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POTENTIAL OF USING TWO *FUSARIUM* SPECIES AND *TRICHODERMA HARZIANUM* AS BIODEGRADING FACTORS OF SOME PESTICIDES IN SOIL AND ORGANIC COMPOST

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Abstract

The aim of this research is to investigate possibility of using some fungi species including *Fusarium oxysporum*, *F. gramen* and *Trichoderma harzianum* as biodegrading agents to remove pesticide residues from soil and organic fertilizers. Five chemical pesticides were used: fungicide Bayfidan, insecticides Bulldok and Oberon, and herbicides Trayf and Mega (used in wheat fields). PDA media was treated with the pesticide product and then inoculated with the three fungi under test individually. The effect of pesticides was measured based on the velocity of the fungus growth in dishes containing pesticide poisoned medium. The results showed that pesticides Trayf and Mega were significantly less affected by the presence of any of the fungi tested, the fungal growth was highly affected showing growth inhibition rates ranged from 74% to 100%. However, the other three pesticides were degraded at various levels by the three tested fungi; they were able to survive and produce in such toxic environment.

Key words : Biodegrading factors, pesticides, organic compost, fungi, toxic environment.

Introduction

The global production of pesticides is 2.5 million tons per year with a total sail price of more than 30 billion US dollars. These quantities are treated worldwide. Numbers of chemicals that fall under the definition of pesticides are numerous to be around 100000. However, out of the total pesticide volume, only 5% or less can reach target pest in the field (Dagim, 2008). Thus, contamination, pollution and residues of toxic pesticides are still problematic and solve challenging. The newly emerged technique of biodegradation or biological treatment is promising technique. This involves using microorganisms such as bacteria and fungi to break down toxic substances and or convert them to less toxic substances by the mean of their enzymes secretion or metabolic processes (Vidali, 2001 and Nerud *et al.*, 2003).

Bioassay on the other hand is considered to be one of the important tools in detecting pesticide residues in soil, it practically may be better than chemical analysis (Al-Zemeti, 2008).

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Studies have shown that microorganisms are very important factors in degrading leading urea derivatives (Al-Zemeti, 2008). These microorganisms including bacteria such as *Bacillus*, *Xanthomonas* and *Pseudomonas* and some fungi such as *Penicillium* and *Aspergillus*, which directly use undesirable and toxic compounds as sources of energy (Al-Zemeti, 2008). Suitable environmental conditions (temperature, moisture and ventilation) help and accelerate the demolition of pesticides leading to more clean and friendly environment (Al-Zemeti, 2008).

Studies on biodegradation of pesticides and toxic substances are still fairly recent. Such studies need to have a detailed knowledge of the principles of biodegradation in order to develop efficient methods for decontamination and thus solving the problem of toxic residues that affect the biodiversity and non-target organisms, especially beneficial ones and reduce their effects on crops. This study, therefore was conducted to evaluate the by microorganisms.

Materials and Methods

Isolation and diagnosis of pesticides degrading fungi from soil and organic fertilizers

Agriculture field soil and two organic fertilizers (rice and wheat compost from Organic Fertilizer Center, Kufa) were used as well as five different pesticides including one fungicide (Bayfidan E.C.), two insecticides (Bulldok E.C. and Oberon S.C.) and two herbicides (Trayf W.G. and Mega E.C.) at commercial concentrations (without dilution). The soil and organic fertilizers were evenly distributed into 300 ml plastic pots and the pesticides degrading fungi were isolated by two methods. First, pots with soil or compost were saturated with the commercial concentration of each pesticide individually with three replicates each and stored in the laboratory condition. Three months later, the fungi were isolated from each pot using the dilution method. The second method was by immersing sterilized sticks (tooth picks) in the concentrated pesticides for each pesticide for 48 hours. Three sticks were then embedded in each the pot of each type of potting soil mix with three replicates of each treatments. The pots were also stored in the laboratory condition for three months. Later, the sticks were pulled off and cultured in 9 cm Petri dishes with P.D.A. to which the Chloramphenicol was added at 250 mg/L. The dishes were incubated at 25±2^{0}C. Three days after, the percentage of occurrence and frequency of each presented fungus was calculated (Johnsen *et al*., 2005). Isolated fungi were diagnosed in Mycology laboratory belongs to the graduate division at Faculty of Agriculture - University of Kufa (Booth, etc, 1988).

Fusarium graminearum, *F. oxysporum* and *Trichoderma harzianum* were among the most prevalent (occurrence frequency) fungi and thus they were further used in experiments of this study.

Pathogenicity of the three fungi under study

Pathogenicity tests were carried out on radish seeds in 9 cm Petri dishes containing water agar medium (W.A.). The dishes were filled with WA and inoculated (cultured) with the three fungi (*F. oxysporum*, *F. graminearum* or *T. harzianum*) and incubated at 25±2° C for 48 h. The dishes were then planted with 20 radish seeds after being NaHCl sterilized (Harman, 2000) each with three replicates for each fungus. Planted dishes were incubated for 7 day and seed germination rates, seed rot, infected seeds and healthy seeds were calculated and recorded (Harman, 2000).

In vitro evaluation of *F. oxysporum*, *F. graminearum* and *T. harzianum* as pesticides biodegrading factors in solid P.D.A. medium.

PDA medium was dissolved and distributed into 250 ml flasks of 100 ml each. The flasks were cooled and pesticides were added according each treatment before medium solidification. Pesticides were used at their manufacture recommended dose. Doses were 0.05 ml/L for Bulldok and Bayfidan, 2.5 ml/L of Mega, 8.3 ml/L for Oberon and 1.6 g/L for Trayf. The control treatment was free of pesticide. 20 milliliters of poisoned and non-poisoned PDA was poured in Petri dishes. Pure newly formed colonies of the three fungi were used for inoculation. Fungi were individually inoculated according to single spore culture procedure with 3 replicates per treatment. Dishes of all the treatments were incubated at 25 ± 2°C. Data of inhibition/stimulating rates were taken where the dish is completely full with the fungal growing mycelia of either control or the poisoned treatments. The percentage of inhibition/ stimulation was calculated according to the Abbot (1925) equation following (Shaban and Al-Malah, 1993).

Experimental design and data analysis

The two experiments were factorial based on complete-randomized-design (CRD) with three replicates. Data were analyzed and analysis of variance ANOVA was performed using GenStat 12th edition computer program. Means were compared with the least-significant-difference (LSD) whenever appropriate at ($P \leq 0.05$) (Gomez, 1984).

Results and Discussion

Pathogenicity of the three fungi under study

The results showed that percentage of seed germination was affected differently by different fungi (table 1). The control and *T. harzianum* treatments resulted in the highest germination rate (100%). *F. oxysporum* decreased germination rate to be 52%. Similar results were found in terms seed rot rate. 48% of the seed were rotten in case of *F. oxysporum* treatment while no seed rot was recorded in both the control and the *T. harzianum* treatments. Seedlings of all the treatments were healthy while infected seedlings were not detected in any of the treatments (table 1).

High seed germination rates were recorded even though in the presence of fungi. This may be due to these fungi under study are not pathogenic and or their secretions are non-toxic with no effects on seed germination (Dewan and Sivaithamparam, 1988). Moreover, fungal secretions may facilitate seed germination as their secreted substances help to breaking down seed outer shell. It has been reported that Cellulase

Table 1 : Pathogenicity test of *Fusarium oxysporum*, *F. graminearum* and *Trichoderma harzianum* and their effect on radish seed germination, seed rot and seedlings health seven days after planting on WA in lab condition.

Treatments	% seed germination	% Seed rot	% Infected seeds	% Healthy seeds
Control	100.00	0.00	0.00	100.00
Fusarium oxysporum	52.00	48.00	0.00	100.00
Fusarium graminearum	54.00	46.00	0.00	100.00
Trichoderma harzianum	100.00	0.00	0.00	100.00
L.S.D.0.05	Treatments=3.30	Germination =3.31	Interaction =6.58	

Values are means of three replicates. Differences among treatments were calculated based on least significant difference LSD ($P \leq 0.05$).

is one of fungal secretions that accelerate seed germination (Vidali, 2001). Other substances which stimulate germination and growth are including Indol Acetic acid (IAA) (Dewan *et al*., 1994; Harman, 2000 and Hafez, 2001) or 2-Carboxymethyl-3-n-hexyl malic acid (Mondal *et al*., 2000).

In vitro evaluation of *F. oxysporum*, *F. graminearum* and *T. harzianum* as pesticides biodegrading factors in solid P.D.A. medium

Results showed that fungal growth was affected at various levels as the pesticide differs. In general, fungal growth was almost always highly inhibited in the first days of the treatment. The inhibition rates decreased as the period increases. Regarding *F. oxysporum*, fungal growth was affected the most by the pesticide Mega followed by Trayf and Bayfidan. Growth inhibition rates by Bulldok and Oberon were almost similar especially during the first two days. However, average of fungal growth inhibition rates after 7 days post treatment was the highest due to Mega (81.36%) and trayf (74.22%) with significant difference from Byfidan (29.22%), Bulldok (27.32%) and Oberon (17.03%).

F. graminearum

As shown in table 3, *F. graminearum* growth was also inhibited at highest by the pesticide Mega compared to the other tested pesticides. similar manner of fungal growth inhibition was also detected in terms of period, the fungal growth was more affected by the first period (1-3 days) than the last four days of the treatments. inhibition rates of fungal growth significantly differed among treatments (pesticides). Averages of growth inhibition rates were almost in similar ranks except in case of Bulldok. unlike *F. oxysporum, F. graminearum* radial growth was more inhibited Bulldok than by Bayfidan. Generally, the five tested pesticides resulted in inhibition rates that ranged from 91.89% to 26.89% in case of *F. graminearum* compared to a range from 81.36% to 17.03% with *F. oxysporum*.

T. harzianum

In the case of bio-control *Trichoderma harzianum*, it was also observed that there were significant differences in the percentage of inhibition among days and pesticides treatments (table 4). The highest rate of inhibition (54%) was recorded in the two first days compared with the seventh day with inhibition rate of 37%. As for treatments (pesticides), the highest percentage of inhibition (100%) was in the treatment of Trayf + *T. harzianum* and Mega + *T. harzianum* followed by Bifida 49% with significant difference (table 4). The least inhibition rates were recorded in Bulldok and Oberon that of 9.67% and 0.42%, respectively.

The results obtained in tables 2, 3 and 4 explain that the chemical pesticides Bulldok, Oberon and Bayfidan affect the fungi directly and within a short time after the fungus cultivation in the poisonous medium. It was noted that the effect of a pesticide decreases gradually, so its effect is higher in the early days and then the effect is reduced until elimination at the end of the period under test. This may be due to the ability of fungus to break down the toxic substance and convert it into a non-toxic substance (source). Mushrooms metabolize pesticides with sequential steps. The organism first secretes enzymes in the presence of the pesticide. These enzymes act in two interrelated ways. In the first, the micro structure of the pesticide is changed to become less toxic than the original compound (source). In the second, the molecule is converted into a more polarized compound and then becomes more soluble in water, then will be much easier to be disposed outside the fungus body. Where most of the chemical pesticides are insoluble in water, oxidation or hydrolysis helps to introduce polar groups to the compound to become more soluble in water. Now this compound is ready to contribute in other reactions, this is called primary metabolism. In most cases the compound from primary metabolism is linked to natural compounds within the tissues of the organism, including sugars (Al-Adil, 2006).

Table 2 : Effect of five different pesticides on fungal growth of *Fusarium oxysporum* on P.D.A. medium, measured by percent inhibition/stimulation of fungal growth.

Treatments	% stimulation/inhibition							Treatments Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Bulldok	40.86	41.41	28.33	27.03	26.64	13.89	13.11	27.32
Trayf	93.54	74.74	73.00	72.49	71.66	66.94	67.21	74.22
Oberon	45.16	19.19	11.33	5.36	0.93	21.12	16.12	17.03
Mega	100.00	93.43	84.00	77.62	76.36	70.16	67.95	81.36
Bayfidan	88.17	45.95	25.00	11.65	0.18	21.33	12.28	29.22
Average	61.28	45.78	36.94	32.35	29.29	32.24	29.44	
L.S.D $_{P\leq0.05}$	Days =0.61			Interaction=0.66				1.63

Values are means of three replicates. Differences among treatments were calculated based on least significant difference LSD ($P\leq0.05$)

Table 3 : Effect of five different pesticides on fungal growth of *F. graminearum* on P.D.A. medium, measured by percent inhibition/stimulation of fungal growth.

Treatments	% stimulation/inhibition							Treatments Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Bulldok	52.84	46.84	43.54	44.88	44.88	27.22	14.11	39.18
Trayf	40.33	47.66	85.00	42.50	42.50	41.50	36.00	47.92
Oberon	34.14	41.86	38.30	37.86	36.11	0.00	0.00	26.89
Mega	100.00	100.00	93.34	91.58	90.77	85.44	82.11	91.89
Bayfidan	86.99	61.46	45.36	37.16	34.44	0.00	0.00	37.91
Average	52.38	49.63	50.92	42.33	41.45	25.69	22.03	
L.S.D $_{P\leq0.05}$	Days =10.54			Interaction=11.39				27.89

Values are means of three replicates. Differences among treatments were calculated based on least significant difference LSD ($P\leq0.05$).

Table 4 : Effect of five different pesticides on fungal growth of *Trichoderma harzianum* on P.D.A. medium, measured by percent inhibition/stimulation of fungal growth.

Treatments	% stimulation/inhibition							Treatments Average
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Bulldok	37.53	30.22	0.00	0.00	0.00	0.00	0.00	9.67
Trayf	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Oberon	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.42
Mega	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Bayfidan	87.40	93.89	55.56	38.88	25.22	24.44	22.66	49.72
Average	54.64	54.01	42.59	39.81	37.53	37.40	37.11	
L.S.D $_{P\leq0.05}$	Days =2.86			Interaction=2.65				7.02

Values are means of three replicates. Differences among treatments were calculated based on least significant difference LSD ($P\leq0.05$).

Except for Mega and Trayf, the three other pesticides tested are recommended where *T. harzianum* is used as bio-control agent. Our findings showed that *T. harzianum* was continuously affected by the two pesticides (Mega and Trayf). The cause of the slow or delayed effect of the pesticide Trayf may be due to the fact that some pesticides require a relatively long time to affect the fungus. In order for the pesticide to be able to influence the fungus, the pesticide must be sufficiently and rapidly spread into the body of the fungus and with an effective effect on the biological processes (Al-Adil, 2006).

Many chemical pesticides can kill the organism by interfering with cellular respiration and energy production

in the mitochondria, or by binding with certain compounds essential to the organism's life, such as nucleic acids (RNA, DNA) and proteins. As a result, cytoplasmic glycolysis, Krebs cycle and oxidative phosphorylation in mitochondria processes will be affected (Al-Adil, 2006). The results showed encouraging in fungal growth cultured in pesticide-poisoned media, as a result of adapting the fungus after exposure to the pesticide more than once. In other words, the fungus is able to convert the toxic substance of the pesticide into a less toxic or non-toxic substance to be used as a nutrient.

The low efficiency of the fungicide on fungi may be attributed to the ability of fungus to tolerate or resist the action of pesticides at certain levels of tolerance or resistance depending on fungus species (Bollen and Scholten, 1971). However, some fungi have been severely affected by pesticides. This may be due to the effect of the pesticide in disrupting activities of some necessary enzymes in the feeding process (Koller *et al*., 1982). Some pesticides inhibit the action of Chtinase and Phosphatase and or affect growth by influencing DNA synthesis and cell division or inhibition of some mitochondrial vital enzymes (Al-Adil, 2006).

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