INFLUENCE OF THE INSECTICIDE DIMETHOATE ON SOME METABOLIC ACTIVITIES OF FIVE ZOOSPORIC FUNGI

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SUMMARY: Using sesame water culture and various doses of Dimethoate, no mycelial growth appeared in cultures at rate above 5 ppm. There was no appreciable effect of the low dose of the insecticide (2.5 ppm) when incorporated into water cultures on vegetative growth, asexual and sexual sporulation of all tested fungi compared with the control. Using liquid media, respiration, mycelial dry weight as well as protease, lipase, amylase and cellulose production were varied depending upon the doses and tested fungal species.

Key Words: Insecticides, dimethoate, zoosporic fungi.

INTRODUCTION

Pesticides are used in modern agriculture to increase production through controlling harmful effects caused by the targets organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops. Pesticides reach to water and accumulate in soil through run-off from treated plants, other sources such as treated seeds, dead sprayed plants, dead grazing animals fed by polluted grasses and irrigation water previously treated by herbicides and molluscicides. Past reviewers have stressed the fact that insecticides do not in general have much effect on soil microbial population and activities, except at concentrations greatly exceeding normal recommended field rates (4). On the other hand, Zoosporic fungi represent an important part of water community that give a hand in degradation of organic matter and nutrients recycling in aquatic habitats and may influenced by direct or indirect addition of pesticides to water environment. The degradative enzymes produced by fungi are important in host infection, food deterioration and breakdown of organic matter. Numerous investigations have been dealt with the effects of various insecticides on growth, enzymatic and other microbial activities of terrestrial fungi (5,6,9,11,14, 22,23,25). However, no available information concerning the effect of insecticides on metabolic activities of Zoosporic fungi. Thus, this work was conducted to evaluate the effect of the insecticide Dimethoate on some metabolic activities of five common Zoosporic fungal species in Egyptian various water areas.

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MATERIALS AND METHODS

Organisms. Five Zoosporic fungal species were tested in this investigation; *Achlya* racemosa Hildebrand, *Dictyuchus monosporus* Leitgeb, *Saprolegnia ferax* (Gruith.) Thuret, *Thraustotheca clavata* (de Bary) Humphrey and *Allomyces arbuscula* Butler.

The organophosphate insecticide Dimethoate [dimethyl S-(N-methylcarbamoylmethyl) phosphorothiolothionate], is produced by Kafr El-Zyat pesticides and chemicals Co. (kz), was employed in this investigation using five levels; 2.5, 5, 10, 15 and 75 ppm.

Vegetative growth and some morphological aspects. Sesame seeds water cultures were used to study the effect of insecticide Dimethoate upon some morphological aspects (vegetative mycelium, sexual and asexual sporulation) of the five tested fungal species. Sesame seeds were autoclaved in a few milliliters of distilled water till the testa raputred exposing the radicle. Two milliliters of a zoospore suspension were inoculated into plates

containing approximately 20 ml of different concentrations of Dimethoate (0.0, 2.5, 5, 10, 15 and 75 ppm) and five sesame seeds. These plates were incubated in 21°C for 20 days during which the colonized sesame seeds were microscopically examined at successive intervals (2, 5, 8, 11, 15 and 20 days).

Respiration, enzymatic activities and mycelial dry weight. The basal liquid medium (K_2HPO_4, 29; KH_2PO, 0.5 g; $MgSO_{4.7}H_{20}$, 1.0 mg; L-cysteine, 0.1 g; tryptone, 9.9 g; biotin, 0.005 mg; thiamine, 0.05 mg/L of distilled water) was employed in this investigation. Some substances were added to the basal medium for the production of protease (glucose, 69; peptone, 69 (18), Lipase (Tween 80, 10 ml; Peptone, 10 g; NaCl2, 59 (12), amylase (soluble starch, 10 g (10) and cellulose (carboxymethylcellulose, 5 g (7).

The medium pH was adjusted to 5.0 with 0.1 HCl. 20 ml of the proper medium was dispersed in 100 ml Erlenmeyer conical flasks and sterilized. Flasks containing various liquid media were amended with the range of insecticide concentrations (0.0, 2.5 and 5 ppm). Each flask was inoculated with 1 ml of spore suspension prepared from 8-day-old cultures, then incubated for 10 days at 21°C. Cultures untreated with Dimethoate served as control. After the incubation period (10 days) the cultures were taken for measuring respiration, mycelial dry weight as well as the protease, lipase, amylase and cellulose activities.

Respiration. It was estimated by the method described by Cheng and Coleman (8) in which cultures were subjected to CO_2-free air current loading CO_2 evolved by the growing cultures. The air current terminates its pathway by bubbling in 0.5 M KoH solution for trapping CO_2 which is then estimated by titration with 0.25 μHCl using phenolphthalein as indicator.

Mycelial dry weight. At the end of experiment (10 days), mycelia were collected, by filtration and placed in the oven at 70°C until constant weight. The dry weight of each treatment was calculated and is expressed as mg/20 ml medium.

Enzymatic activities

Protease activity. The assay procedure adopted by Kunitz (15). A 1% casein solution was prepared in 0.05 M sodium phosphate buffer (pH 7.0), heat-denaturated at 100°C for 15 minutes in a water bath, cooled and used as substrate. To 1 ml of the casein solution, 0.5 ml of culture filtrate was added, mixed the roughly and incubated at 35°C for 3 hours. The reaction was terminated by adding of 3 ml of cold 10% trichloroacetic acid (TCA). The tubes were allowed to stand for one hour at 4°C to allow undigested protein to precipitate. Blanks were made in the same way using boiled filtrate. The tubes were centrifuged at 100 g for 15 minutes. The supernatant fluid was analyzed for digested un-precipitated protein by the method of Lowry's method. Data were expressed as μg BSA-protein/hr/1 ml culture filtrate.

Lipase activity. Cultures filtrate was used for estimation of lipase activity by the method suggested by Urs *et. al.* (26) with some modifications. The reaction mixture consisted of 2 ml tween, 20; 2 ml culture filtrate; 5 ml citrate-phosphate buffer (pH 8.0), 1 ml toluene and incubated at 28°C for 3 hours. The reaction was terminated with 25 ml of absolute ethanol and titrated against 50 mM NaOH using 1% ethanolic phenolphthalein as indicator. The lipase activity was calculated from the difference between the control and experimental titre value. The enzyme activity was calculated as mg free fatty acids/1 ml culture filtrate/1 hr.

Amylase activity. A mixture of 5 ml of the culture filtrate and 5 ml of 2 M Na-acetate acetic and buffer (pH 5.2) containing 1% soluble starch was incubated at 37°C for 2 hours (10). The reducing sugar (RS) released therein were then determined by Nelson's modification of Somogy's method (17) with reference to a standard curve of maltose. The activity was expressed as μg maltose/1 ml/1 hr.

Cellulose activity. Cellulose (Cx-cellulose) activity was measured by incubating a mixture of 1 ml of the culture filtrate and 0.5 ml of 0.5% CMC in 55 mM citrate buffer (pH 5.2) at 37°C for 1 hour (16).

Cellulose 1,4-β cellobiosidase (C1-cellulose) activity was also measured in terms of filter paper activity by incubating a mixture containing 50 mg strip (10x10 cm) of Whatman No. 1 filter paper, 1 ml of 50 mM acetate buffer (pH 5.2) and 1 ml of culture filtrate at 37°C for 1 hour (7). The reaction was terminated by boiling in water bath for 5 minutes and reducing sugars were determined photometrically according to Nelson (17). Results were calculated from glucose standard curve.

Statistical Analysis. Statistical analysis was made by means of one-way analysis of variance (Pc-state computer program). Means were separated by using the Duncans multiple-range test.

RESULTS AND DISCUSSION

Vegetative growth and some morphological aspect. Using sesame water culture and various levels of Dimethoate (0.0, 2.5, 5, 10, 15 and 75 ppm), no mycelial growth occurred in levels above 5 ppm Dimethoate in case of all tested fungal species. The density of all tested fungal mycelia, number of zoosporangia, number of sexual and asexual organs recorded its highest values in the level of 2.5 ppm Dimethoate followed by control. These aspects were severely affected by the high dose (5 ppm) of Dimethoate. In comparison between the tested fungal species, the spores of *Dictyuchus monosporus* and *Achlya racemosa* were more sensitive to the all applied levels of Dimethoate than those of *Saprolegnia ferax, Thraustotheca clavata* and *Allomyces arbuscula.* Abd-Alla and Manci (2) studied the interaction between a *Pythium* and the herbicide stomp and found that the development of oogonia and sporangia was greatly stimulated when 100 ppm Stomp were incorporated with the solid medium whereas subsequent high doses (800-1000 ppm) consistently increased the inhibitory effect on both sexual and

asexual spore-bearing organs. They also reported that Stomp did not significantly retard sporangial formation between the concentrations 100-700 ppm. Our observations came in agreements with the findings of Abdalla (1) and Katan and Eshel (13) using a fungicide and herbicides respectively.

Respiration. Table 1 shows that mycelial respiration on different growth media treated with Dimethoate varied depending upon fungal species, medium used as well as the dose of insecticide. CO_2 production from cultures of *Allomyces arbuscula* and *Achlya racemosa* grown on the basal media amended with tween, starch and carboxymethyl-cellulose was significantly promoted by both treatments (2.5 and 5 ppm). In contrast, CO_2 production inhibited in media supplemented with casein. Also, the mycelial respiration of *A. racemosa* was higher in dose 2.5 than in 5 ppm Dimethoate.

The respiration of *Dictyuchus monosporus* was significantly inhibited with the two doses of Dimethoate on the basal medium amended with tween. It was significantly promoted in media amended with casein, starch and carboxymethyl-cellulose by the two treatments.

In case of *Thraustotheca clavata,* respiration was promoted with the two treatments on medium amended with casein whereas decreased in other media.

Respiration of *Saprolegnia ferax* was significantly inhibited on tween containing media, promoted on casein supplemented media but on other media was promoted with the low dose (2.5 ppm) and inhibited with high one (5 ppm). No available literature about effect of pesticides on respiration of Zoosporic fungi. However several investigation have been dealt with terrestrial fungi. Shonkeir (24) reported that Dimethoate at rates of 13.5 and 67.5 ppm stimulate O_2-uptake by *Aspergillus fumigatus, Cunninghamella echinulata* and *Penicillium funiculosum.* Selecron at rates of 6.4 and 38.4 ppm exerted a significant increase in mycelial respiration (CO_2-evolution) of *A. niger, Mucor racemosus* and *Trichoderma harzianum* especially with the lose dose but on the hand respiration of *P. chrysogenum* and *Stachybotrys chartarum* was inhibited, (19). Anderson (4) concluded that increasing respiration is more likely to be due to stimulation of metabolic activity than to uncoupling of oxidative phosphorylation.

Mycelial dry weight. The mycelial dry weight of

Table 1: Effect of Dimethoate on mycelial dry weight (mg/20 ml media) and respi- ration (μg CO_2/10 mg dry weight/hour) of five Zoosporic fungal species using the basal medium amended with tween (I), casein (II), starch (III) and carboxymethyl cellulose (IV).

Species	Doses (ppm)	Dry weight media								Respiration media							
		I		II		III		IV		ýI		II		III		IV	
Achlya racemasa	0	111.5	b	463.4	a	276.6	c	56.1	c	209.0	a	94.5	a	52.0	b	88.7	c
	2.5	114.0	a	363.4	b	329.3	b	70.4	b	175.0	b	62.5	b	57.5	a	126.5	b
	5	115.2	a	91.3	c	598.0	a	73.0	a	173.0	b	11.0	c	47.0	c	136.4	a
Dictyuchus sterile	0	47.1	a	90.5	c	116.0	c	28.0	c	342.0	a	25.0	b	159.5	a	106.0	a
	2.5	19.0	c	151.3	a	368.7	a	34.5	b	114.0	b	60.4	a	79.0	c	95.5	b
	5	25.3	b	156.2	b	187.9	b	50.2	a	100.0	c	16.0	c	111.5	b	72.4	c
Saprolegnia ferax	0	107.5	c	114.5	c	214.0	b	60.1	b	228.6	a	45.0	a	80.5	c	55.2	c
	2.5	123.2	b	149.0	a	225.0	a	62.4	a	215.3	b	23.4	b	99.0	a	62.6	b
	5	131.4	a	120.2	b	139.0	c	54.5	c	148.0	c	23.0	b	88.5	b	112.3	a
Thraus- totheca clavata	0	324.4	a	269.5	c	101.0	a	39.2	a	95.5	c	17.0	a	42.5	c	45.5	b
	2.5	202.5	c	481.0	b	35.0	b	25.2	b	150.0	a	18.5	a	67.5	a	47.3	b
	5	263.5	b	501.2	a	27.0	c	24.5	b	119.3	b	11.5	b	51.0	b	89.2	a
Allomyces arbuscula	0	26.4	c	79.4	a	120.9	c	30.4	c	104.5	a	115.5	c	114.0	a	115.5	a
	2.5	50.5	b	29.3	c	171.4	b	48.1	a	113.0	a	169.5	b	63.0	b	80.1	b
	5	78.9	a	68.3	b	210.3	a	35.5	b	78.0	b	200.0	a	35.0	c	82.6	b

Each value represents the average of three replicates. Values in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range.

Allomyces arbuscula on casein containing basal medium was significantly increased by both doses of Dimethoate but on the other media the effect turned into inhibitory specially with the high dose (Table 1). The dry weight of *Achlya racemosa* showed two response to Dimethoate application the first was inhibitory on tween and casein amended media but on the other two media, this response turned into pro-motive at least with the low dose. The mycelial dry weight of D. monosporus was significantly decreased by Dimethoate and the inhibition in most cases was confined to the doses except in case of media amended with casein where promoted by 2.5 ppm and retarded by 5 ppm Dimethoate.

On the contrary, dry weight of *Thraustotheca* clavata was significantly induced by insecticide irrespective to either medium or dose used. The highest mycelial dry weight of *T. clavata* was produced at 2.5 ppm in case of media amended with tween, casein and starch but in case of medium amended CMC it was confined to the high dose. In case of S. ferax the mycelial dry weight was decreased on tween and casein media whereas promoted on other media. Literature about effect of pesticides on mycelial growth of Zoosporic fungi are not available. However, Abd-Alla and Manci (2) reported that 50 to 300 ppm of the herbicide Stomp reduce the mycelial dry weight of *Pythium* using liquid media.

Dieldrin at 5 ppm inhibited the growth of *Dictyostelium discoideum* (6). Also, Phosphamidon (1,4 and 8 ppm) inhibited the growth of *Penicillium coryhophilum* (3). *Mycelial growth of Chaetomium globosum* was significantly decreased with cyolane at 10 and 20 ppm. It appeared that most insecticides had inhibitory effect on growth of soil fungi. Increment in growth of some fungi in the presence of insecticides is due to their capability to degrade insecticides especially those of aquatic origin (20, 21).

Enzymatic activities. Protease activity was more pronounced in case of *T. clavata* and *S. ferax* (Table 2). Using the two doses it was promoted in case of T. clavata whereas retarded in case of S. ferax. In case of *A. arbuscula* and *D. monosporus* protease was significantly promoted with the low dose while in case of *A. racemosa* it inhibited with the low dose but not influenced with the high.

Lipase activity of tested fungi was not severely influenced by the insecticide Dimethoate except in case of *A. arbuscula* and *D. monosporus* where it was inhibited but in

Table 2: Effect of Dimethoate on enzyme activities (per 1 ml filtrate/hour) of tested fungi.

Species	Doses (ppm)	Lipase (mg free fatty acid)	Protease (μg BSA protein)	Amylase (μg maltose)	Cx-cellullase (μg glucose)	C_1-cellulase (μg glucose)
Achlya racemosa	0	1.7 a	57.0 a	51.8 a	43.6 c	57.0 a
	2.5	1.9 a	51.0 b	8.0 b	60.4 a	48.0 b
	5	1.6 a	53.5 a	5.9 c	56.5 b	31.0 c
Dictyuchus sterile	0	1.6 c	49.0 b	11.0 b	17.2 c	32.0 a
	2.5	2.5 a	57.3 a	30.7 a	20.7 b	29.5 a
	5	2.0 b	54.5 ab	10.9 b	29.3 a	32.5 a
Saprolegina ferax	0	2.1 a	62.0 a	271.3 a	21.0 b	33.0 a
	2.5	1.5 b	52.0 b	310.4 b	40.0 a	30.2 b
	5	2.1 a	50.0 b	41.2 b	7.0 c	30.8 b
Thraustotheca clavata	0	2.0 a	24.0 c	176.9 a	8.9 c	14.3 b
	2.5	1.7 b	54.0 b	14.7 b	25.1 a	32.0 a
	5	2.1 a	76.5 a	8.5 c	12.9 b	15.5 b
Allomyces arbuscula	0	2.5 a	51.5 b	40.5 c	63.2 a	6.7 b
	2.5	1.8 b	68.3 a	46.3 b	21.7 b	10.4 a
	5	2.3 b	47.0 c	49.4 a	12.7 c	12.3 a

Each value represents the average of three replicates. Values in the same column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range.

case of *D. monosporus* where promoted.

Amylase activity of *A. racemosa* and *T. clavata* was significantly decreased with Dimethoate but was accelerated in case of *A. arbuscula.* The enzyme activity of *D. monosporus* and *S. ferax* was significantly promoted with the low dose whereas the high dose decreased the enzyme activity of the later.

Cx-cellulose of *A. arbuscula* was inhibited by both doses of insecticide was inhibited by both doses of insecticide whereas promoted in case of other tested fungi. The highest activity was in 2.5 ppm Dimethoate in case of *A. racemosa, T. clavata* and *S. ferax.*

No available literature dealing with Zoosporic fungi in this respect. However, some reports revealed that the enzymatic activities of some terrestrial fungi were inhibited with some insecticides (14).

To our knowledge, few reports were published concerning the effect of pesticides on Zoosporic fungi. So that further investigations are necessary to elucidate the fate of these chemicals in water environment based on determination of degradation products of pesticide under static and shaking condition.

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