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Pesticides have an effect on microbial activities (Dehydrogenase, Phosphatase, and Protease) in paddy (Black and alluvial) soils

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Research Article

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ABSTRACT

Chemical pesticides are often employed in agricultural areas in contemporary agriculture to boost crop yield. Insecticides influence the activity and abundance of beneficial soil microbial communities in addition to controlling insect pests. This has severe environmental ramifications. Pesticides have varying effects on soil microbial activity. The effects of carbosulfan and chlorpyrifos (insecticides) and kresoxim-methyl and mancozeb (fungicides) on the enzymatic activities of soil microorganisms in paddy farmed (black and alluvial soils) at varied doses of 1.0, 2.5, 5.0, 7.5, and 10.0 kg/ha were investigated in the laboratory. Pesticides applied at a field rate boosted dehydrogenase activity. The activity of dehydrogenase was reduced by high doses. At all doses tested, the dehydrogenase enzyme rate showed the most inhibition after 24 hours. There was a reduction in phosphate activity at all doses when compared to the control. At 5.0 kg/ha and 2.5 kg/ha, insecticides and fungicides increased protease activity in both soils. Maximum inhibition of protease enzyme rate was reported after 10 days at all doses tested.

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INTRODUCTION

Excessive use of different pesticides (fungicides, herbicides, and insecticides) to efficiently manage a range of pests is becoming a serious concern in contemporary agriculture. The accumulation of pesticide residues in the soil, which culminates in the immersion of these xenobiotic substances, is one of the detrimental outcomes of using pesticides. Another disadvantage of pesticide usage is that it may kill or inhibit not just the target species in agricultural crops, but also non-target species that contribute to soil fertility [1]. However, studies have shown that pesticides have the ability to activate soil enzymes. Singh and Kumar [2] found that acetamipirid increased dehydrogenase activity, whereas Bamaga et al. discovered that carfentrazone-ethyl stimulated acid phosphatase and alkaline phosphatase activity [3]. Because some bacteria species and strains may grow resistant to toxins by establishing defensive mechanisms, microbiological breakdown is the most effective approach for converting dangerous chemical compounds [4]. Soil fertility and biological balance, as well as changes in biological status caused by pollution, are all linked to the amount of enzymes in the soil [5,6]. Because they react fast to environmental stress or changes in management practises, soil enzyme activity are sensitive indicators of soil quality (Sravanthi et al. 2015). The purpose of this study was to explore whether soil enzymes might be employed as soil pollution monitors. In the proddatur and duvvur mandalam kadapa area, four insecticides are regularly utilised. The effects of carbosulfan, chlorpyrifos, kresoxim methyl, and mancozeb on soil enzymatic activity were studied.

Materials and methods

Soils used in the present study

Soil samples were taken from cultivated fields of paddy black and alluvial soil in the Proddatur and duvvur manadalam Kadapa districts, and were taken from a depth of 12 cm, air-dried at room temperature, sieved with a 2 mm sieve before use, and completely mixed to produce a homogeneous composite sample. Soil samples were collected from paddy black and alluvial soil cultivated fields of Proddatur, and duvvur manadalam Kadapa district, were chosen from a depth of 12 cm, air-dried at room temperature and sieved with 2 mm sieve before usage and subjected to mix thoroughly to prepare a homogenous composite sample.

Table 1. paddy soil physico-chemical characteristics considered in this investigation.

Properties	Black soil	Alluvial soil
Sand (%)	50	57.4
Silt (%)	22	25.7
Clay (%)	28	16.9
pH a	8.26	7.8
Water holding capacity (ml g^{-1} soil)	48.8	56
Electrical Conductivity (m. mhos)	0.19	0.31
Organic matter (%) b	0.86	0.126
Total nitrogen (%) c	0.54	0.80
NH_4^+–N(μg^{-1} soil)d	4.42	6.24
NO_2^-–N(μg^{-1} soil)e	5.32	8.23
NO_3^-–N(μg^{-1} soil)f	0.48	0.98

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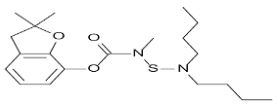
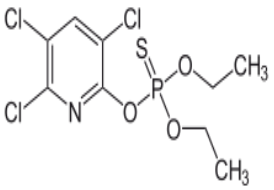
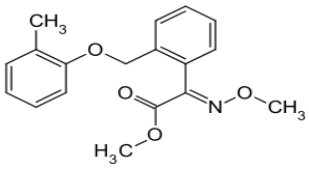
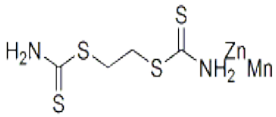
Where a = 1 : 1.25 (Soil : Water); b = Walkley - Black method (Jackson, 1971); c = Micro - Kjeldahl method (Jackson, 1971); d = Nesslerization method (Jackson, 1971); e = Diazotization method (Barnes & Folkard, 1951); f = Brucine method (Ranney and Bart ler, 1972).

Insecticides used in the present study

Bayer scientific India market grades of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb were utilised to investigate the impact of various insecticides and fungicides on paddy soil microbial activity (Table 2).

Table 2: Specifications of the insecticides and fungicides that were utilised in this research.

pesticides	Trade name	Commercial formulation	Chemical Class	Technical grade (%purity)	structure
Carbosulfan	Marshal	25% EC*	Carbomate	99.8	
Chlorpyrifos	Noban	50% EC*	Orgnophosphate	97.54	
sKresoxim Methyl	Ergon	44.3% SC*	Strobilarin	94	
Mancozeb	Sparsh	75% WP*	Dithiocrbomate	95	

Soil enzyme activities

To see how Carbosulfan, Cholropyrifos, Kresoxim-methyl, and Mancozeb (10, 25, 50, 75, and 100 g g-1 soil) affected the activities of soil enzymes, appropriate amounts of soil samples were deposited in test tubes or Erlenmeyer flasks and treated with various insecticide concentrations corresponding to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha-1. As controls, soil samples that had not been treated with insecticides were used. All soil samples in test tubes or flasks were incubated at room temperature (28 4 °C) with a 60 percent water holding capacity (WHC) during the incubation period. The soil samples were taken for enzyme activity testing at the specified intervals (10, 20, 30, and 40 days or 7, 14, 21, 28, and 35 days).

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Assay of dehydrogenase

Casida et al., Rangaswamy et al., and Srinivasulu and Rangaswamy, were used to test the dehydrogenase enzyme (2013) [7]. Five grammes of soil were treated with 0.1 g CaCO3 and 1 ml of 0.18 M aqueous triphenyl tetrazolium chloride, then incubated at 37oC for 24 hours. The reaction mixture was then treated with methanol to extract triphenyl formazan, which was measured at 485 nm in a Thermo Scientific Evolution 201 UV Visible Spectrophotometer. After 7, 14, 21, 28, and 35 days of incubation, dehydrogenase activity was evaluated.

Assay of phosphatase

Soil samples were transported to 100 ml Erlenmeyer flasks, where they were mixed with 0.2 ml toluene, 6 ml 0.1 M maleate buffer (pH 6.5), and 2 ml p-nitrophenyl phosphate disodium salt. The flasks were spun for a few seconds to mix the contents before being stoppedpard and incubated for 30 minutes at 37oC. The reaction was halted by rotating the flask for a few seconds after adding 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH, and the soil suspension was filtered using a Whatman No. 1 filter paper. A UV Visible Spectrophotometer (Thermo Scientific) Evolution 201 was used to measure the released p-nitrophenol in the filtrate at 410 nm.

Assay of protease:

Two grammes of soil samples were incubated for two hours at 30°C in 10 ml of 0.1 M Tris (2-amino-2-(hydroxymethyl)-propane-1, 3-diol at pH 7.5) with sodium caseinate (2 percent w/v). The contents were centrifuged after adding 4 ml of aqueous trichloro acetic acid (17.5 percent w/v) to the mixture. A suitable portion of the supernatant was treated with 1.4 M Na2CO3, and then swirled with 1 ml Folin-Ciocalteu reagent (33.33 percent v/v). After 30 minutes, a blue colour developed, which was measured at 700 nm in a Thermo Scientific Evolution 201 UV Visible Spectrophotometer.

3.5 Statistical analysis

On the basis of soil weight, the enzyme activities of the soil were determined (oven dried). One-way ANOVA was used to evaluate the data, and Duncan's multiple range test (DMRT) was used to contrast the differences (Megharaj et al., 1999 and Jaffer Mohiddin et al., 2013). Using the SPSS statistical software programmed, all statistical analyses were done at ($p \leq 05$).

Results and discussion

Dehydrogenase activity:

Dehydrogenase participates in a number of oxidative processes that aid in the decomposition of organic molecules. During the biological oxidation of organic molecules in the soil, soil dehydrogenase transfers protons and electrons from substrates to acceptors [8]. Insecticide-treated soil samples incubated for 7 days, ranging from 1 to 5 kg ha-1, had significantly higher soil dehydrogenase activity as measured by triphenyl formazan accumulated from triphenyl tetrazolium chloride (TTC). In the case of dehydrogenase, pesticide doses induced a significant increase in enzyme activity in soil samples ranging from 1 to 7.5 kg ha-1 during a 7-day incubation period. Dehydrogenase activity ranged from 6-27 percent, 12-21 percent, 12-31 percent, 15-49 percent in black soil and 8-56 percent, 14-37 percent, 6-25 percent, and 4-32 percent in alluvial soil due to stimulation of dehydrogenase by carbosulfan chlorpyrifos, mancozeb, and kresoxim methyl (Table 3). Dehydrogenase activity was significantly greater in soil samples treated with 2.5 kg ha-1 of both pesticides until 21 days of incubation (Fig. 4). After 35 days of incubation with insecticide-treated soil samples, the enzyme activity was not boosted. Pesticides typically used in rice production, according to the data, increased dehydrogenase activity in black and alluvial soils when administered at field application rates. Dehydrogenase activity was discovered at the maximal dose (41 mg kg-1) and decreased with lower pentachlorophenol additions, according to [9].

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Table 3. After 24 hours, activity of dehydrogenase in black and alluvial soil under the impact of varied concentrations of insecticides and fungicides.

Pesticide Concentration (kg ha^{-1})	Carbosulfan	Chlorpyrifos,	Kresoxim- methyl	Mancozeb
		Black soil		
0.0	114e (100)	114c (100)	114f (100)	114f (100)
1.0	121d (106)	128b (112)	128e (112)	132c (115)
2.5	142a (127)	139a (121)	140b (122)	150b (131)
5.0	131b (114)	110d (96)	150a (131)	170a (149)
7.5	121c (106)	99e (86)	142c (124)	158d (138)
10.0	110e (96)	86f (75)	121d (106)	131e (114)
		Alluvial soil		
0.0	124c (100)	124c (100)	124f (100)	124f (100)
1.0	135b (108)	142b (114)	132c (106)	130c (104)
2.5	194a (156)	171a (137)	149b (120)	142d (114)
5.0	84d (152)	91d (73)	155a (125)	164a (132)
7.5	73e (58)	66e (78)	140d (112)	151b (121)
10.0	64f (51)	78f (62)	129e (104)	138e (290)

*Number of colonies per gram soil = $\frac{\text{No. of colonies x Dilution factor}}{\text{A dry weight of soil}}$

The figures, in brackets, represent proportional percentages of production. The means obtained for each sample in each row preceded by the same letter are not obtained for each sample.

According to the DMR measure, they are substantially different ($P \leq 0.05$) from each other. In the table, the values are the averages of the triplicates.

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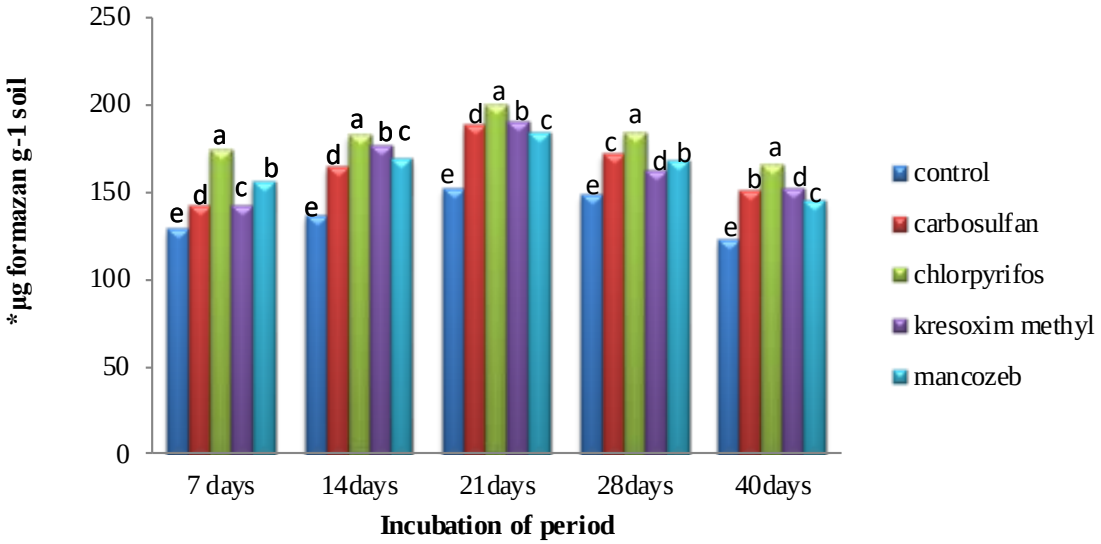
a)



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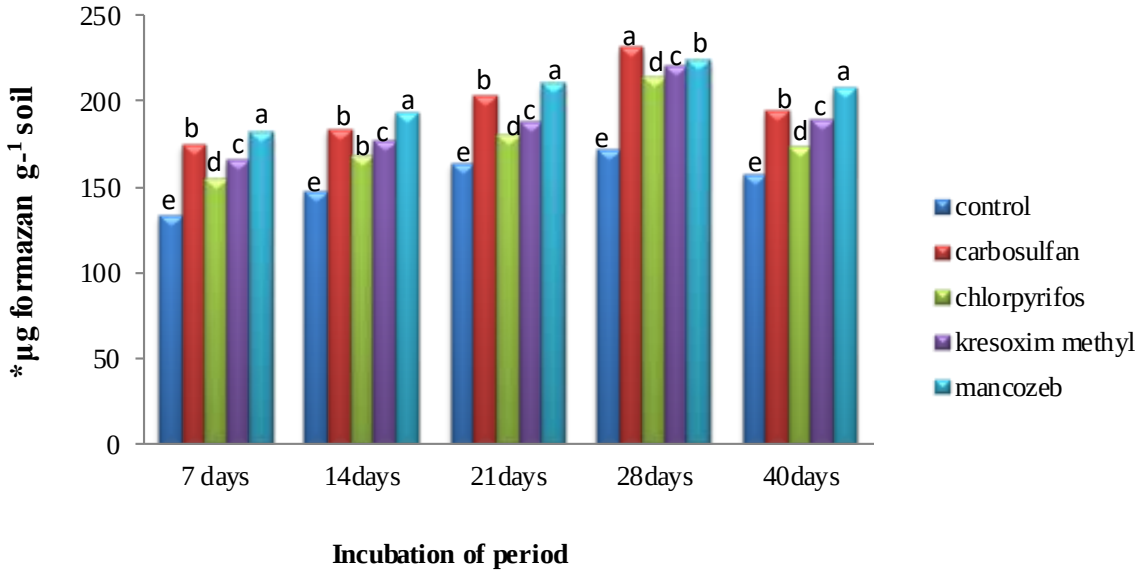

Figure a and b. Influence of insecticides in black and alluvial soil at 2.5 kg ha-1 and fungicides in black alluvial soil 5.0 kg ha-1 on dehydrogenase*activity in paddy soil. Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test. *Values in the table are means of triplicates

Activity of phosphatase:

Phosphatase produces the accumulation of organic phosphorous in soils, which is one of the essential components for plant growth. In the laboratory, phosphophatase activity was measured in cotton black soil under the influence of pesticides at concentrations of 1.0, 2.5, 5.0, 7.5, and 10.0 Kgha-1. The findings of this research are summarised in Table 4. Phosphatase activity in paddy soil samples was gradually increased by insecticides and fungicides (pesticides) at dosages ranging from 1.0 to 2.5 kg ha-1, peaking at 2.5 kg ha-1 for black soil and 5.0 kg ha-1 for alluvial soil. In paddy soil for 10-days incubation, around 11-33 percent, 14-44 percent, 12-41 percent, and 13-44

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percent, and alluvial soil is 12-17 percent, 17-31 percent, 27-50 percent, and 52-73 percent, respectively, and significantly inhibited at higher concentrations of 10.0 Kg ha-1 in both pesticides treatments (Table 4). Phosphatase activity was significantly greater in soil samples incubated for 30 days in black soil and 20 days in alluvial soil with 2.5 and 5.0 kg ha-1 of the four pesticides (Fig c). On the other hand, incubation of pesticide-treated samples for up to 40 days showed no influence on enzyme activity. The present study's results clearly reveal that pesticides routinely used in rice cultivation enhance phosphatase activity in soil when administered at field application rates.

The activity of phosphatase was enhanced in the presence of butachlor [10]. Monocrotophos, chlorpyripfos alone and in combination, monocrotophos + mancozeb, chlorpyripfos + carbendazim, increased phosphatase activity up to 5.0 kg ha-1, according to [7]. Acid phosphatase activity has risen by 1.8 times by the 14th day of incubation with 1 ppm endosulfan by [11]. The activity of phosphatase was enhanced in the presence of butachlor [10].

Table 4. After 10 days, the activity of phosphatase in black and alluvial soil was studied under the impact of varied pesticide concentrations.

Pesticide Concentration (kg ha^{-1})	Carbosulfan	Chlorpyrifos,	Kresoxim- methyl	Mancozeb
		Black soil		
0.0	134f (100)	134f (100)	134f (100)	134f (100)
1.0	149d (111)	154d (114)	164d (112)	152d (113)
2.5	168b (125)	174b (129)	178c (132)	179b (133)
5.0	179a (133)	193a (144)	190a (141)	194a (144)
7.5	154c (114)	169c (126)	182b (135)	164c (112)
10.0	139e (103)	140e (104)	150e (111)	144e (107)
		Alluvial soil		
0.0	156f (100)	156f (100)	156f (100)	156f (100)
1.0	176b (112)	183c (117)	199c (127)	240b (153)
2.5	184a (117)	205a (131)	234a (150)	270a (173)
5.0	165c (105)	190b (121)	214b (137)	230c (147)
7.5	160d (102)	170d (108)	188d (120)	195d (125)
10.0	158e (101)	162e (103)	166e (106)	180e (115)

*Number of colonies per gram soil = $\frac{\text{No. of colonies x Dilution factor}}{\text{A dry weight of soil}}$

The figures, in brackets, represent proportional percentages of production. The means obtained for each sample in each row preceded by the same letter are not obtained for each sample.

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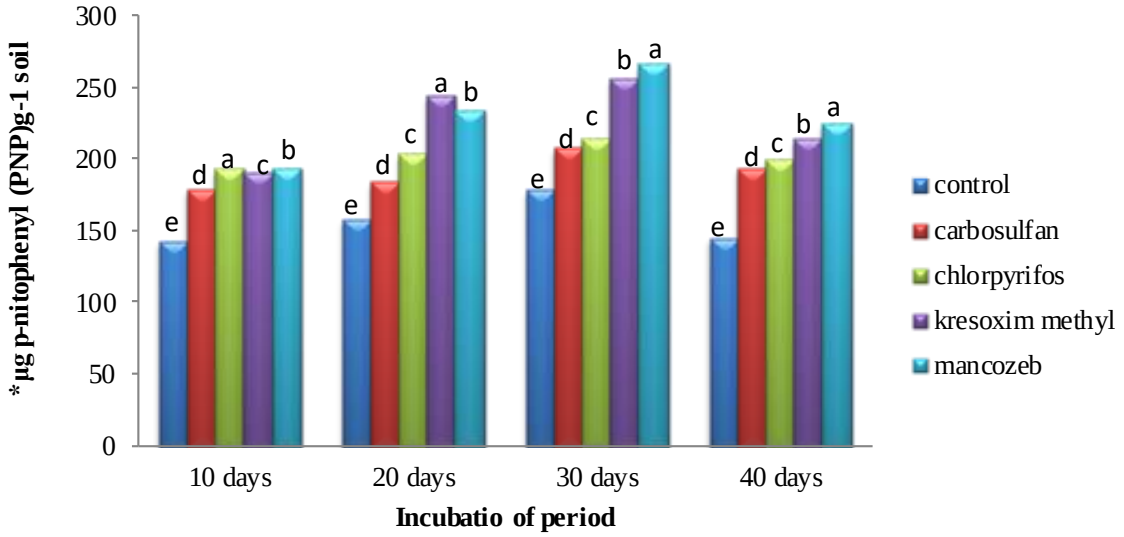
c)



d)

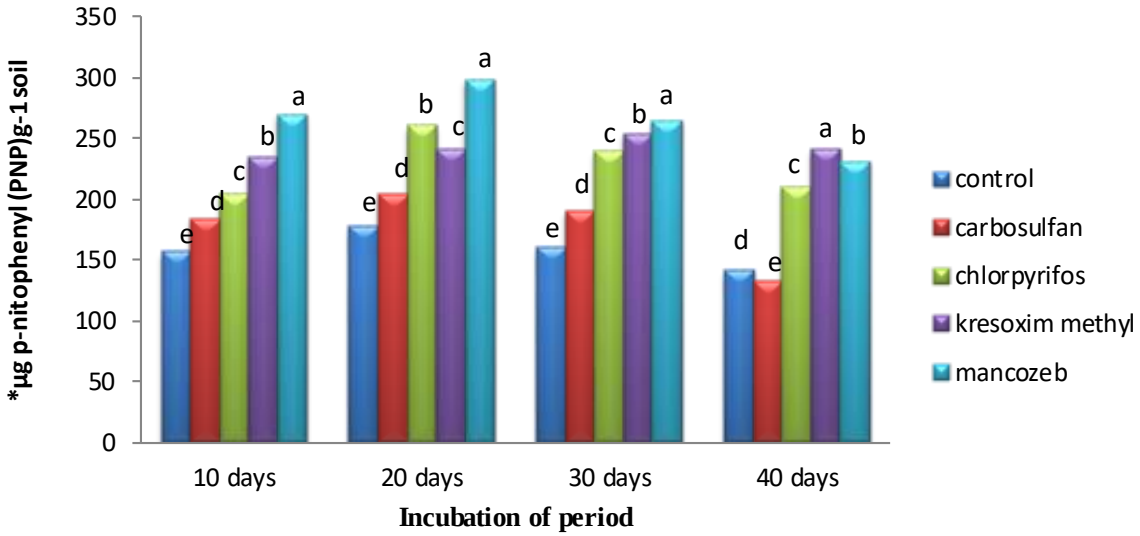


Figure c and d. Influence of pesticides in black soil at 5.0 kg ha-1 and 2.5 kg ha-1 in alluvial soil on phosphatase activity in paddy soil.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test.

*Values in the table are means of triplicates

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Activity of protease:

Protease plays an important part in the nitrogen cycle by hydrolyzing proteninaceous components of organic nitrogen into simpler inorganic amino acids in soils. The findings of this research are summarised in Table 5. Surprisingly, following a 10-day incubation period, stimulatory effects were seen in black soils at 10-50 ppm concentrations. The percentages of increase in protease activity of the two pesticide treatments over control at a 10-day interval (pesticides treated at 10, 25, and 50 g g-1) in paddy soils are as follows: 26-73 percent, 29-58 percent, 48-76 percent, and 62-81 percent in black soil and 8-28 percent, 35-40 percent, 46-66 percent, and 47-65 percent in alluvial soil (Table 5).

The activity of protease was significantly greater in soil samples that received 5.0 Kg ha-1 of the two herbicides until 30 days of soil incubation (Fig e and f). In contrast, incubating insecticide-treated samples for up to 40 days resulted in an increase in enzyme activity. Sabale and Misal [12]discovered that lower levels of endosulfan (0.05 and 0.1 w/v) increased protease activity, but lower amounts of methyl parathion drastically increased enzyme activity (2000). After 21 days of incubation, melathoin protease activity is discovered [13]. Insecticides like kelthane and fenvelerate, according to Omar and Abd-Alla, impede enzyme activity (2000) [14]. Ditera a nematicde, given at 2.5 and 5.0 kg ha-1 for one day, boosted enzyme activity, according to [15].

Table 5. After 10 days, activity of protease in black and alluvial soil under the impact of varied pesticide concentrations.

Pesticide Concentration (kg ha^{-1})	Carbosulfan	Chloropyrifos,	Kresoxim- methyl	Mancozeb
		Black soil		
0.0	405f (100)	405f (100)	405f (100)	405f (100)
1.0	512d (126)	524d (129)	602d (148)	658c (162)
2.5	634b (156)	588c (145)	634c (156)	698b (172)
5.0	704a (173)	641a (158)	715a (176)	734a (181)
7.5	564c (139)	590b (145)	684b (168)	654d (161)
10.0	472e (116)	510e (125)	585e (144)	512e (126)
		Alluvial soil		
0.0	380f (100)	380f (100)	380f (100)	380f (100)
1.0	412c (108)	514b (135)	555d (146)	562d (147)
2.5	490a (128)	534a (140)	602b (158)	594c (156)
5.0	434b (114)	494c (130)	634a (166)	628a (165)
7.5	406d (106)	430d (113)	580c (152)	602b (158)
10.0	390e (102)	404e (106)	470e (123)	540e (142)

*Number of colonies per gram soil = $\frac{\text{No. of colonies x Dilution factor}}{\text{A dry weight of soil}}$

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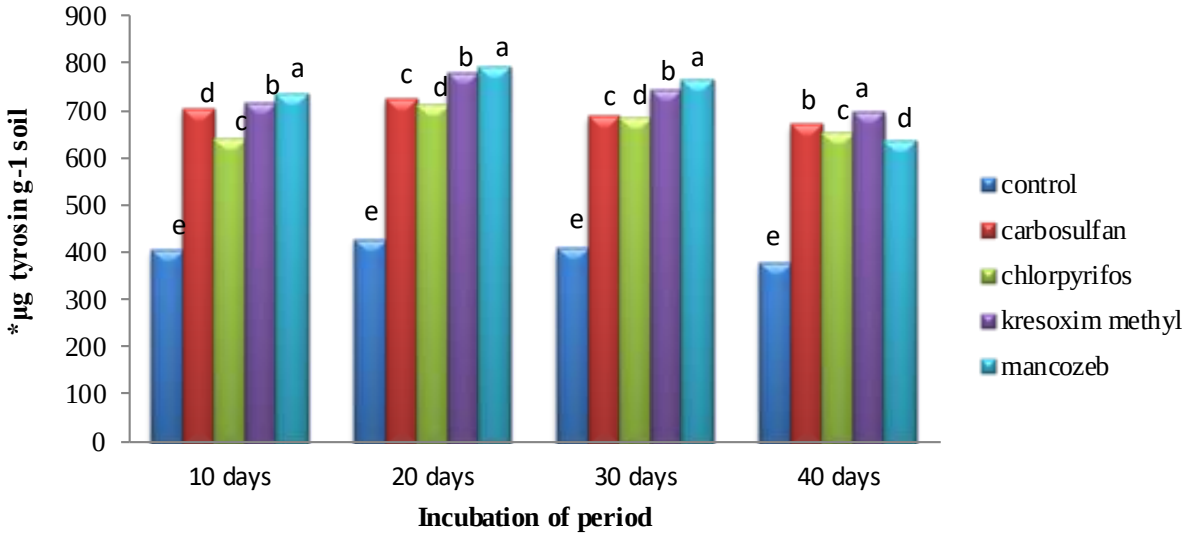

Figure e. Influence of pesticides in black soil at 5.0 kg ha-1 on protease activity in paddy soil.

f)

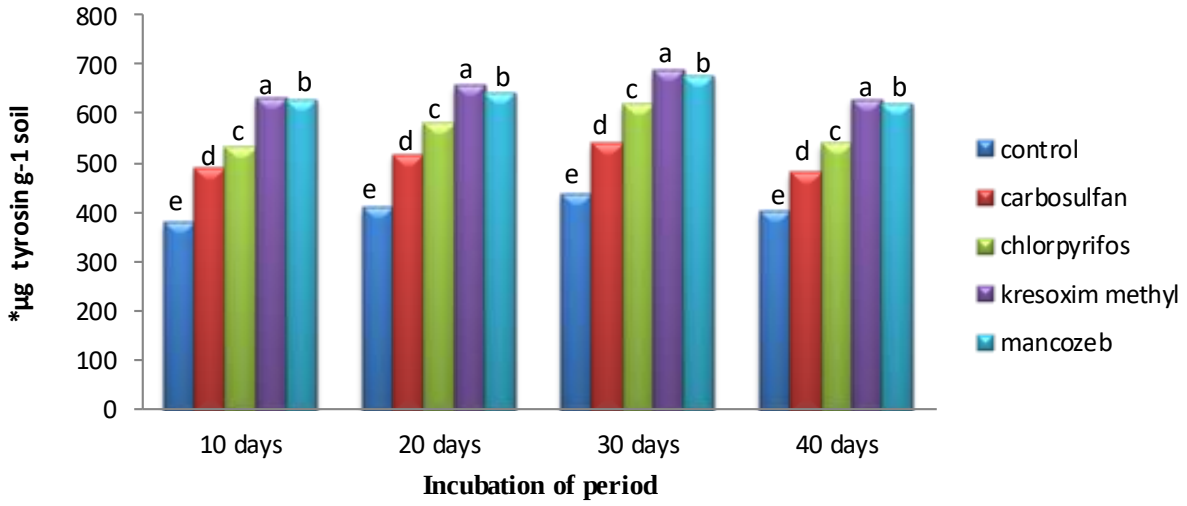

Figure f. Influence of insecticides in alluvial soil at 2.5kg ha-1 and fungicides at 5.0 kg ha-1 on protease activity in paddy soil.

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Means, in each row, obtained for each sampling, followed by the same letter are not significantly different (P≤0.05) from each other according to DMR test.
*Values in the table are means of triplicates.

Conclusion:

Both pesticides (insecticides and fungicides) produced a significant increase in dehydrogenase activity in paddy black soil and alluvial soil at 2.5 and 5.0 kg ha-1, similar to previous enzymes. There is a gradual increase in dehydrogenase activity measured in terms of triphenyl formazan (TPF) accumulation from tryphenyl tetrazolium chloride (TTC) accumulation in pesticide-treated soil samples up to 21 days of incubation. To determine the activity of soil phosphatase, the amount of p-nito phenol released by p-nitro phenyl phosphatase (PNPP) was supplied to soil samples that had been treated with the indicated insecticides. P-nitro phenol emissions are highest in soil samples treated with pesticides weighing 2. 5 and 5.0 kg ha-1, as well as all four pesticides. Throughout the study, the rate of phosphatase activity, like the rates of other soil enzymes, exhibited a consistent pattern of stimulation in both treated and untreated soil samples. In rice soils, the four pesticides boosted protease activity by up to 7.5 kg ha-1. The protease activity of one percent casein modified soil samples was measured in terms of tyrosine produced after 24 hours of incubation at 300° C. Pesticides had the greatest effect on protease activity in black soil (5.0 kg ha-1) and alluvial soil (5.0 kg ha-1) (2.5 kg ha-1). Furthermore, the activity of protease increased significantly until 30 days after incubation when soil samples were treated with stimulatory pesticide dosages.

However, depending on the dosage of interacting pesticides, distinct interactions between pesticides on microbial activities were detected in the present study. These interaction patterns might have a resonant impact on soil enzymes, affecting soil fertility.

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