table are means of three replicates

Table 2 Influence of selected pesticides alone and in combination on the activity of arylamidase in laterite soil after 10 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	105^{a} (100)	105^{a} (100)	105^{a} (100)	105^{a} (100)
1.0	120^{b} (114)	110^{b} (104)	102^{a} (97)	126^{b} (120)
2.5	125^{b} (119)	165^{c} (157)	156^{b} (148)	162^{c} (154)
5.0	192^{c} (182)	126^{d} (120)	110^{a} (104)	142^{d} (135)
7.5	125^{b} (119)	125^{d} (119)	100^{c} (105)	105^{a} (100)
10.0	100^{d} (95)	99^{e} (94)	75^{d} (71)	92^{e} (87)

μg 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. Values represented in table are means of three replicates

arylamidase over time even at 100 μg g^{-1} (10 kg ha^{-1}) soil (Floch et al. 2011). In vertisol soil, monocrotophos with mancozeb showed 40–70 % increase and same combination in laterite soil showed 4–57 % increase in arylamidase activity at the end of 10-day incubation. The combination of chlorpyrifos and carbendazim led to 20–49 % increase in arylamidase activity in vertisol soil and in laterite soil the activity increased from 20 to 54 % over control (Tables 1, 2).

Dehydrogenase activity

The activity of dehydrogenase increased in all pesticide treated soils up to 2.5 kg ha^{-1} than the control after 21 days of incubation (Fig. 2). Monocrotophos either singly or in combination with mancozeb (fungicide) improved the dehydrogenase activity significantly in 21-day incubated soil samples, whereas the enzyme activity gradually decreased with increase in period of incubation up to 35 days. Likewise, individual application of metalaxyl (fungicide) increased the dehydrogenase activity initially in fungicide-treated (40–80 μg g^{-1}) soil and then gradually decreased after 30 days (Sukul 2006). In contrast, Mayanglambam et al. (2005) observed 30 % inhibition in dehydrogenase activity in quinalphos treated soil after 15 days of incubation. Monocrotophos and chlorpyrifos at concentrations ranging from 1.0 to 2.5 kg ha^{-1} increased the dehydrogenase activity gradually and reached maximum at the concentration of 2.5 kg ha^{-1} in both vertisol and laterite soils. Amendment of both soils with monocrotophos and chlorpyrifos above 2.5 kg ha^{-1} resulted in minimum dehydrogenase activity while higher concentrations (7.5 and 10 kg ha^{-1}) showed inhibitory effect indicating antagonistic interaction (Tables 3, 4). Similarly Nweke et al. (2007) also reported that atrazine and northrin (herbicides) stimulated the dehydrogenase activity at lower dose (0.2 %) and inhibited it at higher concentration (0.55 %) in rhizoplane microbial community. After 10 days of incubation, 10 to 52 and 19 to 59, 2 to 59 and 2 to 45 % increase in dehydrogenase activity was observed by the application of pesticides in vertisol and laterite soils respectively, when compared with controls (Tables 3, 4). Likewise, Singh and Kumar (2008) observed that acetamiprid increased dehydrogenase activity up to 22 % after first insecticide application.

The combination of monocrotophos and chlorpyrifos along with mancozeb and carbendazim, respectively, showed increase in dehydrogenase activity at 1.0 and 2.5 kg ha^{-1} of each pesticide in laterite and vertisol soils, but in laterite soil the same combination (chlorpyrifos + carbendazim)

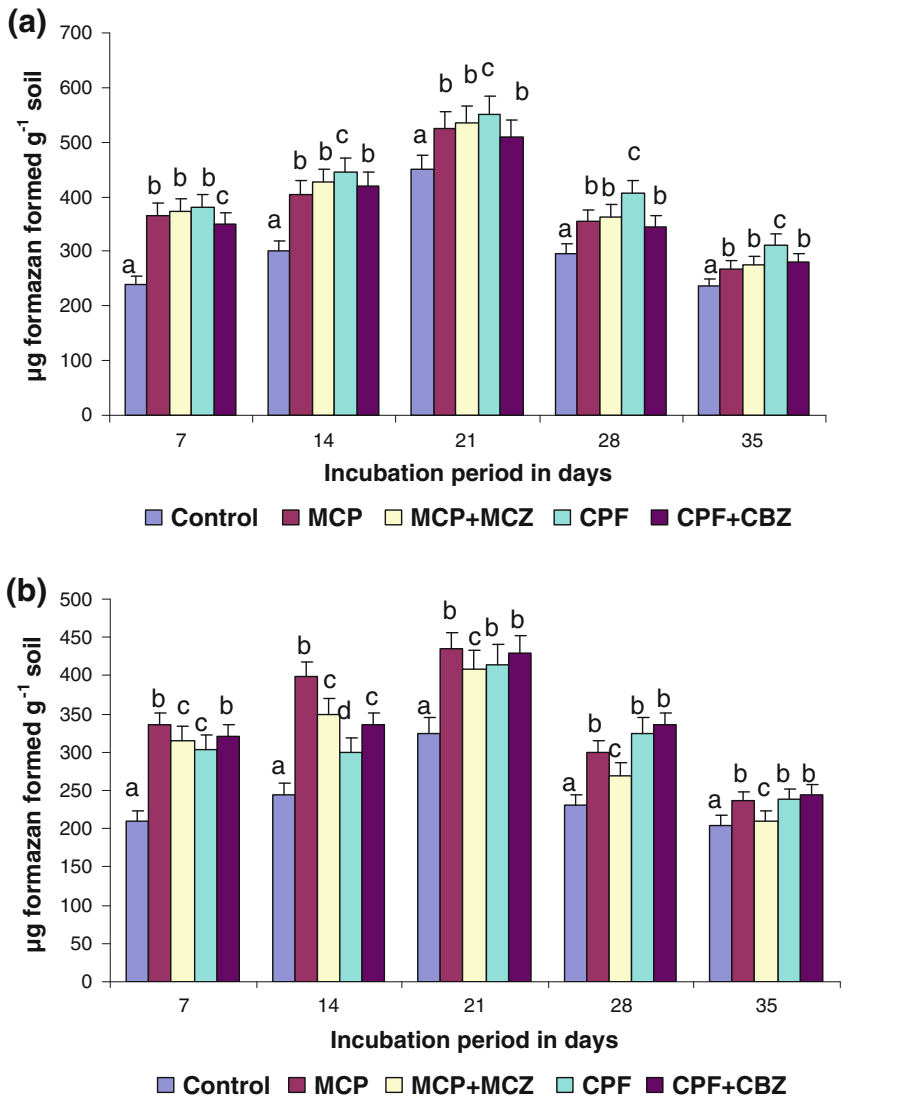

Fig. 2 Influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, on dehydrogenase activity in **a** vertisol soil and **b** laterite soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figures are mean ± standard errors of three replicates

MCP = Monocrotophos, **MCZ** = Mancozeb, **CPF** = Chlorpyrifos, **CBZ** = Carbendazim

increased dehydrogenase activity up to 5.0 kg ha^{-1}. In contrast, dehydrogenase was the most sensitive to mancozeb + dimethomorph even at 15 mg kg^{-1} soil (1.5 kg ha^{-1}) when compared with control in both loamy sand and sandy loam soils (Cycon et al. 2010). On other hand, Yao et al. (2006) reported that the activity of dehydrogenase was enhanced after acetamiprid application for 2 weeks in samples treated with 50 mg kg^{-1} (5.0 kg ha^{-1}) dry soil which was about two fold to that of the control on sample day 28.

In vertisol soil, monocrotophos with mancozeb showed 16–56 % increase and same combination in laterite soil showed 16–50 % enhancement in dehydrogenase activity over control after 7 days of incubation. The combination of chlorpyrifos and carbendazim showed 19–45 % increase of dehydrogenase activity in vertisol soil and in laterite soil the activity increased from 17 to 52 % in comparison to controls (Tables 3, 4). The activity of dehydrogenase was enhanced significantly under the influence of pesticides after 21 days of incubation. Further increase in period of incubation (up to 35 days) decreased the rate of dehydrogenase activity gradually (Fig. 2). The experimental data showed that the enzyme activity decreased gradually by the application of pesticides alone and in combinations above 2.5 or 5.0 kg ha^{-1} in two soils.

Myrosinase activity

Myrosinase activity increased in all individual and binary mixtures of pesticides treated soils up to 7.5 kg ha^{-1} than the controls in 10-day incubated soil samples. The enzyme activity continued up to 20 days and then decreased gradually after 30 and 40 days of incubation (Fig. 3). Monocrotophos either singly or in combination improved the myrosinase activity significantly in 10-day incubated soil

Table 3 Influence of selected pesticides alone and in combination on dehydrogenase activity in vertisol soil after 7 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	240^{a} (100)	240^{a} (100)	240^{a} (100)	240^{a} (100)
1.0	265^{b} (110)	278^{b} (116)	245^{a} (102)	285^{b} (119)
2.5	366^{c} (152)	374^{c} (156)	382^{b} (159)	350^{c} (145)
5.0	315^{d} (131)	305^{d} (127)	280^{c} (117)	225^{d} (93)
7.5	304^{d} (127)	230^{a} (96)	200^{d} (83)	211^{e} (88)
10.0	205^{e} (85)	200^{e} (83)	170^{e} (70)	160^{f} (67)

μg of formazan g^{-1} soil formed after 24 h incubation with triphenyl tetrazolium 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 Duncan's multiple range (DMR) test

Table 4 Influence of selected pesticides alone and in combination on dehydrogenase activity in laterite soil after 7 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	210^{a} (100)	210^{a} (100)	210^{a} (100)	210^{a} (100)
1.0	250^{b} (119)	245^{b} (116)	215^{a} (102)	245^{b} (117)
2.5	335^{c} (159)	315^{c} (150)	305^{b} (145)	278^{c} (152)
5.0	210^{a} (100)	220^{a} (105)	247^{c} (118)	320^{d} (132)
7.5	170^{d} (81)	200^{a} (95)	142^{d} (68)	240^{b} (114)
10.0	120^{e} (57)	155^{d} (74)	96^{e} (46)	160^{e} (76)

μg of formazan g^{-1} soil formed after 24 h incubation with triphenyl tetrazolium 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 Duncan's multiple range (DMR) test. Values represented in table are means of three replicates

samples. 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 5.0 kg ha^{-1} in both the soil samples. Above 5.0 kg ha^{-1}, monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, showed minimum enzyme activity and exhibited negative effect at 10.0 kg ha^{-1} (Tables 5, 6). At the end of the 10-day incubation, 31–75 % increase in myrosinase activity was observed in vertisol soil and 44–100 % increase in laterite soil treated with monocrotophos when compared with control (Tables 5, 6). After 10 days of incubation, 6–31 % increase in myrosinase activity was observed in vertisol soil, and 12–44 % increase in laterite soil treated with chlorpyrifos in comparison to control (Tables 5, 6). The combination of monocrotophos with mancozeb and chlorpyrifos with carbendazim showed increase in myrosinase activity at 1.0 and 5.0 kg ha^{-1} of each pesticide in both soils. However, higher concentrations (10 kg ha^{-1}) of pesticides decreased myrosinase activity indicating antagonistic interaction (Tables 5, 6). In vertisol soil, monocrotophos with mancozeb showed 40–109 % increase and same combination in laterite soil showed 56–112 % in myrosinase activity at the end of 10 days of incubation. The combination of chlorpyrifos and carbendazim showed 3–40 % increase of myrosinase activity in vertisol soil and in laterite soil the activity increased from 24 to 56 % over controls (Tables 5, 6).

All four pesticides exhibited maximum stimulation in enzyme activities at 2.5 or 5.0 kg ha^{-1} throughout the incubation period suggesting synergistic interaction (Fig. 1, 2, 3). Chlorpyrifos alone and in combination with carbendazim at 10 kg ha^{-1} showed 27 and 33 % inhibition in arylamidase activity respectively in vertisol, whereas in laterite soil same pesticides exhibited 29 and 13 % inhibition in enzyme activity in comparison to control indicating antagonistic interaction (Tables 1, 2). Monocrotophos singly and along with mancozeb exhibited 15 and 17 % inhibition in dehydrogenase activity, whereas chlorpyrifos alone and combination with carbendazim showed 30 and 33 % inhibition in dehydrogenase activity, respectively, in comparison to control in vertisol soil. Monocrotophos alone and monocrotophos + mancozeb and chlorpyrifos singly and chlorpyrifos + carbendazim at 10.0 kg ha^{-1} showed 43, 26 and 54 %, 24 % inhibition in dehydrogenase activity respectively in comparison to control in laterite soil (Tables 2, 3). On the other hand, Kalam et al. (2004) observed that individual

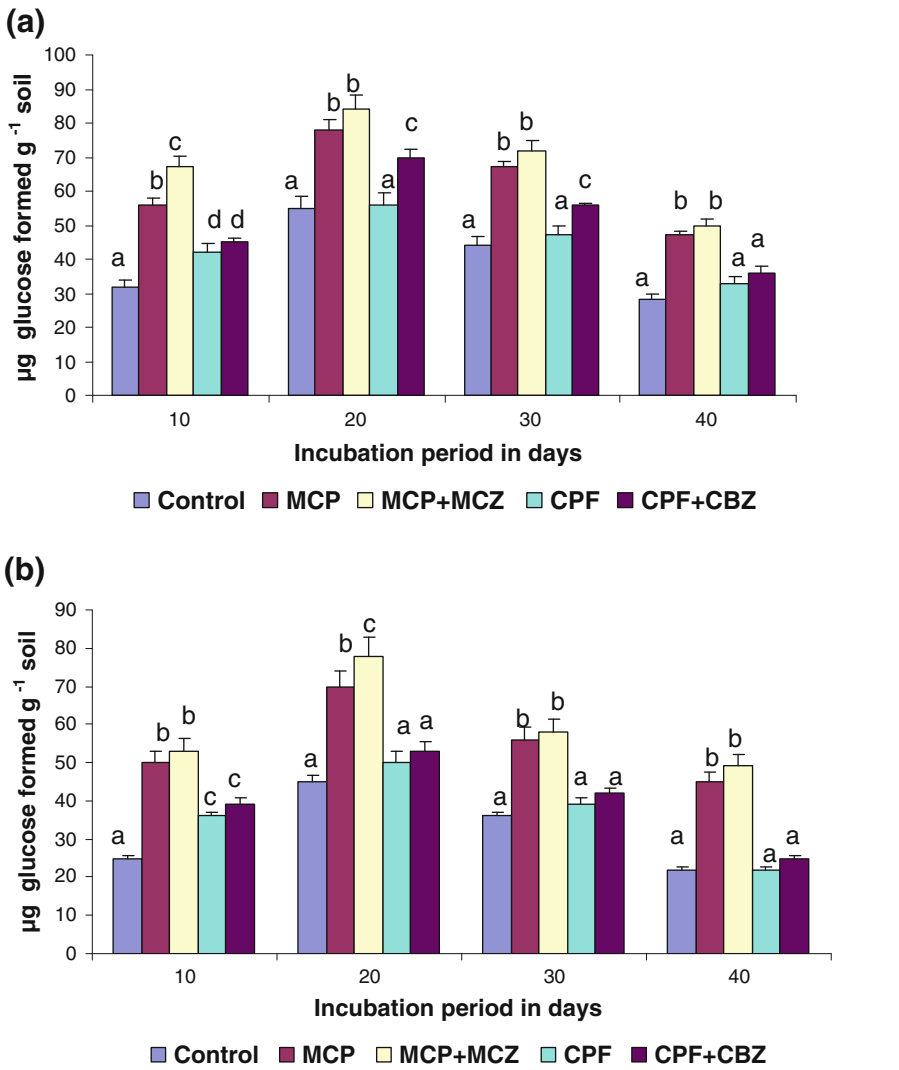

Fig. 3 Influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, on myrosinase activity in **a** vertisol soil and **b** laterite soil at 5.0 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figures are mean ± standard errors of three replicates

MCP = Monocrotophos, **MCZ** = Mancozeb, **CPF** = Chlorpyrifos, **CBZ** = Carbendazim

Table 5 Influence of selected pesticides alone and in combination on the activity of myrosinase[1] in vertisol soil after 10 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	32^{a} (100)	32^{a} (100)	32^{a} (100)	32^{a} (100)
1.0	42^{b} (131)	45^{b} (140)	34^{a} (106)	33^{a} (103)
2.5	48^{b} (150)	50^{b} (156)	36^{b} (112)	39^{b} (122)
5.0	56^{c} (175)	67^{c} (209)	42^{c} (131)	45^{c} (140)
7.5	45^{b} (140)	52^{b} (162)	33^{a} (103)	36^{a} (112)
10.0	29^{a} (90)	32^{a} (100)	30^{a} (93)	27^{d} (84)

µg 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. Values represented in table are means of three replicates

application of profenofos affected the soil dehydrogenase activity by 47 % at higher concentration of 1,000 mg kg^{-1} after 80 days and thereafter, the extent of toxicity decreased slightly.

Conclusion

The results of the present study clearly indicate that application of the insecticides alone and in combination

Table 6 Influence of selected pesticides alone and in combination on the activity of myrosinase in laterite soil after 10 days

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + mancozeb	Chlorpyrifos	Chlorpyrifos + carbendazim
0.0	25^{a} (100)	25^{a} (100)	25^{a} (100)	25^{a} (100)
1.0	36^{b} (144)	39^{b} (156)	28^{a} (112)	31^{b} (124)
2.5	42^{c} (168)	44^{b} (176)	33^{b} (132)	36^{c} (144)
5.0	50^{d} (200)	53^{c} (212)	36^{b} (144)	39^{c} (156)
7.5	39^{b} (156)	39^{b} (156)	30^{b} (120)	37^{b} (148)
10.0	21^{e} (84)	19^{d} (76)	20^{a} (80)	26^{a} (104)

μg 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. Values represented in table are means of three replicates

with fungicides, in cultivation of groundnut, at field application rates (2.5–5.0 kg ha^{-1}) enhanced the activities of arylamidase, dehydrogenase and myrosinase significantly in vertisol and laterite soils. However, higher concentrations (7.5 and 10 kg ha^{-1}) of the pesticides were either innocuous or toxic to the enzyme activities in soils. The most efficient pesticide for the enhancement of arylamidase activity was monocrotophos, in both soils. Chlorpyrifos and monocrotophos alone in vertisol soil and laterite soil, respectively, were more effective on the activity of dehydrogenase. Data from the present study indicate that the most effective combination for myrosinase activity is monocrotophos + mancozeb in both soils. However, very few reports are available on the influence of insecticide and fungicide combinations on the enzyme activities especially myrosinase and arylamidase activities in groundnut-cultivating vertisol and laterite soils for the sake of comparison. Hence, further investigation is needed to evaluate the influence of insecticide and fungicide combinations on the enzyme activities in agricultural soils which are important and affect nutrient cycling and fertility of soils.

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References

Acosta-Martinez V, Tabatabai MA (2000) Arylamidase activity in soils. Soil Sci Soc Am J 64:215–221

Alexander M (1977) Introduction to soil microbiology, 2nd edn. Wiley, New York, pp 113–330

Angus JF, Grander PA, Kirkegaard JA, Desmarchelier JM (1994) Biofumigation: isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant Soil 162:107–112

Antonious GF (2003) Impact of soil management and two botanical insecticides on urease and invertase activity. J Environ Sci Health B 38:479–488

Appel W (1974) Peptidases. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol 2. Academic Press, New York, pp 949–954

Ayyanna T, Arjuna Rao P, Subbaratnam GV, Krishnamurthy Rao BH, Narayana KL (1982) Chemical control of *Spodoptera litura* Fabricus on groundnut crop. Pesticides 16:19–20

Brown PD, Morra MJ (1997) Control of soil-borne plant pests using glucosinolate-containing plants. Adv Agron 61:167–231

Chendrayan K, Adhya TK, Sethunathan N (1980) Dehydrogenase and invertase activities of flooded soils. Soil Biol Biochem 12:271–273

Cycon M, Piotrowska-Seget Z, Kozdroj J (2010) Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. Int Biodet Biodegra 64:316–323

Floch C, Anne-Celine C, Karine J, Yvan C, Steven C (2011) Indicators of pesticide contamination: soil enzyme compared to functional diversity of bacterial communities via biolog ecoplates. Eur J Soil Biol 47:256–263

Getenga NC, Weil RR (2006) Elements of the nature and properties of soils (p. 5). Prentice Hall, Englewood Cliffs

Hiwada K, Ito T, Yokoyama M, Kokubu T (1980) Isolation and characterization of membrane-bound arylamidases from human placenta and kidney. Eur J Biochem 104:155–165

Hiwada K, Yamaguchi C, Inaoka Y, Kokubu T (1977) Neutral arylamidase in urine healthy and nephritic children. Clin Chim Acta 75:31–39

Ismail BS, Yapp KF, Omar O (1998) Effects of metsulfuron-methyl on amylase, urease, and protease activities in two soils. Aust J Soil Res 36:449–456

Jayamadhuri R, Rangaswamy V (2009) Biodegradation of selected insecticides by *Bacillus* and *Pseudomonas* sps in groundnut fields. Toxicol Int 16:127–132

Kalam A, Tah J, Mukherjee AK (2004) Pesticide effects on microbial population and soil enzyme activities during vermicomposting of agricultural waste. J Environ Biol 25:201–208

Kucharski J, Jastrzebska E, Wyszkowska J, Hiasko A (2000) Effect of pollution with diesel oil and leaded petrol on enzymatic activity of the soil. Zesz Probl Poste p Nauk Rol 472:457–464 (in Polish)

Liu J, Xie J, Chu Y, Sun C, Chen C, Wang Q (2008) Combined effect of cypermethrin and copper on catalase activity in soil. J Soils Sed 8:327–332

Mayanglambam T, Vig K, Singh DK (2005) Quinalphos persistence and leaching under field conditions and effects of residues on dehydrogenase and alkaline phosphomonoesterases activities in soil. Bull Environ Contam Toxicol 75:1067–1076

Megharaj M, Boul HL, Thiele JH (1999a) Effects of DDT and its metabolites on soil algae and enzymatic activity. Biol Fertil Soils 29:130–134

Megharaj M, Kookana K, Singleton S (1999b) Activities of fenamiphos on native algae population and some enzyme activities in soil. Soil Biol Biochem 39:1549–1553

Menon P, Gopal M, Parsad R (2005) Effects of chlorpyrifos and quinalphos on dehydrogenase activities and reduction of Fe^{3+} in the soils of two semi-arid fields of tropical India. Agric Ecosyst Environ 108:73–83

Nweke CO, Ntinugwa C, Obah IF, Ike SC, Eme GE, Opara EC, Okolo JC, Nwanyanwu CE (2007) In vitro effects of metals and pesticides on dehydrogenase activity in microbial community of cowpea (*Vigna unguiculata*) rhizoplane. Afr J Biotechnol 6:290–295

Pandey S, Singh DK (2006) Soil dehydrogenase, phosphomonoesterase and arginine deaminase activities in an insecticide treated groundnut (*Arachis hypogaea* L.) field. Chemosphere 63:869–880

Patil RK, Shekarappa (2002) Management of *Spodoptera litura* F on groundnut with newer insecticides. Pestol 26:23–24

Rangaswamy V, Reddy BR, Venkateswarlu K (1994) Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. Agric Ecosyst Environ 47:319–326

Sha JJ (1999) A manual of industrial and chemical production: agricultural chemicals. Chemical Industry Press, Beijing, pp 13–14

Singh DK, Kumar S (2008) Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. Chemosphere 71:412–418

Srinivasulu M, Mohiddin GJ, Subramanyam K, Rangaswamy V (2012) Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (*Arachis hypogeae* L.) soils. Environ Geochem Health 34:365–374

Sukul P (2006) Enzymes activities and microbial biomass in soil as influenced by metalaxyl residues. Soil Biol Biochem 38:320–326

Tu CM (1992) Effect of some herbicides on activities of microorganisms and enzymes in soil. J Environ Sci Health B 27:695–709

Turki AI, Dick AW (2003) Myrosinase activity in soil. Soil Sci Soc Am J 67:139–145

Yao X, Min H, Lu Z, Yuan H (2006) Influence of acetamiprid on soil enzymatic activities and respiration. Eur J Soil Biol 42:120–126