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Full Length Research Paper

Activities of invertase and cellulase as influenced by the application of tridemorph and captan to groundnut (*Arachis hypogaea*) soil

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A laboratory experiment was conducted to study the effect of selected fungicides, tridemorph and captan, at concentrations ranging from 0 to10 kg ha^{-1} on the activity of invertase and cellulase in a vertisol. The activities of invertase and cellulase were significantly more at tridemorph and captan levels of 2.5 and 5.0 kg ha^{-1}, respectively. But at higher concentrations of 7.5 and 10 kg ha^{-1}respectively, tridemorph and captan were toxic to both cellulase and invertase activities. In soil samples receiving 2.5-5.0 kg ha^{-1} of the fungcides, the accumulation of reducing sugar was pronounced more at 20 days, and the activity of the invertase and cellulase was drastically decreased with increasing period of incubation up to 30 and 40 days.

Key words: Fungicides, invertase, cellulase, groundnut, *Arachis hypogaea*.

INTRODUCTION

Groundnut (*Arachis hypogeae* L.) is one of the important, major, profitable oil seed crop grown throughout the year in India (Guha and Chandrasekhar, 2001). It contributes to 41.3% of country's oil seed production (Giraddi et al., 1999). Groundnut is the principal crop grown in Anantapur, a semi-arid district of Andhra Pradesh, India. Complete monocropping of groundnut in high yielding varieties resulted in an eruption of a wide collection of fungi causing damage to groundnut crop in many areas. More than 90 insect pests and mites were found associated with groundnut crop. Due to these insect pests, yield was significantly reduced (Das and Roy, 1995). In order to minimize the crop loss and to attain higher yield, chemical use of fungicide has become an essential input in modern agriculture (Ramakrishna et al., 1997).

Despite the beneficial impact of these fungicides in improving and sterilizing agricultural productivity by the control of pests and diseases, a major portion of these agro chemicals tend to affect the soil biological activity in different ways (Ramakrishna et al., 1997; Nagaraj et al., 1997). Repeated and extensive application of the pesticide ultimately reaches the soil, which in turn may interact with soil organisms and their metabolic activities (Sharma and Roomiro, 2002). Therefore the behavior of the total micro flora and their biological activity (enzyme activities) under continue fungicide input is an important aspect of research of the agricultural ecology (Anderson, 1978; Andreas et al., 2000).

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MATERIALS AND METHODS

Soil

The soil samples (black and red), were collected from fallow groundnut fields of Anantapur district a semi-arid region in Andhrapradesh, India, to a depth of 12 cm were air-dried and sifted through a 2-mm sieve before use.

Application of fungicides

Commercial samples of two fungicides were dissolved in distilled water. Details of the fungicides used in the present study are listed in Table 1. Five gram portion of the soil samples were weighed and dispersed into sterile test tubes (25 x 150 mm). Stock solutions from selected fungicides were added at the rate of 10, 25, 50, 75 and 100 µg g^{-1} soil equivalent to field application rates of 1.0, 2.5, 5.0, 7.5 and 100 kg ha^{-1} respectively. Soil samples without fungicide treatment served as controls. Soil samples were mixed thoroughly

Table 1. Particulars of the fungicides.

S. No	Technical name	Commercial name	Chemical class	Commercial formulation	Sources
1	Tridemorph	Calixin	Morpholine	80%EC*	BASF India Ltd Rhone - poulene house, S.K. Ahirema worly Mumbai-400025
2	Captan	Captaf	Phtalamide	50%WP**	Rallis India Ltd Agro chemicals division Mumbai -400001.

EC*: Emulsifying concentration.
WP**: Wettable powder.

for uniform distribution of fungicide added. Duplicates were maintained for each treatment at room temperature (28 ± 4^{o}C) with 60% water holding capacity throughout the incubation period. After desired intervals of incubation, soil samples were extracted in distilled water for estimation of enzyme activities. Similar model was used earlier by Singaram and Kamala kumari (2000)

Assay of invertase

Soil samples were transferred to 100 ml Erlenmeyer flasks and 1 ml toluene was added to arrest the enzyme activity. After 15 min, 6 ml of 0.2 M acetate phosphate buffer (pH 5.5) containing 18 mM sucrose was added to the soil samples and the flasks were closed with cotton plugs and held for 24 and 48 h at 30°C. Soil extracts were passed through Whatman No.1 filter paper and glucose in the filtrate was assayed (Nelson, 1994).

Assay of cellulase

To determine cellulase enzyme activity in soils, 10 ml of carboxy methylcellulose (CMC) 1% was used as a substrate followed by 10 ml of acetate buffer (pH 5.9) and incubated for 24 h to determine the reducing sugar content in the filtrate (Deng and Tabatabai, 1995). In another experiment, the rate of enzyme cellulase activitiy was determined at 10, 20, 30 and 40 days of soil incubation and further with the suitable substrate.

Statistical analysis

The concentration of the enzyme activity was calculated on a soil weight (oven dried) basis. The fungicide treatment were contrasted with untreated controls and the significant difference (P 0.05) between values of each sampling and each fungicide were performed using Duncan's new multiple range (DMR) test (Rangaswamy et al., 1989).

RESULTS AND DISCUSSION

Invertase activity

Invertase enzyme activity was expressed as the amount of glucose formed from the substrate, sucrose at 24 and 48 h in soils incubated for 10 days under the influence of selected fungicides. The invertase activity was more in black soil than in red soil is due to its high organic matter content (Table 2). In fact two fungicides, tridemorph and captan, at 10, 25, 50 ppm levels individually causes increments, 3 to 8% and 5 to 11% increase in invertase activity over control respectively in black soil at 10 day interval, respectively. Similarly tridemorph and captan at 10, 25, 50 ppm levels significantly cause increments of 5 to 12% and 2 to 13% increase in the invertase activity over control respectively at the same interval in red soil (Table 2).

A different behavior was observed with the activity of invertase, regarding the effect of tridemorph and captan applied at different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) to groundnut cultivated black and red soils. The invertase activity was pronounced more in the case of captan-treated black soil than the tridemorph, and was also slightly increased in captan-treated red soil than the tridemorph (Table 2). Among the two fungicidal treatments, captan produced a distinct stimulation over control, at 48 h incubation in black soil (Table 2). Comparatively, in the present study, the black soil showed higher enzyme activity than the red soil throughout the experiment. It is usually concluded that high enzymatic activities are associated with higher organic matter content.

Tu (1982) reported that two fungicides, triazophos and thiram, when applied at 5 and 10 mg/kg and incubated for three days, stimulated invertase activity. Especially, with triazophos, a phosphoro-thioate triazole, the activity was increased 10-fold. Similar effect was observed with respect to thiram (Tu, 1988). On the contrary, captan and maneb, at the same concentrations and incubation period, had no effect on invertase activity. Tu (1993) demonstrated that captafol and chlorothalonil suppressed Invertase activity for one day temporarily in a sandy loam soil and later on, after 2 days, the inhibitory effect diminished.

Invertase activity was more at a fungicide concentration of 2.5 kg ha^{-1} in black and red soil. Application of these fungicides at 7.5 kg ha^{-1} and 10.0 kg ha^{-1} drastically inhibited invertase activity. The data presented in the Table 2 shows the activity of invertase under influence of

Table 2. Activity of invertase* under the impact of different concentrations of selected fungicides in soils (black and red) for 24 h and 48 h after 10 days.

Concentration of fungicides (kg ha^{-1})	Black soil				Red soil			
	Tridemorph		Captan		Tridemorph		Captan	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
0.0	760a (100)	1120a (100)	760a (100)	1120a (100)	725a (100)	1057a (100)	725a (100)	1057a (100)
1.0	790b (103)	1223b (109)	802b (105)	1228b (109)	766b (105)	1082a (102)	746a (102)	1105b (104)
2.5	885c (112)	1282c (114)	911c (119)	1307c (116)	861c (118)	1135b (107)	894b (123)	1142c (108)
5.0	821d (108)	230b (109)	845d (111)	1247b (111)	805d (111)	1065a (100)	823c (113)	1043a (98)
7.5	702e (92)	1049d (93)	729e (95)	1052d (93)	683e (94)	962c (91)	668d (92)	964d (91)
10.0	649f (85)	922e (82)	678f (89)	941e (84)	624f (86)	825d (78)	604e (83)	845e (79)

*µg glucose per gram soil formed after 24 and 48 h incubation with 18 mM sucrose.
Figures, in parentheses, indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different (P 0.05) from each other according to Duncan's Multiple Range (DMR) test.

Table 3. Influence of selected fungicides at 2.5 kg ha^{-1} on invertase* activity in black soil after 24 and 48 h.

Fungicide	10 days		20 days		30 days		40 days	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control	760a (100)	1120a (100)	925a (100)	1415a (100)	832a (100)	1004a (100)	645a (100)	805a (100)
Tridemorph (2.5 kg ha^{-1})	885b (116)	1282b (114)	1103b (119)	1723b (121)	922b (110)	1320b (131)	668a (103)	820a (101)
Captan (2.5 kg ha^{-1})	911c (119)	1307c (116)	1120b (121)	1755c (124)	941b (113)	1343b (133)	692b (107)	842b (104)

*µg glucose per gram soil formed after 24 and 48 h incubation with 18 mM sucrose.
Means, in each column, followed by the same letter are not significantly different (P 0.05) from each other according to Duncan's Multiple Range (DMR) test.

different concentration of fungicides after 10 days. In all untreated black and red soil samples invertase activity was significantly more at 20 day incubation when compared with 10 day, 30 day and 40 day incubated samples. There was more invertase activity at concentrations of 2.5 kg ha^{-1} (Tables 3 and 4; Figures 1 and 2). Further more this increase in invertase activity was striking when the substrate was exposed to the soil samples for 48 h.

Cellulase activity

Cellulases play an important role as a group of enzymes in global recycling of the most abundant polymer, cellulose in nature. Hence the impact of selected fungicides on cellulase activity in soil was assessed. Results presented in Table 5 shows that cellulase activity in black and red soils (fungicides received at different concentrations) was enhanced significantly by trdemorph and captan up to 5.0 kg ha^{-1} at 10-day interval. The two

Table 4. Influence of selected fungicides at 2.5 kg ha^{-1} on invertase* activity in red soil after 24 and 48 h.

Name of the fungicide	10 days		20 days		30 days		40 days	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Control (0.0)	725a (100)	1057a (100)	905a (100)	1340a (100)	803a (100)	866a (100)	553a (100)	704a (100)
Tridemorph 2.5kg ha^{-1}	861b (118)	1135b (107)	1044b (115)	1658b (123)	903b (111)	1120b (129)	610b (110)	705a (100)
Captan (2.5 kg ha^{-1})	894c (123)	1142b (108)	1060b (117)	1688c (125)	922b (114)	1142b (131)	634b (114)	740b (105)

*µg glucose per gram soil formed after 24 and 48 hrs incubation with 18 mM sucrose.
Means, in each column, followed by the same letter are not significantly different (P 0.05) from each other according to Duncan's Multiple Range (DMR) test.

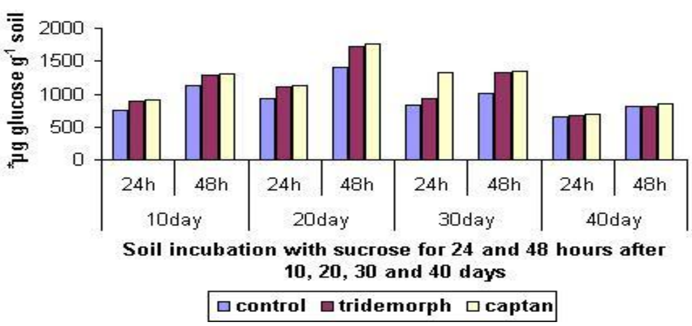

Figure 1. Effect of fungicides (tridemorph and captan at 2.5 kg ha^{-1}) on invertase* activity in black soil after 10, 20, 30 and 40 days.

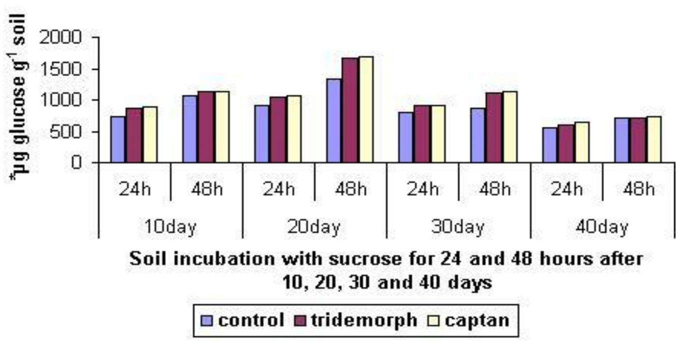

Figure 2. Effect of fungicides (tridemorph and captan at 2.5 kg ha^{-1}) on invertase* activity in red soil after 10, 20, 30 and 40 days.

fungicides caused stimulation in cellulase activity in the black soil by 24 to 66% and 29 to 65 % over control at 1.0, 2.5 and 7.5 kg ha^{-1}, respectively, by the end of 10 days (Table 5). The corresponding stimulations of the red soil during the same period incubation were 19 to 57% and 22 to 69%, respectively (Table 5). From the experimental data it is clear that stimulatory effect was comparatively more in black soil than red soil (Table 5). The stimulatory effect on cellulase activity was highest at 5.0 kg ha^{-1} in both soils (Table 5). Similarly the cellulase activity was reportedly promoted at 50 ppm by pyrazofos (as Afugan) and propiconazole (as Tilt) in soils inoculated with root fungi faba bean pots (Omar and Abd-alla, 2000). Interestingly, high concentrations of 7.5 and 10 kg ha^{-1} levels of two fungicidal treatments had either stimulatory or innocuous effect on cellulase activity, in both soil samples (Table 6) . Similarly, an anthraquic fluvisol soil incubated with the formulated fungicide, hymexazol, for 4 weeks remained unchanged in cellulolytic activity (Katayama and Kuwatsuka, 1991). However, quintozene resulted in initial decrease under flooded soil conditions, whereas under upland conditions, the activity was recovered to control value (Katayama and Kuwatsuka, 1991). The cellulolytic activity of *Helmenthosporium spiciferum* in the presence of the fungicide, vita avax (carboxin) had no significant effect (Singh et al., 1988). On the other hand, captafol significantly inhibited mineralization of cellulose in a sandy loam soil (Atlas et al., 1978). A distinct depression was observed with chlorothalonil, under all conditions tested in both flooded and non-flooded soil (Vincent and Sisler, 1968). Similarly, trichlamide at 10 times recommended field rate (i.e. 400 mg/kg) incubated for 4 weeks under flooded soil conditions inhibited the cellulolytic activity completely (Katayama and Kuwatsuka, 1991). Further Petkar and Rai (1992) demonstrated that five fungicides, captan, cosan, thiram, zineb and sandolex inhibited the cellulase activity, with greater inhibition with increasing fungicidal concentrations. According to Arinze and yubedee (2000), benlate, calixin and captan inhibited the activity of cellulase in *Fusarium monoliforme* isolates.

The data presented (in Table 6; Figures 3 and 4) reveals, that the rate of cellulase activity at the stimulatory concentrations of 5.0 kg ha^{-1}, of two fungicides, for 10, 20, 30 and 40 days of incubation in both soils. Stimulation of cellulase activity by the two selective fungi-

Table 5. Activity of Cellulase* under the impact of different concentrations of selected fungicides in both (black and red) soils for 24 h after 10 days.

Concentration of fungicides (kg ha^{-1})	Black soil		Red soil	
	Tridemorph	Captan	Tridemorph	Captan
0.0	802a (100)	802a (100)	781a (100)	781a (100)
1.0	1010b (124)	1039b (129)	930b (119)	960b (122)
2.5	1063c (132)	1103c (137)	1012c (129)	1058c (135)
5.0	1335d (166)	1324d (165)	1231d (157)	1320d (169)
7.5	932e (116)	954e (118)	805a (103)	862e (110)
10.0	780a (97)	802a (100)	680e (87)	744f (95)

*µg glucose per gram soil formed after 24 h incubation with 1% carboxy methyl cellulose.
Figures in parentheses, indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different (P 0.05) from each other according to Duncan's Multiple Range (DMR) test.

Table 6. Influence of selected fungicides at 5.0 kg ha^{-1} on Cellulase* activity in black and red soil after 24 h.

Name of the fungicide	Black soil				Red soil			
	10 days	20 days	30 days	40 days	10 days	20 days	30 days	40 days
Control (0.0)	802a (100)	1310a (100)	905a (100)	702a (100)	781a (100)	1242a (100)	821 (100)	610a (100)
Tridemorph (5.0kg ha^{-1})	1335b (166)	1962b (149)	1528b 168)	948b (135)	1231b (157)	1741b (140)	1432 (174)	915b (150)
Captan (5.0 kg ha^{-1})	1324b (165)	1991b (151)	1572c (173)	968b (137)	1320c (169)	1762b (141)	1443 (175)	936b (153)

*µg glucose per gram soil formed after 24 h incubation with 1% carboxy methyl cellulose.
Means, in each column, followed by the same letter are not significantly different (P 0.05) from each other according to Duncan's Multiple Range (DMR) test

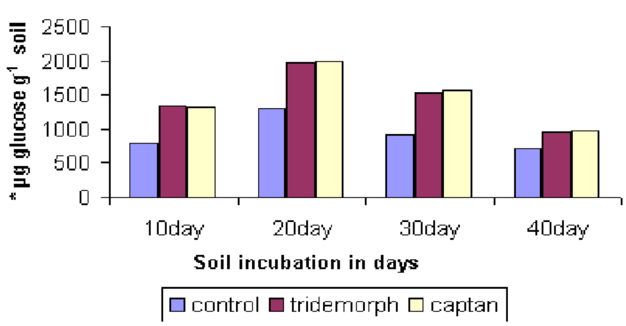

Figure 3. Effect of fungicides (tridemorph and captan at 5 kg ha^{-1}) on cellulase* activity in black soil after 10, 20, 30 and 40 days.

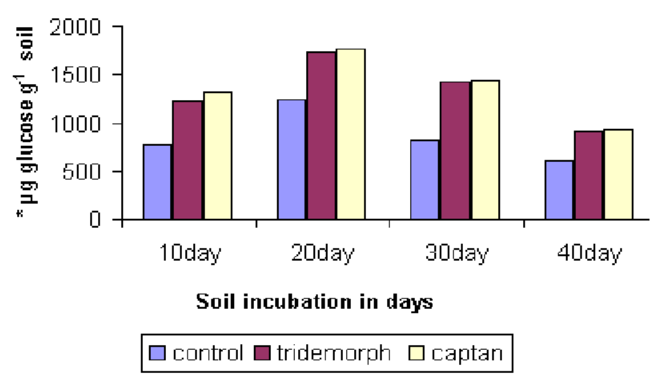

Figure 4. Effect of fungicides (tridemorph and captan at 5 kg ha^{1}) on cellulase* activity in red soil after 10, 20, 30 and 40 days.

cides was observed throughout the incubation period. However, the cellulase activity was pronounced more at 20-day period of incubation. Prolonged incubation (up to 40 days) of fungicide treated soil samples showed either stimulation or no measurable effect on the enzyme activity. Similar observation was made by Katayama and Kuwatsuka (1991) and Jayamadhuri (2002) on the cellulase activity.

Conclusion

The results of the present study clearly indicate that the fungicides widely used in cultivation of groundnut, at field application rates, enhance the activities of invertase and cellulase.

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