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Original article (Orijinal araştırma)

Laboratory assessment for biological control of *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) by entomopathogenic fungi

Tribolium confusum du Val., 1863 (Coleoptera: Tenebrionidae)'un biyolojik mücadelesinde entomopatojen fungusların kullanımının laboratuvar ortamında değerlendirilmesi

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Summary

This research was carried out at the Plant Protection Department, Agricultural Faculty, Ataturk University (Erzurum, Turkey) in 2016. The objective of this study is to determine using as biological control agent against *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) adults of seven entomopathogenic fungal treatments, *Beauveria bassiana*, *Paecilomyces farinosus*, *Isaria fumosorosea*, *Isaria farinosa*, *Lecanicillium muscarium* (2 isolates) and an extract of *L. muscarium*, under laboratory conditions (25±1°C and 75±1% RH). Fungal isolates at two different concentrations ($1x10^5$ and $1×10^7$ conidia/mL) were sprayed on the tested adult insects in Petri dishes. The results demonstrated that the mortality rates of *T. confusum* adults treated with seven entomopathogenic fungi varied from 34.6 to 100% after 10 days of treatment. The entomopathogenic fungi isolates at both $1×10^5$ and $1×10^7$ conidia concentration caused in high mortality levels of *T. confusum* adults. In conclusion, it was observed that tested seven entomopathogenic fungi isolates might have a potential effect to biological control of this stored-product pest.

Keywords: Biological control, entomopathogenic fungi, stored-product insect, *Tribolium confusum*

Özet

Bu çalışma, 2016 yılında Atatürk Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü (Erzurum, Türkiye)'nde yürütülmüştür. Çalışmanın amacı, yedi entomopatojen fungal (*Beauveria bassiana*, *Paecilomyces farinosus*, *Isaria fumosorosea*, *Isaria farinosa*, *Lecanicillium muscarium* (2 izolat) ve *L. muscarium* ekstraktı) izolatlarının, laboratuvar şartlarında **(**25±1°C and 75±1% RH**).** *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) erginlerine karşı biyolojik kontrol ajanı olarak kullanımlarını tespit etmektir. Fungus izolatları petri kaplarında test edilen ergin böceklere karşı iki farklı konsantrasyonda ($1x10^5$ ve $1×10^7$ konidi/mL) sprey şekilde uygulanmıştır. Elde edilen sonuçlar, uygulamadan 10 gün sonra yedi entomopatojen fungus izolatı uygulaması ile *T. confusum* erginlerinin ölüm oranlarının %34.6'dan %100'e kadar değiştiğini göstermiştir. Entomopatojen fungus izolatları $1×10^5$ ve $1×10^7$ konsantrasyonlarında *T. confusum* erginlerinde yüksek seviyede ölüme neden olmuştur. Sonuç olarak, test edilen yedi fungal entomopatojenin depolanmış ürün zararlılarının biyolojik mücadelesi için potansiyel etkiye sahip olabileceği gözlemlenmiştir.

Anahtar sözcükler: Biyolojik mücadele, entomopatojen funguslar, depolanmış ürün zararları, *Tribolium confusum*

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Introduction

Each year throughout the world about 10 to 40% of stored cereal grain is qualitatively and quantitatively damaged by insect pests, especially in tropical and subtropical regions of developing or undeveloped countries (Madrid et al., 1990; Shaaya et al., 1997; Tripathi et al., 2009). Stored foods are destroyed by different groups of insect pests, especially by beetles, moths and mites (Rajendran, 2002). Protecting stored grain and seeds against insect pests is a major challenge in post-harvest processes. Since stored-grain insect pests become widespread throughout the world through human activity and seed transportation, they are considered to have evolved adaptations to different stored foodstuffs. One of the most important common and destructive stored-product insects worldwide is confused flour beetles, *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) (Aitken, 1975; Hodges et al., 1996). Confused flour beetles have an extremely large appetite for a variety of foods, such as food products stored in soils, warehouses, grocery stores, and houses including meal, crackers, beans, spices, pasta, dried pet food, dried flowers, chocolate, nuts and seeds, and even dried museum specimens (Via, 1999; Weston & Rattlingourd, 2000). Also, they are particularly abundant in cereal products, in wheat and flour (Aitken, 1975; Hodges et al., 1996). When they occur in large number, confused flour beetles secrete a chemical mixture that includes quinones, which are carcinogenic, thereby affecting product quality (Hodges et al., 1996). Generally, the control of this pest species relies on fumigants, phosphine and residual grain protectants. However, fumigation, by far the most effective method of grain and grain-product disinfestation, has serious limitations (Mills, 1983; Taylor, 1989; Bell & Wilson, 1995; Bell, 2000; Caddick, 2004).

Increased concern by consumers over grain protectant (organophosphorus and pyrethroid insecticides and fumigants) residues in processed cereal products, the occurrence of insecticide resistant insect strains (Champ & Dyte, 1976; Zettler, 1991; Arthur & Zettler, 1992; Arthur, 1996; Zettler & Arthur, 1997; APRD, 2016) and the precautions necessary to work with chemical insecticides, call for new approaches to control stored-product insect pests.

Entomopathogenic fungi, as both biological control agents and sources of bioactive compounds active against the insect pests, could provide an alternative to chemical pesticides (Isaka et al., 2005; Monlar et al., 2010), since they have low mammalian toxicity, high effectiveness and a natural origin (Moore et al., 2000). Entomopathogenic fungi as natural enemies of insect pests in different ecosystems have high potential to control pests in agroecosystems (Altieri, 1999; Gurr et al., 2003; Tscharntke et al., 2005; Fiedler & Sosnowska, 2007; Jaronski, 2010; Jaber, 2015). There are approximately 90 genera and 700 species of entomopathogenic fungi known (Roberts & Humber, 1981) and the common species of *Beauveria*, *Metarhizium*, *Lecanicillium* and *Isaria* are quite amenable for mass production. Previous studies have mostly focused on *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Rice & Cogburn, 1999; Moore et al., 2000; Dal Bello et al., 2001; Lord, 2001, 2005; Akbar et al., 2004; Batta, 2004, 2005; Kavallieratos et al., 2006; Michalaki et al., 2006; Vassilakos et al., 2006).

The anamorphic entomopathogenic fungi such as *B. bassiana*, *M. anisopliae*, *Lecanicillium muscarium* (Petch) Zare & W. Gams, *Isaria farinosa* (Holmsk.) Fr. (formerly *Paecilomyces farinosus* (Holmsk.) A.H.S. Br. & G. Sm.), *Isaria fumosorosea* Wize (formerly *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.), and *Lecanicillium muscarium* (Zimm.) Zare & W. Gams from the order Hypocreales (Ascomycota) are natural enemies of wide range of insect pests, and these fungi may produce enormous numbers of conidia over many asexual life cycles in a single cultivation season (Roberts & St. Leger, 2004; Rehner, 2005; Gurulingappa et al., 2010). Some of the entomopathogenic fungi (e.g., *B. bassiana*) are endophytic symbionts in maize, potato, cotton, date palm, banana and coffee (Jones, 1994; Wagner & Lewis, 2000; Leckie, 2002; Ownley et al., 2004; Arnold & Lewis, 2005; Gómez-Vidal et al., 2006; Akello et al., 2007; Posada et al., 2007), and could control the insect pests after feeding. The grain loss by *Tribolium castaneum* (Herbst, 1797) treated with *B. bassiana* has been studied by Padin et al. (2002). In their study, *B. bassiana* do not show effective control against the *T. castaneum*. *Beauveria bassiana* mixed with diatomaceous earth as a desiccant insecticide had a synergistic effect on the adults of *Rhyzopertha dominica* (Fabricius, 1792) (Lord, 2001).

The objective of this study was to assess the effectiveness of entomopathogenic fungi (*B. bassiana*, *I. farinosa*, *I. fumosorosea*, *L. muscarium*, *P. farinosus*), collected from different locations and infected insects, against *T. confusum* adults under laboratory conditions. A Mycotal extract of *L. muscarium* was used as a positive control.

Materials and methods

Rearing of test insect

Tribolium confusum adults used as test insects were obtained from a laboratory culture maintained at the Plant Protection Department, Agricultural Faculty, Ataturk University, Erzurum, Turkey, which were initially collected from hard wheat (cv. Seval in grain storage) in 2016 and were reared on cracked wheat grains. The adults were kept in cracked wheat grain under laboratory conditions in cloth mesh covered plastic pots (15 cm diameter, 20 cm high) until used in the experiments as newly emerged adults with mixed sex. Each experiment was conducted with three replicates and 25 adults were used for each replicate. The adults were fed with wheat grains in plastic Petri dishes (9 cm) during laboratory bioassay of entomopathogenic fungi.

Entomopathogenic isolates and preparation

Seven entomopathogenic fungi isolates (*Beauveria bassiana* (ARSEF-4984); *Paecilomyces farinosus* (ARSEF-2538); *Isaria fumosorosea* (ARSEF-4501); *Isaria farinosa* (ARSEF-3580); *Lecanicillium muscarium* (ARSEF-972 and ARSEF-5128), Mycotal extract of *Lecanicillium muscarium* (as positive control) and distilled sterile water with Tween 20 (as negative control) were tested against *T. confusum* adults in this study. Fungal isolates were cultivated in potato dextrose agar (PDA, Oxoid, CM0139) medium at 25°C for two weeks before being used to spray *T. confusum* adults. Conidia harvested from 14-day-old cultures were thoroughly mixed in 3 mL distilled sterile water with 12 µL Tween 20 in screw capped bottles. The suspensions were sieved, diluted and 1 mL sprayed on each replicate consisting of the insects, wheat grains and filter paper in Petri dishes. The sprayed Petri dishes were incubated at 25°C and the alive and dead adults were counted every 48 h for 10 days.

Bioassays

Fungal entomopathogenic treatments were applied at 1×10^5 and 1×10^7 conidia/mL sterile distilled water using PET plastic spray bottles. In each Petri dish, 25 adults of *T. confusum* were fed by wheat grains (30 wheat grains/dish) and incubated at 25±1°C and 75±1% RH in a completely dark growth chamber. The mortality of the adults was evaluated at 48-h intervals for 10 days.

Statistical analysis

The differences among insecticidal activities of the seven tested entomopathogenic fungi isolates were determined according to analysis of variance using the SPSS 17.0 software package. Duncan's test was used for comparison between means. The significance of differences between means were determined at $p < 0.05$.

Results

The seven entomopathogenic fungi isolates were tested against *T. confusum* at two concentrations (1×10^5 and 1×10^7 conidia/mL) and compared with controls. The mortality of *T. confusum* adults varied from 34.6% to 100% 10 days after treatment (Table 1). The mortalities of *T. confusum* adults for positive control (Mycotal extract of *L. muscarium*) and negative control (distilled sterile water with Tween 20) were 34.6% and 4% 10 days after treatment, respectively. There were not significant differences in mortality of *T. confusum* adults 6, 8 and 10 days after treatment. The highest mortalities of *T. confusum* adults were observed for *P. farinosus* (ARSEF-2538) with 100% mortality at 1×10^7 conidia/mL and *I. farinosa* (ARSEF-3580) with 97.3% mortality at 1×10^7 conidia/mL, followed by *I. fumosorosea* (ARSEF-4501), *B. bassiana* (ARSEF-4984) and *L. muscarium* (ARSEF-5128) with 94.6% mortality (Table 1). The lowest mortalities were observed for *I. farinosa* (ARSEF-3580) with 37.3% mortality at 1×10^5 conidia/mL and the

positive control with 34.6% mortality. The mortality of *T. confusum* adults differed between the different spore concentrations for one isolate only, *I. farinosa* (ARSEF-3580). However, the mortality rates at 1×10^5 conidia/mL were generally lower than those at 1×10^7 conidia/mL. All the entomopathogenic fungi caused high levels of mortality of *T. confusum* adults (Table 1).

More than 80% mortality of *T. confusum* adults was observed with 1×10^5 conidia/mL of *P. farinosus* (ARSEF-2538), *B. bassiana* (ARSEF-4984), *L. muscarium* (ARSEF-5180) and *L. muscarium* (ARSEF-972) (Figure 1), while *P. farinosus* (PAF-2538), *I. fumosorosea* (ARSEF-4501), *B. bassiana* (ARSEF-4984), *I. farinosa* (ARSEF-3580) and *L. muscarium* (ARSEF-972) at 1×10^7 conidia/mL caused more than 90% mortality of *T. confusum* adults (Figure 2).

Table 1. Mortality of *Tribolium confusum* exposed to two concentrations of six entomopathogenic fungi isolates and controls over 10 days from treatment (DAT)

Entomopathogenic fungi treatment	Dose	Cumulative mortality (%)[a*]				
		2 DAT	4 DAT	6 DAT	8 DAT	10 DAT
Paecilomyces farinosus (ARSEF-2538)	$1x10^7$	69.3 ± 11.3 cba	73.3 ± 11.3 a	92.0 ± 6.11 a	98.6 ± 1.33 a	100 ± 0.0 a
	$1x10^5$	50.6 ± 15.3 dc	70.6 ± 16.2 a	78.6 ± 9.33 a	81.3 ± 9.61 ba	85.3 ± 7.42 ba
Isaria fumosorosea (ARSEF-4501)	$1x10^7$	86.6 ± 13.3 a	90.6 ± 9.33 a	90.6 ± 9.33 a	93.3 ± 6.66 ba	94.6 ± 5.33 a
	$1x10^5$	66.6 ± 10.6 cba	68.0 ± 10.0 a	70.6 ± 8.74 a	72.0 ± 8.0 cb	72.0 ± 8.0 cb
Beauveria bassiana (ARSEF-4984)	$1x10^7$	76.0 ± 14.4 cba	90.6 ± 3.52 a	92.0 ± 4.00 a	94.6 ± 5.33 ba	100 ± 0.0 a
	$1x10^5$	69.3 ± 17.3 cba	78.6 ± 13.5 a	81.3 ± 12.7 a	81.3 ± 12.7 ba	89.3 ± 8.74 ba
Lecanicillium muscarium (ARSEF-972)	$1x10^7$	81.3 ± 10.6 ba	88.0 ± 10.0 a	88.0 ± 10.0 a	89.3 ± 10.6 ba	90.6 ± 9.33 ba
	$1x10^5$	82.6 ± 11.8 ba	88.0 ± 12.0 a	88.0 ± 12.0 a	88.0 ± 10.0 ba	88.0 ± 10.0 ba
Isaria farinosa (ARSEF-3580)	$1x10^7$	90.6 ± 1.33 a	92.0 ± 0.0 a	93.3 ± 1.33 a	94.6 ± 1.33 ba	97.3 ± 2.66 a
	$1x10^5$	10.6 ± 2.66 fe	13.3 ± 2.66 c	30.6 ± 14.8 cb	90.6 ± 1.33 a	37.3 ± 15.3 d
Lecanicillium muscarium (ARSEF-5128)	$1x10^7$	58.6 ± 16.7 cb	85.3 ± 12.8 a	89.3 ± 5.81 a	90.6 ± 1.33 a	94.6 ± 5.33 a
	$1x10^5$	28.0 ± 2.30 ed	68.0 ± 14.0 a	76.0 ± 10.0 a	81.3 ± 7.42 ba	86.6 ± 7.05 ba
Positive control (*L. muscarium* extract)	$1x10^7$	22.6 ± 3.52 fe	38.6 ± 14.8 b	49.3 ± 11.3 b	58.6 ± 13.10 c	62.6 ± 13.9 c
	$1x10^5$	6.66 ± 2.66 fe	17.3 ± 2.66 cb	26.6 ± 7.05 c	29.3 ± 7.42 d	34.6 ± 7.05 d
Negative control (Tween20+sterile water)	-	0.0 ± 0.0 f	0.0 ± 0.0 c	1.33 ± 1.11 d	3.5 ± 0.78 e	4.0 ± 0.0 e

[a] Mean ± SE of three replicates, each consisting of 25 adults.
[*] Values followed by different letters in the same column differ significantly at $p < 0.05$ according to Duncan Multiple test.

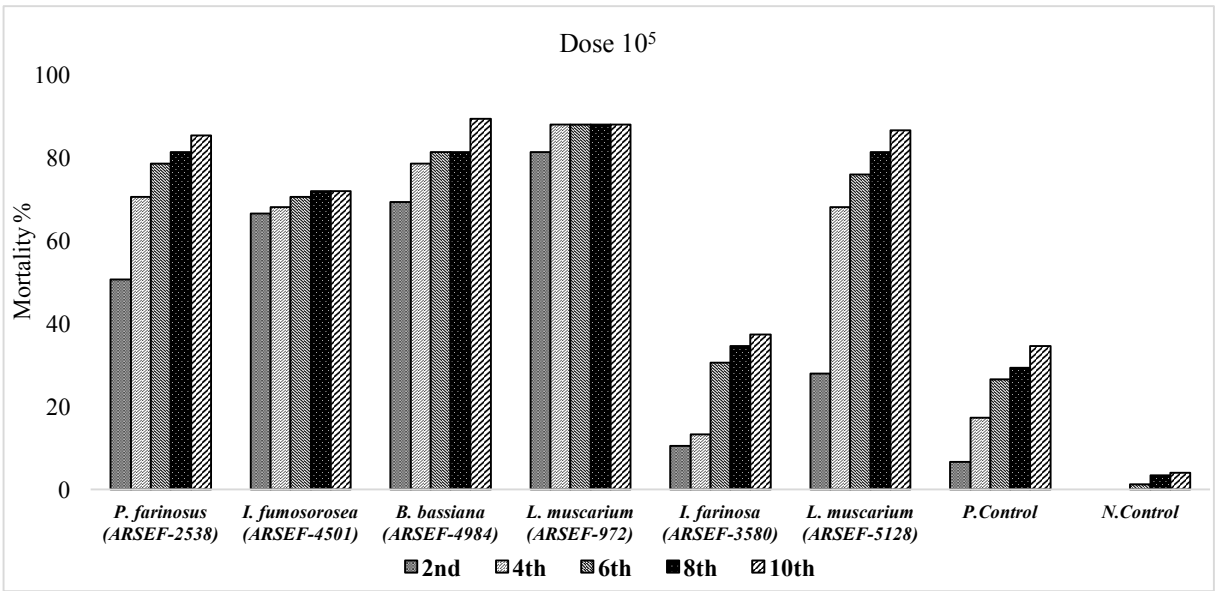

Figure 1. Mortality (%) of *Tribolium confusum* adults exposed to different entomopathogenic fungi isolates at 1×10^5 conidia/mL 2, 4, 6, 8 and 10 days of treatment (ANOVA; $p < 0.05$). The negative control was sterile distilled water with Tween 20 and the positive control a Mycotal extract of *Lecanicillium muscarium*.

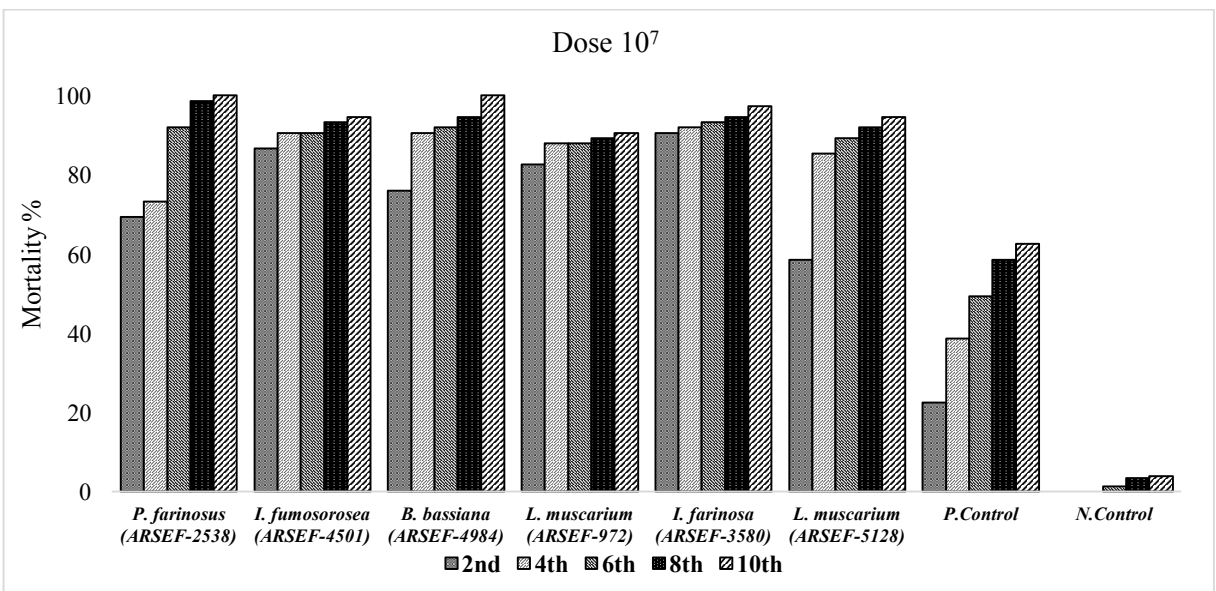

Figure 2. Mortality (%) of *Tribolium confusum* exposed to different entomopathogenic fungi isolates at 1×10^7 conidia/mL 2, 4, 6, 8 and 10 days of treatment (ANOVA test; $p < 0.05$). The negative control was sterile distilled water with Tween 20 and the positive control a Mycotal extract of *Lecanicillium muscarium*.

Discussion

This study determined the mortality of *T. confusum* adults 10 days after exposure to five species of different entomopathogenic fungi. The pathogenicity of *B. bassiana*, *P. farinosus*, *I. fumosorosea*, *I. farinosa*, *L. muscarium* (2 isolates) to beetles was demonstrated by spraying *T. confusum* adults with conidia under laboratory conditions. Mortality of *T. confusum* adults was high, ranging from 37% to 100% across the different entomopathogenic fungi. Specifically, the adult mortalities were 37% with *I. farinosa* (ARSEF-3580) and 89.3% with *B. bassiana* (ARSEF-4984) at $1x10^5$ conidia/mL, and 90.6% with *L. muscarium* (ARSEF-972) and 100% with *B. bassiana* (ARSEF-4984) and *P. farinosus* (ARSEF-2538) at $1x10^7$ conidia/mL.

Many studies indicate that entomopathogens which occupy plant tissues and insects have the potential to interact with insect pest in diverse ways. Entomopathogenic fungi may produce conidia on the plants, where they may contact insects. The fungal metabolites via consumption of plant materials or on the leaf surfaces have the potential to control pest insects. Thus, the role of entomopathogenic fungi as biological control agents in pest management requires further consideration. Inclusion of entomopathogens in IPM appears to be an obvious approach to take advantage of the potential of these fungi. While a number of questions remain to be clarified, published research has demonstrated the potential for the use of entomopathogens in IPM (Padin et al., 1997; Barra et al., 2013).

Recently the use of fungal entomopathogens against grain pests has been gained increasing attention throughout the world and researchers continue to seek highly pathogenic fungal isolates for controlling stored-product insects. In this regard, *Tribolium* species appear as a particularly good candidate for biocontrol by entomopathogenic fungi as was indicated by the survey of Wakil et al., (2014). *Metarhizium anisopliae* inhibited *Sitophilus oryzae* (L., 1763) (Coleoptera: Dryophthoridae) by 73.3% to 86.7% (Batta, 2004). Padin et al. (2002) investigated the insecticidal effects of *B. bassiana* on *T. castaneum, Acanthoscelides obtectus* (Say, 1831) (Coleoptera: Chrysomelidae) and *S. oryzae* by exposing pest-infested wheat and bean seeds to conidia of *B. bassiana* over a long period. In that study, *S. oryzae* was significantly affected from *B. bassiana*, but the other species were not significantly affected after four months. In the present study, *B. bassiana* (ARSEF-4984) had up to 94% mortality four days after treatments with no subsequent increase in mortality increase, which may indicate a rapid decline in efficiency of *B. bassiana* conidia. Contrary to current findings, Rice & Cogburn (1999), recorded a lower efficiency with another *B. bassiana* isolate (22292A); only 31.5% mortality, of *T. castaneum* adult was achieved on 14 days after treatment. Although these differences may be attributed to differences in methods used, there is also likely variation in pathogenicity of different isolates of the fungus was a contributing factor (Zettler, 1991).

Based on the findings of the present study, all isolates performed better at higher dosage 10 days after treatment causing mortality of over 90%. The increasing trend observed in mortalities (with the exception of *B. bassiana*) throughout the experiment is also considered as a good indication of preserved pathogenicity. Among the isolates tested, *I. farinosa* (ARSEF-3580) and *I. fumosorosea* (ARSEF-4501) particularly gave high mortalities from the beginning of experiment. Similarly, *P. farinosus* gave a consistent increase in mortality and had kill all adults by 10 days after treatment. In conclusion, based on their high pathogenicity, these three isolates are considered as good candidates for biocontrol agents against *T. confusum* adults.

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