University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)

https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr.v13ii 10.*

Evaluation of the Efficiency of Fungal Filtrates in Controlling the Khapra Beetle Trogoderma granarium (Everts) (Coleoptera : Dermestidae)

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I. Abstract

This study aimed to isolate and identify fungi associated with the khapra beetle (*Trogoderma granarium*) and to evaluate their potential use in biological control. The results of isolation from dead larvae and adults of the khapra beetle revealed ten different fungal isolates belonging to several genera. These included four isolates of *Aspergillus spp.* (*A. flavus, A. terreus, A. niger,* and *A. parasiticus*), one isolate each of *Beauveria bassiana* and *Cladosporium oxysporum*, two isolates belonging to *Penicillium spp.* (*P. chrysogenum* and *P. crustosum*), and two isolates belonging to *Trichoderma spp.* (*T. asperellum* and *T. longibrachiatum*).

These isolates were identified based on their morphological and microscopic characteristics. The biological efficiency of five fungal isolates (*B. bassiana, P. chrysogenum, P. crustosum, T. asperellum,* and *T. longibrachiatum*) was evaluated using different concentrations of fungal filtrates (25, 50, 75, 100%) to control second stage larvae and adults of insect.

The results showed that the filtrate of *B. bassiana* was the most effective in causing the highest mortality rates at all concentrations for both second stage larvae and adults, reaching 69.96% and 61.95%, respectively. In contrast, *T. asperellum* recorded the lowest mortality rates at all concentrations, reaching 47.94% and 45.97% for larvae and adults.

The results also indicated that fungal filtrates had no significant effect on the germination rate of treated grains. This indicates the potential use of these fungi as safe and effective alternatives in biological control programs for stored insects, and the use of grains as seeds suitable for planting.

Keywords : *Trogoderma granarium, biological control, fungal filtrate*

University of Thi-Qar Journal of agricultural research
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II. Introduction

Stored cereal crops and their products are of great importance for food security, as they provide the calories required for daily consumption and are among the main sources of protein essential for human nutrition (Abdul Hussain & Rashid, 2023). Wheat is one of the most important cereal crops in the world, grown in most countries, and covers 35% of the world's food need (Gad et al., 2024).

During storage, grains are exposed to infestation by many insect pests (Wakil et al., 2014). The khapra beetle, *Trogoderma granarium* (Everts), is one of the most destructive pests of stored cereal (Younus and Karso, 2022). It is one of the main insect pests in storage facilities due to its ability to penetrate seed coats and often feeds on intact grains (Hadi and Sabet, 2024) it infects cereal and causes severe damage (Riaz et al., 2022).

Control depends on The *T. granarium* beetle is currently heavily reliant on chemical insecticides (Kavalieratos et al., 2017). The continued use of chemical pesticides has led to insect resistance, in addition to serious environmental, human, and animal health problems (Karanastasi et al., 2020). This has prompted researchers to seek alternative, safe, and residue-free methods (Mantzoukas et al., 2020). Recently, biocontrol has become a modern research focus for combating stored pests. Mycotoxins have also garnered increasing attention due to their ability to produce acute or non-lethal toxic effects (Sharma and Walia, 2021). Several studies have demonstrated the effectiveness of entomopathogenic fungi as biological control agents against stored insects (Abdelgaleil et al., 2025). The use of *Beauveria bassiana* by Al-Amara (2009) in controlling the khapra beetle led to Second stage larvae and adult mortality rates reached 45.38% and 48.39%, respectively. In another study, 10 fungal species belonging to the genera Penicillium, Lecanicillium, Condenascus, and Cladosporium were used on stored beetles including the *T.granarium* insect, high mortality rates were recorded with these treatments, with the highest percentage observed with the fungus *P.goetzii* (Mantzoukas et al., 2023).

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III. Materials and Methods :

1. Insect Rearing

A pure colony of the khapra beetle, *Trogoderma granarium*, was obtained from the Entomology Laboratory at the College of Agriculture, University of Basra. The insect was identified by Professor Jinan Malik Khalaf using the taxonomic key (N D P, 2022). The insects were reared in culture at a temperature of 30 ± 1 °C and a relative humidity of 60 ± 5% (Al-Iraqi and Suleiman, 2002). They were fed sterile wheat grains of the Abu 99 cultivar. The colony was continuously monitored and renewed after each generation.

2. Isolation of Fungi

Fungi were isolated from the larvae and dead adults of the *T. granarium* by taking four larvae and adults and sterilizing them with 10% sodium hypochlorite for three minutes. They were then washed with sterile distilled water and placed on sterile filter paper to dry. This process was repeated several times, and the plates were kept in an incubator at 25 ± 2°C for seven days. After that, the plates were examined, and the growing fungal colonies were purified of the insect and larvae by taking a disc from the margin of the fungal colony and transferring it with a sterile needle to the center of a new Petri dish containing PDA culture medium.

3. Fungal Identification

The fungi isolated from the larvae and adults of the dead insect *T. granarium* were identified morphologically based on their morphological characteristics, the shape of their spores and spore holders, and the sexual and asexual structures they formed. This identification was performed by Dr. Yahya Ashour, Department of Plant Protection, College of Agriculture, University of Basra, using the following taxonomic keys: Ellis 1971; Domsch et al. (1980); Pitt and Klich (1988); Pitt and Hocking 2009)

4. Preparation of Isolated Fungal Filtrates

Fungi isolated from dead larvae and adults of the *T. granarium* were cultured on potato dextrose broth supplemented with 250 mg/L of the antibiotic chloramphenicol. The filtrate was aliquoted into 250 ml flasks at 150 ml per flask, and the flasks were securely sealed with cotton plugs. The filtrate was then autoclaved at 121°C and 15 psi for 15 minutes. Each flask was then inoculated with a 0.5 cm diameter disc containing 7-day-old fungal colonies grown on PDA medium. The flasks were incubated in the incubator at a temperature of 25 ± 2 °C for 14 days, shaking the flasks every 3-4 days for the purpose of distributing fungal growth. Afterwards, they were filtered using Whatman No. 1 filter paper, then filtered with a Buchner funnel with the help of a vacuum pump and using 0.22 filter paper for the purpose of preventing the passage of fungal spores (Huxham and Lackie, 1988).

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5 . Effect of Fungal Filtrates and Devimethrin EC (10%) on the Mortality Rate of Second stage Larvae and Adults of *Trogoderma granarium*

Ten grams of wheat grains (Abu 99 cultivar) were sterilized in an oven at 50 °C for two hours (Husain et al., 1921). The grains were then placed in plastic Petri dishes (90 mm diameter) and treated with fungal filtrates at a rate of 1 ml per dish using concentrations of 25, 50, 75, and 100% (v/v). The required concentrations were prepared by diluting the fungal filtrate with sterile distilled water for concentrations lower than 100%. Ten larvae were introduced into each dish, with three replicates per treatment.

For the insecticide treatment, sterilized wheat grains were treated with Devimethrin EC (10%) at concentrations of 0.02, 0.04, 0.06, and 0.08%. Each replicate contained 10 g of treated wheat grains per concentration, which were allowed to air-dry before introducing ten second instar larvae per replicate. Each treatment was conducted separately in 90 mm plastic Petri dishes with three replicates per concentration.

The control treatment was sprayed with 1 ml of distilled water only. All treatments were incubated at 30 ± 1 °C and 60 ± 5% relative humidity. Mortality percentages were recorded after 1, 3, 5, and 7 days of treatment. The rate mortality was calculated using the following equation (Al-Mallah and Al-Jubouri, 2006). The data were transformed into angular values for statistical analysis:

Mortality (%) = (Number of dead individuals / Total number of individuals) × 100

For adult insects, ten individuals (5 females and 5 males) were used one day after emergence from the pupal stage. The corrected mortality percentage was calculated using Abbott's formula (Abbott, 1925), and the values were also transformed into angular values for statistical analysis:

Corrected mortality (%) = [(Mortality in treatment − Mortality in control) / (100 − Mortality in control)] × 100

6.**Effect of Fungal Filtrates and Devimethrin EC (10%) on the germination Percentage of Wheat grains**

This experiment was conducted to evaluate the effect of fungal filtrates and the insecticide Devimethrin on the germination rate of wheat grains. The grains were treated by soaking them separately in fungal filtrates and the insecticide for 10 minutes, using the highest concentration 100% for the filtrates and 0.08% for Devimethrin. The grains were then allowed to air-dry under laboratory conditions.

Afterward, ten grains were placed in Petri dishes on moist gauze, with three replicates for each treatment (five fungal filtrates and the insecticide). Moisture was maintained by adding water as needed. For the control treatment, the grains were soaked in water only.

The germination percentage was recorded after 5 days according to the following equation (Shaaban and Al-Mallah, 1993):

Germination (%) = (Number of germinated seeds / Total number of seeds) × 100

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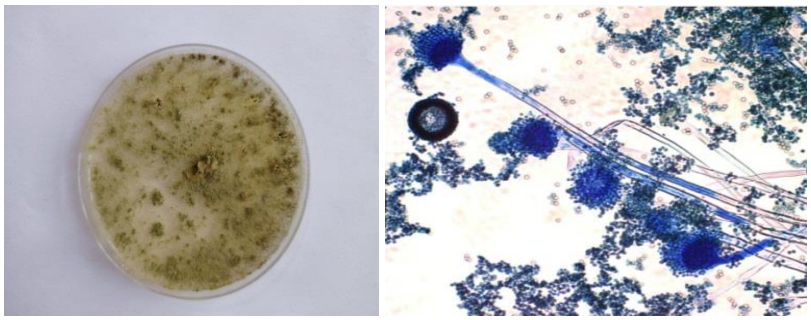
7- Statistical Analysis

All laboratory experiments were conducted according to the design of three-factor and one-factor experiments according to the CRD design. The percentage values of mortality ratios were angularly transformed, and the means were compared according to the least significant difference (LSD) method under a probability level of 0.01 (Al-Rawi and Khalaf Allah, 1980).

IV. Results and Discussion

1- Fungal Isolation and Morphological Identification

The results of the isolation and morphological identification process yielded 10 fungal isolates associated with the larvae and dead adults of *T. granarium*. Four of these isolates belong to the genus *Aspergillus* spp. (*A. flavus*, *A. terreus*, *A. niger*, and *A. parasticus*), one isolate each from *Beauveria bassiana* and *Cladosporium oxysporum*, two isolates from the genus *Penicillium* spp. (*P. chrysogenum* and *P. crustosum*), and two isolates from the genus *Trichoderma* spp. (*T. asperellum* and *T. longibrachiatum*). These are shown in Figures (1-10).



A B

Figure (1) -**A**. *Aspergillus flavus* fungal colony

B. Mycelium of the fungus *A. flavus*

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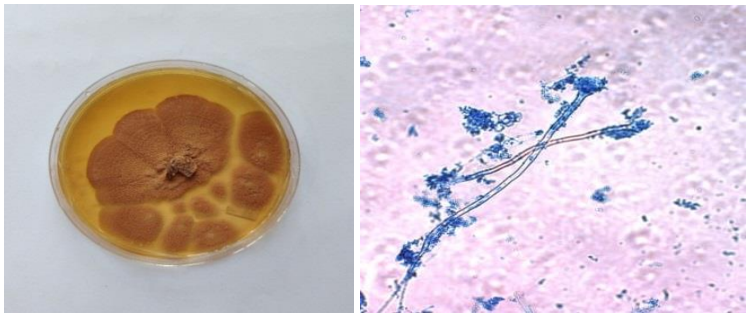
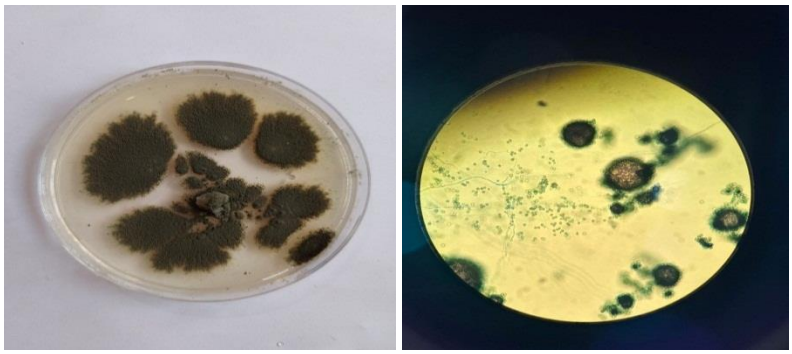


A B

Figure (2) –A. *A. terreus* fungal colony

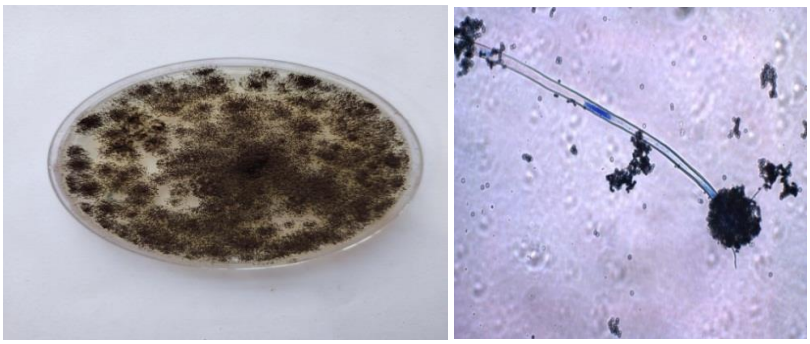
B. Mycelium of the fungus *A. terreus*



A B

Figure (3) –A. *A. Parasticus* fungal colony

B. Mycelium of the fungus *A. Parasticus*



A B

Figure (4) –A. *A.niger* fungal colony

A.niger **B.** Mycelium of the fungus

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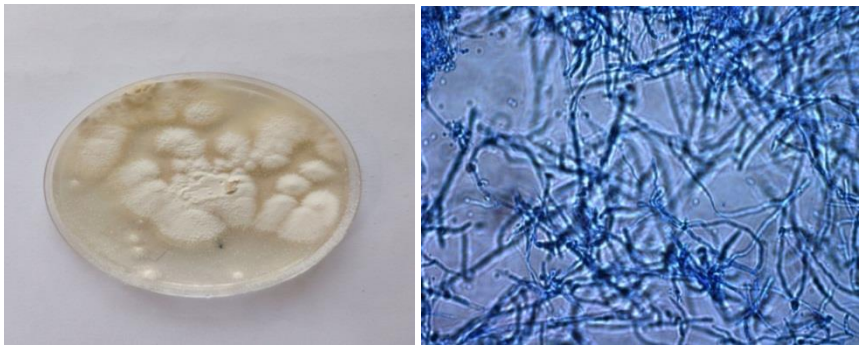
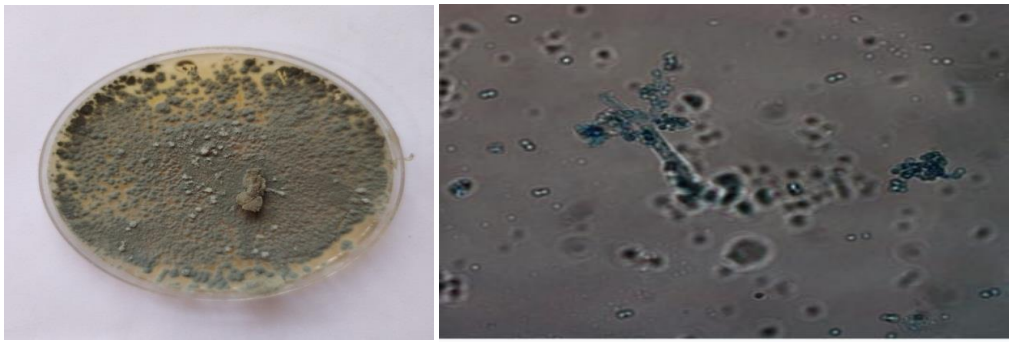


A B

Figure (5) –A. *B.bassiana* fungal colony

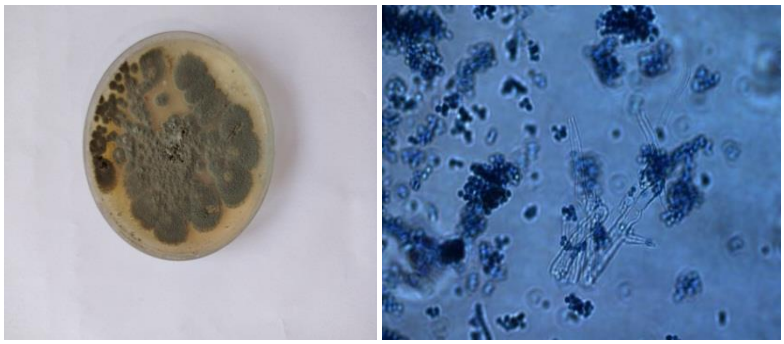
B. Mycelium of the fungus *B.bassiana*



A B

Figure (6) –A. *P .chrysogenum* fungal colony

B. Mycelium of the fungus *P.chrysogenum*



A B

Figure (7) –A. *P . crustosum* fungal colony

B. Mycelium of the fungus *P . crustosum*

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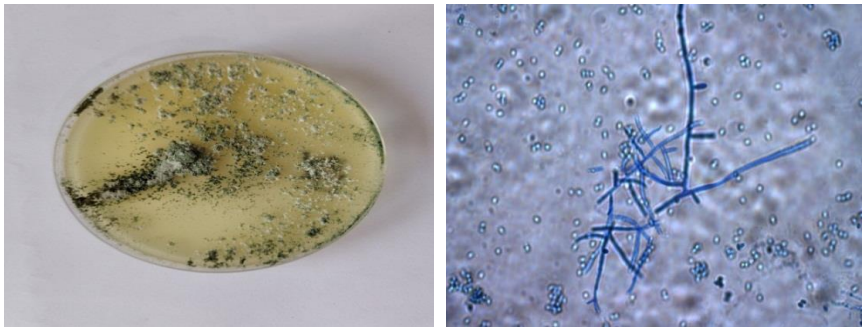
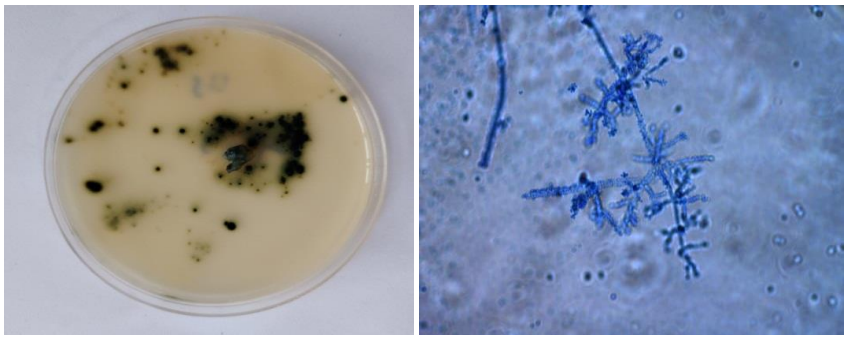


A B

Figure (8) –A. *T. longibrachiatum* fungal colony

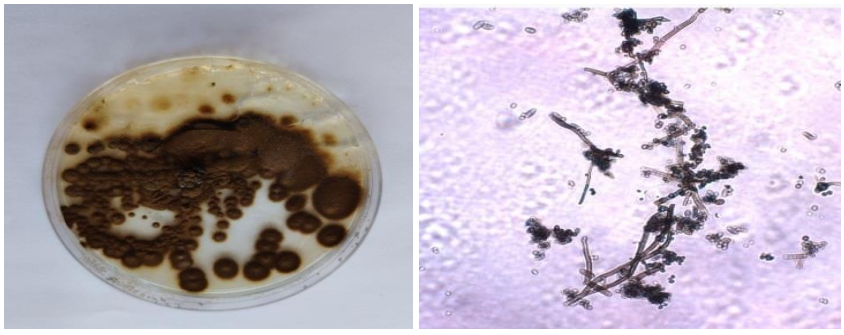
B. Mycelium of the fungus *T. longibrachiatum*



A B

Figure (9) –A. *T. asperellum* fungal colony

B. Mycelium of the fungus *T. asperellum*



A B

Figure (10) –A. *Cladosporium oxysporum* fungal colony

B. Mycelium of the fungus *Cladosporium oxysporum*

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2.Effect of fungal filtrates and the fungicide Ec Devimethrin 10% on the mortality rate of second-stage larvae of *T. granarium*

The results shown in Table (1) indicate the effect of the filtrate and the pesticide on the percentage mortality of second instar larvae, which were 69.96%, 63.39%, 56.90%, 53.76%, 47.94%, and 83.81% for *B. bassiana*, *P. crustosum*, *P. chrysogenum*, *T. longibrachiatum*, *T. asperellum*, and the pesticide Devimethrin, respectively. Statistical analysis revealed significant differences between the treatments at a probability level of 0.01.

The observed mortality can be attributed to the production of secondary metabolites by fungi. These compounds include peptides, enzymes, organic acids, antibiotics, pigments, and others (Lopes et al., 2013). *Beauveria bassiana* produces several secondary metabolites such as Beauvericin, Bassianolide, Beauveriolide, and Bassiacridin, in addition to yellow pigments like Tenellin and Bassianin. The fungus also produces the red non-peptidic pigment oosporein, which consists of biologically active compounds that do not enter the food chain or accumulate in the environment, and are characterized by low toxicity to humans (Ávila-Hernández et al., 2020). Beauvericin is considered one of the most important mycotoxins due to its insecticidal and antibiotic activities, along with other biological effects (Mallebrera et al., 2018).

Species of *Trichoderma* spp. produce secondary metabolites with insecticidal activity, such as Peptaibols, Gliotoxins, and Chitinases, which degrade the insect cuticle and disrupt the molting process (Atta et al., 2025). In addition, *Trichoderma* spp. produce other compounds including Pyrones, Terpenes, Butenolides, Viridin, Harzianopyridone, and Harziandione (Rodríguez-González et al., 2022).

Similarly, *Penicillium* spp. produce several secondary metabolites such as Citrinin, Ochratoxin, Patulin, Rubratoxin, and Penicillic acid, which have been reported to reduce fertility, decrease feeding rates, inhibit larval growth, and affect the nervous system, ultimately leading to increased mortality (Nicoletti et al., 2023).

Mycotoxins cause a range of symptoms in exposed insects, including severe desiccation, abnormal behavior, feeding difficulties, convulsions, and metabolic disturbances, which ultimately lead to insect death (Chu et al., 2017).

The results also demonstrated that the 100% filtrate concentration and 0.08% insecticide concentration were the most effective in causing high mortality rates when applied to wheat grains, with significant differences compared to other concentrations. This increase in mortality is attributed to the higher concentration of fungal toxins, which enter the insect body either orally, through contact with the cuticle, or via the respiratory openings, leading to symptoms of intoxication (Al-Ouboodi and Mohammed, 2024).

Furthermore, the results indicated that the effectiveness of the filtrates increased with time after treatment, as the mortality rate of larvae increased significantly, reaching 44.03%, 56.80%, 72.35%, and 77.34% after 1, 3, 5, and 7 days, respectively. This may be due to the ability of entomopathogenic fungi to produce toxic substances that affect insect pests, with mortality increasing as both concentration and exposure duration increase (Diwan et al., 2009).

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Table (1) Effect of fungal filtrates and pesticide on the percentage of death of second-stage larvae of *T.*

Mean effect of concentrations	Mean effect of treatments	Interaction between treatments and concentration	Percentage mortality of second instar larvae per day				Concentration	Treatments
			7	5	3	1		
54.25	69.96	63.58	79.33	73.33	61.66	40	% 25	***Beauveria bassiana***
55.66		66.55	80.86	75.66	64.66	45	% 50	
65.64		72.69	85.51	79.66	70.86	54.73	% 75	
72.18		77.06	90.34	90.2	83.04	44.66	% 100	
	56.90	49.04	66.08	60	40.08	30	% 25	***Penicillium chrysogenum***
		49.35	68.66	61.1	41	33.33	% 50	
		61.30	79.43	77	49.45	39.33	% 75	
		66.27	74.94	73.74	70.08	46.33	% 100	
	63.39	54.71	73	68.66	45.2	40	% 25	***Penicillium crustosum***
		59.50	78.41	71.66	46.84	41.1	% 50	
		65.60	82.41	78.41	54.2	47.4	% 75	
		71.77	82.98	75.44	71	57.66	% 100	
	47.94	37.25	53.33	46.66	28.33	20.66	% 25	***Trichoderma asperellum***
		42.90	63.66	56.76	31	20.1	% 50	
		53.86	76.12	70	45.16	24.61	% 75	
		57.87	71.2	62.25	55.41	41.94	% 100	
	53.76	43.51	63.1	50	33.11	27.86	% 25	***Trichoderma longibrachiatum***
		48.85	67.66	61.66	35.76	30.33	% 50	
		56.85	78	74.66	47.08	27.66	% 75	
		65.85	76.91	76.57	63.24	46.70	% 100	
	83.81	75.58	84	80	74	64.33	0.02	**Devimethrin**
		82.08	90	85.90	80.1	72.33	0.04	
		83.55	90.24	87.28	81.70	75	0.06	
		94.03	100	100	90.32	85.83	0.08	
	0	0	0	0	0	0		control
			77.34	72.35	56.80	44.03	Mean effect of exposure time	

0.01 –LSD Treatments= 2.46 ، – 0.01LSD Concentration = 2.01 – 0.01 ، LSD Time2.01 =

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granarium

3.Effect of fungal filtrates and 10% Devimethrin fungicide on mortality rates of adult *T. granarium* insects

The results shown in Table (2) indicate the effect of the filtrate and the pesticide on the corrected percentage mortality of adult Khapra Beetle, which were 61.95%, 57.05%, 50.06%, 53.34%, 45.97%, and 78.56% for the treatments *B. bassiana*, *P. crustosum*, *P. chrysogenum*, *T. longibrachiatum*, *T. asperellum*, and the pesticide Devimethrin, respectively. Statistical analysis revealed significant differences between the treatments at a probability level of 0.01. The control treatment had a mortality rate of 2.5%.

The results are consistent with a study conducted by Hassuba et al. (2024), in which four isolates of *Trichoderma* spp. and the fungus *Metarhizium anisopliae* were used, all of which caused significant mortality in *T. granarium* larvae. Similarly, a study by Mantzoukas et al. (2023) evaluated the effect of fungi such as Penicillium, Lecanicillium, Cladosporium, and Condenascus on various economically important beetle species, including *T. granarium*, and reported high mortality rates for all tested insects.

The results also showed that the 100% fungal filtrate and 0.08% Devimethrin concentrations produced the highest mortality rates in adult *T. granarium* when wheat grains were treated, with statistically significant differences compared to other concentrations. Al-Jubouri (2007) noted that insect mortality increases with higher concentrations of fungal filtrates. Moreover, the effectiveness of the filtrates increased over time after treatment, as the adult mortality rates significantly rose with exposure duration, reaching 38.10%, 54.72%, 66.09%, and 71.13% after 1, 3, 5, and 7 days, respectively.

These findings agree with Latif (2010), who reported that exposure time plays a crucial role in biological control, with a positive correlation observed between the exposure period and mortality rate.

University of Thi-Qar Journal of agricultural research

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Table (2) Rate of effect of fungal filtrate and pesticide on the percentage of death of adult *T. granarium* insects

Mean effect of concentrations	Mean effect of treatments	Interaction between treatments and concentrations	Percentage mortality of adult insects per day				Concentration	treatments
			7	5	3	1		
47.48	61.95	52.56	73.51	63.41	53.33	20	% 25	*Beauveria bassiana*
52.85		56.81	76.91	68.33	60.33	21.66	% 50	
59.05		62.61	82.12	71.66	66.66	30	% 75	
70.65		75.85	90.08	90	81.66	41.60	% 100	
	50.06	40.06	55.25	48.36	33.33	23.33	% 25	*Penicillium chrysogenum*
		47.61	60.16	53.41	40.20	33.33	% 50	
		51.72	63.5	63.33	45.08	35	% 75	
		61.70	70.04	66.74	66.70	43.33	% 100	
	57.05	46.28	63.50	58.33	33.33	30	% 25	*Penicillium crustosum*
		51.73	67.54	59.08	43.66	36.66	% 50	
		59.21	73.45	70	48.43	45	% 75	
		70.95	82.08	75	70.08	56.66	% 100	
	45.97	35.05	46.83	46.72	33.33	13.33	% 25	*Trichoderma asperellum*
		39.23	50.16	46.79	40	20	% 50	
		45.81	65.12	51.70	43.66	22.75	% 75	
		56.20	70.04	60.08	53.37	41.66	% 100	
	53.34	44.19	60.12	60	40	16.66	% 25	*Trichoderma longibrachiatum*
		49.18	65.08	65	45	21.66	% 50	
		54.21	70.20	70	48.33	28.33	% 75	
		65.80	76.83	76.66	63.04	46.66	% 100	
	78.56	67.58	76.91	66.75	63.33	60	0.02	Devimethrin
		73.37	77.75	73.08	72	70.66	0.04	
		80.78	90	81.74	78.37	73	0.06	
		93.35	100	100	90.08	83.33	0.08	
	2.5	2.5	10	0	0	0		Control
			71.13	66.09	54.72	38.10		Effect of time

– 0.01L.S.D treatments = 3.67 – 0.01 ، L.S.D Concentration – 0.01، 2.99 = L.S.D Time2.99 =

University of Thi-Qar Journal of agricultural research
Thi-Qar Journal of agricultural research
ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 10.

5.Effect of fungal filtrates and the fungicide Devimethrin 10% on wheat grain germination rates

The results shown in Figure (11) indicate no significant differences between the fungal filtrate and the control treatment in wheat grain germination percentage. The average germination percentages were 66.66%, 86.66%, 86.66%, 83.33%, 80%, and 76.66% for *B. bassiana*, *P. crustosum*, *P. chrysogenum*, *T. longibrachiatum*, *T. asperellum*, and the fungicide Devimethrin, respectively. The average germination percentage for the control treatment was 83.33%. The difference in effect compared to other studies is attributed to the different fungal isolates used.

Khalaf and Banyan (2025) reported that B. bassiana reduced the germination percentage of wheat grains compared to the control treatment because it secretes toxins that may affect vital physiological activities and disrupt the function of some tissues, thereby potentially reducing seed germination. Another study indicated that *P. chrysogenum* improved wheat seed germination by alleviating the effects of salinity stress on wheat (Dargiri et al., 2025).

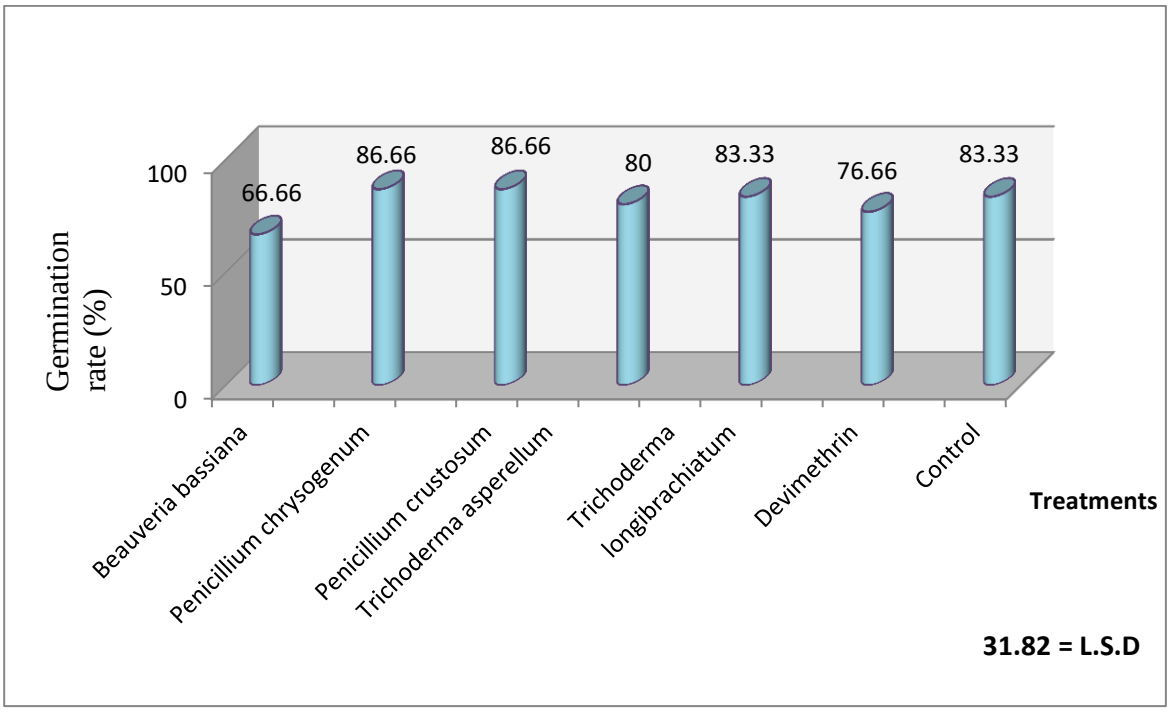

Figure (11) Germination rate (%) of wheat treated with fungal filtrates and Devimethrin insecticide after 5 days at 100% concentration for the filtrates and 0.08% for the insecticide.

ISSN Onlin:2708 -9347, ISSN Print: 2708 -9339 Volume 15, Issue 1 (2026)
https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr.v13ii 10.*

V. Conclusions :

The results of this study indicated the efficiency of fungal filtrates in causing the destruction of the different stages of the Khapra Beetle *T. granarium*. The fungus *B. bassiana* was the most efficient in controlling the insect, with the effect increasing with increasing concentration and exposure time. The fungal filtrates did not significantly affect the germination percentage of the grains, which demonstrates the possibility of using them in protecting stored grains and incorporating them into biological control programs.

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