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Effects of methamidophos on amylase, urease and protease activities in soils from Cameron Highlands

(Kesan metamidofos terhadap amilase, urease dan protease dalam tanah dari Cameron Highlands)

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Key words: amylase, urease, protease, methamidophos

Abstrak

Kesan racun serangga perosak methamidophos terhadap aktiviti enzim tanah iaitu amilase, urease dan protease dalam tanah loam berpasir (tapak 1), tanah loam (tapak 2) dan loam berpasir (tapak 3) dari tanah yang tidak bertanam berdekatan dengan ladang sebagai kawalan dan tanah dari ladang telah dikaji di makmal selama 28 hari. Methamidophos pada kepekatan 10.0 μg/g menyebabkan pengurangan yang ketara terhadap aktiviti amilase sepanjang tempoh kajian terutamanya dalam tanah dari tapak 1 dan tapak 3 (loam berpasir). Pada hari ke-28, aktiviti amilase berkurangan 27% dan 21% berbanding kawalan, masing-masing dalam tanah dari tapak 1 dan 3. Bagaimanapun terdapat kecenderungan untuk pulih selepas hari ke-7 bagi tanah dari tapak 2. Aktiviti protease dalam tanah daripada ketiga-tiga tapak tersebut menunjukkan kecenderungan untuk meningkat apabila tempoh pengeraman dilanjutkan kecuali tanah yang diambil dari ladang. Pengurangan aktiviti urease dikesan dalam tanah dari tapak 1 dan 2 apabila diperlakukan dengan 10.0 μg/g methamidophos. Aktiviti urease berkurangan dalam tanah yang diambil dari ladang berhampiran tapak 2 dan 3.

Abstract

Effects of pesticide, methamidophos on the activities of soil enzymes viz. amylase, urease and protease in sandy loam (site 1), loam soil (site 2) and sandy loam (site 3) from uncultivated sites near vegetable farms as control, and soils from three cultivated farms in Cameron Highlands were evaluated under laboratory conditions for up to 28 days. Methamidophos at 10.0 mg/g caused a marked reduction in amylase activities for the entire period of study, especially in soil from site 1 and site 3 (sandy loam). On day 28, the activity of amylase reduced by 27% and 21% of the control in soil from site 1 and 3. However, in soil from site 2, there is a tendency of recovery after day 7. The protease activity in either soil showed a tendency to increase when the incubation periods were prolonged up to 28 days except in soils taken from the farm. Reduction in activity of urease was observed in site 1 and site 2 when treated with 10 mg/g methamidophos. The urease activities declined in soils taken from the farm near site 2 and site 3.

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Introduction

Methamidophos is a member of the organophosphorous group and is widely used for the control of most insect pests such as *Plutella xylostella* in vegetables at Cameron Highlands, Malaysia and in tobacco cultivations (Cheah et al. 1997). This insecticide is being used before harvesting on a wide spectrum of insects such as Acarina, Diptera, Homoptera dan Lepidoptera. Recently, methamidophos was restricted to control insect pests in coconut and oil palm plantations. As the acerage of oil palm plantations cover a wide area of Malaysian agricultural land, it is of environmental concern to see the impact of the insecticide to the soil.

It was reported that most of the organophosphorous pesticides exhibit varying tendencies to be adsorbed by soil particles, particularly clay and organic constituents due to differences in structures (Green 1974). Most organophosphates are not persistent and the half-lives vary between the soil types. Jui-Hung et al. (1998) observed that methamidophos degraded rapidly with half-lives ranging from 1.11 to 1.61 days and 7.5 to 13.20 days in silt loam and silt clay loam, respectively. Beach et al. (1995) reported that methamidophos would be largely lost through leaching, adsorbed to surface soil at low levels and moderately transported in runoff.

While much is known about the fate, efficacy, and mode of action of methamidophos, scarce quantitative information is available concerning its residual effects on soil enzyme activities, especially in the tropical soils of Malaysia. The insecticide may reach the soil directly as drift after spraying or indirectly after washing by rainfall. Activity of soil enzymes may predict the potential capacity of a soil to perform certain biological transformations of importance to soil fertility (Chendrayan and Sethunathan 1980). Enzymes in soil are involved in many different aspects of the metabolism of soil organic matter. Those involved in the hydrolysis of complex compounds containing nitrogen or phosphorus are of particular interest because they help to release essential elements normally taken up by plants as simple inorganic compounds. Soil urease is the enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Due to the increased usage of urea as a fertilizer in agriculture, soil urease level in conjunction with other tests has been used as one of the indices leading to a better understanding of the fertility of the soil ecosystem. Protease is involved in the initial protein hydrolysis in soil of organic nitrogen to simple amino acids. Amylase catalyzes the hydrolytic depolymerization of polysaccharides. Although many studies have been reported on non-target effects of pesticides in soil (Tu and Miles 1976), knowledge of the action of pesticides on soil enzymes is scarce (Tu 1993), especially in relation to Malaysian tropical soils (Ismail and Ahmad 1994). The present study was initiated to assess effects of methamidophos on activities of amylase, urease and protease in two soil types collected from three sites at Cameron Highlands.

Materials and methods

Soil samples

The soils used in this study were taken from three different sites in Cameron Highlands, namely Kea Farm (Brinchang, site 1), Soon Cheong Farm (Brincang, site 2) and Muniammah Farm (Kuala Terla, site 3). The soils were taken from uncultivated soil which pesticides had never been used. Different samples from the three farms (cultivated with cabbage) were also taken to assess the enzymatic activities in soils subjected to farmers practices. The farmers in these three farms used methamidophos to control insect pest every three months. Five soil cores were taken at 10 cm depth using auger 7 cm in diameter from each site including the cultivated farms. The five samples from each site were mixed thoroughly, kept in black plastic bag and

brought back to the laboratory. The soil characteristics are shown in *Table 1*. Before use, the soil was passed through a 2 mm sieve, placed in black polythene bags and stored at 4 °C. The herbicide tested was Tamaron® (Bayer) containing 43.9% (w/w) methamidophos (O,S-dimethyl phosphoramidothioate).

Treatment of the soils

Moist soil equivalent to 1 kg oven-dried soil was placed in a cylindrical metal drum (30 cm x 27.5 cm) lined with a polythene sheet. The herbicide methamidophos was applied by spraying onto the soil to give a mean final concentration of 0, 0.1, 0.5, 2.0, 5.0 and 10.0 mg/g (on an oven-dried basis). The soil was mixed thoroughly in a rotating drum. The moisture content was then adjusted to 45% of field capacity as described by Ismail and Ahmad (1994). Triplicate samples of 300 g soil per treatment were prepared and incubated in 500 mL flasks at 27 °C. The flasks were covered with aluminium foil and closed with cotton wool. The flasks were opened twice a week to prevent the soil becoming oxygen-deficient and of reduced humidity. The soil moisture levels were checked regularly by weighing and adjusted to 45% of field capacity by adding deionized water where appropriate.

Two g soil samples were removed from the flasks on day 0, 7, 14, 21 and 28 for amylase, urease and protease assays. Day 0 is regarded as the sample taken immediately after each treatment with herbicide and thereafter the day is counted as day 1.

Assay of amylase

The method employed for the assay of amylase was essentially developed by Bernfield (1951). The soil samples (2 g) were transferred to 250 mL Erlenmeyer flasks, and treated with 12.5 mL of 0.02 M phosphate buffer (pH 6.9) containing 1% starch. The flasks were closed with cotton plugs and held for 5 h at 30 °C before determining the amylase activity. Controls had no added substrate. Enzyme amylase activity was determined as reducing sugar equivalent by adding 1.0 mL colouring reagent (1 g 3,5-dinitrosalicylic acid in 20 mL 2N NaOH and 50 mL H_2O. A 30 g potassium-sodium tartarate was added to the mixture and diluted with distilled water to make 100 mL solution) to 1.0 mL supernatant of the treated sample. The mixture was then kept in boiling water for 5 min before adding 10 mL of distilled water and read at 540 nm. The amount of equivalent maltose formed was deduced from the calibration curve prepared to the standard concentration of maltose. One unit

Table 1. Physico-chemical properties of the cultivated soils

Characteristics	Soil		
	Site 1	Site 2	Site 3
pH ($CaCl_2$)	4.0	4.9	5.9
Moisture (%)	29.0	30.4	28.4
Organic C* (%)	3.3	4.3	3.6
Ca (meq/100 g soil)	133.9	100.4	48.9
Na (meq/100 g soil)	28.2	4.1	10.3
Mg (meq/100 g soil)	31.8	8.9	17.5
K (meq/100 g soil)	28.1	2.35	10.2
Sand (%)	60.4	52.4	68.6
Silt (%)	26.7	32.3	21.1
Clay (%)	12.9	15.3	10.3
CEC** (cmol/kg soil)	17.0	20.0	22.8

*C = organic carbon

**CEC = Cation Exchange Capacity

of amylase is considered as equivalent of that 1 μmol maltose liberated in an hour assay.

Assay of urease

Activity of soil urease was determined using methods as described by Broadbent et al. (1958). Urease activity was measured as the release of ammonia from urea in the presence of sodium azide as an inhibitor of microbial proliferation. The yellow colour was read after 15 min at 420 nm in a spectrophotometer.

Assay of protease

The assay of protease activity was based upon that as reported by Rangaswamy et al. (1994), with some modifications in the incubation conditions. Soil (2.0 g) in a conical flask (50 mL) was allowed to stand for 50 min at 30 °C after removal from the incubated flasks. It was then incubated for 2 h at 37 °C with 10 mL of 0.1 M tris (2-amino-2[hydroxymethylmethyl]-propane-1:3-diol), at pH 7.5, containing sodium caseinate (2% w/v). Four mL of aqueous solution (17.5% w/v) of trichloro acetic acid was then added and the mixture was centrifuged. A suitable aliquot of the supernatant was treated with 3 mL of 1.4 M $NaCO_3$ followed by the addition of 1.0 mL Folin-Cicalteau reagent (33.3% v/v). The blue colour was read after 30 min at 700 nm in a spectrophotometer against tyrosine as a standard.

Statistical analysis

The concentrations of the enzymes were calculated on a soil dry weight (oven-dried) basis. Data were subjected to an analysis of variance and means were compared by the LSD test at $p = 0.05$.

Results and discussion

A comparison of amylase activity in the methamidophos-treated soil with that of control soil revealed that activity was significantly high especially at 2.0 mg/g in soil from site 1 when incubation period was prolonged to 28 days (*Figure 1*). During the entire period of incubation, amylase produced remained suppressed in soil treated with either 5 or 10 ug/g and did not show any recovery tendency. With these treatments, the activity was lower than that of control soil irrespective of incubation days. Amylase activity in soil taken from the cultivated farm was higher at day 1 than in control soil. Further incubation reduced amylase activity progressively but in general the activity higher than soils treated with either 5 or 10 ug/g methamidophos.

An increase of methamidophos in the soil from site 2, showed a reducing amylase activity but not in the control soil. Significant decreased in amylase activity was observed at day 14 in soil treated with either 5 or 10 ug/g. In most of the treated soil, the activity of amylase was recovered at day 21 and day 28. Amylase activity in the soil sample taken from the farm showed low amylase activity throughout incubation day. In general, the amylase activity in the cultivated farm soil was lower than the untreated control.

Amylase activity in the soil taken from site 3 and then treated with different concentrations of methamidophos at 0.1, 0.5 and 2.0 μg/g soil showed low activity of amylase at day 14 but recovered at day 21 onward. In contrast, amylase activity was lower than that of the control especially at day 28. Amylase activity in the soil taken from the cultivated farm decreased as the incubation period was prolonged. The activity in cultivated farm soil at day 28 was lower than the control but significantly higher than the activity in soil treated with 5 or 10 ug/g methamidophos.

In the control soil, protease activity *(Figure 2)* seemed to increase as incubation period was prolonged up to 28 days. At day 1, protease activity in the soil treated with either 5 or 10 μg/g was higher than in the control soil but low at day 28. Protease activity in the soil taken from the farm decreased as incubation period was prolonged. It was observed that protease

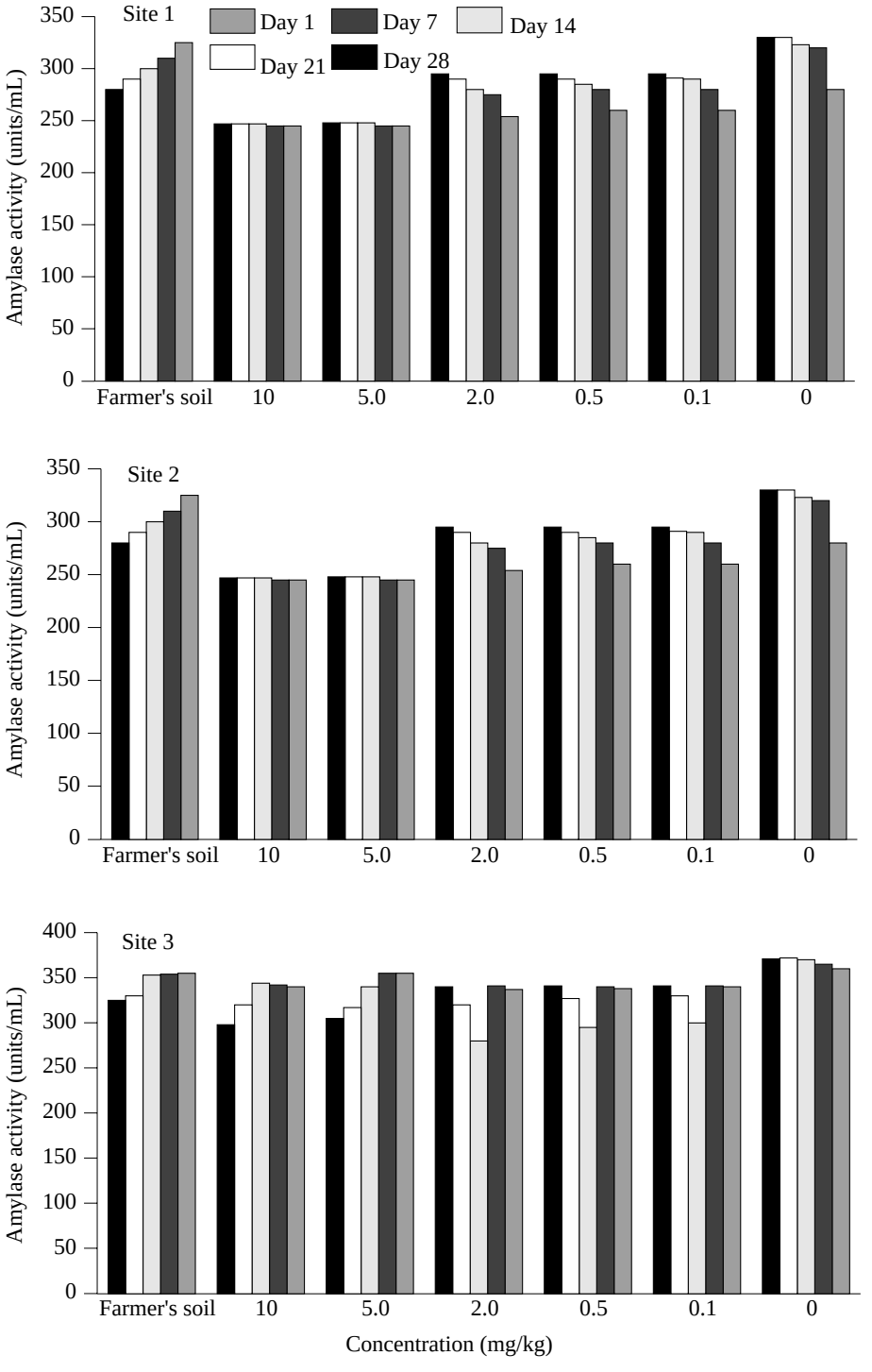

Figure 1. Soil amylase activity from farms and soils treated with different concentrations of methamidophos (Site 1, LSD $_{0.05}$ = 7.5; Site 2, LSD $_{0.05}$ = 9.6; Site 3, LSD $_{0.05}$ = 10.1).

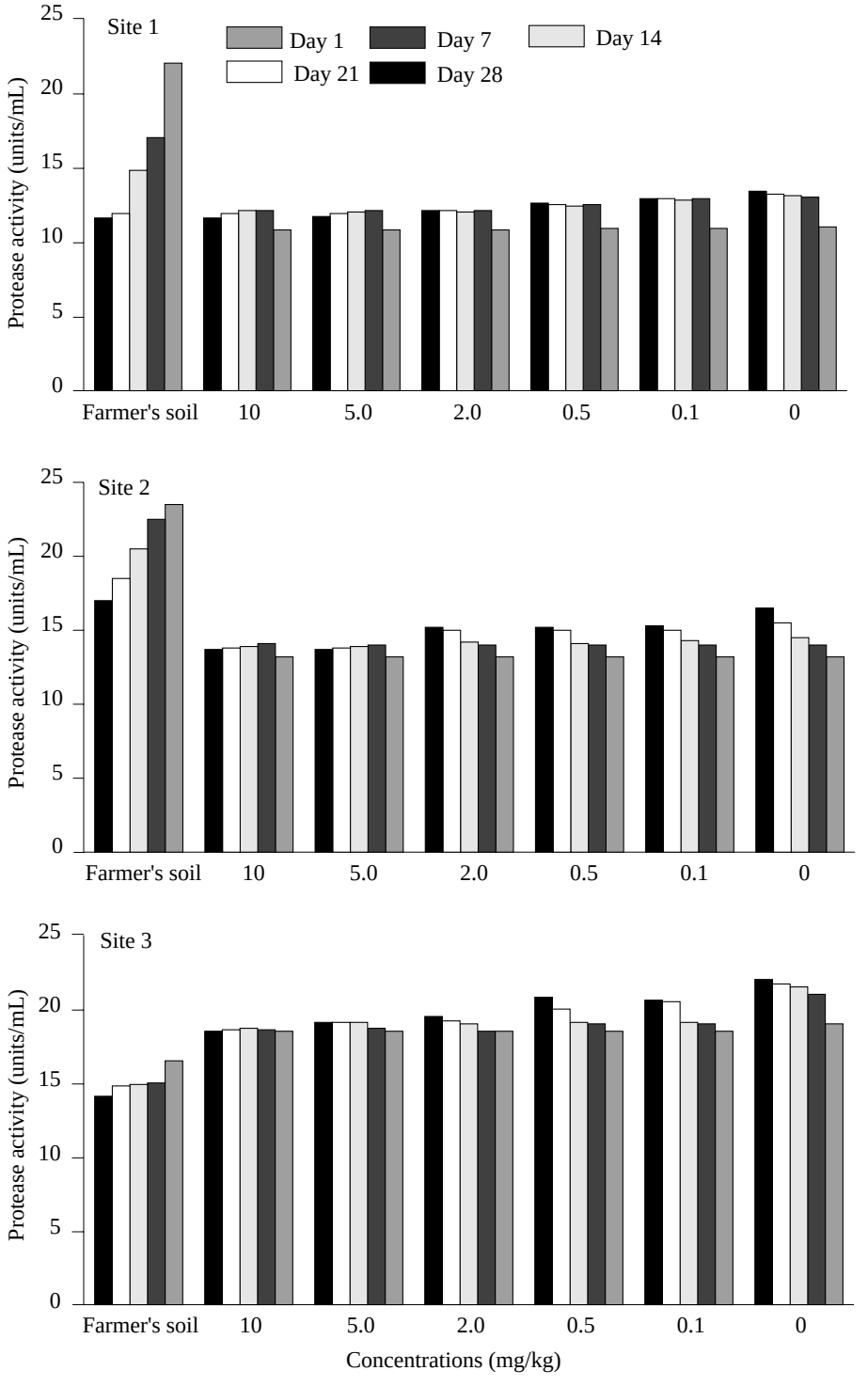

Figure 2. Soil protease activity from farms and soils treated with different concentrations of methamidophos (Site 1, LSD $_{0.05}$ = 1.3; Site 2, LSD $_{0.05}$ = 0.6; Site 3, LSD $_{0.05}$ = 0.4).

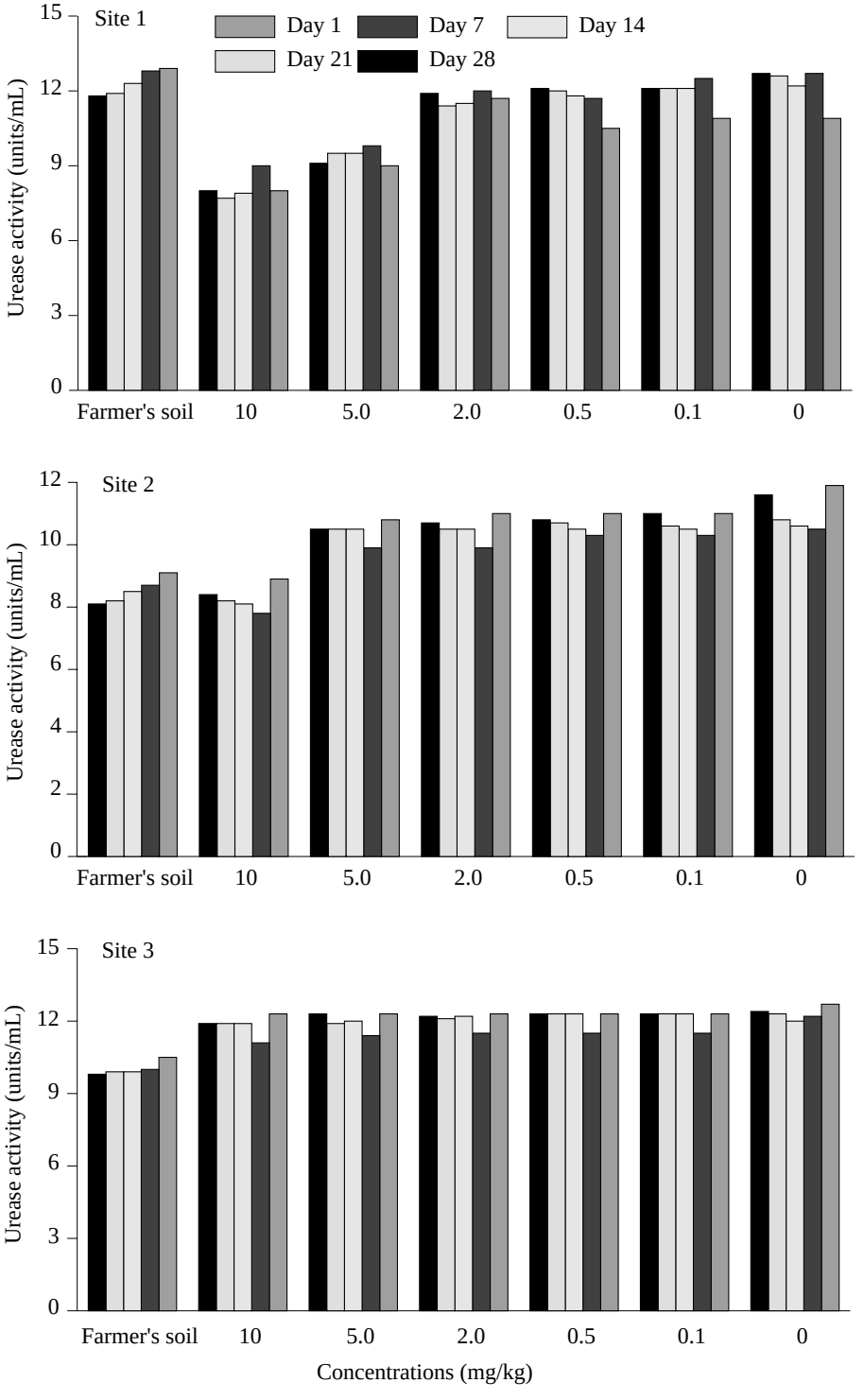

Figure 3. Soil urease activity from farms and soils treated with different concentrations of methamidophos (Site 1, LSD $_{0.05}$ = 2.2; Site 2, LSD $_{0.05}$ = 0.9; Site 3, LSD $_{0.05}$ = 2.1).

activity in the cultivated farm soil at day 21 and day 28 were not much different as those soils treated with 5 or 10 μg/g.

Protease activity in the control soil and soil treated at 0.1, 0.5 and 2.0 mg/g from site 2 increased as incubation was extended to 28 days. However, in soil treated with 5 and 10 mg/g, no significant difference was observed in protease activity at each incubation day and slightly lower than control soil. Protease activity in the soil taken from Soon Cheong Farm showed higher activity than the control. However, the activity decreased progressively as the soil was incubated for up to 28 days.

For soil from site 3, protease activity in soil treated with methamidophos was lower than control soil. The activity recovered in soil treated with 0.1, 0.5 and 2.0 ug/g as incubation period was prolonged. However, protease activity in soil treated with 5 and 10 μg/g were not significantly different irrespective of incubation day. Our result showed that protease activity in soil sample from the cultivated farm was lower than that of the other treatments. However, further incubation decreased protease activity in soil taken from the cultivated farm.

Urease activity *(Figure 3)* in soil from site 1 treated with either 5 or 10 μg/g and then incubated for 14 days or onward was slightly lowered than control soil. In control soil and soil treated at 0.1 and 0.5 μg/g showed tendency to increase the activity at 28 days. However at higher concentration, the activity was not much different among the incubation days. Generally the urease activity in soil from cultivated farm was higher than control soil but decreased as the incubation days was prolonged.

Urease activity was lowered in soil from site 2 treated with 10 μg/g. However, soil treated as high as 5 μg/g were not significantly different as untreated soil. In soil taken from the cultivated farm showed low activity even on day 1 and decreased as the incubation period was prolonged.

In general, the urease activity in treated soil taken from site 3 was lower than the control at day 7. The activity in all the treated soil was not significantly different with that of the control. However, urease activity in the soil sample taken from the cultivated farm was observed to be lower than the control. The activity was slightly decreased as the incubation period increased to 28 days.

Earlier reports showed that pesticides may either enhance or inhibit soil enzyme activity (Mishra and Pradhan 1987; Tu 1993). A marked suppression of amylase activity in the soil treated by insecticides such as monocrotophos, quinalphos, cypermethrin and fenvalerate in groundnut soil was shown by Rangaswamy and Venkateswarlu (1992). Similarly, Tu (1993) reported that 11 pesticides (including herbicides) used in his study inhibited amylase activities after 1 day incubation. Our results have shown in soil from site 1, the amylase activity declined at higher concentrations. On the other hand, Tu (1988) reported that malathion, carbofuran and permethrin at a high level after 3 days were stimulatory in the formation of glucose from added starch. The report also noted that autoclaving could inhibit formation of reducing sugar.

The urease activity was not affected by the low concentration (0.1 mg/g) of methamidophos but reduction in activity was observed at high concentration such as 10 μg/g in soil 1 and 2. It should be noted that under normal condition the concentration of methamidophos in soil was estimated about 0.1 mg/g after application at the recommended rate of 0.5–1.0 kg/ha. Other herbicides such as chlorbromuron and *EPTC* have shown stimulatory effects on urease activity in sandy loam soil (Tu 1993). In contrast, Satpathy and Behera (1993) reported that the insecticide malathion (organophosphorus) had an inhibitory effect on urease activity. Our results have shown that methamidophos reduced urease activity only at higher concentrations in soil from site 1 and 2. Low activity was also observed in cultivated farm soil near site 2 and 3.

Our results showed that protease activity was higher after 7 days incubation in soils from 3 sites. These results were in line with those reported by Satpathy and Behera (1993) for the insecticide malathion, which caused an initial depression of protease activity but showed a recovery tendency around the twenty-first day of incubation. However, protease activities in soils from the three cultivated farms decreased when incubation periods were prolonged.

Farm soils received various treatments including pesticides (directly or indirectly) and chemical fertilizers. Therefore, the activities of soil enzymes such as amylase and urease are very much lower than the control. The mixture of various treatments to soil may cause synergistic effect on enzyme activity. Soils from the areas were not much different in their physico-chemical properties. Therefore, this factor may not have great influence on the behaviour of methamidophos in soils. It is believed that pesticide application and soil management by the farmers in respective farm most probably had an influence on microbial populations and consequently their enzymatic activity.

Reports on the impact of pesticides on soil enzymes show considerable diversity depending upon type of pesticide used, rate and mode of application, climatic factors, composition of soil and organic matter content (Tu 1981; Endo et al. 1982). Therefore, direct comparisons of our results with those reported in the literature are rather difficult. The findings in the present study of the initial suppression followed by subsequent recovery of soil enzymatic activities is in agreement with earlier reports on the characteristics of carbaryl-treated soil (Mishra and Pradhan 1987). Recovery of microbial populations after an initial inhibition may be due to development of microbial resistance or to the dissipation of methamidophos residues in soil (Ismail and Ahmad 1994; Ismail and Lee 1995; Ismail et al. 1996). A significant degradation of pesticides was observed in the tropical soil after 25 days exposure (Ismail and Lee 1995). Pettit et al. (1976) have suggested that fluctuations or changes in soil enzyme activities after pesticide application may be due to the release of intracellular enzymes on the death or lysis of microorganisms.

Studies on the impact of methamidophos on soil enzymes and microbes, especially under tropical conditions, to the best of our knowledge are scarce. Therefore, comparison of the results obtained with those reported under temperate conditions may offer irrelevant conclusion due to the differences in climatic conditions and the different concentrations used. As mentioned earlier, effects of the same pesticides on the same enzymes were different depending on other environmental factors such as soil composition, temperature, moisture and so on. The variations in the effects are due to the fact that, in nature numerous microorganisms exist and each microorganism may respond differently to the herbicidal treatment.

In conclusion, our experiments showed that application of methamidophos at the recommended rate of 30 g a.i./ha which is applied twice a month only temporarily reduced enzymatic activity in the soil. Clark (1977) reported that organophosphorus insecticides such as *DDVP* and *malathion* are mutagenic and capable of alkylating DNA which may alter the synthesis of many enzymes and their activity. Hence the decline and suppression of different enzyme activity can be explained on the basis of adverse effect of malathion on microbial genetic material.

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