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Original article (Orijinal araştırma)

Pathogenicity of some entomopathogenic fungi on wheat weevil *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae)

Buğday biti *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) üzerinde bazı entomopatojen fungusların patojenisitesi

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Abstract

In the present study, the toxicities of seven entomopathogenic fungal isolates, *Beauveria bassiana* (ARSEF-4984), *Isaria farinosa (*ARSEF-3580), *Isaria fumosorosea (*ARSEF-4501), *Lecanicillium muscarium* (ARSEF-5128), *L. muscarium (*ARSEF-972), *Lecanicillium lecanii* (TR38/11) and *L. muscarium* (Ve6), were tested against the adults of granary weevil, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), under laboratory conditions (25±1°C, 75±5% RH and 14h light:10h dark). Studies were conducted in Atatürk University (Erzurum, Türkiye), in 2018. Fungal isolates were sprayed to adults at two different conidial concentrations (1×10^5 and 1×10^7 ml^{-1}). Mortality percentages were observed on the 2nd, 4th, 6th, 8th, and 10th days of treatment. A commercial isolate of *L. muscarium* were used as positive control and sterile water+0.25% Tween 20 used as negative control. The results demonstrated that the mortality rates of *S. granarius* adults treated with entomopathogenic fungi ranged from 1.01% to 98.9% across 10-day period. Higher concentration and longer exposure periods resulted in increasing virulence on the adult individuals. Among the strains tested, at 1×10^7 ml^{-1} concentration, *I. fumosorosea*, *L. muscarium* (ARFES-5128) and *L. lecanii* isolates displayed 97.85%, 94.62% and 93.58% cumulative mortalities respectively, on *S. granarius* adults by the 10th day of the experiment. These three isolates are regarded as highly promising biological control agents.

Keywords: Entomopathogen, fungi, stored grain

Öz

Bu çalışmada, yedi entomopatojen fungus izolatı *Beauveria bassiana*, *Isaria farinosa (*ARSEF-3580), *Isaria fumosorosea (*ARSEF-4501), *Lecanicillium muscarium* (ARSEF-5128), *L. muscarium (*ARSEF-972), *Lecanicillium lecanii* (TR38/11) ve ticari bir *L. muscarium* (Ve6), buğday biti, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) erginlerine karşı laboratuvar şartlarında (25±1°C, 75±5% nisbi nem ve 14sa aydınlık:10sa karanlık) denenmiştir. Çalışma, Atatürk Üniversitesi'nde (Erzurum, Türkiye) 2018 yılında gerçekleştirilmiştir. Fungal izolatlar ergin bireylere iki farklı spor konsantrasyonunda ($1x10^5$ ve 1×10^7 ml^{-1}) spreylenmiştir. Yüzde ölümler uygulamadan sonraki 2., 4., 6., 8. ve 10. günlerde kaydedilmiştir. Pozitif kontrol olarak ticari bir *L. muscarium* izolatı ve negatif kontrol olarak da steril su+Tween20 (0,25%) kullanılmıştır. Sonuçlar, entomopatojen fungus ile uygulanan *S. granarius* erginlerinde, uygulamadan sonraki 10 gün süresince ölüm oranlarının %1,01 ile %98,9 arasında değiştiğini göstermiştir. Yüksek konsantrasyon ve daha uzun maruz kalma süreleri ergin bireylerde artan patojenisite ile sonuçlanmıştır. Uygulanan suşlar arasında, 1×10^7 ml^{-1} konsantrasyonda *I. fumosorosea*, *L. muscarium* (ARFES-5128) ve *L. lecanii* izolatları, denemenin 10. gününde *S. granarius* erginlerinde sırasıyla %97,85%, %94,62 ve %93,58 kümülatif ölüm oranlarına ulaşmıştır. Bu üç izolatın biyolojik mücadele kapsamında yüksek derecede ümitvar oldukları değerlendirilmiştir.

Anahtar sözcükler: Entomopatojen, fungus, depolanmış ürün

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Introduction

Pest infestations of stored grains may result in severe losses in quality, including loss of vigor of seeds, changes in natural color, providing habitat for insects and mites, toxic compounds from microbial pathogens and excretion and remainders of insects. There are about 130 species that were recorded, and majority of storage pests are from the Coleoptera and Lepidoptera families (Proctor, 1994; Hagstrum & Subramanyam, 2009; Morales-Quiros, 2019). Among these, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), also called "wheat weevil, granary weevil or grain weevil", is one of the most common pests of stored grains including wheat, barley, rye, corn, and rice worldwide. Female deposits about 50-400 eggs into cavities on grains and both adult and larvae feed on germ and endosperm of grains. Combining long term storage of grains with relatively short (30-60 days) development duration of the pest (Kirkpatrick & Wilbur, 1965; Campbell et al., 1976; Sinha & Sinha, 1991) and ability to avoid the grains contain their offspring (Danho et al., 2002; Woodbury, 2008), helps to explain the success of the pest.

This pest leads to large economic losses of stored wheat grains after harvesting in the tropical and subtropical regions of the world (Hare, 1980; Isman, 2006). The losses may vary from 10 to 40 percent, particularly in primitive grain stores (Saroukolai et al., 2000). Loss of grain weight and nutritional value are among quantitative and qualitative losses caused by this insect (Moina et al., 1998). Many chemical insecticides and fumigants have been tested to prevent the damage of this pest for years. Chemical insecticides generally provide an advantage as the rapid and efficient control method for *S. granarius* (Emsen et al., 2015; Kordalı et al., 2017). These chemicals present some serious risks such as environmental pollution because of their slow biodegradation, toxic effects on non-target organisms, food safety, pest resistance, direct toxicity to users, and cause depletion of ozone layer (Arthur, 1996; Isman, 2006; Santos et al., 2009; Kesdek et al., 2013; Kücükaydin et al., 2020).

Synthetic pesticide consumption is another disadvantage, considering the high cost-benefit ratio (Roy & Dreja, 1998). Therefore, authorities are hesitant to allow chemical insecticides on stored grains, primarily due to their residues (Thaung & Collins, 1986). Due to negative effects of synthetic insecticides, alternative control strategies are needed under storage conditions and one of the effective and environmentally friendly methods for control of storage pests is utilizing microbial biocontrol.

Entomopathogenic microorganisms generally belong to the groups of fungi, bacteria, protozoan, and viruses. They can cause diseases in infected arthropods, leading to a rapid decline in host arthropod populations. Entomopathogenic fungi are primary microorganisms used against harmful arthropods on culture plants since they are very effective on a wide range of insects, and development of resistance risk is low. They are also safe for non-target organisms and environment, and can adapt to various environmental conditions (Hajek & St. Leger, 1994; Lacey & Goettel, 1995; Inglis et al., 2001; Roy et al., 2001; Zimmermann, 2007). Their role is crucial in biological control of hazardous insects. They have gained the most interest in research for utilizing microbial pesticides. There are more than 100 genera and 700 species containing insect pathogens. They display low mammalian toxicity, being relatively safe for the environment (Roberts & Humber, 1981; Charnley, 1989; Cox & Wilkin, 1996). The common species of *Metarhizium, Beauveria*, *Isaria* and *Lecanicillium* and *Isaria* are quite amenable for mass production. At least 10 entomopathogenic fungi species have been widely used for biocontrol purposes (Hajek & St. Leger, 1994). The use of entomopathogenic fungi in the biological control against pests is an attractive alternative to the use of conventional pesticides, because these fungi are very strong control agents against a wide range of arthropod pests (Khetan, 2001; Sevim et al., 2013).

Entomopathogenic fungi enter the insect cuticle through both physical and enzymatic mechanisms (Erkılıç & Uygun, 1993). The process involves settling of fungus spores on the insect cuticle, followed by their germination and penetration into the cuticle by forming an appressorium (Clarkson & Chamley, 1996; Sevim et al., 2015). In most instances, insect mortality results from the presence of toxic substances rather than mycosis (Charnley, 2003).

There are many studies with the use of entomopathogenic fungi for control of stored product pests (Ferroni, 1977; Domsch et al., 1980; Serale & Doberski, 1984; Hidalgo et al., 1998; Padin et al., 2002; Cherry et al., 2005; Tunaz & Er, 2008; Barra et al., 2013; Emsen et al., 2015; Batta & Kavallieratos, 2017; Komaki et al., 2017; Usanmaz Bozhüyük et al., 2018; Kordali et al., 2021). Several strains were successfully tested on stored grains including maize (Barra et al., 2013), wheat (Batta & Kavallieratos, 2017), rice (Rice & Cogburn, 1999), sorghum (Ekesi et al., 2000), oat (Throne & Lord, 2004) and barley (Aregger, 1992).

The objective of the current investigation was to assess the effectiveness of seven entomopathogenic fungal isolates, namely *Beauveria bassiana* (Bals.-Criv.) Vuill. (ARSEF-4984), *Lecanicillium muscarium* Zare & Gams (ARSEF-972), *Lecanicillium lecanii* Zare & Gams (TR38/11), *Lecanicillium muscarium* (ARSEF-5128), *I. fumosorosea* Wize (ARSEF-4501), *Isaria farinosa* (Holmsk.) Fr. (ARSEF-3580), and *L. muscarium* Ve6 obtained from Mycotal®, for the purpose of controlling adult *S. granarius* in laboratory settings.

Materials and Methods

Insects

The insects tested in this study were collected from the private storage houses and flour mills in Fethiye (Muğla). Species identifications were conducted by Dr. Erol Yıldırım (Atatürk University, Erzurum, Turkey). Wheat grains (common wheat, *Triticum aestivum* L.) were purchased locally. They were stored in a freezer at −20°C to prevent deterioration and were inspected for two days to check for the presence of any arthropod pests prior to use for bioassays. Then, the grains were washed in tap water, allowed to dry, and heated to prevent pre-infestations that may occur prior to the experiments. *S. granarius* individuals were reared to adult stage in laboratory, at 25 ± 1°C, 75±5 relative humidity and L:D=14 h:10 h in the Department of Plant Protection, Atatürk University, Erzurum-Turkey. The adults obtained from rearing cultures were kept in cages containing enough amount of wheat grains. All experiments were implemented under identical conditions.

Fungal entomopathogens

A total of seven entomopathogenic fungi isolates were used in the study. Five of these were obtained from ARSEF collection (USA), *Beauveria bassiana (*4984), *Isaria farinosa (*3580), *I. fumosorosea (*4501), *Lecanicillium muscarium (*5128), *L. muscarium (*972). One isolate was a soil extraction from Kayseri province (*L. lecanii*; TR38/11). One additional isolate (Ve6; *L. muscarium* Ve6) was extracted from a commercial product (Mycotal, Koppert, NL) and used as positive control. Fungal isolates were allowed to grow on potato dextrose agar (PDA, Oxoid, CM0139) medium at 25°C for two weeks, before the spray treatments to *S. granarius* adults. Conidia were harvested from 14-day-old fungi cultures by gently scraping the surface of fungal growth uisng a sterilized glass micro spatula and mixed thoroughly in 3 ml distilled sterile water containing a surfactant, 0.25% (v/v) Tween 20, in screw capped bottles. Conidia viability of EPFs were tested with the following method: Conidia suspension of each isolate was spread onto water agar (15%) plates then incubated at 28°C in the dark for 18 h. The percentage of germinated conidia was examined by examining 50 conidia per plate. Germination capacity was defined as a conidium having a germ tube equal to the length of at least half the width of the conidium (Wyss et al., 2001). The suspensions were then mixed with tween 20 (0.25% v/v) and adjusted to two different (1×10^5 and 1×10^7 conidia ml^{-1}) concentrations by using a haemocytometer, under a light microscope, for subsequent use in the experiments.

Experiment design and application of entomopathogenic fungi

To test the pathogenicity of entomopathogenic fungi isolates, sterile plastic Petri dishes lined with sterile filter paper were used. In each Petri dish, 11 newly emerged of *S. granarius* adults (mixed males and females) were collected with an aspirator and fed with wheat grains (30 wheat grains/dish). The suspensions were sieved, adjusted to two different spore concentrations and 1 ml sprayed on each replicate

containing the insects, wheat grains and filter paper in Petri dishes, under a laminar flow hood and kept there until excess water evaporates. Then Petri dishes were incubated in 25±1°C with 75±5% RH, in 16:8 (L:D) photoperiod. Each assay was repeated three times for each of the doses and exposure time combination. Isolate extraction of *L. muscarium* was used as positive control and distilled sterile water containing 0.25% (v/v) Tween 20 was used as negative control in the study. The experiment was set up as completely randomized design with three replicates, each consisting of three Petri dishes with 11 *S. granarius* adults per Petri dish, as explained above. Alive and dead *S. granarius* adult individuals were counted every 48 h for 10 days, following the treatments. All the experiments were repeated at least twice.

Statistical analyses

Due to the initial uniform population structure, mortality rates from treatments were corrected according to Schneider-Orelli's formula (Puntener, 1981). Differences in virulence between the tested entomopathogenic fungi isolates were determined by analysis of variance using the SAS JMP v9.0 software package. Tukey's HSD test was used for comparison of means for each day and each isolate across the experiment, at $p \leq 0.05$ significance level. To prevent bias, angular transform applied to control-corrected mortality percentages, prior to statistical analyses.

Results

The isolates were tested against *S. granarius* at two different spore concentrations (1×10^5 and 1×10^7 conidia/ml) and results were corrected with control. Statistical tests were performed on control-corrected and transformed mean mortality values from each spore concentration level, individually. In lower concentration ($1x10^5$), means from the eight treatments (including positive control) were found statistically significant for each of the measurement days (10 days after treatment (DAT), $F_{(6,14)} = 8.16$, $p = 0.0006$) and separated to four to five mean groups in each measurement day, across the experiment, by Tukey's HSD test (Table 1). Best performing isolates were TR38/11 and (+) Control for most of the days, in lower spore concentration tests. In this experiment, over 75% control-corrected mortality rates were first achieved by (+) Control and TR38/11 isolates, on the 8th DAT. Highest mortality rates achieved on the 10th day by again the (+) Control isolate (98.9%), followed by TR38/11 (86.0%), 5128 (71.0%) and 4501 (64.5%) isolates (Figure 1). While the TR38/11 isolate scored a 37.5% mortality which was the highest for the beginning of the experiment, there were no statistical differences with (+) Control isolate (Table 1) on the 2nd DAT. The numbers were equalized around 50% about the 6th DAT and (+) Control performed better in the remaining days. The lowest mortality recorded by 3580 isolates (31.2%) on the10th DAT. The performance of 4501 and 4984 isolates were statistically on par, across the experiment (Table 1).

At a lower spore concentration of $1x10^5$, statistical significance was found for each of the measurement days (10 DAT, F (6,14) = 8.16, $p = 0.0006$) among the eight treatments (including the positive control). Tukey's HSD test separated these treatments into four to five mean groups for each measurement day throughout the experiment (Table 1).

The TR38/11 and (+) Control isolates performed best for most of the days in the lower spore concentration experiment, with over 75% control-corrected mortality rates first achieved by these isolates on the 8th DAT. The (+) Control isolate achieved the highest mortality rates on the 10th day (98.9%), followed by TR38/11 (86.0%), 5128 (71.0%), and 4501 (64.5%) isolates (Figure 1). While the TR38/11 isolate had the highest mortality rate at the beginning of the experiment (37.5%), there was no statistical difference with the (+) Control isolate on the 2nd DAT (Table 1). The mortality rates were centered around 50% on the 6th DAT, and the (+) Control isolate performed better in the remaining days. The 3580 isolate had the lowest mortality rate (31.2%) on the 10th DAT. The performance of the 4501 and 4984 isolates were statistically similar throughout the experiment (Table 1).

Table 1. Mean number of cumulative mortalities (±SE) in adults of *Sitophilus granarius* (Coleoptera: Curculionidae) 2-10 days after treatment with spore suspensions of seven entomopathogenic fungi (EPF) isolates adjusted to ($1x10^5$) spore concentration. Each treatment started with a total of 33 adult individuals

EPFs*	Days after treatments									
	2		4		6		8		10	
TR38/11	13.0±1.0	aB**	15.7±2.3	aB	17.7±1.5	abB	22.0±1.0	abAB	28.7±3.8	abA
3580	0.0±0.0	dB	7.0±1.0	bcB	13.0±2.0	bcAB	18.0±2.7	bcAB	24.7±0.6	abcA
4501	4.3±3.2	cC	8.0±3.6	bcBC	15.7±4.0	bAB	18.7±2.1	bcA	22.0±2.0	bcA
4984	3.3±3.2	cD	5.3±3.2	cC	7.0±2.7	cBC	13.3±2.1	cAB	18.3±2.3	cA
5128	5.7±0.6	bcE	9.3±2.3	abcD	18.7±1.2	abC	23.0±2.0	abB	31.3±1.5	aA
972	7.7±2.1	abcC	12.3±2.5	abC	15.7±2.1	abB	18.0±1.7	bcB	22.0±1.0	bcA
(+) Control	10.7±0.6	abE	16.0±1.7	aD	22.0±1.7	aC	26.3±1.5	aB	30.3±0.6	abA
(-) Control	0.7±0.6		1.0±0.0		1.0±0.0		1.0±0.0		1.3±0.6	

* ARSEF isolates are 3580 (*Isaria farinosa*), 4501 (*Isaria fumosorosea*), 4984 (*Beauveria bassiana*), 5128 (*Lecanicillium muscarium*), 972 (*Lecanicillium muscarium*). TR38/11 is a local isolate (*Lecanicillium lecanii*) extracted from Kayseri province soils (Turkey), and (+) control is *Lecanicillium muscarium* Ve6 isolate obtained from a commercial product (Mycotal; Koppert, NL).

** Mean mortality numbers followed by same lowercase letter (s) in each column, or same uppercase letter (s) in each row are statistically not different (Tukey's HSD, P≤0.05)

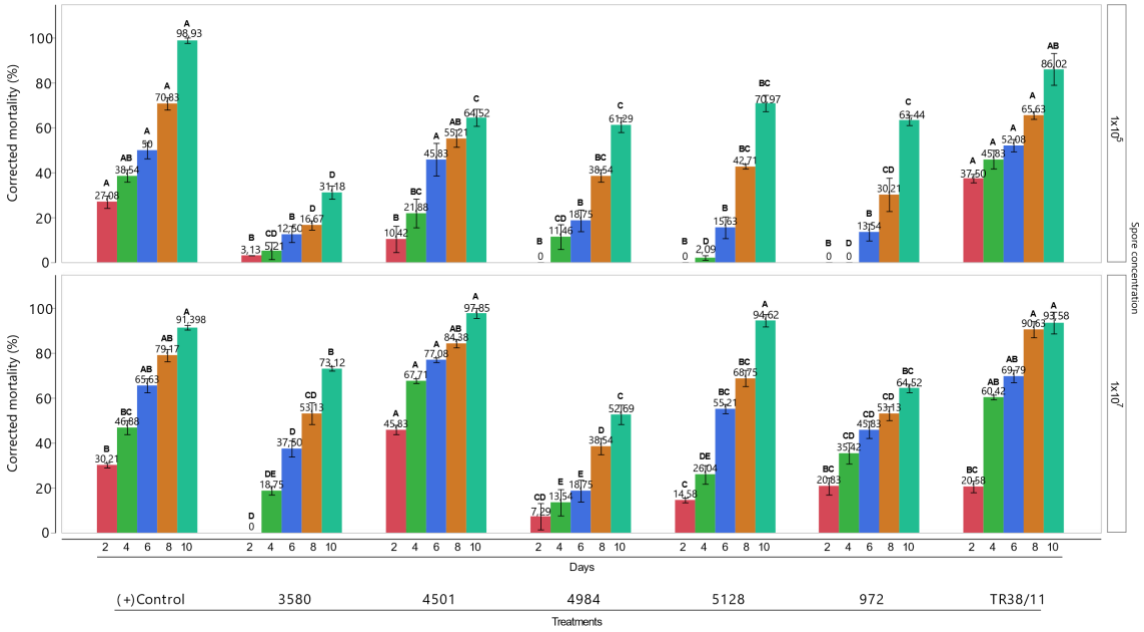

Figure 1. Cumulative mortality rates of seven entomopathogenic isolates (TR38/11: *Lecanicillium lecanii*; 3580: *Isaria farinosa*; 4501: *Isaria fumosorosea*; 4984: *Beauveria bassiana*; 5128: *Lecanicillium muscarium*; 972: *Lecanicillium muscarium*; (+) Control: commercial *Lecanicillium muscarium* Ve6) on *Sitophilus granarius* adults, 2-10 days after treatment (DAT). Different capital letters on same days after treatment (DAT) with each isolate indicates a statistically significant difference for the given DAT, among the isolates (Tukey's HSD, P≤0.05; Bars indicate standard error).

Same statistical tests were also performed for higher concentration ($1x10^7$) and results are presented in Table 2. Similarly, the difference between treatments at each of the measurement days was found statistically significant (10 DAT, $F_{(6,14)} = 28.62$, $p < 0.0001$). Contrary to lower concentration, the number of statistical groups among mean mortality numbers of isolates remained low, typically three to four mean groups formed in many of each measurement days, as seen in Table 2. There was an overall increase in mortalities in the higher concentration, as expected (Figure 1). Three treatments (TR38/11, 4501 and (+) Control) consistently outperformed the others, across the experiment. Mortalities from these three isolates were over 75%, by 8th DAT. Highest mortalities were again obtained by 10th DAT as seen in Figure 1, and isolate 4501 performed best with 97.85%, followed by 5128 (94.63%), TR38/11 (93.58) and (+) Control (91.40%).

Table 2. Mean number of cumulative mortalities (±SE) in adults of *Sitophilus granarius* (Coleoptera: Curculionidae) 2-10 days after treatment with spore suspensions of seven entomopathogenic fungi (EPF) isolates adjusted to ($1x10^7$) spore concentration. Each treatment started with a total of 33 adult individuals

EPFs*	Days after treatments									
	2		4		6		8		10	
TR38/11	7.3±1.2	bD**	20.3±0.6	abC	23.3±1.5	abBC	30.0±2.0	aAB	31.0±2.7	aA
3580	2.0±0.0	cE	2.3±2.6	cD	5.0±2.0	cdC	6.3±1.2	dB	11.7±1.5	cA
4501	15.7±1.2	aD	22.7±0.6	aC	25.7±0.6	aBC	28.0±1.0	abB	32.3±1.2	aA
4984	0.3±0.6	dC	4.7±3.1	cBC	7.0±2.7	cBC	13.3±1.5	cAB	21.0±1.7	bcA
5128	0.0±0.0	dC	1.7±0.6	cC	6.0±2.7	cB	14.7±0.6	cB	24.0±2.0	bA
972	0.0±0.0	dD	1.0±0.0	cCD	5.3±2.1	cBC	10.7±4.0	cdAB	21.7±1.2	bA
(+) Control	9.7±1.6	bD	13.3±1.5	bCD	17.0±2.0	bC	23.7±1.5	bB	32.7±0.6	aA
(-) Control	0.7±0.6		1.0±0.0		1.0±0.0		1.0±0.0		1.3±0.6	

* ARSEF isolates are 3580 (*Beauveria bassiana*), 4501 (*Isaria fumosorosea*), 4984 (*Beauveria bassiana*), 5128 (*Lecanicillium muscarium*), and 972 (*Lecanicillium muscarium*). TR38/11 is a local isolate (*Lecanicillium lecanii*) extracted from Kayseri province soils (Turkey), and (+) control is *Lecanicillium muscarium* (Ve6) obtained from a commercial product (Mycotal; Koppert, NL).

** Mean mortality numbers followed by same lowercase letter (s) in each column, or same uppercase letter (s) in each row are statistically not different (Tukey's HSD, P≤0.05).

Mean mortality by the isolates were initially compared for each of 2, 4, 6, 8 and 10^{th} days after treatment (DAT) with Tukey's HSD, to find out if there are higher performing isolates in earlier days and whether their mortality efficiency persists throughout the experiment.

The last day of the experiment (10 DAT) was the one with highest mortality for all isolates and well separated from the other days for all isolates, with exception of isolate 4501 in 10^5 concentration tests. Comparing both concentration levels, none of the isolates drew a similar differentiation pattern across days, and mortalities from the same isolate were separated differently for each concentration, until the last day of the experiments.

Interactions between time and treatments were also statistically tested. In the individual tests for each of the concentrations, result for 10^5 spore concentration was, $F_{(34,70)} = 30.90$, $p < 0.0001$. The result for 10^7 concentration was, $F_{(34,70)} = 72.65$, $p < 0.0001$. These statistical tests based on control-corrected and post-transformed data indicated that the effect of time on the mortality of treatments were significant.

Discussion

In this study, the mortality rates of *S. granarius* adults were detected for seven isolates of five different entomopathogenic fungi species (*B. bassiana*, *L. lecanii*, *I. fumosorosea*, *I. farinosa*, *L. muscarium (*two isolates) and an isolate extract of commercial *L. muscarium (*as positive control)) across 10 days after the treatment. The pathogenicity tests were performed by spraying *S. granarius* adults at two different conidial concentrations (1×10^5 and 1×10^7) and constant laboratory conditions (at 25±1°C, 75±5% RH, 14h light:10h dark). The mortality rates of *S. granarius* adults were high, ranging from 52.7 to 97.9% among these entomopathogenic fungi isolates by the 10^{th} day after treatments. Specifically, the adult mortalities for the same day at 1×10^5 conidial concentration were minimum 31.2% for *I. farinosa (*3580) and maximum 98.9% for *L. muscarium* ((+) Control). The adult mortalities at 1×10^7 conidial concentration was minimum 52.7% for *B. bassiana (*4984), and 97.9% for *I. fumosorosea (*4501). While the maximums of both concentrations appear equally pathogenic, considering overall increase in mortality rates in higher concentration, and having more than one isolate reaching over 90% mortality, results showed advantages of increased primary inoculum, as indicated in several other studies (Hidalgo et al., 1998; Dembilio et al., 2010).

Against *S. granarius*, many different entomopathogenic strains have been tested for pathogenicity by various researchers so far and majority of them have focused on *B. bassiana* strains, either alone, or

combined with some synergistic compounds, like diatomaceous earth formulations or traditional chemicals (Dal Bello et al., 2000; Athanassiou & Steenberg, 2007) with varying degrees of success. Mantzoukas et al. (2019) tested several strains of *B. bassiana* with $1x10^8$ concentration and mean mortality ranged between 3.3% and 100% among the isolates, as of the 14th day after treatments. In the same study, *I. fumosorosea* isolate reached 36.6% mortality for the same day. Kavallieratos et al. (2014), reported a 100% mortality with $2.11x10^7$ spore concentration of a *B. bassiana* isolate applied to *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) adults, without food, 7 days after treatments. The *B. bassiana* isolate in our study performed poorly among others and reached only 56.7% mortality by the 10th day, under similar environmental and application conditions. The highest mortality rates achieved from the *I. fumosorosea* and two *Lecanicillium* isolates were over 90% by the 10th DAT in the present study. On the contrary, Ak (2019) reports that under 25°C and $1×10^8$ spore concentration, while *S. granarius* mortality sourced from *I. fumosorosea* isolate was 84.2%, *B. bassiana* and *L. muscarium* isolates were lower, 56.1% and 22.8%, respectively, by the seventh day after treatments. The same study also states highest mortality, with a *M. anisopliae* isolate.

Barra et al. (2013) recorded a 10% mortality on *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae) (a pest of stored maize), by treatments of *Purpureocillium lilacinum* (Thom) Luangsa-Ard, Houbraken, Hywel-Jones & Samson, while 90% mortality was recorded with same isolate on another storage pest, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae).

Many studies on the mortality effects of entomopathogenic fungi against *S. granarius* adults were carried out by various researchers worldwide. Among these, *B. bassiana* is considered as one of the most successful entomopathogens. The use of *Beauveria bassiana* to control stored grain pests was studied by some researchers. Rice & Cogburn (1999) demonstrated that *B. bassiana* isolate (22292A) prevented mortalities of the three stored grain pests (*Sitophilus oryzae (*L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica (*Fabricius, 1792) (Coleoptera: Bostrichidae) and *Tribolium castaneum (*Herbst, 1797) (Coleoptera: Tenebrionidae) adults ranging from 80% to 100% on day 21 after treatment. Cherry et al. (2005) recorded that *B. bassiana* 0362 isolate caused mortality from 35.56% to 100% against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae)) adults on the 6th day of treatment. Padin et al. (2002) studied the pesticidal effects of *B. bassiana* on *S. oryzae, T. castaneum* and *Acanthoscelides obtectus (*Say, 1831) (Coleoptera: Chrysomelidae) and adults for a long period. In their study, *B. bassiana* isolate caused significant mortality in *S. oryzae* adults but did not lead to significant mortality in *T. castaneum* and *A. obtectus* adults, after four months. Khashaveh et al. (2011) recorded up to 88.33% mortality effect of *B. bassiana* for *S. granarius* adults. Komaki et al. (2017) investigated that *B. bassiana* isolate resulted in mortalities between 69.3% and 100% at $1x10^5$ and $1x10^7$ doses, on the 2nd, 4th, 6th, 8th and 10th days of treatment on *T. confusum* adults. In our study, *B. bassiana (*4984) caused varying mortality rates at $1x10^5$ (ranging between 10.1-55.5% across the experiment) and $1x10^7$ (1.01- 63.6%) doses on *S. granarius* adults (Table 1).

Yang et al. (2009) stated that *I. farinosa* had mortality effect up to 88% on the *Pissodes punctatus* (Langor & Zhang in Langor, Situ & Zhang, 1999) (Coleoptera: Curculionidae) larvae, pupae, and adults. In another work, *I. farinosa* isolate caused very high mortalities in ovisacs, second larval stage, adult females, and first larval stage of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) at 95% RH and at $1x10^8$ conidia ml^{-1} inoculum concentration, 89.39%, 84.07%, 84.53% and 78.71%, respectively (Demirci et al., 2011). Cabanillas & Jones (2013) indicated that *I. poprawskii* is pathogenic to the immature and adults of glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar, 1821) (Hemiptera: Cicadellidae)). Also, in that study, pathogenicity, and virulence of *I. poprawskii* against *H. vitripennis* pointed out that this fungus could be a promising biocontrol agent to control this pest. It was specified that *Isaria fumosorosea* had a mortality effect on *S. oryzae* adults (Kavallieratos et al., 2014). Komaki et al. (2017) demonstrated that two *Isaria* fungi isolates (*Isaria fumosorosea (*ARSEF-4501 and *Isaria farinosa (*ARSEF-3580)) had mortality rates from 37.3 to 72.0% against *T. confusum* adults at $1×10^5$ and $1×10^7$ doses on the 10th day of treatment.

In the present study, *I. fumosorosea (*ISFUM-4501) and *I. farinosa (*IFA-3580) fungi isolates caused mortalities from 6.06 to 97.9% against *S. granarius* adults at 1×10^5 and 1×10^7 doses after 10 days of treatment. These two studies support each other. It is reported that the isolate of *Paecilomyces* sp. led to between 4.29% and 26.32% mortality against *S. zeamais* adults (Kassa et al., 2002). The same researchers demonstrated *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrichidae) to be more susceptible to the tested entomopathogenic fungi than *S. zeamais*. In this study, *L. lecanii (*TR38/11) fungus isolate caused the mortalities in the 1×10^5 dose (from 39.3% to 86.8%) and $1x10^7$ dose (from 22.2% to 93.9%) on *S. granarius* adults between the 2nd and 10th of treatment (Table 1; Figure 1).

Although these differences may be connected to variances in the methods used, there may be a degree of variation in the virulence of different isolates which might be a contributing factor (Zettler, 1991). According to the findings of the study, all isolates acted better at higher concentration (except *I. farinosa (*IFA-3580) isolate) 10 days after treatment by resulting in mortality of over 60%. The tendency of increase observed in mortalities (except for *B. bassiana (*ARSEF-4984) and *I. farinosa* (ARSEF-3580)) throughout the treatment is also considered as an indicator of consistent pathogenicity. Among the isolates tested, *L. lecanii (*TR38/11) and *I. fumosorosea (*ISFUM-4501) particularly provided higher mortality rates, from the initial stages of the experiments. *L. muscarium (*ARSEF-5128) isolate gave a consistent increase in mortality and resulted in up to 94.9% mortality in *S. granarius* adults by 10 days after treatment.

Conclusions

Various chemical, biotechnical, and microbiological ways to control the entry of storage pests into storages were advised by the researchers, like treating the empty bins, and application of treatment to topmost layer of the stored products (“top-dress” or “cap off”), which helps to prevent emergence or new entries (Mason & Obermeyer, 2010). Aside from strain virulence which was considered as the primary factor for higher pest mortality, this shows the importance of treatment methodology for entomopathogenic fungi in stored conditions and points out the need for further research considering both pathogenicity and application methodology of fungal treatments, under actual storage conditions.

Based on higher virulence they provided, *L. lecanii (*TR38/11), *I. fumosorosea (*ARSEF-4501) and *L. muscarium (*ARSEF-5128) isolates are considered better candidates as biocontrol agents against adults of *S. granarius,* in stored wheat.

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