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Effect of insecticides and fungicides on arylamidase and urease activity in paddy soils

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Abstract The effect of selected insecticides; carbosulfan, chlorpyrifos and fungicides; kresoxim-methyl, and mancozeb on two enzyme activities in paddy (black and alluvial) soils were recorded. The two soils were amended with lower to higher dosage ("1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}") of pesticides and incubated in the laboratory at 37^{o}C for 40 days. Arylamidase and ureaseactivity was measured during the incubation at 10, 20, 30, and 40 days intervals. Carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb stimulated the enzyme activity at lower concentrations at a 10-day interval. Striking improvement in the soil enzyme activities was noted particularly at 2.5 to 5.0 kg $ha^{-1}$of pesticides and persisting for 30 days in both soils. Overall, higher concentrations (7.5 - 10.0 kg ha^{-1}) of carbosulfan, chlorpyrifos, kresoxim-methyl andmancozeb were toxic or innocuous to the enzyme activities.

Keywords: Carbosulfan, Chlorpyrifos, Kresoxim-methyl, Mancozeb, Soil enzymes

Introduction

Soil enzymes have a major role in the mineralization of nutrients and the decomposition of organic materials, which are essential drivers of plant nutrients production. Soil enzyme activities are "sensors" which provide awareness about the microbial status and physicochemical conditions of the soilas well as soil organic matter decomposition (SOM) (Baum *et al*., 2003; Sinsabaugh *et al*., 2008). They have also been used in soil treatment impact studies (Chen *et al*., 2003) and may also apply to the availability of nutrients (Asmar *et al*., 1994; János *et al*., 2011). Rhizosphere enzyme production is typically greater than bulk soil because of greater activities in plant microglyphs or due to the release of root enzymes (George *et al*., 2005; Vilányi *et al*., 2006). Enzymes that compose Zn, such as CO_2 fixation, biological membrane maintenance, protein synthesis, auxin synthesis, and pollen grain formation, regulate many plant processes. The efficiency of added $ZnSO_4$ is only 1 to 4 percent, and most of the zinc added is made

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inaccessible to plants due to several variables such as leaching, fixation (Nair *et al*., 2010). Soil enzymes are of predominant microbial origin and are derived from cell-associated, intracellular, or free enzymes (Burns, 1982). They excel in catalysing numerous fundamental reactions required for soil microorganisms and soil structure stabilisation processes, decomposition, recycling, and nutrient cycling, thereby playing a major role in the protection of the soil biodiversity, physical and chemical properties, fertility, and soil quality (Das and Varma, 2011). The activities of soil enzymes are widely considered to be one of the positive bioindicators of the quality of soil fertility, respond more rapidly than physical variables and/or after some chemical ground shift, and may quantify adjustments arising from natural and anthropogenic disruptions in soil climate (Xian *et al*., 2015). The information available on the interactions between pesticides and soil arylamidase, urease is scarced. Hence, the present study was undertaken to determine the effect of pesticides on soil arylamidase and urease activities in paddy soils.

Materials and methods

Soils used in the present study

Black and alluvial soilswere collected from paddy fieldsof ProddaturandDuvvur (Kadapa district) respectively, from 12 cm depth, air-dried at room temperature, and sieved prior to use with a 2 mm sieve and thoroughly mixed to prepare a homogeneous composite sample.

Table 1. Physico-chemical properties of paddy soil used in the present study

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

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 and Folkard, 1951);f = Brucine method (Ranney and Bartler, 1972).

Insecticides and fungicides used in the present study

To assess the effect of selected insecticides on the enzyme activities of the paddy soil, industrial grades of insecticides; carbosulfan, chlorpyrifos, and fungicides; kresoxim-methyl, and mancozeb were used.

Soil enzyme activities

Theeffectof chosen pesticides, carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb ("10, 25, 50, 75 and 100 μg g^{-1} soil")was examinedon the soil enzyme activities that the required quantityof soil samples weretransferred into the tubes or Erlenmeyer flasks.The tested agrochemicals concentrations were equal to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. The control soil samples were maintained without tested agrochemicals.The soil samples in tubes or flasks with and without tested agrochemicals application were incubated at room temperature (28 ± 4 °C) and water retention capacity was maintained throughout the incubation period.For the assay of enzyme activities, soil samples were taken during the appropriate intervals ("10, 20, 30 and 40 days or 7, 14, 21, 28, and 35 days").

Assay of arylamidase

One gram portion of soil samples in 50 ml Erlenmeyer flasks was taken after 10 days of incubation 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 containers were swirled down for a few seconds, then stopped and placed in an incubator (37 ℃) for 1 hour in a shaker. After the incubation 6 ml ethanol (95%) was applied to each flask. The soil was pooled directly in a centrifugal tube and centrifugated at 17000 × g for 1 minute. The supernatant was moved to a test tube to avoid substratum hydrolyses and the aliquot was added with 1 ml of ethanol, 2 ml of acidified ethanol, and 2 ml of p-dimethylaminocinnamaldehyde (in the second collection of test tubes). After each reagent was applied, the solution was mixed with a vortex blender. The intensity of the resulting red azo compound was measured in a Spectronic-20D spectrophotometer at 540 nm (Hiwada *et al*., 1977 and Acosta-Martinez and Tabatabai, 2000; Srinivasulu and Rangaswamy, 2013").

Assay of urease

The soil ureaseenzyme is mainly involvedin urea hydrolysis. The urease enzyme activity was determined using the Fawcett and Scott (1960) technique and approved by Malkomes (2001). At the desired intervals, one

ml of 3% urea and 2 ml of 0.1 M phosphate buffer (pH 7.1) were added to one gram of soil samples and incubated for 30 minutes at 37℃ in the water bath shaker. Subsequently, the tubes were placed in the ice until the ammonia was extracted with10 ml of 2 M KCl and filtered withWhatman No. 1 filter paper. Fourml of filtrate, 5 ml of phenol sodium nitroprusside solution and 3 ml of 0.03 M sodium hypochlorite solution were added. The mixture was shaken and kept for 30 minutes in the dark, and the formed blue colour was read at 630 nm in a Spectronic20D spectrophotometer.The rate of action of arylamidase and urease enzymes was assayed at 10, 20, 30, and 40 days after soil incubation at the respective tested agrochemicals concentrations.

Results

Arylamidase activity

Arylamidase production increased in all 10-day incubated soils treated with tested agrochemicals when compared with controls, up to 2.5 or 5.0 kg ha^{-1}. The activity of enzymes persisted up to 30 days, then progressively decreased after 40 days (Figure 1 and 2) of incubation. For 10 days, these four tested agrochemicals significantly improved the activity of arylamidase in incubated soils. Carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb treatments gradually increased the production of arylamidase at concentrations in both black and alluvial soils varied from 1,0-2.5 kg ha^{-1}, with an averageconcentration of 2.5 kg ha^{-1}. A maximum volume of 10.0 kg ha^{-1}showed a negative effect on the arylamidase activity with the usage of carbosulfan, chlorpyrifos, and kresoxim-methyl and mancozeb over 2,5 kg ha^{-1} (Table 2). 17-10, 8-66, 35-47, and 38-70 percent increased in arylamidase output in black soil and 6-13, 25-52, 52-70, and 64-80 percent increased in carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb treated alluvial soil which were observed related to control at the end of 10-day incubation (Table 2).

Urease activity

The activity of enzyme urease was improved with relative to the controls under the influence of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 5.0 kg ha^{-1}which amounted in black soil and alluvial soil. But at higher concentration, there was detrimental to urease activity after 10 days of incubation at greater concentrations of 7.5 and 10.0 kg (Table 3).The function of the urease enzyme in ammonia extracted from the preparation of 2.5 kg ha^{-1} and 5.0 kg ha^{-1} soil samples was more evident from urea. While urease activity was greater in black and alluvial soil, carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb were obtained at

5.0 kg ha^{-1}, 56-93, 46-106, 23-76, 43-103percent.Thealluvial soilwas showncarbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb of23-82, 32-102, 23-176, 67-117percent, respectively after incubation for 20 days.Whereas, the highest enzyme activity was demonstrated by carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 5.0 kg ha^{-1} with higher activity in black and alluvial soils (Table 3). In both soils, the enzyme activity reduced substantially at 30 and 40 days of incubation (Figures 3 and 4).

Table 2. Influence of tested agrochemicals on arylamidase activity in black soil and alluvil soil after 10 days

Agrochemicals Concentration (kg ha^{-1})	**Carbosulfan**	**Chlorpyrifos**	**Kresoxim-methyl**	**Mancozeb**
Black soil				
0.0	570c (100)	570c (100)	570d (100)	570e (100)
1.0	630b (110)	620b (108)	770b (135)	790c (138)
2.5	670a (117)	720a (166)	840a (147)	970a (170)
5.0	520d (91)	540d (94)	740c (129)	870b (152)
7.5	470e (82)	430e (75)	620d (108)	720d (126)
10.0	340f (59)	380f (66)	540f (94)	670f (117)
Alluvial soil				
0.0	510d (100)	510e (100)	510f (100)	510f (100)
1.0	480c (106)	640b (125)	780b (152)	840b (164)
2.5	580a (113)	780a (152)	870a (170)	920a (180)
5.0	540b (105)	630c (123)	740c (145)	790c (154)
7.5	420e (88)	580d (113)	680b (133)	630d (123)
10.0	310f (60)	500f (98)	540e (105)	580e (113)

*µg of β-naphthylamine g^{-1} soil formed after one hour incubation with L-leucine β-naphthylamide.

Values in the table are means of three replicates.The numbers in parentheses, indicate relative production percentages. Means in each column followed by same letter are not significantly different ($P \leq 0.05$) from each other according to DMRT.

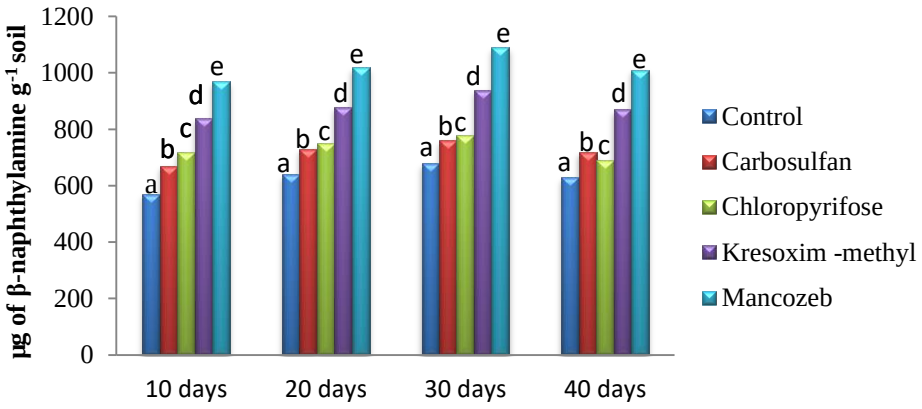

Figure 1. Effect of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 2.5 kg ha^{-1} on arylamidase activity* in black soil

Table 3. Influence of agrochemicals on urease activity in black soil and alluvial soil after 10 days

Agrochemicals oncentration(kg ha^{-1})	**Carbosulfan**	**Chlorpyrifos**	**Kresoxim-methyl**	**Mancozeb**
Black soil				
0.0	300e (100)	300f (100)	300f (100)	300f (100)
1.0	470c (156)	440e (146)	370d (123)	430e (143)
2.5	520b (173)	540c (180)	480b (160)	570b (190)
5.0	580a (193)	620a (206)	530a (176)	640a (213)
7.5	420d (140)	570b (190)	440c (146)	501c (170)
10.0	280f (93)	480d (160)	320e (106)	420d (140)
Alluvial soil				
0.0	340e (100)	340e (100)	340e (100)	340f (100)
1.0	420d (123)	450d (132)	520d (152)	570d (167)
2.5	540b (158)	570b (167)	620c (182)	670b (197)
5.0	620a (182)	690a (202)	720a (211)	740a (217)
7.5	510c (150)	520c (152)	640b (188)	630c (185)
10.0	420d (123)	320f (94)	152d (152)	153e (155)

*µg ammonia g^{-1} soil formed after 30 minutes incubation at 37°C with urea.
Values in the table are means of three replicates.The numbers in parentheses, indicate relative production percentages. Means in each column followed by same letter are not significantly different ($P \leq 0.05$) from each other according to DMRT.

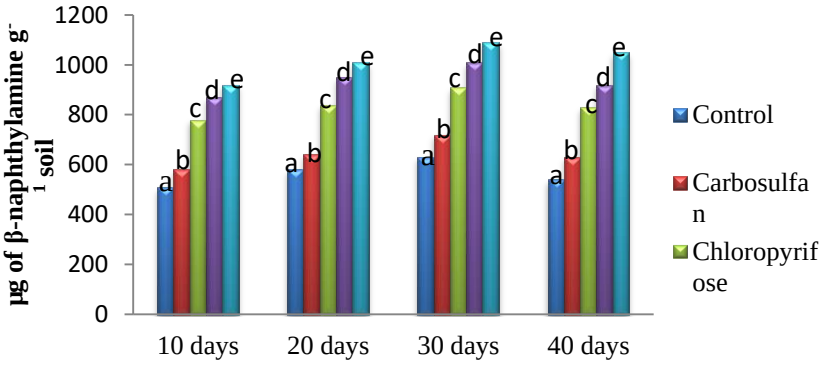

Figure 2. Effect of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 2.5 kg ha^{-1} on arylamidase activity* in alluvial soil

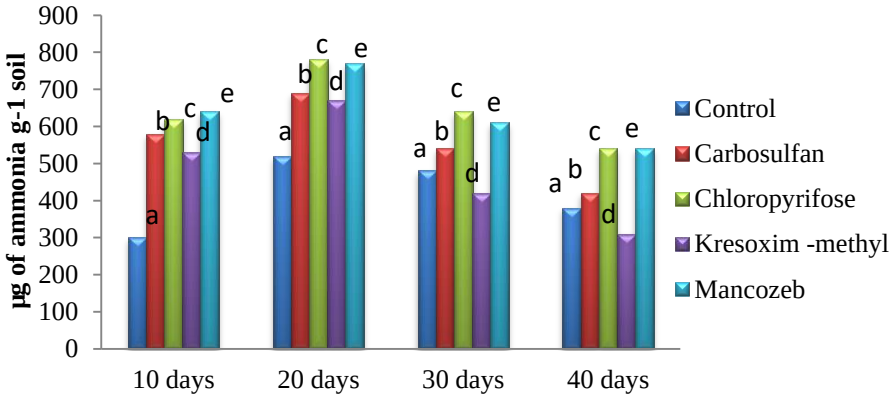

Figure 3. Effect of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 5.0 kg ha^{-1} on urease activity* in black soil

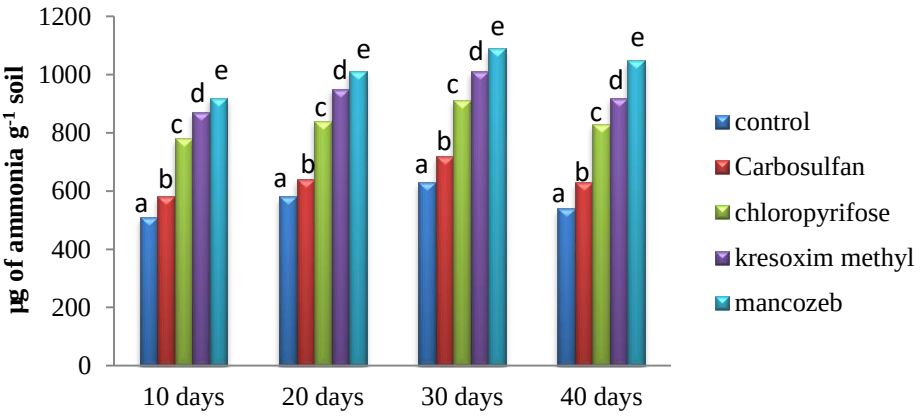

Figure 4. Effect of carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 5.0 kg ha^{-1} on urease activity* in alluvial soil

Discussion

The reduction in paddy's yield is mainly due to the infections caused by pests. Hence, huge amounts of pesticides are used to combat insect pests dseasesand to protect the paddy fields, ultimately the applied pesticides reached to the soil, which changes soil conditions as well as enzymatic activities.Arylamidase catalyzes the releasing of N-terminal aminoacid from peptides and amides and is distributed in the earth's microorganisms as well as plants. The enzyme urease plays an essential function in the release of carbon dioxide and ammonia through urea hydrolysis. The physicochemical characteristics of soils depend primarily on the quality of the soil. The sand percentage in alluvial soils was high (57%) relative to black soils (50%). All soils are alkaline. The percentage of clay in black soils high (28%) relative to alluvial soils (16%). The activity of arylamidase at concentrations from 1.0-2.5 kg ha^{-1}, with an averageconcentration of 2.5 kg ha^{-1} varied in both black and alluvial soils.In both soils, arylamidaseactivity improved by 1 and 2.5 kg ha^{-1} of each pesticide with mancozeb, chlorpyrifos and carbendazim. Similar findings were reported by Srinivasulu and Rangaswamy (2013). Floch *et al.* (2011) contradicted that the development of arylamidase varied with the period ofincubation, but after a sustained time to 12 months revealed 100 μg g^{-1} soil (10 kg ha^{-1}) pesticide incubation.The activity of enzyme urease was improved which related to the controls under the influence of tested agrochemicals, carbosulfan, chlorpyrifos, kresoxim-methyl, and mancozeb at 5.0 kg ha^{-1} in black soil and alluvial soil. Experiments comprising organophosphates, monocrotophos, and quinalphos performed by Rangaswamy and Venkateswarulu (1992) stated thatat 5.0 kg ha^{-1} greatly improved the output of urease activity in black and red soil at 10-day incubation. Sannino and Gianfreda (2001) suggested that pesticides have a complicated impact on the activity of soil urease. After two weeks of incubation, triazophos supported urease production at 5 and 10 mg/kg clay loam soil (Tu, 1981a and 1981b). Chlorimuron ethyl and furadan improved soil urease activity up to 14-18% and 13-21 %, respectively (Yang *et al*., 2006).

The findings obtained in the current study revealed that the tested agrochemicals, insectidides;carbosulfan, chlorpyrifos, and fungicides;kresoxim-methyl, and mancozeb substantially increased the activityofarylamidase up to 2.5 kg ha^{-1} and urease up to 5.0 kg ha^{-1} in black and alluvial soil over 10 days. Furthermore, the improvement in arylamidase was significantly increased until 20-dayincubation and the increasedin urease was significantly marked until 30-dayincubation. Based on the above findings, it is indicated that the tested agrochemicalsapplied at the prescribed amounts in the agricultural system have not influenced the activity of arylamidase and urease.

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