

Exploring the biocontrol efficiency of indigenous entomopathogenic fungus on *Cadra cautella* (Lepidoptera, Pyralidae) larvae in controlled laboratory conditions

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Abstract: *Cadra cautella* (Walker, 1863) is a global pest of agricultural stored commodities, particularly date fruits, in the field and storage. To reduce *C. cautella* populations in stored agricultural goods, it is recommended to use ecologically friendly methods rather than chemical treatments. In this regard, current research evaluated the efficacy of various indigenous strains of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.). Vuill. (1912), fungal isolates against *C. cautella* last-instar larvae under laboratory conditions. The last instar larvae were incubated in Petri plates with dry *Beauveria bassiana* inoculum (1×10^7 conidia/mL) for five minutes per fungal isolate. The susceptibility of *C. cautella* larvae to fungal infection was tested until either 100% mortality across all treatments was achieved or the control larvae had transformed into pupae. The results showed that, compared with the control treatment, all fungal isolates were pathogenic to *C. cautella* larvae, with varying levels of reaction and durations of mortality. After four days of inoculation, the *B. bassiana* isolates BbSA-5, BbSA-8, and BbSA-9 infected treated *C. cautella* larvae at rates of 16.66%, 23.33%, and 43.33%, respectively. The death rate of *C. cautella* larvae increased significantly, reaching 91.66% for BbSA-5 isolates after eight days of the treatment, while BbSA-5 and BbSA-9 isolates achieved 100% mortality after sixteen days. The control treatment resulted in no larval mortality. Furthermore, all *C. cautella* larvae in the control group pupated and successfully matured into adults. It is determined that the examined dry formulations of local fungal isolates have the capacity to protect stored agricultural commodities, including date fruits, from *C. cautella* infestation.

Keywords: stored product pest; almond moth; *Beauveria*; *Metarhizium*; dry formulation

The date palm, *Phoenix dactylifera* (Linnaeus, 1753), is an economically significant fruit tree in warm climates such as Saudi Arabia, Egypt, Iran, Iraq, and the UAE (Ghafari et al. 2019). Saudi Arabia is a major date producer, with 152 734 ha under date palm cultivation and 1 565 830 t of dates

produced (FAO 2021). Date palm fruits are rich in amino acids, carbs, proteins, selenium, potassium, calcium, magnesium, manganese, iron, fiber, and vitamins. This material contains antioxidants, including polyphenols, carotenoids, and phenolic acids (Hussain et al. 2020). Several pests target date

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palms; among the approximately ten major pests that harm date fruits, the almond moth *Cadra cautella* (Walker, 1863) (Lepidoptera, Pyralidae) is one of the most important insects (El Shafei et al. 2022).

The *C. cautella* can infect date fruits both in the field and in storage facilities. Surveys were conducted, and date samples were collected from several fields in 2024 to assess *C. cautella* infestation in freshly harvested dates. It has been discovered that *C. cautella* can infest date fruit over a certain moisture level. When the dates begin to ripen, the insect *C. cautella* infestation usually begins while the dates remain on the trees (unpublished data). This early attack affects the quality and appearance of the dates, necessitating careful protection and monitoring to guarantee a healthy harvest and storage. This pest has a significant economic impact on other stored agricultural commodities as well. Insect species larvae cause significant harm, resulting in global losses of stored products. Almond moth larvae have six instars and a broad host range, which can destroy stored agricultural products. Under normal circumstances, the larval lifespan is 25–30 days. During this time, the larvae cause significant harm to the target goods. A female moth can lay 300–500 eggs over her lifetime, depending on the environmental conditions (Mankin et al. 2014; Husain et al. 2017).

Previous research has used various strategies to control stored pests such as *C. cautella*, including pesticide application (Zettle et al. 1973; Sim et al. 2006), heat treatment (Arbogast 1981; Mohammed et al. 2019), atmospheric modification (Damarh et al. 1997; Rasool et al. 2017; Tütüncü & Emekci 2020), and UV irradiation (Kirkpatrick et al. 1970; Faruki et al. 2007; Sorungbe et al. 2016; Husain et al. 2022). None of these treatments has received well-known recognition as a practical remedy for *C. cautella* management. As a result, effective management requires ecologically responsible solutions. Concerns about the environmental impact of chemical pesticides have led to restrictions in Gulf countries. The focus has shifted toward biological regulation. The current study explores the potential to develop a biopesticide. Biological control has proven effective in combating storage insects. Numerous fungal species, such as *Beauveria bassiana* (Bals. -Criv.) Vuill. and *Metarhizium anisopliae* (Metchnikoff), have specific characteristics; these species have been used as microbial control agents to manage a variety of pests (Srinivasan 2012).

Variations in insect mortality caused by different strains of entomopathogenic fungi have been widely reported (Cruz-Avalos et al. 2019; Fite et al. 2020). For example, *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) mortality ranged from 38% to 91% for *B. bassiana* and 20% to 73% for *M. anisopliae* (Fite et al. 2020), and *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera, Noctuidae) larvae mortality varied from 28% to 100% with different *B. bassiana* strains (Cruz-Avalos et al. 2019). Despite the success seen in lepidopteran pests, stored product pests often show lower susceptibility to EPF (Khidr et al. 2024; Moghasem et al. 2024). In *C. cautella* eggs and 2nd instar larvae. The treatment consisted of 3×10^8 spores/mL. The results showed that *B. bassiana* and *M. anisopliae* had hatchability rates of 62.50% and 69.7%, respectively. Furthermore, larval mortalities of 81% and 89% were observed after 14 days. In a study by El Shafei et al. (2022), larvae treated with 2×10^{11} spores/mL of *Bacillus thuringiensis* (Berliner 1915) died at a 72% rate after 14 days. *C. cautella* larvae and pupae, aged 8 and 15 days, were utilised to assess the pathogenicity of two *B. bassiana* strains, BbSA-1 and BbSA-2. BbSA-1 and BbSA-2 isolates killed 75% and 27% of the 15-day-old larvae, respectively. The BbSA-2 isolate killed 90% and 50% of pupae at one and five days old, respectively (Alwaneen et al. 2020).

The virulence of *B. bassiana* combined with either spinetoram, diatomaceous earth, or nanoparticles has been evaluated against coleopteran species infesting stored products (Wakil et al. 2022, 2023; Sohrabi et al. 2025). However, the indigenous strains of *B. bassiana* need to be fully evaluated for their pathogenicity against *C. cautella* larvae. This study assesses the virulence of native isolates as potential biocontrol agents targeting the almond moth's larval stage.

MATERIAL AND METHODS

Almond moth, *Cadra cautella* rearing

The almond moth larvae used in the present research were reared on a poultry feed-based artificial diet in our entomology laboratory (Husain et al. 2017). To enhance reproductive behaviour and egg production, 10 adult moth pairs were placed in a plastic container measuring 6 cm in diameter and 7 cm in height. This container served as a mat-

ing arena for the couples. A small cut was made on the lateral side of the plastic container, and a piece of cotton soaked in a 10% sugar solution was inserted to provide the adult moths with additional nutrition. Every day, the eggs were collected, placed in 50 g plain plastic cups, and incubated at $25 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. After three to four days, the eggs hatched, and the neonates were placed in a plastic container with artificial feed. Larvae were utilised for fungus inoculation once they reached the age of the last instar.

Entomopathogenic fungi source

The fungal isolates obtained for this study were indigenous and sourced from laboratories in specific localities within the Madinah region of Saudi Arabia. In this study, three fungal isolates of *B. bassiana* (designated as BbSA-5, BbSA-8, and BbSA-9) were employed. For DNA sequencing, fungal DNA was extracted using lysis buffer (Serna-Domínguez et al. 2018), heated, and precipitated in alcohol. PCR amplification was performed to determine the fungal species. A general primer was used to amplify a ribosomal DNA fragment (Shanker et al. 2023). MacroGen, Inc. processed the PCR products, verified the sequence identification, and submitted the data to the National Centre for Biotechnology Information.

DNA sequencing of fungus isolates

The PCR products from each fungal isolate were processed by MacroGen, Inc. (Republic of Korea) to obtain the sequence data. The use of the Basic Local Alignment Search Tool [BLAST(version X)] verified the fungus sequence identification. All fungus sequences were submitted to the National Centre for Biotechnology Information (NCBI), and accession numbers were obtained (Sutanto et al. 2024).

Preparation of fungal cultures *Beauveria bassiana*

Beauveria bassiana conidia were grown on potato dextrose agar (PDA) in 9-cm Petri dishes at 25°C in darkness. All fungal samples reached maximum growth in 15 days. Their concentration was determined by first scraping mature fungal isolate conidia into sterile distilled water in glassware, using 1 mL/L. Tween-80 solution determined their conidia concentration. The suspension was vortexed for 2 min and placed in an orbital incubator (Stuart SI500, Equipment, USA) at $25 \pm 2^\circ\text{C}$ and $85 \pm 2\%$ relative humidity. Then the suspension was filtered

via four sterile muslin layers. The conidial concentration of the stock suspension was measured using an improved Neubauer hemocytometer under a $40\times$ microscope. The fungal isolate plates contain about 10^7 conidia/mL on average. This study used dried conidia from each fungal isolate as the treatment. The dry formulation approach was used to apply fungal isolates onto *C. cautella* larvae (Alwaneen et al. 2020). The "dry formulation" refers to the conidia that were dehydrated and kept before application. To obtain the desired moisture content, we collected conidia from our culture and dried them using a freeze-dryer.

Pathogenicity bioassay on *Cadra cautella* larvae

To assess the susceptibility of *C. cautella* larvae to fungal infection, the larvae were subjected to contamination with dry conidia (1×10^7 conidia/mL) of different fungal isolates. The last instar (6th instar) of *C. cautella* larvae, aged 20–22 days, was exposed to dry *B. bassiana* inoculum in Petri dishes for 5 min. Subsequently, the inoculated larvae were transferred to Petri plates with an artificial diet. In the control treatment, larvae were not contaminated with fungus. The experimental unit consisted of three replicates, each containing five larvae. The larvae subjected to each treatment were thereafter placed in an incubator for 16 days. After the application was submitted, daily observations were conducted. Furthermore, we confirm that all experiments were conducted independently and repeated three times to ensure the reliability of our results. The deceased larvae were placed in sterile Petri dishes with damp filter paper and incubated at $25 \pm 2^\circ\text{C}$ and $85 \pm 2\%$ relative humidity. This step confirmed that the fungal isolates were indeed responsible for the larvae's mortality.

Data collection and statistical analysis

Mortality rates were computed to analyse the comparative effectiveness of various fungal isolates statistically. Daily observations were conducted until the larvae in the control group underwent a transformation and developed into pupae or adult moths. The *C. cautella* larvae killed in all experimental groups were counted to determine the mortality percentage. Killed larvae with fungal growth on their bodies were considered contaminated after incubation. In contrast, death percentages did not include larval individuals without fungal infection. The dependent variable was the

Table 1. Overall mortality trends (mean $\pm$ SE) and comparative performance of *Beauveria bassiana* isolates against *Cadra cautella* last instar larvae at the 4th day of post-treatment

Treatment	Mortality percentages	Statistical analysis (N = 15)
<i>B. bassiana</i> (BbSA-5)	16.66 $\pm$ 0.64 ^{bc}	
<i>B. bassiana</i> (BbSA-8)	23.33 $\pm$ 1.04 ^b	$F = 9.91$, df = 3, 12; $P \leq 0.0014$
<i>B. bassiana</i> (BbSA-9)	43.33 $\pm$ 1.19 ^a	
Control	0 $\pm$ 0 ^c	

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

presence of *C. cautella* larvae, while the major factor was fungus type. SAS was used to analyse the data using one-way analysis of variance (SAS 2009). The least significant difference (LSD) test was used to assess if the means differed. Significance was determined at $P < 0.05$. No data transformations were applied, as the percentage data met the ANOVA assumptions.

RESULTS

The findings indicated that all examined fungal isolates were pathogenic to *C. cautella* larvae, with varying degrees of reaction and fatality rates. The *C. cautella* larvae exhibited a significant susceptibility to all fungal isolates tested, in contrast to the control treatment. This study evaluated various mortality rates and found a statistically signifi-

Table 2. Overall mortality trends (mean $\pm$ SE) and comparative performance of *Beauveria bassiana* isolates against *Cadra cautella* last instar larvae at the 8th day of post-treatment

Treatment	Mortality percentages	Statistical analysis (N = 15)
<i>B. bassiana</i> (BbSA-5)	91.66 $\pm$ 0.47 ^a	
<i>B. bassiana</i> (BbSA-8)	65 $\pm$ 0.75 ^b	$F = 136.97$, df = 3, 12; $P \leq 0.0001$
<i>B. bassiana</i> (BbSA-9)	90 $\pm$ 0.64 ^a	
Control	0 $\pm$ 0 ^c	

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

cant difference in larval mortality after four days ($F: 9.91$; df: 3, 12; $P < 0.0014$) (Table 1).

The *B. bassiana* isolates, namely BbSA-5, BbSA-8, and BbSA-9, induced infection in 16.66%, 23.33%, and 43.33% of *C. cautella* larvae, respectively. After 8 days, there was a notable increase in the mortality rate of *C. cautella*, reaching 91.66%, 65%, and 90%, respectively. This increase was found to be statistically significant ($F = 136.97$, df = 3, 12, $P < 0.0001$) according to the data presented in Table 2.

All the *C. Cautella*-infected larvae, the bodies were changed to a pink color and then over time, the bodies were covered with fungus mycelium, as shown in Figure 1.

On the twelfth day of post-treatment, a significant difference was observed in larval mortality ($F: 263.63$; df: 3, 12; $P < 0.0001$) (Table 3). Larvae treated with isolate BbSA-5 showed the highest mortality (96.66 $\pm$ 0.50%), closely followed

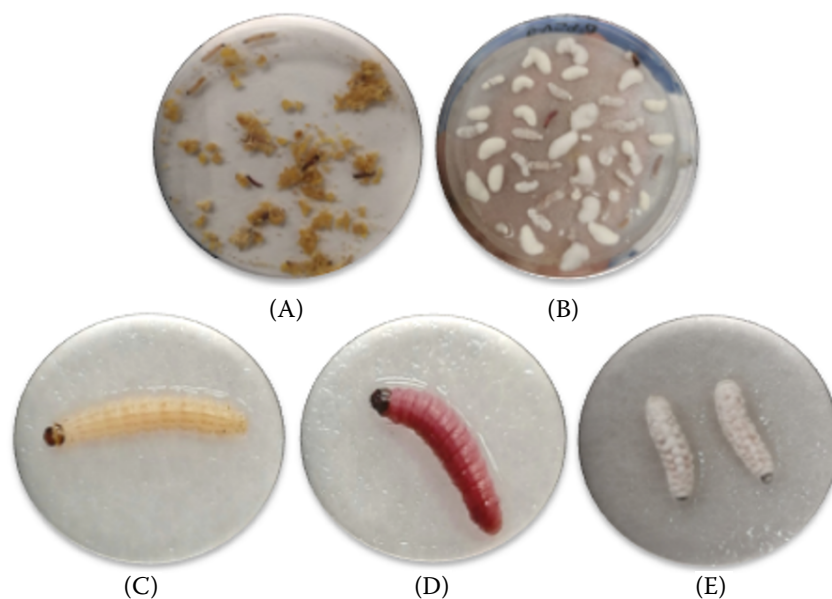


Figure 1. Infection development in *Cadra cautella* larvae after treatment with the fungus *Beauveria bassiana*

A – Larvae infected with *Beauveria bassiana* after 3 days; B – larvae infected with *Beauveria bassiana* after 6 days; C – healthy *C. cautella* larva; D – the infected body of the *C. cautella* larvae were changed to a pink colour; E – the larvae were covered with fungus mycelium.

Table 3. Overall mortality trends (mean $\pm$ SE) and comparative performance of *Beauveria bassiana* isolates against *Cadra cautella* last instar larvae at the 12th day of post-treatment

Treatment	Mortality percentages	Statistical analysis (N = 15)
<i>B. bassiana</i> (BbSA-5)	96.66 $\pm$ 0.50 ^a	
<i>B. bassiana</i> (BbSA-8)	83.33 $\pm$ 0.64 ^b	$F = 263.63$, df = 3, 12; $P \leq 0.0001$
<i>B. bassiana</i> (BbSA-9)	95 $\pm$ 0.25 ^a	
Control	0 $\pm$ 0 ^d	

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

by BbSA-9 (95.00 $\pm$ 0.25%), both of which were significantly greater than the 83.33 $\pm$ 0.64% mortality observed with BbSA-8. No mortality occurred in the untreated control group (0 $\pm$ 0%).

Most fungal isolates caused mortality in 80–95% of *C. cautella* larvae. After 16 days, most fungal isolates caused 87–100% larval mortality in *C. cautella*. A significant difference was observed in larval mortality after sixteen days (F : 247.05; df: 3, 12; $P < 0.0001$) (Table 4).

However, the highest mortality for *C. cautella* larvae was consistent across BbSA-5 and BbSA-9 isolates within each mortality period. In the control treatment, no larval mortality occurred. Moreover, in the control treatment, all *C. cautella* larvae pupated and emerged as adults (Table 4).

DISCUSSION

The results of the present research showed that fungal isolates from local sources can kill a significant number of *C. cautella* larvae. Several studies have examined the use of fungal pathogens as potential methods to control insect pests in stored products to maintain their quality (Batta & Kavallieratos 2018; Mantzoukas et al. 2022). Microbial agents, such as *M. anisopliae* and *B. bassiana*, were tested on *E. cautella* larvae. *M. anisopliae* killed 89% of 2nd instar larvae, and *B. bassiana* killed 81% of larvae after 14 days at concentrations of 3×10^8 conidia/mL (El Shafei et al. 2022). Similar to our study, most *B. bassiana* fungal isolates caused high last-instar larval mortality (85–100%) in *C. cautella* at 1×10^7 conidia/mL after 16 days, and many insect cadavers occurred by the 6th day. In the same way, it was reported that a quantity

Table 4. Overall mortality trends (mean $\pm$ SE) and comparative performance of *Beauveria bassiana* isolates against *Cadra cautella* last instar larvae at 16th day of post-treatment

Treatment	Mortality percentages	Statistical analysis (N = 15)
<i>B. bassiana</i> (BbSA-5)	100 $\pm$ 0.50 ^a	
<i>B. bassiana</i> (BbSA-8)	87 $\pm$ 0.70 ^c	$F = 247.05$, df = 3, 12; $P \leq 0.0001$
<i>B. bassiana</i> (BbSA-9)	100 $\pm$ 0.25 ^{bc}	
Control	0 $\pm$ 0 ^d	

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

of 2×10^9 conidia (powder formulation) caused an infection in all stages of *Lasioderma serricorne* (Fabricius, 1972) (Coleoptera: Anobiidae) (Saeed et al. 2017). In another study, it was shown that *M. anisopliae* isolates killed *C. serratus*; when 0.1 g of conidia was applied per 50 g of peanuts, mortality ranged from 19 to 100% after 10 days (Ekesi et al. 2001). Previous research indicated that 7.7–60.0% of *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae) larvae died after consuming food containing *B. bassiana* (Akbar et al. 2004).

To treat various grain pests, studies have utilised fungal conidia at different concentrations. *M. anisopliae* kills 55% of first instar *Plodia interpunctella* larvae after 5 days at a concentration of 10^6 conidia/mL (Khezripour et al. 2022). The immersed approach has also been used to test different fungal isolates of *B. bassiana* and *M. anisopliae* on *Ephestia kuehniella* (Zeller, 1879) (Lepidoptera: Pyralidae), with *M. anisopliae* causing 11–92% larval mortality and *B. bassiana* causing 17–88% mortality (Faraji et al. 2013). Another *B. bassiana* isolate was tested on *Agrotis ipsilon* (Hufnagel, 1766) (Lepidoptera: Noctuidae) larvae, and after 7 days, 50% of the larvae had died at a concentration of 10^8 , and the mycelium of the fungus had covered the body of the larvae (Gabarty et al. 2014). Pathogenicity was also observed for *B. bassiana*, *M. anisopliae*, and *Isaria fumosorosea* Wize (1904) against *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae) at a concentration of 10^8 conidia/mL. These pathogens caused 0–100% adult mortality after 14 days of food contamination (Kavallieratos et al. 2014). High mortality rates were observed in *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Pyralidae) larvae treated with 1.49×10^5 conidia/mL,

4.05×10^6 conidia/mL, and 2.02×10^8 conidia/mL of *B. bassiana* (Kaur et al. 2014). Entomopathogenic fungi have been demonstrated in the lab and in the field to be effective against various insect pests in stored products (Sabbour 2014).

Additionally, selecting a fungal isolate with a strong pathogenic response is crucial for protecting stored grain from entomopathogenic fungi in a safe manner (Batta 2016). The time of host infection, incubation period, and fungal dispersal all play a role in determining the virulence of entomopathogenic fungi (Shah & Pell 2003). The use of dry fungal spore powder has been proposed as a promising alternative to conventional pest control for stored grains, given the success of entomopathogenic fungi in reducing insect infestations (Rumbos & Athanassiou 2017). The process of selecting entomopathogenic fungi (EPF) performance within the context of an IPM program can increase EPF efficacy in controlling pests (Khun et al. 2020; Kidanu & Hagos 2020). However, environmental and biotic conditions, such as temperature and humidity, affect the success of entomopathogenic fungi in infecting insect pests (Butt et al. 2016; Rumbos & Athanassiou 2017; Mantzoukas et al. 2022).

Indigenous *Beauveria bassiana* isolates were highly pathogenic to *C. cautella* larvae. The fungus can efficiently manage the insect using a series of biological methods. The process begins with the adhesion of conidia to the insect cuticle, followed by germination and penetration, which are aided by cuticle-damaging enzymes (Wakil et al. 2023). This phenomenon, in which fungal biological mechanisms kill host organisms, is well established. Various studies have thoroughly explained that fungi have been used as biological control agents in application strategies to manage agricultural pests (El Shafei et al. 2022; Swedaan et al. 2023; Güner et al. 2025).

CONCLUSION

Current research suggests that indigenous *B. bassiana* fungal isolates could be exploited to manage storage pest populations, such as *C. cautella*. The infection of larvae with a dry formulation demonstrated *B. bassiana*'s efficacy as an entomopathogen. These discoveries have far-reaching consequences for the global protection of stored agricultural commodities and products. Future

research is necessary to explore the practical applications of these findings, and field validation is essential to confirm their efficacy in protecting agricultural commodities during storage.

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