Efficacy of *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium muscarium* against different stages of the flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

Sienaa Al-Zurfi[1*], Ali A. Kareem[1], Alaa T. Alamry[1], Zahraa Jawad Kadhem[1], Gordon Port[2] and Roy Sanderson[2]

[1]Plant Protection Dept. College of Agriculture University of Kerbala, Karbala, Iraq
[2] School of Natural and Environmental Sciences, Newcastle University, United Kingdom
***Corresponding author email: sienaa.m@uokerbala.edu.iq**
https://doi.org/10.59658/jkas.v10i2.1182

Received:
Apr. 01, 2023

Accepted:
Apr. 30, 2023

Published:
June 20, 2023

Abstract

This study evaluated the efficacy of commercial formulations of *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium muscarium* against different stages of *T. castaneum*. All commercial formulations were tested against the insects using a topical spray under laboratory conditions. Insects were sprayed with conidial concentration 3×10^8 conidia/ml of *B. bassiana* or *M. anisopliae* and 2×10^7 conidia/ml of *L. muscarium*. Mortality was recorded daily for seven days while insects were kept on wheat flour. All commercial formulationsshowed an effect on *T. castaneum*; however, *B. bassiana* and *M. anisopliae* were more effective than *L. muscarium* at the concentrations tested. However, susceptibility showed significant differences in percentage mortality at the end of the experiments between first, third, and sixth instar larvae and adults treated with all three commercial formulations. First and third-instar larvae were most susceptible, and sixth-instar larvae and adults were least vulnerable to infection. In all experiments, the temperature had no significant effect on insect mortality caused by *B. bassiana*, *M. anisopliae*, and *L. muscarium*. There was a small difference in the insect mortality level for each fungus between 25°C and 30°C. All fungi were slightly, but not significantly, more effective at 25°C. The strains of all fungi tested caused significant mortality of *T. castaneum* by contact.

Keywords: *Beauveria bassiana, Metarhizium anisopliae, Lecanicillium muscarium, Tribolium castaneum*

* It is part of PhD thesis for the first author.

Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is a cosmopolitan insect pest of storage facilities and processing plants. A significant problem with this insect is the massive economic losses it causes to stored wheat grain [1]. It feeds on the flour of

many grain species, including wheat, barley and corn. In particular, reserved wheat flour is at risk of invasion by *Tribolium* species [2]. This pest causes both qualitative and quantitative damage. Quantitative damage is caused by larval and adult insects feeding on flour and grain, which causes weight loss.

In contrast, qualitative damage is affected by product alteration and loss of nutritional value, and new-generation adults feeding on grains. Therefore, a comprehensive understanding of the biology of *T. castaneum* is necessary for the management of its population. There have been many studies of the life history of *T. castaneum* in different parts of the world. The crucial, most damaging stages in its life cycle are the larval and adult stages when the insects feed most before dispersing [3].

Most *Tribolium* species have been reported as having developed resistance to insecticides [4]. Furthermore, the need to manage these insects, combined with high levels of insecticide resistance, increases the risks of applying chemical insecticides to food. Moreover, the costs associated with chemical control, the need to reduce insecticide inputs in line with changing policy, public opinion, and the shift towards less intensive farming [5] all contribute to the need for alternative control methods. The search for alternative control methods has led to the development of biological agents. Biological control using fungi is a feasible alternative [6,7]. The possibility of using fungi to control pests in stored products has been studied for many years [8,9]. The fungi are very safe for the environment and therefore are considered by many to be promising alternatives for insect control [10,11]. In recent years there has been increasing literature in the UK and Iraq attempting to identify the most effective naturally-occurring fungal pathogens for application to structures for the control of insects in stored products [12,13].

The objectives of the current study were (1) to evaluate the impact of B. bassiana, *M.anisopliae*, and *L. muscarium* against *T. castaneum* (2) to assess the relative susceptibility of adults and different instar larvae to the fungi. Thirdly, to investigate the effect of different temperatures, precisely 25°C and 30°C, which are appropriate for the growth of both the insect and the fungi, on the efficacy of the three fungi above against *T. castaneum*. Finally, to determine the spore concentrations of the entomopathogenic fungi at which 50% of subject insects are killed (LC_{50}) and the time after application at which 50% of subject insects are dead (LT_{50}).

Materials and Methods

Source of insects

Tribolium castaneum adults were mass-reared on fresh flour medium in a glass jar (300 ml) covered with filter paper without light and humidity control. The cultures were kept in a controlled environment at a temperature of 30°C, which helped in increasing insect activity. *T. castaneum* has six larval instars. To determine the presence of these larval instars under laboratory conditions and obtain the required larval stage for each experiment, a study was conducted to differentiate between instars. Using a fine brush, adults were put into a container for two days, the adults were removed, and the containers were left for the eggs to hatch. First, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} instar larval stages were identified by observing moults after the larvae were

put into vented plastic Petri dishes (9 cm × 1.6 cm) covered with their lids and kept at 30°C without light as mentioned in [14].

Sources and providers of fungal suspension

Commercial formulations of *M. anisopliae*, *B. bassiana* and *L. muscarium* were used in the experiment Met52 granules containing *M. anisopliae*, a liquid suspension of *B. bassiana* and a powder of *L. muscarium* were obtained from Fargro Ltd, Toddington Lane, Littlehampton, West Sussex, BN17 7PP UK, Belchim Crop Protection Limited, 1b Fenice Court, Phoenix Park, Eaton Socon, and Koppert BV Ireland respectively. Met52 granules contained 2% w/w *M. anisopliae* var. *anisopliae* strain F52. The liquid suspension was Naturalis-L, an oil dispersion containing 7.16% w/w *B. bassiana* ATCC 74040, and *L. muscarium* was product Mycotal, spore powder containing 16.1% w/w spores with inert ingredients 83.9% w/w. The spore suspension of *B. bassiana* (10 ml) was diluted directly with 100 ml of distilled water in a 500 ml glass beaker. The Met52 granules were prepared into liquid formulation by weighing a certain amount of granules (10 g) using a sensitive balance in the laboratory; then, the granules were mixed with distilled water (100 ml) in a 500 ml glass beaker. To dislodge spores from the granules, the beaker was vortexed. The volume of the mixture was measured, and the spore concentration in the mix was determined using a haemocytometer. The mixture volume and spore level were used to calculate the number of conidia per ml of water [15]. The *L. muscarium* conidia powder (10 g) was weighed as described above and then mixed with distilled water (100 ml) in a 500 ml glass beaker mixed with Tween 20 . The fungal suspension was left for 2-4 h before application to allow the spores to rehydrate and assist in dispersion. The conidial concentration was estimated using a haemocytometer with a light microscope and was adjusted to 3×10^8 spores/ml for both *B. bassiana* and *M. anisopliae* and 2×10^7 spores/ml for *L. muscarium*. This suspension was the primary concentration of spores. Five different concentrations were produced from each of the main suspensions of each fungus for use in the experiments, as mentioned in [14].

Contact application of commercial formulations

In three separate experiments, conidial concentrations of *B. bassiana*, *M. anisopliae* and *L. muscarium* formulation in distilled water were sprayed onto the 1st, 3rd and 6th instar larvae and adults of *T. castaneum*. The bioassay involved treatments with five different spore concentrations from the original suspension and water as a control. *B. bassiana* and *M. anisopliae* were used at concentrations 3×10^4, 3×10^5, 3×10^6, 3×10^7, and 3×10^8 spores/ml, and *L. muscarium* at 2×10^3, 2×10^4, 2×10^5, 2×10^6, and 2×10^7 spores/ml. They are applied using a hand-held sprayer.

There are five replicates for each treatment. Each replicate comprised ten insects in one Petri dish 9 cm in diameter. Each group of insects (50) was directly sprayed with 3 ml of the fungal suspension while standing on a large glass Petri dish (200 cm). Insects were left for 10-15 min to ensure the fungal suspension adhered to the insect bodies. After 15 min, the treated insects were separated into small (9 cm × 1.6 cm) plastic Petri dishes which acted as replicates to assess mortality. The insects in each

replicate were fed a diet of flour using 5 g fresh flour. Records of insect mortality started 24 hours after the treatments were applied and continued for seven days, as mentioned in [14].

Confirmation of fungal infection in beetles

Mortality caused by fungal infection (fungal treatments) and other causes of mortality (control treatments) was recorded daily to day 7 of the experiment. Dead insects were removed after each assessment. They were then cleaned using cotton wool soaked in 75% ethanol before being placed individually in sterilised Petri dishes containing a damp filter paper. After the initial process, the Petri dishes were put into an incubator at a temperature of 25˚C for 8-10 days to retrieve mycelia from the surface of the insect. This was to confirm the fungal infection on the dead insects. After ten days, it was noticed that fungal mycelia were emerging from the bodies of the insects that died from mycosis. Therefore, those insects that did not produce mycelia were also assumed to have died from fungi.

Statistical analysis

Minitab® 17.1.0 (© 2010 Minitab Inc.) was used to compare the efficacy of the three entomopathogenic fungal species against different stages of *T. castaneum* (larvae and adults). Data were transformed using logit transformation to comply with the normality assumption. First, a General Linear Model (GLM) was used to test significant differences between treatments, and then Tukey's multiple range tests were used to separate means. In addition, the probit analysis program computed LC_{50} and LT_{50} values for entomopathogenic fungal species [16] in Minitab® 17.1.0. Finally, the mean and median survival times (MST) for 25˚C and 30˚C combined for each treatment were estimated using the Survival Log Rank Test [17] using SIGMAPLOT program (version 13.0 Systat Software Inc., San Jose, USA).

Results and Discussion

Preliminary testing showed that *B. bassiana*, *M. anisopliae* and *L. muscarium* could infect *T. castaneum* by contact, through the consequent death of the beetles. In addition, fungal mycelia emerging from dead beetles were identified according to known morphological characteristics as the fungal species initially applied to the insects (Figure 1,2).

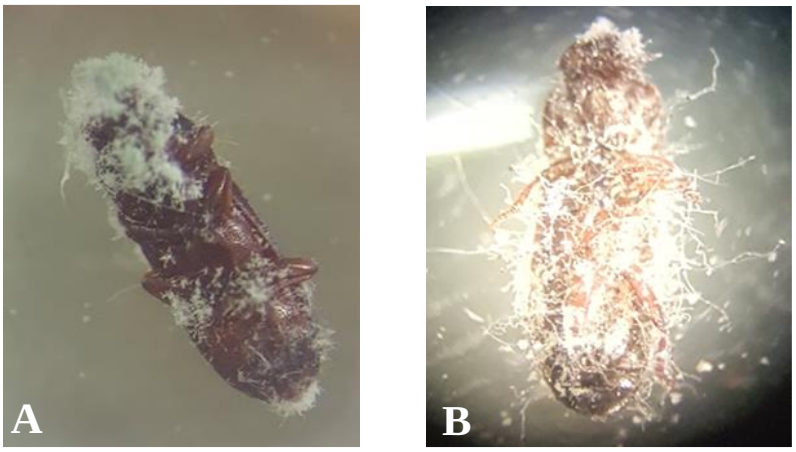

Figure (1): (A) *Tribolium castaneum* with *M. anisopliae* mycelium. (B) *T. castaneum* with *B. bassiana* mycelium.

Experiment 1: The effect of Beauveria bassiana on the mortality of T. castaneum adults and different instar larvae

The first experiment with the conidia suspension of *B. bassiana*, significant differences in *T. castaneum* mortality were noted with different spore concentrations for each of the four insect stages after 7 days of treatment. The effects of the two temperatures, six fungal concentrations (including controls) and days after fungal application were 'fixed factors', and dead beetles were treated as a 'response variate' by analysis of variance (1^{st} instar larvae: $F_{(5, 59)} = 464.87$, $P < 0.05$; 3^{rd} instar larvae: $F_{(5, 59)} = 148.50$, $P < 0.05$; 6^{th} instar larvae ($F_{(5, 59)} = 123.96$, $P < 0.05$ and adults: $F_{(5, 59)} = 134.52$, $P < 0.05$). The highest mortality level was noted with the highest fungal dosage of 3×10^8 spores/ml for the four different insect stages (Figure 2,3). All the larvae and adults were susceptible to *B. bassiana* at higher concentrations. However, susceptibility decreased with the insect's age, indicating that older larvae and adults were more tolerant to the infection. When incubated at 25°C and 30°C first instar larval mortality rates were respectively 98% and 92%, and 90% and 88% for third instar larvae compared to 70% and 68% and 60% and 58% for sixth instar larvae and adults respectively. Furthermore, there was no significant difference between the efficacies at 25°C and 30°C ($F_{1,230} = 0.90$, $P = 0.343$). Mortality did not reach 100% in any of the other treatments. Also, the control mortality of *T. castaneum* larval stages and adults did not exceed 10.0%.

The median survival times (MST) for different insect stages when treated with *B. bassiana*, leading to over 50% mortality, were five days after treatment at 25˚C and 30°C. Survival times for the four different insect stages are shown in Figure (4).

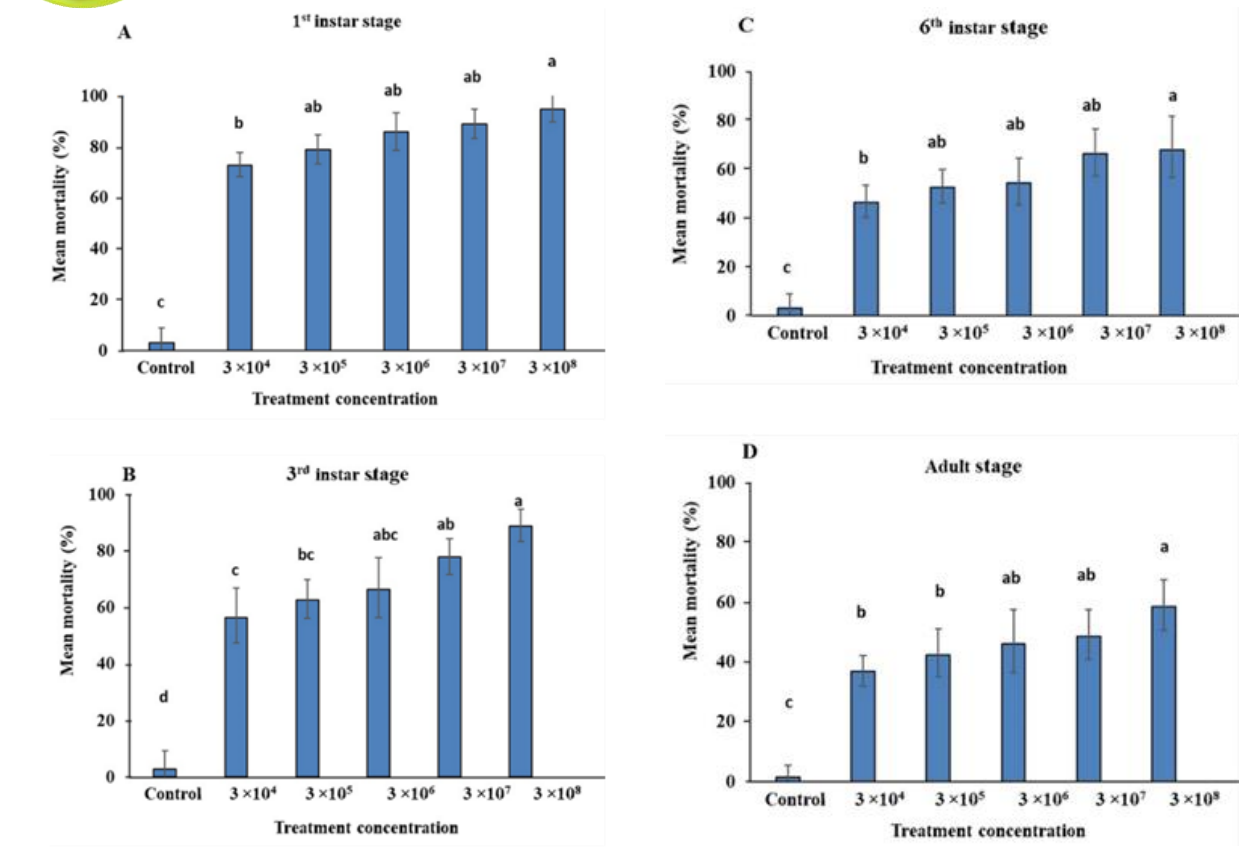

Figure (2): Combined mean percentage mortality at 25˚C and 30°C of *T. castaneum* 1st instar (A), 3rd instar (B), 6th instar larvae (C), and adults (D) after 7 days following treatment with different doses (spore ml-1) of *B. bassiana*. Bars with the same superscript letter are not significantly different (P > 0.005 Tukey test). Error bars are + 1 standard deviation (n = 5).

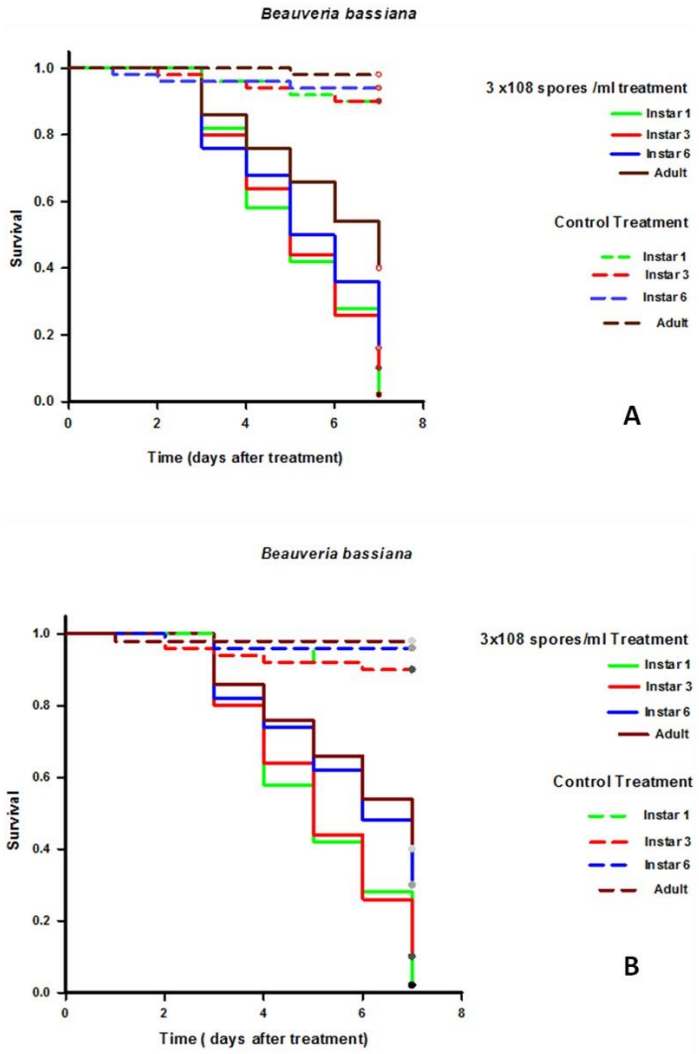

Figure (3): Survival curves of different instar larvae of *T. castaneum* following treatment with 3×10^8 spores/ml of *B. bassiana* at 25ºC (A) and 30ºC (B).

Experiment 2: The effect of *Metarhizium anisopliae* on the mortality of *T. castaneum* adults and different instar larvae

The result of *M. anisopliae* fungus tests showed that high mortality levels were recorded for first and third instar larvae after seven days of treatment (Figure 4 A, B) compared with the sixth-instar larval and adult stages (Figure 4 C and D) at the highest fungal dosage. The highest concentration of 3×10^8 spores/ml was more effective at the end of the experiment, and significant differences were noted with spore doses for each stage. The effect of two temperatures, fungal concentration and days after fungal application, were 'fixed factors', and dead beetles were treated as a 'response variate' by analysis of variance (1^{st} instar larvae: $F_{(5, 59)} = 110.68$, $P < 0.05$; 3^{rd} instar larvae: $F_{(5, 59)} = 85.72$, $P < 0.05$; 6^{th} instar larvae: $F_{(5, 59)} = 88.76$, $P < 0.05$; and adults: $F_{(5, 59)} = 43.06$, $P < 0.05$). The adults were less susceptible than larvae based on mortality rates. Percentage mortality rates at 25°C and 30°C were 52% and 50% for adults and 68% and 66% for 6^{th} instar larvae. 1^{st} instar mortality rates were 86% and 84%, and rates were 82% and 80% for 3^{rd} instar larvae. These mortality rates mean that the old larvae and adults were more tolerant

to fungus than the early larvae. For temperatures, there was no significant difference in the mortality rates ($F(1,230) = 1.30$, $P = 0.255$).

The median survival times (MST) for different insect stages when treated with *M. anisopliae*, leading to over 50% mortality, were six and seven days after treatment at 25˚C and 30°C. Survival times for the four different insect stages are shown in Figure (5).

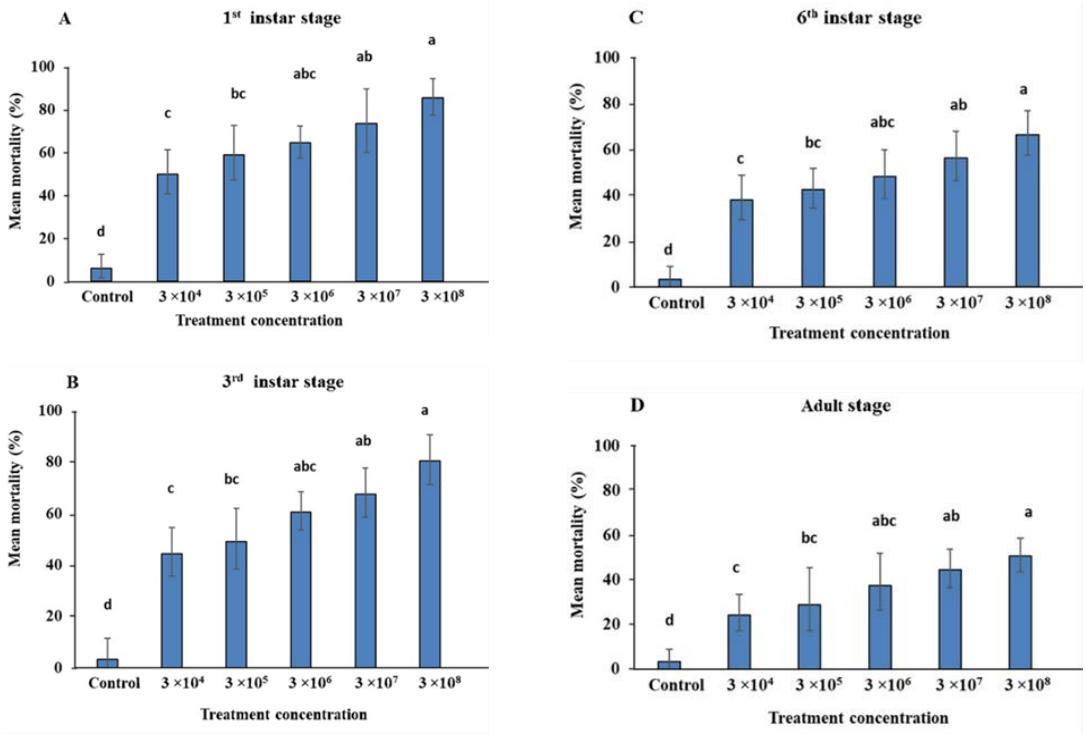

Figure (4): Combined mean percentage mortality at 25˚C and 30°C of *T. castaneum* 1st instar (A), 3rd instar (B), 6th instar larvae (C), and adults (D) after seven days following treatment with different doses (spore ml-1)of *M. anisopliae*. Bars with the same superscript letter are not significantly different (P > 0.001 Tukey test). Error bars are + 1 standard deviation (n = 5).

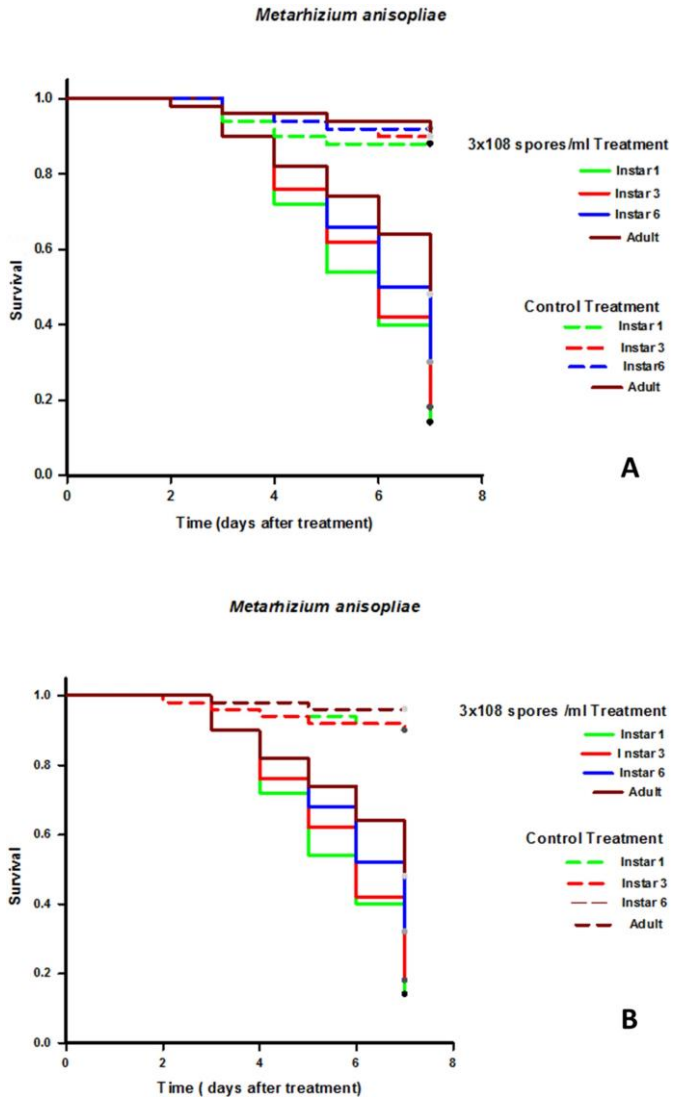

Figure (5): Survival curves of different instar larvae of *T. castaneum* following treatment with 3×10^8 spores/ml of *M. anisopliae* at 25ºC (A) and 30ºC (B).

Experiment 3: The effect of *L. muscarium* on the mortality of *T. castaneum* adults and different instar larvae

The highest concentration of 2×10^7 spores/ml gave high mortality, and significant concentration effects were found for each stage. The effect of two temperatures, fungal concentration and days after fungal application, were 'fixed factors', and dead beetles were treated as a 'response variate' by analysis of variance (1^{st} instar larvae: $F_{(5,59)} = 108.53$, $P < 0.05$; 3^{rd} instar larvae: $F_{(5,59)} = 79.70$, $P < 0.05$; 6^{th} instar larvae: $F_{(5,59)} = 50.94$, $P < 0.05$; and adults: $F_{(5,59)} = 36.99$, $P < 0.05$) when they were incubated at 25°C and 30°C. For each stage, there was a significant difference between the efficacies of the different *L. muscarium* concentrations. However, there were no significant differences between the efficacies of *L. muscarium* at the two different temperatures. Based on mortality rates, the adults were less susceptible than the larvae: percentage mortality rates at 25°C and 30°C were 48% and 46% for adults and 58% and 56% for sixth instar larvae. Mortality rates were 84% and 82% for 1^{st} instar and 78% and 76% for 3^{rd}

instar larvae seven days after treatment (Figure 6 A, B, C and D). The mortality rates above mean that the early larvae stages were less tolerant to fungus than the adults. For temperatures, there was no significant difference in the mortality rates ($F(1,230) = 0.66$, $P = 0.418$).

The median survival times (MST) for different beetle stages, when treated with *L. muscarium* at 25˚C and 30°C, leading to over 50% mortality, were six and seven days after treatment. Survival times for the four different insect stages are shown in Figure (7).

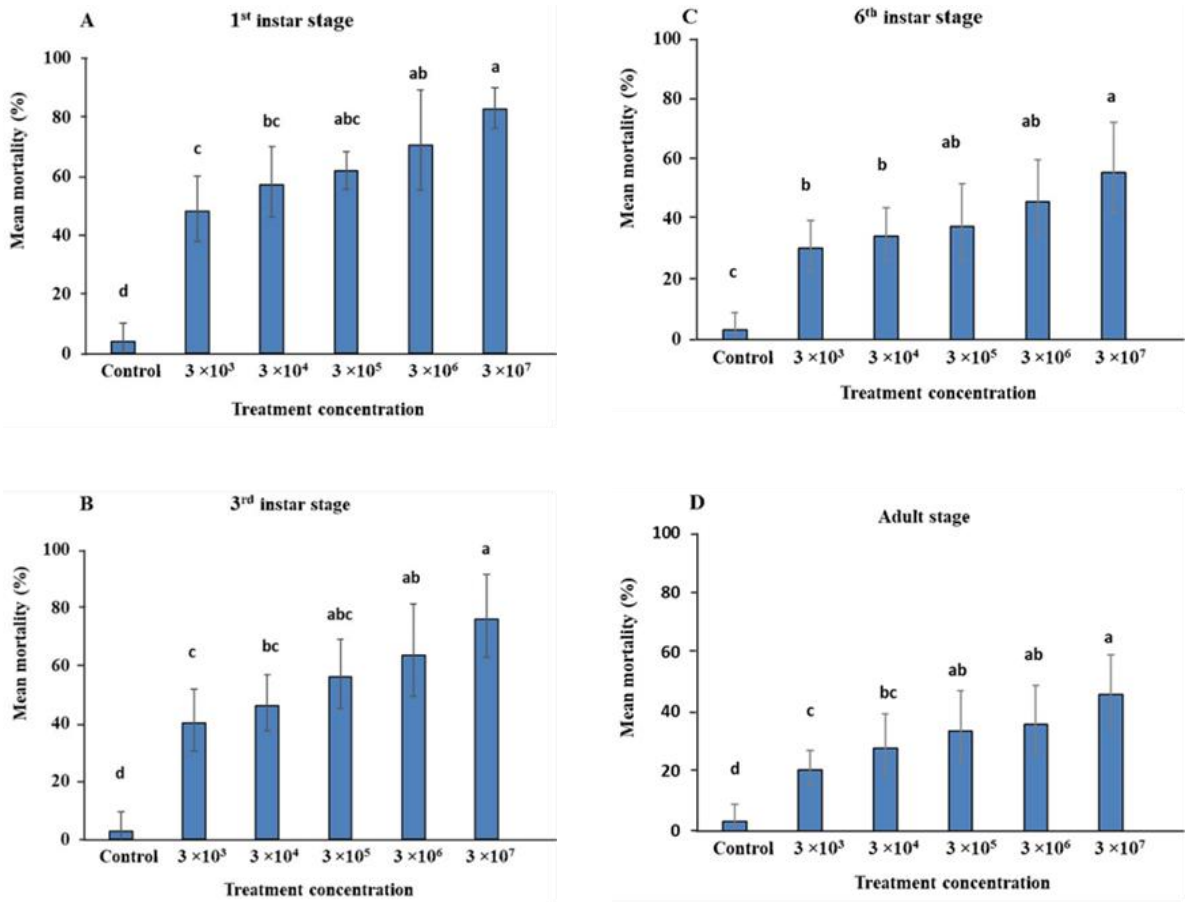

Figure (6): Combined mean percentage mortality at 25°C and 30°C of *T. castaneum* 1st instar (A), 3rd instar (B), 6th instar larvae (C), and adults (D) after seven days following treatment with different doses (spore ml-1) of *L. muscarium*. Bars with the same superscript letter are not significantly different (P > 0.001 Tukey test). Error bars are +1 standard deviation (n = 5).

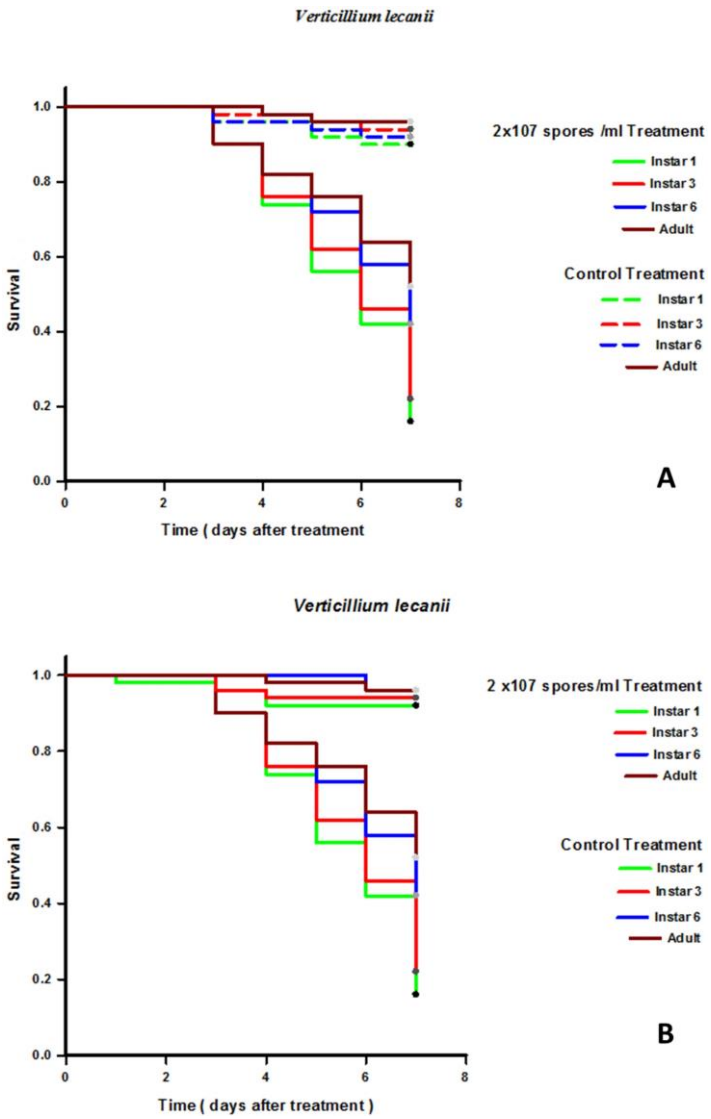

Figure (7) : Survival curves of different instar larvae of *T. castaneum* following treatment with 2×10^7 spores/ml of *L. muscarium* at 25ºC (A) and 30ºC (B).

Compasion of fungi

To conclude, first comparing the efficacy of the fungi, *B. bassiana*, *M. anisopliae* and *L. muscarium* were pathogenic against all larval instars and adults. Secondly, a comparison of susceptibility between life stages showed significant differences in percentage mortality between 1st and 3rd instar larvae from 6th instar larvae and adults infected by *B. bassiana* ($F_{(3,230)} = 40.42$, $P < 0.05$). However, 1st instar larvae were more susceptible. Similarly, there was a significant difference in the mortality of 1st, 3rd, and 6th instar larvae and adults infected by *M. anisopliae* ($F_{(3,230)} = 42.62$, $P < 0.05$) and *L. muscarium* ($F_{(3,230)} = 44.65$, $P < 0.05$). 6th instar larvae and adults were more tolerant to the fungal infection than 1st and 3rd instar larvae (Figure 8). Finally, there was no significant interaction between each fungus's insect stage factors, fungal concentration, and temperatures.

Tribolium castaneum LC_{50} (spores/ml) values at day 5 confirmed that adults were less susceptible to *B. bassiana*, *M. anisopliae* and *L. muscarium* than the larval stages (Figure 8; Table 1). There were differences in the LT_{50} with 3 x 10^6 spore/ml.

It increased with the insect's age, indicating that older larvae and adults were more tolerant to the infection (Table 1). The LT_{50} (days) values of the insects treated with *B. bassiana* were shorter than those treated with other fungi. The LT_{50}s of the adult stage were calculated as 5.7, 6.3 and 6.9 days for *B. bassiana*, *M. anisopliae* and *L. muscarium*, respectively, indicating the superiority of *B. bassiana* over the other two.

Finally, in all experiments, as noted above, the results indicated that temperature had no significant effect on insect mortality caused by *B. bassiana*, *M. anisopliae*, and *L. muscarium.*

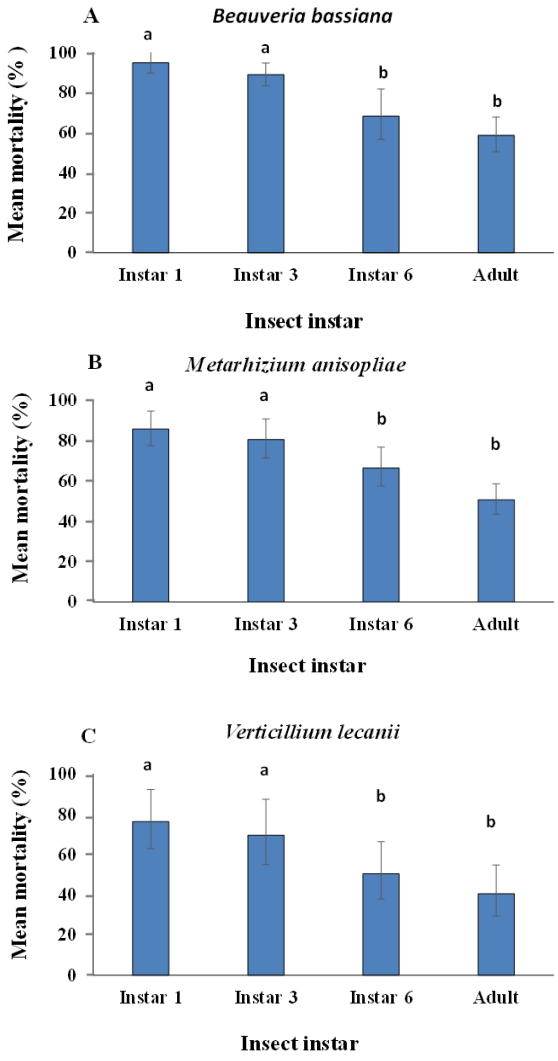

Figure (8): Mean percentage mortality of *T. castaneum* after seven days following treatment with 3×10^8 spores/ml of *B. bassiana* (A), *M. anisopliae* (B) and 2×10^7 spores/ml of *L. muscarium* (C). Bars with the same superscript letter are not significantly different (P >0.001 Tukey test). Error bars are ± 1 standard deviation (n = 5).

Table (1): LC_{50} (spores/ml) (at day 5) and LT_{50} (days) values (at 3×10^6 spores/ml) of *T. castaneum* treated with *B. bassiana, M. anisopliae* and *L. muscarium* fungi.

Life Stages	*B. bassiana*		*M. anisopliae*		*L. muscarium*	
	LC_{50}	LT_{50}	LC50	LT50	LC50	LT50
First instar	3.5×10 4	3.4	4.7 × 10 5	4.2	4.1 × 10 5	4.4
Third instar	4.5 × 10 5	4.0	5.2 × 10 5	4.5	4.7 × 10 5	4.8
Sixth instar	5.6 ×10 5	4.8	6.3 × 10 5	5.1	6.4 ×10 6	5.9
Adult	6.8 × 10 5	5.7	7.9 × 10 5	6.3	7.5 × 10 6	6.9

LC50 value = the number of spores per ml needed for 50 % mortality.
LT50 = time needed (days) by spores to cause 50 % mortality.

This study has demonstrated that the fungi used are capable of causing infection and mortality in *T. castaneum* through contact. All three tested commercial formulations based on *M. anisopliae, B. bassiana,* and *L. muscarium* show potential as biocontrol agents against this stored insect pest. These findings are similar to a study by Erler and Ates [18], which demonstrated that commercial formulations of *M. anisopliae* and *B. bassiana* can cause infection and mortality of the June beetle *Cotinis nitida* (Coleoptera: Scarabaeidae). With contact application, *B. bassiana* at the same concentration caused more mortality than the other fungi. Although the conidial suspensions of *B. bassiana* and *M. anisopliae* caused more mortality than *L. muscarium* when sprayed, *B. bassiana* took less time to kill the beetles based on LT_{50} (days) values (Table 1).

At the highest concentration, the maximum daily mortality caused by *B. bassiana*, *M. anisopliae* and *L. muscarium* occurred on day seven after treatment. The first insect mortality caused by all the concentrations of *B. bassiana*, *M. anisopliae* and *L. muscarium* was observed three days after treatment. These times are similar to the results of other researchers. Bateman [19] stated that insect field mortality caused by pathogens rarely occurs earlier than six days after application. In a field trial, the fungal product *M. anisopliae* var. *acridum* strain IMI 330189 developed by the LUBILOSA project for biological control of grasshoppers and locusts showed the first observable mortality at 7-10 days after application, and the full effects were observed at 14-18 days after application [20].

First and 3rd instar larvae were most susceptible, and 6th instar larvae and adults were least susceptible to infection by all fungi (Figure 8). This result agrees with the observations by Öztürk, Güven [21] that earlier life cycle stages of *Leptinotarsa decemlineata* (Colorado potato beetle) were more susceptible to *B. bassiana* than adults. Entomopathogenic fungi and bioinsecticides in their study were more effective in early larval stages than in fourth larval instars and adults treated by spray methods. Weiser [22] also found that in laboratory cultures of *T. castaneum*, 98 % of the larvae but only 2-3 % of the adults were infected with a parasitic protist,

Farinocystis tribolii. The reason for the low percentage mortality due to *F. tribolii* infection of *T. castaneum* adults has still to be further explored.

The relative susceptibility of different developmental stages of a host depends on the host species and the fungal isolate [23]. According to Rohde, and Alves [24], *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) adults were less susceptible to *B. bassiana* and *M. anisopliae* isolates than the larvae. Also, the susceptibility of *Leptinotarsa decemlineata* larvae to *B. bassiana* decreased with age [25]. The differential susceptibility levels observed in insects infected by entomopathogenic fungi could be related to moulting by the larval stages, which is relevant when the pathogen is inoculated immediately before ecdysis or when the time interval between sequential moults is short [26,27,28].

In general, optimum temperatures for germination, growth, sporulation and virulence of entomopathogenic fungi have been reported to range between 20 and 30°C [29,30,31,32]. Abodarb [33] proved that a temperature lower than the optimum (20-30°C) could retard the development of the fungus and spore production, leading to a reduction in the mortality of insects.

The first stage of infection is when spores are retained on the integument surface, where the formation of the germinative tube initiates the fungi starting to excrete enzymes such as proteases, chitinases, chitobiases, lipases and lipoxygenases [34]. These enzymes degrade the insect's cuticle and help penetration by the mechanical pressure initiated by the appressorium, a functional structure formed in the germinative tube. The second phase of infection occurs inside the insect, where the fungi develop as hyphal bodies that disseminate through the haemocoel and invade several muscle tissues, fat bodies, Malpighian tubes, and haemocytes, leading to the death of the insect 3 to 14 days after infection [34]. Once the insect dies and many nutrients are exhausted, the fungi begin mycelial growth and invade all the host organs. Finally, the spores germinate first, and then hyphae penetrate the cuticle from the interior of the insect and emerge at the surface, where they initiate spore formation under appropriate environmental conditions [35,36,37,38].

The fungi results showed that the mortality of 1st and 3rd instar larvae caused by the fungal doses tested was significantly higher than that of the 6th instar larvae and adults. In addition, the temperatures tested had no significant impact on the efficacy of the fungi. Furthermore, available fungal formulations belonging to *Metarhizium*, *Verticillium* and *Beauveria* could be appropriate biological control agents for controlling *T. castaneum*.

Acknowledgements

The first two authors thank the School of Natural and Environmental Sciences, Newcastle University, United Kingdom, for using their facilities.

References

1) Sallam, M. N. (2008). Insect damage: damage on post-harvest. AGSI/FAO: INPhO Available via.

2) Wakil, W.; A. Javed, and S. Anwar . (2003). Comparison of nutritional losses of insect infested wheat in laboratory and public storages. Pakistan Journal of Arid Agriculture (Pakistan), 6 (1): p. 1-6.

3) Abrol, D. P. and Shankar, U. (Eds.). (2012). Integrated pest management: principles and practice. CABI.p. 386.

4) Boyer, S.; Zhang, H.and Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. Bulletin of entomological research, 102(2): 213-229.

5) Tomizawa, M. and Casida, J. E. (2003). Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annual review of entomology, 48(1): 339-364.

6) Hidalgo, E.; Moore, D. and Le Patourel, G. (1998). The effect of different formulations of Beauveria bassiana on Sitophilus zeamaisin stored maize. Journal of stored products research, 34(2-3): 171-179.

7) Mislit, H. M.; Mohammed, A. A. and Kareem, A. A. (2020). Evaluation of the efficacy of local and commercial entomopathogenic fungal isolates against Oryzaephilus surinamensis (Coleoptera: Silvanidae). Plant Archives, 20(2): 5672-5676.

8) Khan, A. R. and Selman, B. J. (1988). On the mortality of Tribolium castaneum adults treated sublethally as larvae with pirimiphos methyl, Nosema whitei and pirimiphos methyl—N. whitei doses. Entomophaga, 33, 377-380.

9) Attiya, R. L.; Al-Ubori, R. N.; Alrubaye, S. M.; Gani, M. M.; Shakir, A. S. and Duraid, K. A. T. (2020). Physiological Effect of the Water Sources and Salicylic Acid on the Germination and Some Seedling Traits of Sorghum. Indian Journal of Ecology, 47, 275-280.

10) Sahayaraj, K. and Tomson, M. (2010). Impact of two pathogenic fungal crude metabolites on mortality, biology and enzymes of Dysdercus cingulatus (Fab.)(Hemiptera: Pyrrhocoridae). Journal of Biopesticides, 3(Special Issue), 163.

11) Fawaz, S. F. ; Al-Zurfi, S. M.; Shatti, R. H. and Jaafar, H. M. (2020) The larvicidal activity of ethanolic extracts of citrus maxima peels, seeds against mosquito larvae in the laboratory. Ecology, Environment and Conservation, 26(4): 1540-1543.

12) Wakefield, M. E. (2006). Factors affecting storage insect susceptibility to the entomopathogenic fungus Beauveria bassiana. In 9th international working conference on product protection (pp. 15-18).

13) Mohammed, A. A.; Ahmed, F. A.; Younus, A. S.; Kareem, A. A. and Salman, A. M. (2022). Molecular identification of two entomopathogenic fungus Clonostachys rosea strains and their efficacy against two aphid species in Iraq. Journal of Genetic Engineering and Biotechnology, 20(1): 1-8.

14) Al-Zurfi, S. M. A. (2019). Biological control of the red flour beetle, Tribolium castaneum using entomopathogenic fungi (Doctoral dissertation, Newcastle University).

15) Behle, R. W. and Goett, E. J. (2016). Dosage response mortality of Japanese beetle, masked chafer, and June beetle (Coleoptera: Scarabaeidae) adults when exposed to experimental and commercially available granules containing Metarhizium brunneum. Journal of Economic Entomology, 109(3): 1109-1115.

16) Arabi, F.; Moharramipour, S. and Sefidkon, F. (2008). Chemical composition and insecticidal activity of essential oil from Perovskia abrotanoides (Lamiaceae) against Sitophilus oryzae (Coleoptera: Curculionidae) and Tribolium castaneum (Coleoptera: Tenebrionidae). International journal of tropical insect science, 28(3): 144-150.

17) Kaplan, E. L. and Meier, P. (1958). Nonparametric estimation from incomplete observations. Journal of the American statistical association, 53(282): 457-481.

18) Erler, F. and Ates, A. O. (2015). Potential of two entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. Journal of Insect Science, 15(1): 44.

19) Bateman, R. (1997). The development of a mycoinsecticide for the control of locusts and grasshoppers. Outlook on Agriculture, 26(1):13-18.

20) Lomer, C. J.; Prior, C. and Kooyman, C. (1997). Development of Metarhizium spp. for the control of grasshoppers and locusts. The Memoirs of the Entomological Society of Canada, 129(S171): 265-286.

21) Öztürk, H. E.; Güven, Ö. and Karaca, I. (2015). Effects of some bioinsecticides and entomopathogenic fungi on colorado potato beetle (*Leptinotarsa decemlineata* L.). Communications in Agricultural and Applied Biological Sciences, 80(2): 205-211.

22) Weiser, J. A. R. O. S. L. A. V. (1953). Schizogregariny z hmyzu, skodiciho zasobam mouky. I. Věstn. Česk. Spolecnosti Zool., (17): 199-212.

23) Pedrini, N.; Crespo, R. and Juárez, M. P. (2007). Biochemistry of insect epicuticle degradation by entomopathogenic fungi. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 146(1-2):124-137.

24) Rohde, C.; Alves, L. F.; Neves, P. M.; Alves, S. B.; da Silva, E. R. and de Almeida, J. E. (2006). Seleção de isolados de Beauveria bassiana (Bals.) Vuill. e Metarhizium anisopliae (Metsch.) Sorok. contra o cascudinho Alphitobius diaperinus (Panzer)(Coleoptera: Tenebrionidae). Neotropical Entomology, 35, 231-240.

25) Fargues, J.; Delmas, J. C.; Augé, J. and Lebrun, R. A. (1991). Fecundity and egg fertility in the adult Colorado beetle (Leptinotarsa decemlineata) surviving larval

infection by the fungus Beauveria bassiana. Entomologia experimentalis et Applicata, 61(1): 45-51.

26) Ouedraogo, A.; Fargues, J.; Goettel, M. S. and Lomer, C. J. (1997). Effect of temperature on vegetative growth among isolates of Metarhizium anisopliae and M. flavoviride. Mycopathologia, 137, 37-43.

27) Ekesi, S. and Maniania, N. K. (2000). Susceptibility of Megalurothrips sjostedti developmental stages to Metarhizium anisopliae and the effects of infection on feeding, adult fecundity, egg fertility and longevity. Entomologia Experimentalis et Applicata, 94(3): 229-236.

28) Lopes, R. B. and Alves, S. B. (2011). Differential susceptibility of adults and nymphs of Blattella germanica (L.)(Blattodea: Blattellidae) to infection by Metarhizium anisopliae and assessment of delivery strategies. Neotropical Entomology, 40, 368-374.

29) Ekesi, S.; Maniania, N. K. and Ampong-Nyarko, K. (1999). Effect of temperature on germination, radial growth and virulence of Metarhizium anisopliae and Beauveria bassiana on Megalurothrips sjostedti. Biocontrol Science and Technology, 9(2): 177-185.

30) Tefera, T. and Pringle, K. (2003). Germination, radial growth, and sporulation of Beauveria bassiana and Metarhizium anisopliae isolates and their virulence to Chilo partellus (Lepidoptera: Pyralidae) at different temperatures.

31) Dimbi, S.; Maniania, N. K.; Lux, S. A. and Mueke, J. M. (2004). Effect of constant temperatures on germination, radial growth and virulence of Metarhizium anisopliae to three species of African tephritid fruit flies. BioControl, 49, 83-94.

32) Kiewnick, S. (2006). Effect of temperature on growth, germination, germ-tube extension and survival of Paecilomyces lilacinus strain 251. Biocontrol Science and Technology, 16(5): 535-546.

33) Abodarb, A. A. M. (2016). Interactions between the entomopathogenic fungus Lecanicillium muscarium and the parasitoid Aphidius colemani for the control of green peach aphid Myzus persicae under laboratory and field conditions (Doctoral dissertation, University of Reading).

34) Ortiz-Urquiza, A. and Keyhani, N. O. (2013). Action on the surface: entomopathogenic fungi versus the insect cuticle. Insects, 4(3): 357-374.

35) Roberts, D. W. and St Leger, R. J. (2004). Metarhizium spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Advances in applied microbiology, 54(1): 1-70.

36) Maniania, N. K.; Bugeme, D. M.; Wekesa, V. W.; Delalibera, I. and Knapp, M. (2009). Role of entomopathogenic fungi in the control of Tetranychus evansi and Tetranychus urticae (Acari: Tetranychidae), pests of horticultural crops. Diseases of Mites and Ticks, 259-274.

37) Vega, F. E. (2008). Insect pathology and fungal endophytes. Journal of invertebrate pathology, 98(3): 277-279.

38) Vega, F.E. et al. (2012). Fungal Entomopathogens, in Insect Pathology (Second Edition). Elsevier. 171-220.