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A laboratory evaluation of South African strains of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin against adults of *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract

A total of 21 South African strains of *Beauveria bassiana* were evaluated for their virulence against adults of *Lasioderma serricorne* and *Sitophilus zeamais*, under laboratory conditions. In the first bioassay, strains were applied at a single dose of $1x10^8$ conidia ml^{-1}. Adults of *L. serricorne* was more susceptible than those of *S. zeamais*, with 14 strains causing mortality levels > 50.0% on *L. serricorne*, compared to two strains in the case of *S. zeamais*. Six strains that caused mortality levels > 90.0% on *L. serricorne* were compared in a dose-response assay using a water suspension and a powder formulation, with cornflour as the carrier, using five doses ($2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia per volume basis in water (ml^{-1}), and weight basis in the powder formulation (g^{-1})). The water suspension consistently provided a higher mortality than the powder formulation at all concentrations of conidia. 100% mortality was recorded with a water suspension of $2x10^8$ conidia ml^{-1} with Strains 7284, 7769 and 7320. With the powder formulation, the highest mortality of 72.2% was recorded at a dose of 2x108 conidia g^{-1} of cornflour with Strain 7284. In both assays, Strains 7284 and 7769 outperformed the other strains at low doses. These two strains had the lowest LD_{50} values of $7x10^4$ and $1x10^5$ conidia ml^{-1} and the shortest lethal times (LT_{50}) of 2.67 and 2.94d with the water suspension, respectively. The result was similar with the powder formulation but there was not much difference between strains. Strains 7284 and 7769 were highly virulent on *L. serricorne*. However, none of the strains were adequately effective against *S. zeamais*.

Keywords: *Beauveria bassiana*; *Lasioderma serricorne*; *Sitophilus zeamais*; Biocontrol

Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a pest of economic importance in stored products worldwide, particularly of maize (*Zea mays* L.) in tropical and sub-tropical regions Throne [1]. The pest is capable of multiplying to large populations resulting in severe damage to grains in storage Cosmas et al. [2]. An estimated 40.0% of stored maize is lost to *S. zeamais* in Africa Meikle et al. [3], and with severe infestations, maize weevils can cause losses of 90.0% Giga et al. [4]. The cigarette beetle (*Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae)) is found globally in tropical and subtropical areas. This pest is polyphagous, being able to feed on various food sources such as grains, spices, and tobacco Mahroof & Phillips [5].

Fumigants and synthetic insecticides have been used to control pests in grain stores. However, the repeated use of these materials has led to various problems, including insecticide resistance, environmental and human health concerns, mortality of non-target organisms, and chemical residues in foodstuffs Cherry et al. [6]. Therefore, alternative control strategies are being evaluated, including the use of entomopathogenic fungi. Differences in pathogenicity (virulence) among strains against stored product insects have been reported previously in assays with *Beauveria bassiana* (Balsamo) Vuillemin and other entomopathogenic fungi on *S. zeamais* Rondelli et al. [7]; Agoligan et al. [8]; Saeed & Laing [9], *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) Golshan et al. [10]; Golshan et al. [11], *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) da Paz Júnior et al. [12], *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) Kavallieratos et al. [13], *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) Jyothi et al. [14], *Plodia interpunctella* Hubner, *Ephestia cautella* Walker and *E. kuehniella* Zeller (Lepidoptera: Pyralidae) Sabbour et al. [15].

One of the most important steps in the development of a myco-insecticide is the selection of highly pathogenic strains Tefera & Pringle [16]. Little research has been conducted into biocontrol of the cigarette beetle, *L. serricorne* AiYing et al. [17]; Yuan et al. [18]. Therefore, the objectives of this research were to: (i) identify the most pathogenic of 20 novel strains of *B. bassiana* and one commercial strain (ARC R444) against adults of the cigarette beetle, *L. serricorne*, and the maize weevil, *S. zeamais*, in the laboratory; and (ii) to compare the efficacy of six highly pathogenic strains as either a water suspension or a powder formulation against the cigarette beetle *L. serricorne*.

Material and methods

Insect rearing

The initial population of insects was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal. *L. serricorne* was reared on rice grains. *S. zeamais* was reared on yellow maize grains. The grains were stored at -20°C for one week to eliminate unwanted natural infestation. Approximately ten adults of each insect of mixed sexes were placed in 250 ml glass jars, each containing 100g of either grain. The jars were covered with insect nets to facilitate air circulation. The adults were removed from the jars after one week of infestation. Two-day-old adults from each insect were used in the experiments.

Fungi

A total of 21 strains of *B. bassiana* from diverse geographical origins (Table 1) were screened against adults of the cigarette beetle, (*L. serricorne*) and the maize weevil, (*S. zeamais*). Twenty strains of *B. bassiana* were provided by the Plant Protection Research Institute, Agricultural Research Council (PPRI-ARC, South Africa) and the commercial strain of *B. bassiana* was provided by Plant Health Products (Pty) Ltd (PHP).

Table 1: Origin of fungal strains of *Beauveria bassiana* used to study their pathogenicity against the tested insects.

Strains	Site of isolation
B. bassiana 7284	Orchard
B. bassiana 7320	Fallow land
B. bassiana 7768	Oats
B. bassiana 7288	Wheat
B. bassiana 7291	Rooibos
B. bassiana 7297	Vineyard
B. bassiana 7769	Small Grain Institute, Bethlehem
B. bassiana 7832	Field
B. bassiana 7280	Small Grain Institute, Bethlehem
B. bassiana 7302	Rooibos 167
B. bassiana 7772	Sugarcane rows
B. bassiana 7777	Field
B. bassiana 7312	Rooibos 203
B. bassiana 7791	Rooibos field
B. bassiana 7815	Vineyard
B. bassiana 7689	Sugarcane field
B. bassiana 7278	Lawn
B. bassiana 7310	Rooibos 223
B. bassiana 7303	Rooibos 191
B. bassiana 7794	Rooibos 503
B. bassiana R444	Rooibos

Production of conidial suspension

The *B. bassiana* strains were cultured on potato dextrose agar (PDA) (Biolab Merck (Pty) Ltd. The PDA was prepared using 4g potato extract, 20g dextrose and 15g agar in 1L of distilled water, as directed by the manufacturer, in 9cm diameter Petri dishes and incubated at 28°C for 15 days for complete sporulation. The contents (hyphae and conidia) were harvested by flooding the Petri dishes with 15ml distilled water with 0.01 (v/v) Tween 80 and stirring with a glass rod. The adjuvant Tween 80 was added to facilitate the suspension of conidia in distilled water. Samples were vortexed for 3 minutes to split up conidial clumps. Conidia were separated from hyphae by filtration through three layers of cheese cloth. Conidial concentration was determined using a Neubauer Improved Haemocytometer.

Conidial viability

To assess conidial viability, 0.1ml of each sample suspension was pipetted and thinly spread over PDA in Petri dishes using an "L" shaped glass rod and incubated at 28°C for 24h, with three Petri dishes per sample suspension. Conidia were examined at 400x magnification under a light microscope. Germination of a conidia was counted when its germ tube was apparent. All the conidia in each field of view were counted and the percentage germination was calculated. All the strains used in the experiments displayed > 90.0% viable conidia.

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First screening bioassay

All 21 strains of *B. bassiana* were used in the first screening against adults of *L. serricorne* and *S. zeamais* in single-dose bioassays under laboratory conditions at 1x108 conidia ml^{-1}. Thirty < two-day old adults of each insect were treated by immersion for 10 seconds in 5 ml of conidial suspension. Each treatment was replicated three times. Control insects were treated with sterile distilled water with 0.01v/v Tween 80. The treated insects were moved to a plate containing filter paper. The filter paper helped to absorb surplus moisture Adane et al. [19]. After 24h the treated insects were transferred into 250ml glass jars with 50g of rice and kept at 28±2°C and 65±5.0% RH for 10 days. The mortality was counted every two days. Dead insects from each treatment were washed in 70.0% sodium hypochlorite, rinsed in sterile distilled water three times and kept in Petri dishes with wet filter paper at 28±°C to observe fungus outgrowth. The experiment was a 22 x 2 factorial, arranged in a randomized complete block design (RCBD). The bioassay was repeated twice. Percentage mortalities of tested insects were corrected relative to the control using Abbott's formula Abbott [20]. Data was subjected to analysis of variance (ANOVA) using GenStat for Windows, 17th edition Payne et al. [21]. Means were compared using Fisher's Least Significant Difference test at a 5.0% level of significance.

Production of dry conidia

A concentration of 10^8 conidia ml^{-1} was determined using a Neubauer Improved Haemocytometer. Petri dishes with PDA were inoculated by 100µl of fungal suspension and plates were sealed by Parafilm to maintain an adequate moisture level for fungal growth. The inoculated Petri dishes were incubated at 25°C in the dark for 15 days. The Parafilm was then removed from the plates and they were allowed to dry under a laminar flow for 5 days. Conidia were harvested by scraping them off from the surface of the dried medium using a sterile scalpel blade. The harvested fungal tissues were passed twice through a 100 mm diameter sieve (110 µm pore size) to obtain pure powdered conidia. The conidia were stored in sterile sealed bottles at 4°C for further use.

Multiple bioassays using water suspensions and powder formulations

Six strains selected from the first screening bioassay were used in the second bioassay against adults of *L. serricorne*. Five different doses ($2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia ml^{-1}) of conidial suspension in 0.01 Tween 80 aqueous solutions were prepared for each strain. Each dose was replicated three times. For each replicate, thirty < two days old adults were treated by the immersion method as described in Section 2.2.5. The control treatment was sterile distilled water with Tween 80 (0.01% v/v).

For the powder formulation, conidial doses for each strain were prepared from an initial stock of $2x10^9$ conidia g^{-1}, which was measured using a Neubauer Improved Haemocytometer, and diluted with cornflour to $2x10^8$, $2x10^7$, $2x10^6$, $2x10^5$ and $2x10^4$ conidia g^{-1}. Each dose was replicated three times. For each replicate, thirty < two-day old adults of *L. serricorne* were introduced into Petri dishes containing 0.01g of the various conidial doses. A control contained the carrier only. After 1 hour the treated insects were transferred into 250ml glass jars with 50g of rice grains and kept at 28±2°C and 65±5.0% RH for 10 days. Mortality was counted every two days for ten days. Glass jars were placed as per a 6 x 6 x 2 factorial experiment arranged in a randomized complete block design (RCBD). This bioassay was performed twice. Mortality was monitored every two days for ten days, and corrected on the basis of natural mortality observed in the control treatment using Abbott's formula Abbott [20]. Data was subjected to analysis of variance (ANOVA). Means were compared using Fisher's Least Significant Difference test at a 5.0% level of significance. Probit analysis was used to determine the median lethal dose (LD_{50}) and median lethal time (LT_{50}). All these analyses were performed using GenStat for Windows, 17th edition Payne et al. [21].

Results

First screening bioassay

There were significant differences between fungal strains in their virulence on the two insects ($F=64.04$; $P<0.001$), and the mortality levels that they caused ($F= 738.75$; $P<0.001$). The interaction between fungal strains and insects ($F=12.19$; $P<0.001$) was also significant. All *B. bassiana* strains were pathogenic and mycelial growth demonstrated that most of the tested insects died due to the fungus (Figure 1). *L. serricorne* was more susceptible than *S. zeamais* after 10 days of exposure to the 21 strains of *B. bassiana*. Mortality varied as a result of the strains, and the mortality levels ranged from 14.29 to 96.4% and 10.71 to 82.20%, for *L. serricorne* and *S. zeamais*, respectively (Figure 2). Among the tested strains, six strains (7284, 7320, 7768, 7288, 7769 and R444) were the most virulent against *L. serricorne*, with mortality levels > 90.0%; eight strains were moderately virulent, inducing 50.0% to 82.2% mortality, and the remaining seven strains caused mortality < 50.0% (Figure 2). Adults of *S. zeamais* were relatively resistant to *B. bassiana*, with 20 of the strains causing mortality levels of < 51.0%. However, Strain 7769 was more pathogenic on this insect, and caused an 82.2% mortality when applied at a dose of $1x10^8$ conidia ml^{-1}. The commercial Strain R444 used in this bioassay caused <50.0% mortality against *S. zeamais*, but it was highly virulent on *L. serricorne* and caused a 92.9% mortality level (Figure 2.2).

Multiple bioassays using water suspensions and powder formulations

All main effects as well as related interactions were highly significant at $p< 0.001$. The dose response for the water suspensions was linear between $2x10^4$ -$2x10^8$ with the $R^2 > 0.94$. The same occurred with the powder formulations, with the $R^2 > 0.95$ (Figure 3). Dose responses always reach a plateau where increased doses do not increase mortality. In this experiment, the

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plateau was reached at a dose of $2x10^8$ conidia with both the water suspensions per ml or the powder formulations per g. Therefore, a useful practical range is $2x10^4$ - $2x10^8$ conidia ml^{-1}, or conidia g^{-1} ,which is achievable in practice.

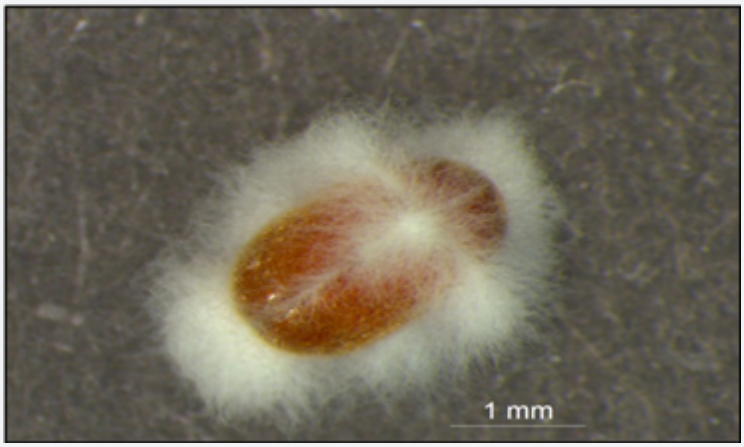

Figure 1: Mycelial growth emerging from an adult of *Lasioderma serricorne* infected by *Beauveria bassiana* Strain 7284.

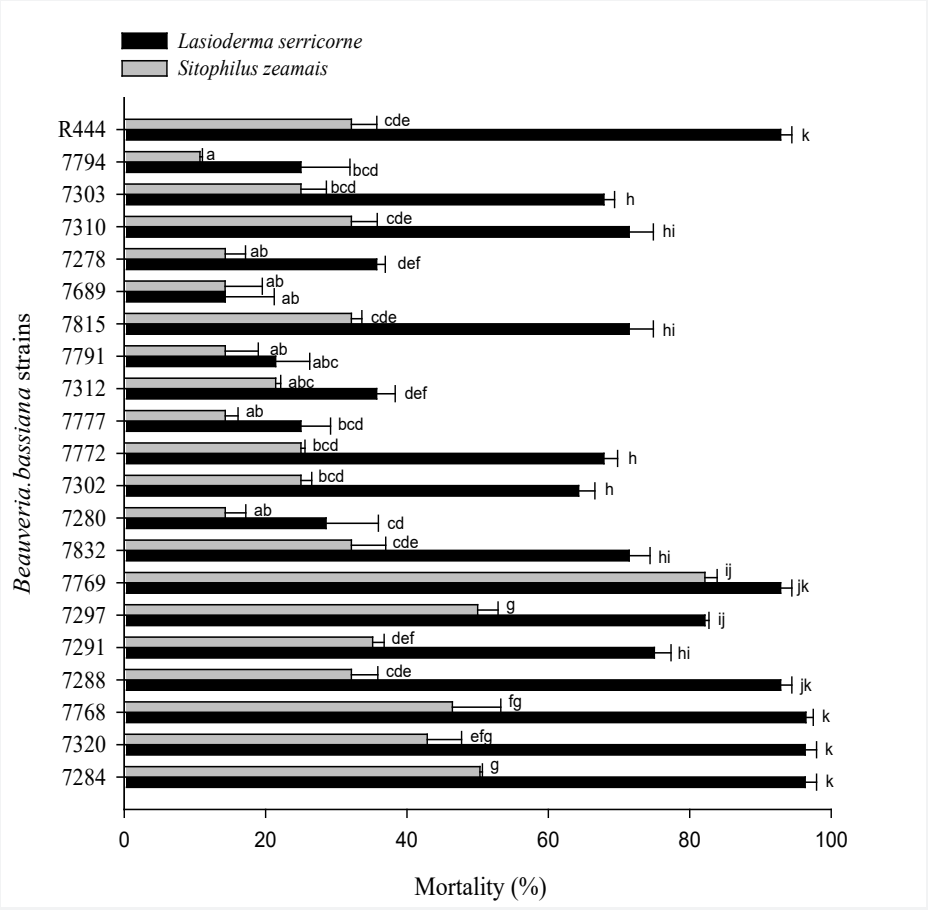

Figure 2: Corrected mortality (Abbott's correction) of adults of cigarette beetle, *Lasioderma serricorne*, and maize weevil, *Sitophilus zeamais*, following 10 days exposure to 21 strains of *Beauveria bassiana* at 1x108 conidia ml-1. Bars represent the standard error.

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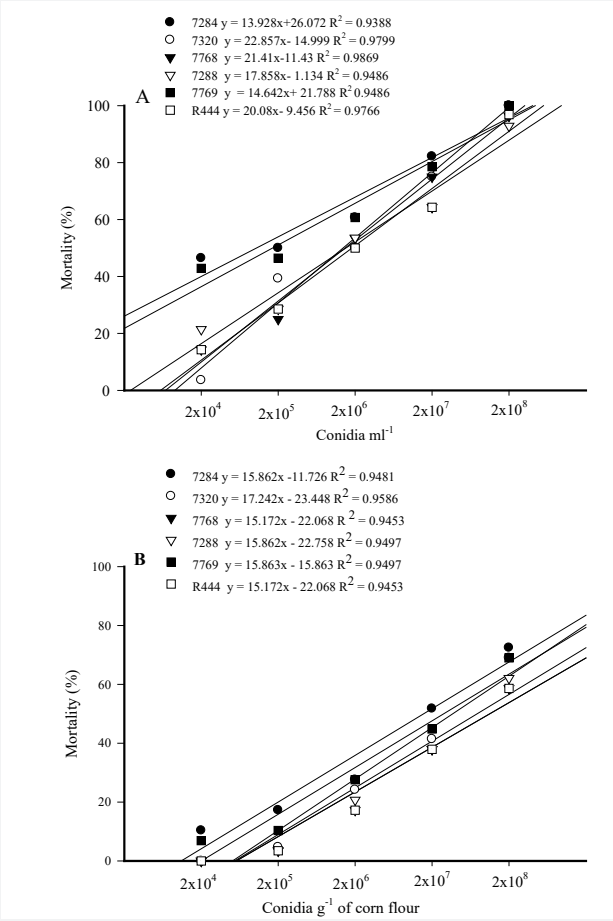

Figure 3: Corrected mortality (Abbott's correction) of cigarette beetle, *Lasioderma serricorne* adults after exposure to different doses of selected *Beauveria bassiana* strains using A = water suspension and B = powder formulation.

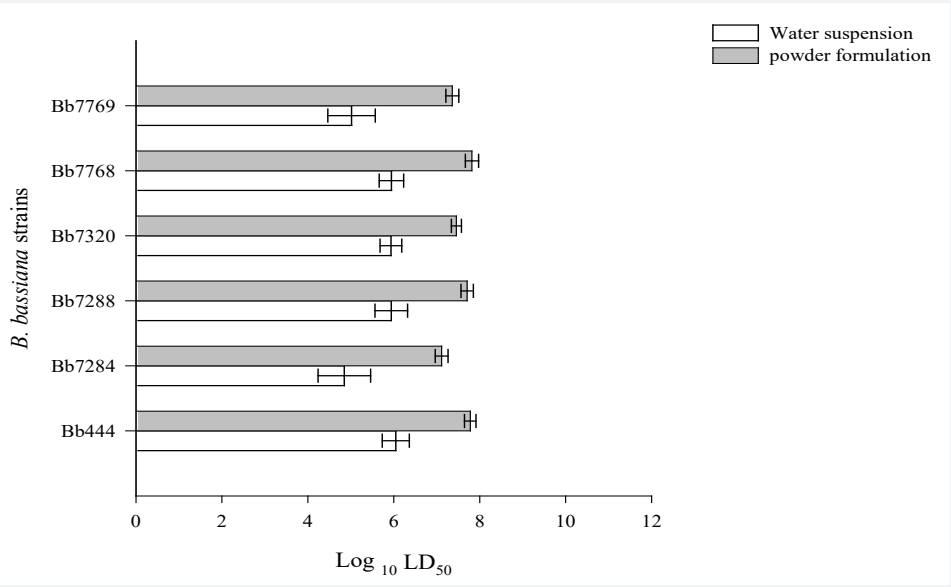

Figure 4: Lethal dose (LD50) of cigarette beetle, *Lasioderma serricorne*, adults upon exposure to water suspensions and powder formulations of six selected *Beauveria bassiana* strains at five different doses. Bars represent the standard error.

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Water suspensions of the six selected strains in the first bioassay against the adults of *L. serricorne* consistently caused higher mortality levels at all doses than the powder formulations. 100% mortality was achieved with a water suspension at $2x10^8$ conidia ml^{-1} with Strains 7284, 7769 and 7320, compared to a high of only 72.2% achieved with a powder formulation at $2x10^8$ conidia g^{-1} using Strain 7284. Using water suspensions, Strains 7204 and 7769 caused higher mortalities at low doses than the other strains, which is important because it is at low doses that successful control in granaries will need to occur. The same two strains were also the most virulent at low doses with the powder formulations (Figure 3). The commercial strain R444 used in this study caused the lowest mortality level of 58.7% against *L. serricorne*, together with Strain 7768, using powder formulations at $2x10^8$ conidia g^{-1}. However, R444 caused a higher mortality (96.5%) against the same insect when used in a water suspension at $2x10^8$ conidia ml^{-1} (Figure 3).

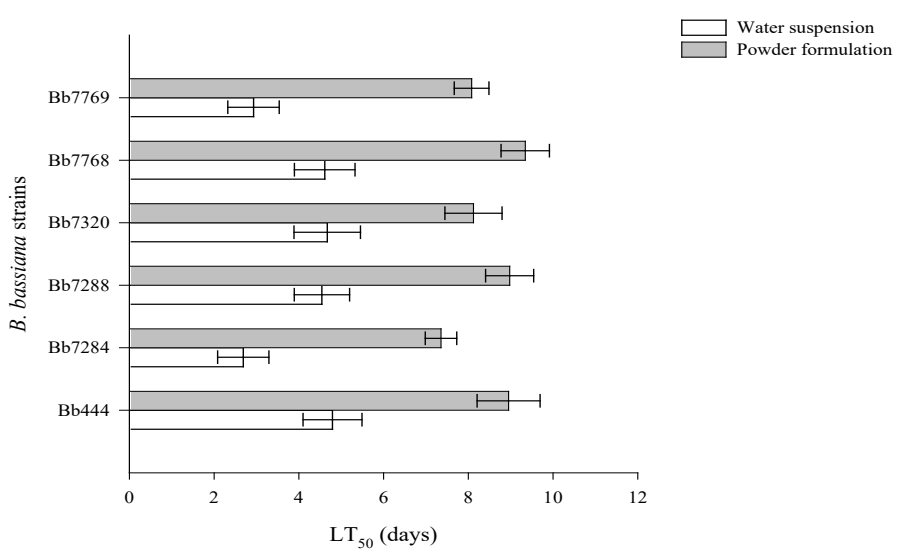

Figure 5: Lethal time (LT_{50}) of cigarette beetle, *Lasioderma serricorne*, adults upon exposure to water suspensions and powder formulations of six selected *Beauveria bassiana* strains at doses of at 2x108 conidia ml-1 or at 2x108 conidia g-1, respectively. Bars represent the standard error.

Probit analyses were performed separately for each strain formulated in a water suspension or powder formulation, and the results are presented in Figure 4 & Figure 5. The 21 *B. bassiana* strains in water suspensions achieved lower LD_{50} and LT_{50} values than they did in the powder formulations.

Using a water suspension, Strain 7284 achieved the lowest LD_{50} value (log (LD_{50} = 4.849)) and the shortest LT_{50} value (LT_{50} = 2.69 days) followed by Strain 7769 (Log (LD_{50} = 5.019) and (LT_{50} = 2.94). The results were similar for powder formulations, but the differences between strains were reduced. The six strains performed relatively well, and even the least virulent strain caused significant mortality levels.

Discussion

The present study evaluated the pathogenicity of 21 strains of *B. bassiana* (including a commercial strain (R444)) applied to adults of *L. serricorne* and *S. zeamais*, with a 10 day of exposure period. Although all the tested strains were pathogenic and caused some mortality of the tested insects, there was a wide range in the levels of virulence. The cause of such variation in virulence can be attributed to differential virulence by the strains, or by differential resistance by the host insects. All the cadavers of treated insects showed outgrowth of fungal mycelium, confirming that the fungus was the main cause of insect death, none being detected in cadavers in the control treatments.

L. serricorne was more susceptible than *S. zeamais* to the tested strains. Among the tested strains, 14 strains achieved more than 50.0% mortality levels against *L. serricorne*, with only seven recording < 50.0% mortality. These results are similar to those of Cherry et al. [22] who found that 100% mortality of *C. maculatus* resulted from exposure to the aqueous suspension of various strains of *B. bassiana* and *M. anisopliae* at a doses of $1x10^8$ conidia ml^{-1} at six and eight days after treatment, respectively.

In contrast, *S. zeamais* was relatively resistant to the 21 strains of *B. bassiana* used in this study, with 20 strains achieving mortality levels < 51.0%, and one strain causing 82.2% mortality. Shams et al. [23] observed a similar pattern with S. granarius , which was less susceptible than *C. maculatus* following exposure to five different conidial concentrations of *B. bassiana* under laboratory

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conditions. In the same context Rondelli et al. [7] reported that all twelve isolates of *B. bassiana* tested against *S. zeamais* adults caused pathogenicity. However, there were highly significant differences among the isolates with respect to virulence, with corrected mortality levels ranging between 19.7 and 72.0%, but only three of the twelve isolates caused mortality levels > 50.0%. Golshan et al. [10] used nine isolates of *B. bassiana* against the adults of *T. castaneum*, and these caused mortalities of between 15 and 60.0%, with only two isolates achieving mortality levels > 50.0%.

The six most virulent strains from the first bioassay were compared in dose response assays of a water suspension, and a powder formulation, using cornflour as the carrier, against adults of *L. serricorne*. The water suspension consistently achieved higher mortality levels at all conidial doses, and lower LD_{50} and LT_{50} values than the powder formulations. The high humidity associated with the water suspensions creates a conducive environment for infection by *B. bassiana* Devi et al. [24]. The higher mortality levels and lower LT_{50} achieved by Strains 7284 and 7769 at the lower doses are important because biocontrol of storage pests in granaries will need to be achieved at similarly low doses. In addition, 100% mortality was also observed with these strains at $2x10^8$ ml^{-1}. Several authors have also reported that using the aqueous conidial suspensions of *B. bassiana* and other entomopathogenic fungi increased the efficacy of these fungi toward target insects and resulted in high mortality levels Teshome & Tefera [25]; Mahdneshin et al. [26]; Faraji et al. [27]; Uçaret al. 2020). Although using water suspension achieves high mortality levels, it would not be practical to use these as a product against grain storage pests because the treatment of grains with aqueous suspensions would create high humidity conditions in storage that would promote the emergence of fungal moulds.

Use of a powder formulation at $2x10^8$ g^{-1} with Strain 7284 resulted in a 72.2% mortality, with higher LD_{50} and LT_{50} values than the water suspension. Nevertheless, powder formulations are more practical for the treatment of stored grains. These results are in agreement with Khashaveh et al. [28] who reported mortality levels of 88.4, 78.4 and 65.0% for *S. granarius*, *Oryzaephilus surinamensis* Linnaeus (Coleoptera: Silvanidae) and *T. castaneum*, respectively, following their exposure to wheat grains treated with a dry formulation of *B. bassiana* (Bb Weevil™, a commercial product containing $2x10^9$ conidia g^{-1}). This was applied at a concentration of 1000 mg kg^{-1}, equivalent to ten times the conidial concentration of conidia tested in this study. Saeed & Laing (2023) reported on a six-month study in which a formulation of *B. bassiana* Strain MS-8 applied in kaolin ($1x10^9$ conidia g^{-1}) at levels of 1 g kg^{-1} of grain performed well, resulting in a low number of live weevils (36 insects/500g of maize grain), a low level of grain damage (14.0%), and a low level of weight loss (7.0%). In contrast, in the untreated control, the number of live insects was 340 insects/500g of maize grain, the level of grain damage was 68.0%, and the weight loss was 51.0%.

Conclusion

In conclusion, *B. bassiana* Strains 7284 and 7769 were the most effective for control of cigarette beetle, *L. serricorne*, either as water suspensions or powder formulations. However, there is a need to screen for more virulent strains against the maize weevil, *S. zeamais*, for use in the management of this pest.

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