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ENTOMOPATHOGENICITY OF *Beauveria bassiana* AGAINST IMMATURE LIFE STAGES OF ALMOND MOTH, *Cadra cautella* (LEPIDOPTERA: PYRALIDAE)

Waleed S. Alwaneen[1], Mureed Husain[2,*] , Koko D. Sutanto[2], Khawaja G. Rasool[2], Khalid Mehmood[2], Muhammad Tufail[3] and Abdulrahman S. Aldawood[2]

[1]National Center for Agricultural Technology (NCAT), King Abdulaziz City for Science and Technology (KACST), P.O. Box 6086, Riyadh, Saudi Arabia; [2]Economic Entomology Research Unit, Department of Plant Protection, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, P.O. Box 2460, Riyadh 11451; [3]Ghazi University, Dera Ghazi Khan, Punjab, Pakistan.
***Corresponding author's e-mail: mbukhsh@ksu.edu.sa**

Agricultural products remain under threat from biotic and abiotic factors from production to storage. For example, stored grains, pulses, legumes, and dates are exposed to the attack by several insect pests during storage. The almond moth, *Cadra cautella* (Walker) is an important cosmopolitan insect that can cause damage to dates under storage conditions which causes significant losses to the famers. We tested the pathogenicity of indigenous entomopathogenic fungus isolates against the immature stages of *C. cautella*. The *Beauveria bassiana* (Balsamo) Vuillemin, BbSA-1 isolate (originally from a coleopteran cadaver), BbSA-2 isolate (from a lepidopteran cadaver), and natural *B. bassiana* (the NBVR isolate; commercial) were screened against different developmental stages. The developmental stages were directly exposed to each fungus isolate at a concentration of 1×10^7 conidia/mL. It was observed that all fungus isolates affected the hatchability of eggs, caused significant larval and pupal mortality. The NBVR isolate was the most pathogenic to *C. cautella* eggs, whereas BbSA-2 demonstrated the highest pathogenicity against *C. cautella* larvae and pupae. The following order of susceptibility was observed: pupae > larvae > eggs. Data from this study indicated the importance of this fungus to be used as environmentally friendly of management tool to control *C. cautella* in dates store houses.
Keywords: Stored pest, *Cadra cautella*, entomopathogenic fungi, *Beauveria bassiana*, infection.

INTRODUCTION

The postharvest losses of agricultural produce are an important food security threat. As with other agricultural crops, date palm has a strong economic significance and is grown in many countries around the world, including the Kingdom of Saudi Arabia (Chao and Krueger, 2007). Kingdom of Saudi Arabia produces dates worth of several billion Saudi Riyals/year (MEWA, 2017) and are grown on 108133 hectares with an annual production of 7,54,761 tons of fruits. Many farmers sell their dates directly whereas others keep their produce in ordinary farm storage to sell these later. Many farmers are unaware of the proper storage and fumigation methods required for dates (Al-Abbad *et al.,* 2011). Although many pests attack dates, the threat of almond moth, *Cadra cautella* (Walker) (Lepidoptera: pyralidae) is extremely acute and necessitates measure to ensure the security and quality (Rostom, 1993; Arbogast *et al.,* 2005; Al-zadjali *et al.,* 2006; Aldawood *et al.,* 2013). The life cycle of *C. cautella* ranges from 50 to 55 d at a temperature of 25°C. The larval stage has six instars and the larval period depends on condition of rearing. The last larval instar is of longer duration than that of the earlier instars. The larvae eat extensively, causing marked damage to stored dates. In the last instars, the larvae emerge from a date, and searches for a suitable location to pupate. The development time and reproductive success depends on the food supply and the environmental rearing conditions (Mankin *et al.,* 2014; Husain *et al.,* 2017a).

A number of control methods are used to control the storage food pests The main management tools include botanical extracts/oil (Ayvaz *et al.,* 2010; Chu *et al.,* 2013), heat treatment (Hulasare *et al.,* 2010), freezing treatment (Collins and Conyers, 2010), and atmospheric modification (Soderstrom *et al.,* 1990, Donahaye *et al.,* 1995, Navarro, 2012; Husain *et al.,* 2017b; Rasool *et al.,* 2017). Nevertheless, none of these methods has been accepted as a promising solution for handling *C. cautella*. Hence, there is a need to find an environmentally benign technique.

Several groups of fungi are infective to insect species. Such entomopathogenic fungi are known to be capable to kill their insect hosts. These fungi are environmentally friendly, easily mass-produced, and cost-effective for insect pest management (Senthamizhlselvan *et al.,* 2010; Latifian *et al.,* 2013). The *Beauveria bassiana* has been reported to be pathogenic to several stored product insect species (Rumbos

and Athanassiou, 2017; Bashir *et al.*, 2018), as well as to field crops (Bayu and Prayogo, 2018; Akutse *et al.*, 2019) and forest products (Ozdemir *et al.*, 2019).

Indigenous isolates of this fungus are reported to be more virulent. In the United Kingdom, 1×10^8 conidia/mL dose of local *B. bassiana* isolates killed 86–100% within 7 days' post treatment (Cox *et al.*, 2004). Sabbour *et al.* (2012) reported that *B. bassiana* was the most effective fungus against the third instar larvae of three pyralid moth species. Rice treated with *B. bassiana* @ 9×10^7 conidia/kg in storage produced 80% mortality in third instar larvae of *Corcyra cephalonica* at 21 days' post exposure (Bashir *et al.*, 2018).

Beauveria bassiana has been used for the management of several coleopteran species relevant to stored agricultural commodities (Throne and Lord, 2004; Athanassiou and Steenberg, 2007; Lord, 2009; Storm *et al.*, 2016). e., the use of *B. bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) entomopathogenic fungi, has been evaluated as a biological control against *C. cautella*.

The present study reports the efficacy of *B. bassiana* against different developmental stages of almond moth, *C. cautella*.

MATERIALS AND METHODS

Cadra cautella larvae rearing: The insects were reared in the Economic Entomology Research Unit (EERU), at 25 ± 1°C, 65 ± 5% RH, and photoperiod: 10:14 h L/D. The test insects were reared on a previously used poultry feed based an artificial diet. Ten pairs of newly emerged adult moths placed inside a 250-g plastic cup and allowed to mate and produce eggs. The neonates were placed in a 50-g capacity plastic cup and the larvae were provided with 2 g of an artificial diet. The artificial diet consists of poultry feed for broiler and layer birds, wheat flour and glycerin already used by (Aldawood *et al.*, 2013).

The larvae were placed inside an incubator and allowed to develop. Larvae of 8- and 15-d were placed in a new 50 g plastic cup, starved for two hours, and then used for bioassay. Some larvae were allowed to fully grow and molt into pupae. 1- and 5-d-old pupae were used in these bioassay.

Beauveria bassiana Saudi Arabian Isolates (BbSA):The fungus *B. bassiana* isolates were obtained from Saudi Arabia's Ministry of Environment, Water, and Agriculture. These isolates were originally collected from the Al-Qathif region. The isolates were coded in the EERU laboratory, grown on a potato dextrose agar (PDA) medium, and purified. The isolates were preliminarily labeled and screened for virulence, and the most virulent isolates were tested against various stages of *C. cautella*. The studied isolates were originally from host cadavers of coleopteran insects (BbSA-1) and lepidopteran insects (BbSA-2). This was a key justification for using these two indigenous isolates, along with the natural *B. bassiana* Indonesia (NBVR) isolate, which is commercially available.

Mass production of Beauveria bassiana: The most virulent entomopathogenic isolates of Saudi Arabian *B. bassiana* (BbSA) were cultivated on autoclaved barley for mass production following the protocol of Latifian *et al.* (2013). Small amounts of BbSA isolate primarily grown using PDA were thoroughly mixed into the barley and inoculated for 15 days at 28 ± 1°C to achieve germination. When the fungus was fully grown, the barley medium was dried in an incubator for one day. With the aid of a hemocytometer, the final concentration of fungus spores was determined to be 1×10^7 conidia/mL.

Bioassay for Cadra cautella eggs: Fresh laid eggs were collected and examined under a microscope to select healthy eggs. For this bioassay we used 1-d- and 3-d-old eggs. The fungus isolates were gently scratched from the mass-cultivated petri dishes and spread on the eggs. One hundred eggs of either age were placed in 50-g plastic cups and all of the fungal isolates under consideration were spread on the eggs. Four replicates were conducted with 100 eggs of either age. The treated eggs were examined daily until all of the eggs in the control treatment had hatched.

***Bioassay against larvae and pupae of Cadra cautella*:** The larvae and pupae of *C. cautella* from the different age groups were directly contaminated with fungus spores of all the tested fungal isolates for 5 minutes. These larvae and pupae were placed in a 50-g plastic cup, and 1-g of diet was provided for the larvae. There were four replicates for each isolate of the fungus with10 larvae and 5 pupae in each replicate. After bioassay, all the cups were kept in an incubator at 26 ± 1°C and 65 ± 5% RH and mortality data were collected.

Data Collection: The data were collected after 72 hours when all eggs in the control group had hatched. Larvae and pupae mortality data were collected until all the individuals in the control treatment had molted to the next stage. The larvae/pupae showing fungus growth symptoms or a lack of movement were softly touched with a camel-hair brush to confirm death. The suspect larvae were also observed under a microscope. Signs of mortality were lack of movement, body shrinkage, and color change (Husain *et al.*, 2015). For larvae that died after treatment, mortality was confirmed post-incubation after observing clear symptoms of fungus hyphae growth on the cadaver body. The dead larvae were found with clear symptoms of mortality resulting from fungus attack. The color of the infected larvae was reddish-pink/brown, whereas the healthy larvae were white/cream. The dead larvae/pupae were collected, sterilized for one min. in 75% ethanol, and incubated at 28°C and 65 ± 5% before fungus growth was confirmed, the growth of hyphae was observed after 24 h of incubation. The entire cadaver body was covered with mycelium growth within three days of incubation. Larvae and pupae that died but did not show the growth of the fungus were not counted as dead by fungus.

A completely randomized design was used. The analysis of variance was done by using SAS Institute, (2009). Larval mortality was considered the response variable and fungus was considered the key effect.

RESULTS

Pathogenic effects against Cadra cautella eggs: Fungus treated *C. cautella* eggs of various ages were observed every day till the eggs in control group had hatched. Although lower hatchability was observed in all treatments against all ages when compared with the control, eggs were not the most vulnerable stage. Although fungus isolates did not show significant difference in hatchability, the results were significantly different than control. The highest egg hatching failure (up to 42%) was observed in 1-d-old eggs treated with NBVR which is an indigenous fungus isolates. The differences in eggs hatchability between the fungus isolate treatment and the control were highly significant (Table 1).

Table 1. Mean mortality (%±SE) of *Cadra cautella* eggs treated with different isolates of *Beauveria bassiana* under laboratory conditions.

Eggs age	Fungus isolates	Mortality (mean ± SE)	ANOVA Parameters			
			N	*F*	df	*P*
1-d-old	BbSA-1	29.00±3.3b	4	24.37	3,15	<0.0001
	BbSA-2	38.25±3.03a	4			
	Natural BVR	42.50±3.30a	4			
	Control	9.25±2.13c	4			
3-d-old	BbSA-1	20.00±1.41c	4	17.96	3,15	<0.0001
	BbSA-2	29.75±2.95b	4			
	Natural BVR	40.25±3.63ba	4			
	Control	10.75±3.44d	4			

Means with the same letter within each age group are not significant ($\alpha = 0.05$).

***Pathogenic effects against Cadra cautella larvae*:** Larval stage is considered to be the most damaging in terms of the destruction of agricultural food resources. *Cadra cautella* has many larval instars; we used larvae of the third and fifth instars to test the pathogenicity of fungus isolates against young and full-grown larvae. The results of larval mortality are presented in Table 2. It is evident from the results that the third instar larvae were more tolerant to the fungus than fifth instars. In the BbSA-2 treatment, highest mortality (75%) was found in the fifth instar larvae, whereas only 27% larvae died in with BbSA-2 treatment.

Pathogenic effects against Cadra cautella pupae: Although pupal stage in insects does not cause any damage, pupae is an important link in the life cycle. There is much physiological activity at the pupal stage. In *C. cautella*, adults begin to mate and start laying eggs within 1 or 2 days eclosion. The results for the effectiveness of the different fungus isolates against pupae are presented in Table 3. The results indicated that 1-d-old pupae were more vulnerable to the fungus than 5-d-old pupae. BbSA-2 treatment showed highest mortality (90%) against 1-d-old pupae whereas mortality was only 50% in 5-d-old pupae.

In general, the BbSA-1 isolate showed lowest mortality against all stages of *C. cautella*, whereas the BbSA-2 isolate proved to be the most pathogenic to both larval and pupal stages. Overall mortality was lowest in larval stages as compared to eggs or pupae. These results indicate that all of the fungus isolates have the potential to be effective in *C. cautella* management.

Table 2. Mean mortality (%±SE) of *Cadra cautella* larvae treated with different isolates of *Beauveria bassiana* under laboratory conditions.

Larvae age	Fungus isolates	Mortality (mean ± SE)	ANOVA Parameters			
			N	*F*	df	*P*
8-d-old	BbSA-1	10.00±4.08bc	4	8.43	3,15	0.0028
	BbSA-2	20.00±5.77ab	4			
	Natural BVR	25.00±2.88a	4			
	Control	0.00±0.00c	4			
15-d-old	BbSA-1	27.50±2.50b	4	59.97	3,15	<0.0001
	BbSA-2	75.00±5.00a	4			
	Natural Bb	37.50±4.78b	4			
	Control	2.50±2.50c	4			

Means with the same letter within each age group are not significant ($\alpha = 0.05$).

Table 3. Mean mortality (%±SE) of *Cadra cautella* pupae treated with different isolates of *Beauveria bassiana* under laboratory conditions.

Pupae age	Fungus isolates	Mortality (mean ± SE)	ANOVA Parameters			
			N	*F*	df	*P*
1-d-old	BbSA-1	10.00±5.77bc	4	41.42	3,15	<0.0001
	BbSA-2	90.00±10.00a	4			
	Natural BVR	25.00±5.00b	4			
	Control	0.00±0.00c	4			
5-d-old	BbSA-1	5.00±5.00b	4	5.23	3,15	0.0154
	BbSA-2	60.00±23.09a	4			
	Natural BVR	10.00±5.77b	4			
	Control	0.00±0.00b	4			

Means with the same letterwithin each age group are not significant ($\alpha = 0.05$).

DISCUSSION

The *Beauveria bassiana* isolates showed pathogenicity against all *Cadra cautella* developmental stages. The NBVR isolate showed high virulence against C. *cautella* eggs as compared to other fungus isolates. In the present study, the NBVR isolate caused 42% egg mortality which corroborates the findings of Lord *et al. (*2009) who reported 40–90% egg mortality in coleopteran species of stored grain insects applied at a rate of 1.0×10^5 conidia/mm^2. The low mortality of *C. cautella* eggs may have been due to the short incubation period. An extended incubation period where we owe the fungus more time to be in storage; may provide ample time for the fungus to enter the developing embryo. *Plodia*

interpunctella eggs and larvae sprayed with *B. bassiana* spores (1.2×10^7conidia/mL), produced maximum mortality of 41 and 17% in eggs and larvae, respectively (Sedehi *et al.,* 2014).

Similarly, *P. interpunctella* larvae treated with *B. bassiana* (1.1×10^7 conidia/mg) mixed into the diet showed 61% mortality (Arooni-Hesari *et al.,* 2015). These results strongly support the findings of this study reporting 42 and 75% mortality in eggs and larvae, respectively. In contrast with beetles, it is worth noting that only a few studies have reported the pathogenicity of fungi against moths in stored products. Several studies have concluded that *B. bassiana* is highly efficient and causes severe mortality in different developmental stages of beetles and weevils that cause heavy damage to stored agricultural commodities (Akbar *et al.,* 2005; Batta, 2008; Golshan *et al.,* 2014; Batta and Kavallieratos, 2018). In the present study, the BbSA-2 isolate was found to be most pathogenic against the larvae and pupae of *C. cautella*. The high mortality of BbSA-2 isolate can be attributed to the fact that it was originally obtained from a moth cadaver. The importance of indigenous fungus isolates has been highlighted in the literature and it has been concluded that indigenous isolates are more likely to respond to local environments (Ak, 2019; Gava *et al.,* 2020).

The fungus penetrates the insect cuticle (Gabarty *et al.,* 2014; Mora *et al.,* 2017). The low mortality in eggs than larvae and pupae may have been due to a strong chorion defense that impedes penetration of the fungus mycelium and leads to low infectivity (Wu *et al.,* 2020). Likewise, the larval body is often coated with wax and scales, whereas *C. cautella* pupae are naked with a simple outer cuticle case that may promote the adherence and penetration of the fungus spores. Results from this study corroborated the findings of Al-Deghairi (2008) who reported infection level of up to 4.49% at 6×10^6conidia/mL dose of B. *bassiana* against whitefly eggs. The insecticidal activity of the *B. bassiana*NBAII-Bb-5a isolate showed 20% larval mortality and 60% ovipositional reduction in *Sitophilus oryzae* (Kaur *et al.,* 2014).

A limited number of research studies explored the insecticidal activities of entomopathogenic fungus isolates against the immature stages of insects. Most studies have reported the efficacy of *B. bassiana* isolates against the adult stages of coleopteran species relevant to stored products (Rumbos *et al.,* 2017; Mantzoukas *et al.,* 2019). It is very important to understand the biology of a specific pest before deciding on pest management strategies (Husain *et al.,* 2020). The present study provides some useful information that can used in *C. cautella* management.

This pest offers a farmer an advantage because unlike coleopteran stored product insect species, the adult does not remain inside food commodities; rather, it flies into the storages arena, copulates, and eggs are laid on the external surface of the food items (Aldawood *et al.,* 2013; Husain *et al.,* 2017a). Similarly, larvae come out of the food during last larval instars before finding an appropriate location for pupation. We would like to highlight the importance of pest behavior in the above-mentioned discussion, which underpins the management options. In short, the eggs are laid on the outside of the food product, larvae are mobile, and pupae are exposed in cracks and crevices; these attributes favor the effectiveness of fungus spores applied as a fine powder, as practiced in this study. Further work is required to explore the efficacy of other fungus isolates and apply this technique to storage in a more realistic manner.

***Conclusion*:** Our results demonstrated the potential of indigenous fungus isolates for *C. cautella* management and suggest that future studies should pay attention to additional aspects like application methods, environment in stores, mass production, residues, and health effects of the fungus. The present results clearly illustrate that with the use of *B. bassiana* isolates in dates storage, *C. cautella* populations could be reduced below threshold level.

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