Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

The bioactivity of *Metarhizium anisopliae* and *Verticillium lecanii* on immature and mature stages of the flour Beetle, *Tribolium castaneum*

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Abstract

Objective

The red flour beetle, *Tribolium castaneum* (Herbst.), is one of the most destructive pests of stored grains and their by-products, creating substantial economic losses worldwide. Its remarkable capability to develop resistance to conventional insecticides, along with increasing concerns about environmental and health hazards related to chemical control, necessitates alternative management strategies. Entomopathogenic fungi (EPFs), natural pathogens of insects, represent a hopeful biocontrol option. This investigation aimed to evaluate the pathogenic activity of two commercially available EPF formulations, *Metarhizium anisopliae* (Met52) and *Verticillium lecanii* (Mycotal), against different developmental stages of *T. castaneum*.

Materials and methods

Laboratory bioassays were managed to evaluate the effectiveness of *M. anisopliae* and *V. lecanii* against second-, fourth-, and sixth-instar larvae, as well as adults of *T. castaneum*. Multiple concentrations of fungal spores were applied, and mortality rates were documented over varying

exposure periods. Mortality percentages were statistically analyzed to identify dose- and stage-dependent susceptibility.

Results

Both EPFs exhibited meaningful pathogenicity against *T. castaneum*, though their effectiveness varied across life stages and concentrations. *M. anisopliae* consistently demonstrated higher virulence than *V. lecanii*. Susceptibility was strongly stage-dependent: second- and fourth-instar larvae were more vulnerable, while sixth-instar larvae and adults showed comparatively higher resistance to fungal infection. Increasing spore concentration and developing exposure duration meaningfully increased mortality rates across all tested stages. A positive correlation was observed between mortality percentage and both fungal concentration and exposure time. Notably, the sixth-instar larvae and adults exhibited the lowest mortality, emphasizing the importance of targeting younger developmental stages for effective control.

Conclusions

The results affirm that *M. anisopliae* and *V. lecanii* possess considerable potential as biological control agents against *T. castaneum*. Among the two, *M. anisopliae* exhibited superior pathogenicity across life stages. The investigation underscores the importance of optimizing fungal concentration and targeting susceptible larval stages to maximize effectiveness. These results support the integration of entomopathogenic fungi into sustainable pest management programs for stored-product pests, reducing reliance on chemical insecticides.

Keywords: entomopathogenic fungi, *Metarhizium anisopliae*, *Tribolium castaneum*, *Verticillium lecanii*

Paper Type: Research Paper.

Citation: Al Shareefi, E., Sahi, N. M., Al-khafaji, N. A., Abdulsada, F. F., & Al-Khafaji, S. A. (2026). The bioactivity of *Metarhizium anisopliae* and *Verticillium lecanii* on immature and mature stages of the flour Beetle, *Tribolium castaneum*. *Agricultural Biotechnology Journal,* 18(2), 313-330.

Agricultural Biotechnology Journal, 18(2), 313-330. DOI: 10.22103/jab.2025.25789.1754

Received: July 06, 2025. Received in revised form: September 18, 2025.

Accepted: September 19, 2025. Published online: February 28, 2026.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.

Introduction

Tribolium castaneum (Herbst), commonly known as the red flour beetle, is a member of the family Tenebrionidae, order Coleoptera (Fathy et al., 2025). This species is among the most notorious stored-product pests, infesting a broad range of grain flours, exclusively wheat and

barley. Wheat flour is especially vulnerable to *T. castaneum* infestations through storage, where large populations can develop and persist (Wakil et al., 2023). The pest is cosmopolitan in distribution, occurring in warehouses, flour mills, grain stores, and feed processing facilities worldwide. Red flour beetles are relatively small, calculating almost 4 mm in body length (Waqas et al., 2015). Although their origins are believed to be Indo-Australian, they have successfully established populations across temperate and subtropical regions (Thakur et al., 2012). Notably, *T. castaneum* can survive adverse winter conditions, exclusively in environments with central heating, which supply favorable microhabitats (Panezai et al., 2019). The beetle is capable of developing between 20 °C and 43 °C, which explains its broad geographic distribution (Al-Zurfi et al., 2023). Morphologically, *T. castaneum* is a glossy, reddish-brown, flattened, and ovoid insect almost 4 mm long (Johnson, 2013; Stack, 2015). Females oviposit directly onto flour or grain particles, producing whitish eggs covered with a sticky secretion that permits adhesion to flour dust. Under optimal conditions, eggs hatch within three to five days. The larvae are cream-colored, cylindrical, and reach about 6 mm in length before pupation (Baldwin & Thomas, 2005). The damage created by *T. castaneum* is both qualitative and quantitative. Loss of quantity Both larvae and adults cause direct feeding, consuming flour and grains leading to a significant reduction in weight (Shankar & Abrol, 2012). Globally, it is assumed that between 5% and 30% of the total grain production around the world is lost every year to infestations of *T. castaneum* and other stored-product pests. Due to such losses, a complete study of the biology and ecology of *T. castaneum* is necessary to designing successful control strategies (Mohammadabadi et al., 2025). Resistance to synthetic insecticides is one of the main problems for controlling populations of *T. castaneum*. Resistance to organophosphates, pyrethroids and other chemical groups is widely reported within *Tribolium* spp. in several studies (Boyer et al., 2012). This resistance not only diminishes the efficacy of chemical control, but also causes alarms for food security and human health by insecticide residues. In addition, chemical products are expensive and cannot be all used over long time due to more restrictive regulations on their use, increasing consumer demand of food free from pesticide residues and aimed at the environmentally friendly agriculture which is promted. The problems presented by these chemicals emphasize the importance of safe, sustainable and economical alternatives to traditional insecticides. Biological control has emerged as a promising alternative for managing stored-product pests. Out of the biocontrol agents tested so far, entomopathogenic fungi (EPFs) have revealed a tremendous potential as biocontrol agents (Shah & Pell, 2003). EPFs are naturally occurring pathogens of insects that infect their hosts via cuticular penetration and subsequently proliferate inside the body, leading to host mortality. Importantly, these fungi are highly specific to insects, pose negligible risks to humans, animals, and plants, and are environmentally benign. Such attributes make them attractive candidates for

integration into pest management strategies. The potential of entomopathogenic fungi as biocontrol agents has been studied for decades. Early investigations demonstrated their capability to suppress pest populations under laboratory conditions. Since then, multiple fungal species have been examined, with *Metarhizium anisopliae* and *Verticillium lecanii* (recently reclassified as *Lecanicillium lecanii*) among the most broadly studied. Liu et al. (2003) recognized *M. anisopliae* as a safe and effective biological control agent with a broad host range. Subsequent investigations affirmed its pathogenicity against different stored-product pests, containing *Sitophilus oryzae* (rice weevil), *Rhyzopertha dominica* (lesser grain borer), *Callosobruchus maculatus* (cowpea weevil), and *T. castaneum* (Khashaveh et al., 2011). Similarly, *V. lecanii* has shown effectiveness against a variety of insect pests, making it another potential candidate for stored-product protection. One of the major limitations of EPFs in field applications is their sensitivity to environmental stressors like ultraviolet (UV) radiation, which can rapidly degrade fungal conidia and reduce. However, this limitation is far less critical in stored-product environments, where conditions like darkness, moderate humidity, and stable temperatures are generally favorable for fungal persistence. For this reason, EPFs may be exclusively well-suited for application in storage systems. Indeed, both *M. anisopliae* and *V. lecanii* have been documented as naturally occurring pathogens of *T. castaneum* in the United Kingdom and other regions. Laboratory and semi-field evaluations have demonstrated their potential to meaningfully reduce *T. castaneum* populations (Kavallieratos et al., 2014; Sabbour, 2014). Given the extensive damage created by *T. castaneum* and the limitations of chemical control, there is a pressing need to evaluate the bioefficacy of entomopathogenic fungi under conditions relevant to storage systems. Furthermore, it is critical to examine the susceptibility of different developmental stages, as larval and adult beetles may vary in their response to fungal infection. Younger larval stages are generally more susceptible, whereas later instars and adults often exhibit greater resistance. Understanding these differences is essential for optimizing application strategies and improving control outcomes. Accordingly, the current investigation investigates the pathogenicity of *M. anisopliae* (Met52) and *V. lecanii* (Mycotal) against multiple developmental stages of *T. castaneum*, containing second-, fourth-, and sixth-instar larvae, as well as adults. The specific objectives are: (1) to evaluate the susceptibility of *T. castaneum* life stages to fungal infection; (2) to evaluate the impression of spore concentration and exposure duration on mortality; and (3) to identify the most effective fungal species and conditions for pest suppression. By addressing these aims, the investigation provides discernments into the potential role of entomopathogenic fungi in sustainable stored-product pest management.

Materials and methods

Collection of samples: Wheat flour samples infested with the red flour beetle, *Tribolium castaneum* (Herbst), were gathered from retail markets in Babylon/Hilla city, Iraq. To ensure the purity of experimental material, only infested samples with no known history of exposure to insecticides were selected. The gathered material was transferred to the laboratory in clean, airtight containers to prevent contamination and escape of beetles through transportation.

Diet preparation: To obtain sufficient numbers of *T. castaneum* at different developmental stages (second, fourth, and sixth instar larvae, as well as newly emerged adults of almost uniform age), a standardized artificial diet was prepared. The diet consisted of 95% whole wheat flour and 5% brewer's yeast (w/w ratio of 95:5). A bulk mixture was prepared by combining 1.9 kg of sterilized whole wheat flour with 100 g of yeast. The mixture was thoroughly blended to ensure homogeneity and was applied for all experiments managed in this investigation. Prior to use, the diet components were sterilized at 105 °C for 1 h to eliminate contaminating microorganisms and insect eggs. The sterilized diet was cooled to room temperature, transferred to airtight containers, and stored in a dry place until needed.

Rearing of *Tribolium castaneum:* Adult beetles of *T. castaneum* were reared on the prepared diet under controlled laboratory conditions. Almost 250 g of the sterilized diet was placed into each of five 1-L pre-sterilized Kilner jars. Thirty adult beetles, gathered from the infested flour samples, were introduced into each jar. The jar openings were covered with fine muslin cloth secured with metal lids to permit aeration while preventing beetle escape. The cultures were maintained in a growth chamber at 32 ± 1 °C and 68 ± 2% relative humidity (RH), with continuous darkness to mimic storage conditions and raise beetle activity. The cultures were checked weekly to monitor development, remove excess debris, and ensure optimal conditions for colony maintenance.

Collection of larvae: The life cycle of *T. castaneum* consists of multiple larval instars, of which six are most commonly observed under laboratory conditions. To obtain larvae of defined ages and instars, ten pairs of newly emerged adults (3-5 days old) were introduced into five 500-mL Kilner jars, each containing 30 g of diet prepared for oviposition. Adults were permited to oviposit for 48 h, after which they were removed to prevent further egg laying. The jars were then inspected daily under a stereomicroscope to detect egg hatching. Newly emerged larvae were maintained in 9 × 1.6 cm sterile plastic Petri dishes at 32 °C in the dark. Almost 5 g of fresh diet was added to each dish to supply adequate nutrition. The larval instars were distinguished based on size, body morphology, and molting documents. Larvae corresponding to the second, fourth, and sixth instars were carefully selected for bioassays. The entire development from first instar to adult emergence needed almost 30 days under the conditions applied.

Sources and preparation of entomopathogenic fungi: Two entomopathogenic fungi were evaluated in this investigation: *Metarhizium anisopliae* and *Verticillium lecanii* (currently *Lecanicillium lecanii*). Both fungi were derived as commercial formulations from ProGreen (WCF Ltd T/A Progreen, Unit 3 Axis Park, Manasty Road, Orton Southgate, Peterborough, PE2 6UP, UK). The *M. anisopliae* formulation, Met52, consisted of granules containing 2% (w/w) viable spores of *M. anisopliae* var. anisopliae strain F52. The *V. lecanii* formulation, Mycotal, was supplied as a spore powder containing 16.1% (w/w) viable conidia, with 83.9% (w/w) inert components. For preparation of suspensions, 10 g of each formulation was weighed applying an analytical balance and suspended in 100 mL of sterile distilled water in 500-mL glass beakers. The mixtures were vortexed vigorously for 5 min to release conidia from the carrier material. For *V. lecanii*, the suspension was permited to stand for 3 h before use to facilitate spore hydration and uniform dispersion. Conidial concentrations were identified applying a Neubauer improved haemocytometer under a light microscope at 400× magnification. The stock suspensions were adjusted to 3 × 10^8 spores/mL for *M. anisopliae* and 2 × 10^8 spores/mL for *V. lecanii*. These suspensions were regarded as primary (stock) concentrations. Serial dilutions were then prepared from the stock suspensions applying the formula C1V1 = C2V2, where C1 represents the stock concentration, C2 the needed concentration, V1 the volume of stock suspension, and V2 the final volume. Three working concentrations were prepared for each fungus: *M. anisopliae*: 3 × 10^4, 3 × 10^6, and 3 × 10^8 spores/mL, and *V. lecanii*: 2 × 10^4, 2 × 10^6, and 2 × 10^8 spores/mL Sterile distilled water served as the control treatment.

Application of fungal suspensions: Fungal suspensions were applied to larvae and adults of *T. castaneum* applying a manual hand sprayer to simulate surface contact exposure. For each treatment, 30 insects (either larvae of a defined instar or adults) were placed on a sterile 100-mm glass Petri dish and sprayed with 4 mL of fungal suspension. Control groups were sprayed with distilled water. Subsequent spraying, insects were left undisturbed for 15-20 min to ensure that conidia adhered to their cuticle surfaces. Thereafter, insects were randomly allocated into three replicates (10 individuals per replicate) and transferred into sterile 9-cm plastic Petri dishes containing 5 g of diet. Each larval instar (second, fourth, sixth) and the adult stage were tested separately. For each stage, 40 insects were divided into four groups: three groups exposed to different fungal concentrations and one control group exposed to distilled water. All treatments were replicated three times.

Incubation and mortality assessment: All treated and control groups were incubated at 32 °C in complete darkness without additional humidity regulation. Mortality was documented daily for 7 days post-treatment, beginning 24 h after exposure. Insects were considered dead when they failed to respond to gentle probing with a fine brush. Dead individuals were removed daily to

prevent secondary contamination. The number of surviving insects was also documented daily to monitor treatment progression. Mortality data were pooled for statistical analysis.

Confirmation of fungal infection: To affirm that insect mortality was due to fungal infection rather than other causes, cadavers were subjected to post-mortem analysis. Dead insects were first surface-sterilized by gently swabbing with cotton buds dipped in 76% ethanol. Sterilized cadavers were then placed individually into sterile Petri dishes lined with moist filter paper to promote fungal outgrowth. The dishes were incubated at 27 °C for 10 days. Fungal mycelia emerging from the cadavers were observed under a stereomicroscope to affirm fungal infection. Cadavers exhibiting external mycelial growth were documented as mycosis-positive. Insects that did not show visible fungal growth but had been exposed to fungal suspensions were also attended infected, based on previous criteria established in mycosis confirmation investigations.

Results

The current investigation demonstrated that both *Metarhizium anisopliae* and *Verticillium lecanii* were pathogenic to *Tribolium castaneum* via direct contact, leading to meaningful mortality across larval and adult stages. Mortality was affirmed to result from fungal infection based on the morphological identification of fungal mycelia emerging from the cadavers. Infected insects exhibited characteristic external fungal growth, with white to greenish mycelia for *M. anisopliae* and white to creamy mycelia for *V. lecanii*, thus affirming the role of the inoculated fungi in insect mortality.

Effect of *Metarhizium anisopliae* on different developmental stages: The pathogenic activity of *M. anisopliae* was evaluated against second-, fourth-, and sixth-instar larvae, as well as adults of *T. castaneum*. Results disclosed a strong positive relationship between spore concentration, exposure time, and mortality. Higher conidial concentrations generated greater mortality, and mortality improved steadily over the seven-day observation period (Figure 1A-D). For second-instar larvae, *M. anisopliae* was highly effective, with mortality reaching 90%, 97%, and 100% at concentrations of 3 × 10^4, 3 × 10^6, and 3 × 10^8 spores/mL, respectively, after 7 days. In contrast, mortality in the untreated control group stayed at 0% throughout the experiment (Figure 1A). Fourth-instar larvae exhibited slightly lower susceptibility compared to the second instar. Mortality rates after 7 days were almost 82%, 87%, and 93% at 3 × 10^4, 3 × 10^6, and 3 × 10^8 spores/mL, respectively (Figure 1B). Control larvae again showed no mortality. In the case of sixth-instar larvae, the pathogenic effect of *M. anisopliae* was comparatively reduced, with mortality reaching 63%, 83%, and 93% at the three concentrations tested (Figure 1C). This illustrates a higher degree of tolerance in older larvae, although meaningful mortality still

occurred at the higher concentrations. Adult beetles were the least susceptible stage tested. Mortality after 7 days was 72%, 78%, and 88% for 3 × 10^4, 3 × 10^6, and 3 × 10^8 spores/mL, respectively (Figure 1D, Figure 4). Control adults showed no mortality. These results propose that while *M. anisopliae* is capable of infecting all developmental stages, its effectiveness is most pronounced in early larval instars.

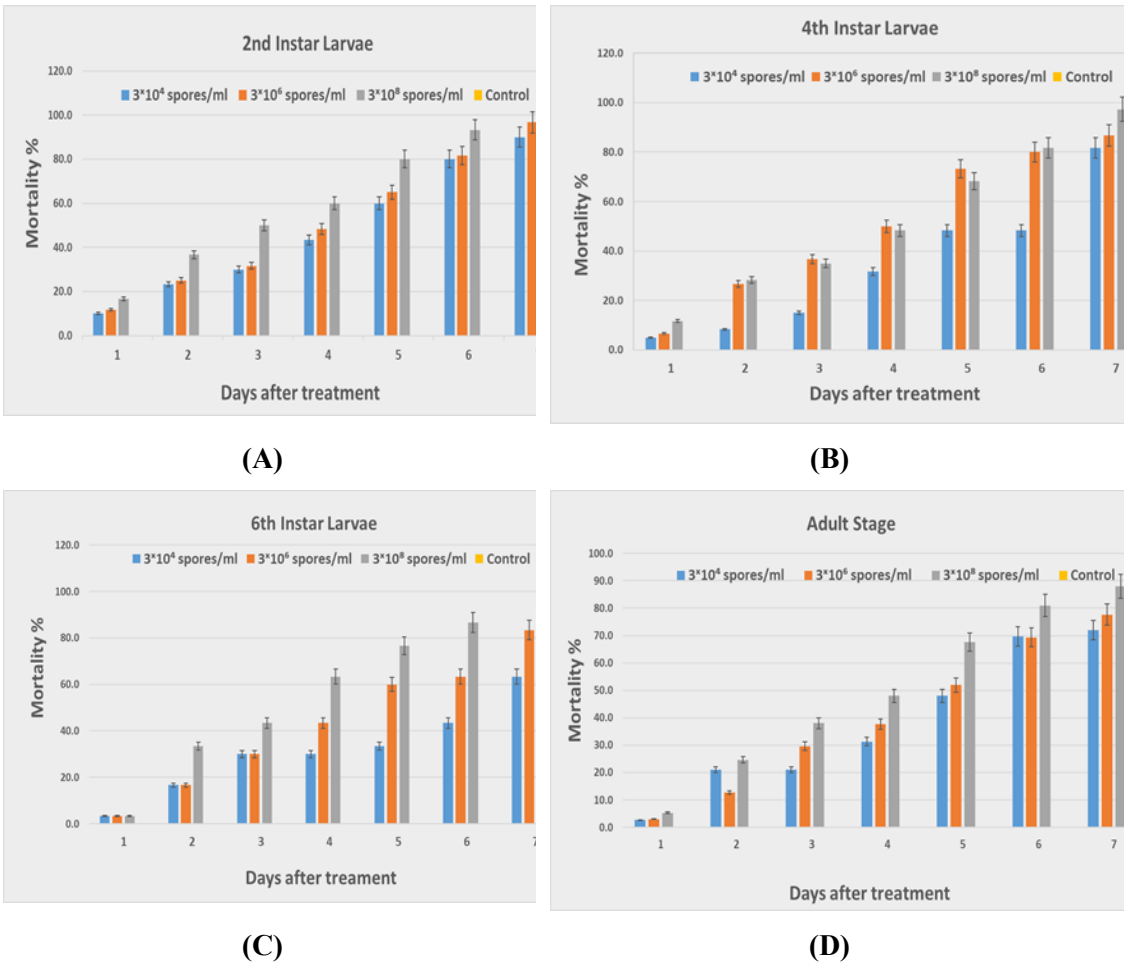

Figure 1. Percentage mortality of the red flour beetle, *Tribolium castaneum*, second-instar larvae (A), fourth-instar larvae (B), sixth-instar larvae (C), and adults (D) after seven days of exposure to different concentrations of *Metarhizium anisopliae*

Effect of *Verticillium lecanii* on different developmental stages: The effectiveness of *V. lecanii* was likewise evaluated against the same developmental stages of *T. castaneum*. Mortality trends again showed a positive correlation with spore concentration and exposure duration (Figure 2A-D). In second-instar larvae, mortality after 7 days reached 81%, 89%, and 90% for the concentrations of 2 × 10^4, 2 × 10^6, and 2 × 10^8 spores/mL, respectively (Figure 2A). Compared with *M. anisopliae*, mortality at the highest concentration was slightly lower,

proposing reduced virulence. Fourth-instar larvae responded differently, with mortality rates of 78%, 87%, and 95% at 2 × 10^4, 2 × 10^6, and 2 × 10^8 spores/mL, respectively (Figure 2B). Interestingly, mortality at the highest concentration was higher than that created by *M. anisopliae* in this stage, denoting that *V. lecanii* may be more effective against intermediate larval instars. The susceptibility of sixth-instar larvae was again lower than that of younger larvae. Mortality after 7 days was 77%, 83%, and 83% at the three concentrations tested (Figure 2C). Unlike *M. anisopliae*, increasing the concentration beyond 2 × 10^6 spores/mL did not meaningfully improve mortality in sixth instars, proposing a plateau in pathogenic activity for this stage. In adults, *V. lecanii* generated mortality rates of 72%, 77%, and 92% at the three concentrations (Figure 2D, Figure 4). Mortality in adults was higher than that created by *M. anisopliae* at the highest concentration, emphasizing some stage-specific differences in fungal virulence. Control mortality stayed 0% for all treatments.

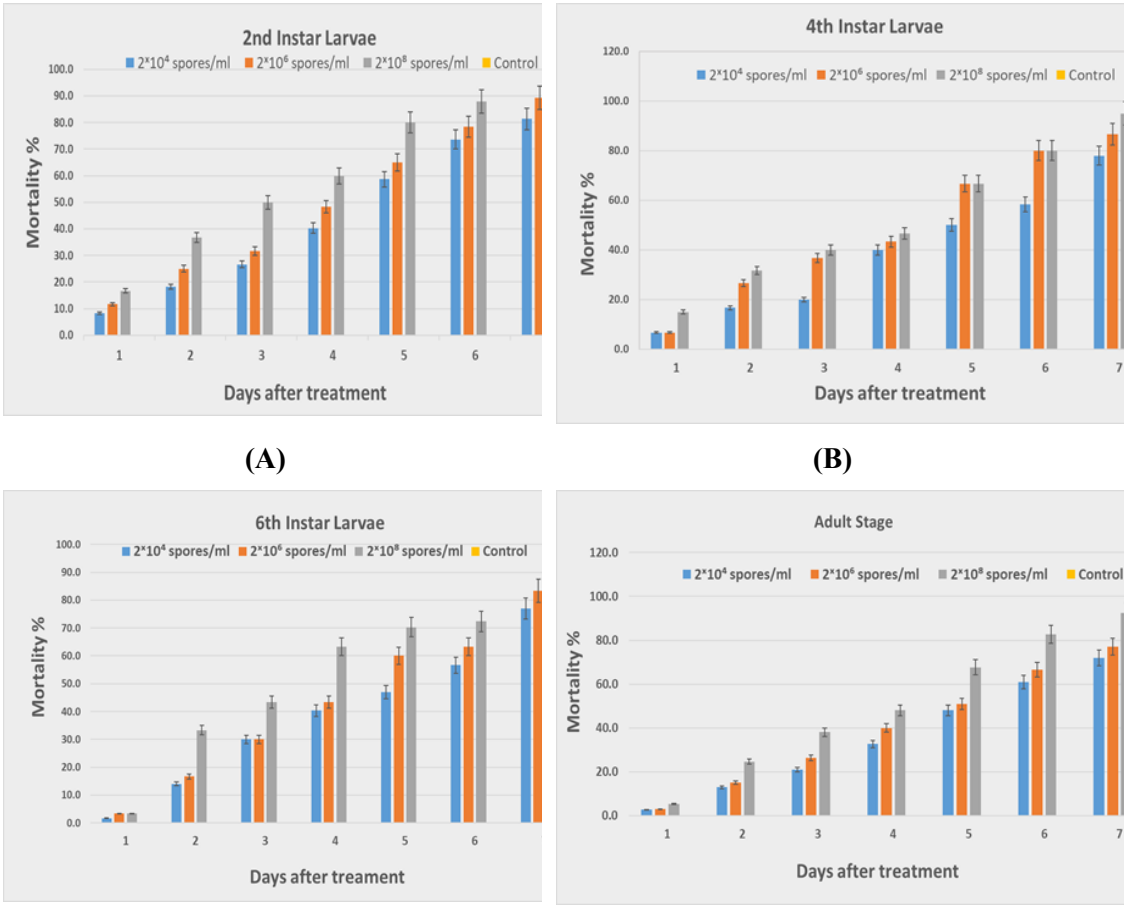

Figure 2. Percentage mortality of *T. castaneum* second-instar larvae (A), fourth-instar larvae (B), sixth-instar larvae (C), and adults (D) after seven days of exposure to different concentrations of *Verticillium lecanii*

Comparative analysis of the two fungi: When comparing the pathogenicity of the two fungi, both *M. anisopliae* and *V. lecanii* were effective in creating substantial mortality of *T. castaneum* across developmental stages (Figure 3). However, separate differences in virulence and stage-specific susceptibility were evident. Overall, *M. anisopliae* was more effective against early larval instars, exclusively the second instar, where complete mortality (100%) was observed at the highest concentration. In contrast, *V. lecanii* exhibited its strongest impact on fourth-instar larvae and adults, achieving 95% and 92% mortality, respectively, at the highest concentration. Sixth-instar larvae and adults consistently showed greater resistance to infection by both fungi compared to earlier larval stages. Nevertheless, mortality stayed substantial, exceeding 80% in most cases at higher concentrations. These results illustrate that while both fungi possess strong pathogenic potential, their relative effectiveness depends on the developmental stage of the host. The overall mean mortality across all treatments affirmed meaningful stage-dependent differences. Younger larvae (second and fourth instars) were more susceptible, while later instars and adults exhibited comparatively higher resistance. Such differences may reflect variations in cuticle thickness, immune defenses, or behavioral responses between life stages.

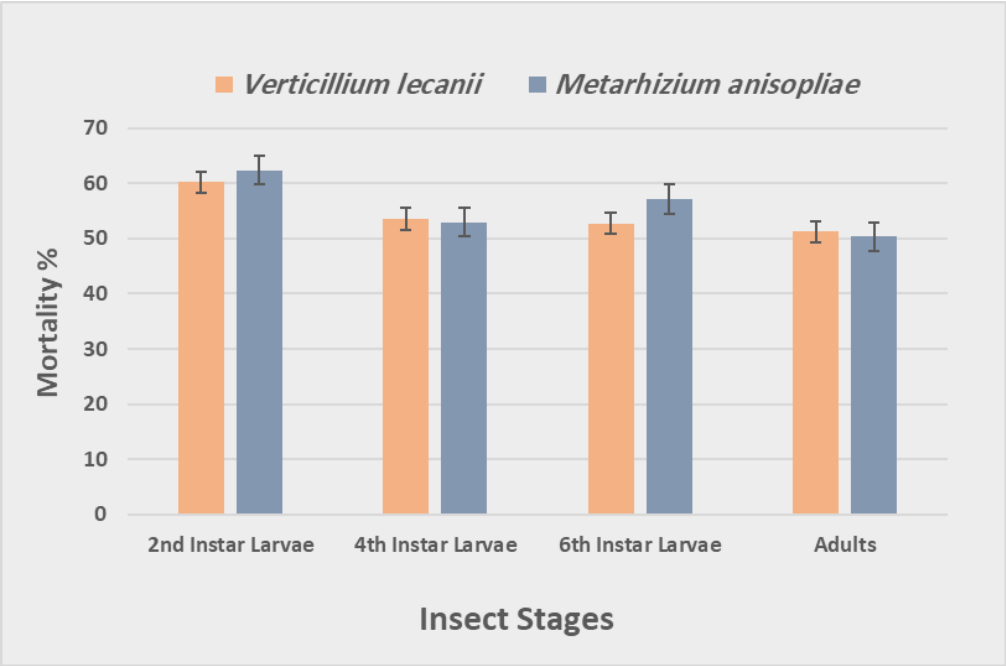

Figure 3. Mean mortality percentage of *T. castaneum* (Herbst) after seven days of treatment with 3 × 10^8 spores/mL of *M. anisopliae* and 2 × 10^8 spores/mL of *V. lecanii*, evaluated in second-, fourth-, and sixth-instar larvae as well as adults

Discussion

The current investigation affirmed that both *Metarhizium anisopliae* and *Verticillium lecanii* can infect and create mortality in *Tribolium castaneum* via direct contact, demonstrating their

potential as biological control agents against this pest. Fungal mycelia emerging from cadavers verified that insect death was created by fungal infection, in line with previous results in other Coleoptera species. For example, Erler and Ates (2015) announced that commercially available formulations of *M. anisopliae* induced meaningful mortality in *Cotinis nitida* (Coleoptera: Scarabaeidae), illustrating the broad applicability of entomopathogenic fungi (EPFs) for insect management.

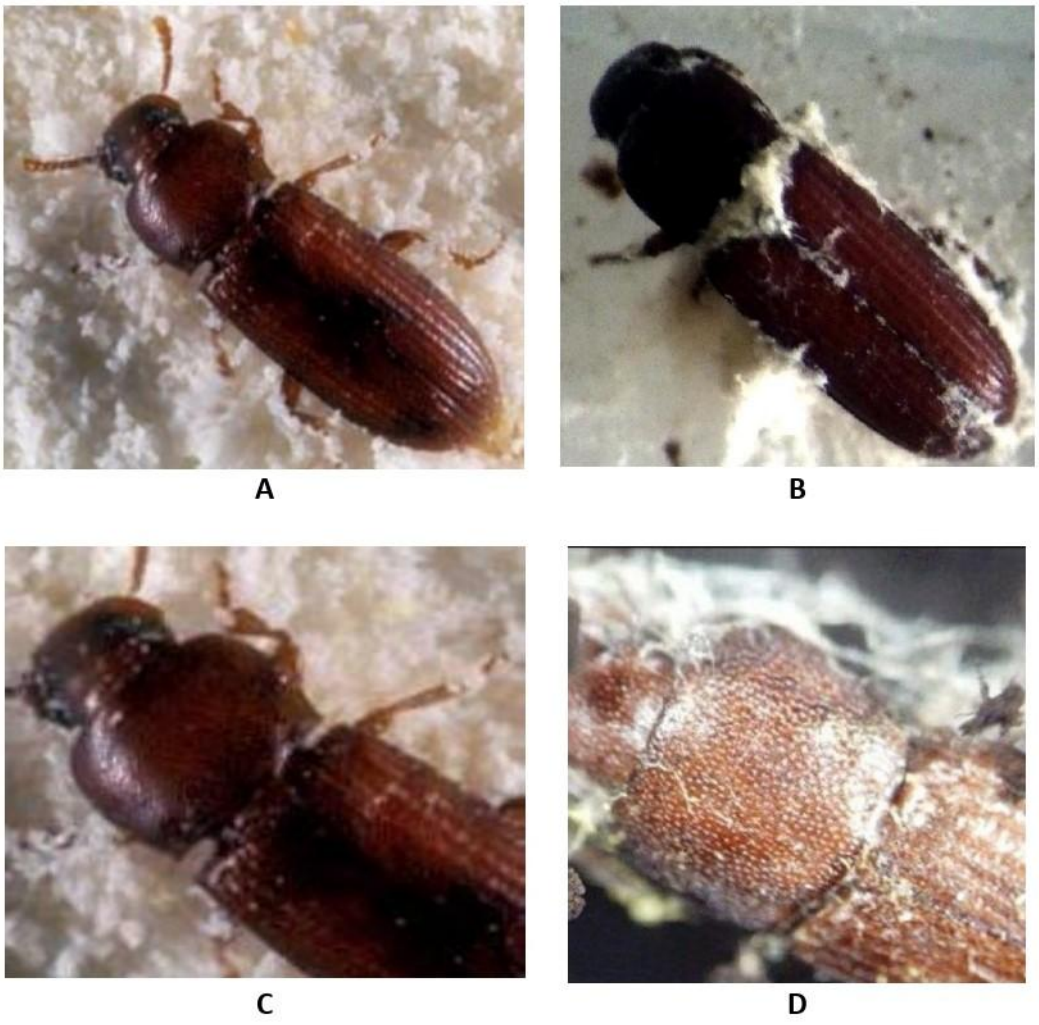

Figure 4. (A, C) Untreated *T. castaneum* prior to fungal exposure. (B, D) Post-mortem development of mycelial and conidial structures on *T. castaneum* cadavers subsequent infection with entomopathogenic fungi.

The observed increases in mortality among second-, fourth-, and sixth-instar larvae of *T. castaneum* are likely related to different physiological and behavioral factors. As larval instars age, their immune responses intensify, containing elevated hemocyte counts, phenoloxidase activity, and antimicrobial peptide production, which can affect fungal virulence. Additionally,

fungal invasion may lead to the accumulation of mycotoxins in the hemolymph, disrupting normal metabolic processes and accelerating mortality (Akmoosh et al., 2023; 2024). Larval lethargy and reduced feeding activity due to fungal infection may further contribute to higher mortality, as energy reserves are depleted more rapidly in infected larvae. Analysis of mortality over time illustrated that, for all tested concentrations, mortality generally became evident around the third day post-treatment, with peak mortality occurring by day seven. Higher concentrations of *M. anisopliae* and *V. lecanii* consistently generated the greatest mortality, affirming a clear dose-dependent response. These results align with the observations of Bateman (1997), who noted that EPFs typically create observable mortality in field insects after different days, with delayed effects reflecting the time needed for fungal germination, cuticle penetration, and internal colonization. Our results also highlight the differential susceptibility of developmental stages to fungal infection. Immature stages, exclusively second- and fourth-instar larvae, were more susceptible to infection than sixth-instar larvae and adults (Figures 1-3). This stage-dependent susceptibility has been announced for other beetle species as well; for instance, Öztürk et al. (2015) demonstrated that early larval instars of the Colorado potato beetle (*Leptinotarsa decemlineata*) were more vulnerable to EPFs than adults, likely due to thinner cuticles and less developed immune defenses. The lower susceptibility of older larvae and adults may reflect a combination of thicker cuticles, increased melanization, and more robust behavioral defenses like grooming or reduced exposure to inoculum. Molting behavior in larvae may also impress susceptibility. If fungal spores contact the insect shortly before ecdysis, some spores may be shed along with the old cuticle, thereby reducing effective infection rates (Lopes and Alves, 2011). This phenomenon may contribute to the differing mortalities among consecutive larval instars. Attachment of the spores seems to be followed by germination and a penetration through the epicuticular layer. The outer cuticle is covered by a thin (1-5 μm) wax layer compound of hundreds of lipophilic and hydrocarbon substances; its composition can vary extensively among individuals at the same stage and even among individuals (Vasquez et al., 2025). This variability may impact the effectiveness of fungal adhesion, germination, and cuticle-degradation and offers a possible mechanistic explanation for variations in susceptibility among larval instars and adults (Ortiz-Urquiza & Keyhani 2013). After penetrating the cuticle, the fungus grows inside the hemocoel and causes systemic infection. The host bite is followed by the death of the host typically in 3-4 day period, which may be evolved depending on fungus strain, inoculum concentration and host developmental types (Al-Zurfi et al., 2023). After mycelia emerges post-mortem from the cadaver surface, new conidia are produced and the life cycle of the fungus is finished. Conidial production is fast and leads to successive rounds of infection, which are an important aspect for successful pest control in storage. Comparison of the two phytopathogens

revealed distinct virulence profiles. The efficacy of *M. anisopliae* was higher in early larval instars, with 100% mortality (only second-instar larvae killed) at the highest concentration. *V. lecanii*, however, was more effective when used against fourth instar larvae and adults reaching 95% and 92% of mortality, respectively. These differences suggest that fungal species choice might have to be adjusted according to the targeted developmental stage in IPM strategies. The overall results demonstrate the significant influence of dose and contact time on attaining acceptable levels of infestation reduction. An increasing concentration of spores and longer exposure resulted in higher percentages of mortality at all stages indicating that the minimum inoculum dosage; necessary for *M. anisopliae* and *V. lecanii* to overcome host resistance are not below those levels were as infected could be successfully elevated. Establishing successful infection. This is consistent with earlier studies that reported inactivated EPF generally require prolonged contact with susceptible hosts for effective control (Shah and Pell, 2003). The results of the study also validate use EPFs in practice in stored grain. In comparison with field conditions, storage systems generally provide stable temperature and humidity to minimize UV-triggered inactivation of spores and persistence of fungi. Thus, both *M. anisopliae* and *V. lecanii* are promising agents for the biocontrol of *T. castaneum* in stored grains.

Conclusions: Mortality rates among second-instar larvae exposed to the 3 fungal dose levels tested were significantly higher than those of fourth and sixth instar larvae, or adults, in all treatments. Both *Metarhizium anisopliae* and *Verticillium lecanii* successfully infected and killed *T. castaneum*, and mortality significantly depended on spore concentration, exposure duration, and developmental stage of the host. *M. anisopliae* was most active in early larval instars, while V. lecanii was more effective toward fourth-instar larvae and adults. These results illustrate that fungal formulations from the genera *Metarhizium* and *Verticillium*, exclusively *V. lecanii*, have considerable potential as biological control agents for the management of *T. castaneum* in stored grain environments. The results also supply a foundation for further investigations to optimize application methods, dosages, and integration with other pest management strategies to raise the sustainability and effectiveness of fungal-based biocontrol.

Author Contributions

Conceptualization and methodology, Al Shareefi Ekhlas; software and validation, Nebras M. Sahi.; formal analysis and investigation, Nada A. Al-khafaji; resources, All authors contributed equally; data curation, Farah F. Abdulsada; writing—original draft preparation, Al Shareefi Ekhlas; writing—review, Nebras M. Sahi, Nada A. Al-khafaji, Farah F. Abdulsada; visualization, F. Abdulsada; supervision, Al Shareefi Ekhlas; project administration, Nebras M. Sahi; funding acquisition, All authors contributed equally.

Data Availability Statement

Data are available from the authors upon reasonable request.

Acknowledgements

The authors gratefully acknowledge the participation of all individuals in this investigation.

Ethical Considerations

The study was carried out with integrity, with no fabrication, falsification, plagiarism, or scientific misconduct.

Funding

No external funding was received for this study from public, commercial, or charitable organizations.

Conflict of Interest

The authors report no conflicts of interest.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸
دانشگاه شهید باهنر کرمان
انجمن بیوتکنولوژی ایران

زیست‌فعالیت قارچ‌های Metarhizium anisopliae و Verticillium lecanii بر مراحل نابالغ و بالغ سوسک آردی Tribolium castaneum

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تاریخ دریافت: ۱۴۰۴/۰۴/۱۶ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۴/۰۶/۲۷ تاریخ پذیرش: ۱۴۰۴/۰۶/۲۸

چکیده

هدف: سوسک آرد قرمز *Tribolium castaneum* (Herbst.) یکی از مخرب‌ترین آفات غلات انباری و فرآورده‌های جانبی آنها است که زیان‌های اقتصادی قابل توجهی در سراسر جهان ایجاد می‌کند. توانایی چشمگیر این آفت در ایجاد مقاومت نسبت به حشره‌کش‌های متداول، همراه با نگرانی‌های فزاینده درباره خطرات زیست‌محیطی و بهداشتی کنترل شیمیایی، ضرورت تدوین راهبردهای مدیریتی جایگزین را ایجاب می‌کند. قارچ‌های بیماری‌زای حشرات (EPFs)، که به طور طبیعی پاتوژن حشرات هستند، گزینه‌ای امیدبخش برای کنترل زیستی محسوب می‌شوند. این پژوهش با هدف ارزیابی فعالیت بیماری‌زایی دو فرآورده تجاری قارچ‌های EPF یعنی *Metarhizium anisopliae* (Met52) و *Verticillium lecanii* (Mycotal) علیه مراحل رشدی مختلف *T. castaneum* انجام شد.

مواد و روش‌ها: آزمون‌های زیستی در شرایط آزمایشگاهی به‌منظور بررسی کارایی *M. anisopliae* و *V. lecanii* علیه لاروهای سن دوم، چهارم و ششم، همچنین حشرات بالغ *T. castaneum* انجام گرفت. غلظت‌های مختلفی از اسپور قارچ‌ها اعمال شد و درصد مرگ‌ومیر در بازه‌های زمانی متفاوت ثبت گردید. داده‌های مربوط به مرگ‌ومیر با استفاده از تحلیل آماری جهت تعیین حساسیت وابسته به غلظت و مرحله رشدی بررسی شدند.

نتایج: هر دو قارچ EPF بیماری‌زایی قابل توجهی علیه *T. castaneum* نشان دادند، اگرچه میزان کارایی آنها در مراحل رشدی و غلظت‌های مختلف متفاوت بود *M. anisopliae* به طور مداوم قدرت بیماری‌زایی بالاتری نسبت به *V. lecanii* نشان داد. حساسیت به شدت به مرحله رشدی وابسته بود: لاروهای سن دوم و چهارم آسیب‌پذیرتر بودند، در حالی که لاروهای سن ششم و حشرات بالغ مقاومت بیشتری در برابر آلودگی قارچی داشتند. افزایش غلظت اسپور و طول مدت مواجهه به طور معنی‌داری مرگ‌ومیر را در تمام مراحل آزمایش‌شده افزایش داد. همبستگی مثبت بین درصد مرگ‌ومیر و هر دو عامل غلظت قارچ و مدت زمان مواجهه مشاهده شد. به‌ویژه، لاروهای سن ششم و بالغین کمترین میزان مرگ‌ومیر را نشان دادند که اهمیت هدف‌گیری مراحل نابالغ‌تر را برای کنترل مؤثر برجسته می‌سازد.

نتیجه‌گیری: نتایج نشان داد که *M. anisopliae* و *V. lecanii* پتانسیل قابل توجهی به‌عنوان عوامل کنترل زیستی علیه *T. castaneum*دارند. در میان این دو قارچ، *M. anisopliae* بیماری‌زایی برتری در مراحل رشدی مختلف از خود نشان داد. این مطالعه بر اهمیت بهینه‌سازی غلظت قارچ و هدف‌گیری مراحل لاروی حساس برای بیشینه‌سازی کارایی تأکید دارد. یافته‌ها از ادغام قارچ‌های بیماری‌زا در برنامه‌های پایدار مدیریت آفات محصولات انباری و کاهش وابستگی به حشره‌کش‌های شیمیایی حمایت می‌کنند.

کلمات کلیدی: قارچ‌های بیماری‌زای حشرات، *Metarhizium anisopliae*، *Tribolium castaneum*، *Verticillium lecanii*

نوع مقاله: پژوهشی

استناد: اخلاص الشریفی، نبرس م. صاحی، نُدا ا. الخفاجی، فرح ف. عبدالساده، سجا ایاد الخفاجی (۱۴۰۵). زیست‌فعالیت قارچ‌های Metarhizium anisopliae و Verticillium lecanii بر مراحل نابالغ و بالغ سوسک آردی Tribolium castaneum. *مجله بیوتکنولوژی کشاورزی*، ۱۸(۲)، ۳۱۳-۳۳۰.

Publisher: Shahid Bahonar University of Kerman & Iranian Biotechnology Society.