EFFECT OF THREE PESTICIDES ON NITROGEN CONTENT OF SOME SOIL FUNGI

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SUMMARY: Nitrogen fractions such as amino-N, peptide-N, ammonia-N, total soluble-N, protein-N as well as total-N of six fungal species exposed to three pesticides were measured. The fungicide Afugan (Pyrazofos) induced a significant increase in the total-N of Trichoderma harzianum and Fusarium solani whereas a significant decrease was observed with Aspergillus niger and Penicillium chrysogenum. Certain doses of the herbicide Brominal (Bromoxynil) caused a significant decrease in the total-N of A. niger, F. solani, T. harzianum and Mucor racemosus. Total-N of Stachybotrys chartarum showed a significant increase in response to Brominal. The insecticide Selecron (Profenfos) caused a significant reduction in the total-N of p. chrysogenum. Other nitrogen fractions showed different responses depending on the type and dose of the pesticide as well as on the fungal species itself.

Key Words : Pesticides, fungi.

INTRODUCTION

The selective effects of different pesticides on microorganism has received a great deal of attention. Information of growth, respiration, cellulose degradation, production of proteins, RNA, DNA as well as other biological activities of fungi subjected to certain doses of fungicides, herbicides or insecticides were given by several investigators (1-4). Afugan (Pyrazophos) is a member of organophosphate fungicides which include also Kitazin, Edifenfos, Risolex etc. As reported by Smit (5) Afugan proved to have systemic, preventive and curative action against a number of powdery mildew fungi including cereals and cucumbers. De Waard (6) found that Pyricularia oryzae and Colletotrichum lindemuthianum were sensitive to Pyrazophos. he also showed that in sensitive fungi Pyrazophos is metabolically converted into two fungitoxic products viz, the phosphate analogue of pyrazophos (PO-Pyrazophos) and 2-hydroxy-5-methyl-6-ethoxy carbonylpyrazole (1,5-a) pyramidine (pp). The latter compound which is believed to be fungitoxic principle, inhibits growth of p. oryzae at pH 5. Brominal (Bromoxynil) is one of the soil through pre-sowing, pre-emergence treatments where they may alter the soil flora and may even affect the other microbial processes adversely of otherwise (7). This herbicide was found to inhibit mycelial growth of Phymatotrichum omnivorum (8). Among the organophosphate insecticides, Selecron (Profenfos) is commonly used in Egypt for controlling the cotton leaf worm. This insecticides was found to be inhibitory to cellulose production, extra-cellular protein as well as mycelial protein of A. niger, Nectria haematococca and T. harzianum.

In this investigation trials were made to shed some lights on the influence of Afugan, Brominal and Selecron on the different nitrogen fractions of six fungal species commonly recovered from Egyptian soil.

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Table 1: Effect of various doses of Afugan on nitrogen contents (μg-N/mg dry weight) of test fungi.

Nitrogen fractions								
Species	Doses μg ml^{-1}	Amino-N	Peptide-N	Ammonia-N	Other Soluble-N	Total Soluble N (A)	Insoluble protein-N (B)	Total-N (A+B)
Aspergillus niger	0	2.15	22.80	0.26	1.41	26.62	6.30	32.92
	3	2.55	15.00*	0.24	1.18	18.97*	6.30	25.27*
	18	1.57	4.11*	0.34	3.79*	9.81*	7.50*	17.31*
Fusarium solani	0	8.40	5.90	1.90	0.35	16.55	14.50	31.05
	3	2.70*	8.60	0.22*	2.78*	14.30*	12.90*	27.20
	18	5.15*	16.55*	0.22*	4.38*	26.30*	12.95*	39.25*
Mucor racemosus	0	4.75	12.80	2.25	0.20	20.00	19.10	39.10
	3	3.35*	11.55	0.18*	7.39*	22.47	18.45	40.92
	18	2.85*	12.80	0.69*	2.13	18.41	20.15	38.62
Penicillium chrysogenum	0	7.70	21.25	1.50	5.20	35.65	20.70	56.35
	3	7.80	15.33*	1.80*	0.85	25.78*	9.90*	35.68*
	18	4.90*	7.55*	1.10*	1.90	15.45*	10.55*	26.00*
Stachybotrys chartarum	0	5.12	9.30	0.68	5.06	20.16	11.50	31.66
	3	3.36*	12.85*	0.57	3.82	20.60	9.10	29.70
	18	3.40*	9.50	0.22*	8.37*	21.49	7.45*	28.94
Trichoderma harzianum	0	1.65	13.50	0.56	0.79	16.50	15.20	31.70
	3	2.40*	20.55*	0.24	1.04	24.23*	19.65*	43.88*
	18	3.10*	18.75*	0.41	5.22	27.48*	13.15*	40.65*

* means significant difference from the control at 5% level. $No^{-}3$-N and $NO^{-}2$-N were undetectable in all treatments for all fungi.

MATERIALS AND METHODS

The pesticides used: The fungicides Afugan is commonly known as Pyrazophos with 30% active ingredients (a.i.). Chemically it is 0,0-diethyl 0-(6-ethoxy-carbonly-5-methyl-pyrazolo [1,5-a] pyrimidin-2-yl)phosphorothioate. The herbicide Brominal is also known as Bromoxynil or Labuctril-25 with 24% a.i. The chemical name is 3,5-dibromo-4-hydroxybenzonirile. Afugan and Brominal were manufactured by Hoechst Orient S.A.A. Cairo R.C.C. 106526 under license of Hoechst AG Frankfurt (Main) Germany. Selecron (Profenfos) is chemically known as 0-(4-bromo-2-chloro-phenyl)0-ethylS-n-propyl-phosphorothioate. The product contains 72% a.i. and is manufactured by Ciba Geigy Limited, Basel, Switzerland. From each pesticide two concentrations were prepared and added to the liquid culture medium to give final concentrations of 3.0 and 18.0 μg a.i. ml^{-1} in case of Afugan, 5.4 and 32.4 μg ml^{-1} with Brominal, and 6.4 and 38.4 μg ml^{-1} with Selecron. These concentrations are equivalent to the recommended field dose and six times field dose except with Afugan where it was incorporated in lower concentrations since the recommended field dose completely eliminated all fungi.

Organisms and culture medium: Six fungal species frequently recovered from soil treated with different doses of pesticides (9) were used and these were Aspergillus niger VAN TIEGH, Fusarium solani (MARTIUS APPEL AND WOLL., Mucor racemosus FRESS, Penicillium chrysogenum THOM, Stachybotrys chartarum (ENRENB. ex LINK) HUGHES and Trichoderma harzianum RIFAI. These fungi were maintained on modified Czapek's-Dox agar of the following composition (g/1): Sucrose 20; KNO_3, 4; NaH_2PO_4, 2; $MgSO_4.7H_2O$, 1; KCI, 0,5; $FeSO_4$, 0.1 and agar, 15. Liquid Czapek's-Dox medium (without agar) was prepared and distributed in 100 ml conical flasks (20 ml/flask). After autoclaving and cooling, the pesticides were incorporated with the doses described before. Each flask was seeded by 1 ml of fungal spore suspension from 7 days old cultures containing 200 colony forming units (CFU). Control flasks (free of pesticides) were also prepared in the same manner. Cultures were set up in duplicates and were incubated at 28°C for 7 days.

Determination of mycelial dry weight: For control and treated cultures, fungal mycelia were harvested by filtration through Whatman No.1 filter paper under low pressure vacuum, washed thrice with distilled water and dried at 80°C for 24 hours and weighed.

Table 2: Effect of various doses of Brominal on nitrogen contents (μg-N/mg dry weight) of test fungi.

Nitrogen fractions								
Species	Doses μg ml^{-1}	Amino-N	Peptide-N	Ammonia-N	Other Soluble-N	Total Soluble N (A)	Insoluble protein-N (B)	Total-N (A+B)
Aspergillus niger	0 5.4 32.4	3.37 3.17 2.20	18.90 10.25* 8.05*	0.24 0.25 0.29	1.65 2.20 3.88	24.52 15.87* 14.42*	6.40 12.95* 9.95*	32.92 28.82 24.37*
Fusarium solani	0 5.4 32.4	8.85 7.20 9.95	5.60 7.70 5.60	0.22 0.57* 0.22	2.28 1.84 2.48	16.95 17.31 18.25*	14.35 14.15 8.70*	31.30 31.46 26.95*
Mucor racemosus	0 5.4 32.4	4.75 3.80* 4.05	12.80 6.35* 7.65*	2.25 1.35 1.00*	0.20 0.20 0.25	20.00 11.70* 12.95*	19.10 13.60* 13.90*	39.10 25.30* 26.85
Penicillium chrysogenum	0 5.4 32.4	4.42 4.65 6.55*	6.50 9.12* 11.90*	0.24 0.65* 0.55*	5.14 2.37* 3.48*	16.30 16.79 22.48*	14.55 8.40* 13.75*	30.85 25.19* 36.23*
Stachybotrys chartarum	0 5.4 32.4	5.12 5.14 5.17	9.30 13.00* 18.70*	0.68 0.59 0.59	4.91 1.28* 6.10	20.01 20.01 30.56*	11.50 15.80* 19.55*	31.51 35.81* 50.11*
Trichoderma harzianum	0 5.4 32.4	1.65 6.04* 6.70*	13.50 5.50* 4.80*	0.57 0.79 0.79	0.79 4.98* 2.71*	16.51 17.31 15.00*	15.20 12.40* 13.15*	31.71 29.71 28.15*

* means significant difference from the control at 5% level. $No^{-}3$-N and $NO^{-}2$-N were undetectable in all treatments for all fungi.

Nitrogen analysis of dried mycelium: According to El-Abyad (10), 50 mg dried fungal mycelium from cultures subjected to pesticide treatments were homogenated in 5 ml borate buffer (pH 8). The homogenate was transferred using small amount of distilled water to small beakers and kept standing overnight before being filtered. The residue was dried at 80°C and the filtrate was made up to 50 ml. The filtrates were employed for estimating the soluble-N fractions ($NH^{+}4$-N, $NO^{-}3$-N, $NO^{-}2$-N), soluble peptide-N, amino-N, other soluble-N (amide-N and acyclic-N) and total soluble-N, whereas the insoluble residues were used for estimation of the protein contents of mycelia.

Estimation of soluble-N fractions: The method used for estimation of $NH^{+}4$-N was Nessler's reagent (11). $NO^{-}3$-N was determined by the phenoldisulphonic acid method (12). $NO^{-}2$-N was determined colorimetrically using sulfanilic acid reagent as described by (13). Peptide-N was estimated according to the method adopted by Lowry *et. al.* (14) and described by Plumner (15) using Folin-ciocalteau reagent, and the calibration curve was obtained using bovine serum albumin as a protein. Measurements were carried out at 750 nm and the peptide-N was calculated by multiplying the obtained values of protein by 16% (16). Amino-N was estimated according to Lee and Takahashi (17) using stannous chloride reagent. The blue violet color was measured at 570 nm and the calibration curve was mad using isoleucine as amino-acid. According to El-Abyad (10), other soluble-N fractions, including the amide-N and acyclic forms (purines and pyrimidines) were calculated by subtracting the summesion of peptide, amino, nitrate, nitrite and ammonia nitrogen from the value of total soluble-N (estimated total soluble-N+$NO^{-}3$-N+$NO^{-}3$-N). The insoluble protein-N was determined by refluxing the dried residue of the homogenated fungal mycelium with 5 ml 5-N-NaOH in a boiling water bath for 5 hours (18). The hydrolysate was made slightly acidic using 2.5 ml 10 $N^{-}H2SO4$ filtered and made up to 50 ml and the amino acid nitrogen of the hydrolysate was estimated as described before. The value of insoluble protein-N to that of soluble nitrogen fractions.

RESULTS AND DISCUSSION

With reference to the data in Table 1 it could be observed that the total nitrogen content of A. niger and

Table 3: Effect of various doses of Selecron on nitrogen contents (μg-N/mg dry weight) of test fungi.

Nitrogen fractions								
Species	Doses μg ml^{-1}	Amino-N	Peptide-N	Ammonia-N	Other Soluble-N	Total Soluble N (A)	Insoluble protein-N (B)	Total-N (A+B)
Aspergillus niger	0 6.4 38.4	3.40 1.80* 1.80*	18.90 14.50* 13.40*	0.24 1.10* 0.90*	1.70 3.40 0.60	24.24 20.80* 16.70*	6.40 6.70 8.70*	30.64 27.50* 25.40*
Fusarium solani	0 6.4 38.4	8.80 6.80* 5.30*	5.60 9.10* 14.20*	0.22 0.55* 1.00*	2.28 2.47* 3.90*	16.90 18.92 24.40*	14.35 11.65* 5.25	31.25 30.57 29.65
Mucor racemosus	0 6.4 38.4	4.75 4.60 2.40*	12.80 8.85* 11.80	2.25 1.60* 0.50*	0.20 3.75* 3.50*	20.00 18.80 18.20	19.10 19.25 20.90	39.10 38.05 39.10
Penicillium chrysogenum	0 6.4 38.4	4.40 3.90 7.80*	6.50 12.10* 7.30	0.23 0.22 0.22	5.20 4.10 3.20	16.33 20.32* 18.52*	14.60 12.10* 13.40*	30.93 32.42* 31.92*
Stachybotrys chartarum	0 6.4 38.4	5.10 1.50* 1.19*	9.30 19.20* 12.60*	0.68 0.50* 0.23*	4.90 0.45* 0.48*	19.98 21.65 14.50*	11.50 8.60 13.05	31.48 30.25 27.55*
Trichoderma harzianum	0 6.4 38.4	1.65 7.78* 3.89	13.50 9.65* 12.85	0.56 0.57 0.89	0.78 3.96* 2.46*	16.50 21.96* 20.89*	15.20 11.15* 12.90*	31.70 33.11 32.99

* means significant difference from the control at 5% level. $No^{-}3$-N and $NO^{-}2$-N were undetectable in all treatments for all fungi.

P. chrysogenum was significantly retarded by the two doses of the fungicide Afugan while that of F. solani and T. harzianum was accelerated by the high dose and the two dose respectively. The total nitrogen content of M. racemosus and S. chartarum was not significantly affected by any dose. Ismail *et. al.* (19) studied the effect of Dupond Benlate (10-1000 ppm) on growth parameters, protein and nucleic acids content of A. fumigatus, Verticillium agaricinum, Helminthosporium oryzae and F. oxysporum f. sp. lycopersici. Their results showed that the fungicide induced an increase in total protein production in all fungi except H. oryzae which showed an increase in RNA content only and concentrations of 10,100 and 200 ppm. On the other hand, they found that DNA content decreased with the increase with Pyricularia oryzae, Yoshinaga *et. al.*, Maeda and coworkers and Misato Kakiki (20-22) found that the growth inhibition concentration of Kitazin (50 ppm) did not interfere with protein and nucleic acid synthesis of the tested fungus. Accordingly, it could be suggested that each type of fungicides has its own specific effect on certain metabolic activities of the sensitive fungal species.

In case of the herbicide Brominal (Table 2), the quantity of peptide-N, total soluble-N and total-N of A. niger was significantly decreased whereas that of insoluble protein-N was increased. Ammonia-N and total soluble-N of F. solani was promoted but insoluble protein-N and total-N was significantly inhibited. In case of M. racemosus, the amount of the different nitrogen fractions was significantly retarded at least by one dose except the amount of other soluble-N (amide-N, purines and pyrimidines) which was not significantly affected. Nitrogen analysis of dried mycelium of P. chrysogenum showed that insoluble protein-N as well as other soluble-N were significantly inhibited by the two doses whereas peptide-N and ammonia-N were significantly promoted by the two doses, and amino-N as well as total soluble-N were also increased by the high dose. Moreover, the total-N of P. chrysogenum

was significantly inhibited by the low dose and promoted by the high dose.

In case of S. chartarum, Brominal was promotive to peptide-N, total soluble-N, insoluble protein-N and total-N but it was inhibitory to other soluble-N.

With the exceptions of amino-N of T. harzianum which increased with the two doses of Brominal, the different nitrogen fractions as well as insoluble protein-N and total-N showed mostly a significant inhibition after treatment. In this respect, Schroder *et. al.* (23) found that nucleic acid synthesis was increased by 140% when Neurospora crassa was grown in media containing 220 ppm 2,4-D. They also reported that protein synthesis in N. crassa was stimulated in the presence of 216 ppm Atrazine. El-Abyad (10) studied the effect of the herbicide Prometryn on metabolic activities of two Fusarium wilt fungi. They found that Prometryn at rates of 128 and 256 ppm significantly reduced the rate of synthesis of carbohydrates and organic nitrogenous compounds by both fungi. They also concluded that protein synthesis was inhibited on the expense of increased total soluble-N that existed mainly in the form of amino-N. On the other hand, inactivation of protein synthesis induced accumulation of amino acids and peptides. Osman *et. al.* (24) reported that the influence of Triazines (Glotix and Igran) on total protein and nucleic acids of A. fumigatus, F. oxysporum f. sp. vasinfectum, H. oryzae and decreased in F. oxysporum and V. agaricinum after treatment with Glotix. Igran was stimulatory to the nucleic acid content of the tested fungi in low concentration and was mostly inhibitory in high levels (1000 ppm).

Nitrogen contents of fungi grown in media treated with 6.4 and 38.4 $\mu g\ ml^{-1}$ of the insecticide Selecron showed different responses (Table 3). Most nitrogen fractions of A. niger showed a significant decrease with both doses of Selecron. The significant increase was observed in ammonia-N and protein-N. In case of F. solani, amino-N and protein-N were significantly reduced whereas peptide-N, ammonia-N and other soluble-N were significantly promoted. The amino-N, peptide-N and ammonia-N of M. racemosus exhibited a significant decrease by both or either doses of Selecron. The promotive response was only observed with other soluble-N. Most nitrogen fractions of P. chrysogenum were significantly increased whereas protein-N was decreased. Nitrogen contents of S. chartarum were mostly retarded at least by the high dose and the exception was peptide-N which showed an increase by both doses of Selecron. In case of T. harzianum, our results showed an increase in amino-N, other soluble-N and total soluble-N after Selecron treatment whereas peptide-N and insoluble protein-N were significantly inhibited. Reports dealing with the effects of insecticides on nitrogen content of fungi are very few. Bushway, Hanks (25) found that Dieldrin at 5 ppm inhibited RNA, DNA and protein synthesis in Dictyostelium discoideum cultures exposed to the insecticide for one hour. More recently, Abdel-Basset *et. al.* (1) found that Selecron was inhibitory to the extra-cellular protein production by three cellulose-decomposing fungi (A. niger, Nectria haematococca and T. harzianum). They also showed that during the period of study (4-20 days), the mycelial protein of N. haematococca and T. harzianum was significantly decreased after treatment with 10 and 50 ppm of Selecron. Mycelial protein of A. niger was almost not significantly affected by the doses of Selecron.

It could be pointed out that pesticides are not always harmful to protein synthesis in fungi. Certain doses of fungicides, herbicides or insecticides increased nitrogen content of some fungi. The total-N of T. harzianum and F. solani was allowed to increase in the presence of sub-lethal doses of Afugan. This increase was coincided with the increase in peptide-N and total soluble-N. The promotive effect of Brominal was observed with S. chartarum where the amounts of peptide-N, total soluble-N as well as insoluble protein-N were significantly higher than the control. The increase in the total soluble-N of P. chrysogenum and T. harzianum after Selecron treatment was correlated with the increase of amino-N in addition to peptide-N or other soluble-N fractions.

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