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Occurrence of Fungal Species and Mycotoxins from Decayed Sugarcane (*Saccharrum officinarum*) in Egypt

Gamalat A. Abd-Elaah* and Samya Soliman A.

Botany Department, Faculty of Science, South Valley University, Sohag, Egypt
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Seventy-three fungal species belonging to forty-three genera were isolated from 40 samples of *Saccharrum officinarum* (collected from Naage-Hamadi canal in Qena Governorate, Egypt). *Aspergillus*, *Trichoderma*, *Mucor* and *Pythium* were the most common genera on the two isolation media. The dominant species of *Aspergillus* were *A. niger*, *A. flavus*, *A. ustus*, *A. terreus* and *A. wentii*. Some species were dominant on 40 g/l sucrose such as *Aspergillus niger*, *A. flavus*, *Emericella nidulans*, *Trichoderma viride*, *Torula herbarum* and *Mamaria echinoeotryoides*, while the dominant species on 10 g/l glucose were *Mucor circinelloides*, *Aspergillus niger*, *Torula herbarum* and *Trichoderma viride*. Mycotoxins including aflatoxins B_1, B_2, G_1 and G_2, zearalenone and diacetoxyscirpenol were detected in the examined samples of *Saccharrum officinarum*. The mycelial growth of *A. flavus*, *A. niger*, *Fusarium moniliforme* and *Torula herbarum* decreased with the increase in Dimethoate concentrations, although 25 ppm was less effective than the higher levels of the insecticide (75~200 ppm). Dimethoate stimulated the activity of Go-T in *A. niger*, *F. moniliforme* and *T. harbarum*, while the Go-T activity was inhibited in *A. flavus* with the Dimethoate treatments.

KEYWARDS: Aquatic and terrestrial fungi, Mycotoxin production, *Saccharrum officinarum*

Sugarcane plant (*Saccharum officinarum*) is the predominant crop used in sugar production in the upper Egypt. Sugar present in the stem of *S. officinarum* represents the main source for fungal growth. Accordingly, the fallen stems in the canal or in the irrigation water during the harvest become a source of pollution for the water and other cultivated plants. The pollution effects depend on the mycoflora grown on the stem and on some environmental factors such as temperature. Mohawed *et al.* (2001) studied the seasonal fluctuations of soil and root surface fungi of sugarcane in the upper Egypt. They isolated 73 species and 5 varieties representing 33 genera using glucose-, cellulose-and sucrose-Czapek's agar media. Abdel-raheem (1999) examined the presence of fresh-water ascomycetes in various decayed plant parts of *Eucalyptus rostrata*, *Phragmites australis* and *Phoenix dactylifera*, collected from the river Nile in Egypt. In addition, El-Sharouny *et al.* (1999) studied the biodiversity and distribution of fungi on submerged wood in the river Nile and irrigation canals in the upper Egypt. Since some molds can produce toxic metabolites (mycotoxins), proliferation of the organisms represents a potential health hazard (Northolt *et al.*, 1995). Therefore, detection of fungal contaminants is essential to ensure safe and high quality food (Bullerman, 1979).

On the other hand, insecticides have been used to control insects and play a significant role in increasing crop production. They commonly affect some of the non-target organisms such as microbial population ranging from inhibitory to stimulatory effects. Dimethoate is a broad-spectrum insecticide and commonly used in sugarcane to control the white fly (Anonymous, 1989). The effect of insecticides on fungal growth and enzymes activity was studied by different workers (Audus, 1960; El-Hissy and Abdel Kader, 1980; Abd-Elaah, 1993).

Materials and Methods

Sugarcane samples. Forty samples of sugarcane stems (*S. officinarum*) were collected during two seasons (winter and summer 2002) from the Naage-Hamadi canal in Qena Governorate in upper Egypt. Each sample was represented by 10 decayed stem parts. The samples were transferred directly to the laboratory for fungal isolation and toxin analyses.

Isolation and identification of fungi from *S. officinarum*. The stem-samples were washed with sterilized distilled water. Each stem was cut into segments (ca. 0.5 cm long) by knife, then each segment was cut into four equal parts. These segments were placed on the surface of two solidified media, glucose (10 g/l) and sucrose (40 g/l) Czapek's agar to which chloromphnicol was added as a bacteriostatic agent (Smith and Dawson, 1944). Five Petri plates of tested medium were used for each stem sample. Plates were incubated at 25°C for one week. For the recovery of aquatic fungi, stem segments from the collected samples were placed in Petri-dishes; 12 cm in

*Corresponding author <E-mail: gamalata@yahoo.com>

diameter (6 replicates). The segments in each Petri-dish were then covered with sterile distilled water (20 *ml*) and 12 sterilized sesame seeds were introduced into each Petri-dish, as employed by El-Nagdy (1986).

The growing fungi were identified, counted as numbers per segment. The identification of fungal genera and species was performed according to Raper and Thom (1949), Gilman (1957), Raper and Fennell (1965), Domsch and Gams (1972), Booth (1971), Pratt and Heather (1973) and Lund (1978). Fungal species recovered from stem samples were purified on suitable media such as glucose-peptone-agar, malt-extract-agar, potato-dextrose-agar, potato-dextrose-yeast-agar, sabouraud's-dextrose-agar and Czapek's-medium.

Mycotoxin extraction from *S. officinarum*. Fifty grams of decayed stems of each sugarcane sample were transferred to 500 *ml* Erlenmeyer flasks containing 150 *ml* of chloroform each and placed in a shaker (200 rpm) for 16 hours, then filtered through filter paper (Whatman No. 1). The chloroform extract was dried over anhydrous sodium sulphate. The remaining stem samples were dried at 50°C over night, followed by re-extraction by 150 *ml* of 90% methanol-water.

Chemical detection of mycotoxins. Thin layer chromatography (TLC) technique was carried out using precoated with Silica Gel type 60, F_{254} (MERCK, Germany). Aflatoxins B_1, B_2, G_1 & G_2; ochratoxins A & B; sterigmatocystin; citrinin; T-2 toxin; diacetoxyscirpenol (DAS); zearalenone; moniliformin and fusarin C were used as standards. The developing solvent systems used were methanol - chloroform (v/v 3 : 97), ethyl acetate-hexane (v/v, 70 : 30), ethanol - chloroform (v/v, 5 : 95) and toluene - acetone - methanol (v/v/v, 50 : 30 : 20). The developed plates were then viewed under UV light (254 and/or 366 nm) and sprayed with reagents for identification according to Gimeno (1976) and Vesonder (1986).

Determination the effect of Dimethoate insecticide on mycelial growth and Go-T activity. The effect of the insecticide Dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate, IUPAC) on mycelial growth and Go-T (glutamic-oxaloacetic transaminase) activity were studied using most commonly occurring fungal species namely; *Aspergillus flavus*, *A. niger*, *Fusarium moniliforme* and *Torula herbarum*. Fifteen Erlenmeyer flasks (250 *ml*), each containing 50 *ml* Czapeck's-Dox liquid medium, were used for each fungus. Triplicate flasks served as control, in which media were amended with 25, 75, 150 or 200 ppm of the insecticide. Each flask was inoculated with 1 *ml* of the spore suspension obtained from seven days old cultures (Czapeck's-Dox medium) of the required fungus. The flasks were then incubated at 27°C for seven days, after which the mycelial filtrates were collected from the flasks by Buchner filtration using hardened filter papers, washed several times with sterile distilled water and weighted. For determination of Go-T activity, ten milligrams of the fresh mycelia were mixed and homogenized with 1.0 *ml* phosphate buffer. The extracts were clarified by centrifugation for 15 min at 8000 ×g and then analyzed for Go-T as described by Reitman and Frankel (1957) using the transaminases kit (Quimica Clinica Aplicada S.A.).

Results and Discussion

The total fungal isolates obtained from 40 decayed sugarcane (*S. officinarum*) samples using glucose or sucrose as a carbon source were listed in Table 1. These results showed that numbers of fungi were greatest on 10 g/*l* glucose medium than 40 g/*l* sucrose medium, while, the number of species isolated on sucrose medium was more diverse than that recovered on glucose medium. In this respect, 26 species belonging to 18 genera were collected from the 40 samples of sugarcane on glucose-medium, while thirty-eight species belonging to twenty-six genera were collected on the sucrose-medium. A total of 48 fungal species belonging to 32 genera were isolated from the 40 samples of sugarcane on both glucose- and sucrose-media during winter and summer seasons. Sixteen out of these species were isolated on both tested media, while 10 species were obtained only on glucose medium and 22 species were isolated only on sucrose medium. The dominant genera on the two types of media were *Aspergillus*, *Trichoderma*, *Mucor*, *Mammaria*, *Torula* and *Cephalosporium*.

Fungi recovered on glucose-agar medium. Twenty-six species belonging to eighteen genera were recovered from 40 decayed sugarcane samples in water canal on glucose-Czapek's agar at 25°C (Table 1). *Aspergillus* was the dominant genus representing 95% of the samples constituting 45.8% of the total number of fungi. It was represented by 7 species of which *A. flavus* (8.78%), *A. niger* (30.4%), and *A. ustus* (4.4%) were of high occurrence. These *Aspergillus* species were also recovered from sugarcane leaves, stem, bagasse and juice by Higgy *et al.* (1977), Sandhu and Sidhu (1980), Sandhu *et al.* (1980), Olufolaji (1986), Sivanesan and Waller (1986), Muhsin and Abdel-Kader (1995), and Abdel-Hafez *et al.* (1995).

The remaining *Aspergillus* species were of moderate to rare occurrence on 20~5% of the samples. These species namely, *A. awamori* (1.1% of the total number of fungi), *A. terreus* (0.6%), *A. wentii* (0.4%) and *A. japonicus* (0.2%). *Trichoderma*, *Emericella*, and *Torula* were the second in occurrence. These were recovered from 85%, 75% and 90% of the tested samples and represented by

Table 1. Fungi isolated from sugarcane on 10 g/*l* glucose and 40 g/*l* sucrose Czapeck's agar media

Genera and species	Glucose-medium				Sucrose-medium			
	TC	TC %	F %	Season*	TC	TC %	F %	Season*
Aspergillus								
A. awamori Nakazawa (Usami)	16	1.1	20.0	W&S	28	2.5	40.0	W&S
A. niger Van Tieghem	433	30.4	95.0	W&S	286	25.6	85.0	W&S
A. ustus (Bain.) Thom & Church	63	4.4	70.0	W&S	8	0.7	20.0	W
A. flavus Link	125	8.8	80.0	W&S	61	5.5	60.0	W
A. versicolor (vuill.) Tiraboschi					19	1.7	20.0	W&S
A. oryzae (Ahlb) Cohn					4	0.4	10.0	W
A. terreus Thom	8	0.6	15.0	W	6	0.5	20.0	W
A. terricola Marchal					2	0.2	5.0	W
A. wentii wehmer	5	0.4	10.0	W				
A. japonicus Saito	2	0.1	5.0	S				
Apodachlya brachynema (Hildebrand)	9	0.6	10.0	W				
Achlya megasperma (Humphrey)	4	0.3	10.0	S	2	0.2	5.0	S
Achlya americana (Humphrey)	2	0.1	5.0	S				
Allomyces macrogynous (Emerson & Willson)	5	0.4	10.0	S				
Acremonium furcatum F.et V.Moreau					2	0.2	5.0	W
Curvularia tetramera (Mckinney) Boedijn	2	0.1	5.0	S	21	1.9	15.0	W&S
Cunninghamella elegans Lendner	8	0.6	10.0	W	17	1.5	30.0	S
Cladosporium herbarum Link ex Fr.					28	2.5	30.0	S
Colletotrichum dematium (Pers.ex Fr.)	56	3.9	45.0	S	6	0.5	10.0	W
Cephalosporium curtipes (Saccardo)					16	1.4	25.0	W&S
Emericella nidulans (Edam.) Vuill	169	11.9	75.0	W&S	28	2.5	25.0	W&S
Eurotium chevaliere Mangin	21	1.5	40.0	W				
Fusarium moniliforme Shled.					14	1.3	25.0	W
Mammaria echinoeotryoides Cesati					29	2.6	40.0	W
Mortierella polycephala Coemans			-		2	0.2	5.0	W&S
Melanospora fallax Zukal	9	0.6	20.0	W	4	0.4	5.0	W
Moncillium mucidum W. Gams	6	0.4	10.0	S				
Mucor circinelloides Van Tiegh					23	2.1	20.0	S
M. heimalis Wehmer	20	1.4	40.0	W&S	83	7.4	50.0	S
M. plumbeus Bon.					2	0.2	5.0	W
M. racemosus Fres.					85	7.6	70.0	S
Gliomastix cerealis (Kart.) Dickinson	14	1.0	20.0	W				
Gymnoascus reessii Baran.					2	0.2	5.0	W
Torula herbarum Link ex Fr.	113	7.9	90.0	W&S	55	4.9	20.0	W
T. grisea Szilvinyi					7	0.6	10.0	W
Trichoderma viride Pers.ex Fr.	276	19.4	85.0	W&S	180	16.1	80.0	W&S
Stemphylium piriforme Wallroth					10	0.9	10.0	S
Syenecephalostrum racemosum (Schroeter)	10	0.7	10.0	W				
Saccharomyces spp.					39	3.5	30.0	W&S
Penicillium luteum (Zukal)					12	1.1	20.0	S
Pilobolus sp Van Tieghem.	26	1.8	35.0	W&S				
Pythium intermedium (de Bary)	19	1.3	30.0	W&S	16	1.4	20.0	W
Py. aphanidermatium (Drechsler & water house)					2	0.2	5.0	W
Verticillium tenerum Link					4	0.4	10.0	S
Rhizopous stolonifer (Fhrenb) Lindat	3	0.2	5.0	W	5	0.5	10.0	W
Humicola fusco-atra Traaen					4	0.4	5.0	W
H. grisea Traaen					4	0.4	5.0	W
Helminthosporium sativum (Pammel, King and Bakke)					2	0.2	5.0	S

TC: total counts, TC%: percentage of total counts, F%: frequency of occurrence.
*S=summer and W=winter.

high occurrence by 19.4%, 11.9% and 7.9% of the total fungal, respectively. Each was represented by one species namely, *Trichoderma viride*, *Emericella nidulans* and *Torula herbarum*. *Colletotrichum dematium* was of high occurrence, it appeared in 45% of the sugarcane samples and constituted 3.93% of the total fungal; this agrees with Brinker and Seigler (1991). *Eurotium chevaliere*, *Mucor heimalis*, *Pythium intermedium* and *Pilobolus* sp. were of

come moderate occurrence (40%, 40%, 30% and 35% of the samples) and constituted 1.5%, 1.4%, 1.3% and 1.8% of the total fungi, respectively. Each of *Gliomastix cerealis* and *Melanospora fallax* were of low occurrence (20% of the samples), matching 1.0% and 0.6% of total fungi, respectively.

The remaining species were of rare occurrence (5~10% of the samples) constituting 0.7~0.1% of the total fungi. These species were *Apodachlya brachynema*, *Achlya megasperma*, *A. americana*, *Allomyces macrogynous*, *Curvularia tetramera*, *Cunninghamella elegans*, *Moncilium mucidum*, *Synecephalostrum racemosum* and *Rhizopous stolonifer*. Several researches reported that some strains of these fungi produced several toxic metabolites (Debeaupuis and LaFont, 1985; Charles *et al.*, 1979; Stinson, 1985; Megalla *et al.*, 1985; Leitao *et al.*, 1989).

Fungal genera and species recovered on sucrose agar. Thirty-eight species belonging to twenty-six genera were recovered from 40 sugercane samples on 40 g/*l* sucrose-Czapek's agar at 25°C (Table 1). Abdel-Hafez *et al.* (1995) isolated 46 species and 2 varieties belonging to 20 genera from 50 sugarcane juice samples on glucose-, sucrose- and cellulose-Czapek's agar at 28°C. The dominant genera were *Aspergillus* (8 species), *Trichoderma* (1 species), *Mucor* (4 species), *Torula* (2 species) and *Cladosporium* (1 species). They occurred in samples at rates 0.5~85.0% of the total samples investigated. In this respect, Abdel-Sater and Sabah Saber (1999) recorded that *Aspergillus*, *Eurotium* and *Penicillium* were the most common genera in dried raisins using 20% sucrose-Czapek's agar at 28°C. These results almost agree with the findings of Abdel-Sater and Ismail (1993), Megalla *et al.* (1985), Ismail (1993) and Aran and Eke (1987) who noted that *Aspergillus* and *Penicillium* were the most common in Egyptian and Turkish foodstuffs, respectively. Of the *Aspergillus*, the most dominant species were *Aspergillus awamori*, *A. niger*, *A. ustus*, *A. flavus*, *A. versicolor*, *A. oryzae* and *A. terreus*. *Trichoderma* (80% of the samples) was second to *Aspergillus* and was represented by one species namely, *T. viride* which constitutes 16.1% of the total count of the isolates. *Mucor* came third and it was represented by four species namely, *M. circinelloides*, *M. heimalis*, *M. plumbeus*, and *M. racemosus* constituting 0.2~7.6% of the total fungi recovered (Table 1). *Torula* was represented by two species namely, *T. herbarum* and *T. grisea* constituting 4.9% and 0.6% of the total fungi recovered, respectively. *Cladosporium herbarum*, *Cunninghamella elegans*, *Saccharomyces* spp., *Fusarium moniliforme* and *Cephalosporium curtipes* were of moderate occurrence, they comprised 1.25~3.49% of the total fungi recovered. The following genera namely, *Curvularia tetramera*, *Mammaria echinoeotryoides*, *Penicillium luteum* and *Pythium intermedium* were represented by low occurrence and constituting 1.1~2.3% of the total fungi recovered. The remaining genera and species were represented by rare occurrence (5.0~10.0%); with a frequency of 0.1~0.8%. *Saccharomyces* spp appeared only on the sucrose medium.

Seasonal fluctuation of the fungal species. The results given in Table 1 showed that 26 species appeared on glucose medium in both summer and winter seasons. The seasonal fluctuation of these species on glucose medium revealed that 9 species appeared in winter, 7 species in summer and 10 species in both seasons. On the other hand, 38 fungal species were recovered on sucrose medium in both summer and winter seasons. 19 species of them were isolated in winter, 10 species in summer and 9 species isolated in both seasons on sucrose medium. Generally, 48 fungal species were recovered from the two seasons on the two tested media. Nineteen out of these species were isolated only in winter and 12 species were isolated only in summer, while 17 species were isolated in both seasons. Seasonal fluctuations of fungi were also studied by El-Hissy (1979), El-Hissy *et al.* (1982) and Steciow (1998).

Mycotoxin production. *Fusarium moniliforme* produced zearalenone and diacetoxyscirpenol toxins (Table 2). These results are in agreement with those of Basch and Mircua (1992). *Fusarium* was recorded as zearalenone producer in Egypt (El-Maraghy, 1984; El-Kady and El-Maraghy, 1982; El-Maghraby and El-Maraghy, 1988; El-

Table 2. Visual estimation of mycotoxins in sugarcane samples

Fungal species	Toxin production					
	Aflatoxins				Zearalenone	Diacetoxy-scripenol
	B_1	B_2	G_1	G_2		
Fusarium moniliforme	−	−	−	−	+	+
Aspergillus flavus	+	+	+	+	−	−
A. niger	−	−	−	−	−	−
A. ustus	−	−	−	−	−	−
Emericella nidulans	−	−	−	−	−	−
Colletotrichum dematium	−	−	−	−	−	−

Table 3. Mycelial fresh weight and GO-T content in the presence of different concentrations of Dimethoate

Conc. (ppm)	*A. flavus*		*A. niger*		*F. moniliforme*		*T. herbarum*	
	Fresh weight (mg)	GOT μl	Fresh weight (mg)	GOT μl	Fresh weight (mg)	GOT μl	Fresh weight (mg)	GOT μl
0.0	4.1	14.0	5.7	8.0	8.0	8.0	7.2	14.0
25	3.8	10.0**	4.0	9.0	5.4*	11.0**	4.7*	26.0**
75	2.6**	10.0**	3.1**	11.0**	4.3**	9.5*	4.0**	12.0
150	1.8**	9.0**	2.0**	9.5**	2.3**	9.5*	2.0**	8.5*
200	1.1**	8.5**	0.9**	9.0	1.5**	7.0	1.1**	6.0**
LSD 0.05	1.13	1.90	1.60	1.01	2.26	1.35	2.10	5.29
0.01	1.48	2.49	2.10	1.32	2.96	1.77	2.75	6.93

*, **: Significant and highly significant values as compared with the control treatment.

Maghraby *et al.*, 1995). Thin layer chromatography analysis revealed the significant amounts of aflatoxins B_1, B_2, G_1 and G_2 in the tested samples produced by *A. flavus* (Table 2). These aflatoxins particularly B_1 are associated with acute poisoning of animal and human (Jukes, 1978), lack of appetite, weight loss, unthrifitness, neurological abnormalities, jaundice of mucous membrane, convulsions and death (Harwig and Munro, 1975), causes damage of chromosomes (El-Zawahri *et al.*, 1977) and carcinogenic for human liver (Smith and Moss, 1985). Based on visual estimation, when the sugarcane samples were subjected to aflatoxin screening, *A. niger*, *A. ustus* and *Emericella nidulans* were not toxin producers. Similarly, the isolated *Colletotrichum dematium* was also not toxin producer. Brinker and Seigler (1991) isolated piceatannol as a phytoalexin from the infected sugarcane with *Colletotrichum falcatum* but not from healthy or wounded sugarcane.

Effect of Dimethoate insecticide on mycelial growth and Go-T activity. Table 3, Figs. 1 and 2 indicate the effect of different concentrations of the insecticide Dimethoate on mycelial growth and Go-T activity in *A. flavus*, *A. niger*, *F. moniliforme* and *T. harbarum*. Generally, the growth of mycelium decreased with the increase in Dimethoate concentrations in all tested fungi. Although 25 ppm was less effective than the higher levels of the insecticide, highly significant decrease in mycelial fresh weight was observed at 75~200 ppm of Dimethoate in all studied fungi, as compared with the control treatment. At low level (25 ppm) of Dimetoate, mycelial growth decreased significantly in *F. moniliforme* and *T. herbarum* while the reduction in mycelial growth of *A. flavus* and *A. niger* did not statistically differ from the control treatment. The results revealed that the fungal growth decreased with the increase in pesticide concentrations, which comes in agreement with Abd-Elaah (1993) who found that Dimethoate sharply reduced the growth of *Saprolegnia ferax*, *Achlya proliferoides* and *Dictyuchus sterilis*. The effect of insecticides on the inhibition of mycelial dry weight of *Aspergillus fumigatus* and *Fusarium moniliforme* was also observed by El-Hissy and Abdel Kader (1980). They reported that the rate of inhibition to be also influenced by the type of the fungus, age of the mycelium and concentration of the pesticides.

The results indicated that the Dimethoate treatments stimulated the Go-T activity in *A. niger* and inhibited it in *A. flavus*, as compared with the respective control values (Table 3 and Fig. 2). The activity of Go-T in *F. moniliforme* was higher than that of the control at 25~150 ppm of the insecticide, while it was inhibited at the higher level (200 ppm). In *T. herbarum*, the lowest level of Dimethoate (25 ppm) greatly stimulated the Go-T activity,

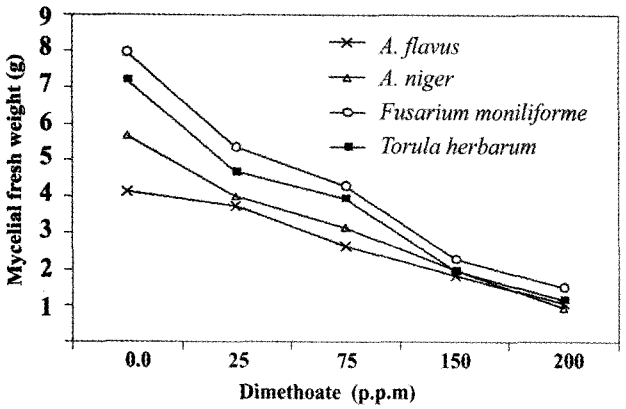

Fig. 1. The effect of Dimethoate on mycelial growth.

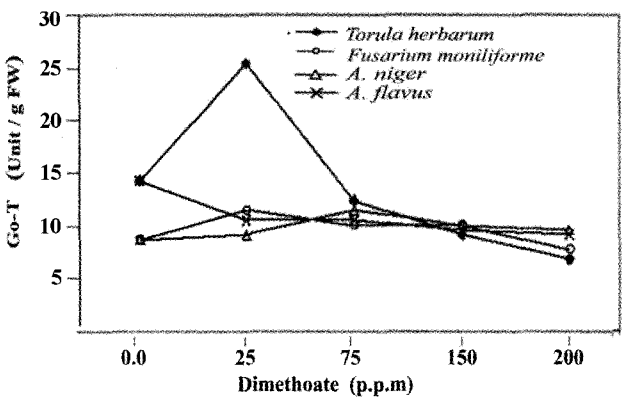

Fig. 2. The effect of Dimethoate on Go-T content.

while its activity decreased with the increase of Dimethoate concentration. The above results revealed that the insecticide Dimethoate stimulated the activity of Go-T in *A. niger*, *F. moniliforme* and *T. herbarum* especially at low doses. The inhibitory effect was prominent in case of *A. flavus*, indicating that this fungus was more sensitive to this insecticide than the other tested fungi. Audus (1960) suggested that microorganisms can develop the ability of degrade pesticides either by enzyme induction or by mutation. Abd-Elaah (1993) found that the activity of Go-T increased in *Saprolegnia ferax* and *Dictyuchus sterilis* by the application of the insecticide Dimethoate and the herbicide Basta.

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