Pesticidal Effect on Soil Enzymes

Jaya Madhuri Ravuri and Rangaswami Venganampalle

***Abstract*----**The activities of three soil enzymes namely amylase, invertase and cellulase were determined by incubating the fungicide treated (1, 2, 5, 5, 7.5 and 10 kg ha^{-1}) and untreated groundnut (*Arachishypogaea* L,) soil samples at 10 days interval. After determining the effective concentration, rate of enzyme activity was estimated adding suitable substrate at 10, 20, 30 and 40 days of soil incubation. Amylase enzyme activity was stimulated maximum at 2.5 kg.ha^{-1} of insecticide concentration respectively. Activity was increased unto 5 kg ha^{-1} level of insecticide it. Whereas at 10 kg ha^{-1}, enzyme activity was strongly inhibited. Increase in invertase and cellulase enzyme activities was more at 5 kg ha^{-1}. Rate of enzyme activities namely amylase, invertase and cellulase under the influence of selected fungicides were enhanced significantly at 20 days of soil incubation, with specific substrate starch for 24 and 72 hours (Amylase), carboxy methyl cellulose for 24 hours (Cellulase), 24 and 48 hours with sucrose (invertase).

***Keywords*---** Enzyme activities, fungicides, groundnut soils.

I. Introduction

ANANTAPUR, a semi arid region of Andhra Pradesh, India although ranks first in area of groundnut (Arachishypogaea L.) cultivation, in the state (1), its productivity is low fluctuating around 9 q/ha on average. One of the main reasons attributed for such situation is insect pest problem, abiotic factors etc., (2,3). Hence fungicides, particularly cabendazim, mancozeb and hexaconazole usage has become an indispensable tool in Indian agriculture to combat various pests on groundnut (4,5). Due to extensive application of these pesticides, ecological stress is created on microbial soil enzyme activities which correlates to soil fertility (6). Hence soil enzyme activity under continuous pesticide input is an important aspect of research in agricultural ecology (7). In the present study, soil enzyme like amylase, cellulase, and invertase were selected because of their immense role in maintaining biodynamics of soil ecosystem (8). The enzymes, amylase, cellulase and invertase were actively involved in soil carbon cycle (7).

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In spite of many recent studies on pesticide – microbe interactions in soil (8.9), and increased fungicide usage, influence of selected fungicides on soil enzyme activities in groundnut soils has not received sample attention. Hence in the present experiment, attempts were made to update the effect of three selected fungicides, dhanustin, dithane M- 45 and contaf on soil amylase, cellulase, and invertase, in groundnut soils, the major crop grown in Anantapur District.

II. Materials And Methods

A. Soils

A black clay soil and a red sandy – clay soil collected from groundnut fields to a depth of 12 cm were used for soil incubation studies. Soils were air dried and sieved through 2 mm mesh before use. These soils were chosen because of the differences in their physic-chemical characteristics (10)

B. Fungicides

Three commercial fungicides, dhanustindithance M-45 and contaf were selected in view of their abundant usage in Indian agriculture to combat various insect pests of groundnut. Source and purity of the selected fungicides are presented earlier (10).

C. Soil incubation studies

Suitable aliquots from stock solutions of the three selected fungicides were applied to five – gram portions of soil samples contained in test tubes (15x150-mrn) to provide final concentrations of 1,2,5, 5.0,7.5 and 10 kg ha^{-1} respectively. Soil samples receiving only distilled water served as controls. Samples were maintained at 60% water holding capacity throughout the experimental phase at 28±40C. Duplicates were withdrawn for analyzing soil enymes. Similar model was used earlier to study pesticide-microbial interactions in (11,12).

D. Assay of soil enzymes

Influence of different concentrations of selected fungicides on amylase, cellulase and invertase was determined based on amount of glucose formed from starch (Amylase), carboxy methyl cellulose (cellulase) and sucrose (Invertase) at 10 days. Further, soils were incubated with specific substrate for 24 and 72 hours (amylase) 24 hours (cellulase) and 24 and 48 hours (invertase) employing the method of (13). Rate of these enzyme activities were calculated at stimulatory concentrations of respective insecticides in treated and untreated soils incubated for 10, 20, 30 and 40 days as described earlier.

E. Statistical analysis

In all cases, the fungicide treatments were contrasted with untreated controls and the significant difference (≤0.05) between values of untreated sampling and fungicide treated samples was performed using Duncan's New Multiple Range (DMR) test adopted by (14).

III. RESULTS

Amylase activity was enhanced by the application of dhanustin, dithane M- 45 and contaf at 1,2.5 and 5 kg ha^{-1} in black and red soils, incubated with starch for 24 and 72 hours, than the untreated control (Tables 1,2). Dhanustin at 7.5 kg ha^{-1} inhibited enzyme activity whereas in dithane M-45, the activity was enhanced. On the other hand contaf at 7.5 kg ha^{-1} stimulated amylase activity for 24 hours.

Dithane M-45 and contaf in black soil after 10 days.but at 72 hours showed innocuous effect in both soils. Experimental results further indicate that amylase activity is appreciably increased in soil samples incubated for 72 hours than 24 hours.

TABLE 1
ACTIVITY OF AMYLASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN,DITHANE M-45 AND CONTAF IN BLACK SOIL AFTER 10 DAYS

Fungicide concentration (kg ha^{-1})	Dhanustin 24 72	Dithane M-45 24 72	Contaf 24 72
0	200a 280a	200a 280a	200a 280a
1	270b350b	310b362b	295b405b
2.5	320c420c	380c430c	390c451c
5.0	250d320d	305b385d	320d374d
7.5	165e265d	260d320a	295b225e
10.0	140f 210e	170e240e	180e 190f

*µg glucose g^{-1} soil.
[a]incubation, in hours, of soil with starch (2% w/w).
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05)from each other according to DMR test.

TABLE II
ACTIVITY OF AMYLASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN, DITHANE M-45 AND CONTAF IN RED SOIL AFTER 10 DAYS

Fungicide concentration (kg ha^{-1})	Dhanustin 24 72	Dithane M-45 24 72	Contaf 24 72
0	190a 270a	190a270a	190a270a
1	240b 340b	290b 345b	270b 390b
2.5	300c 400c	365c 410c	370c 455c
5.0	225d 320d	300b 360b	305d 354 d
7.5	160e 230e	254d 310a	254e 287a
10.0	125f 225e	155e 215d	160f 238e

*µg glucose g^{-1} soil.
[a] incubation, in hours, of soil with starch (2% w/w)
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05)from each other according to DMR test.

Furthermore, significant stimulation, measured in terms of glucose formed from starch was noticed at 2.5 kg ha^{-1} in both soils. Higher concentration of 10 kg ha^{-1} is strictly inhibitory to amylase activity (Tables 1,2). Accumulation of glucose was more significant in all soil samples incubated for 20 days and specifically in soil incubated with substrate for 72 hours which declined progressively on further incubation (Tables 7,8).

TABLE VII
INFLUENCE OF DHANUSTIN, DITHANE M- 45 AND CONTAF AT 2.5 KG HA-1 ON AMYLASE ACTIVITY IN BLACK AND RED SOILS INCUBATED FOR 24 HOURS.

Treatment	**Soil**	**Incubation**	**In**	**Days**
	10	20	30	40
		Black soil		
Control	200a	300a	260a	180a
Dhanustin	320b	410b	350b	305b
Dithane	380c	470c	410c	374c
Contaf	390c	520d	457d	392d
		Red soil		
Control	190a	280a	240a	172a
Dhanustin	300b	395b	330b	295b
Dithane	365c	440c	345b	278bc
Contaf	370c	495d	420c	380c

*µg glucose g^{-1} soil after 24 hours incubation with starch at 28°C.
Means, in each column, followed by the same letter are not significantly different (P ≤0.05) from each other according to DMR test.

TABLE VIII
INFLUENCE OF DHANUSTIN, DITHANE M- 45 AND CONTAF AT 2.5 KG HA-1 ON AMYLASE ACTIVITY IN BLACK AND RED SOILS INCUBATED FOR 72 HOURS.

Treatment	**Soil**	**Incubation**	**In**	**Days**
	10	20	30	40
		Black soil		
Control	280a	420a	350a	290a
Dhanustin	420b	545b	485b	395b
Dithane	430b	552b	492b	410b
Contaf	451c	554b	497b	413b
		Red soil		
Control	270a	400a	315a	280a
Dhanustin	400b	504b	457b	370b
Dithane	410b	524b	468bc	384bc
Contaf	435c	537c	475c	392c

*µg glucose g^{-1} soil after 24 hours incubation with starch at 28°C.

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

Similar to amylase activity, increase in invertase enzyme activity was observed up to 5 kg ha^{-1} in all the fungicidal treatments. But stimulation was more pronounced at 2.5 kg ha-1 of contaf and 5 kg ha^{-1} of dhanustin and dithance M-45 incubated for 48 hours in the two soils (Tables 3,4).

TABLE III
ACTIVITY OF INVERTASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN, DITHANE M-45 AND CONTAF IN BLACK SOIL AFTER 10 DAYS.

Fungicide concentration (kg ha^{-1})	Dhanustin 24	48	Dithane M-45 24 48	Contaf 24 48
0	750a	1115a	700a 1115a	700a 1115a
1.0	780b	1190b	785b 1210b	805b 1220b
2.5	820c	1282c	830c 1285c	904c 1452c
5.0	880d	1370d	894d 1375d	852d 1305d
7.5	710a	1075a	705a 1120a	707a 1107a
10.0	657e	1050e	680e 1090e	665e 1085e

*µg glucose g^{-1} soil.
[a]incubation, in hours, of soil with sucrose (2% w/w)

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

TABLE IV
ACTIVITY OF INVERTASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN, DITHANE M-45 AND CONTAF IN RED SOIL AFTER 10 DAYS.

Fungicide concentration (kg ha^{-1})	Dhanustin 24 48	Dithane M-45 24 48	Contaf 24 48
0	700a 1115a	700a 1115a	700a 1115a
1.0	771b 1185b	780b 1201b	800b 1218b
2.5	815c 1264c	820c 1278c	902c 1451c
5.0	872d 1358d	894d 1376d	846d 1297d
7.5	697a 1066e	700a 1123a	703a 1104a
10.0	651e 1045e	677e 1085e	658e 1078e

*µg glucose g^{-1} soil.
[a]incubation, in hours, of soul with sucrose (2% w/w)
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Compared to amylase activity, glucose formation was much higher in invertase activity for 10 days. Higher concentrations of 7.5 and 10 kg ha^{-1} of the three fungicides were neither stimulatory nor inhibitory in both soils (Tables 3,4). It followed a similar trend of maximum enhancement at 20 days to that of other enzymes (Table 9).

TABLE IX
INFLUENCE OF DHANUSTIN, DITHANE M- 45 (AT 5.0KG HA-1) AND CONTAF AT 2.5 KG.HA-1ON INVERTASE ACTIVITY*

Treatment	**Soil incubation indays**			
	10	20	30	40
	24 48	24 48	24 48	24 48
		Black	**Soil**	
Control	750a 1115a	950a 1480a	855a 1025a	670a 773a
Dhanustin	880b 1370b	1100b 1770b	960b 1350b	695b 885b
Dithane	894b 1375b	1135c 1795c	985c 1375c	705b 888b
Contaf	904b 1452c	1490d 1915d	1015d 1390c	785c 943c
		Red	**Soil**	
Control	700a 1115a	920a 1390a 1085b	830a 970a	585a 750a
Dhanustin	872b 1358b	1680b	915b 1160b	645b 790b
Dithane	894b 1376b	1115c 1715c	940c 1220c	660c 843c
Contaf	902b 1451c	1385d 1885d	970d 1295d	685d 920b

*µg glucose g-1 soil formed after 24 and 48 hoursof incubation with sucrose at 28°C.
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from eachother according to DMR test.

Further, the rate of invertase activity was determined at 10, 20, 30 and 40 days of incubation, only at the stimulatory concentrations of 5.0 kg ha^{-1} of dhanustin and dithance M- 45 and 2,5 kg ha^{-1} of contaf in black and red soils incubated for 24 hours and 48 hours with sucrose (Tables 3,4,9) Evidently, the three selective fungicides had no significant difference in the accumulation of glucose from sucrose up to 40 days. All three fungicides used in the present study, in particular, contafhad more stimulation on invertase activity in soils, when compared to control sample. In fact, the stimulation was maximum at 48 hours incubation rather than 24 hours, throughout the incubation period (Tables 3,4,9).

The impact of selected fungicides (1,0,2.0,5.0,7.5 and 10 kg ha^{-1} on cellulase activity in soils was assessed (Tables 5,6). Cellulase activity in black and red soils was enhanced significantly by the three selected fungicides, dhanustin, dithane M-45 and contaf at 5 kg ha^{-1} of 10 day interval. The three fungicides caused stimulation in cellulase activity in the black soil by 25 – 26, 31-37, 52-54 and 16-21 % at 1, 2, 5, 5.0 and 7.5 kg ha^{-1} respectively by the end of 10 days (Table 5) The corresponding percentage of stimulation in respect of the red soil during the same period of incubation were 14-16, 30-32, 51, 3-12 percent respectively (Table 6).. From the experimental data, it is clear that stimulatory effect was comparatively more in black soil than red soil. (Tables 5,6). Interestingly, high concentrations of even 7.5 and 10 kg ha^{-1} levels of the three fungicidal treatments had no inhibitory effect on cellulase activity, in both soil samples (Tables 5,6).

TABLE V
*ACTIVITY OF CELLULASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN,DITHANE M-45 AND CONTAF IN BLACK SOIL AFTER 10 DAYS.

Concentration (kg.ha^{-1})	Dhanustin	DithaneM-45	Contaf
0	1014a	1014a	1014a
1.0	1275b	1283b	1267b
2.5	1332c	1392c	1388c
5.0	1541d	1570d	1564d
7.5	1180e	1025a	1018a
10.0	995a	1003a	987e

*µg glucose g^{-1} soil formed from carboxy –methyl cellulose (CMC) during 24 hours incubation at 30°C.

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

These stimulatory effect on cellulase activity was maximum at 20 days in both soils exerted by three fungicidal treatments (Table 10).

TABLE VI
*ACTIVITY OF CELLULASE UNDER THE IMPACT OF DIFFERENT CONCENTRATIONS OF DHANUSTIN, DITHANE M-45 AND CONTAF IN RED SOIL AFTER 10 DAYS.

Concentration kg.ha^{-1}	Dhanustin	Dithane M-45	Contaf
0	985a	985a	985a
1.0	1128b	1146b	1138b
2.5	1277c	1298c	1282c
5.0	1485d	1494d	1488d
7.5	1002a	1013e	1018e
10.0	954e	968a	970a

*µg glucose g^{-1} soil formed from carboxy –methyl cellulose (CMC) during 24 hours incubation at 30^{o}C.

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

TABLE X
INFLUENCE OF DHANUSTIN, DITHANE M-45 AND CONTAFAT 5.0 KG HA-1 ON CELLULASE ACTIVITY* IN BLACK AND RED SOILS.

Treatment	**Soil incubation in days**			
	10	20	30	40
		Black	**Soil**	
Control	1014a	1532a	1138a	957a
Dhanustin	1541b	2085b	1647b	1145a
Dithane	1570c	2114c	1742c	1265c
Contaf	1564c	2108c	1738c	1248c
	Red Soil			
Control	985a	1485a	1089a	890a
Dhanustin	1485b	2052b	1624b	1088b
Dithane	1494b	2096c	1688c	1192c
Contaf	1488b	2090c	1645b	1184c

*µg glucose g^{-1} soil formed from carboxy-methyl cellulose (CMC) during 24 hours incubation at 30^{o}C.

Means, in each column, followed by the same letter are not significantly different ($P = 0.05$) from each other according to DMR test.

IV. DISCUSSION

Proliferation in the activity of amylase under the influence of selected fungicides even up to 7.5 kg ha^{-1} coincide with (15). Triazole fungicide accelerated the enzyme activity at 5 and 10 mg/kg in an organic soil (14). The innocuous nature of pesticide treatment at 7.5 kg ha^{-1} on amylase activity was also noticed with that of sulfex at lower concentration of 0.02, 0.05 and 0.1% respectively (15). Further, (16) showed that tridemorph at 50 and 100 mg/kg soil unaffected amylase activity. This was further confirmed by (13) on application of two fungicides captofol and chirothalonil. Contradictory to inhibition of enzyme activity with higher concentration of insecticides treatment was demonstrated at low concentration by (4). with seven fungicides. Brassicol, captan, dithane M-45, fytosan, parasan, sulfex and thiram inhibited amylase activity at low concentration. Among them parasan was highly effective and 100% inhibition was observed even at the lowest concentration of 0.025% over control. Thiram andfytosan inhibited amylase activity at 0.5 and 1.5% . Other fungicides such as brassicol.captan, dithane M-45 inhibited amylase activity at 2%.

The pronounced stimulatory effect on enzyme activity as observed in the present study was also demonstrated by (16). A 10 fold increase in invertase activity was observed on application of triazophos at 5 and 10 mg/kg. On the contrary, captan and maneb at same concentration and incubation period had no effect on invertase activity. In contrast to these innocuous effect.demonstrated that captafol and chirothalonil suppressed enzyme activity for 1 day and recovered after 2 days.

In agreement with present findings of stimulation at 5 kg ha^{-1} of insecticide treatment on cellulase activity, (17) reported that activity was alleviated at 50 ppm of profenos and propiconazole in soils. The no toxic effect of selected fungicides on cellulase activity is also observed by (18) on application of hymexazol. On the other hand, (19) reported inhibition of enzyme activity at higher doses of fungicides. Like wise, (20) observed inhibition of cellulase activity at 10 parts of captafol and folpet. (21, 22) also demonstrated a similar parttern of inhibition by captan, cosan, thiram, Zineb and sandolex. This was further confirmed by the results of (15), in benlate.

V. CONCLUSION

Amylase, invertase and cellulose enzyme activities involved in carbon cycle were not affected with the selected fungicides when applied at field application rate.

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