

# Fungal Biodegradation of Organophosphorus Insecticides and their Impact on Soil Microbial Population

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## Abstract

Impact of organophosphorus insecticides Malathion Profenofos and Diazinon were assessed on soil microbial populations. The degradation characteristics of these organophosphorus insecticides at different concentrations, incubation periods and temperature by an isolated fungal strains *Trichoderma harzianum* and *Metarhizium anisopliae* were investigated. Fungal population was reduced by 56.37, 51.07 and 26.65% at 10 day of application by profenofos, diazinon and malathion respectively, compared to the control. Fungal degradation of profenofos, diazinon and malathion increased with increasing incubation period but at the same time decreased with increasing initial concentrations of insecticides. Using *M. anisopliae*, almost 85.60, 77.20 and 68.15% of initial diazinon was decomposed within 20 days at 10, 20 and 40 mg of diazinon, while profenofos was degraded with 54.70, 62.45 and 63.68 at 20 days at 10, 20 and 40 mg. At 20 mg of initial malathion, more than 90% of the initial concentration was degraded by *M. anisopliae*. After 10 days of incubation, the degradation percentage of diazinon at 20, 25, 30, 35 and 40°C was examined to be 17.85, 35.38, 43.45, 33.85, and 7.80%, while degradation percentage of Profenofos was examined to be 13.60, 30.35, 35.43, 30.10 and 7.56% respectively, similar results of malathion degradation percentage were obtained to be 44.78, 50.65, 60.58, 57.73 and 10.28% at 20, 25, 30, 35 and 40°C respectively with using *M. anisopliae*. Degradation percentage at 35°C was 1.90, 2.21 and 1.29 times faster for diazinon, profenofos and malathion respectively than those at 20°C with using *M. anisopliae*. While degradation percentage at 35°C was 2.07, 1.72 and 1.83 times faster for diazinon, profenofos and malathion respectively than those at 20°C with using *T. harzianum*. On the basis of present findings, these fungal strains can be recommended as potentially effective to protect the environment from the organophosphorus insecticides residues.

**Keywords:** Fungi; Organophosphorus insecticides; Biodegradation; Soil microbial population

## Introduction

Organophosphorus insecticides play an important role in success of modern farming and food production. However, important numerous problems including the pollution of the environment are created from the wide use of organophosphorus pesticides. Organophosphorus insecticides kill soil-dwelling pests of corn such as larval corn rootworms (*Diabrotica* spp.) and cutworms (*Agrotis* spp.). According to Racke and Coats [1], organophosphorus pesticides are regarded as non-persistent. and the most widely used insecticides, accounting for an estimated 34% of world-wide insecticide sales. These compounds possess high mammalian toxicity and it is therefore essential to degrade them from the environments [2]. Numerous problems associated with pesticides application are their possible persistence in the ecosystem and therefore, their possible incorporation into the food chain affects ecosystems and human beings [3].

Profenofos (PFF) an organophosphate (OP) insecticide, O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate, is non-systemic foliar insecticide, a broad spectrum and acaricide. Their effective was and still against a wide range of insects including chewing and sucking insects and mites on various plants [4]. PFF is one of the heavily used insecticide of which its contamination is ubiquitous in an agricultural area and has been widely used for last two decades to control insect pests. Extensive use of this toxic pesticide is leading to serious environmental consequences which impose development of methods to minimize the environmental burden of PFF using an environmental friendly and cost effective approach [5]. Another organophosphorus insecticide, Diazinon [O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate] is commonly used. From study Tomlin [6] diazinon is cholinesterase inhibitor and acts as non-systemic insecticide and acaricide with contact, stomach, and respiratory

action. It is commonly used to kill many insects including sucking and chewing insects and mites in a wide range of crops, ornamentals, lawns, and for domestic purpose. Tu [7] reported that diazinon showed an effect on fungi for the first and second week of incubation, but, subsequently, the populations returned to levels similar to those obtained in the controls. Malathion [S-(1,2-dicarbethoxyethyl)-O,O-dimethyl-di-thiophosphate], also known as carbophos, maldison and mercaptothion is a non-systemic, wide-spectrum organophosphorus insecticide used to control the household and agricultural pests. It was recognized as the first organophosphorus insecticide with highly selective toxicity [8].

During handling and application of organophosphorus insecticides, terrestrial ecosystems specially soil and water, receive large amounts of pesticides [9]. Due to the magnitude of this problem and the lack of a reasonable solution, rapid, effective and ecologically responsible cleaning up method is greatly needed to remove toxic organopollutants [10]. Generally, physical and chemical cleanup technologies are expensive and sometimes not much effective. However, biological techniques such as bioremediation by using microorganisms have been proven very effective [11]. Organophosphorus insecticides biodegradation in soil has been widely recorded [1,12-14]; however, the effects of organophosphorus pesticides on soil microorganisms

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has received less attention. Fungal species were isolated which utilize organophosphate pesticides, viz., phosphorothioic (pirimiphos-methyl and pyrazophos), phosphorodithioic (dimethoate and malathion), phosphonic (lancer) and phosphoric (profenfos) acid derivatives. Insecticides degradation with using fungi was studied *in vitro* and *in vivo* (soil). Numerous fungi including *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *Emericella nidulans*, *Fusarium oxysporum* and *Penicillium chrysogenum* were isolated from pesticide-treated wheat straw. *A. flavus* and *A. sydowii* phosphatases efficiently hydrolyzed pesticides at 300 to 1000 ppm in soil [15]. Degradation of chlorpyrifos in the contaminated soils was found to significantly increase with the addition of *Verticillium* sp. Degradation rates of chlorpyrifos in inoculated soils were faster in comparison with the sterilized soil, previously chlorpyrifos-untreated soil, and previously chlorpyrifos-treated soil under laboratory conditions [16]. Detoxifying OPs with a number of enzymes have been discovered and the majority of them belong to the class of phosphotriesterases (PTE). Various PTEs have been identified included organophosphate hydrolase (OPH), methyl parathion hydrolase (MPH), organophosphorus acid anhydrolase (OPAA), diisopropylfluorophosphatase (DFP), and paraoxonase 1 (PON1), carboxylesterases [17]. All of these enzymes are found to induce the degradation of organophosphate compounds. This work aim to study the impact of three organophosphorus insecticides on soil microorganisms. Two safe fungal species were isolated, purified and identified from soil with history insecticides application, then used to estimate their effect on the biodegradation rate of tested organophosphorus insecticides to give a primitive indication about organophosphorus insecticides mycodegradation.

## Material and Methods

### Collection of soil samples for fungal isolation

Soil samples collected from different sites in Jazan region in king Saudi Arabia having history of repeated insecticide application were used for the isolation of fungal organisms. Samples were collected in sterile polyethylene bags and stored at 3°C before processing.

### Fungi isolation and identification

Serial dilutions were carried out for fungal isolation. Plates containing Potato dextrose agar medium (PDA) were inoculated and incubated at 28°C ± 2°C until the sporulation of fungal colonies occurred. Colonies were picked up and transferred to the same growth medium. The developed fungi were purified using hyphal tips or the single-spore technique and then transferred to slant PDA. The purified fungi were verified, and then two safe common fungi strains were selected and identified.

### Microbial enumeration

After 5, 10 and 20 days of organophosphorus insecticides, total numbers of culturable bacteria, fungi, Nitrogen fixing bacteria and actinomycetes were counted in the treated and control soils. Soil samples (1 g) were placed in test tube containing 9 ml of 0.1% (w/v)

sterile sodium pyrophosphate (pH 7.0) for shaking and preparing serial dilutions. Each group of total bacteria, fungi, actinomycetes and Nitrogen fixing bacteria were estimated by measuring colony forming unit on one-strength Trypticase Soy Broth Agar at 28°C, Rose Bengal Streptomycin Agar, starch nitrate medium and Ashby's mannitol phosphate agar respectively.

### Effect of different concentrations of organophosphorus insecticides on fungal growth

Petri dishes containing a solid culture medium without insecticides (control culture) and a medium supplemented with different concentrations: 20, 40 and 80 mg/l. Fungal mycelia (Disc 0.5 cm) were transferred to the center of the plates containing PDA and incubated at 28°C ± 2°C for 7 days. Growth and the tolerance of the fungi to the insecticides were estimated by the measuring the colony grown on growth medium comparison with the control cultures.

### Fungal biodegradation of organophosphorus insecticides and their analytical procedure

Mineral salt liquid medium supplemented with different concentrations of analytical grade standard of Malathion, Diazinon and Profenofos (10 mg l<sup>-1</sup>) (Figure 1) was used for biodegradation test. Fungi were inoculated in the medium and incubated. Fungal degradation of organophosphorus insecticides were estimated at different incubation period and different temperature. During the experiment, samples were collected periodically after 5, 10 and 20 days intervals of time for estimation of organophosphorus insecticides degradation. Medium without inoculation was maintained under the same conditions and served as control. After incubation period, a known volume of growth medium (100 ml) supplemented with organophosphorus insecticides mixed with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20%) and was transferred into 500 ml separator funnel and partitioned successively three times with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20%). Extract was filtered through a pad of cotton and anhydrous sodium sulfate then evaporated to dryness using a rotary evaporator at 30°C, the residue became ready for chromatographic determination. A Hewlett-Packard, USA serial 6890 gas chromatograph (GC) equipped with Flame Photometric Detector (FPD) operated in the phosphorus mode (526 nm filter) was used for determination of malathion, Profenofos and diazinon residues under the following conditions: Column: PAS-1701, 30 m length x 0.32 mm i.d. x 0.52 µm film thickness. Temperature (°C): Detector: 260, Injector: 240, Column: 185. Gases flow (ml/min.): Nitrogen carrier gas: 3, Hydrogen: 75, Air: 100. The organophosphorus insecticide degradation was calculated by the following equation: X% = (C1-C2)/C1 × 100. Where, X is organophosphorus insecticide degradation; C2 is the concentration of organophosphorus insecticide (mg l<sup>-1</sup>) in the medium that has organophosphorus insecticide degrading fungal strain; C1 is the concentration of organophosphorus insecticide (mg l<sup>-1</sup>) in the medium that does not contain organophosphorus insecticide degrading fungal strain.

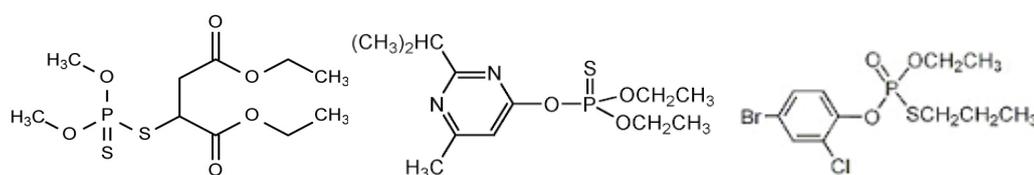


Figure 1: Malathion, Profenofos and diazinon chemical structure from left to right.

## Soil sampling characteristics and processing *in vivo* with insecticides

Soil samples were collected from Agriculture field with no history of insecticides application at at Jazan region a from depth of 5- 20 cm. Soil samples were sieved through a 90-mesh sieve to remove plant material and stones. These samples were brought to the laboratory and autoclaved , then stored at 4°C. Amounts of 200 g of sterile soil were packed in polythene bags. The following variants were set up in triplicate: soil (control), soil + insecticides, soil + insecticides + fungal inoculums. Each of insecticide was added at 400 ppm. Inoculations were done with 2 mL of fungal spore suspension (*T. harizianum* and *M. anisopliae*). The bags were incubated at 30°C for 25 days. CO<sub>2</sub>, pH, CO<sub>3</sub><sup>2-</sup>, Cl and Ca<sup>++</sup>percentage in the treated soil were measured according to standard procedure. Total nitrogen was measured by modified Kjeldahl method [18]. Estimation of released phosphate in soil was detected by Olsen et al. [19].

## Result and Discussion

Extensive use of organophosphorus insecticides causes imbalance in properties of soil, water and air environments and create diverse environmental problem via biomagnifications due to having problem of natural degradation. Currently, microbial degradation is one of the important techniques for degradation of organophosphorus insecticides as well as other pesticide from agricultural soils. It may be supposed that insecticides are not toxic to microorganisms since these microorganisms do not have sensitive targets. The stimulating or repressor effects of of profenofos, diazinon and malathion on soil microbial populations are shown in Table 1. Fungal population was reduced by 56.37, 51.07 and 26.65% at 10 day of application by profenofos, diazinon and malathion respectively, compared to the control. At 20 day of organophosphorus application, their inhibitory effect was disappeared. The response of soil bacteria, nitrogen fixing bacteria and actinomycetes population to by profenofos, diazinon and malathion treatments were almost similar to that of the soil fungi, but surprisingly, it was subsequently recovered to a similar or more level of control particularly with diazinon and malathion application. The significant increase in the numbers of culturable bacteria in soil amended with diazinon was found by Singh and Singh [20]. The obtained results revealed that profenofos, diazinon and malathion possessed an inhibitory effect on soil microorganisms during the initial periods after its application. In agreement with these

results, similar inhibitory effects of profenofos on microbial population also observed in the previous studies [21]. Nazia et al. [22] found that the organophosphorus insecticide had the least adverse effects on the Bacillus population but a significant effect on Methylobacterium. These effects are not drastic but minor in nature and the populations recovered over a period of time. In another study [23], diazinon treatments increased the numbers of culturable bacteria and fungi, however, N-fixing bacteria and nitrifiers (but not denitrifiers) were decreased. Martinez-Toledo et al. [24] stated that the presence of profenofos at 10 to 300 µg/g significantly increased the total number of bacteria and denitrifying bacteria. On the other hand, the population of nitrogen fixing bacteria was suppressed significantly. Nitrifying bacteria and fungal populations decreased initially at concentrations of 100 to 300 µg/g but recovered rapidly to levels similar than those in the control.

In the current study, the impact of insecticides on growth of four fungal species (*Fusarium oxysporum*, *Curvularia lunata* , *Trichoderma harzianum* and *Metarhizium anisopliae*) was studied but the biodegradation of organophosphorus insecticides with the two save fungal species *T. harzianum* and *M. anisopliae* were studied. The obtained results indicated that *T. harzianum* and *M. anisopliae* growth decreased significantly with increasing organophosphorus insecticides (Table 2 and Figure 2), but the decrease in the growth at low concentration (20 mg) of diazinon and malathion was negligible. *F. oxysporum* growth was better than growth of other fungi on medium containing high insecticides this may be explained as the *F. oxysporum* was more resistance to insecticides.

*T. harizinum* growt not significant decreased at low concentration 20 mg of malathion (Table 2). However, at the lower concentration of profenofos, the two fungal species showed greater inhibition, which may indicate that they are less resistant to the profenofos presence. Recently, Abbas and Yadegar [25] reported that the efficacy of the pesticides on the inhibition of the fungal growth and spore germination showed that they were both reduced with the increasing concentration of the insecticide. No significant differences were observed in the spore and hypha diameters of *M. anisopliae* treated with diazinon and malathion except and phialide length at 80 mg of diazinon, and hypha diameter at 40 mg of malathion (Table 3). In *T. harizianum*, phialide

Soil microbial community (CFU)	Application days	Organophosphorus Insecticides		
		Profenofos	Diazinon	Malathion
Fungi(×10 <sup>4</sup> /g dw)	0	31.33 ± 1.53	31.33 ± 1.53	31.33 ± 1.53
	5	14.33 ± 1.53	21.67 ± 1.15	21.67 ± 0.58
	10	13.67 ± 0.58	15.33 ± 0.58	22.98 ± 0.58
	20	18.67 ± 0.58	21.33 ± 1.53	22.67 ± 0.58
Bacteria (×10 <sup>5</sup> /g dw)	0	56.33 ± 1.53	56.67 ± 2.08	56.67 ± 1.15
	5	32.67 ± 2.52	39.00 ± 1.73	40.67 ± 1.15
	10	23.67 ± 1.15	34.33 ± 1.53	32.00 ± 1.00
	20	50.33 ± 0.58	60.67 ± 1.15	64.33 ± 1.15
Nitrogen fixing bacteria (×10 <sup>5</sup> /g dw)	0	25.33 ± 0.58	25.33 ± 0.58	25.33 ± 0.58
	5	24.00 ± 1.00	29.33 ± 1.53	23.00 ± 1.00
	10	21.67 ± 0.58	22.33 ± 0.58	26.67 ± 1.15
	20	34.33 ± 1.53	36.33 ± 1.53	43.33 ± 2.31
Actinomycetes (×10 <sup>5</sup> /g dw)	0	13.00 ± 1.00	13.00 ± 1.00	13.00 ± 1.00
	5	13.67 ± 0.58	12.00 ± 1.00	14.67 ± 0.58
	10	11.00 ± 0.33	15.33 ± 1.53	16.33 ± 0.58
	20	11.00 ± 1.00	17.33 ± 1.15	21.00 ± 1.00

**Table 1:** Colony forming unit of microorganisms in soil treated with different organophosphorus insecticides at different application periods.

(Concentration mg/L)	<i>M. anisopliae</i> colony radius (cm)			<i>T. harzianum</i> colony radius (cm)		
	Diazinon	Malathione	Profenofos	Diazinon	Malathione	Profenofos
0	7.98 ± 0.18a	7.98 ± 0.18a	7.98 ± 0.18a	7.83 ± 0.03a	7.83 ± 0.03a	7.83 ± 0.03a
20	6.40 ± 0.07b	7.43 ± 0.05b	4.03 ± 0.08b	6.20 ± 0.07b	7.78 ± 0.06a	3.93 ± 0.11b
40	3.13 ± 0.05c	5.98 ± 0.05c	2.25 ± 0.10c	3.88 ± 0.05c	7.45 ± 0.03b	1.63 ± 0.05c
80	2.03 ± 0.06d	5.33 ± 0.12d	1.88 ± 0.08d	2.43 ± 0.05d	5.55 ± 0.07c	0.68 ± 0.03d
(Concentration mg/L)	<i>C. lunata</i> colony radius (cm)			<i>F. oxysporum</i> colony radius (cm)		
0	6.05 ± 0.10a	6.05 ± 0.10a	6.05 ± 0.10a	8.08 ± 0.06a	8.08 ± 0.06a	8.08 ± 0.06a
20	4.10 ± 0.07b	4.55 ± 0.09b	2.48 ± 0.08b	7.70 ± 0.12b	8.05 ± 0.05a	4.10 ± 0.04b
40	2.73 ± 0.05c	3.40 ± 0.04c	1.18 ± 0.10c	5.43 ± 0.05c	7.80 ± 0.04b	2.08 ± 0.05c
80	1.95 ± 0.03d	2.08 ± 0.09d	0.78 ± 0.05d	4.00 ± 0.04d	6.90 ± 0.06c	2.05 ± 0.03c

Means followed by the same letters are not significantly different in each insecticide

Table 2: Effect of different concentrations of organophosphorus insecticides on fungal growth.

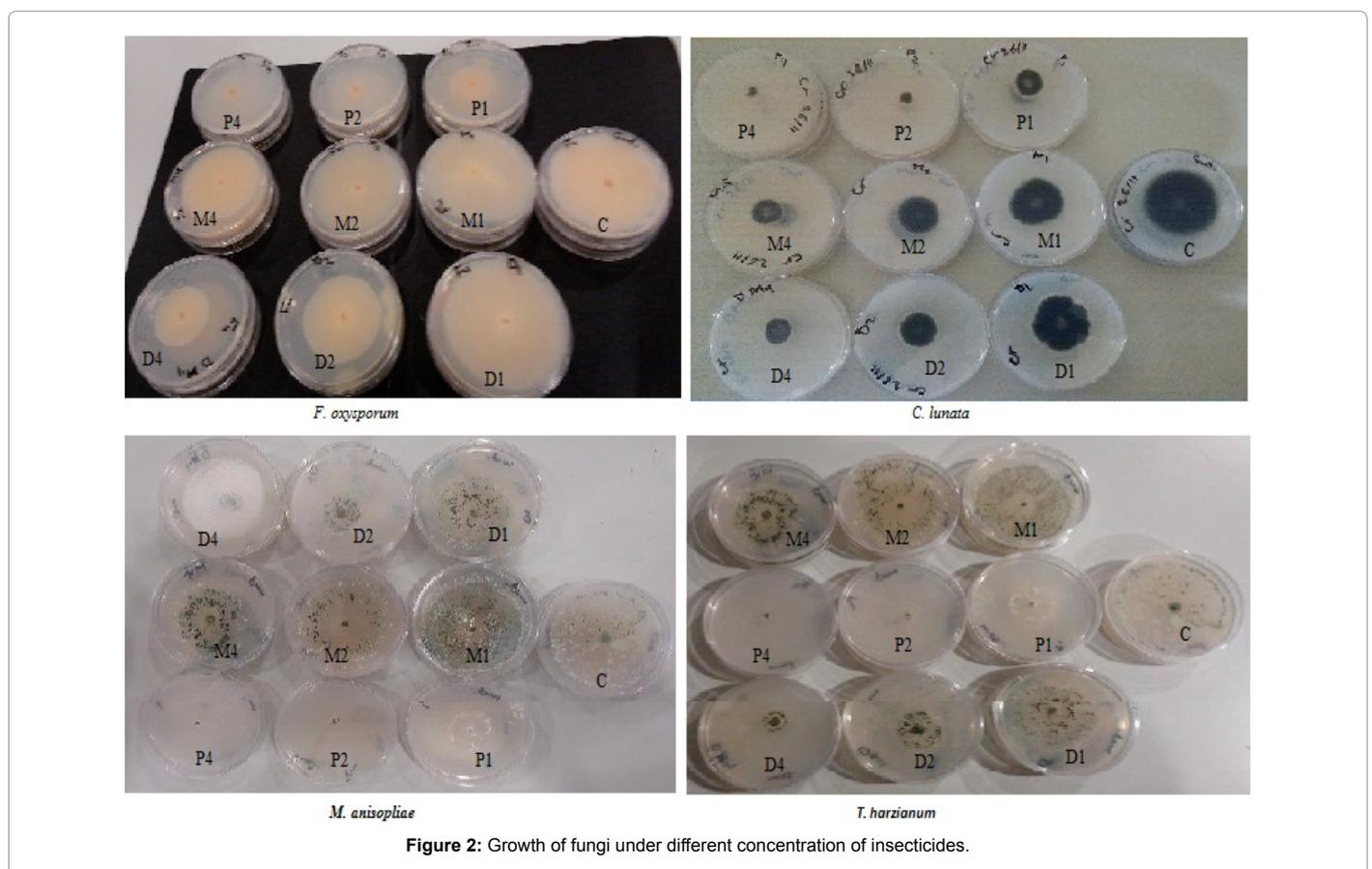


Figure 2: Growth of fungi under different concentration of insecticides.

and hypha diameter were reduced at 40 mg of profenofos treatment, but at diazinon and malathion treatment, non significant differences in the morphological characterization of *T. harzianum* compared with control except phialide length and hypha diameter at 80 mg and 40 mg of diazinon and malathion treatments respectively (Table 4). Overall our results suggest that various pesticides used to protect the plants against weeds and insects could exert an effect on the growth but not on morphological characterization of the *T. harzianum*. *Aspergillus niger*, *A. tamarii*, *A. terreus* and *T. harzianum* grew successfully on the culture media treated with Malathion and Dursban at doses (10, 50 and 100 ppm) but the growth rate varied with the species, the insecticide and the doses [26]. Numerous laboratory studies indicated that the various fungal species or even strains of the same species vary in susceptibility to pesticides [27-29].

Pesticides can be degraded by microbial, chemical and photodegradation processes in the environment. Nonetheless, microbial degradation is considered the major determining factor of the organophosphorus fate in the environment and is often the main process of pesticide degradation in soils, representing the safest, least disruptive and most cost-effective treatment method [30,31]. The results of the present study revealed that fungal degradation of profenofos, diazinon and malathion increased with increasing incubation period but at the same time decreased with increasing initial concentrations of insecticides (Tables 5 and 6). Using *M. anisopliae*, almost 85.60, 77.20 and 68.15% of initial diazinon was decomposed within 20 days at 10, 20 and 40 mg of diazinon (Table 5), while profenofos was degraded with 54.70, 62.45 and 63.68 at 20 days at 10, 20 and 40 mg. Also, from Table 5, it is worthy of note that more than 89.20 and 83.77% of the

Concentration (mg/L)	Diazinon			Malathione			Profenofos		
	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)
0	8.30 ± 0.26a	25.72 ± 2.07b	17.61 ± 0.54a	8.3 ± 0.26a	25.72 ± 2.07a	17.61 ± 0.54a	8.3 ± 0.26b	25.72 ± 2.06ab	17.61 ± 0.54a
20	8.40 ± 0.34a	28.90 ± 1.18b	17.71 ± 0.94a	8.1 ± 0.23a	29.32 ± 2.32a	17.43 ± 0.69a	8.1 ± 0.18b	29.58 ± 2.03a	17.26 ± 0.83a
40	8.40 ± 0.34a	29.70 ± 0.06b	17.38 ± 0.95a	8.4 ± 0.31a	28.80 ± 1.56a	15.27 ± 0.83b	9.5 ± 0.43a	21.20 ± 0.0b	16.50 ± 1.35a
80	8.70 ± 0.34a	39.20 ± 0.00a	18.52 ± 1.10a	8.5 ± 0.34a	28.83 ± 2.12a	19.25 ± 0.51a	-ve	-ve	-ve

Means followed by the same letters are not significantly different in each insecticide; -ve, growth not detected

Table 3: Micromorphological characterization of *M. anisopliae* at different concentrations of organophosphorus insecticides.

Concentration (mg/L)	Diazinon			Malathione			Profenofos		
	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)	Spore diameter (µm)	Phialide (µm)	Hypha diameter (µm)
0	8.7 ± 0.37ab	29.75 ± 0.89c	15.64 ± 0.64a	8.7 ± 0.37a	29.75 ± 0.89b	15.64 ± 0.64b	8.7 ± 0.37a	29.75 ± 0.89a	15.64 ± 0.64a
20	8.4 ± 0.27ab	29.94 ± 1.45b	14.96 ± 0.83a	8.5 ± 0.17a	34.22 ± 1.28a	15.41 ± 0.75b	7.7 ± 0.26b	28.13 ± 1.06a	16.17 ± 0.43a
40	7.3 ± 0.75b	24.18 ± 1.30b	15.19 ± 0.54a	9.3 ± 0.26a	26.87 ± 1.09b	16.50 ± 0.70ab	7.7 ± 0.15b	26.41 ± 1.18a	16.52 ± 0.71a
80	9.0 ± 0.25a	34.02 ± 1.29a	16.35 ± 0.81a	9.2 ± 0.51a	29.22 ± 1.70b	18.01 ± 0.65a	0.0 ± 0.0c	0.00 ± 0.0b	0.00 ± 0.0b

Means followed by the same letters are not significantly different in each insecticide

Table 4: Micromorphological characterization of *T. harizianum* at different concentrations of organophosphorus insecticides.

Concentration (mg/L)	Incubation days	Diazinon		Profenofos		Malathion	
		Rest concentration	Degradation %	Rest concentration	Degradation %	Rest concentration	Degradation %
10	5	5.60 ± 0.07	44.00	7.73 ± 0.23	22.70	5.17 ± 0.07	48.30
	10	2.48 ± 0.14	75.20	5.81 ± 0.14	41.90	2.83 ± 0.17	71.70
	20	1.44 ± 0.10	85.60	4.53 ± 0.04	54.70	1.08 ± 0.03	89.20
20	5	15.49 ± 0.13	22.55	16.77 ± 0.11	16.15	11.76 ± 0.01	41.20
	10	11.63 ± 0.06	41.85	12.74 ± 0.06	36.30	8.87 ± 0.12	55.65
	20	4.56 ± 0.10	77.20	7.51 ± 0.36	62.45	1.81 ± 0.06	90.95
40	5	34.83 ± 0.06	12.93	36.59 ± 0.50	8.53	27.74 ± 0.24	30.65
	10	22.62 ± 0.28	43.45	25.83 ± 0.17	35.43	15.77 ± 0.11	60.58
	20	12.74 ± 0.06	68.15	14.53 ± 0.27	63.68	6.49 ± 0.41	83.77

Table 5: Biodegradation of organophosphorus insecticides by *Metarhizium anisopliae* at different concentrations and incubation periods.

Concentration (mg/L)	Incubation days	Diazinon		Profenofos		Malathion	
		Rest concentration	Degradation %	Rest concentration	Degradation %	Rest concentration	Degradation %
10	5	3.42 ± 0.06	65.80	4.23 ± 0.07	57.70	2.90 ± 0.09	71.00
	10	2.35 ± 0.29	76.50	5.17 ± 0.03	57.70	1.85 ± 0.14	81.50
	20	0.82 ± 0.06	91.80	2.17 ± 0.11	78.30	0.94 ± 0.05	90.60
20	5	10.24 ± 0.11	48.80	14.82 ± 0.11	25.90	8.76 ± 0.16	56.20
	10	6.73 ± 0.20	66.35	9.24 ± 0.11	53.80	5.92 ± 0.11	70.40
	20	2.36 ± 0.12	88.20	3.21 ± 0.05	83.95	1.65 ± 0.05	91.75
40	5	27.79 ± 0.02	30.53	30.77 ± 0.06	23.08	25.11 ± 0.07	37.23
	10	17.36 ± 0.47	56.60	22.15 ± 0.05	44.63	11.59 ± 0.02	71.03
	20	6.08 ± 0.01	84.80	11.58 ± 0.31	71.05	3.79 ± 0.01	90.56

Table 6: Biodegradation of organophosphorus insecticides by *T. harizianum* at different concentrations and incubation periods.

initial malathion was degraded within 20 days at 10 and 40 mg. At 20 mg of initial malathion, more than 90% of the initial concentration was degraded by *M. anisopliae*.

*T. harizianum* was able to degrade malathion with more than 90% at 20 days at all used concentrations in mineral medium (Table 6). Karanth [32] stated that the potential of microorganisms to degrade and remove pesticides from environment has also been successfully attempted. Profenofos was more resistant than other insecticides to degradation by *T. harizianum* followed with diazinon. According to Chalamala et al. [33] *Aspergillus niger*, as an attractive alternative to other conventional technique may be utilized for the bioremediation of malathion contaminated residue soils. *Aspergillus* sp. showed tolerance limit of 800 mg l<sup>-1</sup> of malathion and degraded 300 mg l<sup>-1</sup> within 24 h of incubation [34]. In addition, Chalamala et al. [33] worked on

mycodegradation of Malathion by using *A. niger* and got 86.72% of degradation. Recently, research activities in this area have shown that a diverse range of microorganisms are capable of degrading malathion [35]. *Trichoderma harzianum* and *Rhizopus nodosus* were able to degrade 70-80% of the (Chloropyrifos and Ethion in 21 days period of incubation [36]. In a mineral salt medium, removal in the level of profenofos of 86.81% was obtained within 48 h of incubation with using *Pseudomonas aeruginosa* [37].

The effects of different temperatures (20, 25, 30,35 and 40°C) on diazinon, profenofos and malathion biodegradation in the mineral salts medium are presented in Tables 7 and 8). After 10 days of incubation, the degradation percentage of diazinon at 20, 25, 30,35 and 40°C was examined to be 17.85, 35.38, 43.45, 33.85, and 7.80%, while degradationpercentage of Profenofos was examined to be 13.60,

Temperature °c	Diazinon		Profenofos		Malathion	
	Rest concentration	Degradation %	Rest concentration	Degradation %	Rest concentration	Degradation %
20	32.86 ± 0.03a	17.85	34.56 ± 0.10a	13.60	22.09 ± 0.03a	44.78
25	25.85 ± 0.04b	35.38	27.86 ± 0.02b	30.35	19.74 ± 0.04b	50.65
30	22.62 ± 0.28c	43.45	25.83 ± 0.17c	35.43	15.77 ± 0.11c	60.58
35	26.46 ± 0.06b	33.85	27.96 ± 0.03b	30.10	16.91 ± 0.07c	57.73
40	36.88 ± 0.01d	7.80	36.97 ± 0.02d	7.56	35.89 ± 0.02d	10.28

Table 7: Effect of Temperature on biodegradation by *M. anisopliae* at initial concentration 40 (mg/L) for 10 days.

Temperature °c	Diazinon		Profenofos		Malathion	
	Rest concentration	Degradation %	Rest concentration	Degradation %	Rest concentration	Degradation %
20	28.82 ± 0.01	27.95	29.05 ± 0.05	27.38	23.60 ± 0.01	41.00
25	18.89 ± 0.02	52.78	25.07 ± 0.05	37.33	12.63 ± 0.01	68.43
30	17.13 ± 0.06	57.18	22.15 ± 0.05	44.63	11.59 ± 0.02	71.03
35	16.89 ± 0.02	57.78	21.18 ± 0.02	47.05	10.05 ± 0.05	74.88
40	21.95 ± 0.04	45.13	31.98 ± 0.01	20.05	32.80 ± 0.01	18.00

Table 8: Effect of Temperature on biodegradation by *T. harizianum* at initial concentration 40 (mg/L) for 10 days.

Soil Treatment with insecticide	Moisture %	pH	E.C. (ppm.)	CO <sup>3-</sup> %	CO <sub>2</sub> %	CL %	Ca <sup>++</sup> %	N %	Phosphorus µg/g soil
Control	2.25	7.52	289	5.25	2.50	0.054	0.075	26.85	1.90
Soil + Diazinon	2.29	7.36	265	5.24	2.50	0.055	0.085	26.85	2.30
Soil +Diazinon + <i>M. anisopliae</i>	2.13	7.15	259	5.27	2.59	0.055	0.051	26.85	34.22
Soil +Diazinon + <i>T. harizianum</i>	2.52	7.01	251	5.29	2.76	0.055	0.031	26.85	52.20
Soil +Malathion	2.31	7.30	278	5.19	2.50	0.064	0.085	37.59	3.60
Soil +Malathion + <i>M. anisopliae</i>	1.62	7.25	251	5.34	2.78	0.068	0.065	26.85	33.22
Soil +Malathion + <i>T. harizianum</i>	1.85	7.09	285	5.25	2.69	0.062	0.105	26.85	54.20
Soil +Profenofos	1.92	7.37	331	5.24	2.50	0.054	0.065	37.59	1.50
Soil +Profenofos + <i>M. anisopliae</i>	2.14	7.33	295	5.26	2.55	0.054	0.085	53.70	20.50
Soil +Profenofos + <i>T. harizianum</i>	2.04	7.34	279	5.27	2.58	0.036	0.065	48.33	22.25

Table 9: Chemical characterization of soil sample amended with insecticides ( 30 mg kg<sup>-1</sup>)and inoculated with fungi.

30.35, 35.43, 30.10 and 7.56% respectively, similar results of malathion degradation percentage were obtained to be 44.78, 50.65, 60.58, 57.73 and 10.28% at 20, 25, 30, 35 and 40°C respectively with using *M. anisopliae*. Degradation percentage at 35°C was 1.90, 2.21 and 1.29 times faster for diazinon, profenofos and malathion respectively than those at 20°C with using *M. anisopliae*. While degradation percentage at 35°C was 2.07, 1.72 and 1.83 times faster for diazinon, profenofos and malathion respectively than those at 20°C with using *T. harizianum*. There was no significant difference between degradation percentage of diazinon and profenofos at 25 and 35°C with using *M. anisopliae*. The results showed that the increase in temperature enhances the degradation rate of diazinon, profenofos and malathion up to 30°C and 35°C with using *M. anisopliae* and trichoderma respectively. Recently, it has been reported by many researchers that optimization of different environmental factors can play a vital role in accelerating the process of insecticides biodegradation [38,39].

The pollution of soil by the use of pesticides has become a serious condition all over the world and therefore the chemical insecticides residues in soils are a public safety concern. Since the conditions in soil are much more complex than those in synthetic media, the ability for pesticide degradation in soil was investigated in our study. Results of soil treatment with the three insecticides (Diazinon, Diazinon and Profenofos) and inoculated with *T. harizianum* and *M. anisopliae* revealed changes of the chemical analysis of soil (Table 9). Microorganisms may have a major effect on the persistence of most pesticides in soil [40]. In the current study there is considerable variation among the fungi under study in their ability to degrade the insecticides. Further, there was no relationship between nitrogen content of soil and the degradation of any of the pesticides except nitrogen content in case

of profenofos degradation, these may be due to presence of nitrogen in profenofos. On the other hand, there were clear relationships between the abilities of the fungi to degrade the insecticides and phosphorus content of soil, where soluble phosphorus increased distinctly under the action of *T. harizianum* and *M. anisopliae* (Table 9) indicating that fungi have the capacity to degrade insecticides in soil. Recently, Adelowo et al. [41] stated that release of phosphate ion was an indication that the first step in organophosphonates degradation by fungus was cleavage of the Carbon Phosphorus (C-P) bond. According to Fang et al. [16] Degradation rates of insecticide chlorpyrifos in inoculated soils with microorganisms were 3.61, 1.50 and 1.10 times faster in comparison with the sterilized soil, previously chlorpyrifos-untreated soil. CO<sub>2</sub> percentage in the soil treated with insecticides and inoculated with fungi was highest than in control, these reflected the activity of the total soil microbial populations. The same correlation was reported in study by Boyle [42].

## Conclusion

The fungal strains isolated from the contaminated sites with insecticides especially *T. harizianum* and *M. anisopliae* species showed the ability to degrade organophosphorus insecticides.

The optimal conditions for organophosphorus insecticides degradation by *T. harizianum* and *M. anisopliae* were 35 and 30°C respectively. These strains could be beneficial as a fungal inoculum for efficient biodegradation of insecticides.

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