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Original article (Orijinal araştırma)

Efficacy of native entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) against *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae)

Yerel entomopatojen fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae)'nın *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) üzerindeki etkinliği

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Abstract

Helicoverpa armigera (Hübner, 1808) (Lepidoptera: Noctuidae) is a significant agricultural pest with resistance to conventional synthetic insecticides. The present study, conducted in 2024 at Bolu Abant Izzet Baysal University, Biological Control Laboratory, investigated the pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) against six larval stages of *H. armigera*. Three conidial concentrations (1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}) were evaluated. This study analyzed the effects of varying spore concentrations of *B. bassiana* on the mortality of *H. armigera* larvae, considering days post-application, dose, and larval stage. Lethal concentrations (LC_{50} and LC_{90}) and lethal time values (LT_{50} and LT_{90}) were calculated using probit analysis. Mortality increased with dose and was highest in early instars, particularly at 1×10^8 conidia ml^{-1}, where LT_{50} and LT_{90} values for first instars were 4.05 and 8.10 days, respectively. Older instars exhibited lower mortality rates. LC_{50} and LC_{90} decreased with increasing concentrations, achieving 100% mortality in the first and second instars across all doses. Third instars displayed dose-dependent mortality, with LC_{50} and LC_{90} values of 6.88×10^8 and 1.94×10^4 conidia ml^{-1}, respectively. Higher spore concentrations reduced LT_{50} and LT_{90}, enhancing mortality rates in younger larvae. These findings underscore the efficacy of *B. bassiana* against early larval stages, emphasizing the importance of application timing for effective biological control and its potential role in integrated pest management strategies.

Keywords: *Beauveria bassiana,* biological control, biopesticide, entomopathogenic fungus, *Helicoverpa armigera*

Öz

Helicoverpa armigera (Hübner, 1808) (Lepidoptera: Noctuidae), sentetik insektisitlere karşı direnç geliştirmiş önemli bir tarımsal zararlıdır. Bu çalışmada, entomopatojenik fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae)'ın *H. armigera*'nın altı larva dönemine karşı etkinliği 2024 yılında Bolu Abant Izzet Baysal Üniversitesi, Biyolojik Mücadele Laboratuvarı'nda incelenmiştir. Üç farklı konidiyal konsantrasyon (1×10^6, 1×10^7 ve 1×10^8 konidi ml^{-1}) kullanılarak ölüm oranları değerlendirilmiştir. Uygulama sonrası günler, doz ve larva dönemleri göz önünde bulundurularak, *B. bassiana*'nın farklı spor konsantrasyonlarının etkileri analiz edilmiştir. Probit analizi ile öldürücü konsantrasyonlar (LC_{50} ve LC_{90}) ve öldürücü zaman değerleri (LT_{50} ve LT_{90}) hesaplanmıştır. Ölüm oranlarının, artan dozlarla yükseldiği ve özellikle genç larva dönemlerinde belirgin olup, özellikle birinci larva döneminde 1×10^8 konidi ml^{-1} konsantrasyonunda, LT_{50} ve LT_{90} değerleri sırasıyla 4,05 ve 8,10 gün olarak kaydedilmiştir. Daha ileri dönemlerde ise ölüm oranlarının düştüğü tespit edilmiştir. LC_{50} ve LC_{90} değerleri, artan konsantrasyonlarla azalarak birinci ve ikinci dönem larvalarda tüm dozlarda %100 ölüm sağlamıştır. Üçüncü dönem larvalar doz-bağımlı bir tepki göstermiş ve LC_{50} ile LC_{90} değerleri sırasıyla 6.88×10^8 ve 1.94×10^4 konidi ml^{-1} olarak bulunmuştur. Daha yüksek spor konsantrasyonları, LT_{50} ve LT_{90} sürelerini kısaltarak özellikle genç larvalarda ölüm oranlarını artırmıştır. Bu bulgular, *B. bassiana*'nın genç larva dönemlerine karşı etkinliğini, etkili biyolojik mücadele için uygulama zamanlamasının önemini ve entegre zararlı yönetimi stratejilerinde potansiyel rolünü vurgulamaktadır.

Anahtar sözcükler: *Beauveria bassiana,* biyolojik mücadele, biyopestisit, entomopatojen fungus, *Helicoverpa armigera*

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Introduction

Helicoverpa armigera (Hübner, 1808) (Lepidoptera: Noctuidae) is a highly destructive, polyphagous pest that affects over 100 plant species, including several economically significant crops such as maize, common bean, cotton, tobacco, soybean, chickpea, and various vegetables (Talekar et al., 2006; Cunningham & Zalucki, 2014; Koca & Kaçar, 2024). Over the past few decades, the pest has significantly expanded its geographic range, with reports from regions across Europe, Africa, South America, Asia, and Australasia (Kriticos et al., 2015; Arnemann et al., 2016). Adult *H. armigera* demonstrates notable migratory potential, with recorded long-distance dispersals extending up to 2.000 kilometers (Feng et al., 2009; Jones et al., 2015; Riaz et al., 2021). These moths are particularly attracted to the reproductive stages of host plants, with the larvae predominantly feeding on reproductive parts such as flowers and pods (Liu et al., 2010). This feeding behavior, focused on yield-forming organs, contributes to the severe economic losses observed in a wide range of crops affected by *H. armigera* (Zalucki et al., 1986). Therefore, its infestations are responsible for substantial yield losses, often necessitating costly control measures. Additionally, the species exhibits a remarkable ability to develop resistance to chemical insecticides, further complicating management strategies (Fitt, 1989; Walsh et al., 2022). The extensive use of broad-spectrum chemical insecticides has led to the rapid development of resistance in *H. armigera* populations, particularly against pyrethroids, endosulfan, carbamates, and organophosphates (Ahmad et al., 1999, 2003). This resistance poses significant challenges for sustainable pest control and highlights the need for alternative approaches.

The management of *H. armigera* involves multiple strategies, including transgenic insect-resistant crops, pheromone traps, light traps, chemical pesticides, and biological control agents (Riaz et al., 2021). Among these methods, biological control plays a critical role within Integrated Pest Management (IPM) programs, as it reduces the reliance on synthetic pesticides, particularly due to its eco-friendly and sustainable application (Kaçar et al., 2023). In IPM programs, biological control agents like entomopathogenic fungus (EPF) are integrated with other strategies such as crop rotation, resistant varieties, and selective pesticide use. This multi-faceted approach ensures a reduction in chemical pesticide applications and delays the development of resistance in pest populations (Bale et al., 2008). Entomopathogenic fungi, especially *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) have emerged as highly promising biological control agents due to their ability to infect and kill the pest at various larval stages effectively (Wagner & Lewis, 2000; Younas et al., 2017; Altinok et al., 2019). As a widespread entomopathogenic fungus and obligate parasite, *B. bassiana* is the causal agent of white muscardine disease in insects (Deans & Krischik, 2023). These fungi are known to infect and kill *H. armigera* larvae by adhering to and penetrating the insect's cuticle through their conidial spores, ultimately leading to the host's death via internal colonization and degradation of the hemolymph (Nguyen et al., 2007; Goettel & Glare, 2010).

Considering the potential of entomopathogenic fungi as biocontrol agents, they present an effective alternative to traditional synthetic insecticides within an integrated pest management framework aimed at controlling field populations of *H. armigera*. These biocontrol agents disrupt the physiology, behavior, and development of their host insects, providing a strategic advantage in managing resistant pest species (Michereff-Filho et al., 2008). Unlike chemical pesticides, which can lead to non-target effects and environmental contamination, entomopathogenic fungi like *B. bassiana* offer an environmentally friendly alternative. They are host-specific, leave minimal residues, and contribute to the conservation of natural enemy populations (Vega et al., 2009). Moreover, their compatibility with other IPM tactics highlights their strategic importance in sustainable agriculture (Kaçar et al., 2023).

The present study aimed to evaluate the effects of the entomopathogenic fungus *B. bassiana* as a biocontrol agent against *H. armigera,* investigating the effects across different larval instars and spore concentrations. It is hypothesized that the native strain of *B. bassiana* will significantly reduce *H. armigera* larval populations in vitro, showcasing its potential for broader field applications in an IPM framework. The results of this study will contribute to the ongoing efforts to integrate EPFs into pest management frameworks, reducing the reliance on chemical insecticides and promoting sustainable crop protection.

Materials and Methods

Mass rearing of *Helicoverpa armigera*

The mass rearing of *H. armigera* was conducted under controlled laboratory conditions, with the temperature maintained at 25±1°C, relative humidity (RH) at 65±5%, and 14:10 h light/dark (L/D) photoperiod. Larvae were provided with an artificial diet specifically formulated to support optimal growth (Southland Products Inc., USA). The diet consists of toasted soybean flour, stabilized wheat germ, sugar, vitamins, and mineral salts. Cannibalism among the larvae was mitigated by rearing individuals from the third instar in separate plastic vials. After pupae had developed, they were transferred to ventilated containers to allow adults to emerge. Newly emerged adults were fed a 20% honey solution and maintained under the same conditions. The overall rearing protocol was adapted from Armes et al., (1992), with some modifications. The six larval instars of *H. armigera* (L1, L2, L3, L4, L5, and L6) were determined based on head capsule width and overall size (Queiroz-Santos et al., 2018).

Isolation and identification of entomopathogenic fungi

Insect cadavers of *H. armigera* larvae suspected to be infected with entomopathogenic fungi were collected in maize fields and surface sterilized to eliminate external contaminants. Initially, each cadaver was submerged in 1% sodium hypochlorite solution for 2 minutes, followed by rinsing in 70% ethanol for 1 minute. Subsequently, cadavers were rinsed three times in sterile distilled water to remove any chemical residues. Once cleaned, the insect cadavers were placed individually on sterile filter paper within Petri dishes containing Potato Dextrose Agar (PDA) medium (Sigma Aldrich, St. Louis, MO, USA) supplemented with 0.01% streptomycin to prevent bacterial contamination. The Petri dishes were incubated at 25±2°C with 65±5% RH in darkness for 7–10 days to allow fungal growth. Fungal colonies emerging from the insect tissues were regularly monitored, and those displaying typical morphological characteristics of *B. bassiana.* Once isolated, individual colonies were transferred to fresh PDA plates for further purification and maintenance. Purified isolates were stored at 4°C until further identification and use in bioassays (Inglis et al., 2012; Zimmermann, 2007). To confirm the identification, the internal transcribed spacer (ITS) and the partial *β-tubulin* (*tub2*) loci were amplified and sequenced using primers ITS1/ITS4 (White et al., 1990) and BT2a/BT2b (Glass & Donaldson, 1995) according to Güney et al. (2022). The sequence homology searches were conducted using the Basic Local Alignment Search Tool (BLAST: http://blast.ncbi.nlm.nih.gov/) against the nucleotide database of the NCBI GenBank to identify the closest available reference sequences.

Conidial suspension of *Beauveria bassiana*

Beauveria bassiana isolate used in this study was mass-cultured PDA medium, supplemented with 1% yeast extract to enhance fungal growth. Plates were incubated at 25±2°C with 65±5% RH under a 14:10 h L/D photoperiod for 14 days, allowing for conidial production. Once mature, conidia were harvested by gently scraping the surface of the culture plates with a sterilized spatula. The collected conidia were then air-dried at room temperature and stored at 4°C in sterile containers until further use. To prepare the bioassay, the dry conidial powder was suspended in sterile distilled water supplemented with 0.01% Tween-80 (Merck, Darmstadt, Germany) to achieve uniform spore dispersion. The suspension was passed with two layers of sterile cheesecloth to remove any mycelial fragments, ensuring a uniform spore suspension.

The resulting suspension was thoroughly mixed using a vortex mixer for 1 minute to break any spore clumps. Spore concentrations were adjusted by performing serial dilutions to achieve the desired concentrations of 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}. Spore viability was assessed by incubating a small aliquot of the suspension on fresh PDA plates at 25°C for 24 hours, and the germination rate was confirmed to be above 90% prior to each bioassay. The Bb_Dzc01 isolate was selected among the isolates tested for further assays.

Insect bioassay

The entomopathogenic fungus, *B. bassiana* was utilized for this study, and spore suspensions were prepared in sterile distilled water containing 0.01% Tween 80 as a surfactant. Three spore concentrations were tested: 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}. These concentrations were freshly prepared prior to each bioassay to ensure viability and consistency. The larvae were exposed to the fungal conidial suspensions through immersion. Each larva was individually immersed in a 100 ml conidial suspension of the respective concentration for 10 seconds, ensuring uniform exposure. After immersion, larvae were allowed to dry air on sterile filter paper for 30 minutes before being transferred to experimental places. The bioassay protocol was adapted from Tahir et al. (2019) and Alwaneen et al. (2024). For the L1 and L2 larval instar, each treatment group consisted of 5 larvae per Petri dish (90 mm diameter), with five replicates per concentration. For the L3, L4, L5, and L6 larvae, due to cannibalistic behavior (Kakimoto et al., 2003), only one larva was placed per Petri dish, with five replicates per concentration. Each replicate was repeated five times, ensuring a total of 25 larvae per concentration for each instar. Control groups consisted of larvae immersed in a solution containing 0.01% Tween 80 without fungal conidia. After treatment, all Petri dishes were sealed with a parafilm and maintained in a climate-controlled chamber at 25±1°C and 65±5% RH under a 14:10 h L/D photoperiod. The larvae were provided with a fresh artificial diet every two days to ensure adequate nutrition. Mortality was recorded after 1, 3, 5 ,7, 10 and 14 days, and dead larvae were removed and transferred to moist filter paper in a separate Petri dish to encourage fungal outgrowth and confirm cause of death.

Statistical analysis

Mortality rates were adjusted for control mortality using Abbott's formula (Abbott 1925), to account for natural death rates in the control groups. Corrected mortality rates were then used for further analysis. Before performing analysis of variance (ANOVA), the normality and homogeneity of variances were checked using the Shapiro-Wilk and Levene's tests, respectively. All data was not normally distributed. The percentage of mortality data collected at various time intervals was subjected to arcsine square root transformation to normalize the data. To statistically compare the effects of EPF applications on mortality, a three-way ANOVA was conducted, followed by Tukey's HSD test for multiple comparisons among the spore concentrations (1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}). Probit analysis was performed to estimate the lethal concentrations (LC_{50} and LC_{90}) and lethal times (LT_{50} and LT_{90}) for each larval instar and spore concentration. The fiducial limits for the LC and LT values were calculated with a 95% confidence interval. All statistical analyses were conducted using SPSS software version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). For effective visualization of the data, graphical representations were generated using GraphPad Prism version 6.04 (GraphPad Software, San Diego, CA, United States). All statistical analyses were conducted on arcsine-transformed data, while graphs represent raw (untransformed) data for easy interpretation.

Result

In this study, the Bb_Dzc01 isolate obtained from *H. armigera* larvae in Düzce was identified as *B. bassiana* with 100% identity for the ITS region (541bp), and *tub2* (291 bp). The resulting sequences were deposited in GenBank under the accession numbers PQ826424 for ITS and PQ857545 for *tub2*. The effects of different spore concentrations of *B. bassiana* on the mortality of *H. armigera* larvae were assessed by

considering factors such as day, dose, and larval stage. The analysis results indicate that each factor (day, dose, and larval stage) and its interactions (day×dose, day×larval stage, dose×larval stage, and day×dose×larval stage) significantly impacted mortality rates (Table 1).

Table 1. Results of the factorial analysis for mortality of all larval stages of *Helicoverpa armigera* exposed to *Beauveria bassiana*

Source	*df*	Sum of Squares	*F* Ratio	*p*
Day	5	2543.421	216.255	<0.0001
Dose	3	1669.359	236.563	<0.0001
Larval stage	5	1439.679	122.409	<0.0001
Day * Dose	15	870.991	24.685	<0.0001
Day * Larval stage	25	576.879	9.809	<0.0001
Dose * Larval stage	15	549.150	15.564	<0.0001
Day * Dose * Larval stage	75	512.369	2.904	<0.0001
Error	576	1354.892	-	-
Total	719	9516.741	-	-

Mortality rates showed significant variation across days post-application ($F_{5, 576}$ = 216.255, p < 0.0001), doses applied ($F_{3, 576}$ = 236.563, p< 0.0001), and larval stages ($F_{5, 576}$ = 122.409, p < 0.0001), with significant interactions observed among these factors. A significant day-by-dose interaction ($F_{15, 576}$ = 24.685, p < 0.0001) revealed changes in dose effectiveness over time. The interaction between the day and larval stage ($F_{25, 576}$ = 9.809, p < 0.0001) suggested that the impact of time on mortality varied by developmental stage. Similarly, a dose-by-larval stage interaction ($F_{15, 576}$ = 15.564, p < 0.0001) indicated dose effectiveness differences across stages. A three-way interaction among the day, dose, and larval stage ($F_{75, 576}$ = 2.904, p < 0.0001) highlighted the complex interaction in which timing, spore concentration, and developmental stage collectively shaped mortality outcomes. These findings demonstrate the significant influence of dose, application timing, and larval stage on *H. armigera* mortality under *B. bassiana* exposure, with combined interactions among these factors.

Figure 1 presents the mortality rates of *H. armigera* larvae at different instars when exposed to varying spore concentrations (1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}) of *B. bassiana*. Tukey's HSD test results revealed statistically significant differences among treatments and larval stages. Data analysis showed a clear pattern of higher mortality in younger instars compared to older instars, indicating that susceptibility to *B. bassiana* decreases as larvae progress through their developmental stages. At the lowest concentration of 1×10^6 conidia ml^{-1}, mortality rates were notably higher in the first and second instars, with a marked decline in effectiveness observed in the later stages, particularly in the fifth and sixth instars. Although overall mortality rates were slightly higher than at the lower concentration, this trend was still visible at 1×10^7 conidia ml^{-1}, where early instars were maintained to show higher mortality than more mature stages. At the highest concentration, mortality was consistently high in early instars yet still reduced considerably in the fifth and sixth instars, underscoring the reduced susceptibility of older larvae. The results indicated that increasing the spore concentration enhanced the overall mortality rate, though the effect was particularly remarkable in the younger instars.

The results in Table 2 demonstrate the lethal concentration (LC_{50} and LC_{90}) values of *B. bassiana* applied to different larval stages of *H. armigera* over time. For each larval stage, LC_{50} and LC_{90} values decreased with an increase in days post-application, indicating a time-dependent increase in fungal pathogenicity. In particular, younger instars (L1, L2, and L3) were more susceptible to *B. bassiana*, as reflected by the relatively lower LC_{50} and LC_{90} values compared to older instars (L4, L5, and L6). For instance, mortality rates reached 100% at the 1×10^7 and 1×10^8 concentrations 10 days after treatment in first-instar larvae. At the lowest concentration, a mortality rate of 96% was observed by the end of two weeks (Figure 2). For first-instar larvae (L1), the estimated LC_{50} and LC_{90} values at the end of the two

weeks were 3.65×10^3 and 2.03×10^5, respectively. In second-instar larvae (L2), mortality rates reached 100% by 14 days across all concentrations, with no statistically significant differences observed ($\chi2 = 7.93$, df = 13, $p = 0.848$). 10 days post-application, mortality rates were 96% (1×10^8), 72% (1×10^7), and 60% (1×10^6), respectively (Figure 2). For second-instar larvae, the estimated LC_{50} and LC_{90} values after 10 days were 5.86×10^5 and 5.13×10^7. For third-instar larvae (L3), mortality rates by the end of 14 days were 100% at 1×10^8, 84% at 1×10^7, and 72% at 1×10^6 (Figure 2). The estimated LC_{50} and LC_{90} values for the third instar were 6.88×10^8 and 1.94×10^4. In fourth-instar larvae (L4), mortality rates at two weeks post-application were 84%, 48%, and 32% from the highest to the lowest concentrations (Figure 2). The average LC_{50} and LC_{90} values at the end of 14 days were 6.32×10^6 and 3.88×10^8, respectively. Finally, the mortality rates of older (L5 and L6) instar larvae were significantly lower than those of earlier stages and were statistically grouped as similar.

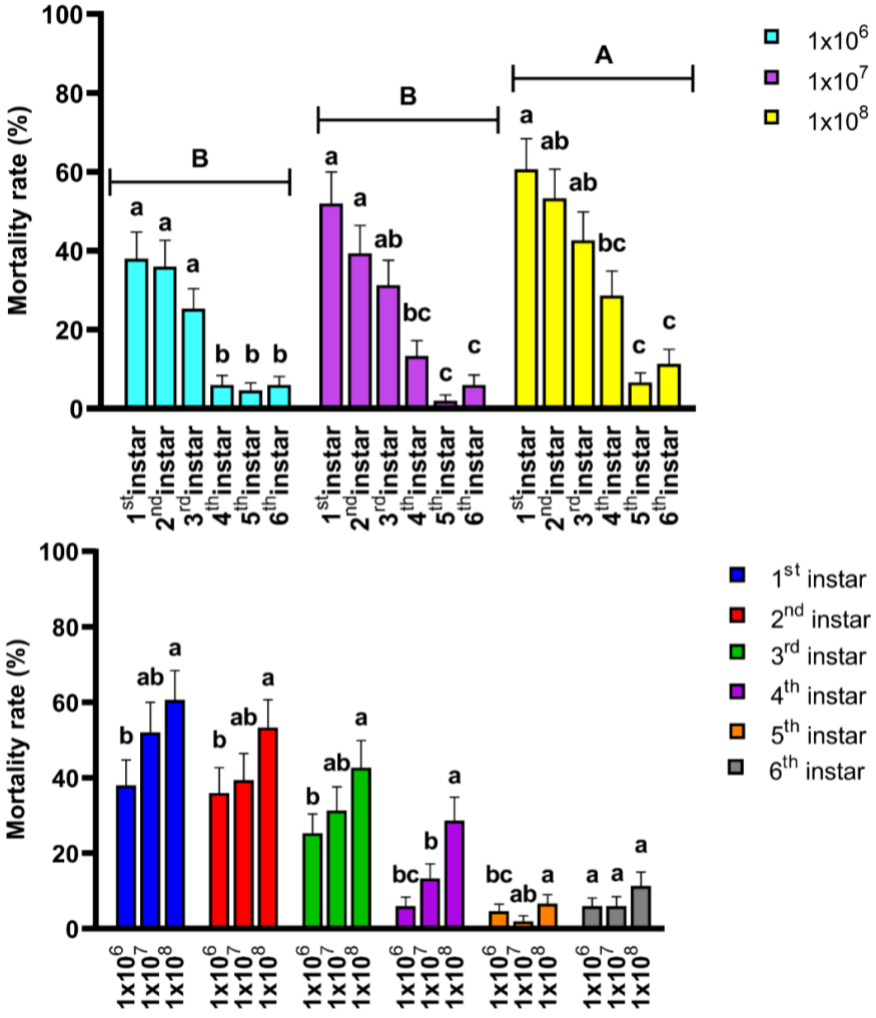

Figure 1. Mortality rates (±SE) of *Helicoverpa armigera* larvae across six instars treated with different concentrations of *Beauveria bassiana*. Significant differences between concentrations and larval instars as indicated by different lowercase letters. Significant differences between fungal concentrations were indicated by the uppercase letters (Tukey's HSD at $p < 0.05$).

Table 2. Lethal concentration (LC_{50} and LC_{90}) values of *Beauveria bassiana* tested against larval stages of *Helicoverpa armigera*

Larval Stage	Days	LC_{50} (conidia ml^{-1})	LC_{90} (conidia ml^{-1})	Slope±SE	Intercept±SE	$\chi 2$ (*df* = 13)	*p*
L1	3	6.62×10^{15}	5.61×10^{22}	0.18±0.07	-2.93±0.47	233.46	0.0001
	5	9.58×10^{7}	2.13×10^{11}	0.38±0.04	-3.06±0.29	221.40	0.0001
	7	9.81×10^{5}	3.82×10^{7}	0.80±0.05	-4.82±0.35	156.39	0.0001
	10	9.63×10^{5}	2.55×10^{6}	3.02±0.43	-18.10±2.60	147.96	0.0001
	14	3.65×10^{3}	2.03×10^{5}	0.74±0.19	-2.61±1.19	93.95	0.0001
L2	3	2.85×10^{8}	1.10×10^{9}	2.17±0.49	-18.46±3.98	83.61	0.0001
	5	5.09×10^{9}	6.68×10^{14}	0.25±0.04	-2.43±0.30	272.46	0.0001
	7	1.10×10^{7}	1.41×10^{10}	0.41±0.04	-2.90±0.28	92.48	0.0001
	10	5.86×10^{5}	5.13×10^{7}	0.66±0.05	-3.80±0.34	103.91	0.0001
	14	6.77×10^{1}	2.56×10^{2}	0.50±0.76	-0.08±4.73	7.93	0.848
L3	3	1.30×10^{8}	3.28×10^{8}	3.18±0.86	-25.81±6.90	326.49	0.0001
	5	6.53×10^{8}	1.43×10^{12}	0.38±.044	-3.38±0.31	562.24	0.0001
	7	3.94×10^{8}	1.62×10^{12}	0.35±0.04	-3.04±0.30	538.04	0.0001
	10	4.64×10^{6}	1.70×10^{12}	0.05±0.04	-0.33±0.27	314.86	0.0001
	14	6.88×10^{8}	1.94×10^{4}	0.28±0.04	-2.49±0.29	368.82	0.0001
L4	3	-	-	-	-	-	-
	5	2.07×10^{12}	4.86×10^{15}	0.38±0.09	-4.68±0.72	231.48	0.0001
	7	2.96×10^{8}	3.32×10^{9}	1.22±0.11	-10.34±0.82	155.52	0.0001
	10	6.53×10^{7}	1.57×10^{9}	0.93±0.05	-7.26±0.39	184.09	0.0001
	14	6.32×10^{6}	3.88×10^{8}	0.72±0.04	-4.87±0.30	147.55	0.0001
L5	3	-	-	-	-	-	-
	5	-	-	-	-	-	-
	7	-	-	-	-	-	-
	10	5.80×10^{6}	1.04×10^{7}	0.63±0.06	-1.61±0.44	232.39	0.0001
	14	9.62×10^{10}	2.09×10^{17}	0.20±0.04	-2.22±0.31	198.87	0.0001
L6	3	-	-	-	-	-	-
	5	-	-	-	-	-	-
	7	-	-	-	-	-	-
	10	1.29×10^{18}	1.29×10^{28}	0.12±0.05	-2.16±0.39	176.30	0.0001
	14	1.39×10^{8}	1.26×10^{12}	0.32±0.04	-2.64±0.29	155.02	0.0001

* Each concentration was tested using 25 larvae, divided into five replicates, each consisting of five larvae.

** Cells left blank in the table indicate instances where no or fewer mortality events were observed, and thus values could not be calculated for the respective days post-treatment.

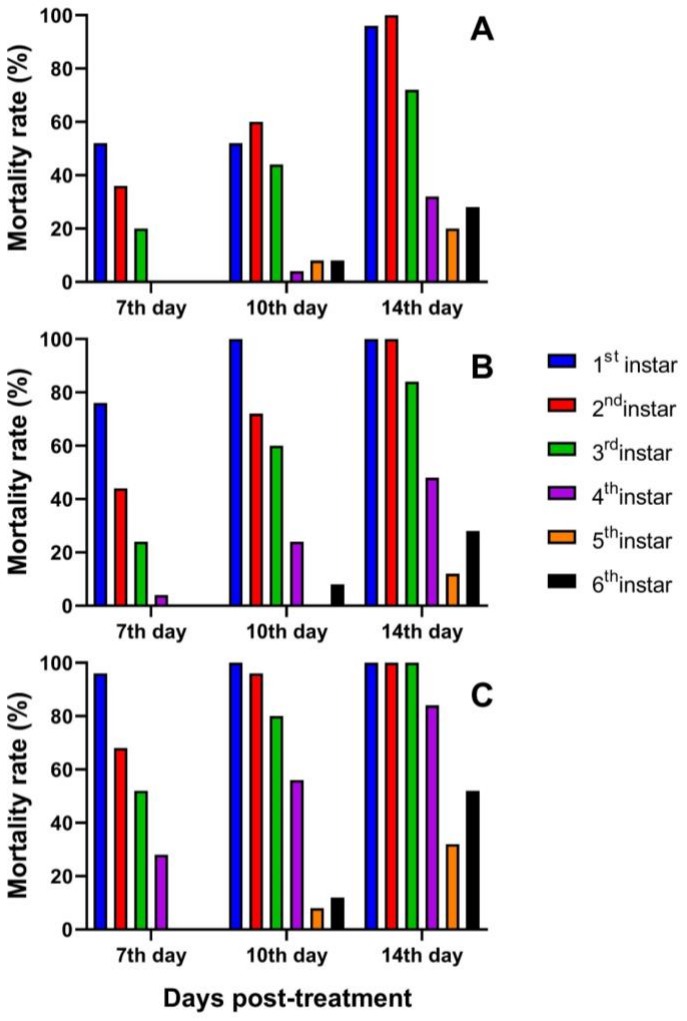

Figure 2. Mortality rates of *Helicoverpa armigera* larvae for six instars at three doses of *Beauveria bassiana* on the 7th, 10th, and 14th days post-treatment (A: $1x10^6$, B: $1x10^7$, C: $1x10^8$ conidia ml^{-1}).

The estimated LT_{50} and LT_{90} values based on the mortality trends across different concentrations were presented in Table 3. Increasing conidial concentrations reduced the LT_{50} and LT_{90} estimates and increased the larval mortality percentages (Figure 3). The LT_{50} of *B. bassiana* against larval instars of *H. armigera* ranged from 7.53 to 20.87 days at $1×10^6$ conidia ml^{-1}, while at the highest concentration, values varied from 4.05 to 18.13 days. Likewise, LT_{90} values at the lowest concentration ranged from 13.06 to 39.01 days, compared to 8.10 to 32.79 days at the highest concentration. The results revealed a concentration-dependent effect on the time required to achieve 50% (LT_{50}) and 90% (LT_{90}) mortality, with shorter LT values observed at higher concentrations for each larval stage. For instance, in the first instar (L1), LT_{50} decreased from 7.53 days at $1×10^6$ conidia ml^{-1} to 4.05 days at $1×10^8$ conidia ml^{-1}, while LT_{90} reduced from 14.99 days to 8.10 days over the same concentration range, indicating a rapid mortality effect at higher fungal doses. Similarly, in the second instar (L2), LT_{50} values decreased from 7.84 to 4.99 days with increasing concentration, showing that younger larvae respond more quickly to higher fungal doses. LT_{90} values for this instar ranged from 10.45 to 13.06 days. In the case of third-instar larvae, LT_{50} values for the $1×10^6$, $1×10^7$, and $1×10^8$ concentrations were 10.43, 8.82, and 7.50 days, respectively, while the LT_{90} values were 21.65, 16.57, and 13.80 days.

Table 3. Estimates of lethal time (LT_{50} and LT_{90}) values of *Beauveria bassiana* tested against larval stages of *Helicoverpa armigera*

Larval Stage	Concentration (conidia ml^{-1})	LT_{50} (days) (CI)	LT_{90} (days) (CI)	Slope±SE	Intercept±SE	χ^2 (*df*=28)	*p*
L1	1×10^6	7.53 (6.50-8.77)	14.99 (12.10-21.91)	4.28±0.15	-3.75±0.13	535.61	0.0001
	1×10^7	5.50 (5.09-6.01)	8.09 (7.32-9.39)	7.80±0.28	-5.81±0.22	328.27	0.0001
	1×10^8	4.05 (2.66-5.51)	8.10 (5.89-16.69)	4.25±0.13	-2.58± 0.09	1885.68	0.0001
L2	1×10^6	7.84 (7.31-8.42)	13.06 (11.75-15.09)	5.78±0.20	-5.17±0.18	194.13	0.0001
	1×10^7	7.32 (6.81-7.87)	11.76 (10.63-13.52)	6.23±0.21	-5.38±0.19	222.53	0.0001
	1×10^8	4.99 (3.34-6.79)	10.45 (7.51-24.39)	3.99±0.12	-2.79± 0.10	1868.96	0.0001
L3	1×10^6	10.43 (9.37-11.92)	21.65 (17.56-30.25)	4.03±0.16	-4.11±0.15	241.69	0.0001
	1×10^7	8.82 (7.87-10.01)	16.57 (13.78-22.44)	4.68±0.17	-4.42±0.16	376.48	0.0001
	1×10^8	7.50 (7.00–8.10)	13.80 (12.90–14.75)	4.75±0.15	-4.30± 0.04	1846.92	0.0001
L4	1×10^6	18.90 (16.47-24.14)	34.46 (26.31-57.29)	4.91±0.40	-6.27±0.43	98.02	0.0001
	1×10^7	14.52 (12.35-19.72)	28.05 (20.41-57.69)	4.48±0.23	-5.21±0.24	385.96	0.0001
	1×10^8	9.31 (8.66-10.07)	15.44 (13.73-18.27)	5.83±0.21	-5.65± 0.20	211.39	0.0001
L5	1×10^6	20.87 (17.16-32.60)	39.01 (26.96-94.69)	4.71±0.43	-6.22±0.46	135.87	0.0001
	1×10^7	27.77 (19.09-37.82)	53.46 (27.97-57.45)	4.50±0.66	-6.50±0.73	175.55	0.0001
	1×10^8	18.13 (16.02-22.29)	32.79 (25.66-50.74)	4.98±0.38	-6.26± 0.41	93.16	0.0001
L6	1×10^6	18.92 (16.48-24.14)	34.54 (26.37-57.21)	4.90±0.40	-6.25±0.42	96.31	0.0001
	1×10^7	18.92 (15.87-28.02)	34.54 (24.58-78.52)	4.90±0.40	-6.25±0.42	179.31	0.0001
	1×10^8	14.70 (12.24-23.04)	25.67 (18.33-74.94)	5.29±0.28	-6.18±0.29	675.44	0.0001

* Each concentration was tested using 25 larvae, divided into five replicates, each consisting of five larvae.

The trend continues across all instars, though older larval stages (L4, L5, and L6) generally exhibited higher LT_{50} and LT_{90} values, reflecting their enhanced resistance to fungal infection compared to younger instars. For example, in fourth-instar larvae (L4), LT_{50} ranged from 9.31 days at 1×10^8 conidia ml^{-1} to 18.90 days at 1×10^6 conidia ml^{-1}, demonstrating the extended time required to induce mortality in more mature larvae. Finally, for fifth (L5) and sixth-instar larvae (L6), where no statistically significant differences were observed, LT_{50} values at the 1×10^6 conidia ml^{-1} ranged from 18.92 to 20.87 days, with LT90 values ranging from 34.54 to 39.01 days. At the highest concentration, LT_{50} values varied between 14.70 and 18.92 days, while LT_{90} values ranged from 25.67 to 32.79 days. In summary, the first instar larvae exhibited the shortest LT_{50} and LT_{90} periods at the highest concentration (1×10^8 conidia ml^{-1}), with values of 4.05 and 8.10 days, respectively. Larval mortality rates increased while both LT_{50} and LT_{90} periods decreased as the concentration increased (Table 3). The dose effect was particularly significant in the third and fourth instar larvae, with the highest dose resulting in LT_{50} and LT_{90} values of 7.50 and 13.80 days for the third instar and 9.31 and 15.44 days for the fourth instar, respectively (Table 3, Figure 3).

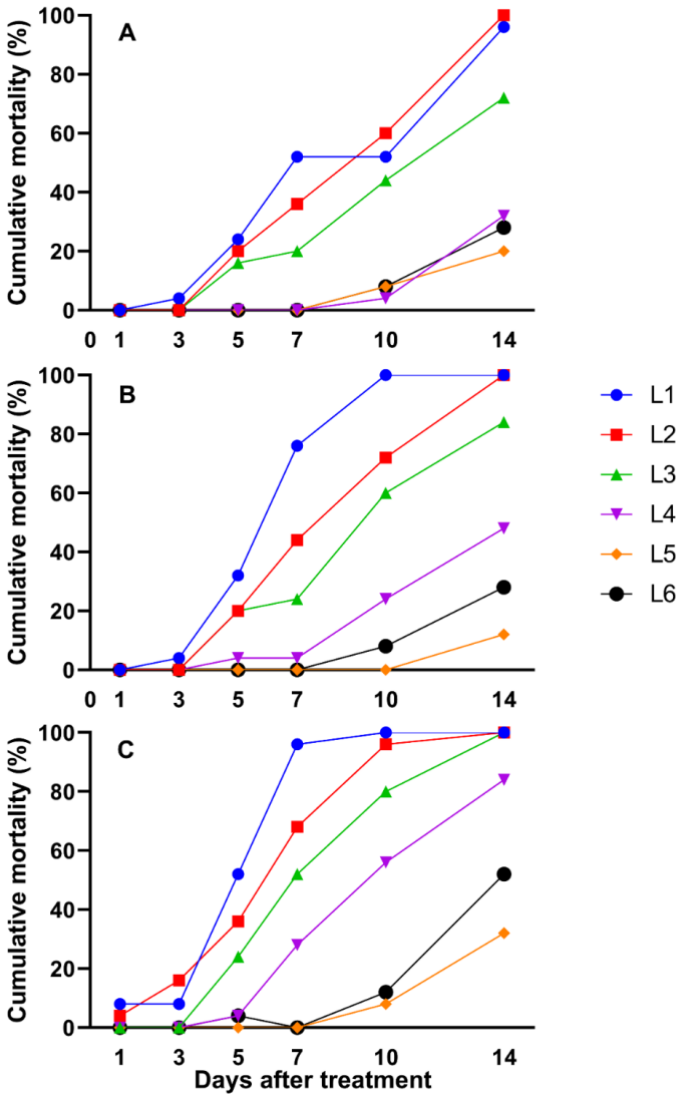

Figure 3. The efficacy of *Beauveria bassiana* against larval stages (L1, L2, L3, L4, L5, L6) of *Helicoverpa armigera* (A: 1×10^6 conidia ml^{-1}, B: 1×10^7 conidia ml^{-1}, 1×10^8 conidia ml^{-1}).

Discussion

The present study highlights the effectiveness of the entomopathogenic fungus *B. bassiana* against various larval stages of the highly destructive polyphagous pest, *H. armigera*. Our findings from the screening study demonstrated *B. bassiana* as pathogenic to the larvae of *H. armigera*. Previous studies have indicated the potential of entomopathogenic fungi, particularly *B. bassiana*, in controlling *H. armigera*. (Sandhu et al., 2001; Nguyen et al., 2007; Alwaneen et al., 2024). However, exotic strains of entomopathogenic fungi developed for pest control may not exhibit effectiveness against insect pests, influenced by the ecological and genetic conditions of the fungal strain (Bidochka et al., 1998). Therefore, it is essential to study the spatial distribution and prevalence of local entomopathogenic fungi to integrate them into pest management programs effectively (Tahir et al., 2019). Differences in virulence among fungal strains of the same species can result from genetic variations associated with their geographical distribution (Coates et al., 2002). Conducting laboratory screenings is crucial to identify virulent strains before their application in field conditions (Cherry et al., 2005).

In our study, *B. bassiana* infected all larval stages of *H. armigera*. However, the younger instars (L1, L2, L3) were particularly susceptible to fungal infection. These larval stages reached 100% mortality at a concentration of 1×10^8 conidia ml^{-1}, while the fourth larval stage (L4) was less susceptible (%84) compared to the first three larval instars. In the older larval stages (L5 and L6), the mortality rates remained low percentages. Insects exhibit varying susceptibility to infection by entomopathogenic fungi of the Hyphomycetes class across different developmental stages (Inglis et al., 2001). Wilson et al. (2001) reported that the level of resistance in caterpillars against pathogens and parasites is positively associated with the occurrence of melanism in the cuticle and midgut. Melanin protects the insect's cuticle, thereby preventing the penetration of parasites and pathogens. Previous studies consistently highlight the higher efficacy of *B. bassiana* against younger instars across various insect species. For example, melanin levels, which contribute to cuticle severity, vary significantly across the larval stages of the cotton leafworm, *Spodoptera littoralis* Boisd*.* (Lepidoptera: Noctuidae), with a notable increase from the late fourth to mid-sixth instar, especially when larvae were reared individually (Lee & Wilson 2006). This increase in cuticle melanin can correlate with greater resistance to fungal infections in older stages. Similarly, Hafez et al. (1997) reported that the younger instars of the potato tuber moth, *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae), exhibited greater susceptibility to *B. bassiana* compared to the later larval stages, while Vandenberg et al. (1998) observed that third and fourth larval stages of the *Plutella xylostella* (L., 1767) (Lepidoptera: Plutellidae) were more vulnerable than second instars. In the case of *H. armigera* larvae, Nguyen et al. (2009) reported high mortality in second to fifth instars when treated with 1×10^7 conidia ml^{-1}, with second instars showing the shortest lethal time, possibly due to their brief developmental duration of about two days. Similarly, Tahir et al. (2019) found that the *B. bassiana* isolate WG-18 was highly effective against younger instars, though fourth instars exhibited moderately lower mortality compared to second instars. Alwaneen et al. (2024) also noted reduced pupation and adult emergence rates in second instar *H. armigera* treated with *B. bassiana* compared to treatments at the fourth instar, further supporting the trend of higher susceptibility in earlier stages. Increasing spore concentrations not only improved the mortality rate but also accelerated the speed of action, as reflected by reduced LT_{50} and LT_{90} values at higher doses. This indicates that higher fungal doses can provide more rapid control, particularly in the early larval stages where *B. bassiana* susceptibility is greatest (Altinok et al., 2019). However, older instars showed greater resistance, suggesting that biological control may be less effective as larvae mature. The composition of their outer integument becomes less penetrable to fungal infections as larvae mature. This reduced susceptibility may explain the higher mortality observed in early instars, where the fungus can invade more effectively (Idrees et al., 2021; Bosa et al., 2024). Targeting these younger stages could thus maximize the impact of *B. bassiana* in integrated pest management programs.

The results further underscore the potential of entomopathogenic fungi as sustainable alternatives to chemical pesticides. Excessive chemical pesticide has led to environmental contamination, harm to non-target species, and increased pest resistance (Ahmad et al., 2003; Walsh et al., 2022). In contrast, *B. bassiana* offers an eco-friendly approach that preserves natural predators and promotes agroecosystem biodiversity (Meyling & Eilenberg, 2007). Integrating biological agents into IPM strategies contributes to reducing pest resistance and decrease dependence on chemical controls (Bale et al., 2008). Entomopathogenic fungi such as *B. bassiana* were increasingly recognized for their role in pest management due to their ability to infect and kill target insects through natural infection processes (Goettel & Glare, 2010; Inglis et al., 2012). Additionally, they can be applied in various formulations (e.g., sprays, wettable powder, or even seed treatments) to control pest populations effectively within IPM frameworks (García-Estrada et al., 2016; Mascarin & Jaronski, 2016; Darsouei et al., 2024). Furthermore, various plants have been shown to be colonized by *B. bassiana* as an endophyte, a relationship linked with decreased herbivore damage. For example, endophytic *B. bassiana* has been linked to lower feeding by pests such as *Ostrinia nubilalis* Hübner, 1796 (Lepidoptera: Crambidae) in maize, *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae) in tomato (Jaber & Ownley, 2018). Fungal endophyte infections in plants

negatively impact insect development (Christian et al., 2020; Silva et al., 2020) but also deter pest feeding. For instance, the larvae of *Helicoverpa gelotopoeon* Dyar, 1921 (Lepidoptera: Noctuidae) and *H. zea* prefer uninfected plants over those harboring fungal endophytes (Russo et al., 2019; Castillo Lopez & Sword, 2015). Likewise, *B. bassiana*-infected maize shows reduced feeding by *Sesamia calamistis* Hampson, 1910 (Lepidoptera: Noctuidae) (Cherry et al., 2004) and *O. nubilalis* (Bing & Lewis, 1991). Overall, this study provides key insights for incorporating *B. bassiana* into IPM strategies, particularly through targeted application in the early larval stages to improve pest control. Future research should explore the efficacy of *B. bassiana* across diverse environmental conditions and crop systems, supporting its broader adoption as a fungal biopesticide in sustainable agriculture.

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