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Efficacy of two species of entomopathogenic fungi against the stored-grain pest, *Sitophilus granarius* L. (Curculionidae: Coleoptera), via oral ingestion

Yacoub Ahmad Batta

Abstract

Sitophilus granarius is a serious stored-grain insect that can destroy the whole grain of cereal crops. The present research demonstrated that two species of entomopathogenic fungi (EPF), *Beauveria bassiana* and *Metarhizium anisopliae*, killed the treated adults of *S. granarius* when applied via oral ingestion. Mortality of *S. granarius* was shown by growth and development of the ingested conidia in the insect gut causing death of infected insects. During bioassays, the fungus conidia were mixed with wheat flour then provided to the insects for feeding on the mixture for 24 h. The insects were surface-treated with a contact fungicide (Merpan®) to kill the external conidia that could penetrate the cuticle and develop to kill the infected insects. Results indicated that the most effective ratio of mixing fungus conidia with the wheat flour was 0.1:2.0 (g/g) where this ratio should contain 2.75×10^8 conidia/g of *M. anisopliae* mixture or 2.6×10^8 conidia/g of *B. bassiana* mixture. Ingested conidia of each fungus caused a first mortality of *S. granarius* adults after 10 and 12 days of *B. bassiana* and *M. anisopliae* treatments, respectively. They also caused 50% mortality after 2.787 and 3.047 weeks of treatments for the same fungi, respectively. The novelty of this research is in adjusting and applying a technique of disinfection of insect cuticle with a fungicide and then verifying the presence of applied fungi in the infected insects. The practical application of this method was discussed.

Keywords: Biocontrol, *Sitophilus granaries*, *Beauveria bassiana*, *Metarhizium anisopliae*, Surface sterilization

Background

Entomopathogenic fungi (EPF) are potential biocontrol agents of many insect pest species (Batta 2007; Gabarty et al. 2014 and Han et al. 2014). One of the main groups of agricultural insect pests that can be effectively controlled by EPF is the stored-grain insects (Batta 2016). For examples, the yellow meal worm, *Tenebrio molitor* L. (Batta et al. 2010); confused flour beetle, *Tribolium confusum* Jacquelin du Val.; corn weevil, *Sitophilus zeamais* Motschulsky and lesser grain borer, *Rhyzopertha dominica* Fabricius (Barra et al. 2013); rice weevil,

Sitophilus oryzae L. (Kavallieratos et al. 2014) and rice meal moth, *Corcyra cephalonica* Stainton (Kaur et al. 2014). The mode of action of these fungi is through germination of conidia that adheres to the outer surface of target insect cuticle producing germ tube that can penetrate the cuticle towards the insect hemocoel. Penetrating germ tube multiplies forming mycelium in the interior portion causing eventually death of the infected insect (Pedrini et al. 2007; Zhang et al. 2008; Stephou et al. 2012; Ortiz-Urquiza and Keyhani 2013). On the cadavers of dead insects due to infection with EPF, the internal mycelium penetrates the cuticle to the outside then grows on the cuticle surface producing large quantity of conidia at favorable conditions of temperature and relative humidity (Fig. 1a, b). The efficacy of applied

Correspondence: yabatta@najah.edu

Laboratory of Plant Protection, Department of Plant Production and Protection, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, West Bank, Palestine

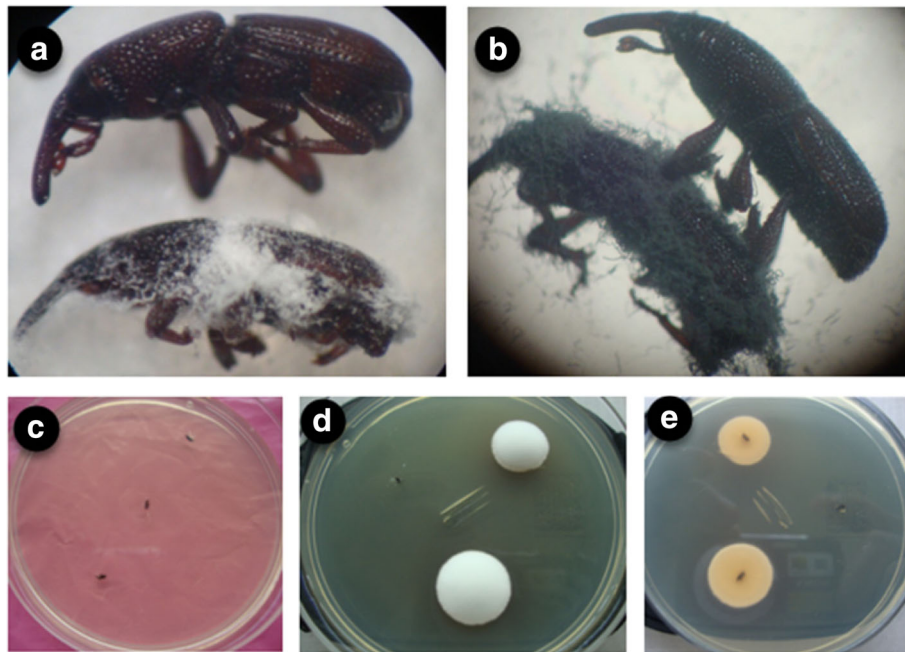


Fig. 1 Infection and isolation of two species of EPF from adults of *Sitophilus granarius* (strain SG5), **a** Infection of *S. granarius* adult (lower picture) with *Beauveria bassiana* (strain BB2) exhibited by white superficial mycelium growth, upper picture is uninfected or healthy. **b** Infection of *S. granarius* adults (lower picture) with *Metarhizium anisopliae* (strain MA1) exhibited by olive green superficial mycelium growth, upper picture is uninfected or healthy. **c** Isolation technique of ingested conidia of *B. bassiana* or *M. anisopliae* from treated adults of *S. granarius* after being superficially disinfected then plated using a selective medium (SDA + Chloramphenicol). **d-e** Growth of *B. bassiana* on the plate surface with a selective medium (SDA + Chloramphenicol) (upper and lower views of fungus growth as a white dense mycelia growth on the plate's surface in case of positive isolation)

EPF depends on many factors, especially the susceptibility of insect species and virulence of the strain or isolate of EPF (Michalaki et al. 2006; Ahmed 2010 and Han et al. 2014).

To the best of our knowledge, there were no reports about the effect of EPF on stored-grain insects via oral ingestion. Therefore, the general objective of the present study is to demonstrate if the EPF conidia incorporated via oral ingestion by *S. granarius* adults could be able to generate mortality, whereas the specific objectives are the following: (i) to develop an effective technique for a superficial disinfection of EPF fungus conidia that may adhere to the outer surfaces of insect cuticle then germinate and penetrate into the hemocoel, (ii) to develop an effective technique of isolation for testing the viability of ingested conidia or its developing mycelium thus showing viability of these fungal structures, (iii) since the death of infected insects is an indication on viability of ingested conidia, the mortality of treated insects will be taken as an indication of the effectiveness of applied technique, and (iv) to prepare mixtures of fungus conidia of EPF and wheat flour then feed them to the target insects to ensure that the ingested conidia will kill the infected insects.

Methods

Isolates of *Metarhizium anisopliae* and *Beauveria bassiana*
MA1 and BB2 are the isolates of *M. anisopliae* and *B. bassiana* that were used, respectively, in the conducted bioassays. They were originally isolated from infected adults and larvae of the ground beetle, *Carabus nemoralis* L. (Carabidae: Coleoptera). The isolation was done, at first, on a selective medium: Sabouraud Dextrose Agar (SDA) + Chloramphenicol (250 mg/L) then, pure cultures of these isolates were obtained from monosporic cultures on the same culture medium.

Strain of *Sitophilus granarius*

Male and female adults of *S. granarius* (strain SG5) were used in the conducted bioassays. They were obtained from insect cultures kept in the insectary of Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus, Palestinian Territories. *S. granarius* was reared on wheat and rice grain in plastic pots, covered with pieces of cheese cloth to permit a good aeration during the rearing process.

Mixtures of fungal isolates and wheat flour

Mixtures of conidia of each isolate (MA1 and BB2) and wheat flour were prepared. This is because, during their growth on the culture medium, *M. anisopliae* and *B. bassiana* form a dry dense crust of conidia on the medium surface within 2 weeks of incubation at 20 °C and 12 h of light period. The crust which was olive-green in *M. anisopliae* and white in *B. bassiana* could be easily removed from the surface of culture plate by scraping them, using a sterile scalpel. The scraped conidia could be then thoroughly mixed with wheat flour (as a palatable commodity for stored-grain insects) in order to be used for feeding the adults of *S. granarius*. Mixtures of dry fungus conidia and wheat flour were prepared, using different ratios of both ingredients as the following: T1 (0.2 g of fungus conidia mixed with 2.0 g of wheat flour or 0.2:2.0 ratio, g/g), T2 (0.1 g of fungus conidia to 2.0 g of flour or 0.1:2.0 ratio, g/g), T3 (0.05 g of fungus conidia to 2.0 g of flour or 0.05:2.0 ratio g/g), and T4 (0 g of fungus conidia to 2.0 g of flour or 0:2.0 ratio, g/g). The last ratio which had only wheat flour substrate was used as a control treatment for testing the effect of the substrate. The prepared mixture with the highest ratio of fungus conidia and wheat flour (T1) contained (5.5×10^8 conidia/g of *M. anisopliae* mixture and 5.2×10^8 conidia/g of *B. bassiana* mixture), whereas the other mixtures T2 and T3 of the two fungi were prepared by making successive dilutions of the highest ratio by a factor of one half, respectively. T4 was used as a control since it contains only the substrate (flour). The concentration of fungus conidia in the highest ratio mixture (T1) was measured by suspending and homogenizing 1 g of the mixture in 50 ml of sterile distilled water then counting the conidial number in the suspension using a hemocytometer.

Bioassays

Two grams of each one of the above-mentioned mixtures: T1, T2, T3, T4, were transferred into small plastic pots (10 cm diameter and 7 cm depth), and then 20 adults of *S. granarius* (strain SG5) were introduced into each pot. Four pots, representing four replicates, were used in each treatment (mixture type). Sixteen pots were used, in total, in bioassays conducted for each EPF species. Each pot was covered with a piece of cheese cloth as described before. The insects were left for feeding on the contents of each mixture for 24 h, then the insects, in each replicate, were separated immediately and treated superficially with a contact fungicide such as Merpan® (active ingredient Captan, concentration 800 g/kg of wettable granular formulation). An aqueous suspension containing 0.2% Merpan® (w/v) was prepared and then used for the superficial treatment so the application rate of the fungicide was 2.0 g/L (w/v). The

treatment was done by spraying the insects with the fungicide suspension, using ULV spraying technique in a closed chamber. The fungicide was used as a secondary agent to prevent the conidial germination of *M. anisopliae* or *B. bassiana* on insect cuticle. Target conidia in this treatment may come from the mixtures used for feeding insects. However, the ingested conidia during feeding were not affected by the fungicide treatment. Treated insects with the fungicide were then placed in new plastic pots similar to the previous ones but containing 10 g of healthy rice grain each then incubated in a growth chamber at 20 °C and 12 h of light period for 7 weeks. Briefly, after the feeding period and the transfer of insects, each pot contained 20 treated adults of *S. granarius* and 10 g of healthy rice grain and covered with a piece of cheese cloth as described before. The rate of application was calculated at 20 g of mixture per kilogram of grain. Each treatment was repeated three times to confirm the reproducibility of the results.

Evaluation of the treatment efficacy

To evaluate the efficiency of the fungicide treatment and to demonstrate the viability of ingested conidia, the following techniques and procedures were applied: (i) testing the viability of ingested conidia by *S. granarius* adults after feeding on the mixtures described above by isolating the ingested fungi on a selective culture medium (SDA + Chloramphenicol 250 mg/L) within the period of incubation (6 to 7 weeks) so a positive or negative isolation of fungi could be obtained and (ii) counting the dead *S. granarius* adults/replicate/treatment over a period of 7 weeks, following superficial treatment, where the mortality percentage was calculated and then used for plotting the linear relationship between the time post treatments and mortality percentage.

To ensure that the mortality of insects observed within the period of 7 weeks of incubation was caused by the effect of ingested conidia of tested fungi, the following plating technique of isolation was applied: (i) the dead insects resulted from feeding on mixtures of fungus conidia and wheat flour were first superficially disinfected by dipping them in a solution of 2.0% sodium hypochlorite for 30 s to exclude any bacterial contamination or mold growing during fungal growth on culture medium, (ii) the insects were rinsed three times with sterile distilled water to wash the residues of disinfecting solution then left for drying up under aseptic conditions, and then (iii) the air-dried insects were aseptically placed in plates with selective medium (SDA + Chloramphenicol 250 mg/L) (Fig. 1c). This is for testing the viability of ingested conidia or mycelium developing from these conidia. Therefore, a positive or negative isolation was recorded, according to the growth of these fungi on the

culture medium within 10 days of incubation of plates at 20 °C and 12 h light per day.

Statistical analyses

One-way ANOVA was carried out to test significant differences between the means of mortality percentage of treated *S. granarius* adults by *M. anisopliae* after 7 weeks of treatment or by *B. bassiana* after 6 weeks of treatment, using different mixtures of fungus conidia and wheat flour. Scheffe test was used for comparison of the above-mentioned means. Data obtained on mortality percentage of *S. granarius* by the two fungi were also used for plotting a linear relationship between the mortality percentage and the time of incubation after treatments, using different ratios of fungus conidia and wheat flour. Regression equations and R^2 values for each ratio used were calculated and provided with its corresponding regression line. Comparisons of regression lines were made according to the regression analysis.

Results and discussion

Isolation of ingested EPF from *S. granarius* adults treated superficially with Merpan®

Isolation of ingested conidia or its developing mycelium from *S. granarius* adults fed on mixtures of EPF conidia + wheat flour and treated with Merpan® was positive, if the fungus mycelium grows on the surface of culture plates (Fig. 1d, e). For the mixtures of *M. anisopliae* conidia + wheat flour, positive isolations were obtained starting from the third day, following the feeding time for treatments T1, T2, and T3, until the first death of treated adults on 12th day for T1 and 13th day for T2 and T3 (Table 1). For the mixtures of *B. bassiana*

conidia + wheat flour, the isolations were also positive starting from the third day, following the feeding time for treatments T1, T2, and T3 until the first death of treated adults on 10th day for T1, 11th day for T2, and 12th for T3 (Table 2). For untreated *S. granarius* adults with Merpan®, the isolation of *M. anisopliae* was positive from the second day, following the feeding time for treatments T1, T2, and T3, until the first death of treated adults on the seventh day for T1, eighth day for T2, and ninth day for T3 (Table 1). For *B. bassiana*, the isolation was also positive starting the second day, following the feeding time for the treatments T1, T2, and T3 until the first death of treated adults on the fifth day for T1, seventh day for T2, and eighth for T3 (Table 2). The applied technique demonstrated its effectiveness by inhibiting the germination and penetration of external EPF conidia existing on insect surface, but it did not affect the conidia ingested by insects so that we can reveal the presence of internal, hemocoelic fungus vegetative cells, as well as any potentially viable conidia in the insect alimentary tracts. No previous studies are available on the lethal effect of ingested conidia of EPF in the stored-grain insects, but few earlier studies are available on other insect species that indicated the possibility of such effect in the nymphs of the grasshopper, *Melanoplus sanguinipes* F., when fed on bait substrate of *B. bassiana* (Inglis et al. 1996).

Mortality of *S. granarius* adults fed on conidia of EPF

Results indicated that *S. granarius* adults, fed on *M. anisopliae* or *B. bassiana* conidia mixtures + wheat flour, died due to ingesting the conidia of the tested fungi. This effect was shown by the daily counting of dead

Table 1 Isolation of ingested fungus conidia (or mycelium developing from them) of *Metarhizium anisopliae* from infected adults of granary weevil, *Sitophilus granarius*, after feeding them on mixtures of *M. anisopliae* conidia + wheat flour for 24 h then incubated after surface treatment with Merpan® on healthy rice grains at 20 °C

Time (days) for isolation the fungus ^c	Mixture ratios of <i>M. anisopliae</i> conidia and wheat flour (w/w) ^a							
	T1 (0.2:2.0 g/g)		T2 (0.1:2.0 g/g)		T3 (0.05:2.0 g/g)		T4 (0:2.0 g/g)	
	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b
1 day	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d
2 days	–	+	–	+	–	+	–	–
3 days	+	+	+	+	+	+	–	–
7 days	+	+	+	+	+	+	–	–
8 days	+	+	+	+	+	+	–	–
9 days	+	+	+	+	+	+	–	–
12 days	+	+	+	+	+	+	–	–
13 days	+	+	+	+	+	+	–	–

^aFour replicates were used per treatment and each replicate contains 20 adults of *S. granarius*

^bTreated or untreated adults of *S. oryzae* with the contact fungicide Merpan®

^cTime in days from treatment with Merpan® to starting of fungus isolation on a selective culture medium

^d+ means positive isolation of the fungus on a selective culture medium (Sabouraud Dextrose Agar + Chloramphenicol 250 mg/L); – means negative isolation of the fungus

Table 2 Isolation of ingested fungus conidia (or mycelium developing from them) of *Beauveria bassiana* from infected adults of granary weevil, *Sitophilus granarius*, after feeding them on mixtures of *B. bassiana* conidia + wheat flour for 24 h then incubated after surface treatment with Merpan® on healthy rice grains at 20 °C

Time (days) for isolation of the fungus ^c	Mixture ratios of <i>B. bassiana</i> conidia and wheat flour (w/w) ^a							
	T1 (0.2:2.0 g/g)		T2 (0.1:2.0 g/g)		T3 (0.05:2.0 g/g)		T4 (0:2.0 g/g)	
	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b	Treated ^b	Untreated ^b
1 day	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d	– ^d
2 days	–	+	–	+	–	+	–	–
3 days	+	+	+	+	+	+	–	–
5 days	+	+	+	+	+	+	–	–
7 days	+		+	+	+	+	–	–
8 days	+		+	+	+	+	–	–
10 days	+		+	+	+	+	–	–
11 days	+		+	+	+	+	–	–
12 days	+		+	+	+	+	–	–

^aFour replicates were used per treatment and each replicate contains 20 adults of *S. granarius*

^bTreated or untreated adults of *S. oryzae* with the contact fungicide Merpan®

^cTime in days from treatment with Merpan® to starting of fungus isolation on a selective culture medium

^d+ means positive isolation of the fungus on a selective culture medium (Sabouraud Dextrose Agar + Chloramphenicol 250 mg/L); – means negative isolation of the fungus

insects over a period of incubation after feeding that extended up to 7 weeks for *M. anisopliae* (Fig. 3) and up to 6 weeks for *B. bassiana* (Fig. 4). Mixtures of conidia of the two fungi with wheat flour at ratios 0.2:2.0 and 0.1:2.0 (w/w) caused 100% mortality in treated adults while mixture at ratio 0.05:2.0 (w/w) caused mortality of 50% for *M. anisopliae* and 55% for *B. bassiana* (Fig. 2). Mortality rates in the control treatments were 9 and 10% for *M. anisopliae* and *B. bassiana*, respectively (Fig. 2). Statistical analysis indicated that there were

significant differences among the means of mortality percentages in the different mixtures after 7 weeks of the treatment for *M. anisopliae* [F value = 11.874 (sig. 0.001), df = 2 and 12 and subset for $\alpha < 0.01$] and 6 weeks of the treatment for *B. bassiana* [F value = 21.000 (sig. 0.001), df = 2 and 12 and subset for $\alpha < 0.01$]. By comparing the above means to the control mean, significant differences ($P < 0.05$) were obtained, especially with the mixtures of 0.2:2.0 or 0.1:2.0; therefore, these mixtures are considered the most effective ones with insignificant

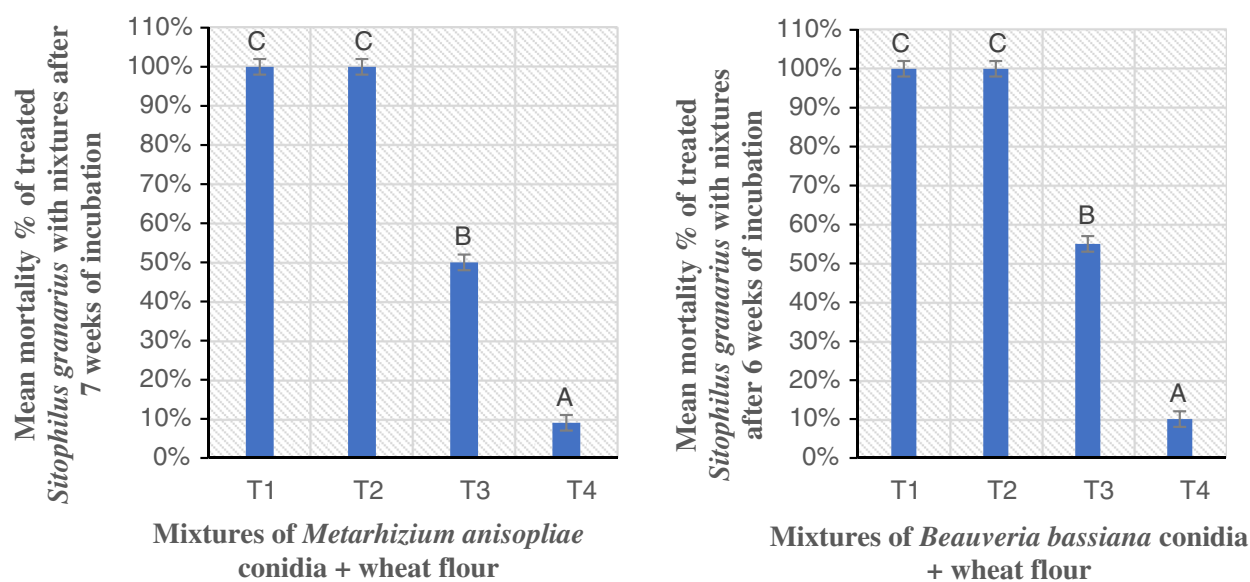


Fig. 2 Mortality of *Sitophilus granarius* adults. Means followed by the same letter are insignificantly different at $P = 0.05$ using ANOVA and Scheffe for means comparison. Ratios of mixtures (w/w) are T1 = 0.2:2.0; T2 = 0.1:2.0; T3 = 0.05:2.0; T4 = 0:2.0

differences. The mixture T2 (ratio 0.1:2.0) of both species of EPF was recommended to be used for the control of *S. granarius* adults. This mixture should be adjusted during preparation to contain 2.75×10^8 conidia/g of mixture for *M. anisopliae* (one half of the highest concentration for this species in the mixture of T1: 5.5×10^8) and 2.6×10^8 conidia/g of mixture for *B. bassiana* (one half of the highest concentration for this species in the mixture T1: 5.2×10^8). The application rate of the recommended mixture was calculated at 20 g/kg grain. Regression analysis of the data indicated that the calculated regression equations and R^2 values of the mixtures of *M. anisopliae* conidia + wheat flour were as follows: for T1, $y = 14.405x + 0.8333$, $R^2 = 0.9019$; T2, $y = 15.179x + 3.75$, $R^2 = 0.9595$; T3, $y = 6.5476x + 2.0833$, $R^2 = 0.7703$; T4, $y = 3.035x + 0.9141$, $R^2 = 0.6352$ (Fig. 3), whereas that of *B. bassiana*, they were as follows: for T1, $y = 14.821x + 3.3929$, $R^2 = 0.8348$; T2, $y = 16.786x + 3.2143$, $R^2 = 0.9208$; T3, $y = 8.3929x + 0.5357$, $R^2 = 0.7784$; T4, $y = 2.3214x + 0.8929$, $R^2 = 0.5633$ (Fig. 4). Based on the regression equation of the recommended mixture (T2) in both fungi indicated above, the time needed for killing 50% of treated adults of *S. granarius* was calculated at 3.047 weeks for *M. anisopliae* and 2.787 weeks for *B. bassiana*.

Results indicated that the mortality rate of adults of *S. granarius* was caused by the development of ingested conidia; the evidence on this conidial development was the positive isolation of these fungi from the internal portion of treated insects by plating method, using a selective culture medium (SDA + Chloramphenicol) (Fig. 1d, e). However, the way by which the ingested conidia develop inside the insect's gut and kill the host insects is not known exactly, probably that these conidia germinate in the gut, then the germ tube of germinated conidia penetrates the gut walls to enter the haemocoel where they can grow profusely and kill the infected insect but this explanation needs to be proved or demonstrated in future studies. Until present, no reports are available on the effect of ingested conidia of EPF in killing the stored-grain insects. However, earlier studies revealed similar results but in other insect species. For example, Kramm and West (1982) reported that plating hindgut contents of workers of termites (*Reticulitermes* sp.) that were exposed to *Metarhizium*, *Gliocladium*, and *Beauveria* on culture medium indicated the presence of living conidia in their gut, following their ingestion. Also, Lacey et al. (1988) indicated that viable conidia of *M. anisopliae* were detected in the gut of *Culex quinquefasciatus* larvae that

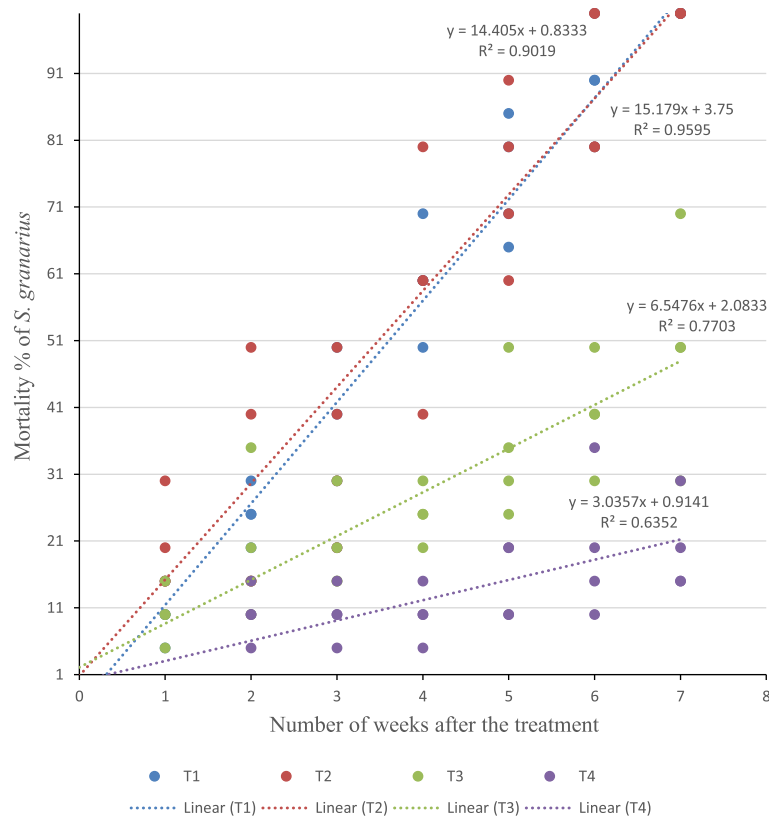


Fig. 3 Mortality of adults of granary weevils (*Sitophilus granarius*; strain SG5) as a result feeding on mixtures of *Metarhizium anisopliae* (strain MA1) conidia + wheat flour for 24 h. Ratios of mixtures (g/g) are T1 = 0.2:2.0; T2 = 0.1:2.0; T3 = 0.05:2.0; T4 = 0.2:0

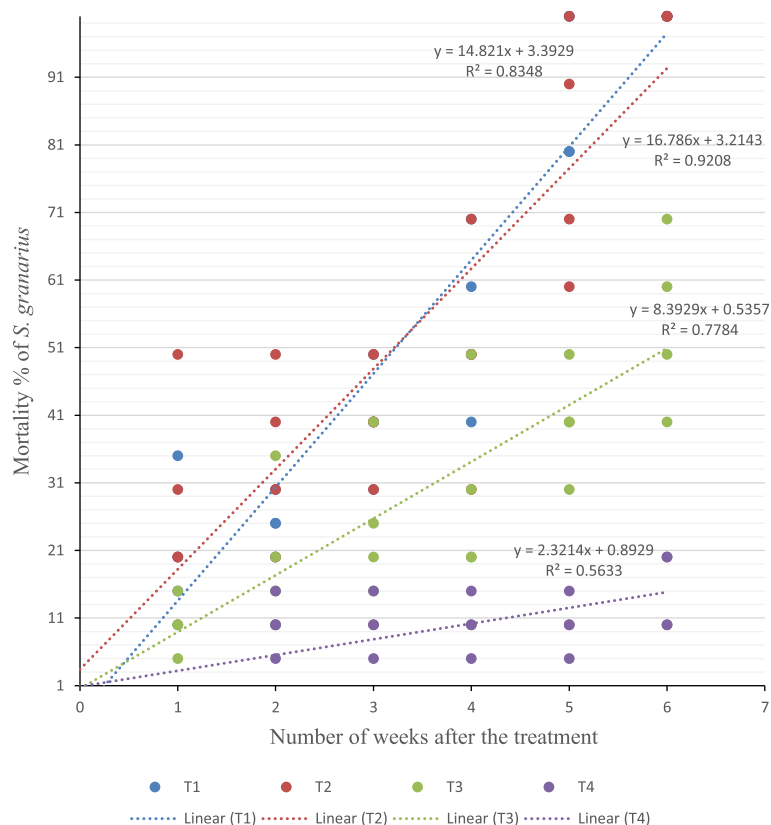


Fig. 4 Mortality of adults of the granary weevils (*Sitophilus granarius*; strain SG5) as a result feeding on mixtures of *Beauveria bassiana* (strain BB2) conidia + wheat flour for 24 h. Ratios of mixtures (g/g) are T1 = 0.2:2.0; T2 = 0.1:2.0; T3 = 0.05:2.0; T4 = 0.2:0

were fed on detergent-wetted conidia. Moreover, Miranpuri and Khachatourians (1991) found that the most preferred site for fungal development of *B. bassiana* after ingestion by the larvae of *Aedes aegypti* was the larval gut and germination of the ingested conidia and their development in the gut caused the death of host larvae. Viable conidia were also recovered in the frass of grasshopper nymphs *Melanoplus sanguinipes* after ingestion of lettuce and bran substrates inoculated with *B. bassiana* conidia (Inglis et al. 1996).

It is important to mention that recent reports are available on the effect of endophytic EPF that live internally in the plants such as *M. anisopliae* that lives in *Brassica napus* and practices control effect against the larvae of *Plutella xylostella* (Batta 2013), *B. bassiana* in *Vitis vinifera* that exercises adverse effect against various sucking insects (Rondot and Reineke 2017), and *B. bassiana* in durum wheat plants that exercises reducing effect against *Spodoptera littoralis* (Sanchez-Rodriguez et al. 2017). Therefore, the lethal effect of these endophytic fungi on target insects is shown by oral ingestion of plant sap in sucking insects or by eating the plant parts in chewing insects. The sap or plant parts that contain these fungi affect target insect by oral ingestion but not

by cuticular penetration, so these fungi can play an important role in the control of insects that attack host plants of these fungi.

It was observed that the development of mycelium resulted from ingested conidia until the death of hosting insects of *S. granarius* was slow in comparison to the development of the mycelium of the same tested fungi that resulted from germination and penetration of external conidia through the insect cuticle. This is because our results indicated that 100% mortality of adults of *S. granarius* was achieved within 6 to 7 weeks after the treatment, due to application of mixtures of conidia of *M. anisopliae* or *B. bassiana* with wheat flour compared to (93.3%) mortality of adults of *Rhyzopertha domnica*, within 1 week due to the cuticular penetration by external germinated conidia applied by spraying the insects with conidial suspension of *M. anisopliae* (Batta 2005). Although a long period of time was needed to achieve 100% mortality in the treated *S. granarius* adults, the applied method is characterized by the following traits: easy to prepare and apply, cheap since it uses a cheap substrate of wheat flour as a carrier of the fungal conidia, and it can be used in grain stores by mixing the grain with flour substrate carrying the fungus conidia, in

addition to the possibility of transmission of infection from one generation of target insects to another where the first infections are usually produced by the first introduction of fungi through sporulation of cadavers.

From a practical point of view, the purpose of using the above-mentioned technique is to be an alternative mean to the chemical control of target insects infesting grains in the stores. For using the technique in grain stores, the following steps are recommended to be done: (i) preparing the mixture of fungus conidia and wheat flour at the recommended ratio (0.1:2.0 w/w, respectively) giving that this mixture should contain 2.75×10^8 conidia/g of mixture for *M. anisopliae* and 2.6×10^8 conidia/g of mixture for *B. bassiana*, and (ii) distributing the prepared mixture thoroughly in the store when it is empty or by mixing it with the grain using the recommended application rate of 20 g of mixture per 1 kg of grain.

Conclusions

A technique for disinfecting the insect cuticle with a fungicide and verifying the presence of EPF in infected host insects after feeding on a substrate containing these fungi was described and applied. The growth and development of these fungi after ingestion caused death of infected insects. This would be a newly recorded interaction between this type of specific host and associated pathogens. Possible application of the results in pest management programs of the target stored-grain insect as an alternative mean to chemical control was discussed.

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Competing of interests

The author declares that he/she has no competing interests.

Authors' contributions

The author carried out the bioassays then collected the data and performed the statistical analysis. The author prepared the manuscript and revised it then read and approved the final version.

Ethics approval and consent to participate

Not applicable.

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