White Rot Fungi to Decompose Organophosphorus Insecticides and their Relation to Soil Microbial Load and Ligninolytic Enzymes

Aisha M. H. Al-Rajhi,[a,*] Amna A. Saddiq,[b] Khatib Sayeed Ismail,[c] Tarek. M. Abdelghany,[d] Abeer M. Mohammad,[e,*] and Samy Selim [f,*]

The functional and structural features of microbial load in soil are influenced by the presence of insecticides. This study examined the impact of two organophosphorus insecticides, dimethoate and parathion on the microbial load of soil. The colony count of fungi, actinomycetes, bacteria, and nitrogen fixing bacteria was reduced after insecticides application at 7 and 14 days, but at 28 days the colony count began to increase. The growth of two white rot fungi including *Pleurotus sajor-caju* and *Phanerochaete chrysosporium* was affected by parathion, which was reflected by a decrease in colony radius to 1.85±0.05 and 0.75±0.06 cm, respectively, and by dimethoate, reflected by a decrease in colony radius to 3.33±0.12 and 1.85±0.05 cm, respectively at 40 mg/L compared to colony radius at control 7.90±0.12 and 7.50±0.06 cm, respectively. The applied low concentration (10 mg/L) encouraged *P. sajor-caju* and *P. chrysosporium* to remove up to 87.7% and 81.8% of dimethoate, respectively, and 69.20 and 68.30% of parathion, respectively compared with the decomposition at high dose (40 mg/L) at 28 days. The presence of insecticides induced the production of ligninolytic enzymes lignin peroxidase, laccase, and manganase peroxidase.

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Contact information: a: Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; b: Department of Biology Science, College of Science, University of Jeddah, Jeddah, Saudi Arabia; c: Department of Biology, Faculty of Science, Jazan University, Jazan 45142, Saudi Arabia; d: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo 11725, Egypt; e: Biology Department, University Collage of Aldarb, Jazan University, P.O. Box 114, Jazan 45124, Saudi Arabia; f: Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia;
** Corresponding authors: amoalrajhi@pnu.edu.sa* (A.M.H.A.), *sabdulsalam@ju.edu.sa* (S.S.), *ayousuf@jazanu.edu.sa* (A.M.M.)

INTRODUCTION

Exposure to pesticides poses both acute and long-term health risks to people. For instance, long-term low-dose pesticide exposure results in immunological suppression, hormone disruption, lowered IQ, anomalies in reproduction, and carcinoma (Faraj *et al.* 2024). Because of their quick solubility in fat and ability to bioaccumulate in non-target animals, they pose a major health risk to living systems (Zhang *et al.* 2023). Herbicides, fungicides, and insecticides are the most often used pesticides; in wealthy nations, herbicides make up around half of all pesticides used, whereas in underdeveloped countries, insecticides are the most used pesticides (Itelima *et al*. 2018). Organophosphorus insecticides are a subset of insecticides that are crucial to the success of contemporary

agricultural and food production. However, widespread usage of organophosphorus pesticides causes a number of significant issues, such as disturbance of ecosystems, contamination of soil, and groundwater pollution by theses pesticides (El Alfy and Faraj 2017). Among the many issues surrounding the use of pesticides is the potential for them to stay in the environment, which means that they might enter the chain of food and have an influence on both humans and ecosystems (Dhiman *et al*. 2024). One of the main substances causing pollution and degradation of soil and groundwater, especially in the vicinity of agricultural areas, is organophosphorus insecticide (Jayashree and Vasudevan 2006). Parathion, also known as O,O-dimethyl O-4-nitrophenyl phosphorothioate, is an insecticide extensively utilized for managing chewing and sucking insects in field crops, fruits, vegetables, and livestock (Guyton *et al*. 2015). Additionally, it is used for eradication of mosquitoes, household insects, flies, and animal parasites. Organophosphorus compounds, such as dimethoate, have been used as insecticides and acaricide. These compounds act in a non-systemic manner on the stomach and respiratory organs, as well as acting as an inhibitor of acetylcholinesterase. It is commonly applied to kill numerous insects such as chewing and sucking insects (Shalaby *et al.* 2024).

The breakdown of organophosphorus insecticides by some microorganisms in soil has been reported (Abd El-Mongy and Abd El-Ghany 2009). However, the influences of organophosphorus-type insecticides on the soil microorganisms have received insufficient attention. According to recent investigation, several insecticides, namely prallethrin, pyrethroids, carbamate propoxur, and permethrin were degraded by several strains of *Trichoderma* (Mendarte-Alquisira *et al.* 2024). The degradation level of insecticides depends on several factors, among which are time, the kind of used microorganism, the insecticide type, and the applied concentration (Góngora-Echeverría *et al.* 2020). Numerous fungi have been isolated and characterized that can degrade organophosphorus compounds, for examples *Aspergillus* and *Penicillium* species (Matúš *et al.* 2023), *Aspergillus niger* (Mohapatra *et al.* 2024), *Purpureocillium lilacinum*, *Trichoderma reesei*, and *T. koningiopsis* (Mueen *et al.* 2024), and white rot fungi (Torres-Farradá *et al.* 2024). Microbial detoxification of organophosphorus insecticides has been reported, resulting from a number of enzymes such as methyl parathion hydrolase, organophosphate hydrolase, organophosphorus acid anhydrolase, and diisopropylfluorophosphatase (Zhao *et al.* 2021; Kiruthika *et al.* 2024; Pashirova *et al.* 2024). Evaluation of the impact of insecticides on soil microbial load, as well as the effect of insecticides on fungi and their decomposition were the aims of this study.

EXPERIMENTAL

Insecticides and Fungi Used

Dimethoate ($C_5H_{12}NO_3PS_2$) and parathion ($C_{10}H_{14}NO_5PS$) were obtained from Sigma Company (St. Louis, MO, USA). Two fungal species, namely *Pleurotus sajor-caju* and *Phanerochaete chrysosporium*, were obtained from Prof. Tarek Mohamed.

Microbial Load in Treated soil by Dimethoate and Parathion

Untreated agricultural soil (3 kg) with any insecticides for the previous one year was collected from Wadi Hanifah, Diriyah, Riyadh Saudi Arabia and divided into three equal quantities. The first part was treated by dimethoate and the second was treated by

parathion *via* a spraying process, while the third did not get treated. The soil samples were kept in dark incubators at 28 ± 2 °C for a maximum period (28 days) to perform microbial analysis. The moisture % of the untreated soil and treated with dimethoate and parathion was recorded, which provided 2.36, 2.56, and 2.66%, respectively. The microbial load in the examined soil was evaluated 7, 14, and 28 days after the insecticides appliction to determine the effect of insecticides on microbial load, as well as the effect of microorganisms on the applied insecticides. Total numbers (colony forming unit) of culturable fungi (using Potato Dextrose Agar), bacteria (using nutrient agar medium), Nitrogen fixing bacteria (NFB) (using Ashby's mannitol phosphate agar) and actinomycetes (using starch nitrate agar medium) were recorded *via* serial dilution protochol utilizing.

Effect of Different Doses of Dimethoate and Parathion on Fungal Growth

The solidified culture medium in Petri dishes not containing any insecticides was used as the control culture. Another set of dishes contained a medium with varying doses of 10, 20, and 40 mg/L of insecticides separately. Fungal mycelia discs (6 mm) of active margin colonies of *Pleurotus sajor-caju* and *Phanerochaete chrysosporium* were transplanted separately to the surface of the agar plates (at center of plate). Subsequently, the dishes were placed in an incubator at 28 ± 2 °C for a period of 7 days. The growth of the fungi was assessed by comparing the size of the colonies on the growth medium with those on the control cultures.

Fungal Decomposition of Dimethoate and Parathion

Different doses of dimethoate and parathion (10, 20, and 40 mg/L) were added individually to mineral salt liquid medium. Then, two dics (6mm) of agar inoculated with *Pleurotus sajor-caju* and *Phanerochaete chrysosporium* were added individually to mineral salt medium and incubated for 7, 14, and 28 days at 25 ℃. At the end of each incubation period, the growing fungus was removed to obtain clear broth medium in order to measure the rest quantity of insecticides and estimate the decomposition of insecticides *via* a gas chromatograph equipped with a Flame Photometric Detector (GC-FPD) (Hewlett-Packard, USA serial 6890). The rest quantity of insecticide (RQI, %) was calculated as follows.

$$\text{RQI\%} = \frac{\text{Quantity of insecticides after incubation period}}{\text{Quantity of insecticides at initial of incubation period}} \times 100 \quad (1)$$

$$\text{Insecticides decomposition (\%)} = [100 - \text{RQI\%}] \quad (2)$$

Ligninolytic Enzymes Assay in Medium Amended With Dimethoate and Parathion

The liquid medium of mineral salt was autoclaved and supplied with 40 mg/L of an analytical grade standard of insecticides, namely dimethoate or parathion. After each fungus was added to a flask containing medium, the flasks were cultured for 14 days at 30 °C. After the conclusion of the incubation period, the metabolised medium was then filtered through filter paper and used for the assay of enzymes namely laccase, manganase peroxidase (MnPase) (Garzillo *et al.* 2001), and lignin peroxidase (LIP) (Singh and Chen 2008).

Statistical Examination

Standard Error (± SE) was assessed *via* calculation of the mean three results of each experiment.

RESULTS AND DISCUSSION

The widespread application of organophosphorus insecticides has led to an imbalance in the characteristics of water, soil, and air environments, resulting in various environmental issues through biomagnification, primarily due to the challenges associated with natural degradation. At present, microbial degradation has emerged as a significant method for breaking down organophosphorus insecticides and other pesticides in soils cultivated with various plants. It can be inferred that these insecticides are not harmful to the microorganisms, as these organisms lack sensitive targets. The induction or inhibitor effects of dimethoate and parathion on soil microbial loads have been reported (Table 1).

Table 1. Microorganisms Load in Treated Soil with Dimethoate and Parathion at Different Periods of Application

Soil microbial load (CFU)	Application days	Insecticides		Untreated soil
		Dimethoate	Parathion	
Fungi (10^4 /g)	0	42.43±1.53	42.43±1.53	42.43±1.53
	7	32.77±1.15	24.92±0.50	42.33±0.53
	14	26.43±0.58	21.32±0.51	38.45±0.66
	28	32.43±1.53	25.72±0.50	37.66±1.50
Actinomycetes (10^5 /g)	0	25.20±1.00	25.20±1.00	25.20±1.00
	7	23.30±1.00	22.33±0.12	25.20±0.57
	14	27.66±1.53	25.54±0.25	27.25±0.50
	28	29.45±1.15	27.66±0.10	27.66±0.33
Bacteria (10^6 /g)	0	67.33±2.08	67.33±0.19	67.33±0.19
	7	50.10±1.73	41.43±0.85	70.12±1.33
	14	45.43±1.53	37.72±0.75	73.01±1.25
	28	71.87±1.15	41.67±1.10	72.66±0.66
Nitrogen fixing bacteria (10^6 /g)	0	26.35±0.58	26.35±0.58	26.35±0.58
	7	31.43±1.53	16.56±1.15	27.25±0.25
	14	23.43±0.58	12.33±1.12	28.35±1.33
	28	37.43±1.53	13.36±1.05	25.66±1.21

Fungal load (colony count) was reduced to 26.43±0.58 and 21.32±0.51 at 14 day of application by dimethoate and parathion respectively, compared their number (42.43±1.53) at control (untreated soil). The fungal colonies decreased with increasing the application days of insecticides up to 28 days, however the colony number began to increase by the 28th day, but it was still less than that at control. This indicated that the applied insecticides were degraded during this period. The response of nitrogen fixing bacteria, actinomycetes, and bacterial population to dimethoate and parathion applications were like to that of the soil fungi. After application of insecticides, these populations increased to the same count or above at control. For instance, in comparision to control (total count was 67.87±2.08) the total colony of bacteria reached to maximum number 71.87±1.15 and 41.67±1.10 at 28 days of dimethoate and parathion application respectively. Actinomycetes started to increase at 14 days with number more than control. This result indicated that actinomycetes were highly resistant to insecticides. The response

of nitrogen fixing bacteria to both insecticides was different. The parathion was more effective than dimethoate with respect to colony count at different perids of application. These outcomes are consistent with Al-Ani *et al*. (2019) who concluded that the biomass and number of bacteria increased in the presence of certain insecticides while decreased with others, also, who concluded that the variance of the microorganisms depended on the type and length of application period. The observations in this study were similar to those of Kilonzi and Otieno (2024). The viable count declination could be assigned to the influences of insecticides toxicity on microbial development.

The growth of white rot fungi, namely *Pleurotus sajor-caju* and *Phanerochaete chrysosporium*, at different doses of organophosphorus insecticides dimethoate and parathion was visualized, and the sizes of the colonies are shown in Table 2. As the dose increased, the growth decreased, but with different levels of inhibition depending on insecticides type and used fungus. Parathion was more effective than dimethoate on both fungi. Thus, the colonies radius of *P. sajor-caju* and *P. chrysosporium* was 1.85 ±0.05 and 0.75±0.06 cm, respectively, using parathion and 3.33±0.12 and 1.85±0.05 cm, respectively, using dimethoate at 40 mg/L. According to Góngora-Echeverría *et al.* (2020), the tolerance of microorganisms to insecticides depends on species, insecticide kind, and applied dose. Also, the different levels of growth may depend on the ability of the fungus to degrade the insecticides *via* enzymes and its utilization as a carbon source.

Table 2. Effect of Different Dose of Dimethoate and Parathion on Fungal Growth

Dose (mg/L)	Colony Radius (cm)			
	Pleurotus sajor-caju		***Phanerochaete chrysosporium***	
	Dimethoate	Parathion	Dimethoate	Parathion
0	7.90±0.12	7.90±0.12	7.50±0.06	7.50±0.06
10	7.43±0.05	4.03±0.33	7.25±0.04	3.93±0.10
20	4.98±0.05	2.25±0.25	5.40±0.05	1.63±0.20
40	3.33±0.12	1.85±0.05	4.50±0.06	0.75±0.06

The ability of both *P. sajor-caju* and *P. chrysosporium* to remove dimethoate and parathion at different periods and concentrations is shown in Table 3. As the incubation period increased, the decomposition increased at all tested concentrations of both applied insecticides and using two fungal species. High decomposition (32.8 and 28.30% of dimethoate and parathion by *P. sajor-caju*, respectively, and 55.80 and 47.70% of dimethoate and parathion by *P. chrysosporium*, respectively) was obtained at applied low concentration (10 mg/mL) compared to decomposition (5.60 and 3.15% of dimethoate and parathion by *P. sajor-caju*, respectively; 18.03 and 13.08% of dimethoate and parathion, by *P. chrysosporium*, respectively) at high concentration (40 mg/L) at the same period of incubation (7 days). The decomposition of dimethoate was higher than that of parathion, particularly using *P. sajor-caju.* This indicates that these fungi possess mechanisms for insecticides degradation. Kumar *et al.* (2017) mentioned that the degradation process is affected by the period of the microorganisms exposed and dose residue of the insecticide. The findings showed that the two fungi in the present study were able to degrade insecticides over the course of 28 days. As noted by Yadav *et al.* (2016), the application of bacteria to degrade the insecticides has gained more investigation than fungi because of their high rate of growth. Unfortunately, the degradation ability of bacteria has been found to decline in the course of application time, unlike fungi (Abd El-Ghany *et al.* 2016). It was shown that *P. chrysosporium* under stress condition of osmosis, could degrade

pesticide mixtures (Fragoeiro and Magan 2005).

Table 3. Biodegradation of Organophosphorus Insecticides by *Pleurotus sajor-caju* and *P. chrysosporium* at Different Concentrations and incubation Periods

Dose (mg/L)	Incubation days	*P. sajor-caju*				*P. chrysosporium*			
		Dimethoate		**Parathion**		**Dimethoate**		**Parathion**	
		Rest concen-tration	Decom-position (%)	Rest concen-tration	Decom-position (%)	Rest concen-tration	Decom-position (%)	Rest concen-tration	Decompo-sition (%)
10	7	6.72± 0.23	32.8	7.17± 0.07	28.30	4.42± 0.06	55.80	5.23± 0.07	47.70
	14	3.81± 0.14	61.9	5.83± 0.17	41.70	3.35± 0.29	66.50	4.17± 0.03	58.30
	28	1.23± 0.04	87.7	3.08± 0.03	69.20	1.82± 0.06	81.80	3.17± 0.11	68.30
20	7	17.72± 0.11	13.65	19.76± 0.01	1.20	13.24± 0.11	33.80	17.82± 0.11	10.90
	14	9.54± 0.06	52.30	11.87± 0.12	40.65	10.73 ±0.20	46.35	12.24± 0.11	38.80
	28	4.51± 0.36	77.45	5.81± 0.06	70.95	4.36± 0.12	78.20	6.21± 0.05	68.95
40	7	37.76± 0.50	5.60	38.74± 0.24	3.15	32.79± 0.02	18.03	34.77± 0.06	13.08
	14	30.83± 0.17	22.93	32.77± 0.11	18.08	27.36± 0.47	31.60	29.15± 0.05	45.75
	28	10.74± 0.06	73.15	12.53± 0.27	68.66	8.08± 0.01	79.80	9.58± 0.31	76.05

The white rot fungi are considered to be a rich source of ligninolytic enzymes (Al-Rajhi *et al.* 2023, 2024). The production of ligninolytic enzymes including Lip, Laccase and MnPase by *P. sajor-caju* and *P. chrysosporium* was induced in the presence of insecticides, as shown in Table 4, documenting a relation between these enzymes and insecticides degradation. According to numerous studies, laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP) encourage the breaking down of several pesticides (Kannan *et al.* 2023). Liu *et al.* (2023) found that insecticide chlorpyrifos was removed with level more than 50% via 250 U/L of fungal laccase; therefore, their results indicated this enzyme considered good candidate for insecticides degradation. Several aromatic and nonaromatic compounds were oxidized *via* laccase which are employed as hydrogen donors. Mendarte-Alquisira *et al.* (2024) mentioned that fungal growth in liquid culture amended with insecticides after 8 days, the insecticide induced the content of extracellular protein and activity of peroxidase.

Table 4. Ligninolytic Enzymes and their Relation to Insecticide Degradation by Fungi

Enzymes (U/mL)	Fungi	Enzymes (U/mL)		
		Control	**Dimethoate**	**Parathion**
Lip	*P. sajor-caju*	0.98±0.11	1.55±0.03	1.25±0.03
	P. chrysosporium	0.87±0.03	0.95±0.11	0.75±0.05
Laccase	*P. sajor-caju*	4.30±0.04	5.33±0.03	6.32±0.06
	P. chrysosporium	3.55±0.02	3.89±0.12	4.20±0.04
MnP	*P. sajor-caju*	0.68±0.04	1.90±0.04	0.78±0.07
	P. chrysosporium	0.67±0.10	1.45±0.05	0.58±0.05

CONCLUSIONS

1. The studied white rot fungi were found to possess high efficacy for dimethoate and parathion decomposition by up to 87.7 and 70.95%, respectively, based on treatments with *Pleurotus sajor-caju*. Likewise, dimethoate and parathion decomposition were up to 79.80 and 76.05%, respectively, when treated by *Phanerochaete chrysosporium*.
2. The production of ligninolytic enzymes was induced in the presence of insecticides, which reflected the vital role of these enzymes in the biodegradation process.

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