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INTERACTION AMONG SOIL MYCOFLORA AMENDED WITH SEQUENTIAL APPLICATION OF THREE DIFFERENT PESTICIDES (SYSTOATE, AFUGAN, K-OTHRINE) ON DIFFERENT SOIL SAMPLES

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Abstract

The changes in soil mycoflora in response to sequentially applied pesticides (Systoate, Afugan, K-othrine) were studied at specific time intervals of soil incubation (3, 7, 14 and 21 days). Different concentrations of each pesticide were used. In sequential application of three pesticides inhibition of fungal population was observed. Systoate at lower concentration cause an increase in mycoflora but higher concentration of Systoate show inhibitory effect. Most significant decrease in fungal population was observed at 3rd, 7th and 14th day while at 21st day an increase was observed showing that negative effect subsided after two weeks. Similar is the case when K-othrine was applied but when Afugan was applied decrease in fungal colonies were observed at 3rd and 7th day but at 14th day of incubation severe decrease was observed but at 21st day of incubation again increase in mycoflora occurred. The effect of pesticide on soil mycoflora was almost similar on three soil samples collected from different fields.

From these soil samples twelve fungal species were isolated and morphologically characterized. Among these fungal species six belonged to *Aspergillus*, two to *Mucor*, two to *Penicillium*, one belonged to *Rhizopus* and one to *Helminthosporium*. Out of this *Aspergillus* was found to be more dominant and was relatively most frequent throughout incubation period.

Introduction

Soil microflora and microbial functions are affected by several physical, chemical and biological factors. One of the recent developments in the agriculture is the use of pesticides (fungicide, herbicide and insecticides). All types of soil microorganisms are susceptible to different types of interaction with pesticides and their functions are affected in different ways by different chemicals. Sharma and Sexana (1974) reported a stimulation of bacterial and fungal population due to herbicide application. Agnithori, 1974 reported the inhibition of soil fungi due to application of fungicides.

Pesticidal compounds can be toxic if they are present in high concentration, but at low concentration they are not toxic and these microorganisms have ability to decompose toxic substances. Certain pesticides stimulate growth of some bacteria (Nelson and Tisade, 1999; Tyun aeva *et al*., 1974; Alexander, 1985)

The side effects of pesticides on the soil microflora were studied by several authors (Anderson, 1978; Duah-Yentumi and Johnson, 1986; Wardle and Parkinson, 1990; Perucci *et al.*, 2000). Pesticides may affect the microbial population by controlling the survival and reproduction of individual species. On the other hand, several microorganisms were reported to degrade some pesticides (Hata *et al.*, 1986; Topp *et al.*, 2000; De Lorenzo *et al.*, 2001; Morgan and Watkinson, 1989).

Materials and Methods

Three soil samples were collected from the area of Mureed (Chakwal District). Soil sample I was taken from wheat field, sample II from Jawar field and soil sample III from Brassica field. The pesticides which were used included two insectides: K-Orthrine and Systoate while a fungicide named Afugan.

Different Physico-chemical analysis of the soil were performed like particle size analysis (Bouyoucos Method, 1962), pH (Anon., Hand Book No. 60, 1954), Water Holding Capacity (USDA Hand Book No. 60), Organic Matter Estimation(Walkley & Black, 1934) and Total nitrogen percentage (Bremner & Mulvaney, 1982).

Three sets of plastic bottles were setup each labeled as soil sample I, II and III respectively having C, T1, T2 and T3 treatment in each set. So, total 12 bottles were used in experiment. 50 g portion of soil and different dilutions of K-Othrine, Systoate and Afugan were added in each bottle sequentially, which are given below:

Control	50 g soil + 8.5ml distill water
T1	50 g soil + 2.5ml Systoate sol + 6.0 ml distilled water
T2	50 g soil + 4.5ml Afugan sol + 4.0 ml distilled water
T3	50 g soil + 8.5 ml of K-Othrine sol.

Stock solutions for all the three pesticides and their different dilutions were prepared. 1 ml from each dilution was transferred to agar Petri plates having chloramphenicol added in it. Dilution plate method (Booth, 1971) was used for fungal count. Colonies were counted considering each colony as a single organism. Total viable count was find out by the following formula:

Total viable count = Average number of colonies x Dilution factor

For Hyphea and spore structure, slants were also prepared by adding 5 ml of malt agar media in test tubes. Isolated fungal colonies were transferred on to the slants with the help of inoculating needle from the Petri plates. Conidia were taken on slide with methylene blue then observed under microscope and sketched with the help of Camera Lucida. Different fungal species were identified by the help of available literature.

Results and Discussion

The physico-chemical analysis was done for all the soil samples showing a slight difference in their physical and chemical characteristics (Table 1).

Table 1. The physico-chemical analysis of all three soil samples showed following results.

	Sample I	Sample II	Sample III
Sand	73%	72%	74%
Silt	16%	17%	17%
Clay	11%	11%	9%
Texture Class	Loamy sand	Loamy sand	Loamy sand
pH	7.85	7.90	8.10
Organic matter	0.74%	0.47%	0.068%
Nitrogen	0.037%	0.023%	0.003%
Water holding capacity	8.5ml	8.5ml	8.5ml

The tables 2, 3 and 4 showed the positive effects of Systoate on soil mycoflora as the fungal population declined as compared to control during 3-14 days of incubation than slightly increased after 21st day as compared to proceeding days.

However in the Afugan addition, during 3-7 days the number of fungal colonies increased than suddenly decreased at 14th day and at 21st day, again increase was observed. Increase in fungal population indicates the negative effect of Afugan subside at 21st day of incubation which indicated that fungal spores became resistant to pesticide with the passage of time under control environmental conditions i.e. temperature and water holding capacity (Table 5). The effect of K-Othrine showed fungal population decrease at 3rd day of incubation as compared to control than further decrease at 7th day and at 14-21 days again increase in number was observed as compared to 3rd and 7th day.

Table 2. Total viable counts for fungi in sample I amended with Systoate, Afugan and K-Othrine.

Treatments	Solutions	Amount of solutions	No of fungi X 10-4 Days of incubation			
			3 days	7 days	14 days	21 days
C	Water	8.5ml	58	60	62	70
T1	Systoate	2.5 µl/g	40	42	42	47
T2	Afugan	4.5µl/g	40	45	32	49
T3	K-othrine	8.5µl/g	30	25	29	38

Table 3. Total viable counts for fungi in sample II amended with Systoate, Afugan and K-Othrine.

Treatments	Solutions	Amount of solutions	No of fungi X 10-4 Days of incubation			
			3 days	7 days	14 days	21 days
C	Water	8.5ml	48	42	44	49
T1	Systoate	2.5 µl/g	36	38	46	50
T2	Afugan	4.5µl/g	29	36	30	37
T3	K-othrine	8.5µl/g	28	34	32	36

Table 4. Total viable counts for fungi in sample III amended with Systoate, Afugan, and K-Othrine.

Treatments	Solutions	Amount of solutions	No of fungi X 10-4 Days of incubation			
			3 days	7 days	14 days	21 days
C	Water	8.5ml	55	49	48	58
T1	Systoate	2.5 µl/g	15	35	39	48
T2	Afugan	4.5µl/g	25	29	21	38
T3	K-othrine	8.5µl/g	29	24	35	42

The increase in fungal population was observed in all the control treatments, with the increase in days of incubation. In soil sample I, No of fungi has a thorough increase with the increased days of incubation, showing highest no of fungi at 21st day. In soil sample II, the decrease in number of fungi at 7th day was observed, however, highest number of fungus was observed at 21st day of incubation. Soil Sample III showed the highest number of fungi at 21st day and the lowest number was seen on 14th day (Plates 1 and 2).

Of the 12 fungal species observed, 6 belonged to *Aspergillus*, 2 to *Mucor*, 1 belonged to *Helminthosporium*, 1 to *Rhizopus* and 2 belonged to *Pencillium*. *Aspergillus* species were present in large number. Their predominance in Pakistan's soils is often reported (Azam & Malik, 1983; Azam *et al.*, 1985; Malik *et al.*, 1982). The relative resistance of *Aspergillus* to pesticides application may also be seen in terms of their tolerance to different kinds of stresses ensuring better survival and competitive ability.

Reported literature suggests that these fungi are highly tolerant to salts (Malik *et al.*, 1982) and pH (Yasmeen & Azam, 1989). Most of the *Aspergillus* species utilizes soluble carbohydrate. *Mucor* species were also present but lesser in number because they can also use only simple carbon compounds as its carbon sources enable to utilize hemicellulose and cellulose. *Rhizopus* species were also present which decompose cellulose. *Helminthosporium* and *Pencillium* were also observed.

Table 5. Morphological characteristics and fungi isolated from soil.

Fungal species	Colony characteristics
Aspergillus sp.	Colonies were pale green in color. Conidiophore course, smooth walled and uncolored. Conidial heads clavate with spore masses sterigmate uniseriate, numerous and crowded
Aspergillus sp.	Colonies were black green almost black with white surrounding of separate branching hyphae conidiophores non septate, usually enlarging upward and broading into elliptical, hemispherical or globose vesicle bearing Philades. Philades are in two series. Conidia varying in colour and form n unbranched chain
Aspergillus sp.	Colonies were lime green in color. Conidiophore rose separately from the substratum. Conidia globose, yellowish green in color. Vegetative mycelium consists of septate hyphae.
Aspergillus sp.	The color of colonies was brownish black or almost black conidiophores hyaline to brown, typicaaly smooth and usually heavy walled vesicle globose and light to dark brown color. Sterigmata in one or two series
Aspergillus sp.	Colony blue green with the bluish effect prominent, velvety with some aerial interlacing and trailing hyphae. Conidiophores colorless, smooth and thick walled. Heads radiate or globose. Phialides in two series. Conidi globose
Aspergillus sp.	Reverse of colony purplish red conidial heads short, columner stalks with smooth wall conidia globose
Helminthosporium sp.	Colonies were velvety, greenish black, mycelium dark brown, multiseptate, conidiophores bent, terminal conidia are present in groups conidia elongate, straight round at both the ends 5 to 12 times separate are clearly visible
Mucor sp.	Colonies blackish thick walled mycelium. Sporangiophore branched containing chlamydospores. The remnants of the sporangial wall in the upper dehisced sporangium
Mucor sp.	Colonies whitish-yellow, mycelium cottony. Sporangiophore unbranched, sporangia brownish. Columella spherical, with flat base. Spores regularly ellipsoidal, hyaline smooth
Penicillium sp.	Septate branching mycelium which usually is uncolored, septate, aerial conidiophores that was perpendicular to and walled off from submerged hyphae from which they arise, branched or unbranched, brush like spore bearing heads with sterigmata born in clusters. Conidia are light brown in color
Peniciliium sp.	Colonies green aerial portion velvety. Reverse of colony was yellow. Conidiophore arises directly from the substratum, slightly swollen at apex, conidia globose green in color and smooth. (Gilman, 1957)
Rhizopus sp.	Non septate has stolons and rhizoids; sporangiophores arise at the nodes, where rhizoids also formed. Sporangia are large and usually black. Abundant cottony mycelium. No sporangioles

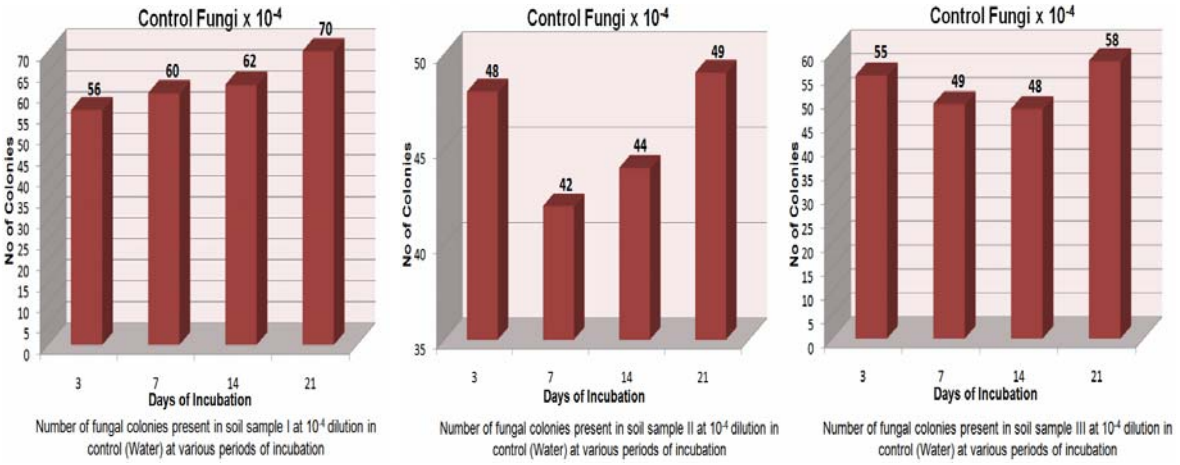

Plate 1. Fungal colonies observed in control treatment.

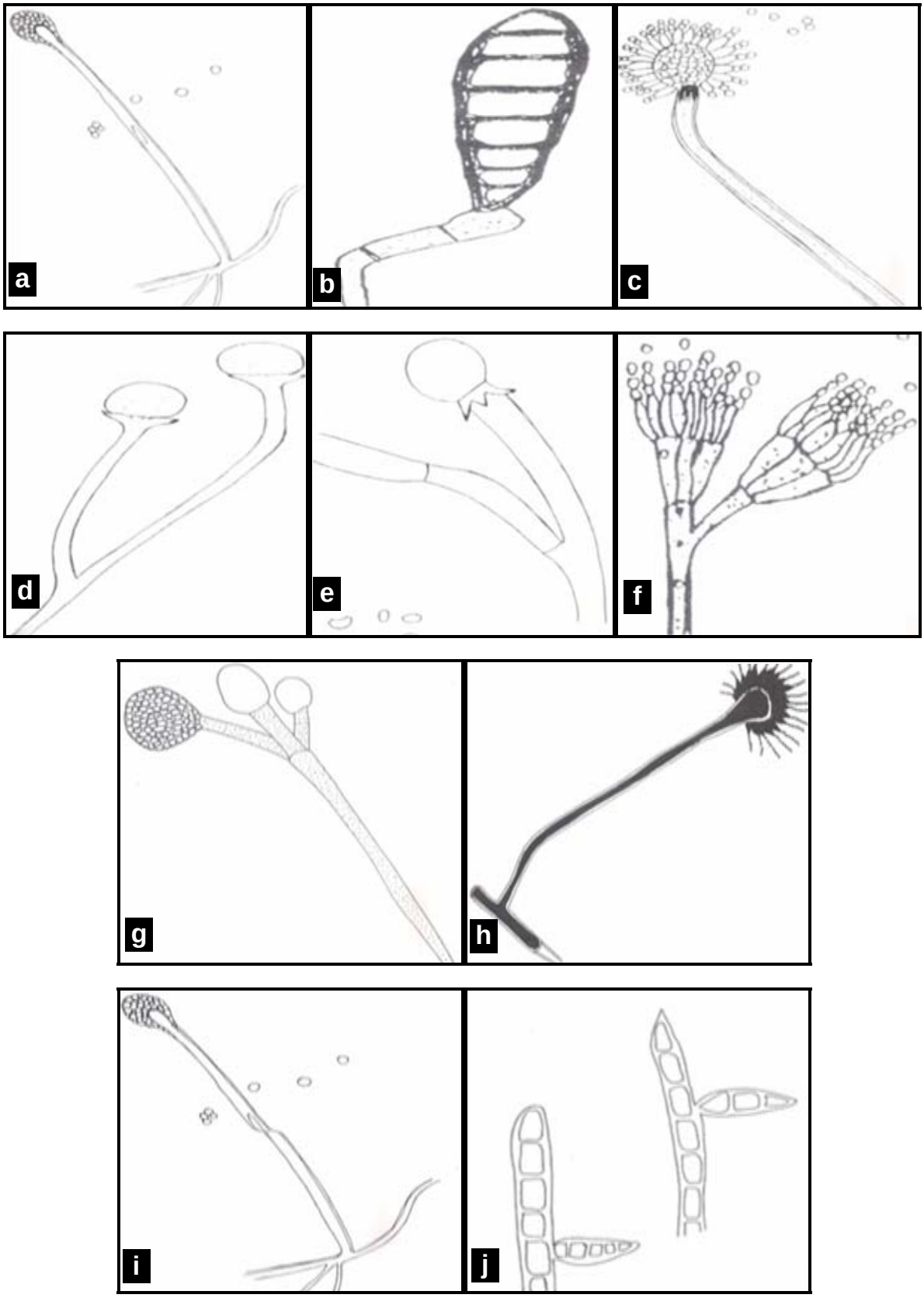

Plate 2. Fungal species isolated from the soil. **a)** *Rhizopus* sp**. b)** *Helminthosporium* sp**. c)** *Aspergillus* sp**. d)** *Mucor* sp**. e)** *Mucor* sp**. f)** *Penicillium* sp. **g)** *Mucor* sp**. h)** *Aspegillus* sp**. i)** *Rhizopus* sp**. j)** *Helminthosporium* sp**.**

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