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Isolation and Identification of Strains of the Entomopathogenic Fungus *Beauveria bassiana*, and Screening their Virulence Against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *Ephestia cautella* Walker (Lepidoptera: Pyralidae) and *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae)

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Abstract

Thirteen soil samples were collected from Ukulinga Research Farm, Pietermaritzburg, South Africa. Entomopathogenic fungi were isolated by baiting the soil samples with waxmoth larvae (Galleria mellonella L). The most abundant pathogenic species were Beauveria bassiana (28 isolates) and Metarhizium anisopliae (4 isolates). Beauveria bassiana was isolated most frequently from larvae baited in soil from maize fields. The 28 B. bassiana strains were evaluated for their virulence on the adults of Sitophilus zeamais, under laboratory conditions. In the first bioassay, strains were applied at a single dose of 2x108 conidia g-1, using kaolin as the carrier. Mortality levels range from 10.0% to 80.0%. The three strains that caused mortality levels >70.0% on S. zeamais were then evaluated in dose response assays on S. zeamais, Ephestia cautella L3 larvae, and Lasioderma serricorne L3 larvae and adults, at doses of 2x105, 2x106, 2x107 and 2x108 conidia g-1. Strain MS-8 consistently caused higher mortality levels on the three insects than other two strains. The same strain achieved the lowest LD50 and LT50 values against the three insects, followed by Strains MS-3 and MS-4. Generally E. cautella L3 larvae was the most susceptible test insect for Strain MS-8, followed by L. serricorne adults, S. zeamais and L. serricorne L3 larvae. The fungal strains MS-3, MS-4 and MS-8 were sequenced, followed by BLAST searches for their identity. Alignments were performed and a Maximum Likelihood tree was developed to infer phylogeny. The Strains MS-3 and MS-4 were identical to each other, but were 99% identical to MS-8. All three sequences had 99% identity with 100% coverage to type material of B. bassiana. A phylogentic analysis of the strain sequences in relation to B. bassiana and several other Beauveria species sequences showed strong bootstrap support (92%) for their membership of the B. bassiana clade.

Keywords: Entomopathogenic Fungus; Beauveria bassiana; Sitophilus zeamais; Lasioderma serricorne; Ephestia cautella

Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is an economically important pest of stored grains in tropical regions. Grain infestation by this pest typically begins in the field, but the greatest damage occurs during storage [1,2]. In tropical countries, grain losses ranging from 20% to 90% have been reported as a result of maize weevil infestations [3]. The almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae),

is another major stored-product pest. It occurs in both tropical and temperate regions and attacks grains, nuts, dried fruits, and a wide variety of other stored products [4]. The cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), is a cosmopolitan pest distributed throughout tropical and subtropical regions. This insect commonly infests stored food products, including dried fruits, grains, cereal flour, and animal feed [5].

Fumigants and synthetic insecticides are commonly used to prevent insect-related losses during grain storage. However, their use raises concerns regarding insecticide resistance, chemical residues in food products, increasing costs, and environmental pollution [6,7]. Therefore, it is essential to explore alternative control strategies, such as entomopathogenic fungi (EPFs), against stored-product insect pests [8]. *Beauveria bassiana* (Balsamo) Vuillemin is a promising fungal biocontrol agent due to its generally low toxicity to non-target animals and humans. This fungus occurs naturally in soils worldwide and infects a wide range of insect species, causing white muscardine disease. Several isolates of *B. bassiana* have been formulated and registered as commercial biopesticides against various insect pests (Feng et al. 1994). However, Batta [9] reported that no commercial EPF products had been registered specifically for the biological control of stored-grain insects.

Considerable genetic variation exists among isolates of *B. bassiana*. Their pathogenicity and virulence against different arthropods, as well as their enzymatic and molecular characteristics, vary among isolates [10,11]. Selecting highly virulent isolates adapted to local agroecosystem conditions is therefore an important step in developing effective mycoinsecticides [12].

Accurate identification of fungal species can be difficult and, in some cases, impossible based solely on morphological characteristics. Morphological criteria have traditionally been used to identify and classify *Beauveria* spp. However, distinguishing between *Beauveria* species remains challenging because of the considerable morphological and genetic variability within the genus [13,14]. Consequently, several molecular techniques have been developed to improve the identification and differentiation of Beauveria isolates [15-18].

Numerous studies have demonstrated the potential of *B. bassiana* isolates as microbial control agents against stored-product insect pests [19-23]. However, comparative information on the virulence of locally isolated strains across different stored-product pest species and developmental stages remains limited. In particular, evaluating the dose-dependent responses of multiple pest species to selected virulent strains may provide valuable information for identifying promising candidates for fungal biopesticide development. Therefore, the objective of this study was to isolate and identify indigenous entomopathogenic fungi from various soil sources and to evaluate their virulence against *S. zeamais* adults, *E. cautella* third-instar larvae, and *L. serricorne* third-instar larvae and adults.

Material and Methods

Soil samples

Soil samples were collected in the winter season at the beginning of May 2014 at Ukulinga Research Farm, Pietermaritzburg (KwaZulu-Natal Provence) (latitude 29. 67 S, longitude 30.41 E, 809 meters above sea level). Thirteen sampling points ≥ 1 Km apart were selected. The sampling protocol was: (i) the soil surface layer was removed; (ii) at each point five samples were taken in the rhizosphere at a depth of 5-15 cm and 10 -15 m between each sample with a volume of around 500g; these samples were mixed together to form one 2500g composite sample; (iii) the samples were placed in plastic bags and stored in a fridge at 4oC; (iv) the samples were processed within two weeks of collection.

Isolation of fungi

Entomopathogenic fungi were isolated using the bait-trap method with larvae of the wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) (Zimmermann 1986), which were reared on artificial media according to [24].

Each composite soil sample was sieved through a 0.5 cm mesh to remove roots and other solid materials. For each composite soil sample, three replicates of 200 g were placed into 250 mL glass jars and moistened with distilled water. Five third- or fourth-instar larvae (approximately four weeks after hatching) were introduced into each glass jar using sterile forceps. The jars were then covered with insect netting to facilitate air circulation.

The glass jars were incubated in the dark at 25°C for two weeks. The jars were checked every three days, and dead larvae were removed from the soil and immersed in a 1% sodium hypochlorite solution for 3 min for surface sterilization, followed by rinsing in sterile distilled water for 4 min. The dead larvae were then placed individually in 9 cm Petri dishes containing moist filter paper. The Petri dishes were sealed with Parafilm, incubated at 25°C, and examined every two days until fungal growth was observed. The presence of fungal mycelium on infected wax moth larvae was considered indicative of infection by entomopathogenic fungi. Following sporulation, conidia were collected from the insect surface using a sterile loop and streaked onto potato dextrose agar (PDA) plates containing 0.05 g/L streptomycin and ampicillin. The fungi were identified according to Humber [25].

Insect rearing

The initial insect populations were obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal. *S. zeamais* and *E. cautella* were reared on yellow maize grains, while *L. serricorne* was reared on rice grains. The grains were

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initially stored at -20°C for one week to eliminate unwanted natural infestations. Approximately ten adults of each insect species, of mixed sexes, were placed in 250 mL glass jars, each containing 100 g of the respective grains. The jars were covered with insect netting to facilitate air circulation and maintained under experimental conditions of 28 ± 2°C and 65 ± 5% relative humidity (RH). The adults were removed from the jars after one week of infestation. Adults of *S. zeamais*, third-instar (L3) larvae of *E. cautella*, and both third-instar larvae and adults of *L. serricorne* were used in the experiments.

First screening bioassay

All 28 *Beauveria bassiana* strains were used in the initial screening bioassay. Dry conidia of each strain were produced by inoculating Petri dishes containing potato dextrose agar (PDA) with 100 µL of a fungal suspension at a concentration of 10^8 conidia mL^{-1}, determined using a Neubauer Improved Hemocytometer. The inoculated plates were sealed with Parafilm to maintain adequate moisture and incubated at 25°C in the dark for 15 days. Subsequently, the Parafilm was removed, and the plates were allowed to dry under laminar airflow for five days. Conidia were harvested by gently scraping the surface of the dried culture medium using a sterile scalpel blade. The harvested fungal material was passed twice through a sieve (100 mm in diameter, 110 µm mesh size) to obtain dry conidial powder. The harvested conidia were stored in sterile, sealed bottles at 4°C until further use.

Each strain was formulated using kaolin as a carrier at a concentration of 2×10^8 conidia g^{-1}, determined using a Neubauer Improved Hemocytometer. Thirty adult *S. zeamais*, less than two days old, were introduced into 250 mL glass jars containing 100 g of maize grain. Three replicates were used for each strain, while the control was treated with kaolin carrier only. Insect mortality was recorded every two days over a period of ten days. Dead insects were examined for fungal outgrowth to confirm that mortality was associated with fungal infection. The experiment was arranged in a randomized complete block design (RCBD).

Multiple screening bioassays

The best three *B. bassiana* strains that caused mortality levels greater than 70.0% against *S. zeamais* in the initial screening bioassay were selected for evaluation at different doses in the second bioassay. For each strain, conidia were prepared from an initial stock of 2×10^9 conidia g^{-1} formulated in kaolin, with the concentration determined using a Neubauer Improved Hemocytometer. The stock formulation was then diluted with kaolin to obtain concentrations of 2×10^8, 2×10^7, 2×10^6, and 2×10^5 conidia g^{-1}. Each dose was replicated three times. The control was treated with the carrier only. For each replicate, 30 adults of *S. zeamais*, 30 third-instar (L3) larvae of *E. cautella*, and 30 third-instar larvae and adults of *L. serricorne* were placed separately into glass jars containing 100 g of rice grain following treatment with the respective formulation. The experiment was arranged as a 3 × 4 × 5 factorial experiment in a randomized complete block design (RCBD).

Identification of fungal strains by sequence analysis

The three best selected B. bassiana strains, MS-3, MS-4, and MS-8, were sent to Inqaba Biotech (South Africa) for bidirectional sequencing of the ITS1 and ITS2 regions. The sequences were examined for quality using FinchTV, and regions of suitable quality were imported into BioEdit [26]. Consensus sequences (approximately 470 bp) were generated and used to perform BLAST searches against type material in the GenBank database for tentative identification. A total of 11 aligned ITS sequences derived from type material of Beauveria/Cordyceps species were downloaded from GenBank. These sequences, together with the consensus sequences of the three strains, were aligned in BioEdit using ClustalW. The aligned sequences were imported into MEGA7 [27]. The most appropriate DNA substitution model for Maximum Likelihood (ML) analysis was determined, and an ML phylogenetic tree was constructed using 1,000 bootstrap replicates.

Statistical analysis

Insect mortality data were corrected relative to the control using Abbott's formula [28]. The corrected mortality data were subjected to analysis of variance (ANOVA). Treatment means were compared using Fisher's least significant difference (LSD) test at a 5% significance level. Probit analysis was performed to estimate the median lethal dose (LD_{50}) and median lethal time (LT_{50}). All statistical analyses were conducted using GenStat for Windows, 17th edition [29].

Results

Soil samples

B. bassiana was more frequently isolated than *M. anisopliae* from the soil samples examined. The highest frequency of *B. bassiana* isolation was recorded in soil collected from the maize field (33.34%), followed by grassland subjected to annual burning for 65 years (GAB; 26.67%). The lowest isolation frequency (6.67%) was recorded in soil samples collected from Horse Pasture 1, the Chicken Farm, and Cattle Pasture 1 (Figure 1).

First Screening bioassay

Highly significant differences in pathogenicity against *S. zeamais* were observed among the 28 tested *B. bassiana* strains ($F = 106.06$; $P < 0.001$).

All tested strains caused mortality in S. zeamais, with mortality levels ranging from 10.0% to 80.0%. Among the 28 strains, three (MS-3, MS-4, and MS-8) caused mortality levels of at least 70.0%, while four strains (MS-22, MS-27, MS-25, and MS-28) caused mortality levels ranging from 50.0% to 63.0%. The remaining 21 strains caused mortality levels below 50.0% (Figure 2).

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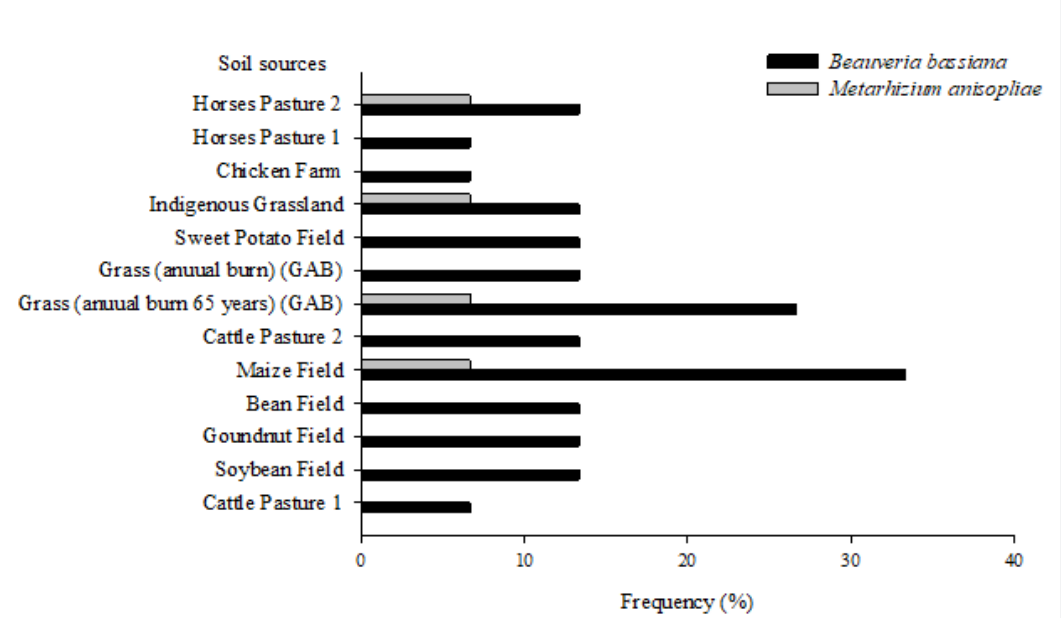


Figure 1: Frequency of isolation of Beauveria bassiana and Metarhizium anisopliae from soil samples.

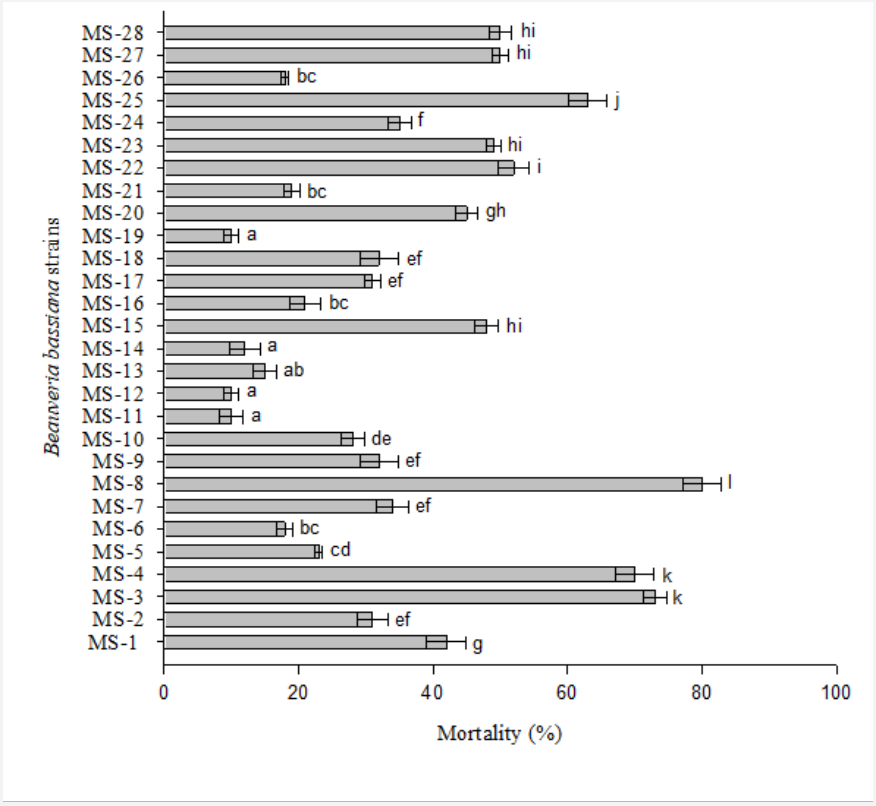


Figure 2: Corrected mortality (Abbott's correction) of maize weevil, Sitophilus zeamais, following 10 days of exposure to 28 Strains of Beauveria bassiana at 2x108 conidia g-1 in a kaolin carrier. Bars represent the standard error.

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Strain MS-8 caused the highest mortality (80.0%), followed by MS-3 (73.0%) and MS-4 (70.0%) (Figure 2). Scanning electron microscopy (SEM) observations revealed germination of MS-8 conidia and penetration of germ tubes into the cuticle of *S. zeamais*, followed by fungal outgrowth and sporulation on the insect body (Figure 3).

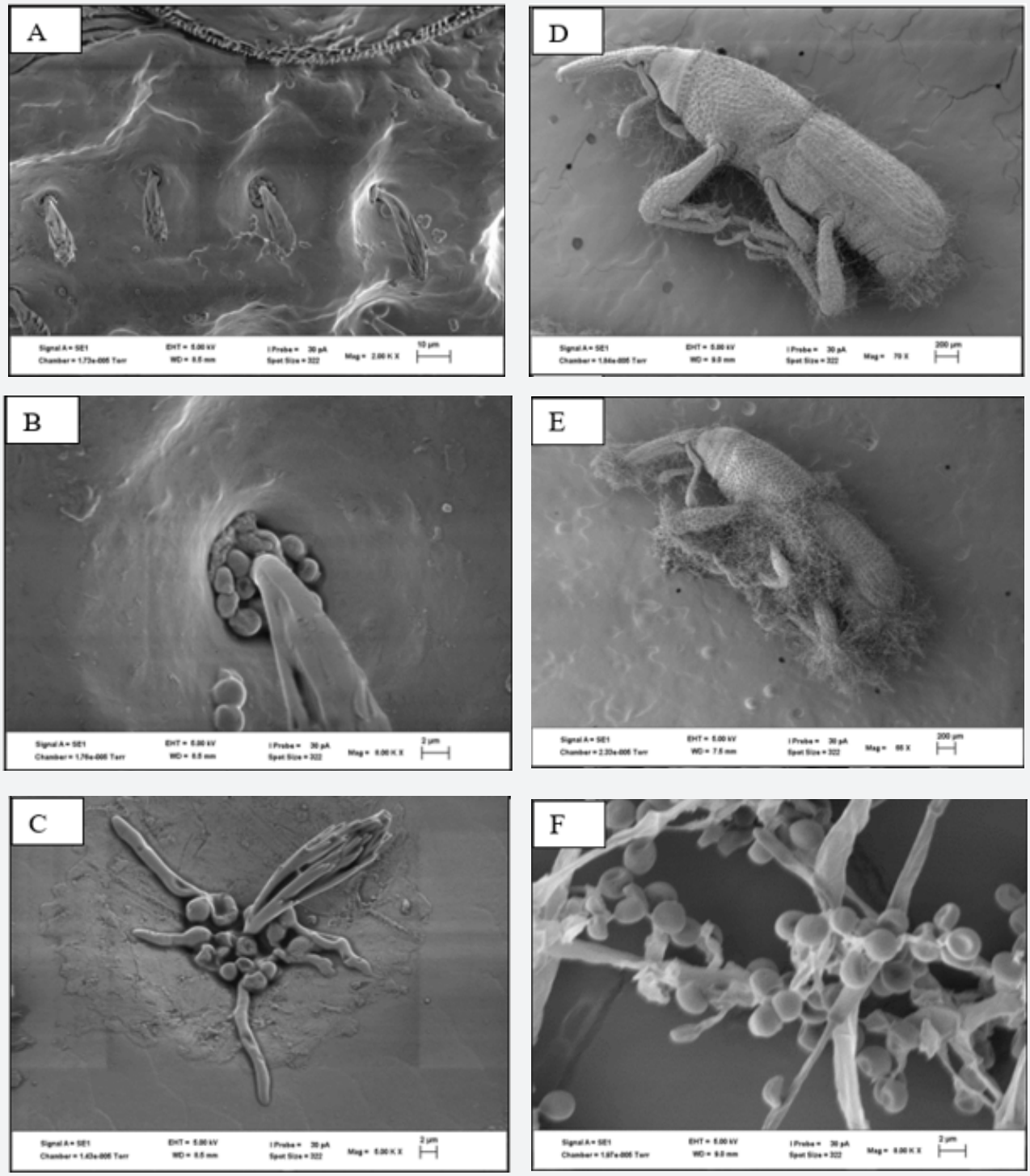


Figure 3: Scanning electron micrographs of Beauveria bassiana Strain MS-8 on Sitophilus zeamais (A-F). On Day 1 no germination of conidia was found (A and B); germination of conidia and penetration of germ tube were seen on Day 2 (C); mycelia emerged from the insect body on Day 5 (D); E and F show intense sporulation on insect legs by Day 10.

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Multiple Screening bioassays

Highly Significant differences in insect mortality were observed among *B. bassiana* strains ($F(2, 94) = 92.29$; $P < 0.001$), conidial doses ($F(3, 94) = 673.76$; $P < 0.001$), and the tested insect groups ($F(3, 94) = 88.97$; $P < 0.001$). The interaction between conidial dose and insect group was also significant ($F(9, 94) = 4.33$; $P < 0.001$). In contrast, the interactions between strain and dose ($F(6, 94) = 1.71$; $P = 0.128$), strain and insect group ($F(6, 94) = 1.41$; $P = 0.217$), and strain, dose, and insect group ($F(18, 94) = 0.46$; $P = 0.969$) were not significant.

The entomopathogenic activity of *Beauveria bassiana* strain MS-8 was evidenced by the emergence of fungal mycelia from the bodies of dead insect. Approximately five days after death, mycelial growth was observed emerging from the intersegmental regions and subsequently spreading over the insect body. Third-instar larvae of *L. serricorne* and *E. cautella* treated with strain MS-8 exhibited reddish discoloration after death (Figure 4).

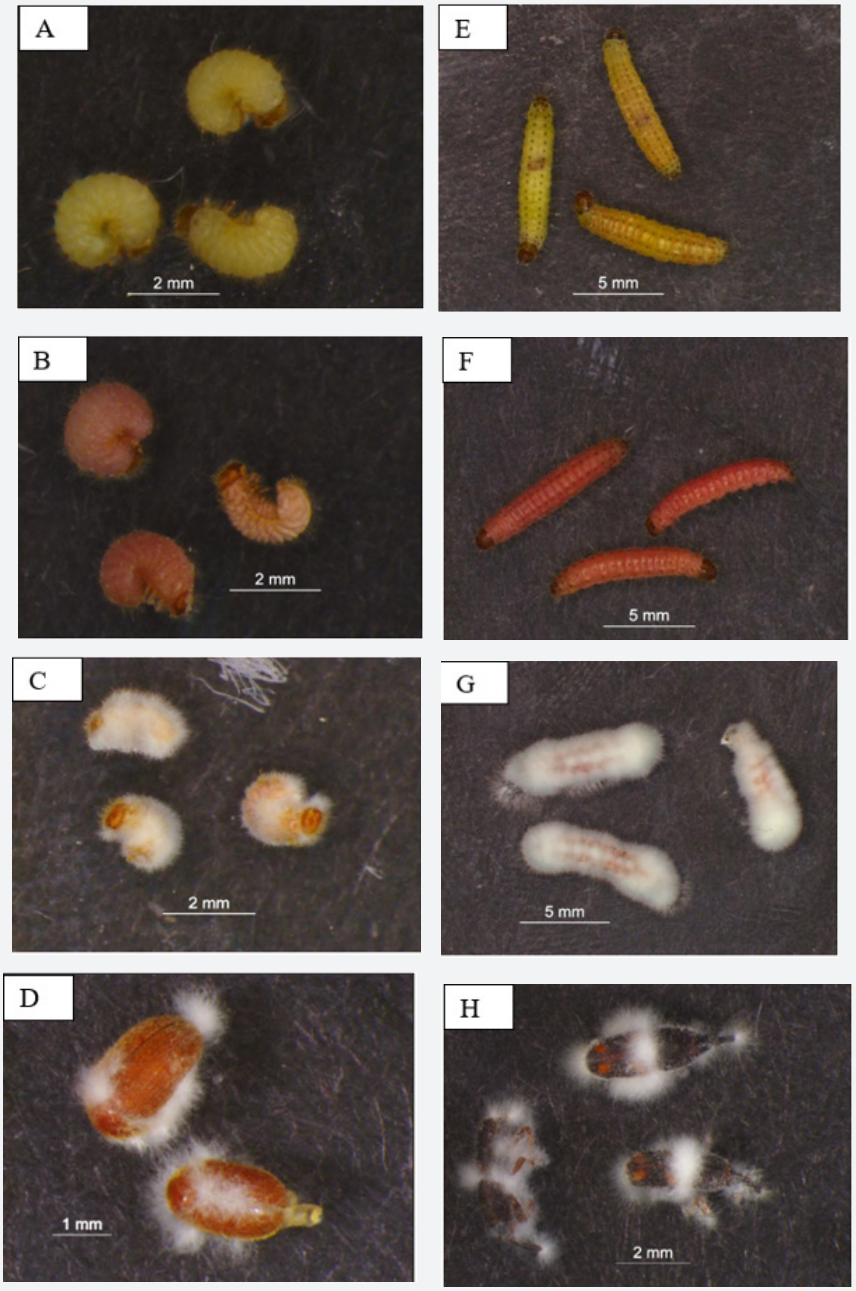


Figure 4: Stereo microscope micrographs of Beauveria bassiana Strain MS-8 on Lasioderma serricorne L3 larvae (A = Control; B = red coloration typical of B. bassiana infection; C = mycelial growth); L. serricorne adults (D = mycelial growth); Ephestia cautella L3 larvae (E = Control; F = red coloration; G = mycelial growth); and Sitophilus zeamais (H = mycelial growth).

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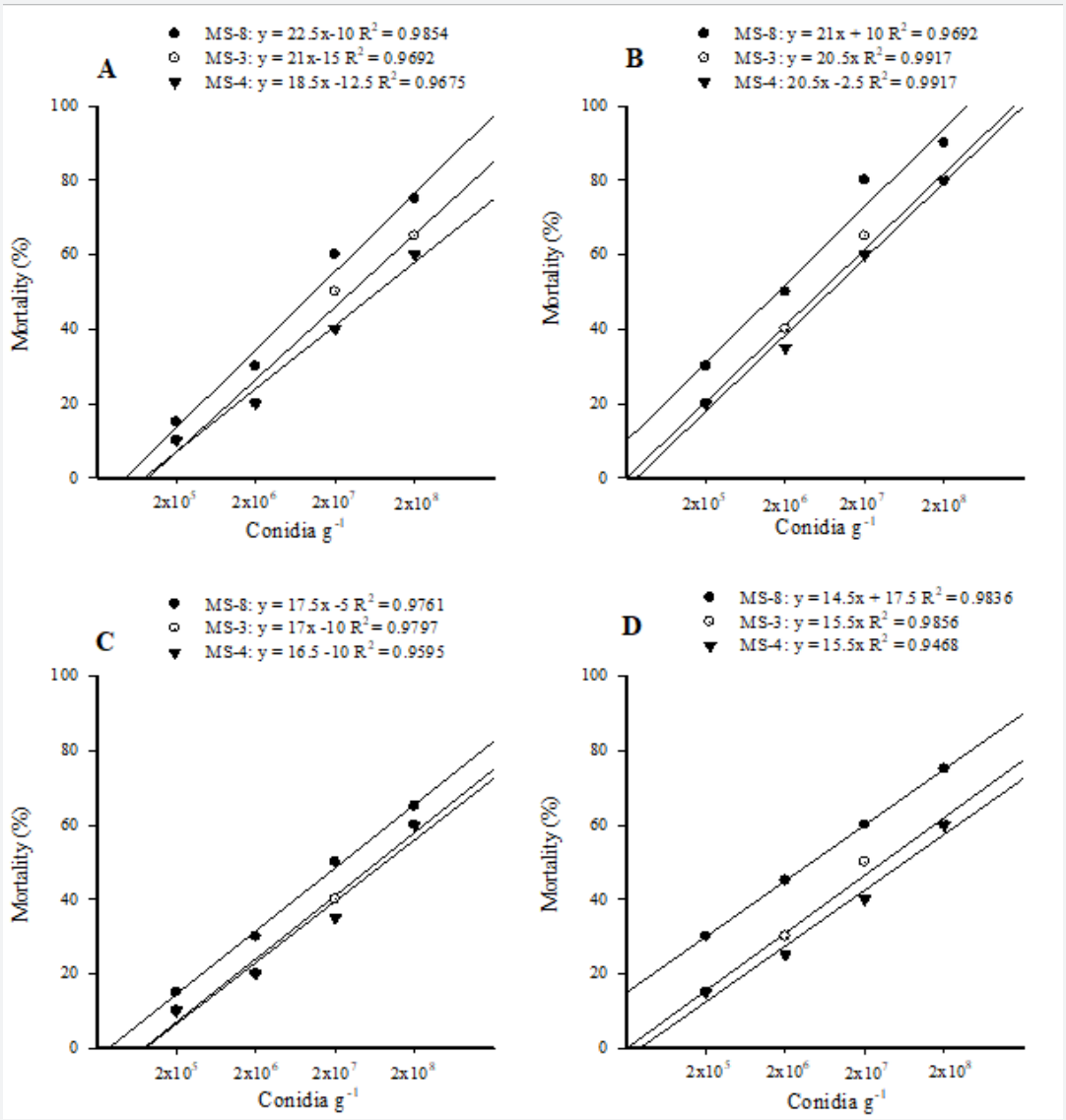


Figure 5: Corrected mortality (Abbott's correction) of A = Sitophilus zeamais adults, B = Ephestia cautella L3 larvae, C = Lasioderma serricorne L3 larvae and D = Lasioderma serricorne adults, upon exposure to three Beauveria bassiana strains applied at doses of 2x105, 2x106, 2x107 and 2x108 conidia g-1 formulated in a kaolin carrier, observed 10 days after treatment.

Insect mortality increased with increasing conidial dose across all tested strains and insect groups, with linear dose–response relationships reported (R^2 > 0.94). Strain MS-8 consistently produced higher mortality than strains MS-3 and MS-4 across the tested doses and insect groups (Figure 5). At the highest dose of 2×10^8 conidia g^{-1}, strain MS-8 caused mortality levels of 90.0%, 76.05%, 75.0%, and 65.0% in third-instar larvae of *E. cautella*, adults of *L. serricorne*, adults of *S. zeamais*, and third-instar larvae of *L. serricorne*, respectively. Among these insect groups, *E. cautella* larvae exhibited the highest susceptibility, whereas *L. serricorne* larvae showed the lowest susceptibility. At lower conidial doses, *L. serricorne* adults were more susceptible than *S. zeamais* adults (Figure 5).

Probit analysis was performed separately for each insect group to estimate the median lethal dose (LD_{50}) and median lethal time (LT_{50}). Strain MS-8 consistently exhibited the lowest LD_{50} and LT_{50} values, followed by strains MS-3 and MS-4. Across the tested insect groups, the lowest LD_{50} and LT_{50} values were reported for *E. cautella* third-instar larvae, followed by *L. serricorne* adults, *S. zeamais* adults and *L. serricorne* third-instar larvae (Figures 6 and 7).

Identification of fungal strains by sequence analysis

Sequence analysis using BioEdit revealed that *B. bassiana* strains MS-3 and MS-4 had identical ITS sequences, whereas their sequences shared 99.1% identity with that of strain MS-8.

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BLAST searches against GenBank revealed sequence identities exceeding 97% with several species within the genus *Beauveria*. All three strains showed the highest sequence identity (99%) with reference sequences of *B. bassiana,* although their levels of similarity to other *Beauveria* species varied (Table 1).

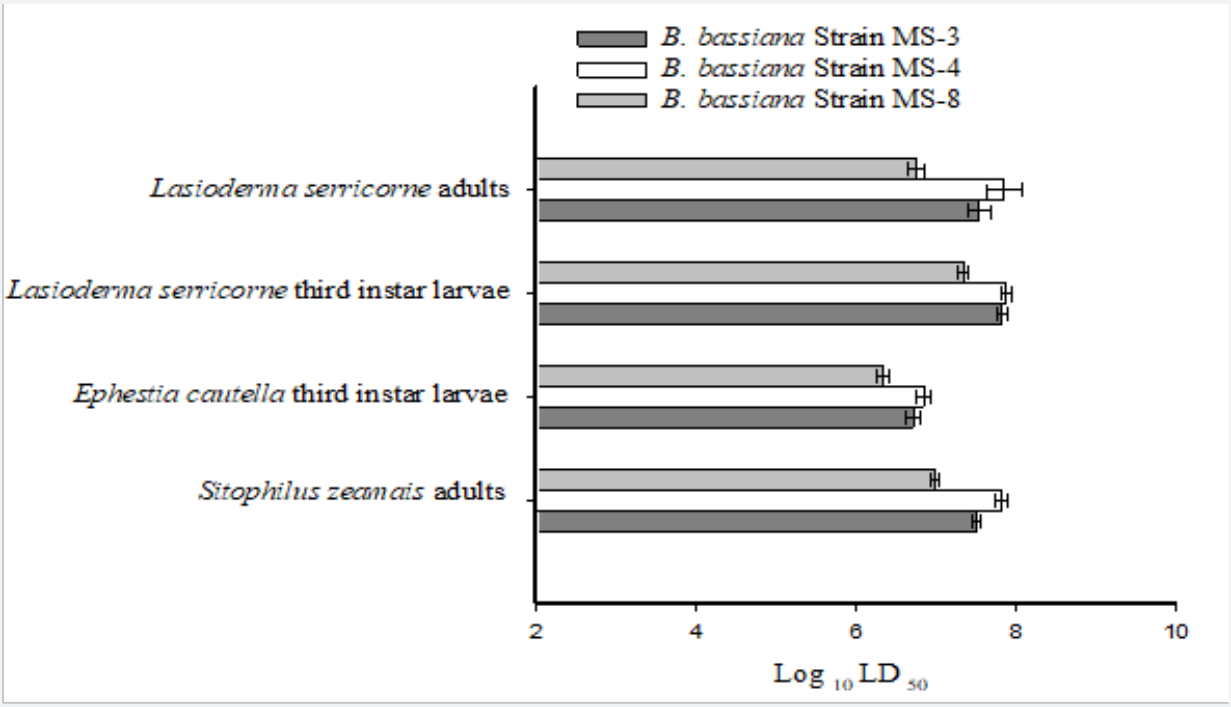


Figure 6: Lethal dose (LD50) of the three tested insects upon exposure to three B. bassiana strains formulated in kaolin at doses of 2x105, 2x106, 2x107 and 2x108 conidia g-1.

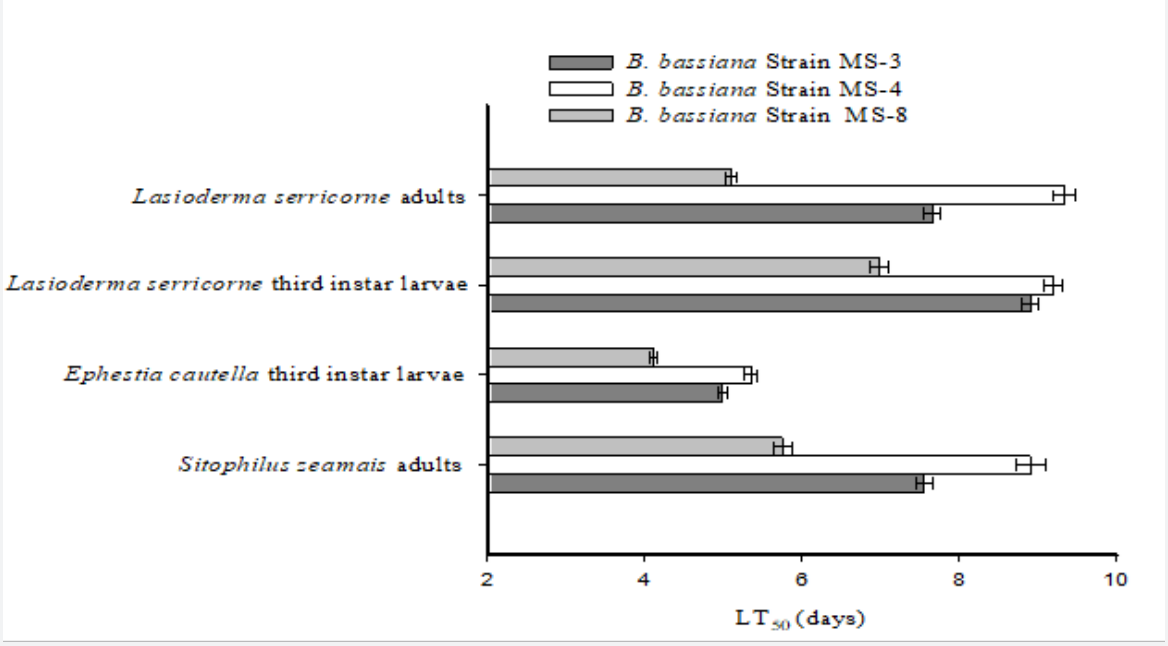


Figure 7: Lethal time (LT50) of the three tested insects upon exposure to three B. bassiana strains formulated in kaolin at a dose of 2x108 conidia g-1.

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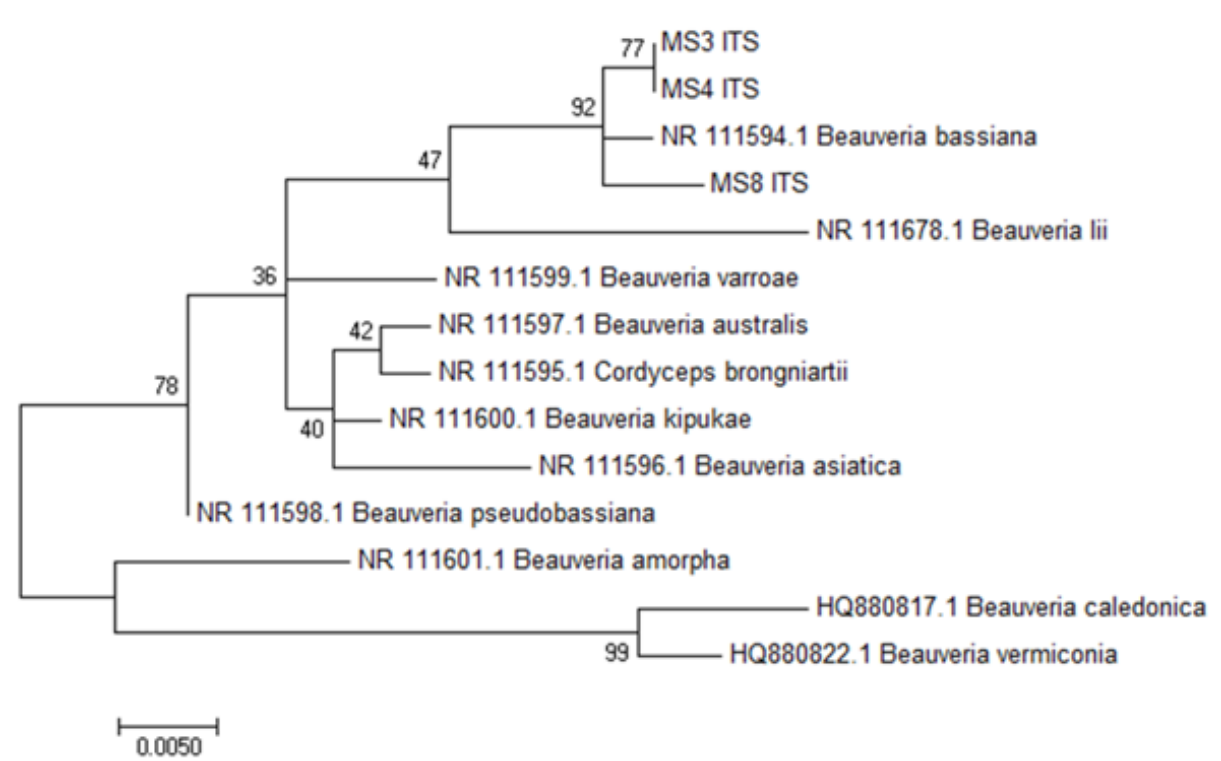


Figure 8: ML tree of MS-3, MS-4 and MS-8 with 11 other type-material sequence accessions from the NVCBI Genbank database. Accession numbers are shown for Genbank serquences. The scale for branch length indicates the number of substitutions per site and bootstrap values are shown at the nodes.

Table 1: Selected GenBank sequence matches for *Beauveria bassiana* strains MS-3, MS-4, and MS-8 based on BLAST analysis of ITS sequences. Only matches to reference type material with 100% query coverage and at least 97% sequence identity were included.

Strains	Identity	Species	Genbank Accession no.
MS-3 and MS-4	99%	*Beauveria bassiana*	NR_111594.1
	98%	*Beauveria varroae*	NR_111599.1
		Beauveria australis	NR_111597.1
	97%	*Beauveria lii*	NR_111678.1
		Beauveria kipukae	NR_111600.1
		Beauveria pseudobassiana	NR_111598.1
		Cordyceps brongniartii	NR_111595.1
MS-8	99%	*Beauveria bassiana*	NR_111594.1
	97%	*Beauveria varroae*	NR_111599.1
		Beauveria australis	NR_111597.1
		Beauveria lii	NR_111678.1
		Beauveria kipukae	NR_111600.1
		Beauveria pseudobassiana	NR_111598.1
		Cordyceps brongniartii	NR_111595.1

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Maximum Likelihood (ML) phylogenetic analysis yielded a tree with a log-likelihood value of −949.1464 (Figure 8). The analysis was performed using the Tamura–Nei substitution model [30] with a discrete gamma distribution comprising five categories to account for variation in evolutionary rates among sites (+G; parameter = 0.0500). The dataset included 14 nucleotide sequences, consisting of the three selected strains and 11 reference sequences retrieved from GenBank. After excluding positions containing gaps or missing data, 452 nucleotide positions were retained for the phylogenetic analysis.

Discussion

The recovery of entomopathogenic fungi from soil samples using *G. mellonella* larvae as insect baits supports the effectiveness of this method for detecting soil-associated entomopathogenic fungi [31,32]. The suitability of the Galleria-bait method has also been demonstrated in more recent studies involving the isolation of indigenous entomopathogenic fungi from agricultural soils [33,34]. In the present study, *B. bassiana* was isolated more frequently than M. anisopliae, accounting for more than 80% of the fungal isolates recovered. This finding is consistent with that of Hassan et al. [35], who identified *B. bassiana* as the predominant entomopathogenic fungus in soil samples collected from selected sites in northern Iraq. Similarly, Asensio et al. [36] reported that *B. bassiana* was the most frequently isolated entomopathogenic fungus in Alicante Province, Spain, occurring in 32.8% of the soil samples examined. However, differences in the recovery of entomopathogenic fungal species may partly reflect the selectivity of the insect-bait method, particularly the susceptibility of the bait insect to different fungal species.

The virulence of *B. bassiana* against insect hosts may be influenced by several factors, including host behaviour, developmental stage, nutritional status, and physiological characteristics, as well as variation among fungal strains. Differences in the physiological characteristics of fungal strains and their responses to environmental conditions may also contribute to variation in pathogenicity [37]. In the present study, considerable variation in virulence was observed among the 28 *B. bassiana* strains evaluated against *S. zeamais* adults at a dose of 2 × 10^8 conidia g^{-1}, with mortality ranging from 10.0% to 80.0%. Strains MS-8, MS-3, and MS-4 were the most effective, causing mortality levels of 80.0%, 73.0%, and 70.0%, respectively. These findings are consistent with those of Adane [38], who reported differences in the virulence of *B. bassiana* strains against *S. zeamais*, with appreciable mortality observed at conidial concentrations exceeding 1 × 10^7 conidia mL^{-1}. Similar variation in the efficacy of entomopathogenic fungal isolates has been reported against other stored-product beetles, further emphasizing the importance of selecting highly virulent strains for biological control [39]. Such variation may be associated with differences in fungal infection mechanisms, including the activity of cuticle-degrading enzymes. A study involving Plodia interpunctella larvae reported positive associations between fungal virulence and protease and lipase activities [40]. Furthermore, secondary metabolites produced by *B. bassiana* may contribute to fungal pathogenesis and interactions with host immune defences [41]. Although these mechanisms were not directly investigated in the present study, they provide plausible explanations for the differences in mortality observed among the tested strains. Overall, the substantial variation in mortality among strains tested under the same experimental conditions highlights the importance of strain-level screening when selecting promising fungal candidates for the biological control of stored-product insect pests.

The dose response bioassays demonstrated that *B. bassiana* strain MS-8 consistently caused higher mortality than strains MS-3 and MS-4 across the tested conidial doses and insect groups. At the highest dose (2 × 10^8 conidia g^{-1}), MS-8 caused mortality levels of 90.0%, 76.05%, 75.0%, and 65.0% in third-instar larvae of *E. cautella*, adults of *L. serricorne*, adults of *S. zeamais*, and third-instar larvae of *L. serricorne*, respectively. Strain MS-8 also exhibited the lowest LD_{50} and LT_{50} values, confirming its greater virulence among the three strains evaluated. The highest susceptibility was observed in *E. cautella* larvae, whereas *L. serricorne* third-instar larvae were the least susceptible. Kaur et al. [42] reported mortality below 50.0% in Corcyra cephalonica larvae treated with *B. bassiana* at 2.2 × 10^8 spores mL^{-1}. In contrast, Zhu et al. [43] reported 100% mortality in second-instar larvae of *L. serricorne* treated with *B. bassiana* strain Bb050722 at 1.46 × 10^8 spores mL^{-1}. Differences between these findings and the present study may reflect variation in fungal strains, insect species or developmental stages, and application methods. In particular, the use of dry conidia formulated with kaolin in the present study, rather than conidial suspensions, may have influenced insect exposure and subsequent mortality.

For adults of *S. zeamais* and *L. serricorne*, *B. bassiana* strain MS-8 exhibited LD_{50} values of 9 × 10^6 and 5 × 10^6 conidia g^{-1}, respectively, with corresponding LT_{50} values of 5.8 and 5.2 days. Teshome and Tefera [44] reported an LT_{50} of 4.0 days for *S. zeamais* treated with *B. bassiana* strain PPRC-GC at 1 × 10^8 conidia mL^{-1}. Similarly, Kassa et al. [45]reported an LD_{50} of 2.04 × 10^6 conidia mL^{-1} for *S. zeamais* exposed to *B. bassiana* strain PPRC-HH. Although these studies demonstrate the susceptibility of *S. zeamais* to different *B. bassiana* strains, direct comparisons of LD_{50} values are limited by differences in conidial formulation and application units. The present study used dry conidia formulated with kaolin, whereas the cited studies reported concentrations expressed in conidia mL^{-1}. The use of kaolin as a carrier for dry conidia may offer a practical approach for applying *B. bassiana* to stored grains. However, further investigations are required to determine how this formulation influences conidial viability, distribution within the grain mass, persistence, and insect mortality under different storage conditions.

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BLAST analysis indicated that strains MS-3, MS-4, and MS-8 were closely related to *B. bassiana*, with approximately 99% sequence identity to reference sequences. Strains MS-3 and MS-4 had identical ITS sequences and showed 99.1% identity with MS-8. Nilsson et al. [46] cautioned against relying exclusively on fixed sequence similarity thresholds for fungal identification, while Nilsson et al. [47] highlighted variation in intraspecific ITS divergence among fungi. Phylogenetic analysis further supported the BLAST results by placing all three strains within the *B. bassiana* clade. Together, these findings support their identification as *B. bassiana*, although additional molecular markers could provide greater taxonomic resolution.

The effectiveness of *B. bassiana* against stored-product pests depends not only on strain virulence but also on the formulation used. Meikle et al. [48] reported that an oil-based formulation of *B. bassiana* reduced populations of the larger grain borer, Prostephanus truncatus, but did not completely prevent grain losses. This finding highlights the importance of selecting highly virulent strains and developing suitable formulations to improve the practical efficacy of fungal biocontrol agents. In the present study, strain MS-8 demonstrated promising potential for further formulation development and evaluation under grain storage conditions.

Conclusion

B. bassiana strain MS-8 emerged as the most promising candidate for the biological control of the tested stored-product pests, exhibiting higher virulence than the other strains evaluated. However, further research is required to optimize mass production, formulation, and storage stability, assess efficacy against a broader range of stored-product pests, and evaluate safety to non-target organisms before its potential development as a commercial mycoinsecticide.

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