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Influence of Some Biological Control Measures on *Tribolium castaneum* (Coleoptera: Tenebrionidae)

Karima S. Khater[1*], Ibrahim F. Shoukry[1], Abdel-Aziz E. Abdel-Aziz [2], and Nora A. Saad[2].
[1]Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt
[2]Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

ABSTRACT

Tribolium castaneum (Herbst) is a highly destructive pest that poses a significant threat to grains and their derived products, such as flour, on a global scale. In this study, the effects of two entomopathogenic fungi (EPF), namely *Beauveria bassiana and Metarhizium anisopliae,* as well as the predator bug *Xylocoris flavipes* (Reuter), on *T. castaneum* were investigated. The results of the study demonstrated that *B. bassiana* exhibited greater toxicity towards the third larval instar of *T. castaneum* compared to *M. anisopliae.* Conversely, *M. anisopliae* displayed higher toxicity against the adult stage of the pest. Furthermore, when assessing the bioassay preference of *X. flavipes* for controlling different immature stages of *T. castaneum*, the egg stage was found to be the most preferred target. The preference of *X. flavipes* can be ranked in descending order as follows: eggs stage, third larval instar, and pupal stage. Additionally, it was observed that the predation of *T. castaneum* immatures by *X. flavipes* resulted in a significant reduction in the population of the pest's first generation. The findings suggest that *B. bassiana*, *M. anisopliae*, and *X. flavipes* demonstrate suppressive effects on the biology of *T. castaneum*. As a result, these eco-friendly agents have the potential to be incorporated into Integrated Pest Management (IPM) programs as effective measures against this economically damaging insect pest.
Keywords: *B. bassiana;* Biological control*; M. anisopliae, T. castaneum; X. flavipes.*

INTRODUCTION

The major challenge confronting humanity is the issue of food security, particularly in the tropics and subtropics where the climatic conditions provide an appropriate environment for a wide range of insects. This requires more great efforts to overcome population consistency of the various insect pests in order to ensure adequate food supply.

In developing nations, the problem of insect pests is exacerbated by the ever- increasing demand for food as a result of explosive growth in human population (Nyamandi and Maphos, 2013; Onuminya *et al.*, 2018). Insect pests are the major limiting factor in crop production, being responsible for the loss of about one-quarter of crop yield worldwide (Rajashekar *et al*., 2012). Furthermore, insect infestations significantly lead to qualitative and quantitative losses in ware-housed- stored food grains (Singh *et al.*, 1997; Pimentel *et al*., 2007; Akmal *et al*., 2020) due to the humid and warm environments that threaten the economy and food security (Ekeh *et al*., 2013). The rust-red flour beetle *Tribolium castaneum* (Herbst), is a prevalent and harmful pest all over the world (Perkin and Oppert, 2019). It is cosmopolitan in tropical areas experiencing a catastrophic economic meltdown (Hamed and Khattak, 1985). *T. castaneum* is a serious pest of cereals and foodstuffs items derived from them, such as flour and dried fruits (Khattak *et al.*, 1999; Dars *et al.*, 2009; Uçar *et al.*, 2020). It is a secondary stored grain pest that causes significant losses to grains that has have been damaged by other stored insect species (LI and Arbogast, 1991). The infested grains acquire a distinct disagreeable odor as a result of insect's excreta and exu-dates, rendering them unpalatable and unfit for human consumption and nutritional uses (Anisa, 1971; Atwal, 1976).

The widespread use and misuse of conservative insecticides and fumigants has resulted in ecosystem disruptions, increased application costs, pest resurgence, pesticide resistance, and lethal effects on non-target organisms (Okonkwo and Okoye, 1996; Isman 2006; Philips and Throne, 2010; Nenaah and Ibrahim, 2011). There is an urgent need to safeguard stored food from the threat of attack by these insects by using eco-friendly alternative insecticides since they can destroy huge quantities, particularly during long-term storage (Pimentel and Pimentel, 1978).

Biological control strategy is one component of the integrated pest management that has been used successfully in controlling stored product pests (Flinn, 1998). Entomopathogenic fungi (EPF) are inexpensive, widely available, less harmful, and less poisonous to animals (Phillips and Throne, 2010). More than 700 insect pathogenic fungus have been identified (Sandhu *et al*., 2012; Erper *et al.*, 2016). The use of the (EPF) has been shown to be a promising and environmentally acceptable strategy because they are naturally occurring, safe for the environment, and have no adverse effect on non-target species and plants (Zimmermann, 1993; Cox and Wilking, 1996).

In this regard, the most common insect pathogenic fungi used for the management of various stored-product insects include *Beauveria bassiana* and *Metarh-izium anisopliae* (Rumbos and Athanassiou, 2017). The EPF, *B. bassiana*, and *M. anisoplia*e have been empl-oyed in biological management of a wide range of stored goods insect pests (Padin *et al*., 2002; Akbar *et*

* *Corresponding author: K_S_kamel@hotmail.com*

al., 2004). The fungus *B. bassiana* has a clear influence on the 2nd instar larvae and adults of *T. castaneum* (Akmal *et al*., 2020). Michalaki *et al*. (2006) investigated the anti-*T. confusum* larval impact of *M. anisopliae*. EPF infects their insect hosts through cuticle penetration and spreads throughout the haemolymph. After killing the infected host, the fungus can extrude from the host cadaver and generate more green spores, increasing the fungus's population and increasing the likelihood of other insects being inoculated (Gabarty *et al.,* 2014). Fungi secrete secondary metabolites inside host tissue which led to mortality (Zhang *et al.,* 2020a).

The Store Bug, *X. flavipes* (Reuter) (Hemiptera: Anthocoridae) is a cosmopolitan polyphagous insect predator known for its ability to control various species of stored product pests (Berger et al., 2017). It achieves this by selectively preying on the immature stages of external-feeding lepidopteran and coleopteran insects that lack consistent sclerotization and do not possess a shaggy appearance (LeCato and Davis, 1973). Several researchers have reported repression of various pest communities (Arbogast, 1976; Brower and Press, 1992; Brower *et al*., 1996; Rahman *et al.,* 2009). Intensification population *Corcyra cephalonica* was suppressed by *X. flavipes* in stored rice grain (Saikia and Borkakati 2020). Therefore, the aim of this study is to investigate the effectiveness of two entomopathogenic fungi, namely *B. bassiana* and *M. anisopliae*, as well as the predatory bug *X. flavipes*, in controlling the harmful stored product pest, *T. castaneum*.

MATERIALS AND METHODS

Tested grains

The wheat grains (*Triticum aestivum*) utilized in this study were acquired locally and sieved to remove any irregular kernels and other foreign materials. Grains were then sterilized in a deep freezer at -5^{o}C for two weeks before application to eliminate any potential insect pest infestation (Ileke and Bulus, 2012). Sterilized grains were allowed to be air dried in the laboratory for a sufficient period (about 2 hours) to equilibrate their moisture content and prevent mould growth (Adedire *et al.,* 2011; Saad, 2017).

Tribolium castaneum propagation

A stock culture of *T. castaneum* was established by selecting 100 pairs of adults (1-3 weeks old) and placing them in glass jars (500 ml) containing 200 g of crushed, sterilized, and conditioned wheat grains as a suitable substratum for laying eggs. Jars were covered with muslin cloth and tied with double rubber bands to allow air to pass through while preventing contamination and escape of beetles. Beetles were fed on crushed wheat grains and kept in an incubator for about two weeks at 28±1° C, 65±5% R.H. and 16: 8 h L: D. The infested crushed wheat grains were sieved through a technical grain sieve with a diameter of 3.0 mm, and the parent beetles were removed. The crushed wheat grains with eggs were reintroduced into the jars, and the incubator was allowed to breed until new adults emerged. Newly emerged adults (1-3 weeks old) of both sexes were selected and employed in the tests. (Rozman *et al.,* 2007).

Entomo-pathogenic fungi

To test the efficacy of commercially produced formulations of EPF, *B. bassiana*, and *M. anisopliae* against the 3rd larval instar and adults of *T. castaneum*, 10% wettable powder (10% WP) was used.

Predator (*X. flavipes*)

In this study, the predatory hemipteran bugs (*X. flavipes*) (Hemiptera: Anthocoridae) adult stages were used against *T. castaneum* immature stages. The anthocorid predator was reared using the approach of Afifi's (1984), with various modifications. Three pairs of anthocorid predator adult stage were reared on *T. castaneum* eggs and larvae by placing 100 individual *T. castaneum* adult stage (as a source of eggs) and diff-erent larval instars inside glass jars (500 ml) containing 200 g of sterilized and conditioned flour for prey feeding.

To avoid cannibalism, more *T. castaneum* larvae were introduced to such jars daily. Because *X. flavipes* was unable to penetrate finely particulate commodities and particle size was an important factor in the predation process, strips of corrugated filter paper or wood mulch were placed inside each jar to serve as an ovipositional site, as confirmed by (Lecato, 1975; Press *et al*., 1978). After 7 days, the parent adults were removed and flour containing predator eggs was incubated. The fifth nymphal instars of the predator were collected and transferred into another jar. The following experiments were conducted on the newly emerged adults. The rearing jars of rearing were kept in the incubator at 28±1° C and relative humidity of 65±5%.

Assessing the biocontrol potential of entomopathogenic Fungi

Comparative Efficacy of B. bassiana and M. anisopliae against T. castaneum Larvae and Adults

The efficacy of *B. bassiana* and *M. anisopliae* on third instar larvae and adults of *T. castaneum* was assessed. Sample of 10 g of crushed wheat grains was added in a glass tubes (1x7.5 cm) and mixed with series concentrations of 0.8, 0.6, 0.4, 0.2, and 0.1% (w/w) [0.8, 0.6, 0.4, 0.2, and 0.1 gm of dry conidia (1x 106 spore/gm) equivalent to $8x10^5$, $6x10^5$, $4x10^5$, $2x10^5$, and $1x10^5$ conidia per 100 gm. Each prepared tube containing treated grains supplied with 25 individuals *T. castaneum* third larval instars. In the case of adult treatment, twenty-five *T. castaneum* adults (1-3 weeks old) were inserted into each prepared tube containing treated grains. Ten grams of untreated crushed wheat grains were used as a control treatment. The tubes were wrapped with a piece of muslin held in position by a rubber band before being placed in an incubator at constant temperatures of 28±1° C and 65±5% R.H. Three replicates of both treated and untreated grains were used. The mortality rate was calculated three, five,

seven, fourteen, and twenty-one days after the target stage was first introduced. The number of F1 progeny was evaluated 35 days after larval treatment, and 50 days after adult treatment.

Investigating the predatory bug's influence on the development of *T. castaneum*

Predation preference of X. flavipes towards different immature stages of T. castaneum

To investigate the predation preference of the anthocorid predator *X. flavipes* towards different juvenile stages of *T. castaneum*, including the 1-3 days old egg stage, 3rd larval instar, and pupal stage, a prey choice test was conducted based on the method outlined by LeCato and Davis (1973) with certain modifications. Three newly emerged predatory adults were put into glass tubes (1x7.5 cm), each containing 10 gm of crushed wheat grains infested with fixed number (50) of *T. castaneum* immature stages. The studied *X. flavipes* individuals were starved for 24 hours to enhance their odds of encountering prey (Hodgson and Aveling, 1988). The tubes were wrapped with piece of muslin cloth and fixed with rubber bands in the incubator at 28 ±1°C and relative humidity of 65 ±5%. Control treatments were prepared using tubes infested with the same number of eggs (1-3 days old, 3rd larval instar, and pupal stage) without *X. flavipes*. Four replicates were prepared for both treatments and controls. After 24 hours of feeding, the number of individuals consumed by *X. flavipes* adults was calculated and recorded.

Effect of X. flavipes on adult emergence of T. castaneum

The efficacy of the *X. flavipes* predator as a bio-agent in suppressing the progeny of *T. castaneum* was determined using a non-choice test with slight modifications, this test was performed on egg stage (1-3) days old, 3rd larval instar, and pupal stage (separately) according to Boraei (2010). After *X. flavipes* were starved for 24 hours, different numbers of newly emerged predatory adults of 20, 15, 10, and 5 individuals were introduced (separately) in a glass tube (1x7.5 cm) containing 10 g of crushed wheat grains infested with a fixed number (50) for each of the tested immature stages of *T. castaneum*. The tubes were wrapped in muslin cloth and held with rubber bands before being placed in an incubator at constant temperatures of 28±1° C and relative humidity of 65±5%. The same protocol was conducted without individuals of *X. flavipes* in the control experiment. Both the treatment and control studies were replicated four times.

Statistical analysis

All data recorded were in four replicates. Abbott's formula (Abbott, 1925) was employed to adjust mortality percentages, while Duncan's multiple range tests (Duncan, 1955) were used to compare means at a significance level of 0.05. The statistical analysis of the obtained results was performed using the ANOVA procedure in the SAS statistical software package (SAS Institute Inc., Cary, NC, USA).

RESULTS

Effect of entomopathogenic fungi

On third larval instar

Data presented in Table (1) indicated that increasing concentrations of entomopathogenic fungi and exposure times clearly resulted in a gradual increase in larval mortality percentages of *T. castaneum* and decrease in adult emergence percentage. Larval mortalities differed significantly ($p \leq 0.0001$) among the tested concentrations (0.1, 0.2, 0.4, 0.6 and 0.8% (w/w) *B. bassiana*) 3 days post application with average percentages of 1.3±0.12, 2.7±0.12, 4.0±0.23, 5.3±0.17 and 6.7±0.12, respectively. On the 21st day after exposure, the respective average mortality percentages were increased to 10.8± 0.12, 12.3±0.17, 15.4± 0.12, 20±0.23 and 26.2±0.11. The same trend of significant ($p \leq 0.0001$) was recorded for *M. anisopliae* using the same series of concentrations, with the average number of larval mortalities being 1.3±0.06, 2.7±0.12, 4.0±0.12, 4±0.17, and 6.7±0.17 after three days. Similarly, the mean percentage number of larval mortalities, compared to the control, increased significantly ($p \leq 0.000$) to 6.0±0.17, 7.6±0.17, 12.4±0.23, 15.2±0.26 and 25.8±0 on the 21st days after exposure. As for the effect of the tested treatments on the adult emergence, *B. bassiana* had the most pron-ounced influence on the adult emergence, scoring 89.8% reduction in emerged adults, while *M. anisopliae* scored 79.7% by the same dosage comp-ared to the control trial, at reduction in emerged adults of 0.8% (Table 1).

On adult

As shown in Table (2), it is evident that the application of *B. bassiana* powders did not result in any mortality in the adult stage on the third and fifth days after exposure, across all tested concentrations. However, there was a clear positive relationship between mortality and the duration of exposure. On the 21st day following exposure to *B. bassiana* concentrations of 0.1, 0.2, 0.4, 0.6, and 0.8% (w/w), the mean nu-mber of adult mortality significantly increased ($p \leq 0.01$) to 1.4± 0.11, 2.9± 0.17, 5.7± 0.17, 10± 0.17, and 11.4± 0.17, respectively. Meanwhile, *M. aniso-pliae* at 0.1 and 0.2% (w/w) did not cause any mortality on the third and fifth days after exposure. However, the mean number of mortalities increased significantly ($p \leq 0.01$) with the increase of exposure period to 4.3± 0.23 and 7.1± 0.20 after 21st days of treatment. Concentrations of 0.4, 0.6, and 0.8% (w/w) resulted in 1.3±0.06, 1.3± 0.12, and 2.7± 0.12 adult mortality on the third day of exposure and signify-cantly increased ($p \leq 0.01$) to 10± 0.17, 11.4± 0.17, and 15.7± 0.23 mortality on the 21st day of exposure (Table 2). The highest reduction percentage in emerging adults (F1)

was 51.3 % and 83.1% after applying concentration of 0.6 and 0.8 of *B. bassiana*, respect-tively. Identical results were recorded for the same concentrations of *M. anisopliae* the reduction percent in emerging adult reached to 69.0 and 71.8 %, respectively.

Effect of releasing different densities of X. flavipes adult in adult emergence of T. castaneum

The efficacy of adult stage of *X. flavipes* as a bio-agent in reducing F1 progeny after predation on different immature stages of *T. castaneum* was studied and the obtained data were presented in Table (3). In general, there is an inverse relationship between the mean number of emerging adults following immature predation and the number of predatory bugs. The use of 20 bug individuals resulted in adult emergence of 0.25±0.3, 0.5±0.3 and 14.5±0.9 for egg stage, 3rd larval instar and pupae, respectively. Meanwhile, the lowest density of bug resulted in 1±0.6, 0.75±0.3, 31±0.8 adult emergences following predation on egg stage, 3rd larval instar and pupae, respectively. The mean number of emerged adults and percent of reduction did not demonstrate a significant difference between predator insect densities for both eggs and the third larval instar, but the differences were obvious for pupal stage. The percentage of reduction in emerging adults after releasing different densities of *X. flavipes* adults showed its maximum values, at 99.4%, 98.8% and 68.5% for eggs, 3rd larval instar and pupal stage, respectively following the release of 20 bug individuals.

Predation preference of X. flavipes on immature stages of T. castaneum

Treatments of different immature stages of *T. castaneum* with three individuals of *X. flavipes* adults at concentration, resulted in a significant (P<0.05) increase in mean number of immature stages consumed at 22.8±0.6,12.8±0.8 and 2.8±0.3 on eggs, larvae, and pupae that equivalent to 45.5%, 25.5% and 5.5% predation percentage, respectively after 24 h. post releasing of the predator bug (Table 4). The results revealed that the highest predation % was recorded for eggs, followed by the third larval instar and lastly by the pupal stage. The preference of X. flavipes for *T. castaneum* immature stages can be organized in the following descending order: eggs stage> third larval instar> pupal stage.

DISCUSSION

Biological control strategies are now thought to be useful in the realm of pest management. They naturally occur with low mammalian toxicity. Our findings demonstrated that *T. castaneum* mortality was primarily concentration dependent, increasing as concentrations increased but not significantly in adult insects. The results clearly indicated that both tested fungi led to higher mortality percent in third instar larvae compared with adult stage at all the tested concentrations. *B. bassiana* elicited a higher mortality percentage against third instar larvae at the end of exposure while *M. anisopliae* showed a higher mortality percentage in the case of adults.

The current study's findings are consistent with those of Akbar *et al.* (2004), who observed that *B. bassiana* inoculation has low effect on *T. castaneum* adults. *B. bassiana* showed low virulence towards *Tribolium* species (Wakefield, 2006; Golshan *et al*., 2014). High dose requirement of *B. bassiana* for stored grain pests has been reported for *T. castaneum* (Padin *et al.*, 2002; Cherry *et al*., 2005). After the seventh day of inoculation, Akmal *et al.* (2020) found that *B. bassiana* had a poor infectivity on *T. castaneum* adults. Long exposure and the highest concentrations of B. *bassiana* had been required against *T. castaneum* (Rizwan *et al.*, 2019). *T. castaneum* adults and larvae were sensitive to higher concentrations of *B. bassiana* Meanwhile, long lived adults' stages exhibit clear resistance to infection (Abid Al-Zufri, 2019). Bashir *et al*. (2018) demonstrated the effectiveness of a commercial formulation of *B. bassiana* (Racer® BB 1.15% WP) against rice meal moth, *Corcyra cephalonica*, and red flour beetle, *T. castaneum*. The low virulence of tested entomopathogenic fungi towards *T. castaneum* could be attributed to the fact that *Tribolium* species are known to create defensive quinones that may hinder fungal germination (Golshan *et al.*, 2014).

According to these findings, the conidial powders of the two investigated fungi maintained higher virulence against the third larval instar of *T. castaneum* than the adult. As well as *M. anisoplia* had greater bio-insecticidal efficacy against both the third larval instar and the adult of *T. castaneum* than *B. bassiana*. Similar results reported that, when *B. bassiana* or *M. anisopliae* were applied to *S. zeamais*, the latter resulted in increased mortality and a lower median survival time (Teshome and Tefera, 2009). *M. anisopliae* was extremely efficient against *T. confusum* larvae because its larvae eat and develop in the exterior region of the seed, increasing the probability of picking up conidia (Kavallieratos *et al.*, 2006).

Teshome and Tefera (2009) found that increased the fungal concentration of both *M. anisopliae* and *B. bassiana* isolates against *S. zeamais* increased the amount of conidia attached to the insect. The weak progression of infection at lower doses of *B. bassiana* caused lower mortality of *Plutella xylostella* (Yoon *et al.*, 1999). In the current study also found a high reduction percentage in F1 progeny after inoculation with both fungi. Similarly, *M. anisopliae* significantly inhibited adult emergence and produced few progenies after adult treatment, as reported by Kavallieratos *et al.* (2006), who estimated that very few *T. confusum* progenies were detected in wheat treated with a high rate of *M. anisopliae*.

Fungi spores develop on the cuticle of the insect host, enter it, and disseminate throughout the body. Furthermore, they can secrete lytic enzymes including protease, chitinase and lipase that may play a significant

Table (1): Comparative efficacy of fungal treatments using *B. bassiana* and *M. anisopliae*, separately, on *T. castaneum* 3rd larval instar. Mortality percent was assessed at different time intervals ranging from 3 to 21 days, after which the survived emerged adults were counted, and the reduction percent was calculated compared to control. All data recorded are presented as mean ± SE (standard error).

Treatments	Dose Conc. (W/W)	Mortality %					Mean number of survived adults	Reduction in emerged adults (%)
		Treatment intervals (day)						
		3	5	7	14	21		
Control	-	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	19.7±0.29^{a}	-
Beauveria bassiana	0.1	1.3±0.12^{e}	1.3±0.03^{e}	1.4±0.05^{e}	9.1±0.17e	10.8±0.12^{e}	4.0±0.57^{b}	79.7
	0.2	2.7±0.12^{d}	2.7±0.17^{d}	2.8±0.11^{d}	10.6±0.12^{d}	12.3±0.17^{d}	3.7±0.28^{c}	81.2
	0.4	4.0±0.23^{c}	4.1±0.05^{c}	4.2±0.15^{c}	12.1±0.17c	15.4±0.12^{c}	3.0±0.57^{d}	84.8
	0.6	5.3±0.17^{b}	5.5±0.17^{b}	5.6±0.17^{b}	18.2±0.12^{b}	20.0±0.23^{b}	2.3± 0.17^{e}	88.3
	0.8	6.7±0.12^{a}	6.8±0.17^{a}	7.0±0.11^{a}	19.7±0.12^{a}	26.2±0.11^{a}	2.0±0.57^{f}	89.9
LSD 0.05	-	0.443	0.385	0.363	0.402	0.441	0.442	-
***p* value**	-	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	-
Control	-	0.0±0.0^{e}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	19.7±0.29^{a}	-
Metarhizium anisopliae	0.1	1.3±0.06^{d}	2.7±0.12^{e}	2.7±0.17^{e}	5.8±0.17e	6.0±0.17^{e}	5.7±0.38^{b}	71.1
	0.2	2.7±0.12^{c}	4.0±0.17^{d}	4.1±0.12^{d}	7.2±0.12d	7.6±0.17^{d}	5.0±0.57^{c}	74.6
	0.4	4.0±0.12^{b}	6.7±0.17^{c}	8.2±0.23^{c}	8.7±0.1c	12.4±0.23^{c}	4.7±0.12^{d}	76.1
	0.6	4.0±0.17^{b}	8.0±0.17^{b}	9.6±0.12^{b}	10.1±0.12b	15.2±0.26^{b}	4.3±0. 17^{e}	78.2
	0.8	6.7±0.17^{a}	10.7±0.12^{a}	13.7±0.17^{a}	17.4±0.7a	25.8±0.37^{a}	4.0± 0.58^{f}	79.7
LSD 0.05	-	0.377	0.429	0.47	0.391	0.71	0.396	-
p value	-	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	-

Means followed by the same letter, per column for each fungus, are not significantly different at level $p \leq 0.05$, based on Duncan Multiple Range test and LSD test.

Table (2): Comparative efficacy of fungal treatments using *B. bassiana* and *M. anisopliae*, separately, on adult stage of *T. castaneum*. Mortality percent was assessed at different time intervals ranging from 3 to 21 days. All data recorded are presented as mean ± SE (standard error). In control group, no treatment was applied.

Treatments	Conc. % (W/W)	Mortality %					Mean number of survived adults	Reduction % in emerged adults
		Treatment intervals (day)						
		3	5	7	14	21		
Control	**0.0**	0.0±0.0^{e}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	35.5± 0.20	0.0
Beauveria bassiana	**0.1**	0.0±0.0^{e}	0.0±0.0^{e}	1.3±0.12^{e}	1.4±0.12^{e}	1.4±0.11^{e}	20.3± 0.35^{a}	42.8
	0.2	0.0±0.0^{e}	0.0±0.0^{e}	2.3±0.17^{d}	2.8±0.11^{d}	2.9±0.17^{d}	18.7± 0.12^{b}	47.3
	0.4	0.0±0.0^{e}	0.0±0.0^{e}	3.5±0.11^{c}	4.2±0.14^{c}	5.7±0.17^{c}	18.0± 0.69^{c}	49.3
	0.6	0.0±0.0^{e}	0.0±0.0^{e}	4.3±0.17^{b}	5.6±0.05^{b}	10±0.17^{b}	17.3± 0.28^{d}	51.3
	0.8	0.0±0.0^{e}	0.0±0.0^{e}	5.3±0.17^{a}	8.3±0.10^{a}	11.4±0.17^{a}	6.0± 0.34^{e}	83.1
LSD 0.05	**-**	-	-	0.429	0.676	0.459	0.433	-
***p* value**	**-**	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	-
Control	**0.0**	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{f}	35.5± 0.20^{a}	0.0
Metarhizium anisopliae	**0.1**	0.0±0.0^{c}	0.0±0.0^{d}	1.4±0.11^{e}	2.9±0.23^{e}	4.3±0.23^{e}	12.3± 0.23^{b}	65.4
	0.2	0.0±0.0^{c}	0.0±0.0^{d}	2.8±0.03^{d}	5.7±0.17^{d}	7.1±0.20^{d}	12.0± 0.18^{bc}	66.2
	0.4	1.3±0.06^{b}	1.3±0.12^{c}	4.2±0.12^{c}	7.1±0.12^{c}	10±0.17^{c}	11.7± 0.43^{c}	67
	0.6	1.3±0.12^{b}	2.7±0.12^{b}	5.6±0.20^{b}	8.6±0.12^{b}	11.4±0.17^{b}	11.0± 0.23^{d}	69
	0.8	2.7±0.12^{a}	6.7±0.17^{a}	9.7±0.23^{a}	10±1.0^{a}	15.7±0.23^{a}	10.0± 0.05^{e}	71.8
LSD 0.05	**-**	0.221	0.622	0.445	0.432	0.671	0.322	-
***p* value**	**-**	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	-

Means followed by the same letter, per column for each fungus, are not significantly different at level $p \leq 0.05$, based on Duncan Multiple Range test and LSD test.

Table (3): Predation percentage on immature stages of *T. castaneum* by three individuals of *X. flavipes* adults after 24 hr. All data recorded are presented as mean ± SE (standard error).

No. of *X. flavipes* individuals	Mean number of survived emerged adults			Reduction % in emerged adults		
	Developmental stage					
	Eggs	Larvae (3rd instar)	Pupae	Eggs	Larvae (3rd instar)	Pupae
Control	40.5±1.9^{a}	41.50±0.9^{a}	46.00±0.0^{a}	–	–	–
5	1.00±0.6^{bc}	0.75±0.3^{b}	31.00±0.8^{b}	97.50	98.20	32.60
10	0.75±0.5^{b}	0.75±0.5^{b}	24.00±0.7^{c}	98.10	98.20	47.80
15	0.50±0.3^{b}	0.75±0.3^{b}	17.30±0.9^{d}	98.80	98.20	62.50
20	0.25±0.3^{b}	0.50±0.3^{b}	14.50±0.9^{e}	99.40	98.80	68.50

Means followed by the same letter, per column for each fungus, are not significantly different at level $p \leq 0.05$, based on Duncan Multiple Range test.

Table (4): Predation percentage on immature stages of *T. castaneum* by three individuals of *X. flavipes* adults after 24 hrs. All data recorded are presented as mean ± SE (standard error) of four replicates.

No. of *X. flavipes* individuals	Mean number of survived emerged adults			Reduction % in emerged adults		
	Developmental stage					
	Eggs	Larvae (3rd instar)	Pupae	Eggs	Larvae (3rd instar)	Pupae
Control	0.0	0.0	0.0	-	-	-
3	22.8±0.6[a]	12.8±0.8[b]	2.8±0.3[c]	45.5	25.5	5.5

Means followed by the same letter, per raw, are not significantly different at level $p \leq 0.05$, based on Duncan Multiple Range test.

part in the process of tissue damage and enable hyphal growth (Sheppared and Filler, 2015; Wang *et al.,* 2021). They can also kill pests by producing neuromuscular toxic metabolites that hurt the central nervous system, resulting in paralysis at the end of infection (Butt *et al.,* 2001; Benítez *et al.,* 2004; Sandhu *et al.,* 2012; Jennifer *et al.,* 2014). Microbial biocontrol agents applied to stored grain insects can be an effective and environmentally benign alternative technique. Most entomopathogenic fungi are more selective to target insects and have lower human toxicity (Islam *et al.,* 2021), allowing them to be used effectively in pest management programs.

The findings of the choice test after releasing a specific number of X. flavipes individuals revealed that the highest preference of *X. flavipes* towards immature stages of *T. castaneum* was recorded for eggs stage then third larval instar and lastly, pupal stage. It is obvious that *X. flavipes* might manage the *T. castaneum* beetle infestation by suppressing its life cycle, hence preventing buildup of pest population. The efficiency of *X. flavipes* in suppressing immature stages of *T. castaneum* can be arranged in the following descending order; eggs stage> third larval instar >pupal stage. As a result, this natural enemy plays a substantial role in lowering damage produced by *T. castaneum* through reducing the number of F1 progeny of immature stages this insect pest, such results could be incorporated in an integrated pest management program for *T. castaneum* control.

The difference in size and cuticle hardening in the immature stages may have contributed to predator choice, as the eggs and third larval stages were more accessible than pupae. Arbogast (1979) obtained similar results, declaring that the preference of *X. flavipes* for prey was based on prey size, vestiture, degree of sclerotization, and defensive behaviour. Lecato (1976) reported that *X. flavipes* consumed more *T. castaneum* than *A. megatoma* regardless of whether the two species were subjected separately or together to some factors like larger size, heavier sclerotization and heavier integument of *A. megatoma*. Because of their small sizes, *X. flavipes* consumed more *C. pusillus* larvae (small and large) than *T. castaneum* and *T. confusum* larvae (Rahman *et al.,* 2009). The early stages of larger species and the late stages of the smaller ones, due to the size and thrashing movement that intimidate predator, *X. flavipes* when attack *T. castaneum* and *P. interpunctella* larvae furthermore hirsute nature of *L. serricorne* larvae and media adhered to their bodies reduced predation (LeCato and Davis, 1973). The eggs and neonate larvae of *A. obtectus* were the only accessible immature stages by *X. flavipes* among many species and predation was greater on them than on any adult bruchids (Sing and Arbogast, 2008 a). As well, *Anisopteromalus calandrae* has great efficacy for control of *Lasioderma. serricorne* larval and pupal stages infestation (Guo *et al.,* 2021).

The current results of the non-choice test after the introduction of different densities of *X. flavipes* demonstrated that *X. flavipes* has the ability of suppressing the progeny of *T. castaneum*. These findings corroborated those reported by (Brower and Mullen, 1990; Russo *et al.,* 2004), who mentioned that *X. flavipes* is a predator capable of regulating storage pests through feeding on eggs, larvae and pupae of beetles and moths that infest stored product. Similarly, *X. flavipes* was completely able to suppress the populations of *T. castaneum*, *O. surinamensis* and *E. cautella* **(**Lecato *et al.,* 1977).

According to Press *et al.* (1975), *X. flavipes* was able to control the population of *T. castaneum* infesting peanut kernels in bins. It was apparent that increasing predator densities had a favorable effect on both predation rate and reduction percent in F1 progeny. It is possible that the small size and sclerotization of pupae contributed to the lower suppression percentage compared to other immatures, as well as the large disparity in predator densities in terms of devoured prey. Thus, the pupal stage predation could have been triggered by the non-choice test, in which only pupae were accessible to prey. Similarly, (LeCato, 1976; Sing and Arbogast, 2008a) said that *X. flavipes* was capable of successfully attacking huge, sclerotized prey when more accessible prey was unavailable, and this was observed with all bruchid species. *X. flavipes* reduced *R. dominica* progeny in wheat more than *S. oryzae*. This could be because larvae of *R. dominica* are usually found inside grain kernels injured by other species, but *S. oryzae* larvae remain inside the kernel permanently (Adarkwah *et al.,* 2019).

CONCLUSION

The results of this research have confirmed the potential application of entomopathogenic fungi, specifically *B. bassiana* and *M. anisopliae*, along with the predatory bug *X. flavipes*, for effectively controlling *T. castaneum*. These findings indicate that the utilization of these natural agents holds promise as an alternative tactic in combating stored product pests. Implementing such materials in pest management strategies offers a promising approach towards achieving the future global vision of promoting a vibrant society with a focus on a healthy lifestyle. This can be accomplished by reducing reliance on traditional insecticides and adopting natural mediators.

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تأثير بعض عوامل المكافحه البيولوجيه علي خنفساء الدقيق الحمراء *Tribolium castaneum* (غمدية الأجنحة : Tenebrionidae)

كريمة شكري خاطر[1]، ابراهيم فتحي شكري[1]، عبد العزيز السيد عبد العزيز[2]، نورا عدلي سعد[2]
[1]قسم علم الحيوان، كلية العلوم، جامعة الزقازيق، الزقازيق، مصر
[2]معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى، مصر

الملخـص العربـي

خنفساء الدقيق الصدئيه، *Tribolium castaneum* (هيربست)، هي آفة واسعة الانتشار وشديدة الضرر في جميع أنحاء الكون. وهي آفة خطيرة تصيب الحبوب ومنتجاتها الغذائية كالدقيق. تمت دراسة تأثير اثنين من الفطريات الممرضة للحشرات , *Beauveria bassiana* (EPF) و*Metarhizium anisopliae*، بالإضافة إلى حشرة مفترسة *Xylocoris Flavipes* (Reuter)، على *T. castaneum* أشارت النتائج إلى أن *B. bassiana* كان أكثر سمية في الطور اليرقي الثالث لـ *T. castaneum* من *M. anisopliae* ، في حين كان الأخير هو الأكثر سمية الحشرات البالغة. أظهر تفضيل الاختبار الحيوي باستخدام *X. flavipes* للتحكم في مراحل مختلفة غير ناضجة من *T.castaneum* أن مرحلة البيض كانت الأكثر تفضيلاً. يمكن تنظيم تفضيلات *X. flavipes* بالترتيب التنازلي التالي: مرحلة البيض، والطور اليرقي الثالث، ومرحلة العذراء. وقد وجد أيضًا أنه تم تحقيق انخفاض كبير في الجيل الأول من *T. castaneum* بعد افتراس الكائنات غير الناضجة بواسطة *X. flavipes* أشارت القدرة القمعية لفطري *B. bassiana* و *M. anisopliae* و *X. flavipes* على بيولوجيا *T. castaneum* إلى أنه يمكن اعتبارهما عوامل صديقة للبيئة ضد هذه الآفة الحشرية الخطيرة اقتصاديًا، ويمكن إدراجهما في قائمة برامج المكافحه المتاكامله للافات.