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ORIGINAL RESEARCH ARTICLE

Efficacy of entomopathogenic fungi for the management of *Trogoderma granarium* Everts on wheat grains

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Abstract

Four species of *Trichoderma* genius (*T. harzianum*, *T. citrinoviride*, *T. viride* and *T. asperellum*) and *Metarhizium anisopliae* were assessed for their effects on larval mortality and progeny production of *Trogoderma granarium* Everts (Coleoptera: Dermestidae). The fungal strains were tested at 2.0×10^6, 2.0×10^7, 2.0×10^8 and 2.0×10^9 spores/kg. The larval mortality of *T. granarium* was recorded after 7, 14 and 21 days of treatment. The emerged adults (F_1) from treated larvae were counted after 30 days of treatment, while F_2 and grain weight loss were examined after 80 days of treatment. In general, increasing of spore concentration of all fungal strains enhanced the larval mortality. After 21 of treatment, all fungal strains induced notable larval mortality particularly at the highest concentration (2.0×10^9 spores/kg) except *T. viride* (19.4%). The highest larval mortality was observed in treatments with *M. anisopliae* (82.1%) and *T. citrinoviride* (69.0%) at 2.0×10^9 spores/kg, respectively. Furthermore, all spore concentrations of tested fungi decreased the emerged adults (F_1 and F_2) with *M. anisopliae*, *T. citrinoviride* and *T. harzianum* being the most potent, particularly at 2.0×10^9 spores/kg. After 80 days, *M. anisopliae* and *T. citrinoviride* at 2.0×10^9 spores/kg induced the highest protection of wheat grains against larvae of *T. granarium* with weight loss of 0.7 and 1.3%, respectively. The outcomes of the present study indicate that the two fungi, *M. anisopliae* and *T. citrinoviride* are highly effective seed protectants against *T. granarium* and could be applied in the integrated pest management programs (IPM) of khapra beetle as effective biological control agents.

Keywords Khapra beetle · Biological control · Fungal strains · Wheat grains · Insecticidal efficacy

Introduction

Cereal crops, such as wheat, barley and rice are important grain crops in Egypt as well as different regions of the world (Matouk et al. 2017). The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is one of the most destructive primary insect pests of stored cereal and other grain crops in tropical and subtropical zones (Barzin et al. 2019; Kavallieratos et al. 2019). Larvae of khapra beetle feed on whole and broken grains causing a huge loss in weight, germination, and quality of infested cereal grains, particularly at heavy infestation (Rajput et al. 2015; Athanassiou et al. 2019). The control of khapra beetle is currently based mainly on the use of chemical insecticides (Kavallieratos et al. 2017; Kavallieratos and Boukouvala 2018). The continuous application of chemical insecticides for the control of stored products insects induced high risks on the environment, and human and animal health (Karanastasi et al. 2020). Hence, alternative control methods have been applied for management of khapra beetle, such as bio-insecticides, modified atmospheres, essential oils and entomopathogenic fungi (Vassilakos et al. 2019; Kavallieratos et al. 2020; Ali et al. 2022; Iqbal et al. 2022).

Entomopathogenic fungi are biological control agents and a useful tool in the integrated pest management systems of many insect pests (Skinner et al. 2014). This biological control method could be a promising alternative for the control of stored product pests owing to its several properties and advantages, such as compatibility with dry conditions in grain storage systems, broad spectrum of insect hosts, mass-production on industrial scale, simple application

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techniques, relative safety for humans, animals and the environment as well as no toxic residues on treated stored products (Rumbos and Athanassiou 2017; Batta and Kavallieratos 2018). Recently, the entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have been applied in the integrated pest management strategies of stored product insects attacking wheat, maize and rice either alone or in combination with other methods (Riasat et al. 2011; Luz et al. 2012; Wakil et al. 2015; Rumbos and Athanassiou 2017; Ak 2019; Mehdi and Al-Fadili 2021). Zidan (2014) found that treatment of stored wheat with the combinations of diatomaceous earth or spinosad with *B. bassiana* or *M. anisopliae* increased the mortality of *Sitophilus oryzae* compared with their application alone. Ak (2019) showed that treatment of wheat grains with *B. bassiana* and *M. anisopliae* at 1×10^8 conidia/ml caused 93.7 and 90.4% adult mortality of *S. oryzae*, respectively, after 7 days of treatment. Rizwan et al. (2019) concluded that the application of *B. bassiana* (1×10^8 conidia/kg) combined with diatomaceous earth (400 mg/kg) on wheat grains was highly effective against *Tribolium castaneum* (Herbst) and caused 88.13% mortality after 21 day exposure.

The fungal strains of *Trichoderma* spp. are broadly recognized as biological control agents applied against many plant diseases and pathogens (Fiorentino et al. 2018; Poveda 2021). Despite of many of *Trichoderma* spp. have been applied as safe method for the control of stored product insect pests (Rodríguez-Gonzalez et al. 2018; Gad et al. 2020a, b, 2021; Abdelgaleil et al. 2021) no information is available on effectiveness of *Trichoderma* spp. against *T. granarium*. Thus, the current study focuses on the evaluation of the potential of *Trichoderma* spp. and *Metarhizium anisopliae* as biocontrol agents against *T. granarium* on stored wheat grains under laboratory conditions. The effects of four strains of *Trichoderma* spp. (*T. harzianum*, *T. citrinoviride*, *T. viride* and *T. asperellum*) and *M. anisopliae* on mortality of larvae, adult emergence, and weight loss and damage caused by *T. granarium* on wheat grains were evaluated.

Materials and methods

Insect culture

Stock culture of *Trogoderma granarium* was obtained from Plant Protection Research Institute, Giza, Egypt. Insect colony has been maintained in our laboratory for several years without exposure to insecticides in glass jars (13 cm diameter × 17 cm height) covered by fine mesh cloth to allow proper ventilation. The insect was reared on whole wheat, var. Giza 168, with 10.9% moisture content under environmental conditions of 30 ± 2 °C, 65 ± 5% r.h. and continuous darkness. The larvae with 2–4 mm size were used in bioassays (Athanassiou et al. 2015; Kavallieratos et al. 2016).

Isolation and identification of *Trichoderma* spp

Four strains of *Trichoderma* spp. (*T. harzianum*, *T. citrinoviride*, *T. viride* and *T. asperellum*) have been isolated from Egyptian soil using serial dilution technique according to Naher et al. (2019). Mixture containing 10 g soil samples and 100 ml sterilized distilled water (SDW) was shaken at 100 rpm for 10 min. using a rotary shaker. Then, serial dilution of soil suspension was carried out to isolate *Trichoderma* spp colonies. Soil suspension (1 ml) was added to 10 ml of SDW to prepare a dilution of 10^{-1}. Subsequent dilutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) were prepared by mixing 1 ml of each solution with 9 ml SDW. Then, 1 ml from each dilution was transferred to sterilized petri plates containing Rose Bengal Agar (RBA) medium (Khang et al. 2013) and incubated for 5–7 days at 25 ± 2ºC. The plates were observed daily and the emerged colonies were taken and further purified on potato dextrose agar (PDA). The PDA slants were prepared from pure cultures and kept at 4 ºC. Morphological properties (conidiophore branching patterns, phialide arrangement, and conidia shape and size) (Gams and Bissett 1998; Kumar and Sharma 2011) were taken as criteria for the identification of the four strains of *Trichoderma* spp.

Isolation and propagation of *M. Anisopliae*

White grubs, *Pentodon bispinosus* Kuster, larvae have been collected from Golf playground, Katameya, Cairo, Egypt and used as a source for isolation *M. anisopliae* fungus. A solution of sodium hypochlorite (2%) was used for sterilization of the surface of dead larvae. After rinsing twice with SDW and drying water, larvae were placed on Petri plates containing PDA supplemented with streptomycin sulfate at concentration of 100 µg ml^{-1}. The plates were incubated at 25 ± 2 °C. The emerged fungal hyphae and sporulation were sub-cultured by moving to new PDA plates and kept for 15 days at 25 ± 2 °C (Ayala-Zermeño et al. 2015). The PDA slants of pure cultures were kept at 4 ºC until use.

Preparation of fungal spore suspensions

To the growing cultures of fungal strains on PDA, 10 ml of SDW containing Tween-80 (0.01%) in was added to the surface of each Petri dish plate. The spore suspension was collected in a sterile glass vial (50 ml) after rubbing the surface of the medium with a glass rod. The resulting spore

suspension was vortexed for 5 min and filtrated through a layer of sterilized cheese cloth. Spore concentrations of 2.0×10^6, 2.0×10^7, $2.0\times10^8$and 2.0×10^9 spores/ml were prepared with aid of a haemocytometer and used in the bioassay experiments.

Bioassays

Efficacy of four strains of *Trichoderma* spp. and *M. anisopliae* were evaluated against larvae of *T. granarium* by admixing with whole wheat grains (Gad et al. 2020b). The concentrations of *Trichoderma spp.* and *M. anisopliae* were prepared in distilled water. The fungal strains were assessed at 2.0×10^6, 2.0×10^7, 2.0×10^8 and 2.0×10^9 spores/kg (Abdelgaleil et al. 2021). Fifty grams of the sterilized wheat grains (var. Giza 168 with 10.9% moisture content) were placed in each glass jar (400 ml). Fungal spores (1 ml) were uniformly mixed with 50 g wheat grains inside each jar to give final concentrations of 2.0×10^6, 2.0×10^7, 2.0×10^8 and 2.0×10^9 spores/kg. After mixing with wheat grains, jars were shaken for 2 min for a complete distribution of the fungal spores throughout the grain mass. In the control jars, 1 ml of distilled water was homogeneously distributed on wheat grains in each jar. Afterward, forty *T. granarium* larvae were introduced in each jar. Three replicates of each concentration and control were used. All treatments were reserved under environmental conditions of 26 ± 2 °C and 65 ± 5% r.h. The percentages of larval mortality of *T. granarium* were calculated after 7, 14 and 21 days after treatment. On the other hand, the larvae exposed to wheat grains treated with different concentrations of tested fungal spores were examined daily until complete adult emergence and the numbers of emerged adults were calculated and termed as F_1 progeny production. Then, the jars were kept under the same rearing conditions for another 50 days and the number of emerged adults were recorded and expressed as F_2 progeny production after 80 days. The reduction (%) in the number of progeny was calculated using the following equation:

$\% = (1 - x/y) \times 100.$

where x = the number of adults emerging in the treatment; y = the number of adults emerging in the control.

Then, the treated and untreated wheat grains were sieved and the powders were discarded. The weight of remaining wheat grains in treatments and control was recorded to obtain the weight loss percentages after 80 days. The weight loss percentage was calculated from the following formula:

$\%\ \text{weight loss} = ((Wu–Wi)/Wu) \times 100.$

where Wu = weight of uninfested wheat grains; Wi = weight of infested wheat grains of control and treatment (Gad et al. 2020b).

Table 1 Mean mortality (%±SE) of *Trogoderma granarium* larvae after exposure for 7 days to wheat grains treated with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Larval mortality (% ± SE) after 7 days			
	2.0×10^6	2.0×10^7	2.0×10^8	2.0×10^9
Control	0.0 ± 0.0b	0.0 ± 0.0b	0.0 ± 0.0d	0.0 ± 0.0b
T. harzianum	0.0 ± 0.0b	10.4 ± 1.2ab	19.9 ± 1.8ab	23.2 ± 0.7ab
T. citrinoviride	1.4 ± 0.9b	13.2 ± 0.9ab	26.4 ± 1.4a	34.6 ± 9.1a
T. viride	0.0 ± 0.0b	1.6 ± 1.1ab	8.9 ± 0.6c	12.9 ± 1.2ab
T. asperellum	0.0 ± 0.0b	13.0 ± 4.8ab	16.8 ± 0.8b	20.4 ± 2.5ab
M. anisopliae	20.7 ± 0.7a	31.3 ± 3.9a	24.8 ± 1.7ab	33.8 ± 0.3a
F	44.3	3.96	87.6	5.7
P	< 0.01	0.024	< 0.01	< 0.01

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12)

Table 2 Mean mortality (%±SE) of *Trogoderma granarium* larvae after exposure for 14 days to wheat grains treated with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Larval mortality (% ± SE) after 14 days			
	2.0×10^6	2.0×10^7	2.0×10^8	2.0×10^9
Control	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
T. harzianum	6.4 ± 1.1c	24.3 ± 3.3ab	36.8 ± 7.2ab	38.6 ± 1.2ab
T. citrinoviride	26.3 ± 2.3b	26.8 ± 2.5ab	35.2 ± 1.9ab	62.6 ± 3.7a
T. viride	6.2 ± 1.0c	11.7 ± 1.2bc	13.3 ± 0.9b	17.8 ± 1.3bc
T. asperellum	5.3 ± 1.3c	41.4 ± 2.1ab	43.6 ± 4.9a	46.3 ± 2.7ab
M. anisopliae	41.6 ± 2.4a	58.8 ± 8.6a	62.4 ± 5.6a	75.8 ± 7.7a
F	60.8	12.1	25.2	17.4
P	< 0.01	< 0.01	< 0.01	< 0.01

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12)

Data analysis

Arcsine transformation of larval mortality and weight loss of wheat grains data was carried out before statistical analysis. Analysis of variance (ANOVA) (Kleinbaum et al. 1998) was run for all data to obtain the differences between treatments using Tukey's HSD test (Haynes 2013) at a significance level < 0.05 (SPSS, Chicago, IL, USA).

Results

Effect of fungal spores on larval mortality of *T. granarium*

The effects of different concentrations of fungal spores of *Trichoderma* spp. and *M. anisopliae* on the mortality of *T. granarium* larvae are present in Tables 1, 2 and 3. The results

Table 3 Mean mortality (%±SE) of *Trogoderma granarium* larvae after exposure for 21 days to wheat grains treated with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Larval mortality (% ± SE) after 21 days			
	2.0×10^6	2.0×10^7	2.0×10^8	2.0×10^9
Control	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0c
T. harzianum	6.4 ± 1.1c	24.3 ± 3.3ab	36.8 ± 7.2bc	46.3 ± 1.5ab
T. citrinoviride	29.0 ± 4.0b	32.8 ± 5.5ab	35.2 ± 1.9bc	69.0 ± 4.2ab
T. viride	9.4 ± 1.5c	11.7 ± 1.2bc	13.3 ± 0.9c	19.4 ± 0.2bc
T. asperellum	7.7 ± 0.7c	48.1 ± 2.6ab	54.8 ± 4.4ab	57.1 ± 3.5ab
M. anisopliae	47.0 ± 2.1a	65.8 ± 9.7a	71.0 ± 7.7a	82.1 ± 7.1a
F	55.4	11.8	25.0	11.5
P	< 0.01	< 0.01	< 0.01	< 0.01

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12)

show that the larval mortality of *T. granarium* was significantly increased with increasing the concentration of fungal spores and exposure period. The highest larval mortality of *T. granarium* was obtained at concentration 2.0×10^9 spores/kg of *T. citrinoviride* (34.6%), followed by *M. anisopliae* (33.8%), *T. harzianum* (23.2%), *T. asperellum* (20.4%) and *T. viride* (12.9%) after 7 days of exposure (Table 1). At the highest tested concentration (2.0×10^9 spores/kg), *M. anisopliae* (75.8 and 82.1%) induced the highest rate of larval mortality, followed by *T. citrinoviride* (62.6 and 69.0%), *T. asperellum* (46.3 and 57.1%), *T. harzianum* (38.6 and 46.3%) and *T. viride* (17.8 and 19.4%) after 14 days and 21 days of treatment, respectively (Tables 2 and 3).

Effect of fungal spores on progeny production of *T. granarium*

Treatment of wheat grains with different fungal spore concentrations of *Trichoderma* spp. and *M. anisopliae* decreased emerged adults (F_1) of *T. granarium* (Table 4). The number of emerged adults in control was 40.6 ± 4.1. The fungal strains (*M. anisopliae* and *T. citrinoviride*) induced the highest reduction in F1 progeny at all tested concentrations. The maximum reduction of *T. granarium* adult F_1 progeny was recorded at concentration of 2.0×10^9 spores/kg of *M. anisopliae* (5.6 ± 1.4), followed by *T. citrinoviride* (10.0 ± 2.2), *T. harzianum* (16.6 ± 1.0), *T. asperellum* (18.3 ± 1.6) and *T. viride* (27.3 ± 0.6). After 80 days of exposure, all fungal strains at the spore concentrations of 2.0×10^7, 2.0×10^8 and 2.0×10^9 spores/kg significantly inhibited adult progeny (F_2) compared with control treatment (867.0 ± 37.9) (Table 5). The highest suppression of *T. granarium* F_2 was observed in the treatment with *M. anisopliae* (19.0 ± 0.4) at concentration 2.0×10^9 spores/kg, followed by *T. citrinoviride* (24.0 ± 2.8), *T. harzianum* (145.3 ± 8.8), *T. asperellum* (306.7 ± 18.0) and *T. viride* (374.6 ± 17.7) at the same concentration.

Effect of fungal spores on weight loss of wheat grains

The results indicated that the untreated wheat grains (control) infested with larvae of *T. granarium* were significantly more damaged than treated wheat grains with spores of different fungal strains. The percentage of weight loss in untreated wheat grains was 54.3% after 80 days of exposure (Table 6). Weight loss and damage of wheat grains caused by *T. granarium* were gradually declined with the increase of spore concentration. At the concentration of 2.0×10^9 spores/kg, *M. anisopliae* and *T. citrinoviride* were the most effective fungi for the protection of wheat grains against damage caused by *T. granarium* with the lowest weight loss of 0.7 and 1.3%, respectively, followed by *T. harzianum* (7.6%), *T. asperellum* (18.2%) and *T. viride* (32.2%).

Table 4 Adult production (F_1) of *Trogoderma granarium* emerged from larvae exposed to wheat grains treated with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Adult emergence of F_1 (± SE)							
	2.0×10^6		2.0×10^7		2.0×10^8		2.0×10^9	
	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)
Control	40.6 ± 4.1a	0.0	40.6 ± 4.1a	0.0	40.6 ± 4.1a	0.0	40.6 ± 4.1a	0.0
T. harzianum	38.0 ± 0.4ab	5.0	25.3 ± 0.5bc	36.6	26.3 ± 1.4ab	34.2	16.6 ± 1.0bc	58.3
T. citrinoviride	23.3 ± 3.9bc	41.7	22.0 ± 1.8bc	45.0	21.0 ± 0.7bc	47.5	10.0 ± 2.2c	75.0
T. viride	35.0 ± 1.1ab	12.5	29.0 ± 0.4ab	27.5	28.6 ± 0.9ab	28.3	27.3 ± 0.6ab	31.6
T. asperellum	37.3 ± 0.6ab	6.7	25.7 ± 1.7bc	35.8	21.0 ± 3.2bc	47.5	18.3 ± 1.6bc	54.2
M. anisopliae	14.0 ± 0.4c	65.0	12.6 ± 1.6c	68.3	8.6 ± 3.0c	78.3	5.6 ± 1.4c	85.8
F	9.7		9.6		8.4		17.3	
P	< 0.01		< 0.01		< 0.01		< 0.01	

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12), R = Reduction

Table 5 Adult production (F_2) of *Trogoderma granarium* emerged from larvae exposed to wheat grains treated with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Adult emergence of F_1 (± SE)							
	2.0×10^6		2.0×10^7		2.0×10^8		2.0×10^9	
	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)	No. adults / 50 g	R (%)
Control	867.0 ± 37.9a	0.0	867.0 ± 37.9a	0.0	867.0 ± 37.9a	0.0	867.0 ± 37.9a	0.0
T. harzianum	619.0 ± 18.0a	28.6	584.0 ± 8.9b	32.6	405.0 ± 40.8b	53.3	145.3 ± 8.8 cd	83.2
T. citrinoviride	460.6 ± 15.4ab	46.9	179.3 ± 11.2c	79.3	103.0 ± 13.5c	88.1	24.0 ± 2.8d	97.2
T. viride	508.3 ± 21.9ab	41.4	500.0 ± 14.7b	42.3	515.6 ± 22.8b	40.5	374.6 ± 17.7b	56.8
T. asperellum	501.3 ± 16.2ab	42.2	466.0 ± 45.7b	46.2	416.6 ± 15.1b	51.9	306.7 ± 18.0bc	64.6
M. anisopliae	87.0 ± 5.3b	89.9	73.6 ± 4.5c	91.5	28.0 ± 7.1c	96.8	19.0 ± 0.4d	97.8
F	6.9		55.4		67.3		77.4	
P	< 0.01		< 0.01		< 0.01		< 0.01	

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12), R = Reduction

Table 6 Mean weight loss (% ± SE) of wheat grains after 80 days of treatment with five entomopathogenic fungal strains at different spore concentrations

Treatment (Concentration, spores/kg)	Weight loss (% ± SE) after 80 days			
	2.0×10^6	2.0×10^7	2.0×10^8	2.0×10^9
Control	54.3 ± 2.0a	54.3 ± 2.0a	54.3 ± 2.0a	54.3 ± 2.0a
T. harzianum	44.8 ± 5.6a	40.8 ± 3.9ab	34.9 ± 4.2b	7.6 ± 0.2c
T. citrinoviride	39.3 ± 7.9a	11.3 ± 3.3c	4.5 ± 0.7c	1.3 ± 0.9d
T. viride	47.8 ± 1.1a	38.5 ± 0.5ab	40.2 ± 0.1ab	32.2 ± 0.6b
T. asperellum	43.6 ± 4.5a	37.0 ± 1.7b	34.4 ± 1.5b	18.2 ± 2.2bc
M. anisopliae	4.7 ± 1.5b	2.3 ± 0.1c	1.5 ± 0.5c	0.7 ± 0.5d
F	10.4	34.4	62.4	61.9
P	< 0.01	< 0.01	< 0.01	< 0.01

Values within each column followed by the same letter are not significantly different ($P < 0.05$, df = 5, 12)

Discussion

The insecticidal efficacy of different entomopathogenic fungi on some stored grain insects including *T. granarium* has been evaluated by several researchers (Khashaveh et al. 2011; Gad et al. 2020a, b; Abdelgaleil et al. 2021; Mehdi and Al-Fadili 2021; Iqbal et al. 2022; Shahbazi et al. 2022). Nevertheless, this is the first study describing insecticidal efficacy of three strains of *Trichoderma* (*T. citrinoviride*, *T. viride* and *T. asperellum* against larvae of *T. granarium* in stored wheat grains. Our results demonstrate that the larval mortality of *T. granarium* increased with the increase of spore concentration of all fungal strains. After 21 days of treatment, all fungal strains caused notable larval mortality particularly at the highest concentration (2.0×10^9 spores/kg) except *T. viride*. The highest level of larval mortality was achieved at the highest concentration (2.0×10^9 spores/kg) of *M. anisopliae* and *T. citrinoviride*. It was also clear that larvae of *T. granarium* were more susceptible to *M. anisopliae* and *T. citrinoviride* than other tested fungal strains. These findings are matched with previous studies demonstrating that the fungal strains had the ability to induce high rate of larval and adult mortality of several stored grain insects. For example, Gad et al. (2020a, b) indicated that the treatment with *T. harzianum* at concentration of 2.1×10^7 spores/kg induced 57.3% mortality of *(A) obtectus* adults after 7 days and 68.0% mortality of *S. oryzae* adults after 21 days. The same fungus has been tested against the *C. maculatus* and *C. chinensis* adults and the mortality percentages were 75.2 and 76.4%, respectively at concentration of 1.0×10^7 spores/kg, after 7 days of exposure (Abdelgaleil et al. 2021). Mehdi and Al-Fadili (2021) found that the larval mortalities of *T. granarium* were 86.67 and 50% when larvae treated with 1.0×10^9 conidia/ml of local and imported isolates of *M. anisopliae* respectively, and 86.67 and 66.67% when larvae treated with 1.0×10^9 of local and imported isolates of *(B) bassiana* respectively after 14 days. Iqbal et al. (2022) tested *M. anisopliae* against larvae of *T. granarium* and found that the fungal strain at concentration of 1.0×10^8 conidia/ml caused larval mortality of 98.3% after 14 days.

Our results demonstrate that the tested entomopathogenic fungi might be valuable for the biological control of khapra beetle particularly *M. anisopliae* and *T. citrinoviride*, because of their capability to penetrate the insect cuticle and use the insect bodies as source of nutrients for growth and propagation (Gonzalez et al. 2020; Iqbal et al. 2022). The insect mortality may also due to secondary metabolites produced by fungal strains, such as peptaibols which have been reported to be produced by *T. harzianum* and showed pronounced insect toxicity (Charnley and Collins 2007; Shakeri and Foster 2007; Rahim and Iqbal 2019). Similarly, antifeeding properties of natural compounds isolated from fungal strains of *T. citrinoviride* have been described against *Corcyra cephalonica* and *Schizaphis graminum* (Evidente et al. 2008; Vijayakumar et al. 2016; Vijayakumar and Alagar 2017).

Our results also indicate that all treatments with spore concentrations of tested fungi reduced the adult F_1 and F_2

production of *T. granarium* after 80 days of exposure. Moreover, adult F_1 and F_2 production of *T. granarium* was highly suppressed at the highest tested concentration (2.0×10^9 spores/kg) of *M. anisopliae*, *T. citrinoviride* and *T. harzianum*. Several reports explained the effect of different spore concentrations of tested fungal strains on the reduction of the progeny of stored product insects. For instance, Wakil et al. (2015) found that concentration of 2.78×10^6 conidia/kg of *M. anisopliae* reduced progeny of *S. oryzae* 3-fold in comparison with untreated rice grains after 62 days. Gad et al. (2020a, b) showed that concentration of 2.1×10^7 spores/kg of *T. harzianum* induced strong suppression of *A. obtectus* progeny (94.5%) after 60 days of exposure and reduced progeny of *S. oryzae* (101.3 ± 4.9 beetles), compared with untreated wheat grains (516.0 ± 2.8 beetles) after 90 days. Abdelgaleil et al. (2021) stated that concentration of 1.0×10^7 spores/kg of *T. harzianum* reduced progeny of *C. chinensis* (69.1%) after 45 days. The high suppression of progeny of *T. granarium* for two generations observed in this study may be due to the quick death of larvae after treatments or to the adverse effect of these tested fungal strains on eggs and newly hatched neonate larvae (Athanassiou et al. 2005). Also, the highest concentration of tested fungi was highly effective to protect wheat grains against damage caused by *T. granarium*. The two fungi, *M. anisopliae* and *T. citrinoviride*, displayed the highest potential to protect weight loss of 0.7 and 1.3% after 80 days, respectively. Similarly, the tested fungi have been proved to be effective to protect stored commodities against the damage induced by other insect species (Padin et al. 2002; Rodríguez-Gonzalez et al. 2020; Gad et al. 2020a, b, 2021; Abdelgaleil et al. 2021).

Although the biological control agents may not be as efficient as chemical insecticides the use of entomopathogenic fungi for the management of stored product insects is very important approach to reduce environmental pollution, food contamination and delay the development of insect resistance. The results of the present study encourage the use of entomopathogenic fungi in integrated pest management programs for the control of khapra beetle. In this regard, a combination of virulent fungal isolates with low concentrations of insecticides may also be advantageous for practical use against stored grain insect pests. Taking into account most of insecticides used for the management of stored product insects showed week effectiveness against *T. granarium* at the recommend rates. The results of current study show that *Trichoderma* spp. and *M. anisopliae* are highly effective as grain protectants against damage caused by of *T. granarium* and may offer a solution for the feasible management of *T. granarium* in stored wheat.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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