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ORIGINAL ARTICLE

EFFECT OF ORGANIC AND PHOSPHATE FERTILIZATION ON SURVIVAL OF BIOLOGICAL CONTROL *TRICHODERMA HARZIANUM* AND *ASPERGILLUS TERRUS* IN SOIL

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Abstract: This research conducted to the study of the effect of levels of organic manure (poultry manure) (0, 5, 10, 15) ton/ha and phosphate fertilizer (super calcium phosphate 46% P_2O_5/ha) (0, 60, 120, 180) kg/ha on survival. Bio-control pesticides (*Trichoderma harzianum* and *Aspergillus terrus*) in soil. The results showed the ability of Bio-control pesticides to survive in the soil at different levels, where the highest average of fungal colonies was 34.1 × 510 spores/1 g soil for *T. harzianum* isolate of challenge when added 15 tons/ha of organic fertilizer. While the highest average of fungal colonies was 45.3 × 510 spores /1 g of soil for *A. terrus* pesticide when added 180 kg P_2O_5 /ha of calcium superphosphate fertilizer. The results also showed a decrease in the numbers of colonies of the bio-control fungi during the period of their stay in the soil for both fertilizers used.

***Key words*:** Organic Fertilization, Fungal Colonies, Bio-control pesticides.

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1. Introduction

The world is taking strides towards implementing strategies for using biopesticides in order to preserve human health and maintain biological and ecological balance from the dangers of using chemical pesticides, whose excessive use has had a profound impact on human health, where it has caused the emergence of many chronic and fatal diseases such as types of cancer. different and recently spread all over the world. The use of plant biopesticides has received a great deal of attention, which made them of great importance in integrated control [Harman (2000)]. In Iraq, interest in this topic has increased, especially after its success in combating some pathogens and pests with efficiency comparable to that of chemical pesticides [Heydari and Pessarakli (2010)]. What is taken of this control strategy is its unstable performance due to the varying environmental conditions in which the control agent operates on the one hand and its varying efficiency in combating all pathogens. One of the important agricultural operations that must be carried out in plants is the process of fertilizing with nutrients that improve plant growth and increase its yield, and organic fertilizers are one of the most important sources of supplying soil and plants with nutrients, which is due to the importance of adding them to the soil in improving its physical and chemical properties. Hao *et al*. (2008) indicated that adding organic fertilizers to the soil increases the organic matter in it and increases the number and activity of microorganisms. Since the soil is the main factor affecting the level of fungi inoculation, including the species of the genus Trichoderma, which is the focus of attention of many researchers who have contributions to biological control through the use of

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fungi [Pandya and Saraf (2010)]. The fungus *Trichoderma harzianum* is one of the most important species of Trichoderma in bio control because of its genes that destroy the cell wall of pathogens, which inhibit the production of DNA and protein and stop the production of some important enzymes [Benzohra *et al*. (2011), Salih *et al.* (2021)]. The fungus *Aspergillus terrus* Thom is one of the bio control factors by having a high efficiency in the production of important secondary metabolic compounds in biotechnology, especially in the agricultural field. Three anti-fungal compounds were identified, two of which recorded high inhibition against A. fumigates [Awaad *et al*. (2012)]. The fungus *A. terrus* is distinguished by its presence in the soil, as the fungus was able to form colonies on the inner stone bodies and penetrate the inner layer of the stone body of soil fungi. Also, the dissolution of the plasma membrane was observed in some cells infected with the fungus *A. terreus* [Melo *et al*. (2006), Manea *et al.* (2021)]. The aim of this research is to determine the appropriate application average of organic manure (poultry manure) and phosphate manure (super calcium phosphate) and the interaction between them in ability to survive of the biological resistance pesticides *Trichoderma harzianum* (isolate of Tahadi pesticide) and the fungus *Aspergillus terreus* in the soil.

2. Materials and Methods

2.1 Preparation of compost

For the purpose of preparing the organic fertilizer, poultry waste was used, where it was buried in pit of dimensions (2.5 × 1.5 × 1 m), to which chemical fertilizers were added (15 kg of urea, 25 kg of potassium sulfate, and 25 kg of calcium superphosphate and 10 kg of agricultural sulfur per ton of organic waste) with continued moistening of the mixture twice a week and the process of stirring and for three times during a period of one month between one and the other to ensure mixing the components and increasing decomposition. After the maturity of the waste, which is inferred by the low temperature of the waste and the disappearance of the ammonia smell and the transformation of the fertilizer to brown, a chemical analysis was conducted for the organic fertilizer and according to the methods described in Chapman and Pratt (1961) sterilization of the fertilizers using an autoclave at a temperature of 121°C and a pressure of 15 pounds. -2 for one hour, and re-sterilization in the same methods day after 24 hours.

2.2 Preparation of the culture medium

Take 39 g of the ready-made Potato Dextrose Agar (PDA) medium produced by Himedia, India and dissolve in an amount of distilled water in a glass flask and complete the volume to reach one liter. Chloramphenical medium was added at an amount of 250 mg/L, then distributed in 250 ml glass flasks, the nozzles of which were sealed with an airtight cotton stopper, and sterilized by autoclave at a temperature of 121°C and a pressure of 15 kg for 20 minutes, then the medium was allowed to cool and the pH adjusted to 7, then poured into Petri dishes according to the purpose of the experiment.

2.3 Isolation and identification of bio control fungi

Fungus *Trichoderma harzianum*: The isolate of the fungus *Trichoderma harzianum* was obtained from the biocide, the challenge of production of the Agricultural and Biological Research Department, Baghdad (active substance, spores and mycelium of the fungus *T. harzianum)*. The pesticide was used in a mixture with water at a ratio of 10 g/100 ml and cultured by decantation method in Petri dishes containing PDA medium and incubated at a temperature of 25°C for five days and after the emergence of colonies. Isolation of fungus colonies was conducted on PDA culture medium to obtain pure colonies of fungus, characterized based on taxonomic characteristics.

Fungus *Aspergillus terreus*: The method mentioned by Hussaini *et al*. (2007) was adopted with modification. Samples were taken from infected apple fruits that showed symptoms of black and brown rot and placed in sterile Petri dishes and poured (40) ml/ dish of PDA culture medium at 25°C, then shake horizontally to mix and leave to solidify. The dishes were incubated in the incubator at 25°C for two weeks. Dishes were tested after three days of incubation and continued monitoring. Isolation of the fungus colonies was conducted on PDA culture medium to obtain pure colonies of fungus, characterizing fungus according to the taxonomic keys given in Thom and Raper (1939).

Effect of adding organic fertilizer and super calcium phosphate fertilizer on numbers of fungal colonies of bio control pesticides. A sandy mixture was used, which was sterilized by spraying the soil with formaldehyde at a concentration of 5% well until it reached the stage of wetness, then it was covered with plastic for a week, after which the plastic was lifted and left for ventilation for 10-15 days. It was distributed in pottery pots with a

Table 1: The effect of adding organic fertilizer on the numbers of fungal colonies of the bio-control pesticides *Trichoderma harzianum* and *Aspergillus terreus* spore/1 g soil (10^5) during (1, 3, 5 months) after application.

Bio-control pesticide	Period after addition/month	Number of colonies of fungal spores/1 g of soil (10^5) at levels of organic fertilizer addition ton/ha				Average
		0 (control)	5	10	15	
Trichoderma harzianum	1	20.3	31.8	39.5	44.6	34.1
	3	6.8	15.7	17.4	20.8	15.2
	5	9.5	16.2	18.1	22.9	16.7
	Average	12.2	21.2	25.0	29.4	
LSD (0.05)		Addition level =2.4		Period = 2.2		
Aspergillus terreus	1	20.3	27.8	30.2	35.6	28.5
	3	6.8	12.4	14.9	21.4	13.9
	5	9.5	15.3	18.2	24.7	17.0
	Average	12.2	18.5	21.1	27.2	
LSD (0.05)		Addition level =2.6		Period = 2.4		

diameter of 27 cm and at a rate of 5 kg of soil for each pot. Then, organic fertilizers were added with good mixing at levels (5, 10, 15) ton/ha and four levels of phosphate fertilizer (super calcium phosphate 46% P_2O_5/ha) (60, 120, 180) kg/ha, with the control treatment remaining without adding organic and phosphate fertilizers. The soil was moistened, then the culture medium was added, then the culture medium containing PDA containing the bio-control pesticides *T. harzianum* isolate of Tahadi pesticide or the isolate of the fungus *A. terreus* at the age of 7 days was added using one plate with a diameter of 9 cm for each pot after cutting it into small pieces up to 1 cm 3 and mixed well with the soil. Then the soil was irrigated and the pots were covered with perforated polyethylene bags and left for three days, after which wheat seeds were cultivated.

Barcelona was classified at 20 seeds per pot, with all basic agricultural operations to serve the crop equally for all treatments. The Dilution Plate Method was used to PDA to calculate the number of reproductive units per gram of dry soil, where a suspension of soil was made and 1 ml of dilutions (10^{-4} and 10^{-5}) was taken and placed in a petri dish with a diameter of 9 cm and the culture medium was poured over it PDA with moving dishes a capillary movement to homogenize the distribution of dishes. The dishes were left to harden and then incubated upside down at $\pm$25°C. The results were recorded after 10 days of incubation and the number of reproductive units per gram of dry soil was estimated by multiplying the number of colonies by the inverted dilution and during the period (1, 3, 5) months of adding the two bio-control pesticides *T. harzianum* and *A. terreus*.

2.4 Statistical Analysis

The results of the research were analyzed according to the factorial experiment model and according to the Randomized Complete Block Design, the Least significant difference (L.S.D) test was used under the probability level of 0.05 to test the significance of the results [Al-Sahoki and Waheeb (1990)]. The data of the experiment were statistically analyzed using the Statistical Analysis System.

3. Results and Discussion

3.1 Effect of adding organic fertilizer on the effectiveness of the bio-control pesticides *Trichoderma harzianum* and *Aspergillus terreus*

The results of isolation from the soil by dilution method showed the ability of the pesticides *T. harzianum* and *A. terreus* to remain in the soil and the root area at different levels during the period of 1, 3 and 5 months after addition, as the results shown in Table 1 indicated that the addition of organic fertilizer recorded an increase in the survival average of the bio control fungi by increasing the levels of adding the organic fertilizer. The number of fungal colonies increased with the increase in the level of addition, where the number of colonies reached (21.2, 25.0 and 29.4) and (18.5, 21.1 and 27.2) × 10^5 spores / 1 g of soil for the two pesticides *T. harzianum* and *A. terreus* and the levels of addition were 5, 10 and 15 tons/ha, respectively compared with 12.2 × 10^5 spores / 1 g of soil in the control treatment. The results in Table 1 indicate the treatment of adding 15 tons/ha of organic fertilizer excelled and gave the highest significant increase in the number of fungal colonies of the

Table 2: Effect of adding calcium super phosphate fertilizer on the numbers of fungal colonies of biocontrol pesticides *Trichoderma harzianum* and *Aspergillus terreus* spore/1 g soil (10^5) during (1, 3, 5 months) after application.

Bio-control pesticide	Period after addition/month	Number of fungal colonies with spores/1 gm of soil (105) at levels of superphosphate fertilizer addition (kg P_2O_5/ha).				Average
		0 (control)	60	120	180	
Trichoderma harzianum	1	20.3	26.6	40.5	45.3	33.2
	3	6.8	17.8	22.0	22.6	17.3
	5	9.5	21.9	23.1	28.8	20.1
	Average	12.2	25.9	28.3	32.9	
LSD (0.05)		Addition level =2.8			Period = 2.4	
Aspergillus terreus	1	20.3	40.1	48.6	50.7	39.9
	3	6.8	21.4	26.7	30.3	21.3
	5	9.5	27.8	31.3	43.6	28.1
	Average	12.2	30.8	36.0	41.5	
LSD (0.05)		Addition level =2.7			Period = 2.6	

biological resistance pesticides *T. harzianum* and *A. terreus* after a month of addition, as it reached (44.6 and 35.6) × 10^5 spores /1 g of soil for the two pesticides, respectively, compared with (22.9 and 24.7) × 10^5 spores/1 g of soil for the two pesticides after 5 months of addition, respectively. The increase in the number of fungal colonies by increasing the level of organic fertilizer addition was in agreement with what was mentioned by Al-Naim (2002) that adding organic fertilizers leads to an increase in organic matter in the soil and thus improves its properties and improves water absorption by the roots.

Organic fertilizers work on improving the soil properties, restoring the microbial balance of the soil, and activating its bio processes. It is also noted that the numbers of fungal colonies of the two pesticides decrease with the length of their stay in the soil, and the preservation of its vitality and survival in the soil and the root area varies according to the type of isolate used.

3.2 Effect of adding calcium superphosphate fertilizer (kg P_2O_5/ha) on the effectiveness of the bio-control pesticides *Trichoderma harzianum* and *Aspergillus terreus*

The number of colonies of the biocontrol fungi *T. harzianum* and *A. terreus* recorded a direct increase with the increase in the levels of addition of calcium superphosphate fertilizer (kg P_2O_5/ha), as the number of fungal colonies reached (41.5 and 32.9) × 10^5 Spores/ 1 g soil of biocontrol fungi *A. terreus* and *T. harzianum*, adding 180 kg P_2O_5/ha of calcium superphosphate fertilizer, respectively, while the addition level of 60 kg P2O5/ha achieved a rate of (30.8 and 25.9) × 10^5 spores/1 g soil for biocontrol fungi *T. harzianum* and *A. terreus*, respectively (Table 2). Table 2 also shows a decrease in the number of colonies of the biological resistance fungi *A. terreus* and *T. harzianum* with an increase in the period, as the highest rate in the number of fungal colonies reached (39.9 and 33.2) × 10^5 spores/ 1 g of soil a month after the addition and for the two pesticides, respectively. While the numbers of fungal colonies decreased reach (28.1 and 20.1) × 10^5 spores/ 1 gm of soil for biocontrol fungi *A. terreus* and *T. harzianum* after 5 months of addition, respectively. The increase in the numbers of fungal susceptibility when adding calcium phosphate fertilizer may be due to the role of the fungi used to produce some antibiotics and organic acids through the cycles of nutrients, including nitrogen, phosphorous, potassium, iron, sulfur and copper [Faddoul and Nafaa (2009)]. It is noticed from Tables 1 and 2 that the addition of calcium superphosphate fertilizer had the greatest effect on the number of colonies of the bio control fungi *T. harzianum* and *A. terreus* compared to the addition of organic fertilizer, as it achieved the highest increase in the number of fungal colonies at the highest level of addition of the two fertilizers amounting to (45.3) and 50.7) × 10^5 spores/1 g of soil for bio control fungi *T. harzianum* and *A. terreus* for the level of addition of 180 kg P_2O_5/ ha of calcium superphosphate fertilizer, respectively, compared to the treatment of adding 15 tons/ha of organic fertilizer amounted to (44.6 and 35.6) × 10^5 Spores/1 gm of soil for bio control fungi *T. harzianum* and *A. terreus*, respectively.

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