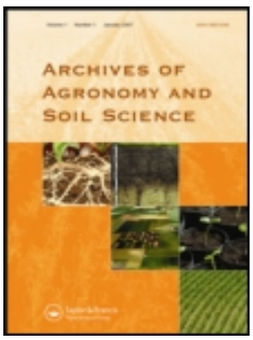

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Effect of carbofuran on enzymatic activities and growth of tomato plants in natural, fertilized and vermicompost-amended soils

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The effects of carbofuran, a widely used carbamate pesticide, on soil enzymatic activities such as fluorescein diacetate hydrolysis (FDAH), dehydrogenase, and acid and alkaline phosphatases were studied at different time intervals in unamended soil and soil amended with inorganic fertilizers and vermicompost, cropped with tomato plants. The results showed that all enzymatic activities varied with carbofuran application rates and increased significantly up to 1.0 kg active ingredient (a.i.) ha^{-1} dose of carbofuran. The most significant increase was observed at 0.20 kg a.i. ha^{-1} dose both in unamended and amended soils. This showed that carbofuran was not toxic to all enzymatic activities studied upto 1.0 kg a.i. ha^{-1} dose of carbofuran in both systems. A significant decrease in all enzymatic activites were observed at higher dose of carbofuran both in unamended and amended soils relative to their respective controls. Highest enzymatic activities were observed in vermicompost amended soil and minimum in fertilized soil compared to control. The results indicated that the growth of tomato plants was significantly higher at 0.20 kg a.i. ha^{-1} dose of carbofuran in all the cases and followed the order: fertilized soil > vermicompost amended soil > natural soil and was positively correlated with the enzyme activities.

Keywords: carbofuran; enzymatic activities; fertilizers; vermicompost

Introduction

The soil environment is composed of biotic and abiotic components. The biotic components contain an enormous number of diverse living organisms, assembled in complex and varied communities, whereas the abiotic component contains organic and mineral matter. Biological properties of soils, for example soil respiration, microbial biomass, nitrogen mineralization capacity and enzyme activities, have been proposed as indicators of soil quality and health (Reddy et al. 1987; Dick 1994; Dick and Breakwell 1996; Bendick and Dick 1999). Soil enzymes are soil active proteins playing an important role in soil health and are involved in the decomposition of organic matter, cycling of plant nutrients, energy transfer and environmental quality. Measurement of specific enzymatic activities may be useful in determining soil biological activity, which might then be used as an index of soil fertility (Perucci 1992; Cladwell 2005; Winding et al. 2005).

Pesticides, mineral fertilizers, composts and vermicompost are commonly used in farming systems. The effects of certain pesticides (Anderson et al. 1981; Lal and Lal

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1988; Perucci 1992; Perucci and Scarponi 1994; Perucci et al. 2000; Chen et al. 2001; Omar and Abdel-Sater 2001; Araujo et al. 2003; Wyszkowska and Kucharski 2004), inorganic fertilizers (Luo and Sun 1994; Simek et al 1999; Jia et al. 2001; Lalfakzuala et al. 2008) and vermicompost (Delgado-Moreno and Pena 2009; Fernandez-Bayo et al. 2009) on enzymatic activities of soils have been reported. Pesticides generally appear to have no adverse effect on soil enzymes except at concentrations exceeding recommended rates. Conflicting data have been published about the impact of inorganic fertilizers on enzyme activities. Some research has suggested that enzymatic activity decreases with inorganic fertilizer application (Simek et al. 1999; Lalfakzuala et al. 2008). However, Yang et al. (2000) reported that chemical fertilizers stimulate enzymatic activities. Vermicompost, generally applied to low organic carbon content soils, plays a positive role because it increases the organic matter content in the soil (Delgado-Moreno and Pena 2009; Fernandez-Bayo et al. 2009). Martens et al. (1992) reported that enzyme activities in organic-matter-amended soil increased an average of two- to fourfold compared with unamended soil. Chang et al. (2007) reported that enzymatic activities significantly increased in compost-treated soils compared with fertilizer-treated soil according to a study concerning the effects of different application rates of organic fertilizer on soil enzyme activity and microbial population.

Recently, Menon et al. (2005), Sukul (2006), Stepniewska et al. (2007), Jastrzebska and Kucharski (2007), Piotrowska-Seget et al. (2008), Cacers et al. (2009) and Rasool and Reshi (2010) have reported the effect of different pesticides on dehydrogenase, acid and alkaline phosphatases activities and fluorescine diacetate hydrolysis in soils.

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methyl carbamate) is a systemic nonionic broad spectrum carbamate insecticide/nematicide, which is widely used on the Indian subcontinent to control nematodes in soils. Considerable work has been done on the effect of carbofuran for the control of nematode population in soils (DiSanzo 1981; Singh et al. 1986; El-Banhawy et al. 1998), but work on the effect of carbofuran on the enzyme activities of soils is very scanty (Rodell et al. 1977; Kale and Raghu 1990; Kalam and Mukherjee 2001). Rodell et al. (1977) studied the effects of several insecticides, including carbofuran, on nitrogenase activity by *Azotobacter vinelandii* and soyabean nodules. Kale and Raghu (1990) studied the effect of carbofuran on soil phosphatases and aryl sulfatase activities, and showed that carbofuran had no marked inhibitory effect on these enzymes. Kalam and Mukherjee (2001) reported an inhibitory effect of carbofuran on the dehydrogenase activity of soils.

A lot of work has been done on single effects of pesticides, fertilizers and vermicompost on soil enzymatic activities, however, no information is available on how soil enzyme activities respond to combined application of these agrochemicals.

In this study, we investigated the effect of varying doses of carbofuran on enzymatic activities [fluorescein diacetate hydrolysis (FDAH), dehydrogenase, acid and alkaline phosphatases] and the growth of tomato plants in natural, fertilized and vermicompost-amended alluvial soil.

Materials and methods

Soil and chemicals

In this study, an alluvial sandy loam soil, which was obtained from village Rehmapur in the district of Aligarh (U.P.) India, was used. Geographically, Aligarh

is located between 27.88°N and 78.08°E at an average elevation of 178 m.a.s.l. Soil texture (% sand, % silt and % clay) was estimated using the International Pipette Method (Piper 1950). The pH in 1:2.5 soil/water samples, percentage of $CaCO_3$ content and bulk density were determined using the methods described by Jackson (1958). The percentage of organic carbon/organic matter, cation-exchange capacity (CEC) and surface area of the soil were determined using the methods proposed by Walkley and Black (1947), Ganguli (1951) and Dayal and Hendricks (1950), respectively, and are given in Table 1.

Carbofuran (98.97% purity) was supplied by the M\S Rallis Agrochemicals Station (Bangalore, India). A stock solution of carbofuran (500 μg mL^{-1}) was prepared by dissolving the requisite amount of carbofuran in acetone.

Inorganic fertilizers used in this study include urea, diammonium phosphate (DAP) and muriate of potash (MOP), and were obtained from E. Merck Chemicals Ltd, India.

Vermicompost was obtained from Dangas Vermi Biotech Pvt. Ltd (Aligarh, India), and was prepared by treating cowdung with epigeic earthworms (*Eisenia foetida*). The composition of vermicompost included 11.5% organic matter (OC), 1.5% N, 1.8% P, 2.0% K.

All other chemicals and reagents were of AR grade and were obtained from E. Merck and CDH Chemicals Ltd India.

Soil amendment with inorganic fertilizers and vermicompost

To study the effect of inorganic fertilizers and vermicompost, the soil was amended with urea, DAP, MOP (N:P:K, 90:60:60 kg ha^{-1}) and vermicompost (5.0 t ha^{-1}), respectively.

Experimental design

Soil samples for this study were collected randomly from a field in the village of Rehmapur, in Aligarh district, India, by taking thin slices from the surface layer (0–15 cm) using a spade, as outlined by Jackson (1958). The composite soils were air dried in the shade, cleaned by removing plant material and other debris and passed through a 2-mm (4–8 mesh cm^{-1}) sieve.

Table 1. Physicochemical properties of the studied soil.

Soil properties	Rehmapur soil
Sand (%)	62.26
Silt (%)	29.74
Clay (%)	8.00
Organic matter (%)	0.724
Organic carbon (%)	0.420
Texture	Sandy loam
pH	7.8
Cation-exchange capacity[cmol(p +)] kg^{-1}	65
$CaCO_3$ (%)	3.50
Bulk density (g cm^{-3})	1.33
Surface area (m^2 g^{-1})	266

A pot experiment was conducted under greenhouse conditions (average temperature 21°C day and 12°C night, relative humidity 60–68%) at the Department of Botany, Aligarh Muslim University, Aligarh, India during October 2009 to February 2010 to study the effect of varying doses of carbofuran on enzymatic activities and the growth of tomato plants in unamended soil and in soil amended with inorganic fertilizers and vermicompost. Three treatments of carbofuran doses (0.2, 1.0 and 5.0 kg a.i. ha^{-1}) were applied in natural (umamended) soil and in amended soils using 5 mL acetone, assuming 2×10^6 kg soil ha^{-1} to a depth of 15 cm (bulk density 1.33 g cm^{-3}). Untreated soil samples were also processed in a similar way by adding only 5 mL acetone. The details of the treatments are as follows:

T_1, natural soil (control);
T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1};
T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1};
T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1};
T_5, control + fertilizers;
T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1};
T_7, fertilized soil + 1.0 kg a.i. carbofuran ha^{-1};
T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1};
T_9, control + vermicompost;
T_{10}, vermicompost-amended soil + 0.2 kg a.i. carbofuran ha^{-1};
T_{11}, vermicompost-amended soil + 1.0 kg a.i. carbofuran ha^{-1};
T_{12}, vermicompost-amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

All the treatments including control were replicated three times.

One healthy and uniform 15-day-old tomato seedling (*Lycopersicon esculentum*, var. K-25) grown in autoclaved soils was transplanted into each pot. The pots were irrigated with equal amounts of water throughout the experiment, as and when required. The pots were arranged in a greenhouse on a bench in a randomized fashion.

Soil samples were drawn at 0, 5, 10, 15, 30, 60, 90 and 120 days post carbofuran application and stored at 4°C for the analysis of soil enzymes activities. The FDAH, dehydrogenase, acid and alkaline phosphatases activities were estimated using the methods proposed by Adam and Duncan (2001), Casida et al. (1964) and Tabatabai and Bremner (1969), respectively. Each value given in the tables is the average of three observations.

After 120 days, the plants were uprooted and brought to laboratory for biometric observation. The shoots and roots of the plants were separated, and shoot and root lengths and fresh weights of shoot and roots were measured. The shoots and roots of the plants were dried in an oven at 60 ± 1°C and weighed.

Statistical analysis

The data on the effect of varying doses of carbofuran on soil enzymatic activities was subjected to analysis of variance (ANOVA) and mean comparison was carried out using the least significant difference (LSD) test at $p < 0.05$. Simple correlation analysis was performed to correlate different enzymatic activities with plant growth parameters using Pearson's correlation test. In all cases, statistical analyses were performed with the software package SPSS 11.0 for Windows.

Results

Enzymatic activities

Results on the effects of varying doses (0.20, 1.0 and 5.0 kg a.i. ha^{-1}) of carbofuran at different time intervals on FDAH, dehydrogenase, acid phosphatase and alkaline phosphatase activities in soil alone and soil amended with inorganic fertilizers and vermicompost are presented in Tables 2–5.

In response to the doses of carbofuran applied (Tables 2–5), a significant increase in all the enzymatic (FDAH, dehydrogenase, and acid and alkaline phosphatases) activities was observed up to the 1.0 kg a.i. ha^{-1} dose of carbofuran, but the most significant increase was observed at 0.20 kg a.i. ha^{-1} carbofuran dose. By contrast, at a higher dose (5.0 kg a.i ha^{-1}) a significant decrease in all enzymatic activities was observed in both unamended and amended soils compared with control treatments (T_1, T_5 and T_9).

All enzymatic activities studied were higher in vermicompost-amended soil than in unamended soil and fertilizer-amended soil at varying doses of carbofuran (Tables 2–5).

A significant increase in dehydrogenase activitiy was observed at 10 days, whereas significant increases in FDAH, acid and alkaline phosphatases activities were observed up to 15 days. Thereafter, all enzyme activities gradually declined to the end of the experiment in all the cases.

The initial differences observed among the treatments with application of fertilizers or vermicompost are taken into account by using normalized (C/C_0) values to check the relative increments in dehydrogenase activity over time. The normalized data (C/C_0) are given in Table 6.

Growth of plants

The effects of varying doses of carbofuran on growth parameters such as shoot/root length and shoot dry weight/root dry weight of tomato plants are shown in Figure 1. An increase in plant growth was observed up to the 1.0 kg a.i. ha^{-1} dose but the largest increase in plant growth was observed at 0.20 kg a.i. ha^{-1}, in both unammended soil and soil amended with fertilizers and vermicompost. At a higher application rate, i.e. 5.0 kg a.i. ha^{-1}, a decrease in plant growth was observed, and plants exhibited phytotoxicity in the form of marginal leaf scorching in unamended soils and fertilizer- and vermicompost-amended soils.

Discussion

Enzymatic activities

The increase in FDAH activity up to 1.0 kg a.i. ha^{-1} dose of carbofuran (Table 2) in unamended soil may be due to the utilization of carbofuran as an available substrate, thereby rapidly stimulating microbial activity. Our results are in accordance with the work of Zelles et al. (1985) and Araujo et al. (2003) who reported an increase in FDAH activity in presence of certain pesticides (lindane, captan, atrazine, glyphosate) in soils. The decrease in FDAH activity at a higher dose is may be due to a direct interaction with the number or activity of microorganisms, which changes intra- and extracellular enzyme activities. This is in agreement with the findings of Das et al. (2008) who studied the effect of novaluron on microbial biomass, respiration and fluorescein diacetate hydrolysing activity in tropical soils.

Table 2. Effect of different doses of carbofuran on fluorescein diacetate hydrolysis activity (μg fluorescein released h^{-1} g^{-1} soil) in soil alone and soils amended with fertilizers and vermicompost at different time intervals under field conditions.

	Fluorescein diacetate hydrolysis activity (μg fluorescein released h^{-1} g^{-1} soil)									
	Days									
Treatments	0	5	10	15	30	60	90	120	Mean	LSD (5%)
T_1	11.71	13.65	14.62	18.33	11.30	11.49	10.55	9.39	12.63	1.262
T_2	11.71	17.25	19.20	20.64	12.51	12.38	12.11	10.59	14.54	1.238
T_3	11.71	15.75	17.25	19.74	11.70	12.21	11.19	9.99	13.69	1.299
T_4	11.71	13.20	13.75	14.70	10.71	10.55	10.03	8.46	11.63	1.301
Mean	11.71	14.96	16.20	18.35	11.47	11.65	10.97	9.60		
LSD (5%)		0.297	0.272	0.408	0.252	0.259	0.261	0.385		
T_5	11.10	11.90	12.75	15.51	9.66	8.97	8.22	6.96	10.63	0.250
T_6	11.10	15.75	16.50	19.74	12.03	10.41	9.54	8.46	12.94	1.218
T_7	11.10	12.67	13.50	16.80	10.56	10.05	9.21	7.56	11.43	0.309
T_8	11.10	11.40	11.81	14.10	9.38	7.98	7.23	6.36	9.92	0.268
Mean	11.10	12.79	13.21	16.53	10.40	9.35	8.55	7.33		
LSD (5%)		0.272	0.291	0.342	0.266	0.222	0.833	0.320		
T_9	13.50	15.37	18.00	19.74	13.17	12.40	11.70	10.29	14.27	0.256
T_{10}	13.50	19.98	22.50	23.28	14.01	14.14	13.98	11.49	16.61	0.679
T_{11}	13.50	16.01	21.00	21.74	13.70	13.40	12.18	10.89	15.30	0.661
T_{12}	13.50	14.75	18.00	16.92	12.84	11.65	10.98	9.30	13.49	0.621
Mean	13.50	16.52	19.80	19.92	13.38	12.89	12.21	10.49		
LSD (5%)		0.303	1.330	0.331	0.365	0.385	0.309	0.347		

Note: T_1, natural soil (control); T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1}; T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1}; T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1}; T_5, control + fertilizers; T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1}; T_7, fertilized soil + 1.0 kg a. i. carbofuran ha^{-1}; T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1}; T_9, control + vermicompost; T_{10}, vermicompost amended soil + 0.2 kg a.i. carbofuran ha^{-1}; T_{11}, vermicompost amended soil + 1.0 kg a.i. carbofuran ha^{-1}; T_{12}, vermicompost amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

Table 3. Effect of different doses of carbofuran on dehydrogenase activity (μg Triphenyl formazon (TPF) day^{-1} g^{-1} soil) in soil alone and amended with fertilizers and vermicompost at different time intervals under field conditions.

	Dehydrogenase activity (μg TPF day^{-1} g^{-1} soil)									
	Days									
Treatments	0	5	10	15	30	60	90	120	Mean	LSD (5%)
T_1	0.70	1.33	1.54	1.37	1.06	1.01	0.084	0.063	1.06	0.494
T_2	0.70	1.44	1.80	1.52	1.20	1.12	0.99	0.74	1.19	0.522
T_3	0.70	1.39	1.66	1.44	1.13	1.09	0.97	0.72	1.13	0.534
T_4	0.70	1.30	1.43	1.33	0.98	0.84	0.80	0.54	1.00	0.309
Mean	0.70	1.36	1.60	1.42	1.09	1.02	0.90	0.65		
LSD (5%)		0.043	0.084	0.048	0.042	0.045	0.120	0.085		
T_5	0.50	1.08	1.41	1.04	0.89	0.76	0.064	0.57	0.861	0.485
T_6	0.50	1.19	1.59	1.55	1.02	0.95	0.82	0.67	1.030	0.610
T_7	0.50	1.15	1.57	1.45	0.97	0.89	0.80	0.63	0.995	0.515
T_8	0.50	1.06	1.20	1.10	0.80	0.69	0.67	0.50	0.815	0.429
Mean	0.50	1.120	1.44	1.28	0.920	0.822	0.732	0.592		
LSD (5%)		0.088	0.157	0.091	0.068	0.122	0.086	0.047		
T_9	0.89	1.42	1.70	1.44	1.15	1.20	1.07	0.84	1.21	0.795
T_{10}	0.89	1.55	1.92	1.65	1.32	1.37	1.35	0.98	1.37	0.698
T_{11}	0.89	1.50	1.85	1.55	1.29	1.27	1.28	0.90	1.31	0.531
T_{12}	0.89	1.33	1.50	1.37	1.06	1.04	1.03	0.81	1.13	0.424
Mean	0.89	1.45	1.74	1.50	1.20	1.22	1.18	0.88		
LSD (5%)		0.060	0.118	0.108	0.042	0.055	0.069	0.053		

Note: T_1, natural soil (control); T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1}; T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1}; T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1}; T_5, control + fertilizers; T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1}; T_7, fertilized soil + 1.0 kg a. i. carbofuran ha^{-1}; T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1}; T_9, control + vermicompost; T_{10}, vermicompost amended soil + 0.2 kg a.i. carbofuran ha^{-1}; T_{11}, vermicompost amended soil + 1.0 kg a.i. carbofuran ha^{-1}; T_{12}, vermicompost amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

Table 4. Effect of different doses of carbofuran on alkaline phosphatase activity (μg p-nitrophenol (PNP) h^{-1} g^{-1} soil) in soil alone and soils amended with fertilizers and vermicompost at different time intervals under field conditions.

	Alkaline phosphatase activity (μg PNP h^{-1} g^{-1} of soil)									
	Days									
Treatments	0	5	10	15	30	60	90	120	Mean	LSD (5%)
T_1	78.60	97.92	120.00	152.30	95.34	68.23	62.14	50.45	90.62	0.877
T_2	78.60	101.10	137.00	159.52	97.50	71.30	65.73	52.97	95.46	0.974
T_3	78.60	99.80	125.00	157.14	96.25	70.27	63.94	51.46	92.80	0.966
T_4	78.60	95.50	120.00	150.00	91.00	65.60	60.90	47.42	88.62	1.310
Mean	78.60	98.58	125.50	154.74	95.02	68.85	63.17	50.57		
LSD (5%)		1.440	1.720	0.916	0.776	0.714	1.140	0.848		
T_5	69.96	76.80	103.00	135.71	85.51	61.00	45.41	39.85	77.15	1.360
T_6	69.96	88.32	121.00	157.41	92.09	68.23	53.51	45.40	86.99	1.120
T_7	69.96	85.32	113.75	150.00	90.00	67.21	50.79	42.80	83.72	1.210
T_8	69.96	80.64	105.00	144.00	80.00	64.67	50.19	39.85	79.28	1.290
Mean	69.96	83.52	110.68	146.78	89.40	65.27	49.89	41.97		
LSD (5%)		1.010	1.600	0.980	1.720	1.160	0.873	1.312		
T_9	81.68	124.60	127.50	164.20	97.50	83.50	72.30	52.97	100.53	0.860
T_{10}	81.68	130.50	146.25	178.57	106.17	95.73	78.88	56.50	109.28	1.000
T_{11}	81.68	128.00	137.50	169.00	105.09	91.65	75.29	54.44	105.33	1.170
T_{12}	81.68	113.92	120.00	160.00	94.25	75.36	65.73	50.45	95.17	1.030
Mean	81.68	123.50	132.81	167.94	100.75	86.56	73.05	53.59		
LSD (5%)		1.230	1.090	1.030	1.080	1.075	1.140	0.901		

Note: T_1, natural soil (control); T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1}; T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1}; T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1}; T_5, control + fertilizers; T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1}; T_7, fertilized soil + 1.0 kg a. i. carbofuran ha^{-1}; T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1}; T_9, control + vermicompost; T_{10}, vermicompost amended soil + 0.2 kg a.i. carbofuran ha^{-1}; T_{11}, vermicompost amended soil + 1.0 kg a.i. carbofuran ha^{-1}; T_{12}, vermicompost amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

Table 5. Effect of different doses of carbofuran on acid phosphatase activity (μg PNP h^{-1} g^{-1} soil) in soil alone and soil amended with fertilizers and vermicompost at different time intervals under field conditions.

	Acid phosphatase activity (μg PNP h^{-1} g^{-1} of soil)									
	Days									
Treatments	0	5	10	15	30	60	90	120	Mean	LSD (5%)
T_1	42.53	62.70	82.00	111.40	61.78	54.70	37.64	32.29	60.63	1.00
T_2	42.53	65.00	84.30	119.17	63.97	57.73	42.81	35.31	63.85	1.44
T_3	42.53	63.70	83.50	114.80	63.97	56.54	40.63	33.59	62.40	1.17
T_4	42.53	58.24	80.60	106.17	57.41	48.21	31.67	30.27	56.88	1.27
Mean	42.52	62.41	82.60	112.88	61.78	54.29	38.18	32.86		
LSD (5%)		0.801	1.220	1.39	1.90	1.09	1.28	0.545		
T_5	39.09	49.92	80.62	95.34	53.52	53.57	35.80	27.74	54.45	1.08
T_6	39.09	54.40	82.50	114.80	59.60	59.52	39.63	30.77	60.03	1.02
T_7	39.09	53.76	81.60	102.90	59.60	55.16	37.85	30.77	57.59	0.912
T_8	39.09	48.00	78.75	86.67	51.40	50.50	25.00	26.74	50.76	0.877
Mean	39.09	51.52	80.86	99.92	56.03	54.68	34.57	29.00		
LSD (5%)		1.260	0.798	1.030	1.010	0.920	1.330	1.440		
T_9	53.72	64.00	93.75	126.76	78.74	61.30	43.66	34.81	69.59	1.148
T_{10}	53.72	67.20	101.25	139.76	76.86	69.60	51.98	40.80	75.14	1.006
T_{11}	53.72	65.92	95.00	130.00	83.11	65.47	47.20	37.48	72.24	0.943
T_{12}	53.72	60.16	91.25	113.75	73.81	58.33	40.63	31.78	65.42	1.230
Mean	53.72	64.32	95.31	127.56	78.13	63.67	45.86	36.21		
LSD (5%)		1.050	0.627	1.350	1.640	1.320	1.120	1.230		

Note: T_1, natural soil (control); T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1}; T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1}; T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1}; T_5, control + fertilizers; T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1}; T_7, fertilized soil + 1.0 kg a. i. carbofuran ha^{-1}; T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1}; T_9, control + vermicompost; T_{10}, vermicompost amended soil + 0.2 kg a.i. carbofuran ha^{-1}; T_{11}, vermicompost amended soil + 1.0 kg a.i. carbofuran ha^{-1}; T_{12}, vermicompost amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

Table 6. Relative increment (C/C_0) of dehydrogenase activity at t_{max} (10 days) at varying doses of Carbofuran with respect to control.

Treatment	Dehydrogenase activity		
	t_o	t_{max} (10 days)	Relative increment C/C_0
T_1	0.70	1.54	2.20
T_2	0.70	1.80	2.57
T_3	0.70	1.66	2.37
T_4	0.70	1.43	2.04
T_5	0.50	1.41	2.82
T_6	0.50	1.59	3.18
T_7	0.50	1.57	3.14
T_8	0.50	1.20	2.40
T_9	0.89	1.70	1.91
T_{10}	0.89	1.92	2.16
T_{11}	0.89	1.85	2.08
T_{12}	0.89	1.50	1.69

Note: T_1, natural soil (control); T_2, natural soil + 0.2 kg a.i. carbofuran ha^{-1}; T_3, natural soil + 1.0 kg a.i. carbofuran ha^{-1}; T_4, natural soil + 5.0 kg a.i. carbofuran ha^{-1}; T_5, control + fertilizers; T_6, fertilized soil + 0.2 kg a.i. cabofuran ha^{-1}; T_7, fertilized soil + 1.0 kg a. i. carbofuran ha^{-1}; T_8, fertilized soil l + 5.0 kg a.i. carbofuran ha^{-1}; T_9, control + vermicompost; T_{10}, vermicompost amended soil + 0.2 kg a.i. carbofuran ha^{-1}; T_{11}, vermicompost amended soil + 1.0 kg a.i. carbofuran ha^{-1}; T_{12}, vermicompost amended soil + 5.0 kg a.i. carbofuran ha^{-1}.

The higher FDAH activity in vermicompost-amended soil in comparison with unamended soil (Table 2) can be ascribed to the fact that the addition of vermicompost causes an increase in the amount of hydrolytic enzyme associated with exogenous microorganisms grown during the vermicomposting process. This is in agreement with the work of Perucci (1992), Garcia-Gil et al. (2000), Perucci et al. (2000), Ros et al. (2003), Crecchio et al. (2004), Bastida et al. (2008), Sanchez-Monedero et al. (2008) and Iovieno et al. (2009) who reported that the organic amendment of soils increases enzymatic activities. The decrease in FDAH activity in fertilizer-amended soil relative to unamended soil (Table 2) is also in agreement with the work of Iovieno et al. (2009) who reported the effect of organic and mineral fertilizers on respiration and enzymatic activities in two Mediterranean horticultural soils.

A significant increase in dehydrogenase activity at the lower (0.20 kg. a.i. ha^{-1}) carbofuran dose might be attributed to the use of carbofuran as a source of electrons and energy by soil enzymes/microorganisms. The results are in accordance with the work of Accinelli et al. (2002) and Gundi et al. (2005) who reported the effects of formulated herbicides and insecticides on microbial population, biomass and dehydrogenase activity in soils. A significant decrease in dehydrogenase activity at the higher (5.0 kg a.i. ha^{-1}) carbofuran dose is due to the inhibitory effect of carbofuran on soil microorganisms. These findings support earlier studies (Dzantor and Felsot 1991; Perucci and Scarponi 1994; Stepniewska et al. 2007) of the effect of glyphosate, imazethapyr and fonofos on the reduction of dehydrogenase activity in soils.

A significant increase in dehydrogenase activity was observed up to 15 days, but the most significant increase was observed at 10 days, thereafter, activity generally declined up to the end of the experiment in all the cases (Table 3). This might be due

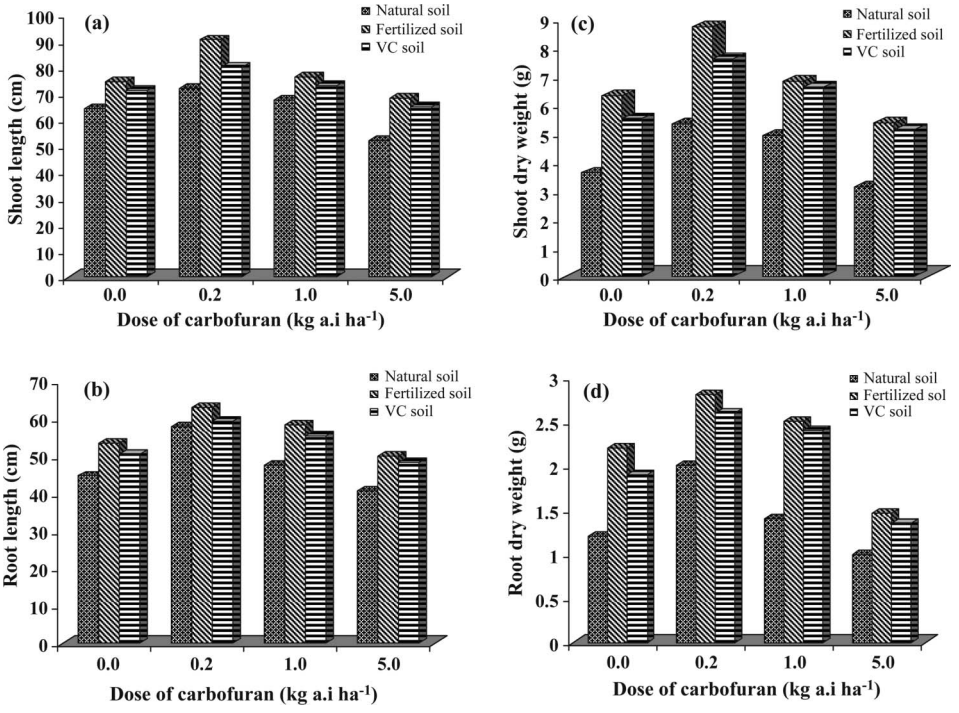

Figure 1. Effect of varying doses of carbofuran on growth parameters: (a) shoot length, (b) root length, (c) shoot dry weight, and (d) root dry weight of tomato plants in natural soil and soil amended with fertilizers and vermicompos.

to the presence of a number of carbofuran degraders in the rhizosphere soil, leading to rapid degradation of carbofuran in the soil (Plangklang and Reunsang 2008).

Maximum dehydrogenase activity was observed in vermicompost-amended soil followed by unamended and fertilizer-amended soil (Table 3). The increase in dehydrogenase activity in vermicompost-amended soil relative to unamended soil is, perhaps, because addition of vermicompost increases soil organic matter content, which can supply available energy. The increase in organic matter content by addition of vermicompost accelerates the growth of microorganisms and enzyme cell multiplication, which improve enzyme composition and activity (Luo and Sun 1994; Li et al. 2000). Dick et al. (1988) also reported that the application of organic manure increases soil enzymatic activity and microbial biomass.

A significant decrease in dehydrogenase activity (Table 3) in fertilized soil compared with unamended (control) soil is due to the fact that dehydrogenase activity is highly sensitive to the inhibitory effects associated with larger amount of fertilizers. Kukreja et al. (1991) reported that dehydrogenase activity was lower in treatments receiving inorganic N, which is attributed to the presence of NO_3^- and NO_2^- that serve as alternate electron acceptors; NO_3^- and NO_2^- are obviously formed on the transformation of urea in soil. Similar results were reported by Marinari et al. (2000) who studied the influence of organic and mineral fertilizers on soil biological and physical properties.

A significant increase in both acid and alkaline phosphatase activities up to 1.0 kg a.i. ha^{-1} carbofuran (Tables 4 and 5) relative to control (T_1, T_5 and T_9) may

be because carbofuran significantly delayed the release of *p*-nitrophenol from p-nitrophenyl phosphate substrate, compared with carbofuran-free soil (control). At the higher (5.0 kg a.i. ha^{-1}) dose of carbofuran both acid and alkaline phosphatase activities were significantly lowered in all cases relative to carbofuran-free soil; this is attributed to the release of orthophosphates from lysed cells, which may act as a competitive inhibitor of phosphatases in soils (Tabatabai 1994). The inhibitory influence of herbicide triflurotox 250 EC on soil phosphatase activity was also shown by Nowak et al. (2000). These results are in partial agreement with the work of Omar and Abdel-Sater (2001) and Wyszkowska and Kucharski (2004) who reported that phoshatase activities increased at a lower rate of pesticide application and decreased at a higher rate.

Increase in both phosphatase activities in vermicompost-amended soil (Tables 4 and 5) in comparison with unamended and fertilizer-amended soils can be, perhaps, explained by the fact that soil microorganisms degrade organic matter through the production of diverse extracellular enzymes after application of vermicompost to soil. Arancon et al. (2006), Ferreras et al. (2006) and Tejada and Gonzalez (2009) have shown an increase in enzymatic activities after addition of different vermicomposts to soil. Similar results were reported by Goyal et al. (1999) who reported that the incorporation of organic amendments to soil influences soil enzymatic activity. The added vermicompost may contain intra- and extracellular enzymes, which stimulate microbial activity in soil. The lowered acid and alkaline phosphatase activities in fertilizer-amended soil are in line with the work of Chang et al. (2007) who reported that both phosphatase activities of compost-treated soil were significantly higher than those in fertilizer-treated soil.

The normalized data suggest that for dehydrogenase activity, the initial increment (C/C_0) in the enzyme activity over time was highest for 0.2 kg a.i. ha^{-1} carbofuran (Table 6). This may be due to the utilization of carbofuran by microorganisms as a carbon source. This can be understood by the fact that some carbofuran-degrading bacteria are methylotropic organisms capable of hydrolysing carbofuran and utilizing methylamine as a sole source of C for growth (Hanson and Hanson 1996). This result can be related to previous findings (Turco and Konopka 1990; Sakata et al. 1992; Das and Mukherjee 2000) reporting the utilization of insecticides and their degradation products for deriving energy and nutrients for microorganism growth and development.

In the natural, fertilizer- and vermicompost-amended soils, the initial increments (C/C_0) were generally the same for all enzymatic activities, except dehydrogenase activity (Tables 6). The initial increments for dehydrogenase activity (Table 6) were highest in fertilizer-amended soils. Dehydrogenase activity in fertilizer-amended soil was lower at the start of experiment because of the inhibitory effects of nitrogenous fertilizers (Laud and Paul 1973). As time progressed, nitrogenous fertilizers either degraded or were taken up by the plants; hence there was an abrupt increase in dehydrogenase activity, which led to highest C/C_0 values.

Growth of plants

The increase in plant growth up to 1.0 kg a.i. ha^{-1} carbofuran in all systems may be due to the fact that carbofuran increases ammonification and decomposition of organic matter in soils (Das and Mukherjee 1998). The maximum increase in plant growth at 0.2 kg a.i. ha^{-1} carbofuran may be because carbofuran increases the

enzymatic activities to a maximum at this dose, which adds to the fertility of soil (Tables 2–5). The higher growth of plants in all carbofuran treatments in fertilized soil relative to unamended and vermicompost-amended soil may be because NPK fertilizers supply higher amounts of nutrients (not reported). The decrease in plant growth parameters in vermicompost-amended soil relative to fertilized soil is attributed to slow release of NPK (Makinde and Ayoola 2010).

Simple correlation analysis was undertaken to compare the activities of FDAH, dehydrogenase, acid and alkaline phosphatase activities with plant growth parameters. The analysis gave rise to correlation coefficients ranging from 0.150 to 0.296. These values showed positive correlations between enzymatic activity and plant growth parameters, but were not sufficiently high to establish straightforward relationships between plant growth parameters and enzymatic activities.

Conclusions

Studies on the effect of carbofuran on enzymatic activities suggest that the use of carbofuran at 0.2 and 1 kg a.i. ha^{-1}, alone or in co-application with inorganic fertilizers and vermicompost is recommended for long-term soil fertility and crop productivity. This study also reveals that pesticides along with other agricultural inputs, in addition to their role in increasing agricultural outcome, may alter soil fertility. Thus, this study emphasizes the fact that the effect of pesticides on soil microbial activities should be evaluated before they are recommended for agricultural use.

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