https://doi.org/10.17221/131/2023-PPS

Isolation, molecular identification, and efficacy of indigenous entomopathogenic fungus isolates against *Tribolium confusum* (Coleoptera: Tenebrionidae) larvae and adults

Koko Dwi Sutanto[1], Mureed Husain[1], Khawaja Ghulam Rasool[1], Mohammed Ibrahim Elbashir[2], Richard W. Mankin[3], Abdalsalam Osman Omar[1], Abdulrahman Saad Aldawood[1]*

[1]*Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia*
[2]*Department of Biopesticides and Biofertilizers – Environment and Natural Resources Research Institute, Khartoum, Sudan*
[3]*United States Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural and Veterinary Entomology, Gainesville, USA*

**Corresponding author: ksutanto@ksu.edu.sa*

Citation: Sutanto K.D., Husain M., Rasool K.G., Elbashir M.I., Mankin R.W., Osman A.O., Aldawood A.S. (2024): Isolation, molecular identification, and efficacy of indigenous entomopathogenic fungus isolates against *Tribolium confusum* (Coleoptera: Tenebrionidae) larvae and adults. Plant Protect. Sci., 60: 258–266.

Abstract: Entomopathogenic fungi (EPF) represent the future of pest control, as they have the capacity to induce serious infections in insects, trigger epizootics, and effectively manage insect populations. The study aimed to identify and test indigenous EPF isolates against *Tribolium confusum* using soil samples from Northwestern and central Saudi Arabia. *Galleria mellonella* larvae were used as insect bait to collect EPF from soil samples from date palm groves, fruit groves, and maise fields. Only 27.3% of soil samples tested positive for palm grove-originating EPFs. Polymerase chain reaction (PCR) identified the cultured fungi as *Metarhizium anisopliae* and *Beauveria bassiana*. *Tribolium confusum* pathogenicity was assessed using nine local fungal isolates. Different fungus isolates were tested for their pathogenicity on larval and adult *T. confusum*. The results showed that *M. anisopliae* MaSA-2, MaSA-3, and MaSA-4 had infection rates of 85%, 85%, and 75%, respectively, in last-instar *T. confusum* larvae and 5%, 10%, and 20% in adults within seven days. On the other hand, *B. bassiana* BbSA-4 showed a 100% infection rate in *T. confusum* larvae, while BbSA-5, BbSA-6, BbSA-7, BbSA-8, and BbSA-9 had infection rates of 70%, 80%, 80%, 75%, and 95%, respectively. In terms of adult mortality rates, *B. bassiana* BbSA-4, BbSA-5, BbSA-6, BbSA-7, BbSA-8, and BbSA-9 had rates of 25%, 35%, 35%, 20%, 20%, and 40%, respectively. The research findings show that indigenous *B. bassiana* and *M. anisopliae* fungus isolates successfully eliminated *T. confusum* larvae, although their effectiveness against adults was limited.

Keywords: biological control; EPF; *Beauveria bassiana*; *Metarhizium anisopliae*; pathogenicity

Tribolium confusum Jacquelin du Val 1863 (Coleoptera: Tenebrionidae) is a prevalent pest that affects stored foodstuffs globally (Athanassiou et al. 2016; Baldwin & Fasulo 2003). *Tribolium con-*

Supported by the Researchers Supporting Project, King Saud University, Riyadh, Saudi Arabia, Project No. RSP2024R107.

https://doi.org/10.17221/131/2023-PPS

fusum is an important stored grain pest and has been extensively studied to develop more effective pest control methods (Campbell et al. 2022). Using chemical pesticides is the standard practice for reducing insect populations in warehouses (Stejskal et al. 2015). Different countries use chemical control measures like deltamethrin and phosphine as fumigants to mitigate the damage caused by stored-grain insects during warmer months (Mantzoukas et al. 2022). The majority of grain storage facilities use fumigation for pest control (Canhilal 2016). Unfortunately, this occurrence has resulted in environmental contamination and building resilience in insect pests (Rajendran 2020). Therefore, scientists have been searching for control strategies that are less harmful to the environment and have no residues that are toxic to humans (Subramanyam & Hagstrum 2012). The review study also gives an overview of old, innovative, and developing approaches for controlling urban pests in the stored-product and food industries. However, bioprotection, which covers non-living nature-based compounds and live biocontrol agents, is proposed to describe naturally-based pest management systems (Stejskal et al. 2021).

Entomopathogenic fungi (EPF) are microorganisms that are harmless to non-targeted animals and environmentally safe (Zimmermann 2007), and they are currently being explored as biopesticides and potential alternatives to chemical pesticides (Mahankuda & Bhatt 2019). By germinating in insects' cuticles, fungal spores lead to the insects' demise through lack of nourishment, fungal toxins, and organ dysfunction (Islam et al. 2021). Numerous studies have examined the use of these pathogens to eradicate insects that infest stored products while ensuring the quality of the products themselves (Batta & Kavallieratos 2018; Mantzoukas et al. 2020). Two entomopathogenic fungi, *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff) Sorokīn is vital in controlling beetle pests in stored products (Mantzoukas et al. 2023). Their implementation in food storage facilities prevents economic losses and promotes an environmentally friendly approach. Recent research has made significant strides in identifying highly virulent fungal strains for crop storage, thus expanding their potential for future use (Perinotto et al. 2012; Saldarriaga Ausique et al. 2017). Their effectiveness against other pests, such as *Sitophilus oryzae* has been underscored by a recent study (Rumbos & Athanassiou 2017; Batta & Kavallieratos 2018).

Researchers typically study EPF possibilities for specific insect pests by collecting soil samples from their natural habitats (Asensio et al. 2003), and their pathogenicity varies depending on host infection time, incubation period, and fungus development acceleration (Shah & Pell 2003). Unfortunately, there is a lack of data on the effectiveness of indigenous EPFs against important stored pests such as *Tribolium confusum*. Our objective was to assess the efficacy of nine EPF isolates indigenous to Saudi Arabia by evaluating the pathogenicity of each isolate against *T. confusum* larvae and adults.

MATERIAL AND METHODS

Confused flour beetle rearing

The larvae of the *Tribolium confusum*, commonly known as the confused flour beetle, were obtained from the insect-rearing laboratory at King Saud University. They were reared on ground maise (Fardisi 2015) and maintained in an incubator (Steridium, Germany) with precise conditions, including a temperature of 25 ± 2 °C, a relative humidity (RH) of 85 ± 2%, and a light-darkness cycle of 12 h each. After a month, approximately one hundred adult insects in a 1:1 ratio were transferred to new plastic containers, and the larvae were separated from the food using sieve plates designed to capture the final-stage larvae. These larvae were then utilised for pathogenicity testing.

Entomopathogenic fungus isolation and identification

Soil sampling. There were 33 farm soil samples from Tabouk, Madinah, Al-Jouf, and Riyadh, Saudi Arabia (Table 1). Trees and crops grown on these farms include date palms (*Phoenix dactylifera* L.), olives (*Olea europaea* L.), mangos (*Mangifera indica* L.), lemons (*Citrus limon* L.), pomegranates (*Punica granatum* L.), and maise (*Zea mays* L.). The top 20 centimetres of soil were sampled for each 1 kg sample (Pérez-González et al. 2014; Shanker et al. 2023). For further study, 150 g samples were collected using shovels by hand into watertight plastic bags and transported to the lab, where they were placed in labelled polypropylene soil containers (15 × 9.5 × 7.5 cm) for EPF isolation testing.

https://doi.org/10.17221/131/2023-PPS

Table 1. Detailed information about collecting soil samples to explore native entomopathogenic fungi species from different regions in the Kingdom of Saudi Arabia

No.	Province	City	Vegetation type	Soil temp./RH	GPS
1	Al Jouf	Qariyat	date palm	28 °C/42%	31°17'19.6"N 37°22'20.2"E
2		Qariyat	date palm	27 °C/42%	31°17'52.6"N 37°21'58.2"E
3		Basita	olive	28 °C/48%	30°15'50.8"N 38°14'27.9"E
4		Basita	olive	27 °C/57%	30°15'50.8"N 38°14'27.9"E
5		Basita	olive	26 °C/60%	30°9'38.2"N 38°20'13.6"E
6		Qariyat	date palm	25 °C/47%	31°16'46.2"N 37°22'20.2"E
7		Basita	maize	26 °C/50%	30°15'50.8"N 38°14'27.9"E
8		Qariyat	olive	28 °C/40%	31°16'22.1"N 37°21'31"E
9		Qariyat	olive	28 °C/54%	31°16'39.7"N 37°21'23.1"E
10	Tabuk	Qaryat Ain	pomegranate	28 °C/47%	27°38'16.5"N 36°49'14.2"E
11		An Nashifah	date palm	28 °C/60%	27°1'6.6"N 37°17'26.2"E
12		Qaryat Ain	date palm	29 °C/45%	27°38'16.5"N 36°49'14.2"E
13		Qaryat Ain	date palm	27 °C/45%	27°38'16.4"N 36°49'9.4"E
14		Qaryat Ain	date palm	28 °C/59%	27°38'16.5"N 36°49'14.2"E
15		An Nashifah	date palm	29 °C/60%	27°1'10.6"N 37°16'44.4"E
16		Al Wajh	date palm	28 °C/60%	26°14'22.9"N 36°28'53.4"E
17		Al Umlaj	date palm	27 °C/40%	25°05'17.2"N 37°20'6.3"E
18		Al Umlaj	date palm	28 °C/50%	25°01'56.9"N 37°20'6.6"E
19		Al Umlaj	mango	27 °C/50%	25°05'11.8"N 37°20'13.1"E
20		Al Umlaj	mango	28 °C/47%	25°05'6.1"N 37°20'16.4"E
21		Al Umlaj	mango	27 °C/50%	25°05'6.1"N 37°20'16.4"E
22		Al Umlaj	lemon	28 °C/60%	25°5'24.4"N 37°19'32.2"E
23		Al Wajah	date palm	28 °C/60%	26°15'11.1"N 36°31'15.7"E
24	Madinah	As Sovirqiya	date palm	26 °C/60%	23°20'43.4"N 40°18'30.1"E
25		Al Faraa	date palm	25 °C/55%	24°58'8.2"N 38°02'54.5"E
26		As Sovirqiya	date palm	28 °C/40%	23°19'23.3"N 40°14'55.2"E
27		As Sovirqiya	date palm	30 °C/43%	23°19'32.6"N 40°16'51.7"E
28		As Sovirqiya	date palm	29 °C/47%	23°20'43.4"N 40°18'30.1"E
29		Bir al Mashi	date palm	30 °C/52%	24°6'37.9"N 39°34'31.9"E
30		As Sovirqiya	date palm	27 °C/45%	23°19'23.3"N 40°14'55.2"E
31		As Sovirqiya	date palm	26 °C/47%	23°18'59.6"N 40°15'21.6"E
32	Riyadh	Huraymila	date palm	30 °C/40%	25°07'277"N 46°06'970"E
33		Huraymila	date palm	30 °C/40%	25°07'307"N 46°06'110"E

Isolation of entomopathogenic fungi. We used baiting techniques to collect EPFs from soil containers and closely monitored *G. mellonella* larvae daily for any signs of death (Zimmermann 2007). Any dead larvae were cleaned, sterilised, and placed on moistened filter paper in Petri dishes (Whatman No. 1, UK). The samples demonstrating promising results were meticulously analysed, and the infections induced by EPF were comprehensively evaluated. Subsequently, PDA cultures were incubated using a Stuart SI500 incubator (Tequipment, Long Branch, New Jersey) at a temperature of 25 ± 2 °C and a relative humidity (RH) of 85 ± 2% to elucidate the molecular profile of the EPF.

Molecular identification of fungi

Extraction of DNA and amplification by polymerase chain reaction (PCR). Fungus DNA was extracted in lysis buffer (Serna-Domínguez et al. 2018), heated to 94 °C for 60 min, and then precipi-

https://doi.org/10.17221/131/2023-PPS

Table 2. Percentages of soil samples from which entomopathogenic fungi were recovered using *Galleria mellonella* bait

Province	No. of collected samples	No. of samples with entomopathogenic fungi		% samples containing:		
		Beauveria bassiana	*Metarhizium anisopliae*	*B. bassiana*	*M. anisopliae*	Either *spp.*
Madinah	9	5	0	55.6	0.0	55.6
Tabouk	13	0	2	0.0	15.4	15.3
Al-Jouf	9	1	1	11.1	11.1	22.2
Riyadh	2	0	0	0.0	0.0	0.0
Total	33	6	3	18.2	9.1	27.3

tated in alcohol. PCR amplification was conducted to determine the fungus species present. A general primer of the internal transcribed spacer (ITS) region, e.g., ITS5 Forwards (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 Reverse (3' TCCTCCGCTTATTGATATGC 5') (Shanker et al. 2023), was used to amplify a ribosomal DNA fragment for further study.

DNA sequencing of fungus isolates. The PCR products of each fungus isolate were processed by Macrogen, Inc. (Seoul, Republic of Korea) to obtain the sequenced data. The Basic Local Alignment Search Tool (BLAST) verified the identification of the fungus sequence. All fungus sequences were submitted to the National Centre for Biotechnology Information (NCBI).

Pathogenicity test on *Tribolium confusum* larvae and adults

Pathogenicity was assessed by employing dry conidia inoculation (approximately 1×10^7 conidia/mL). Three replicates of five adult and late-stage larvae of *T. confusum* were individually exposed to the fungus inoculum for 10 min and then placed in disposable Petri dishes with 5 g of artificial diet. Three replicates were also performed for the control group, each consisting of five *T. confusum* larvae and adults that were not inoculated. Mortality of *T. confusum* larvae and adults was monitored daily for seven days post-inoculation, and the mortality percentage was recorded.

Statistical analysis

The percentages of *T. confusum* larval and adult mortality were analysed using a one-way analysis of variance (ANOVA) in the SAS program (version 9.2). The results were then subjected to Tukey's Test for Statistical Significance to determine if there were any significant differences in the means, with a significance level set at $P < 0.05$.

RESULTS

Entomopathogenic fungi isolation and identification

Only 27.3% of soil samples had EPF (Table 2). *Beauveria bassiana* and *Metarhizium anisopliae* isolates were detected at Madinah and Al Jouf however only *M. anisopliae* was from Tabouk. Madinah and Al Jouf had the highest EPF rates, notably *B. bassiana* and *M. anisopliae*. Madinah had 55.6% soil samples positive for both EPFs, indicating a substantial occurrence of these fungi. Entomopathogenic fungi were found in 22.2% of Al Jouf samples. Tabouk has less EPF than Madinah and Al Jouf. Only *M. anisopliae* was found in Tabouk soil samples at 15.3%. Entomopathogenic fungi were not detected in Riyadh soil samples, indicating that the environment or other factors may not favour EPF availability. The percentage of soil samples containing EPF in Saudi Arabia varied by region.

Using insect baiting with *G. mellonella*, we isolated and identified two EPF species using PCR. Nine EPF isolates were detected using ITS 4 and ITS 5 primers. The amplified ITS region genetic sequences of each fungal isolate were revealed by sequencing. The fungal species closely matched public database reference sequences. These isolates' DNA sequences were thoroughly analysed and compared to NCBI sequences. Table 3 lists all GenBank-submitted EPF sequences.

Soil samples from Madinah, Saudi Arabia, show promise for EPF recovery. Date palm grows in Madinah positive soil tests. Entomopathogenic fungi have been found in recoverable soil samples from Al Jouf and Tabouk, similar to Madinah. These samples have different vegetation than Madinah. Olive trees dominate Al Jouf and Tabouk. The EPF were almost mainly discovered in soil samples from date palm tree farming regions, primarily in Madinah. *Metarhizium anisopliae* isolates were also recov-

https://doi.org/10.17221/131/2023-PPS

Table 3. Accession numbers for fungus isolate sequences

Species	Isolate code	Accession No.
Beauveria bassiana	BbSA-4	OQ630462
	BbSA-5	OQ621412
	BbSA-6	OQ630470
	BbSA-7	OQ626223
	BbSA-8	OQ625860
	BbSA-9	OQ626217
Metarhizium anisopliae	MaSA-2	OQ625883
	MaSA-3	OQ625886
	MaSA-4	OQ641237

ered from date palm and olive tree cultivation areas but at different sites. None of the other soil samples from crops — mango, pomegranate, lemon, or maise had EPFs availability. Table 4 shows positive EPF from soil samples of different vegetation kinds.

Pathogenicity of entomopathogenic fungi on *Tribolium confusum* larvae

Beauveria bassiana and Metarhizium anisopliae pathogenicity against *T. confusum* late-instar larvae were shown in Table 5. After seven days, nine different isolates of *M. anisopliae* (MaSA-2, MaSA-3, and MaSA-4) and *B. bassiana* (BbSA-4, BbSA-5, BbSA-6, BbSA-7, BbSA-8, and BbSA-9) induced mortality rates of 85, 85, 75, 100, 70, 80, 80, 95, and 95% in *T. confusum* larvae, respectively. Compared to other fungus isolates, *B. bassiana* has relatively high larval mortality percentages of 70–100%, while *M. anisopliae* have 75–95%. There was a high incidence of mortality for *T. confusum* larvae when exposed to dry conidial fungi, and both *B. bassiana*, as shown in Figure 1, and *M. anisopliae*, as shown in Figure 2, showed fungus infection signs.

Table 5. Mortality percentages (mean ± SE) of *Tribolium confusum* larvae caused by infection with *Metarhizium anisopliae* and *Beauveria bassiana*

Treatment	Mortality (%)	Statistical analysis
BbSA-4	100 ± 0^{a}	F = 18.2, DF = 9, 30; P < 0 .0001
BbSA-5	70 ± 5.7^{c}	
BbSA-6	80 ± 8.1^{bc}	
BbSA-7	80 ± 8.1^{bc}	
BbSA-8	95 ± 5^{ab}	
BbSA-9	95 ± 5^{ab}	
MaSA-2	85 ± 5^{abc}	
MaSA-3	85 ± 5^{abc}	
MaSA-4	75 ± 5^{c}	
Control	15 ± 5^{d}	

Means followed by the same letters within a column do not differ significantly (P < 0.05)

Pathogenicity of entomopathogenic fungi on *Tribolium confusum* adults

Beauveria bassiana and *Metarhizium anisopliae* pathogenicity against adults is shown in Table 6. Nine *M. anisopliae* (MaSA-2, MaSA-3, and MaSA-4) and *B. bassiana* (BbSA-4, BbSA-5, BbSA-6, BbSA-7,

Table 4. Positive soil samples for entomopathogenic fungi recovery in different provinces with their respective vegetation types

Province	Vegetation type	No. of soil sample	No. of positive soil sample	Fungus isolate code
Madinah	date palm	9	1	BbSA-4
			1	BbSA-6
			1	BbSA-7
			1	BbSA-8
			1	BbSA-9
Tabouk	date palm	7	1	MaSA-3
			1	MaSA-4
	lemon	1	–	–
	mango	4	–	–
	pomegranate	1	–	–
Al-Jouf	date palm	3	1	BbSA-5
	olive	5	1	MaSA-2
	maise	1	–	–
Riyadh	date palm	2	–	–

https://doi.org/10.17221/131/2023-PPS

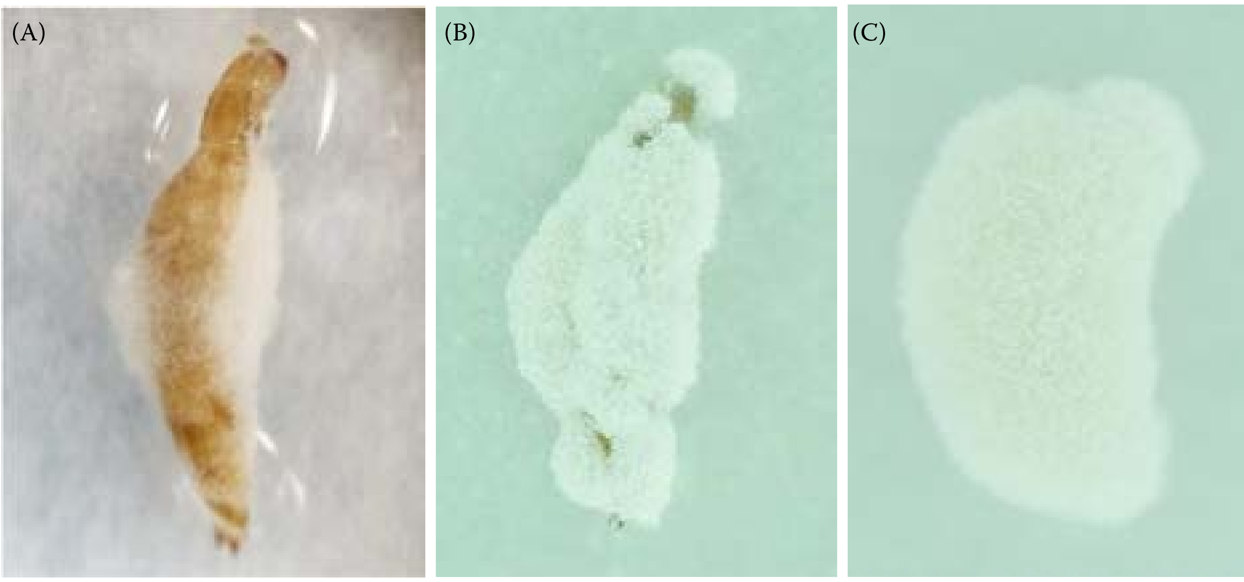

Figure 1. Visualisation of the growth of the fungus *Beauveria bassiana* on larvae of *Tribolium confusum* after 4 days (A), 5 days (B), and 6 days (C) following larvae infection

BbSA-8, and BbSA-9) isolates caused 5, 10, and 20% mortality and 25, 35, 35, 20, 20, and 40% mortality after seven days. Compared to other fungi isolates, *M. anisopliae* kills below 25% of *T. confusum* adults and 50% of *B. bassiana*. Adult *T. confusum* mortality was lower. *B. bassiana* (BbSA-9) killed more adults than *M. anisopliae* isolates. Adult *T. confusum* did not change colour after infection, but sporulation occurred several days later (Figure 3).

DISCUSSION

Based on previous studies on the isolation of EPF, a significant number of microbiologists have successfully recovered members of the *Beauveria* and *Metarhizium* genera by utilising the greater wax moth as a lure (Keller et al. 2003). Moreover, in this study, EPF isolates were found in the soil of different types of vegetation, including date palms (*Phoenix dactylifera* L.) and olives (*Olea europaea* L.). At the same time, in other plant areas, such as maise (*Zea mays* L.), mangos (*Mangifera indica* L.), and pomegranates (*Punica granatum* L.) were not detected.

The results of our study revealed the presence of six isolates of *Beauveria bassiana* and three isolates of *Metarhizium anisopliae* fungus within the EPF. Many investigations have examined soil from different countries for EPF isolates. Insect baiting and soil dilution revealed *Beauveria* (26%) and

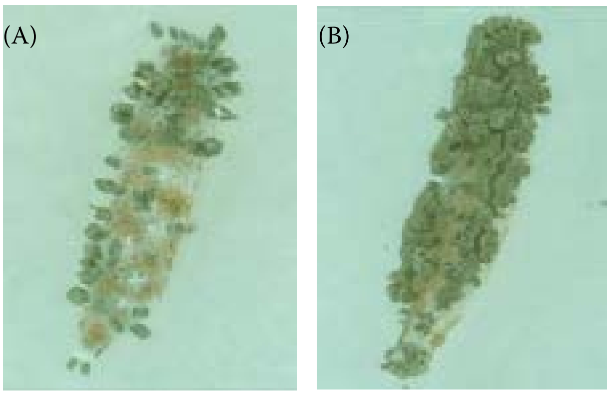

Figure 2. Visualisation of the growth of the fungus *Metarhizium anisopliae* on larvae of *Tribolium confusum* after 4 days (A) and 6 days (B) following larvae infection

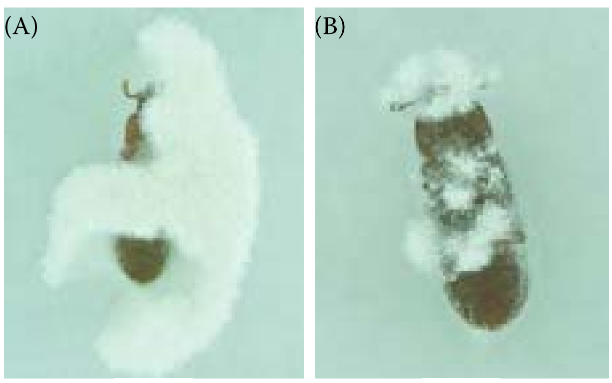

Figure 3. Visualisation of the growth of the *Beauveria bassiana* (A) and *Metarhizium anisopliae* (B) fungi on adult *Tribolium confusum* after infection

https://doi.org/10.17221/131/2023-PPS

Metarhizium (33%) EPFs from 240 vineyard soils in Australia (Korosi et al. 2019). Certain Mexican soil samples contained *Beauveria* (112) and *Metarhizium* (9) (Pérez-González et al. 2014). Approximately 96% of the analysed soil samples from specific regions in Canada were found to contain species of *M. anisopliae*. The remaining samples exhibited the presence of various species belonging to the Beauveria genus, such as *B. brongiartii* (Sacc.) Petch 1926, *B. bassiana*, *P. fumosoroseus* (Wize) A.H.S. Br. & G. Sm. 1957, and *Conidiobolus sp*. (Bref. 1884 em. Humber 1989). These findings were observed in 91% of the 266 soil samples (Keller et al. 2003). *Beauveria bassiana* and *Metarhizium anisopliae* were the most prevalent of 13 *Paecilomyces spp*. isolates. *Metarhizium anisopliae* dominated agricultural soils regardless of type or pH (Bidochka et al. 1998). Similarly, in Finland, *M. anisopliae* (15.6%), *B. bassiana* (19.8%), *Paecilomyces farinosus* (9.2%), and *Purpureocillium fumosoroseus* (Thom) Luangsa-ard, Hou-braken, Hywel-Jones & Samson (2011) (1%) were found in natural and agricultural soils from 590 insect-baited soil samples. Entomopathogenic fungi were found in 33.2% of soil samples, including 15 *B. pseudobassiana* (S.A. Rehner & R.A. Humber 2011), 12 *B. bassiana*, 11 *M. robertsii* (Metchnikoff) Sorokin (1883), and 13 *M. brunneum* Petch, 1935 (Gürlek et al. 2018).

Our study showed that indigenous EPF isolates killed a host of *T. confusum* larvae but few adults. According to previous studies on the pathogenicity of other insect pests, *B. bassiana*, through food contamination at different concentrations, killed *T. castaneum* (Herbst, 1797) at the adult stage by less than 6–8.3% and at the larval stage by 7.7–60% (Akbar et al. 2004). Entomopathogenic fungal virulence varies by species and strain, while rice weevil vulnerability changes by developmental stage. *Beauveria bassiana* is the most effective species, with adults having the highest resistance (Eski & Gezgin 2022). The study found that *B. bassiana*, *P. lilacinum*, and *Fusarium Sp.* Link (1809) at 10^9 conidia/mL killed adult rice weevils 100% after 14 days (Atmaca et al. 2022). Most indigenous EPF, like *B. bassiana*, were more effective for late-instar mealworm larvae and pupae (Eski & Gezgin 2022). Moisture may have affected *B. bassiana*-driven *T. confusum* adult mortality (Lord 2007).

Entomopathogenic fungi deployed high-pathogenicity fungus conidia formulations to treat stored grain pests (Batta & Kavallieratos 2018). Dry fungus spore formulation as powder may be the best option, as demonstrated by the efficacy of EPF in preventing stored grain pests (Rumbos & Athanassiou 2017). Pathogenicity and integration of EPFs with other storage pest management strategies require research on fungus-based pest management solutions, particularly conidia formulation (Mantzoukas et al. 2023). Entomopathogenic fungi formulation influences conidial viability, absorption, and mortality against stored-product insects (Rumbos & Athanassiou 2017). Easy application, better storage, and biocontrol efficacy are essential for a successful mycopesticide (Wraight et al. 2001). Fewer liquid formulations have been tried than other carriers (Batta 2016). Alternative formulations include talc-based dustable powder oil suspension and fat pellets (Smith et al. 1999). Invert emulsion formulations have higher mortality rates than aqueous conidial suspensions (Batta et al. 2010). According to other studies, combining *Metarhizium robertsii*, diatomaceous earth, and lambda-cyhalothrin was highly effective in protecting wheat against three pests. The combined treatment outperformed single treatments, lowering pest mortality rates (Wakil et al. 2024).

Additionally, the combination of diatomaceous earth and indoxacarb was identified as the most effective, leading to higher mortality and reduced progeny. Conversely, *Beauveria bassiana* showed the lowest mortality and the highest offspring. It was also found that diatomaceous earth and indoxacarb were superior against all species, ultimately reducing overall progeny (Wakil et al. 2023).

CONCLUSION

The study's findings reveal that indigenous *Beauveria bassiana* and *Metarhizium anisopliae* fungus isolates effectively killed *T. confusum* larvae but showed limited effectiveness against adults.

REFFERENCES

Akbar W., Lord J.C., Nechols J.R., Howard R.W. (2004): Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273–280.

Asensio L., Carbonell T., López-Jiménez J.A., López-Llorca L.V. (2003): Entomopathogenic fungi in soils from Alicante province. Spanish Journal of Agricultural Research, 3: 37–45.

Athanassiou C.G., Kavallieratos N.G., Campbell J.F. (2016): Capture of *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae) in floor traps: the effect of previous captures. Journal of Economic Entomology, 109: 461–466.

Atmaca S., Yüksel E., Canhilal R. (2022): Evaluation of Turkish isolates of entomopathogenic fungi against the adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Uluslararasi Tarım ve Yaban Hayati Bilimleri Dergisi, 6: 444–452.

Baldwin R., Fasulo T.R. (2003): Confused flour beetle, *Tribolium confusum* Jacquelin du Val (Insecta: Coleoptera: Tenebrionidae) and red flour beetle, *Tribolium castaneum* (Herbst) (Insecta: Coleoptera: Tenebrionidae). UF/IFAS Extension: Gainesville.

Batta Y., Murdoch G., Mansfield S. (2010): Investigations into the formulation and efficacy of entomopathogenic fungi against larvae of yellow mealworm ('*Tenebrio molitor*' L., Coleoptera: Tenebrionidae). General and Applied Entomology: The Journal of the Entomological Society of New South Wales, 39: 5–8.

Batta Y.A. (2016): Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology, 26: 1171–1183.

Batta Y.A., Kavallieratos N.G. (2018): The use of entomopathogenic fungi for the control of stored-grain insects. International Journal of Pest Management, 64: 77–87.

Bidochka M.J., Kasperski J.E., Wild G.A. (1998): Occurrence of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in soils from temperate and near-northern habitats. Canadian Journal of Botany, 76: 1198–1204.

Campbell J.F., Athanassiou C.G., Hagstrum D.W., Zhu K.Y. (2022): *Tribolium castaneum*: A model insect for fundamental and applied research. Annual Review of Entomology, 67: 347–365.

Canhilal R. (2016): The use of entomopathogens in the controlling of insect pests of stored product. Scientific Papers. Series A. Agronomy, 59: 235–240.

Eski A., Gezgin M.M. (2022): Susceptibility of different life stages of *Tenebrio molitor* (Coleoptera: Tenebrionidae) to indigenous entomopathogenic fungi. Journal of Stored Products Research, 98: 102008.

Fardisi M. (2015): Investigation of dried distillers grains with solubles (DDGS) susceptibility to red flour beetle (*Tribolium castaneum* (Herbst)) infestation [PhD. thesis]. Purdue University.

Gürlek S., Sevim A., Sezgin F.M., Sevim E. (2018): Isolation and characterisation of *Beauveria* and *Metarhizium spp.* from walnut fields and their pathogenicity against the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). Egyptian Journal of Biological Pest Control, 28: 1–6.

Islam W., Adnan M., Shabbir A., Naveed H., Abubakar Y.S., Qasim M., Tayyab M., Noman A., et al. (2021): Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests. Microbial Pathogenesis, 159: 105122.

Keller S., Kessler P., Schweizer C. (2003): Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae*. BioControl, 48: 307–319.

Korosi G.A., Wilson B.A., Powell K.S., Ash G.J., Reineke A., Savocchia S. (2019): Occurrence and diversity of entomopathogenic fungi (*Beauveria spp*. and *Metarhizium spp*.) in Australian vineyard soils. Journal of Invertebrate Pathology, 164: 69–77.

Lord J.C. (2007): Enhanced efficacy of *Beauveria bassiana* for red flour beetle with reduced moisture. Journal of Economic Entomology, 100: 1071–1074.

Mahankuda B., Bhatt B. (2019): Potentialities entomopathogenic fungus *Beauveria bassiana* as a biocontrol agent: A Review. Journal of Entomology and Zoology Studies, 7: 870–874.

Mantzoukas S., Kitsiou F., Natsiopoulos D., Eliopoulos P.A. (2022): Entomopathogenic fungi: interactions and applications. Encyclopedia, 2: 646–656.

Mantzoukas S., Lagogiannis I., Karmakolia K., Rodi A., Gazepi M., Eliopoulos P.A. (2020): The effect of grain type on virulence of entomopathogenic fungi against stored product pests. Applied Sciences, 10: 2970.

Mantzoukas S., Lagogiannis I., Kitsiou F., Eliopoulos P.A. (2023): Entomopathogenic action of wild fungal strains against stored product beetle pests. Insects, 14: 91.

Pérez-González V.H., Guzmán-Franco A.W., Alatorre-Rosas R., Hernández-López J., Hernández-López A., Carrillo-Benítez M.G., Baverstock J. (2014): Specific diversity of the entomopathogenic fungi *Beauveria* and *Metarhizium* in Mexican agricultural soils. Journal of Invertebrate Pathology, 119: 54–61.

Perinotto W., Angelo I., Golo P., Quinelato S., Camargo M., Sá F., Bittencourt V. (2012): Susceptibility of different populations of ticks to entomopathogenic fungi. Experimental parasitology, 130: 257–260.

Rajendran S. (2020): Insect Pest Management in Stored Products. Outlooks on Pest Management, 31: 24–35.

Rumbos C.I., Athanassiou C.G. (2017): Use of entomopathogenic fungi for the control of stored-product insects: can fungi protect durable commodities? Journal of Pest Science, 90: 839–854.

Saldarriaga Ausique J.J., D'Alessandro C.P., Conceschi M.R., Mascarin G.M., Delalibera Júnio, I. (2017): Efficacy of entomopathogenic fungi against adult *Diaphorina citri* from laboratory to field applications. Journal of Pest Science, 90: 947–960.

Serna-Domínguez M.G., Andrade-Michel G.Y., Arredondo-Bernal H.C., Gallou A. (2018): Two efficient methods for isolation of high-quality genomic DNA from entomopathogenic fungi. Journal of Microbiological Methods, 148: 55–63.

Shah P., Pell J. (2003): Entomopathogenic fungi as biological control agents. Applied Microbiology and Biotechnology, 61: 413–423.

Shanker R., Prajapati M.R., Singh R.P., Singh R., Singh J., Kumar P. (2023): Isolation, molecular characterisation of indigenous *Metarhizium anisopliae* (Metchnikoff) isolate, using ITS-5.8 s rDNA region, and its efficacy against the *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 33: 23.

Smith S.M., Moore D., Karanja L.W., Chandi E.A. (1999): Formulation of vegetable fat pellets with pheromone and *Beauveria bassiana* to control the larger grain borer, *Prostephanus truncatus* (Horn). Pesticide Science, 55: 711–718.

Stejskal V., Hubert J., Aulicky R., Kucerova Z. (2015): Overview of present and past and pest-associated risks in stored food and feed products: European perspective. Journal of Stored Products Research, 64: 122–132.

Stejskal V., Vendl T., Aulicky R., Athanassiou C. (2021): Synthetic and natural insecticides: Gas, liquid, gel and solid formulations for stored-product and food-industry pest control. Insects, 12: 590.

Subramanyam B., Hagstrum D.W. (eds) (2012): Alternatives to Pesticides in Stored-Product IPM. Springer Science & Business Media: Berlin/Heidelberg, Germany.

Wakil W., Kavallieratos N.G., Eleftheriadou N., Haider S.A., Qayyum M.A., Tahir M., Rasool K.G., Husain M., et al. (2024): A winning formula: sustainable control of three stored-product insects through paired combinations of entomopathogenic fungus, diatomaceous earth, and lambda-cyhalothrin. Environmental Science and Pollution Research, 31: 1–15.

Wakil W., Kavallieratos N.G., Nika E.P., Ali A., Yaseen T., Asrar M. (2023): Two are better than one: the combinations of *Beauveria bassiana*, diatomaceous earth, and indoxacarb as effective wheat protectants. Environmental Science and Pollution Research, 30: 41864–41877.

Wraight S., Jackson M., De Kock S. (2001): Production, stabilisation and formulation of fungal biocontrol agents. Fungi as Biocontrol Agents: Progress, Problems and Potential: 253. doi: 10.1079/9780851993560.0253

Zimmermann G. (2007): Review on safety of the entomopathogenic fungus *Metarhizium anisopliae*. Biocontrol Science and Technology, 17: 879–920.

Received: December 9, 2023
Accepted: April 8, 2024
Published online: June 5, 2024