University of KwaZulu-Natal

Biological Control of Three Grain Storage Pests: Maize Weevil, *Sitophilus zeamais* (Motschulsky), Almond Moth, *Ephestia cautella* (Walker) and Cigarette Beetle, *Lasioderma serricorne* (Fabricius), Using Novel Strains of *Beauveria bassiana* (Balsamo) Vuillemin in Powder Formulation

By

Mohamed Baha Eldeen Eltayed Elhadi Mohamed Saeed

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Thesis Summary

Since the 1950's agrochemicals have been used to control storage grain pests. However, there are concerns about pest resistance and the environmental hazards of these insecticides. Biological control has the potential to reduce populations of storage pests in a safe and sustainable manner.

Twenty one previously isolated South African strains of *Beauveria bassiana* were evaluated for their virulence against the adults of *Lasioderma serricorne* and *Sitophilus zeamais*, under laboratory conditions. The six best strains identified from the previous screening were evaluated against the adults of *Lasioderma serricorne* in dose response assays using water suspensions and powder formulations with cornflour as the inert carrier. Strains 7284 and 7769 were highly virulent on *L. serricorn*e with both formulations. However, it was necessary to screen for more virulent isolates to control *S. zeamais*.

Twenty eight isolates of *B. bassiana* were isolated from soil using *Galleria mellonella* as a trap insect. Soil samples were collected from Ukulinga Research Farm, Pietermaritzburg, South Africa. These strains were evaluated for their effects on the adults of *S. zeamais*, at a single dose of conidia at $2x10^8$ g^{-1} in kaolin as the carrier under laboratory conditions. The three best *B. bassiana*, Strains MS-3, MS-4 and MS-8, were sent to Inqaba Biotech (South Africa) for forward and reverse strand sequencing of the ITS1 and ITS2 region. All three strains were shown to be *B. bassiana*. These strains were then subjected to dose response assays against *S. zeamais*, *Ephestia cautella* L3 larvae and *L. serricorne* L3 larvae and adults. Strain MS-8 outperformed other strains in controlling the tested insects.

Kaolin and cornflour were compared as carriers for conidia of *B. bassiana* Strain MS-8 in tests against *S. zeamais, E. cautella* L3 larvae and *L. serricorne* L3 larvae and adults. Kaolin was a consistently better carrier for the fungal conidia than cornflour against the three insects, except *E. cautella* L3 larvae for which both carriers had a similar effect on mortality. Microscopic studies using scanning electron microscope (SEM) showed that kaolin abraded the epidermis of *S. zeamais* and *L. serricorne* adults, whereas cornflour did not cause any change in the epidermis of tested insects.

Seven *B. bassiana* strains were tested for their pathogenicity against the eggs of *L. serricorne*. The two best strains, MS-8 and 7284 were formulated at 0.03g conidia kg^{-1} of grain in kaolin at doses of 0.0, 0.5, 1, 2, and 3g kg^{-1} of grain. These formulations were evaluated against *L.*

serricorne on rice grains. Strain MS-8 performed better than Strain 7284 at all doses of kaolin. The same strain at a kaolin dose of 3g kg^{-1} of grain caused the lowest levels of adults emerged (10.0%), grain damage (1.7%) and weight loss (0.55%) after 45 days of storage. When unformulated, the same dose of conidia performed poorly for the same parameters.

Short term (45 days) and long term (180 days) evaluation studies were conducted against *E. cautella* on rice grains using Strain MS-8 at dose of 0.03g conidia kg^{-1} of grain formulated in various doses of kaolin. After 45 days, Strain MS-8 at the highest dose of kaolin caused the highest level of larval mortality 90.0% and the lowest numbers of adult emerged of 1.6 adult/100g of rice grains. It also caused the lowest levels of webbed grain (2.0%), grain damage (3.0%) and grain weight loss (1.8%). As expected, the poorest performances for these parameters were observed in the untreated control treatment (UTC).

Long term evaluations of 180 days of Strain MS-8 at dose of 0.03 g conidia kg^{-1} of grain formulated in kaolin at doses of 1, 2, 3, and 4g kg^{-1} of grain was conducted against *S. zeamais* on maize grain. Strain MS-8 at a kaolin dose of 1g kg^{-1} of grain caused the lowest number of live insects (36 insects/500g of maize grain), and the lowest levels of grain damage (14.0%) and weight loss of (7.0%) compared to a count of 340 insects/500g of maize grain, 68.8% grain damage and 51.0% weight loss in the untreated control treatment (UTC).

Keywords: biocontrol, grain storage pests, *Sitophilus zeamais*, *Lasioderma serricorne*, *Ephestia cautella*, kaolin, cornflour, maize, rice, efficacy

Declaration

I, **Mohamed B. Saeed**, declare that:

i. The research reported in this thesis, except where otherwise indicated, is my original work.

ii. This thesis has not been submitted for any degree or examination at any other university.

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M.B. Saeed (Candidate)

Prof. M.D. Laing (Supervisor)

Dr. R.M. Miller (Co- Supervisor)

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Thesis Introduction

Stored-product insects and mites cause considerable post-harvest losses, estimated to range from 9% in developed countries to 20% or more in developing countries (Pimentel 1991). Pest infestations decrease the value of the commodity by contaminating it with insect wastes, faeces, webbing, and metabolic by-products (Lord et al. 2007). They also increace the contamination of food products with fungi that generate mycotoxins (Sinha & Sinha 1992). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae), and the cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) are among the most important stored grain insects in the tropical regions, particularly on maize (*Zea mais* Linnaeus) and rice (*Oryza sativa* Linnaeus) (Mahroof & Phillips 2008; Abebe et al. 2009; Shehu et al. 2010). Concerns over insecticide resistance, residues, and environmental effects, together with changes in legislation, have led to a decline in available pesticides to control stored grain pests. Alternative pest control strategies are required to maintain the levels of grain quantity and quality, and in anticipation of growing global demand for staple foods.

Entomopathogenic fungi are possibly one of the most promising alternatives to traditional pesticides. Entomopathogenic fungi are naturally occurring organisms, are environmentally safe and have low mammalian toxicity (Zimmermann 2007). *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycotina: Hyphomycetes) has been tested with success against various stored-product insect species in both laboratory and field trials (Lord 2001; Padin et al. 2002; Akbar et al. 2004; Dhuyo & Ahmed 2007; Jyothi et al. 2014).

Despite positive research results, no microbial control agents based on entomopathogenic fungi are currently available for grain storage on a commercial scale (Batta 2016). Perceptions that such products provide lower efficacy than conventional chemical control measures may be one barrier to their commercialisation. These perceptions may be prevented by improving the formulation and delivery of bio-insecticides.

Inert dusts, such as mineral clays and silica powders, kill arthropods by removing the epicuticular lipid layers, causing water loss through the cuticle (Subramanyam & Roesli 2000). These dusts have been widely used for stored product pest control around the world (Golob 1997; Kahn & Damicone 2008). Kaolin, an inert dust, is a common, silica-based clay mineral used in industrial manufacturing and the production of ceramics such as porcelain (Murray 2000). Kaolin has displayed insecticidal activity on stored product insects (Arthur &

Puterka 2002; Mahmoud et al. 2010), but it needs high application rates in admixture with grain (5–10 g kg^{-1} of grain) when used as a standalone product (El-Sayed et al. 2010). Such application rates would have an unacceptable impact on grain quality parameters such as appearance, bulk density and flow ability. Other types of inert dusts such as charcoal, oven ash, chalk powder, and wheat flour have also been evaluated as carriers of conidia of *Metarhizium anisopliae* (Metsch.) Sorokin. It has been suggested that some of them can prolong their shelf-life (Batta 2004). There are some examples where the combination of an entomopathogenic fungus with the inert dust such as kaolin and cornflour has been tested on storage pests. Samodra & Ibrahim (2006) observed that conidia of *B. bassiana* formulated in kaolin was more efficacious against larvae of the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), than when the fungus was formulated in tapioca flour or was left unformulated.

The referencing system used in the chapters of this thesis is based on the Harvard system of referencing (De Montfort University), and follows the specific style used in the journal *Florida Entomologist* (Florida Entomological Society).

The thesis is in the form of discrete research chapters, each following the format of a stand-alone research paper. This is the dominant thesis format adopted by the University of KwaZulu-Natal because it facilitates the publishing of research from a thesis far more easily than the older monograph form of a thesis. As such, there is some unavoidable repetition of references and some introductory information between chapters.

General objective

To isolate and select highly virulent strains of entomopathogenic fungi to be used in the development of a mycoinsecticide against storage grain pests.

Specific objectives

a. To review the available literature on the use of the entomopathogenic organisms such as fungi, bacteria and nematodes for the biocontrol of stored grain insects, with specific reference to *Beauveria bassiana* (Balsamo) Vuill.

b. To screen previously isolated strains of *B. bassiana* for the biocontrol of *Sitophilus zeamais* and *Lasioderma serricorne*.

c. To isolate and identify novel strains of *B. bassiana* for the biocontrol of *S. zeamais*, *Ephestia cautella* L3 larvae and *L. serricorne* L3 larvae and adults.

d. To compare two carriers of *B. bassiana* conidia, kaolin and cornflour, for the formulation of *B. bassiana* conidia by testing formulated conidia against *S. zeamais* adults, *Ephestia cautella* L3 larvae and *L. serricorne* L3 larvae and adults.

e. To evaluate the effects of the best *B. bassiana* strain formulated with various doses of the best carrier against *L. serricorne* on rice grains.

f. To evaluate the effect of a fixed dose of the best *B. bassiana* strain formulated in various conidial doses in the best carrier against *E. cautella*, on rice grains in short-term and long-term studies.

g. To evaluate the effect of a fixed dose of the best *B. bassiana* strain formulated in various doses of the best carrier against *S. zeamais*, in maize grains, in a long term study.

References

Abebe F, Tefera T, Mugo S, Beyene Y, Vidal S. 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). African Journal of Biotechnology 8: 5937-5943.

Akbar W, Lord JC, Nechols JR, Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Arthur FH, Puterka GJ. 2002. Evaluation of kaolinite-based particle films to control *Tribolium* species (Coleoptera: Tenebrionidae). Journal of Stored Products Research 38: 341-348.

Batta Y. 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. Crop Protection 23: 103-108.

Batta YA. 2016. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology 26: 1171-1183.

Dhuyo AR, Ahmed S. 2007. Evaluation of fungus *Beauveria bassiana* (Bals.) Infectivity to the larger grain borer Prostephanus trncatus (Horn.). Pakistan Entomologist 29: 77-83.

El-Sayed F, El-Zun H, El-latif AA, Nasr M. 2010. Insecticidal effect of some inert dusts against thRee OF stored grain insects at Kafr El-Sheikh governorate. Journal of Plant Protection and Pathology 1: 959-972.

Golob P. 1997. Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research 33: 69-79.

Jyothi P, Rao N, Lakshmipathy R. 2014. Laboratory evaluation of entomopathogenic fungus alone or in combination with edible oils on progeny adult build-up of lesser grain borer on stored paddy. Asian Journal of Bio Science 9: 139-145.

Kahn BA, Damicone JP. 2008. Kaolin particle film product applications before harvest begins may not improve marketable yields of fresh tomatoes. HortTechnology 18: 144-147.

Lord JC. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94: 367-372.

Lord JC, Campbell JF, Sedlacek JD, Vail PV. 2007. Application and evaluation of entomopathogens for managing insects in stored products, *In* Lacey LA, Kaya HK [eds.], Field Manual of Techniques in Invertebrate Pathology. Springer, Dordrecht, Netherlands.

Mahmoud AG, El-Sebai O, Shahen A, Marzouk A. 2010. Impact of kaolin-based particle film dusts on *Callosobruchus maculatus* (F.) and C. chinenesis (L.) after different storage periods of treated broad bean seeds. Julius-Kühn-Institut, 425: 638-646.

Mahroof RM, Phillips TW. 2008. Life history parameters of *Lasioderma serricorne* (F.) as influenced by food sources. Journal of Stored Products Research 44: 219-226.

Murray HH. 2000. Traditional and new applications for kaolin, smectite, and palygorskite: a general overview. Applied Clay Science 17: 207-221.

Padin S, Dal Bello G, Fabrizio M. 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research 38: 69-74.

Pimentel D. 1991. World resources and food losses to pests, *In* Gorham JR [ed.], Ecology and Management of Food-Industry Pests. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Samodra H, Ibrahim YB. 2006. Effectiveness of selected entomopathogenic fungi in packed rice grain at room temperature against *Corcyra cephalonica* Stainton. ASEAN Journal on Science and Technology for Development 23: 183-192.

Shehu A, Obeng-Ofori D, Eziah VY. 2010. Biological efficacy of Calneem TM oil against the tropical warehouse moth *Ephestia cautella* (Lepidoptera: Pyralidae) in stored maize. International Journal of Tropical Insect Science 30: 207-213.

Subramanyam B, Roesli R. 2000. Inert dusts, *In* Subramanyam B, Hagstrum D [eds.] Alternatives to Pesticides in Stored-Product IPM. Springer, New York, USA.

Zimmermann G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 17: 553-596.

Chapter One

Literature Review

1.1 Stored-grain pests

Storage of grains is challenged by a number of biotic constraints, the most important being insects (Ngamo et al. 2007). The most common insect pests of stored products belong to the orders Coleoptera and Lepidoptera (Bekele et al. 1997). The former is the largest insect order and contains the most important stored product pests. Post-harvest pests can be primary or secondary. Primary pests are those which possess strong mouthparts and able to attack intact grains, while secondary pests have weak mouthparts and attack damaged grains or grain products (El-Aziz 2011). The most important stored grain insects in Africa are presented in Table 1.1. Besides these, there are other insect pests that do not breed in stored grain but their presence in the grain stores is harmful because they degrade the quality of the grain. They do not cause much direct feeding damage to food grains but create noxious smell and debris. These insects include cockroaches, ants, crickets, silverfishes, psocids and termites (Upadhyay & Ahmad 2011).

1.2 Damage and economic losses caused by stored grain pests

A primary global challenge for agricultural research, development and policy is how to feed over 9.1 billion people with safe food by the year 2050 (Parfitt et al. 2010). While considerable attention has been directed toward increasing food production by 50–70% to meet this target, one important and complementary factor that has often been forgotten is reducing food loss and food waste (Hodges et al. 2011). A recent report by the World Bank (World Bank 2011) revealed that significant volumes of food are lost each year after harvest in sub-Saharan Africa (SSA), the value of which is estimated at USD 4 billion for grains alone. Reducing post-harvest loss (PHL) has positive impacts on the environment and climate as it enhances farm-level productivity and reduces the utilization of production resources or expansion into fragile ecosystems to produce food that will be lost and not consumed by humans (Hodges et al. 2011). Most of the losses occur during cereal storage can be attributed to biotic agents, such as insects, diseases, and rodents (Rana 1977), but the role of insects is predominant. For instance, in Zimbabwe, *S. zeamais* caused losses of 56% when maize was stored for a period of 150 days (Derera et al. 2014). Bhanderi et al. 2015 reported a 61% loss sorghum grain 180 days after being infested with *Sitophilus oryzae* In India (Table 1.2).

Table 1.1 Major stored grain insects in Africa (baised on data from Upadhyay & Ahmad 2011)

No	Common name	Latin name	Order	Family	Crop	Geography	Type
1	Maize weevil	*Sitophilus zeamais* (Motsch.)	Coleoptera	Curculionidae	Mainly cereals and some pulses	across Africa	IPP
2	Rice weevil	*Sitophilus oryzae* (L.)	Coleoptera	Curculionidae	Mainly cereals and some pulses	across Africa	IPP
3	Granary weevil	*Sitophilus granarius* (L.)	Coleoptera	Curculionidae	Mainly cereals and some pulses	mostly North Africa	IPP
4	Cowpea beetle	*Callosobruchus chinensis* (L.)	Coleoptera	Bruchidae	Pulses	mostly East Africa	IPP
5	Cowpea beetle	*Callasobruchus maculatus* (L.)	Coleoptera	Bruchidae	Cowpeas and some beans	mostly East, West Africa and middle east	IPP
6	Bean beetle	*Bruchus incarnates* (Boh.)	Coleoptera	Bruchidae	Legumes	East Africa	IPP
7	Broad bean beetle	*Bruchus rufimanus* (Boh.)	Coleoptera	Bruchidae	Legumes	North Africa	IPP
8	Pea weevil	*Bruchus pisorum* (L.)	Coleoptera	Bruchidae	Legumes	Middle East (Sudan)	IPP
9	Groundnut seed beetle	*Caryedon serratus* (Oliv.)	Coleoptera	Bruchidae	Peanuts	Middle East	IPP
10	Groundnut bruchid	*Caryedon gonagra* (F.)	Coleoptera	Bruchidae	Pulses	East and West Africa	IPP
11	Mexican bean weevil	*Zabrotes subfasciatus* (Boh.)	Coleoptera	Chrysomelidae	Beans, cowpea	West Africa and Southern Africa	IPP
12	Red flour beetle	*Tribolium castaneum* (Herb.)	Coleoptera	Tenebrionidae	Cereals and pulses	across Africa	EPP
13	Confused flour beetle	*Tribolium confusum* Jacquelin du Val	Coleoptera	Tenebrionidae	Cereals and groundnut	mostly East Africa	EPP
14	Khapra beetle	*Trogoderma granarium* (Evert.)	Coleoptera	Dermestidae	Cereals	across Africa	EPP

Table 1.1 Continued.

No	Common name	Latin name	Order	Family	Crop	Geography	Type
15	Lesser grain borer	*Rhyzopertha dominica* (Fabr.)	Coleoptera	Bostrichidae	Mainly cereals and some pulses	North, West and middle east Africa	IPP
16	Larger grain borer	*Prostephanus truncatus* (Horn.)	Coleoptera	Bostrichidae	Maize and some pulses	mostly West Africa	IPP
17	Rusty grain beetle	*Cryptolestes ferrugineus* (Stephens)	Coleoptera	Laemophloeidae	Cereals	across Africa	EPP
18	Cigarette beetle	*Lasioderma serricorne* (Fabr.)	Coleoptera	Anobiidae	Pulses and rice	mostly East and West Africa	IPP
19	Drug store beetle	*Stegobium paniceum* (L.)	Coleoptera	Anobiidae	Cereals and pulses	mostly North Africa	IPP
20	Saw-toothed grain beetle	*Oryzaephilus surinamensis* (L.)	Coleoptera	Silvanidae	Cereals and some pulses	mostly south Africa and middle east	SP
21	Merchant grain beetle	*Oryzaephilus mercator* (Fauvel)	Coleoptera	Silvanidae	Groundnut, Rice	East Africa	SP
22	Rice moth	*Corcyra cephalonica* (Staint.)	Lepidoptera	Pyralidae	Cereals	mostly West Africa	EPP
23	Almond moth	*Ephestia cautella* (Walker)	Lepidoptera	Pyralidae	Cereals and pulses	across Africa	EPP
24	Mediterranean flour moth	*Ephestia kuehniella* (Zeller)	Lepidoptera	Pyralidae	Cereals	across Africa	EPP
25	Warehouse moth	*Ephestia elutella* (Walker)	Lepidoptera	Pyralidae	Cereals	mostly North Africa	EPP
26	Angoumois grain moth	*Sitotroga cerealella* (Oliv.)	Coleoptera	Pyralidae	Cereals	mostly North Africa	IPP

* IPP = Internal primary pests. EPP = External primary pests. SP = Secondary pests

Table 1.2 Weight loss (%) caused by various species of stored grain pests in different crops.

Crop	Pests	Weight loss (%)	Storage period (days)	Country	Reference (s)
Wheat	*Sitotroga cerealella* (Oliv.)	5.52 – 13.75	45	Pakistan	Shafique et al. 2006
Maize	*Sitophilus zeamais* (Motsch.)	8.35	45	Zimbabwe	Muzemu et al. 2013
Maize	*Sitophilus zeamais* (Motsch.)	19 - 56	150	Zimbabwe	Derera et al. 2014
Maize	*Sitophilus zeamais* (Motsch.)	1.1 – 10.9	56	Ethiopia	Tefera et al. 2013
Sorghum	*Sitophilus zeamais* (Motsch.)	0.30 – 12.40	64	Ethiopia	Goftishu & Belete 2014
Maize	*Ephestia cautella* (Walker)	2.03	35	Ghana	Shehu et al. 2010
Cowpea	*Callosobruchus maculatus* (L.)	9 - 25	37	Nigeria	Amusa et al. 2013
Cowpea	*Callosobruchus chinensis* (L.)	1.7 – 6.9	180	India	Choudhary et al. 2015
Maize	*Sitotroga cerealella* (Oliv.)	6.4- 14	42	India	Muthukumar et al. 2015
Sorghum	*Sitophilus oryzae* (L.)	48.38 – 61.11	180	India	Bhanderi et al. 2015
Maize	*Prostephanus truncatus* (Horn.)	00 – 7.7	60	Nigeria and Ghana	Nwankwo et al. 2014
Rice	*Sitophilus oryzae* (L.)	2.57 – 3.81	28	Ghana	Badii et al. 2013

Besides causing losses of grain weight, pest attack on stored grains is also linked to mycotoxin contamination and poisoning (Table 1.3). Mycotoxin contamination (especially aflatoxin and fumonisin production by *Aspergillus flavus* (Link) and *Fusarium verticillioides* (Sacc.), respectively makes grain unsafe for food and animal feed (Tefera 2012).

Table 1.3 Fungal species that produce mycotoxin in crops

Fungal species	Crop	Mycotoxin	References
Fusarium spp.	Maize	zearalenone (ZON), α- and β-zearalenols (α- and β-ZOL)	Adejumo et al. 2007
Fusarium sporotrichioides (Sherb.)	Maize, wheat and rice	trichothecenes	Mateo et al. 2002
Fusarium spp.	Maize	fumonisin B_1	Bankole et al. 2003
Aspergillus sp.	Maize	aflatoxins B_1, B_2, G_1, and G_2	Hennigen & Dick 1995
Aspergillus flavus (Link)	Sorghum	aflatoxins	Divakara et al. 2014
Penicillium verrucosum	Wheat	ochratoxin A	Lund & Frisvad 2003

1.3 *Sitophilus zeamais* biology and behaviour

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is a small snout beetle with adults reaching lengths of between 3 and 3.5 mm. The maize weevil has well-developed wings and can fly fast, covering great distances. The female weevils excavate shallow pits in the seed coat of grains, and lay a single egg inside, sealing it with a waxy plug. Typically one egg is laid per kernel (Lathrop 1914; Gomez et al. 1982), but occasionally more than one adult may emerge. If multiple eggs are laid, larvae compete with active aggression between the seed occupants (Guedes et al. 2010). Upon hatching, the single fleshy grub develops, growing and feeding within a single grain. The larva moults into a naked white pupa and later the adult eventually emerges via an emergence hole, which may subsequently be enlarged by feeding of the adult inside, or by other storage insects (Longstaff 1981). The life cycle of the maize weevil averages 35 days at 27°C (80.6°F). Sharifi & Mills (1971)

found it to have a maximum development time of 110 days at 18°C (64.4°F). Survivorship of all immature life stages is highest at 25°C (77°F) (Throne 1994).

1.4 *Lasioderma serricorne* biology and behaviour

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), ranks as one of the most serious pests of stored products globally (Kim et al. 2003). Adult tobacco beetles vary in colour and size depending upon the type of food and atmospheric conditions encountered during their development. Insects are reddish-brown in colour, are typically 2-4 mm long and weigh between 1.6-4.4 mg (Ashworth 1993). The beetle is oval, with head and prothorax bent downwards; the head is deflexed and almost completely obscured from above, giving the insect a humped-convex appearance. Both larvae and adults are covered with fine hairs. Adult elytra (wing covers) have no markings (Ashworth 1993). The antennae are serrate (saw-like). The segments are practically identical and are of the same thickness from base to tip. This feature is used as a diagnostic tool to differentiate this insect from those with similar appearances (Ashworth 1993).

This pest usually flies during the late afternoon, at dusk and during darkness when the temperatures are above 18^{o}C, but sometimes in daylight on dull days (Ashworth 1993). The sex pheromones of *L. serricorne*, anhydroserricornin (2,6-diethyl-3,5-dimethyl-3,4-dihydro-2*H*-pyran), and serricornin (4,6-dimethyl-7-hydroxynonan-3-one), are produced by adult females, and elicit a strong attractive response by adult males (Coffelt & Burkholder 1972; Levinson & Levinson 1986).

After mating and gestation each female lays between 45 and 116 waxy-shelled eggs, deposited singly within the infested commodities over a number of days. These hatch at temperatures above 20^{o}C after six to seven days (Powell 1931; Lefkovitch & Currie 1967; Retief 1988). Larvae pass through three moults, with four larval instars. During the first instar, larvae are most active (mobile) and suffer the highest mortality rates (Ashworth 1993). Larvae are creamy or white in colour and are covered with fine hairs. The larvae pupate within a cell made from food and waste material (Ashworth 1993). The emerged adults are capable of flying and can live for 2-6 weeks, but do not feed during the adult stage (Minor 1979). The development of *L. serricorne* depends on the temperature and relative humidity of its environment and its diet (Howe 1957).

1.5 *Ephestia cautella* biology and behaviour

Adults of the almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae), have light gray wings with straight dark lines, most notably near the distal edge of the forewing (Burges & Haskins 1965). Adults oviposit directly in the infested commodity. Eggs are similar in shape, size, and nature to the Indian meal moth, but are slightly gray instead of white. Almond moth larvae are similar to those of the Indian meal moth and raisin moth but differ by the presence of round, black pinacula (Solis 2006). Development is fastest at 30–32°C and 70–80% R.H., taking 29–30 days from the oviposition to the emergence of the adult moth. This period is extended with lower temperatures and humidities, reaching 145 days at 15.5°C and 70% R.H., and 51 days at 30°C and 20% R.H. (Burges & Haskins 1965). The duration of all developmental stages is highly affected by the temperature, relative humidity, and availability of food (Subramanyam & Hagstrum 1993).

In natural lighting, adult emergence, copulation and oviposition are chronological. When dusk occurs between 17.00–18.00 hours, then highest emergence for both sexes occurs between 15.00–16.00 hours; copulation activity is greatest in the period 17.00–18.00 hours and oviposition commences at midnight (Steele 1970).

1.6 Control of stored grain pests

1.6.1 Cultural control

Traditional methods usually provide cheap and practical ways of post-harvest handling of crops. During storage, some traditionally used materials are often added to the grain, aiming to reduce pest activity. Inert dusts, for example, are often added in variable amounts to the stored product. Abrasion of the cuticles of storage insects by the dust particles leads to desiccation and hampers the development of the pest (Parkin 1955; Golob 1997).

Between crops, food grain store houses should be cleaned thoroughly, and dirt, egg shells and dead larvae should be removed. Broken infested grains should be removed and burnt (or composted) before new grains are stored in the storeroom. All cracks and crevices made in the floors walls and ceiling of the store should be filling up with cement (Upadhyay & Ahmad 2011).

Infestation of grain may start in the field. For instance, *C. chinensis* infests beans in the field (Messina 1984). Timely harvesting can therefore ensure that weevils are not carried into the store along with the beans.

1.6.2 Botanical control

Many botanicals, such as plant essential oils and their chemical constituents, have been reported for their inhibitory activities against insect pests (Upadhyay & Jaiswal 2007; Ayvaz et al. 2010). The essential oils of many plant species are known to have repellent and insecticidal activities (Table 1.4).

1.6.3 Host resistance

The use of resistant varieties is crucial in successful grain preservation, especially in tropical storage. It is a cheap and effective option of pest management, and requires little or no scientific knowledge by the farmers. Plant breeders have made substantial progress in developing maize varieties that combine pest resistance with high yield and palatability. This resistance, when used with other control options in an integrated manner, can lead to effective and sustainable post-harvest pest management (Arnason et al. 1992).

Insect pest resistance in crops is mostly based on four important mechanisms: (i) antixenosis, (ii) antibiosis, (iii) tolerance, and (iv) escape. Tolerance and escape are resistance mechanisms relevant for field infestations but not for storage insect pests of grain crops. However, the processes of resistance involve morphological, physiological and/or biochemical mechanisms, which range from simply mimizing the effects of insect attachment, to adversely effcting the insects' cellular processes, growth and development (Singh 2002). Or prevent them from the using host plant for food shelter or oviposition.

Table 1.4 Effects of some plant essential oil activities against stored grain pests.

Family	Scientific name	Action	Insect	Claims	Author
Zingiberaceae	*Elletaria cardamomum* (L.)	Contact, fumigant, and antifeedant	*S. zeamais* egg larvae and adults of *T. castaneum*	Both insects were equally susceptible to contact toxicity. For fumigant toxicity, *S. zeamais* adults were more than twice as susceptible as *T. castaneum* adults. 12-day larvae were more tolerant than the adults to the contact toxicity of the oil and, 12-16 day larvae were much more tolerant than the adults to the fumigant action. No feeding deterrence against *T. castaneum*. However, slight feeding deterrence against *S. zeamais*.	Huang et al. 2000
Myrtaceae	*Eucalyptus* sp.	Fumigant	*S. oryzae*	GC-MS analysis of essential oil from eucalyptus showed it to be rich in 1,8-cineole (81.1%), limonene (7.6%) and pinene (4.0%). Treatment of *S. oryzae* with each of these terpenes showed 1,8-cineole to be most active (LD50=23.5 µl l^{-1} air).	Lee et al. 2001
Asteraceae	*Artemisia absinthium* *A. santonicum* *A. spicigera*	Contact and fumigant	*S. granarius*	All of the essential oils tested were found to be toxic to adults of *S. granarius*. with 80–90% mortality of *S. granarius* at a dose of 9 µl l^{-1} air after 48 h of exposure	Kordali et al. 2006
Myrtaceae	*Eucalyptus intertexta* *Eucalyptus sargentii* *Eucalyptus camaldulensis*	Fumigant	*C. maculatus* *S. oryzae* *T. castaneum*	The essential oil of these species has potent fumigant toxicity against the tested insects.	Negahban & Moharramipour 2007

Table 1.4 Continued.

Family	Scientific name	Action	insect	Claims	Author
Lamiaceae	*Ocimum gratissimum* (L.)	Fumigant and repellent	*S. oryzae* *T. castaneum* *O. surinamensis* *R. dominica, C. chinensis*	Fumigant toxicity and repellence of *O. gratissimum* oil and its constituents b - (Z)-ocimene and eugenol were effective against all tested insects except for *T. castaneum*, which was more tolerant.	Ogendo et al. 2008
Anacardiaceae	*Pistacia lentiscus* (L.)	Fumigant	*Ectomyelois ceratoniae* Zeller *E. kuehniella*	*P. lentiscus* essential oil contained terpinene-4-ol (23.32%), terpineol (7.12%) and b caryophyllene (22.62%) as major compounds. Fumigant toxicity tests showed that *P. lentiscus* oil was more toxic to *E. kuehniella* than *E. ceratoniae*.	Bachrouch et al. 2010
Lamiaceae	*Salvia leriifolia*	Contact	*L. serricorne*	Tested plant was collected at two developmental stages. The mortality of 3-10 days adults increased with rates of oils and exposure time. May oil were more toxic than March oil against cigarette beetle.	Geranmayeh & Hashemi 2014
Myrtaceae	*Eucalyptus procera*	Contact and fumigant	*C. maculatus* *T. castaneum*	*C. maculatus* and *T. castaneum* were very susceptible to the essential oil of *E. procera* in both contact and fumigant bioassays.	Nouri-Ganbalani et al. 2016

Antixenosis is expressed as non-preference of the insect pest because the plant is unsuitable as a host for reproduction or feeding due to the presence of morphological or biochemical factors. Morphologically, varieties with smooth, soft and thin seed coats may be more preferable for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed et al. 1989; Shaheen et al. 2006), but there is still some controversy and doubt about considering these features as universal indicators of resistance (Lale & Kolo 1998; Somta et al. 2007). For instance, Desroches et al. (1995) found that the seed coat in faba bean (*Vicia faba*) acts as a physical barrier against penetration by *C. chinensis* and *C. maculatus.* They found that only 45–58% of the neonate larvae penetrated the seed coat to reach the cotyledons. A similar type of resistance against *C. maculatus* has also been reported in cowpea (Edde & Amatobi 2003).

Antibiosis causes adverse effects for larvae of storage pests feeding on seed of a resistant host plant, and may be the result of morphological, physiological and biochemical features of the host plant. Antibiosis may kill the insect pest or affects it is biology. Nhamucho et al. (2014) observed that, in general, a high protein content contributed towards resistance while a high starch content contributed to susceptibility. They concluded that an antibiosis mechanism contributed to postharvest resistance in maize to the larger grain borer (LGB) (*Prostephanus truncates* Horn (Coleoptera: Bostrichidae)).

1.6.4 Thermal control

1.6.4.1 Low temperature

Stored-product pests are primarily thermophilic in nature, i.e., their growth and survivability are greatly influenced by temperature. The lower developmental threshold for most stored-product pests is approximately 18^{o}C (Howe 1965). The optimum developmental range of many stored-grain insect pests is approximately 25–33^{o}C (Table 1.5). For most species there is a range of temperatures covering some 3-4^{o}C at which the rate of increase is greatest, usually at a humidity of 60% or more (Howe 1965). Low temperatures are of greater significance. For every species there is a minimum constant temperature threshold below which development ceases, and for many, there is also a low humidity that is lethal.

The use of aeration to reduce the grain bulk temperature to below optimum development thresholds of stored-product insect pests is an IPM-based approach to manage insects in bulk grains (Hagstrum et al. 1999). In a simulation study on the feasibility of aeration management

of *S. zeamais* populations in maize stored in the southern and northern United States using recorded weather data, Arthur et al. (1998, 2001) showed that aeration dramatically reduced the predicted number of *S. zeamais* in all geographical zones compared to the population levels in unaerated corn. Ileleji et al. (2007) observed that chilled aeration could be used to suppress *S. zeamais* progeny in stored maize by rapid cooling of the grain to ≤ 15.0°C during periods of warm ambient temperatures. The effectiveness of aeration can be increased in tropical climates or in the summer in temperate climates by cooling the air with refrigeration units. Low temperatures reduce the rates of development, feeding, fecundity and survival (Longstaff & Evans 1983). Imai & Harada (2006) observed that the reproductive cycle of the cigarette beetle, *L. serricorn*e, can be blocked by temperatures of less than 18°C, and that tobacco stored in such conditions will not become infested, even if eggs are deposited by invading adults. Clean dried fruit and nuts that were stored at 10°C and undisturbed for at least 4 weeks should be relatively free of Indian meal moth (Johnson et al. 1997).

Table 1.5 Response of stored-product insects to temperature (Banks & Fields 1995; Fields & Muir 1995)

Zone	Temperature (°C)	Effects
Lethal	>62	Death in less than 1min
	50 to 62	Death in less than 1h
	45 to 50	Death in less than 1d
	35 to 42	Population dies out
Sub-optimum	35	Development stops
	33 to 35	Slow development
Optimum	25 to 33	Maximum rate of development
Sub-optimum	20 to 25	Slow development
	13 to 20	Development slow or stops
Lethal	3 to 13	Death in days (unacclimatized) and movement stops
	-10 to -5	Death in weeks to months if acclimatized
	-25 to -15	Death in minutes. Insects freeze

1.6.4.2 High temperature

The use of elevated temperatures, also termed heat treatment, has long been documented as an effective approach for managing stored-product insects infesting food-processing facilities (Dean 1911; Fields & White 2002). The mechanism of heat treatment is to raise the ambient temperature of the entire facility or a portion of it, to 50 to 60ºC and to hold these elevated temperatures for 24 to 36 h to facilitate heat distribution throughout the entire space of the facility for effective disinfestation (Mahroof et al. 2003).

Heat treatments can be provided through hot water dips, vapour heat or hot dry air, but an optimal combination of time and temperature is necessary to provide the desired control, without significant losses in the quality of the product (Finkelman et al. 2006; Ben-Amor et al. 2016). Pražić-Golić et al. (2011) observed that for all three species of *Sitophilus* the use of a temperature of 50°C was more effective when the pests were outside the wheat grains. For example, in an empty storage facility, the control period was 110 min for *S. granarius*, 130 min for *S. oryzae* and 160 min for *S. zeamais*, to achieve total progeny reduction. These periods were extended when grain was infested. Exposure of *L. serricorne* eggs, young larvae, old larvae, pupae, and adults to fixed periods of exposure to 46, 50, and 54ºC and 22% RH showed that eggs are the most heat-tolerant stage (Yu et al. 2011). Therefore eggs should be used in bioassays for gauging heat treatment effectiveness, because treatments aimed at controlling eggs should be able to control all other *L. serricorne* life stages. The lethal time to reduce survival by 50% (LT_{50}) at 42ºC for eggs, larvae, pupae and adults were 18, 57, 78 and 71 h, respectively (Loganathan et al. 2011). In general, the conditions needed to control the target insect should not affect the quality of the grain, but the margin of safety before damage occurs can be narrow, necessitating close control of the heating process and subsequent cooling (Banks & Fields 1995).

1.6.5 Chemical control

Fumigation has historically played a major role in insect pest elimination in stored products. Phosphine and methyl bromide (MeBr) have been the two widely used fumigants for stored product protection worldwide (Rajendran & Sriranjini 2008). The most commonly used fumigant is phosphine, but it is use becoming limited because of increasing evidence that stored product insects are becoming resistant to the compound; this has been observed in

more than 45 countries (Bell & Wilson 1995). Methyl bromide has been found to be an ozone depleting substance and therefore it is being phased out completely.

The application of fumigant mixtures has been recognized as a means of overcoming the disadvantages of using a single fumigant. A combination of fumigants is advisable because none of the common fumigants, used alone, possesses ideal characteristics for pest elimination (Navarro et al. 1986; Athié et al. 1998).

Organochlorines are persistent in the environment and are known for bioaccumulating or building up in sediments, plants and animals. DDT (dichlorodiphenyltrichloroethane) was a popular pesticide used to control stored product pests in Brazil. However, resistance to DDT was reported in Brazilian populations of the maize weevil by the early 1990s (Guedes et al. 1995).

When organochlorines were restricted, the use of organophosphorus compounds, especially malathion, for control of stored grain insects increased greatly. The heavy use of malathion to control stored products pest has led to worldwide resistance by several species such as *T. castaneum* (Champ & Campbell-Brown 1970; Saleem & Shakoori 1989) and *R. dominica* (Guedes et al. 1996). Therefore, this compound has been replaced by other organophosphate insecticides including pirimiphos-methyl, chlorpyrifos-methyl, dichlorvos, etrimfos and fenitrothion (Boyer et al. 2012). However, cross resistance to the entire organophosphates family is common and involves common biochemical steps in the resistant pests.

Pyrethroids have proven to be successful alternatives to traditional organophosphorus insecticides, given that they are cost-effective and generally less toxic to mammals and generally more effective (Guedes et al. 1995; Arthur 1996). However, resistance exists in this case too (Collins 1990; Arthur 1996; Araújo et al. 2011).

A more recently released group of insecticides used on stored foods is the Spinosyns (or Naturalyte) class, such as spinosad and spinetoram, which are derived from the fermentation of a soil actinomycete, *Saccharopolyspora spinosa* (Bacci et al. 2016). Like the organophosphates, this class of insecticides disrupts nerve transmission by disrupting acetylcholine activity, but it does it uniquely by disrupting its binding sites in synapses, specifically in Lepidoptera, Thysanoptera, and some Coleoptera and Diptera, making them relatively safe to non-target insects and to mammals. However, resistance has developed in

some lepidopterans, coleopterans and dipterans (Li et al. 2007; Awan et al. 2012; Bacci et al. 2016).

1.6.5.1 Human health and environmental issues

The use of chemicals in modern agriculture has significantly increased productivity (Isman 2006). But it has also significantly increased the concentration of pesticides in food and in our environment, with associated negative effects on human and environmental health. Annually there are millions of cases of pesticide poisonings worldwide (Richter 2002). It is also recognised that pesticides can cause significant chronic health effects, including cancer, neurological effects, diabetes and respiratory diseases. These health effects vary depending on the levels of exposure and the type of exposure. Typically, the effects are different for farmers who are directly exposed to pesticides, compared to those for farmers' relatives or people living in rural areas who are less directly exposed. There are also effects on consumers through pesticide residues in food.

Cancer associated with pesticide exposure is one of the most studied topics related to pesticide toxicity during the last decade (Weichenthal et al 2010). For example, prostate cancer is the most common cancer diagnosed among men in the United States, accounting for an estimated 28.5% of all cancers diagnosed in men in 2012, possibly as a result of exposure to pesticides (Siegel et al. 2012). Others types of cancers have been investigated for their potential link to pesticides such as childhood leukaemia (Ward et al. 2009; Turner et al. 2011), adult leukaemia (Zeeb & Blettner 1998), and breast cancer (Snedeker 2001; Teitelbaum et al. 2007).

Various studies have been carried out on the effects of pesticide exposure on neurological function. Farm workers with decreasing levels of performance may be related to chronic exposure to pesticides (Kamel et al. 2003). Pesticides or pesticide metabolites associated with diabetes include: hexachlorobenzene (Glynn et al. 2003; Codru et al. 2007), DDT (Everett et al. 2007), and its metabolite DDE (dichlorodiphenyltrichloroethylene) (Lee et al. 2006, 2007; Rignell-Hydbom et al. 2007). Studies on respiratory diseases and their link to pesticide exposure have shown an increased risk of respiratory diseases such as asthma (Ernst 2002) and wheeze (Fieten et al. 2009).

Movement of pesticides from the sites of application to non-target regions creates three problems. It causes an economic loss to farmers, inefficient control of pests, and possible

environmental contamination (Waite et al. 2002), where pesticides contaminate soil, water, turf, and other vegetation. In addition to killing insects or weeds, pesticides can be toxic to a host of other organisms including birds, fish, beneficial insects, and non-target plants (Khalaf-Allah 1999; Johnsen et al. 2001; Turgut 2003; Boatman et al. 2004).

1.6.5.2 Resistance to chemical pesticides

The evolution of pesticide resistance is a growing problem in stored product protection. More than 600 species of plant feeding insect pests had developed resistance to insecticides by 1996 (Sharma et al. 2001). Insecticide resistance by various stored product insects has been reported worldwide (Perez-Mendoza 1999; Fragoso et al. 2003; Ribeiro et al. 2003; Benhalima et al. 2004; Pereira et al. 2009; Jittanun & Chongrattanameteekul 2014). Resistance to pesticides among insect populations occurs when applications of the same insecticide are used over multiple generations of the pest. Consequently, susceptible individuals are removed from the population and resistant individuals remain to reproduce generations that can no longer be controlled with that insecticide (Riley & Sparks 2006).
The development of cross-resistance (to different members of the same pesticide group) and multi-resistance (to different pesticide groups) in insect strains of many important insect species is a serious concern all over the world (Zettler & Cuperus 1990; Chaudhry 1997). For example, malathion resistance in *T. castaneum* was recorded in eastern Australia by Champ and Campbell-Brown in 1970 and in Pakistan by Saleem and Shakoori in 1989. Resistance to malathion in Brazilian and U.S. populations of *R. dominica* were reported by Guedes et al. (1996). In Brazil, cross resistance to DDT and pyrethroids (deltamethrin, cypermethrin and permethrin) was verified in *S. zeamais* (Guedes et al. 1995; Fragoso et al. 2005; Araújo et al. 2011). Phosphine resistance was found in adults of 22 Brazilian populations of *S. zeamais* (Pimentel et al. 2009). Survivalship of the life stages of the cigarette beetle, *L. serricorne*, was recorded after treatment with phosphine (Rajendran & Narasimhan 1994; Sağlam et al. 2015). Attia (1981) observed that resistance to DDT, cyclodienes, Benzene hexachloride (BHC) and organophosphorus insecticides has developed in *P. interpunctella, E. cautella* and *E. kuehniella* in Australia.

Development of resistance to an insecticide usually leads to applications of higher doses of the same insecticide, increases in the number of pesticide applications or to the use of new products. Increases in the use of pesticides not only raise the cost of pest control, but also causes more environmental pollution and greater hazards to human health. Therefore, it is

necessary to find out safer alternative control strategies, such as the use of microbial control agents against stored grain pests.

1.6.6 Biological control

Biological control is the application of living organisms to control pests. Pathogens, parasitoids (insect parasites), and predators have been investigated with regards to stored product protection. Many insect pathogens, including fungi, bacteria, protozoa, and viruses, infect stored product insects (Table 1.6). Some of these organisms are highly pathogenic and kill the insect by rapid infection. Others, like the protozoa, adversely affect the development or fertility of the insects.

Entomopathogenic fungi have been tested with success against several stored product insect species in both laboratory and field tests (Mahdneshin et al. 2011; Sabbour 2014). *Bacillus thuringiensis* is a Gram-positive spore forming bacterium that has an entomopathogenic activity (Tounsi et al. 2005). Various strains synthesizes delta-endotoxins (or Cry proteins), which are specifically toxic for different insect orders (Bravo et al. 2011).

Entomopathogenic nematode (EPN) are obligate pathogens that have free-living third stage infective juveniles (IJs) that kill insects by releasing their symbiotic mutualistic bacteria (*Xenorhabdus* in *Steinernema* sp. and *Photorabdus* in *Heterorhabditis* sp.). The IJs invade their hosts via natural body openings and then release their bacterial symbionts that cause death within a few days (Kaya & Gaugler 1993; Gaugler 2002). The families Steinernematidae and Heterorhabditidae have received attention for the control of stored-product pests (Mbata & Shapiro-Ilan 2005).

Many viruses have been reported for their potential to control stored product insect pests. Most of these viruses attack moths, and a few have been reported for beetles. Viruses are generally species-specific. Larvae of Indian meal moth are susceptible to infection by a granulovirus, *Plodia interpunctella* GV (*Pi*GV), which has been assessed as a potential control agent (McGaughey 1975; Consigli et al. 1983). Recently, a naturally occurring Nucleopolyhedrovirus from the Mediterranean flour moth, *Ephestia kuehniella,* was identified, which may be used in biological control (Yaman et al. 2015).

Many species of protozoa naturally infect stored product insects and often play a major role in regulating population growth. One important and common consequence of protozoan infection is a reduction in the number of offspring produced by infected insects. Although

protozoan pathogens play a significant role in the natural limitation of insect populations, few appear to be suited for development as insecticides.

1.6.6.1 Advantage and limitations of biological control

Biological control has many advantages as a pest control method, particularly when compared with insecticides. Although the advantages of biological control are many, there are also disadvantages (Cox & Wilkin 1996; Usta 2013) as disscused below.

Advantages

- Safety to human and other target organisms. The safety offered by microbial insecticides is their greatest strength.
- The toxic action of microbial insecticides is often specific to a single group or species of insects, and this specificity means that most microbial insecticides do not directly affect beneficial insects (including predators or parasites of pests) in treated areas.
- Some biocontrol agents may be able to seek out pests inaccessible to many others methods.
- These fit readily into an integrated approach to pest control.
- The development of resistance to non-microbial biocontrol agents is likely to be slower than to chemical agents due to multiple modes of action.
- A biocontrol agent can multiply if the target pests are in large numbers.
- In some cases, the pathogenic microorganisms can become established in a pest population or its habitat and provide control during subsequent pest generations or seasons.

Disadvantage

- Heat, desiccation (drying out), or exposure to ultraviolet radiation reduces the effectiveness of most microbial insecticides. Consequently, proper timing and application procedures are especially important for some products.
- Special formulation and storage procedures are necessary for some microbial pesticides. While these procedures may complicate the production and distribution of certain products, storage requirements do not seriously limit the handling of microbial insecticides that are usually available.

- Because several microbial insecticides are pest-specific, the potential market for these products may be limited. Their development, registration, and production costs cannot be spread over a wide range of pest control sales. Consequently, some products are not widely available or are relatively expensive (insect viruses, for example).
- They are usually not suitable for dealing with heavy established infestations.

Table 1.6 Entomopathogenic organisms with the potential to control grain storage pests

Organism Latin name	Target insects	Method of application	Comments	Authors
Isaria fumosorosea (Wise) *Nomuraea rileyi* (Farlow) *Lecanicillium (Verticillium) lecanii* (Zimmerman)	*T. castaneum* *T. confusum*	Tested fungi were used alone or in combination with diatomaceous earth (DE). DE alone applied as powder. The target insects were fed on a semi-artificial diet contaminated with the different dose of fungi.	DE was the most effective treatments against the two tested insects. *I. fumosorosea* was the most effective alone against *T. castaneum*. *T. castaneum* was susceptible to *N. rileyi.* DE combinations with tested fungi had synergistic effects.	Sabbour 2014
Beauveria bassiana (Balsamo) Vuillemin *Metarhizium anisopliae* (Metsch) Sorokin	*C. maculatus*	Immersion bioassay method was used at 27±1 °C and 60 ± 5% R.H. under laboratory conditions.	*B. bassiana* had higher virulence than *M. anisopliae* against adults of cowpea weevil.	Mahdneshin et al. 2011
Bacillus thuringiensis (kurstaki)	*E. kuehniella*	Ten third instar larvae were transferred to a sterile bottle containing 1 mg flour mixed with toxin at a desired concentration.	*B. thuringiensis* crystals containing a mixture of Cry1Aa, Cry1Ac, and Cry2Aa displayed toxicity with an LC_{50} and LC_{95} of 109.7 and 463.0 ng of toxin per mg flour, respectively, when used individually or in combination. Cry1Aa, Cry1Ac, and Cry2Aa showed significantly lower activity.	Tounsi et al. 2005

Table 1.6 Continued.

Organism Latin name	Target insects	Method of application	Comments	Authors
Heterorhabditis bacteriophora (Poinar) *Heterorhabditis megidis* (Poinar) *Steinernema carpocapsae* (Weiser) (two strains) *Steinernema feltiae* (Filipjev) (Two strain)	*L. serricorne* *T. confusum*	The bioassays were conducted in plastic Petri dishes containing 10 g of semolina and cracked wheat, respectively. The substrate was inoculated with respective doses of nematodes in 1 ml of water. Then. 10 individuals were introduced into each dish.	In the case of *L. serricorne* adults, one strain of *S. carpocapsae* caused 15.6 and 58.9% mortality after four and eight day's exposure, respectively. However, larval mortality of *L. serricorne* did not exceed 19% in all treatments tested. Similarly, larval mortality of *T. confusum* was low, reaching 15.2 and 22.4% after four and eight day's exposure, respectively, at the highest dose tested.	Rumbos & Athanassiou 2012
Fusarium avenaceum (Strain 10A)	*S. oryzae*	Spraying a conidial suspension of the fungus onto: i) adult insects of *S. oryzae* before their introduction into grains placed in plastic pots; or ii) The inner surfaces of plastic pots (treated before introduction of insects and wheat grains); or iii) grains already placed in plastic pots (treated before introduction of insects).	The highest mortality level caused by *S. oryzae* was observed after direct spraying with the conidial suspension before introduction. Whereas the lowest mortality level was observed after treatment the inner surface of plastic pots.	Batta 2012

1.7 *Beauveria bassiana* (Balsamo) Vuillemin

Beauveria bassiana (Balsamo) Vuillemin, a filamentous fungus, belongs to a class of insect pathogenic deuteromycetes. *B. bassiana* was the first reported insect pathogen, originally isolated from the silkworm, *Bombyx mori* L. (Lepidoptera Bombycidae), by Agostino Bassi in 1834 (Feng et al. 1994), and has subsequently become the most extensively studied and exploited fungal entomopathogen (Hajek & St. Leger 1994) Strains of *B. bassiana* are highly adapted to particular host insects. Many strains of *B. bassiana* spp. have been isolated from a variety of insects worldwide, which are of potential importance in agriculture and health management of pests. *B. bassiana* grows naturally in soils throughout the World. It causes white muscardine disease. *B. bassiana* has displayed potential to control storage grain pests (Meikle et al. 2001; Akbar et al. 2004).

1.7.1 Mechanism of action of *Beauveria bassiana*

Pathogenesis of insect hosts due to *B. bassiana* occurs mainly through infection via the integument, though it may also enter through the respiratory system (Feng et al. 1994). Penetration through the host cuticle is the mode of entry for most entomopathogenic fungi. During fungal infection, the first step prior to penetration is the conidial attachment to the host cuticle (Holder & Keyhani 2005; Dong et al. 2009). Upon contact, the conidia bind to the cuticle and initiate a developmental program that includes the production of specialized infection structures, such as germ tubes and penetrant hyphae (Boucias & Pendland 1991; Hajek & Eastburn 2003). Penetration of the fungus through the cuticle is influenced by different factors such as ambient moisture levels and the occurrence of inhibitory factors (fatty acids or melanin) within the cuticle (Inglis et al. 2001). The fungus utilizes a combination of hydrolytic enzymes such as proteases, chitinases, and lipases, and mechanical action to penetrate the cuticle (Leger et al. 1986; Bidochka et al. 1987; Xiao et al. 2012).

Once the insect haemocoel is colonized by the hyphal bodies of the fungus, host death results from a combination of events, including depletion of nutrients, physical obstruction, invasion of organs, and the release of toxins (Vey et al. 2001) such as beauvercin, bassianin, bassianolide, beauverolides, tenellin, oosporein (Strasser et al. 2000), bassiacridin (Quesada-Moraga & Alain 2004), and several analogues of beauvercin such as beauvercin A (Gupta et al. 1995). Soon after the host dies, and under favourable environmental conditions, fungal

hyphae emerge from the cadaver, produce conidiogenous cells, and sporulate on the host surface and new conidia are liberated (Inglis et al. 2001).

While hoping to challenge selected pests with entomopathogenic fungi, it is essential to understand the pests' biology, in order to identify which stage of the pests' life cycles are the most appropriate to target. Knowledge of feeding or management behaviours of the different developmental stages may provide information useful in the design of bioassays, where targeting the most suitable growth phase within the insect life cycle is a critical part of such evaluations *in vitro*.

1.7.2 Environmental requirement for infection of *Beauveria bassiana*

Biocontrol strategies based on entomopathogenic fungi are not only dependent upon interactions between the host and the pathogen, but also on the environment to which they are exposed. A variety of factors including temperature, relative humidity (RH), light, air, nutrient availability and host physiological status influence fungal pathogenicity (Padmini & Padmaja 2010). Of the various factors, temperature and humidity are the most important environmental factors affecting survival and efficacy of entomopathogenic fungi (Bugeme et al. 2008). Temperature significantly affects growth, germination, survival and virulence of the pathogens (Kiewnick 2006; Bugeme et al. 2008). In general, optimum temperatures for germination, growth, sporulation and virulence of entomopathogenic fungi have been reported to range between 20 and 30°C (Ekesi et al. 1999; Tefera & Pringle 2003; Dimbi et al. 2004; Kiewnick 2006). Vassilakos et al. (2006) observed that *B. bassiana* was more effective at 26°C than at 30°C in stored wheat grain against both *R. dominica* and *S. oryzae*.

Humidity affects rate of pathogenicity, being essential for fungal germination, infection and sporulation on insect cadavers (Luz & Fargues 1997; Devi et al. 2005). Cox et al. (2004) found that high levels of insect mortality could only be achieved under conditions of high humidity and when insects were treated directly with conidia. In contrast, Akbar et al. (2004) found that mortality of *B. bassiana* treated *T. castaneum* was not significantly different at 56% or 75% R.H., but the trend was for higher mortality at the lower R.H. The mortality of *R. dominica* treated with *B. bassiana* was enhanced by reduced moisture (Lord 2005). In addition, Lord (2007) demonstrated that dry stored-grain conditions are favourable for *B. bassiana* activity.

1.7.3 *Beauveria bassiana* as biocontrol agents (BCAs) against storage insects

B. bassiana has been tested with success using various formulations against stored grain pests in both laboratory and field trials (Hidalgo et al. 1998; Lord 2001; Akbar et al. 2004; Dhuyo & Ahmed 2007; Kaur et al. 2014) (Table 1.7).

Table 1.7 Efficacy of various strains of *Beauveria bassiana* against stored grain pests

Strain of *B. bassiana*	Target insect	Method of application	Main results	Reference
B. bassiana (NBAII-Bb-5a)	*C. cephalonica*	Treatment of insect larvae with different concentrations of conidial suspension.	*B. bassiana* was effective at highest conidial concentration. Fewer adults emerged due to treatment.	Kaur et al. 2014
B. bassiana No.274	*P. truncates*	Treatment of *P. truncatus* stages with different concentrations of conidial suspension.		Dhuyo & Ahmed 2007
B. bassiana (unnamed strain)	*P. interpunctella*	Six concentrations of conidial suspension applied against eggs and larvae of *P. interpunctella*.	Significant effects in the different concentrations of the fungus on eggs and larvae under fixed conditions (temperature = 25 ± 2°C and RH = 55 ± 5%).	Sedehi et al. 2014
B. bassiana (unnamed strain)	*S. zeamais*	Applying three formulations of *B. bassiana* to grain then mixing them to be in a direct contact with the adult insects. Formulations used were: dustable powder (DP); oil suspensions (OSs) containing a mixture of mineral oil; and maize oil and hydrogenated rapeseed oil pellet (HP).	The OS formulation provided the highest level of control. OS also preserved the viability of introduced conidia for longer.	Hidalgo et al. 1998

Table 1.7 Continued.

Strain of *B. bassiana*	Target insect	Method of application	Main results	Reference
B. bassiana (Beauvarin®)	*C. maculatus* and *S. granarius*	Five concentrations of conidial suspension applied against the tested insects.	*B. bassiana* was consistently more virulent against *C. maculatus* than *S. granarius*.	Shams et al. 2011
B. bassiana (unnamed strain)	*T. castaneum* adults and larvae	Combinations of DE and *B. bassiana* were mixed thoroughly with the wheat grains infested with *T. castaneum*.	Adults did not show a dose response to *B. bassiana* alone or to combination with DE. However, the combination of *B. bassiana* + DE has positive effects against *T. castaneum* larvae.	Akbar et al. 2004
B. bassiana (GHA)	*R. dominica* and *O. surinamensis*	Mixing different amounts of *B. bassiana* conidia + DE dusts with grains infested with *R. dominica* and *O. surinamensis*.	Treatment with combination mixtures caused significantly higher mortality in the adult insects than that with *B. bassiana* alone or DE alone.	Lord 2001
B. bassiana (Bb050722)	2nd instar larvae of *L. serricorne*	Treatment the larvae of *L. serricorne* with different concentrations of conidial suspension under the condition of 18℃, 21℃, 25℃ and 28℃.	100% larval mortality with lowest LD_{50} value of 3.6 days was observed under 25ºC.	Zhu et al. 2009

1.7.4 Safety of *Beauveria bassiana*

The safety of entomopathogenic fungi such as *Metarhizium anisopliae*, *B. bassiana* and *B. brongniartii*, for mammals and humans is of primary concern. Several papers have been published on their allergic, pathogenic or toxic risks for humans and mammals (Semalulu et al. 1992; Tucker et al. 2004; Westwood et al. 2005). A subsequent review by Zimmermann (2007) found that these organisms posed only a minimal risk to man, domestic animals, wildlife and non-target invertebrates. Information regarding vertebrate safety tests is included in the registration process of commercial *Beauveria* products and has been conducted with many isolates of *B. bassiana*. Both species of *B. bassiana* and *B. brongniartii* have been confirmed to be largely non-toxic and non-infectious to vertebrates. However, in a few cases with immune-compromised patients, infections by *B. bassiana* have also been observed (Zimmermann 2007).

However, despite its wide host range, evidence to date is that *B. bassiana* has little impact on non-target organisms, especially when isolate selection and spacio-temporal factors are taken into consideration (Seiedy et al. 2015; Wu et al. 2016) (Vestergaard et al. 2003). In another study, the application of strains of *B. bassiana* (Bb 5335) and *M. anisopliae* (Ma7965) had little impact on non-target insects, such as natural enemies and beneficial soil insects, making the biocontrol suitable as biological control agents of targeted insect pests (Thungrabeab & Tongma 2007).

1.7.5 Culturing of the biocontrol agents, and their mass production

The choice of an appropriate and economic medium that supports rapid growth without loss of virulence for a number of generations is one of the basic requirements for the mass production of fungi for microbial control of insect pests (Prasad & Pal 2014).

B. bassiana is easily grown on a wide range of conventional mycological media. A wide variety of agar formulations have been developed to induce sporulation and the growth of fungi. Among the culture media commonly used, Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) are the most suitable for *B. bassiana* (Kamp & Bidochka 2002).

Numerous methods exist for the production of both fungal conidia and blastospores, which are relatively resistant to various environmental factors. Infective propagules may be produced in both solid and liquid media. Blastospores are produced in liquid medium, while conidia are mainly produced in solid-state culture (aerial conidia). In addition, conidia can

also be produced under submerged culture, depending on the carbon and nitrogen source of the culture medium (Thomas et al. 1987; Holder et al. 2007), or with the use of a biphasic system, in which the fungus is first grown under submerged conditions initially and then allowed to grow under solid-state conditions (Thomas et al. 1987). The most commonly selected substrate for production of conidia by *B. bassiana* has been rice grains (Jenkins et al. 1998; Taylor et al. 2013; Xie et al. 2013).

Ultimately, the amenability of *B. bassiana* to mass production by both solid substrate and liquid medium renders it highly suitable for development as a mycoinsecticide.

1.8 Formulations

The development of an appropriate formulation is a critical component in helping a biocontrol agent to germinate and infect the target host (Brar et al. 2006; de Faria & Wraight 2007). Moreover, commercial biopesticides must be economic to produce, have high residual activity, be easy to handle, stable in storage, mix and apply, and be consistently effective in controlling the target pest (Brar et al. 2006). There are two types of EPF formulations: (i) liquid formulations that contain conidia suspended in oily liquid ingredients such as invert emulsions; and (ii) dry formulations that contain conidia mixed with other dust ingredients such as diatomaceous earth (DE) (Batta 2016b).

1.8.1 Liquid formulations

Various liquid formulations have been developed. For instance, Hidalgo et al. (1998) investigated the effect of different liquid formulations of *B. bassiana*, such as a mixture of two oils (mineral oil and corn oil) against the adults of the maize weevil, *S. zeamais*, in stored maize and reported high levels of mortality for most of the formulations tested. Similarly, invert emulsion formulations (water in oil type) have been tested as a promising approach for entomopathogens (Batta 2003, 2005, 2007; Batta et al. 2010; Batta 2016a).

Overall, the formulation of EPF using liquid formulations may be necessary to achieve a high level of efficacy against targeted insects on grain under storage conditions. However, the number of successful and effective types of liquid formulations which have been tested under storage environments is very limited, since they are mostly concentrated on oil-based liquid formulations of which an invert emulsion may be the most appropriate.

1.8.2 Dry formulations

The admixture of the dried conidia of EPF with natural dusts such as diatomaceous earth, oven ash, charcoal, chalk powder and wheat flour have been studied, either by mixing them with grains infested with the stored-grain insects, or by treating the inner surfaces of containers before introducing the grains or insects (Akbar et al. 2004; Batta 2004; Michalaki et al. 2006; Vassilakos et al. 2006; Athanassiou & Steenberg 2007; Riasat et al. 2011). In contrast, little is known about use of the other types of natural dusts, such as kaolin, corn flour, talc and tapioca flour as carriers for the conidia of EPF for the control of stored grain pests (Abdel-Raheem et al. 2015).

1.9. Commercialization

To date, no commercial products of EPF have been registered for the biocontrol of stored-grain insects (Batta 2016b). However, many commercial formulations of EPFs have been developed for crop pest management (Table 1.8). Among the 171 products of EPF developed, products based on *B. bassiana* represent 33.9% of total, *M. anisopliae* products were 33.9%, and *I. fumosorosea* and *B. brongniartii* products represented 5.8 and 4.1%, respectively (de Faria & Wraight 2007). A total of 28 products (all based on *Hirsutella thompsonii* Fisher) have been exclusively developed as acaricides and claimed to control mites and ticks. Approximately 43.0% of these products were developed by South American companies and institutions (de Faria & Wraight 2007).

In South Africa, biopesticides incorporating EPFs as their active ingredient have been registered for the control of whitefly and red spider mite on bean (*Phaseolus vulgar* Linnaeus), tomato (*Solanum lycopersicum* Linnaeus), brinjal (*Solanum melongena* Linnaeus) and cucumber (*Cucumis sativas* Linnaeus) (Eco-Bb® - PHP, South Africa), and thrips and diamondback moth (Broadband® - BASF, South Africa).

Table 1.8 Examples of commercial *Beauveria bassiana* products formulated for insects and acarine control in different countries

Brand name	Formulation	Target pests	Crop	Company	Country
Mycotrol	WP / ES / OF	whiteflies/aphids/thrips	field crops	Mycotech	USA
Naturalis	ES	sucking insects	glasshouse crops	Troy BioScience	USA
Conidia	WDG	coffee berry borer	coffee	AgrEvo	Germany
Myco-Jaal	SC	diamondback moth	cabbage	Pest Control India (Pvt) Ltd	India
Biosoft	WS	*Helicoverpa* and sucking pests	several crops	Agriland Biotech Ltd	India
BotaniGard	ES / WP	whiteflies, mealybugs, aphids, thrips and many other insects	greenhouse applications	Mycotech	USA
Eco-Bb	WP	whitefly and spider mite	bean, tomato, cucumber	Plant Health Products (Pty) Ltd	South Africa
Broadband®	EC	Mite, whitefly, thrips and diamondback moth	several crops	BASF (Pty) Ltd	South Africa

wettable powder (WP); emulsifiable oil suspension (ES); oil flowable (OF); water-dispersible granule (WDG); suspension concentration (SC); water soluble (WS); emulsifiable concentrate (EC).

References

Abdel-Raheem M, Ismail I, Rahman RA, Farag N, Abdel I. 2015. Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.), as biological control agents on some stored product insects. Journal of Entomology and Zoology Studies 3: 316-320.

Adejumo T, Hettwer U, Karlovsky P. 2007. Survey of maize from south-western Nigeria for zearalenone, α-and β-zearalenols, fumonisin B1 and enniatins produced by *Fusarium* species. Food Additives and Contaminants 24: 993-1000.

Ahmed K, Khalique F, Afzal M, Tahir M, Malik B. 1989. Variability in chickpea (*Cicer arietinum* L.) genotypes for resistance to *Callosobruchus maculatus* F. (Bruchidae). Journal of Stored Products Research 25: 97-99.

Akbar W, Lord JC, Nechols JR, Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Amusa OD, Ogunkanmi AL, Bolarinwa K, Ojobo O. 2013. Evaluation of four cowpea lines for bruchid (*Callosobruchus maculatus*) tolerance. Journal of Natural Sciences Research 3: 46-52.

Araújo RA, Williamson MS, Bass C, Field LM, Duce IR. 2011. Pyrethroid resistance in *Sitophilus zeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel. Insect Molecular Biology 20: 437-445.

Arnason J, Gale J, De Beyssac BC, Sen A, Miller S, Philogene B, Lambert J, Fulcher R, Serratos A, Mihm J. 1992. Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). Journal of Stored Products Research 28: 119-126.

Arthur FH. 1996. Grain protectants: current status and prospects for the future. Journal of Stored Products Research 32: 293-302.

Arthur FH, Throne JE, Maier DE, Montross MD. 1998. Feasibility of aeration for management of maize weevil populations in corn stored in the southern United States: model simulations based on recorded weather data. American Entomologist 44: 118-123.

Arthur FH, Throne JE, Maier DE, Montross MD. 2001. Impact of aeration on maize weevil (Coleoptera: Curculionidae) populations in corn stored in the northern United States: simulation studies. American Entomologist 47: 104-111.

Ashworth JR. 1993. The biology of *Lasioderma serricorne*. Journal of Stored Products Research 29: 291-303.

Athanassiou C, Steenberg T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40: 411-416.

Athié I, Gomes RA, Bolonhezi S, Valentini SR, De Castro MFPM. 1998. Effects of carbon dioxide and phosphine mixtures on resistant populations of stored-grain insects. Journal of Stored Products Research 34: 27-32.

Attia F. 1981. Insecticide resistance in pyralid moths of grain and stored products. *General and Applied Entomology:* The Journal of the Entomological Society of New South Wales 13: 3-8.

Awan DA, Saleem MA, Nadeem MS, Shakoori AR. 2012. Toxicological and biochemical studies on spinosad and synergism with piperonyl butoxide in susceptible and resistant strains of *Tribolium castaneum*. Pakistani Journal of Zoology 44: 649-662.

Ayvaz A, Sagdic O, Karaborklu S, Ozturk I. 2010. Insecticidal activity of the essential oils from different plants against three stored-product insects. Journal of Insect Science 10: 1-13.

Bacci L. Lupi D, Savoldelli S, Rossaro B. 2016. A review of Spinosyns, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. Journal of Entomological and Acarological Research 48: 40-52.

Bachrouch O, Jemâa JMB, Wissem AW, Talou T, Marzouk B, Abderraba M. 2010. Composition and insecticidal activity of essential oil from *Pistacia lentiscus* L. against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Journal of Stored Products Research 46: 242-247.

Badii K, Asante S, Adarkwa C. 2013. Varietal differences in the susceptibility of new rice for Africa (NERICA) to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). African Journal of Agricultural Research 8: 1375-1380.

Bankole S, Mabekoje O, Enikuomehin O. 2003. *Fusarium* spp. and fumonisin B1 in stored maize from Ogun State, Nigeria. Tropical Science 43: 76-79.

Banks J, Fields P. 1995. Physical methods for insect control in stored-grain ecosystems, *In* Jayas DS, White NDG, Muir WE [eds.], Stored-Grain Ecosystems. Marcel Dekker, New York, USA.

Batta Y. 2003. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes). Crop Protection 22: 415-422.

Batta Y. 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. Crop Protection 23: 103-108.

Batta Y. 2005. Control of the lesser grain borer (*Rhyzopertha dominica* (F.) Coleoptera: Bostrichidae) by treatments with residual formulations of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes). Journal of Stored Products Research 41: 221-229.

Batta Y. 2007. Biocontrol of almond bark beetle (*Scolytus amygdali* Geurin-Meneville, Coleoptera: Scolytidae) using *Beauveria bassiana* (Bals.) Vuill. (Deuteromycotina: Hyphomycetes). Journal of Applied Microbiology 103: 1406-1414.

Batta Y, Murdoch G, Mansfield S. 2010. Investigations into the formulation and efficacy of entomopathogenic fungi against larvae of yellow mealworm (*Tenebrio molitor* L Coleoptera: Tenebrionidae). General and Applied Entomology 39: 5-8.

Batta YA. 2012. The first report on entomopathogenic effect of *Fusarium avenaceum* (Fries) Saccardo (Hypocreales, Ascomycota) against the rice weevil (*Sitophilus oryzae* L.: Curculionidae, Coleoptera). Journal of Entomological and Acarological Research 44: 51-55.

Batta YA. 2016a. Invert emulsion: Method of preparation and application as proper formulation of entomopathogenic fungi. MethodsX 3: 119-127.

Batta YA. 2016b. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology 26: 1171-1183.

Bekele A, Obeng-Ofori D, Hassanali A. 1997. Evaluation of *Ocimum kenyense* (Ayobangira) as source of repellents, toxicants and protectants in storage against three major stored product insect pests. Journal of Applied Entomology 121: 169-173.

Bell C, Wilson S. 1995. Phosphine tolerance and resistance in *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Journal of Stored Products Research 31: 199-205.

Ben-Amor R, Dhouibi MH, Aguayo E. 2016. Hot water treatments combined with cold storage as a tool for *Ectomyelois ceratoniae* mortality and maintenance of Deglet Noor palm date quality. Postharvest Biology and Technology 112: 247-255.

Benhalima H, Chaudhry M, Mills K, Price N. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research 40: 241-249.

Bhanderi G, Radadiya G, Patel D. 2015. Screening of sorghum genotypes against rice weevil (*Sitophilus oryzae* L.). International Journal of Agricultural Sciences 11: 93-98.

Bidochka MJ, Pfeifer TA, Khachatourians GG. 1987. Development of the entomopathogenic fungus *Beauveria bassiana* in liquid cultures. Mycopathologia 99: 77-83.

Boatman ND, Brickle NW, Hart JD, Milsom TP, Morris AJ, Murray AW, Murray KA, Robertson PA. 2004. Evidence for the indirect effects of pesticides on farmland birds. Ibis 146: 131-143.

Boucias DG, Pendland JC. 1991. Attachment of mycopathogens to cuticle, *In* Carry T, Harvey CH [eds.], The Fungal Spore and Disease Initiation in Plants and Animals. Springer, New York, USA.

Boyer S, Zhang H, Lempérière G. 2012. A review of control methods and resistance mechanisms in stored-product insects. Bulletin of Entomological Research 102: 213-229.

Brar SK, Verma M, Tyagi R, Valéro J. 2006. Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process Biochemistry* 41: 323-342.

Bravo A, Likitvivatanavong S, Gill SS, Soberón M. 2011. *Bacillus thuringiensis*: a story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology 41: 423-431.

Bugeme DM, Maniania NK, Knapp M, Boga HI. 2008. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. Experimental and Applied Acarology 46: 275-285.

Burges H, and Haskins K. 1965. Life-cycle of the tropical warehouse moth, *Cadra cautella* (Wlk.), at controlled temperatures and humidities. *Bulletin of Entomological Research* 55: 775-789.

Champ B, Campbell-Brown M. 1970. Insecticide resistance in Australian *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) II: Malathion resistance in eastern Australia. Journal of Stored Products Research 6: 111-131.

Chaudhry M. 1997. Review a review of the mechanisms involved in the action of phosphine as an insecticide and phosphine resistance in stored-product insects. Pesticide Science 49: 213-228.

Choudhary MD, Bharpoda T, Deb S, Choudhary S. 2015. Screening of cowpea (*Vigna unguiculata*) varieties against *Callosobruchus chinensis* under storage condition. Journal of Food Legumes 28: 55-58.

Codru N, Schymura MJ, Negoita S, Environment ATF, Rej R, Carpenter DO. 2007. Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environmental Health Perspectives 115: 1442-1447.

Coffelt JA, Burkholder W. 1972. Reproductive biology of the cigarette beetle, *Lasioderma serricorne*. 1. Quantitative laboratory bioassay of the female sex pheromone from females of different ages. Annals of the Entomological Society of America 65: 447-450.

Collins PJ. 1990. A new resistance to pyrethroids in *Tribolium castaneum* (Herbst). Pesticide Science 28: 101-115.

Consigli R, Tweeten K, Anderson D, Bulla L. 1983. Granulosis viruses, with emphasis on the GV of the Indian meal moth, *Plodia interpunctella*. Advances in Virus Research 28: 141-173.

Cox P, Wilkin D. 1996. The Potential use of Biological Control of Pests in Stored Grain.

Cox P, Wakefield M, Price N, Wildey K, Chambers J, Moore D, De Muro MA, Bell B. 2004. The potential use of insect-specific fungi to control grain storage pests in empty grain stores. Home-Grown Cereals Authority HGCA, London, UK.

Home-Grown Cereals Authority HGCA, London, UK.

de Faria MR, Wraight S P. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43: 237-256.

Dean GA. 1911. Heat as a means of controlling mill insects. Journal of Economic Entomology 4: 142-161.

Derera J, Pixley KV, Giga DP, Makanda I. 2014. Resistance of maize to the maize weevil. III. Grain weight loss assessment and implications for breeding. Journal of Stored Products Research 59: 24-35.

Desroches P, El Shazly E, Mandon N, Duc G, Huignard J. 1995. Development of *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) (Coleoptera: Bruchidae) in seeds of *Vicia faba* L. differing in their tannin, vicine and convicine contents. Journal of Stored Products Research 31: 83-89.

Devi KU, Sridevi V, Mohan CM, Padmavathi J. 2005. Effect of high temperature and water stress on in vitro germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. Journal of Invertebrate Pathology 88: 181-189.

Dhuyo AR, Ahmed S. 2007. Evaluation of fungus *Beauveria bassiana* (Bals.) infectivity to the larger grain borer *Prostephanus truncatus* (Horn.). Pakistan Entomologist 29: 77-83.

Dimbi S, Maniania NK, Lux SA, Mueke JM. 2004. Effect of constant temperatures on germination, radial growth and virulence of *Metarhizium anisopliae* to three species of African tephritid fruit flies. BioControl 49: 83-94.

Divakara ST, Aiyaz M, Hariprasad P, Nayaka SC, Niranjana SR. 2014. *Aspergillus flavus* infection and aflatoxin contamination in sorghum seeds and their biological management. Archives of Phytopathology and Plant Protection 47: 2141-2156.

Dong C, Zhang J, Huang H, Chen W, Hu Y. 2009. Pathogenicity of a new China variety of *Metarhizium anisopliae* (*M. anisopliae* var. *dcjhyium*) to subterranean termite *Odontotermes formosanus.* Microbiological Research 164: 27-35.

Edde P, Amatobi C. 2003. Seed coat has no value in protecting cowpea seed against attack by *Callosobruchus maculatus* (F.). Journal of Stored Products Research 39: 1-10.

Ekesi S, Maniania N, Ampong-Nyarko K. 1999. Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti*. Biocontrol Science and Technology 9: 177-185.

El-Aziz SEA. 2011. Control strategies of stored product pests. Journal of Entomology 8: 101-122.

Ernst P. 2002. Pesticide exposure and asthma. American Journal of Respiratory and Critical Care Medicine 165: 563-564.

Everett CJ, Frithsen IL, Diaz VA, Koopman RJ, Simpson WM, Mainous AG. 2007. Association of a polychlorinated dibenzo-p-dioxin, a polychlorinated biphenyl, and DDT with diabetes in the 1999–2002 National Health and Nutrition Examination Survey. Environmental Research 103: 413-418.

Feng M, Poprawski T, Khachatourians GG. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology 4: 3-34.

Fields PG, Muir WE. 1995. Physical control, *In* Subramanyam B, Hagstrum DW [eds.], Integrated Management of Insects in Stored Products. Marcel Dekker, New York, USA.

Fields PG, White ND. 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects 1. Annual Review of Entomology 47: 331-359.

Fieten KB, Kromhout H, Heederik D, de Joode BVW. 2009. Pesticide exposure and respiratory health of indigenous women in Costa Rica. American Journal of Epidemiology 169: 1500-1506.

Finkelman S, Navarro S, Rindner M, Dias R. 2006. Use of heat for disinfestation and control of insects in dates: laboratory and field trials. Phytoparasitica 34: 37-48.

Fragoso DB, Guedes RNC, Rezende ST. 2003. Glutathione S-transferase detoxification as a potential pyrethroid resistance mechanism in the maize weevil, *Sitophilus zeamais*. Entomologia Experimentalis et Applicata 109: 21-29.

Fragoso DB, Guedes RNC, Peternelli LA. 2005. Developmental rates and population growth of insecticide-resistant and susceptible populations of *Sitophilus zeamais*. Journal of Stored Products Research 41: 271-281.

Gaugler, R. (2002). Entomopathogenic Nematology. CABI Publishing, Wallingford, UK.

Geranmayeh J, Hashemi SM. 2014. Contact toxicity of the essential oils from *Salvia leriifolia* Beth (Lamiaceae) against *Lasioderma serricorne* (F.). Biharean Biologist 8: 106-108.

Glynn AW, Granath F, Aune M, Atuma S, Darnerud PO, Bjerselius R, Vainio H, Weiderpass E. 2003. Organochlorines in Swedish women: determinants of serum concentrations. Environmental Health Perspectives 111: 349-355.

Goftishu M, Belete K. 2014. Susceptibility of sorghum varieties to the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). African Journal of Agricultural Research 9: 2419-2426.

Golob P. 1997. Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research 33: 69-79.

Gomez L, Rodriguez J, Poneleit C, Blake D. 1982. Preference and utilization of maize endosperm variants by the rice weevil. Journal of Economic Entomology 75: 363-367.

Guedes RNC, Lima JG, Santos JP, Cruz CD. 1995. Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Journal of Stored Products Research 31: 145-150.

Guedes RNC, Dover BA, Kambhampati S. 1996. Resistance to chlorpyrifos-methyl, pirimiphos-methyl, and malathion in Brazilian and US populations of *Rhyzopertha dominica* (Coleopera: Bostrichidae). Journal of Economic Entomology 89: 27-32

Guedes NMP, Guedes RNC, Campbell JF, Throne JE. 2010. Contest behaviour of maize weevil larvae when competing within seeds. Animal Behaviour 79: 281-289.

Gupta S, Montllor ., Hwang YS. 1995. Isolation of novel beauvericin analogues from the fungus *Beauveria bassiana*. Journal of Natural Products 58: 733-738.

Hagstrum DW, Reed C, Kenkel P. 1999. Management of stored wheat insect pests in the USA. Integrated Pest Management Reviews 4: 127-143.

Hajek A, St. Leger RJ. 1994. Interactions between fungal pathogens and insect hosts. Annual Review of Entomology 39: 293-322.

Hajek AE, Eastburn CC. 2003. Attachment and germination of *Entomophaga maimaiga* conidia on host and non-host larval cuticle. Journal of Invertebrate Pathology 82: 12-22.

Hennigen MR, Dick T. 1995. Incidence and abundance of mycotoxins in maize in Rio Grande do Sul, Brazil. Food Additives and Contaminants 12: 677-681.

Hidalgo E, Moore D, Le Patourel G. 1998. The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize. Journal of Stored Products Research 34: 171-179.

Hodges RJ, Buzby JC, Bennett B. 2011. Postharvest losses and waste in developed and less developed countries: opportunities to improve resource use. The Journal of Agricultural Science 149: 37-45.

Holder DJ, Keyhani NO. 2005. Adhesion of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana* to substrata. Applied and Environmental Microbiology 71: 5260-5266.

Holder DJ, Kirkland BH, Lewis MW, Keyhani NO. 2007. Surface characteristics of the entomopathogenic fungus *Beauveria* (*Cordyceps*) *bassiana.* Microbiology 153: 3448-3457.

Howe R. 1957. A laboratory study of the cigarette beetle, *Lasioderma serricorne* (F.) (Col., Anobiidae) with a critical review of the literature on its biology. Bulletin of Entomological Research 48: 9-56.

Howe R. 1965. A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. Journal of Stored Products Research 1: 177-184.

Huang Y, Lam S, Ho S. 2000. Bioactivities of essential oil from *Elletaria cardamomum* (L.) Maton. to *Sitophilus zeamais* Motschulsky and: *Tribolium castaneum* (Herbst). Journal of Stored Products Research 36: 107-117.

Ileleji KE, Maier DE, Woloshuk CP. 2007. Evaluation of different temperature management strategies for suppression of *Sitophilus zeamais* (Motschulsky) in stored maize. Journal of Stored Products Research 43: 480-488.

Imai T, Harada H. 2006. Low-temperature as an alternative to fumigation to disinfest stored tobacco of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). Applied Entomology and Zoology 41: 87-91.

Inglis GD, Goettel MS, Butt TM, Strasser H. 2001. Use of hyphomycetous fungi for managing insect pests, *In* Butt TM, Jackson C, Magan N (eds.), Fungi as Biocontrol Agents: Progress, Problems and Potential. CABI Publishing, Wallingford, UK.

Isman M B. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annal. Reviewof Entomolology. **51**, 45-66.

Jenkins NE, Heviefo G, Langewald J, Cherry AJ, Lomer CJ. 1998. Development of mass production technology for aerial conidia for use as mycopesticides. Biocontrol News and Information 19: 21N-32N.

Jittanun C, Chongrattanameteekul W. 2014. Phosphine resistance in Thai local strains of *Tribolium castaneum* (Herbst) and their response to synthetic pheromone. Kasetsart Journal (Natural Science) 48: 9-16.

Johnsen K, Jacobsen CS, Torsvik V, Sørensen J. 2001. Pesticide effects on bacterial diversity in agricultural soils a review. Biology and Fertility of Soils 33: 443-453.

Johnson J, Valero K, Hannel M. 1997. Effect of low temperature storage on survival and reproduction of Indian meal moth (Lepidoptera: Pyralidae). Crop Protection 16: 519-523.

Kamel F, Rowland AS, Park LP, Anger WK, Baird DD, Gladen BC, Moreno T, Stallone L, Sandler DP. 2003. Neurobehavioral performance and work experience in Florida farmworkers. Environmental Health Perspectives 111: 1765.

Kamp A, Bidochka M. 2002. Conidium production by insect pathogenic fungi on commercially available agars. Letters in Applied Microbiology 35: 74-77.

Kaur S, Thakur A, Rajput M. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Journal of Stored Products Research 59: 185-189.

Kaya, HK, Gaugler R. 1993. Entomopathogenic Nematodes. Annual Review of Entomology 38: 181-206.

Khalaf-Allah S. 1999. Effect of pesticide water pollution on some haematological, biochemical and immunological parameters in *Tilapia nilotica* fish. *DTW.* Deutsche Tierärztliche Wochenschrift 106: 67-71.

Kiewnick S. 2006. Effect of temperature on growth, germination, germ-tube extension and survival of *Paecilomyces lilacinus* Strain 251. Biocontrol Science and Technology 16: 535-546.

Kim SI, Park C, Ohh MH, Cho HC, Ahn YJ. 2003. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Stored Products Research 39: 11-19.

Kordali S, Aslan I, Çalmaşur O, Cakir A. 2006. Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus*

granarius (L.) (Coleoptera: Curculionidae). Industrial Crops and Products 23: 162-170.

Lale N, Kolo A. 1998. Susceptibility of eight genetically improved local cultivars of cowpea to *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in Nigeria. International Journal of Pest Management 44: 25-27.

Lathrop FH. 1914. Egg-laying of the rice weevil, *Calandra oryzae* Linn. Ohio Journal of Science 14: 321-328.

Lee BH, Choi WS, Lee SE, Park BS. 2001. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Protection 20: 317-320.

Lee DH, Lee IK, Song K, Steffes M, Toscano W, Baker BA, Jacobs DR. 2006. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes results from the National Health and Examination Survey 1999–2002. Diabetes Care 29: 1638-1644.

Lee DH, Lee IK, Steffes M, Jacobs DR. 2007. Extended analyses of the association between serum concentrations of persistent organic pollutants and diabetes. Diabetes Care 30: 1596-1598.

Lefkovitch L, Currie JE. 1967. Factors affecting adult survival and fecundity in *Lasioderma serricorne* (F.) (Coleoptera, Anobiidae). Journal of Stored Products Research 3: 199-212.

Leger RS, Charnley A, Cooper R. 1986. Cuticle-degrading enzymes of entomopathogenic fungi: mechanisms of interaction between pathogen enzymes and insect cuticle. Journal of Invertebrate Pathology 47: 295-302.

Levinson A, Levinson H. 1986. Restrained pheromone responses of male tobacco beetles (*Lasioderma serricorne* F.) to 2S, 3S-anhydroserricornin in presence of 4S, 6S, 7R-serricornin. Journal of Applied Entomology 101: 282-287.

Li Z, Liu S, Liu Y, Ye G. 2007. Temperature-related fitness costs of resistance to spinosad in the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutelidae). Bulletin of Entomological Research 97: 627-635.

Loganathan M, Jayas D, Fields P, White N. 2011. Low and high temperatures for the control of cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in chickpeas. Journal of Stored Products Research 47: 244-248.

Longstaff B, Evans D. 1983. The demography of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), submodels of age-specific survivorship and fecundity. Bulletin of Entomological Research 73: 333-334.

Longstaff BC. 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A Critical Review. Protection Ecology 2: 83-130.

Lord JC. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94: 367-372.

Lord JC. 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biological Control 34: 180-186.

Lord JC. 2007. Desiccation increases the efficacy of *Beauveria bassiana* for stored-grain pest insect control. Journal of Stored Products Research 43: 535-539.

Lund F, Frisvad JC. 2003. *Penicillium verrucosum* in wheat and barley indicates presence of ochratoxin A. Journal of Applied Microbiology 95: 1117-1123.

Luz C, Fargues J. 1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*, pathogenic to *Rhodnius prolixus*. Mycopathologia 138: 117-125.

Mahdneshin Z, Vojoudi S, Ghosta Y, Safaralizadae MH, Saber M. 2011. Laboratory evaluation of the entomopathogenic fungi, Iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin against the control of the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). African Journal of Microbiology Research 5: 5215-5220.

Mahroof R, Subramanyam B, Eustace D. 2003. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. Journal of Stored Products Research 39: 555-569.

Mateo J, Mateo R, Jimenez M. 2002. Accumulation of type A trichothecenes in maize, wheat and rice by *Fusarium sporotrichioides* isolates under diverse culture conditions. International Journal of Food Microbiology 72: 115-123.

Mbata GN, Shapiro-Ilan DI. 2005. Laboratory evaluation of virulence of heterorhabditid nematodes to *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). Environmental Entomology 34: 676-682.

McGaughey WH. 1975. A granulosis virus for Indian meal moth control in stored wheat and corn. Journal of Economic Entomology 68: 346-348.

Meikle WG, Cherry AJ, Holst N, Hounna B, Markham RH. 2001. The effects of an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), *Sitophilus zeamais* Motschulsky (Col.: Curculionidae), and grain losses in stored maize in the Benin Republic. Journal of Invertebrate Pathology 77: 198-205.

Messina FJ. 1984. Influence of cowpea pod maturity on the oviposition choices and larval survival of a bruchid beetle *Callosobruchus maculatus*. Entomologia Experimentalis-et Applicata 35: 241-248.

Michalaki MP, Athanassiou CG, Kavallieratos NG, Batta YA, Balotis GN. 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* Duval larvae: Influence of temperature, relative humidity and type of commodity. Crop Protection 25: 418-425.

Minor M. 1979. Do adult cigarette beetles feed? International Tobacco 23: 61-64.

Muthukumar M, Ragumoorthi K, Balasubramani V, Vijayakumar A. 2015. Screening of sixteen maize hybrids for resistance to *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). Journal of Entomological Research 39: 303-310.

Muzemu S, Chitamba J, Goto S. 2013. Screening of stored maize (*Zea mays* L.) varieties grain for tolerance against maize weevil, *Sitophilus zeamais* (Motsch.). International Journal of Plant Research 3: 17-22.

Navarro S, Carmi Y, Kashanchi Y, Shaaya E. 1986. Malathion resistance of stored-product insects in Israel. Phytoparasitica 14: 273-280.

Negahban M, Moharramipour S. 2007. Fumigant toxicity of *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* against stored-product beetles. Journal of Applied Entomology 131: 256-261.

Ngamo T, Ngatanko I, Ngassoum M, Mapongmestsem P, Hance T. 2007. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. African Journal of Agricultural Research 2: 173-177.

Nhamucho E, Mugo S, Kinyua M, Gohole L, Mulima E, Tefera T. 2014. Antibiosis mechanism of resistance to larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in maize. Journal of Entomology 11: 248-260.

Nouri-Ganbalani G, Ebadollahi A, Nouri A. 2016. Chemical composition of the essential oil of *Eucalyptus procera* Dehnh. and its insecticidal effects against two stored product insects. Journal of Essential Oil Bearing Plants 19: 1234-1242.

Nwankwo E, Egwuatu R, Okonkwo N, Boateng B. 2014. Screening of ten maize varieties, *Zea mays* (L.) for resistance against *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) from different zones of Nigeria and Ghana. Academic Journal of Entomology 7: 17-26.

Ogendo J, Kostyukovsky M, Ravid U, Matasyoh J, Deng A, Omolo E, Kariuki S, Shaaya E. 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. Journal of Stored Products Research 44: 328-334.

Padmini PCP, Padmaja V. 2010. Impact of different relative humidities on *in vitro* growth and sporulation of entomopathogenic fungal isolates of *Beauveria* species. International Journal of Pharmaceutical and Biological Archives 1: 355-359.

Parfitt J, Barthel M, Macnaughton S. 2010. Food waste within food supply chains: quantification and potential for change to 2050. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365: 3065-3081.

Parkin E. 1955. Progress in the control of insects infesting stored foodstuffs. Annals of Applied Biology 42: 104-111.

Pereira C, Pereira E, Cordeiro E, Della Lucia T, Tótola M, Guedes R. 2009. Organophosphate resistance in the maize weevil *Sitophilus zeamais*: magnitude and behavior. Crop Protection 28: 168-173.

Perez-Mendoza J. 1999. Survey of insecticide resistance in Mexican populations of maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 35: 107-115.

Pimentel M, Faroni LDA, Guedes R, Sousa A, Tótola M. 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 45: 71-74.

Powell TE. 1931. An ecological study of the tobacco beetle, *Lasioderma serricorne* Fabr., with special reference to its life history and control. Ecological Monographs 1: 333-393.

Prasad C, Pal R. 2014. Mass production and economics of entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* on agricultural and industrial waste. Scholars Journal of Agricultural Veternary Science 1: 28-32.

Pražić-Golić M, Andrić G, Kljajić P. 2011. Effects of 50^{o}C temperature on *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) and *Sitophilus zeamais* (Motsch.). Pesticidi i fitomedicina 26: 221-227.

Quesada-Moraga E, Alain V. 2004. Bassiacridin, a protein toxic for locusts secreted by the entomopathogenic fungus *Beauveria bassiana*. Mycological Research 108: 441-452.

Rajendran S, Narasimhan K. 1994. Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. International Journal of Pest Management 40: 207-210.

Rajendran S, Sriranjini V. 2008. Plant products as fumigants for stored-product insect control. Journal of Stored Products Research 44: 126-135.

Rana, P. 1977. Report on the studies of different types of storage structures in Nepal. Nepalese Journal of Agriculture 12: 203-215.

Retief E. 1988. The cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae): a serious herbarium pest. Bothalia 18: 97-99.

Riasat T, Wakil W, Ashfaq M, Sahi ST. 2011. Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhyzopertha dominica* on stored wheat. Phytoparasitica 39: 325-331.

Ribeiro B, Guedes R, Oliveira E, Santos J. 2003. Insecticide resistance and synergism in Brazilian populations of *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Stored Products Research 39: 21-31.

Richter E. 2002. Acute human pesticide poisonings, *In* David Pimentel [ed.], Encyclopedia of Pest Management. Marcel Dekker, Basel, Switzerland.

Rignell-Hydbom A, Rylander L, Hagmar L. 2007. Exposure to persistent organochlorine pollutants and type 2 diabetes mellitus. Human and Experimental Toxicology 26: 447-452.

Riley DG, Sparks AN. 2006. Insecticide resistance management for diamondback moth in Georgia. the University of Georgia College of Agricultural and Environmental Sciences. http://athenaeum.libs.uga.edu/bitstream/handle Accessed 10/10/2016.

Rumbos CI, and Athanassiou CG. 2012. Insecticidal effect of six entomopathogenic nematode strains against *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) and *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae). Journal of Stored Products Research 50: 21-26.

Sabbour M. 2014. Efficacy of some microbial control agents and inorganic insecticides against red flour beetle *Tribolium castaneum* and confused flour beetle, *Tribolium*

confusum (Coleoptera: Tenebrionidae) integrated protection of stored products. IOBC-WPRS Bulletin 98: 193-201.

Sağlam Ö, Edde PA, Phillips TW. 2015. Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with phosphine. Journal of Economic Entomology 108: 2489-2495.

Saleem M, Shakoori A. 1989. Toxicity of malathion, permethrin and cypermethrin against resistant and susceptible strains of *Tribolium castaneum* (Herbst.). Pakistan Journal of Zoology (Pakistan) 21: 347-360.

Sedehi A, Sedaghatfar E, Modarres Najafabadi S. 2014. Studies on effect of the *Beauveria bassiana* on eggs and larvae of *Plodia interpunctella*. Canadian Journal of Basic and Applied Sciences 2: 40-45.

Seiedy M, Tork M, Deyhim F. 2015. Effect of the entomopathogenic fungus Beauveria bassiana on the predatory mite Amblyseius swirskii (Acari: Phytoseiidae) as a non-target organism. Systematic and Applied Acarology 20: 241-250.

Semalulu S, MacPherson J, Schiefer H, Khachatourians G. 1992. Pathogenicity of *Beauveria bassiana* in mice. Journal of Veterinary Medicine, Series B 39: 81-90.

Shafique M, Ahmad M, Chaudry MA. 2006. Evaluation of wheat varieties for resistance to angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae). Pakistan Journal of Zoology 38: 7-10.

Shaheen F, Khaliq A, Aslam M. 2006. Resistance of chickpea (*Cicer arietinum* L.) cultivars against pulse beetle. Pakistan Journal of Botany 38: 1237-1244.

Shams G, Safaralizadeh MH, Imani S, Shojai M, Aramideh S. 2011. A laboratory assessment of the potential of the entomopathogenic fungi *Beauveria bassiana* (Beauvarin®) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). African Journal of Microbiology Research 5: 1192-1196.

Sharifi S, Mills RB. 1971. Radiographic studies of *Sitophilus zeamais* Mots. in wheat kernels. Journal of Stored Products Research 7: 195-206.

Sharma H, Sharma K, Seetharama N, Ortiz, R. 2001. Genetic transformation of crop plants: Risks and opportunities for the rural poor. Current Science 80: 1495-1508.

Shehu A, Obeng-Ofori D, Eziah VY. 2010. Biological efficacy of Calneem TM oil against the tropical warehouse moth *Ephestia cautella* (Lepidoptera: Pyralidae) in stored maize. International Journal of Tropical Insect Science 30: 207-213.

Siegel R, Naishadham D, Jemal A. 2012. Cancer statistics, 2012. CA: A Cancer Journal for Clinicians 62: 10-29.

Singh BD. 2002. Plant Breeding: Principles and Methods. Kalyani Publishers, New Delhi, India.

Snedeker SM. 2001. Pesticides and breast cancer risk: a review of DDT, DDE, and dieldrin. Environmental Health Perspectives 109: 35.47.

Solis M. 2006. Key to selected Pyraloidea (Lepidoptera) larvae intercepted at US ports of entry: revision of Pyraloidea in keys to some frequently intercepted Lepidopterous larvae by Weisman 1986. http://digitalcommons.unl.edu/systentomologyusda/. Accessed 25/10/2016.

Somta P, Ammaranan C, Ooi PAC, Srinives P. 2007. Inheritance of seed resistance to bruchids in cultivated mungbean (*Vigna radiata*, L. Wilczek). Euphytica 155: 47-55.

Steele R. 1970. Copulation and oviposition behaviour of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae). Journal of Stored Products Research 6: 229-245.

Strasser H, Abendstein D, Stuppner H. 2000. Monitoring the distribution of secondary metabolites produced by the entomogenous fungus *Beauveria brongniartii* with particular reference to oosporein. Mycological Research 104: 1227-1233.

Subramanyam B, Hagstrum D. 1993. Predicting development times of six stored-product moth species (Lepidoptera: Pyralidae) in relation to temperature, relative humidity, and diet. European Journal of Entomology 90: 51-64.

Taylor B, Edgington S, Luke B, Moore D. 2013. Yield and germination of the entomopathogenic fungus *Beauveria bassiana* when grown on different rice preparations. Journal of Stored Products Research 53: 23-26.

Tefera T, Pringle K. 2003. Germination, radial growth, and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae* isolates and their virulence to *Chilo partellus* (Lepidoptera: Pyralidae) at different temperatures. Biocontrol Science and Technology 13: 699–704.

Tefera T. 2012. Post-harvest losses in African maize in the face of increasing food shortage. Food Security 4: 267-277.

Tefera T, Demissie G, Mugo S, Beyene Y. 2013. Yield and agronomic performance of maize hybrids resistant to the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Crop Protection 46: 94-99.

Teitelbaum SL, Gammon MD, Britton JA, Neugut AI, Levin B, Stellman SD. 2007. Reported residential pesticide use and breast cancer risk on Long Island, New York. American Journal of Epidemiology 165: 643-651.

Thomas K, Khachatourians G, Ingledew W. 1987. Production and properties of *Beauveria bassiana* conidia cultivated in submerged culture. Canadian Journal of Microbiology 33: 12-20.

Throne JE. 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. Environmental Entomology 23: 1459-1471.

Thungrabeab M, Tongma S. 2007. Effect of entomopathogenic fungi, *Beauveria bassiana* (Balsam.) and *Metarhizium anisopliae* (Metsch.) on non target insects. KMITL Science and Technology Journal 7: 8-12.

Tounsi S, Dammak M, Rebaî A, Jaoua S. 2005. Response of larval *Ephestia kuehniella* (Lepidoptera: Pyralidae) to individual *Bacillus thuringiensis* kurstaki toxins and toxin mixtures. Biological Control 35: 27-31.

Tucker D, Beresford C, Sigler L, Rogers K. 2004. Disseminated *Beauveria bassiana* infection in a patient with acute lymphoblastic leukemia. Journal of Clinical Microbiology 42: 5412-5414.

Turgut C. 2003. The contamination with organochlorine pesticides and heavy metals in surface water in Küçük Menderes River in Turkey, 2000–2002. Environment International 29: 29-32.

Turner MC, Wigle DT, Krewski D. 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Ciencia and Saude Coletiva 16: 1915-1931.

Upadhyay RK, Jaiswal G. 2007. Evaluation of biological activities of *Piper nigrum* oil against *Tribolium castaneum*. Bulletin of Insectology 60: 57-61.

Upadhyay R, Ahmad S. 2011. Management strategies for control of stored grain insect pests in farmer stores and public ware houses. World Journal of Agricultural Sciences 7: 527-549.

Usta C. 2013. Microorganisms in Biological Pest Control-A Review (Bacterial Toxin Application and Effect of Environmental Factors). IN TECH Open Access Publisher, Rijeka, Croatia.

Vassilakos T, Athanassiou C, Kavallieratos N, Vayias B. 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth

against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. Biological Control 38: 270-281.

Vey A, Hoagland RE, Butt TM. 2001. Fungi as biocontrol agents: progress, problems, and potential, *In* Butt TM, Jackson C, Magan N [eds.], Biocontrol Agents. CABI Publishing, Wallingford, UK.

Waite D, Cessna A, Grover R, Kerr L, Snihura A. 2002. Environmental concentrations of agricultural herbicides. Journal of Environmental Quality 31: 129-144.

Ward MH, Colt JS, Metayer C, Gunier RB, Lubin J, Crouse V, Nishioka MG, Reynolds P, Buffler PA. 2009. Residential exposure to polychlorinated biphenyls and organochlorine pesticides and risk of childhood leukemia. Environmental Health Perspectives 117: 1007-1013.

Weichenthal S, Moase C, Chan P. 2010. A review of pesticide exposure and cancer incidence in the Agricultural Health Study cohort. Environmental Health Perspectives **118**, 1117-1125.

Westwood GS, Huang SW, Keyhani NO. 2005. Allergens of the entomopathogenic fungus *Beauveria bassiana*. Clinical and Molecular Allergy 3: 1-8.

World Bank (2011). Missing Food: The Case of Post-Harvest Grain Losses in Sub-Saharan Africa. Washington, DC: http://siteresources.worldbank.org/INTARD/Resources/MissingFoods10_web_final1.pdf. Accessed 30/10/2016.

Wu S, Gao Y, Smagghe G, Xu X, Lei, Z. 2016. Interactions between the entomopathogenic fungus Beauveria bassiana and the predatory mite Neoseiulus barkeri and biological control of their shared prey/host Frankliniella occidentalis. Biological Control 98: 43-51.

Xiao G, Ying SH, Zheng P, Wang ZL, Zhang S, Xie XQ, Shang Y, Leger RJS, Zhao GP, Wang C. 2012. Genomic perspectives on the evolution of fungal entomopathogenicity in *Beauveria bassiana*. *Scientific Reports*, Published online 2012 July. Doi 10.1038/srep 00483.

Xie L, Chen HM, Yang JB. 2013. Conidia production by *Beauveria bassiana* on rice in solid-state fermentation using tray bioreactor. Advanced Materials Research 610: 3478-3482.

Yaman M, Acar KF, Radek R. 2015. A nucleopolyhedrovirus from the Mediterranean flour moth, *Ephestia kuehniella* (Lepidoptera: Pyralidae). Applied Entomology and Zoology 50: 355-359.

Yu C, Subramanyam B, Flinn PW, Gwirtz JA. 2011. Susceptibility of *Lasioderma serricorne* (Coleoptera: Anobiidae) life stages to elevated temperatures used during structural heat treatments. Journal of Economic Entomology 104: 317-324.

Zeeb H, Blettner M. 1998. Adult leukaemia: what is the role of currently known risk factors? Radiation and Environmental Biophysics 36: 217-228.

Zettler LJ, Cuperus GW. 1990. Pesticide resistance in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. Journal of Economic Entomology 83: 1677-1681.

Zhu Y, Fang L, Chen B, Li ZY. 2009. Virulence of *Beauveria bassiana* against the instar larvae of *Lasioderma serricorne* (Fabricius). Journal of Yunnan Agricultural University 24: 42-46.

Zimmermann G. 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology 17: 553-596.

Chapter Two

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

Abstract

A total of 21 South African strains of *Beauveria bassiana* were evaluated for their virulence against the adults of *Lasioderma serricorne* and *Sitophilus zeamais*, under laboratory conditions. In the first bioassays, strains were applied at a single dose of $1x10^8$ conidia ml^{-1}. *L. serricorne* was more susceptible than *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 dose response assays 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. 100% mortality was recorded with a water suspension at $2x10^8$ 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 $2x10^8$ conidia g^{-1} of cornflour with Strain 7284. In both assays Strains 7284 and 7769 outperformed others at low doses. The same strains achieved 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 2.94 days 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. serricorn*e. However, it was still necessary to screen for more virulent isolates to control *S. zeamais*.

2.1 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 1994). The pest is capable of multiplying to large populations resulting in severe damage to grains (Cosmas et al. 2012). An estimated 40.0% stored maize is lost to *S. zeamais* in Africa (Meikle et al. 2001). And with severe infestations,

maize weevils can cause losses of 90.0% (Giga et al. 1991). The cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), is cosmopolitan, especially 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 2008).

Fumigants and synthetic insecticides have been used to control pests in grain stores. The repeated use of these materials has led to serious problems, including insecticide resistance, environmental and human health concerns, mortality of non-target organisms, and chemical residues in foodstuffs (Cherry et al. 2004). Therefore alternative control strategies based on the use of entomopathogenic fungi are being evaluated.

Differences in pathogenicity (virulence) among isolates against stored product insects have been previously reported in assays with *Beauveria bassiana* (Balsamo) Vuillemin and other entomopathogenic fungi on *S. zeamais* (Rondelli et al. 2012), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Golshan et al. 2013; Golshan et al. 2014), *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) (da Paz Júnior et al. 2012), *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) (Kavallieratos et al. 2014), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) (Jyothi et al. 2014), *Plodia interpunctella* Hubner, *Ephestia cautella* Walker and *E. kuehniella* Zeller (Lepidoptera: Pyralidae) (Sabbour et al. 2012).

One of the most important steps in the development of a myco-insecticide is the selection of highly pathogenic strains (Tefera & Pringle 2008). Littlie research has been conducted into biocontrol of the cigarette beetle, *L. serricorne* (AiYing et al. 2009; Yuan et al. 2009). 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 a water suspension or a powder formulation against the cigarette beetle *L. serricorne*.

2.2 Material and methods

2.2.1 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[1]. *S. zeamais* was reared on yellow maize grains[2]. The grains were stored at -20ºC for one week to eliminate unwanted natural infestation. Approximately ten adults of each insect of mixed sexes were placed in 250 ml glass jars[3], each containing 100g of either grain. The jars were covered with insect nets[4] 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.

2.2.2 Fungi

A total of 21 strains of *B. bassiana* from diverse geographical origins (Table 2.1) were bioassayed 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[5] (PPRI-ARC, South Africa) and the commercial strain of *B. bassiana* was provided by Plant Health Products[6] (Pty) Ltd (PHP).

2.2.3 Production of conidial suspension

B. bassiana strains were cultured on potato dextrose agar[7] (PDA) [Biolab Merck (Pty) Ltd] in 9 cm diameter Petri dishes[8] and incubated in 28ºC for 15 days for complete sporulation. The contents (hyphae and conidia) were harvested by flooding the Petri dishes with distilled water with 0.01 (v/v) Tween 80 and stirring with a glass rod. 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 Hemocytometer[9].

[1] Milled white rice, Osman's Taj Mahal, 180 Sirdar Road, Clairwood, Durban, South Africa.
[2] Smith Animal Feed, Unit 8 Davlen Pk, 11 Halsted Rd, M'Kondeni, Pietermaritzburg, 3201. The seed morphology was intermediate between flint and dent and the source was Brazil but the variety was unknown.
[3] Victoria Packaging c.c. Agricultural Packaging Equipment and Safety Wear, 298 Victoria Road, Pietermaritzburg 3201, South Africa.
[4] Film Flex Plastic Natal cc, Unit 10, Shepstone Park, Cnr. Shepstone and Blasé Roads, New Germany, South Africa.
[5] Agricultural Research Council, 1134 Park Street, P.O. Box 8783, Hatfield, Pretoria 0001.
[6] Plant Health Products (Pty) Ltd., P.O. Box 207, Nottingham Road, South Africa.
[7] Merck (Pty) Ltd 1, Friesland Drive, Longmeadow Business Estate, Modderfontein, 1645, South Africa.
[8] Prestige Laboratory Supplies CC, 9 Marshall Dr, Durban, 4302, South Africa.
[9] Hirschmann®, Pietermaritzburg, South Africa.

2.2.4 Conidial viability

To assess the conidial viability, 0.1ml of each sample suspension was pipetted and thinly spread over the PDA in Petri dishes using an "L" shaped glass rod and incubated at 28^0C for 24 hour, with three Petri dishes per sample suspension. Conidia were examined at 400x magnification and germination was counted when the germ tube was apparent. All the conidia in each field of view were counted and percentage germination was calculated. All the isolates used in the experiments displayed > 90.0% viable conidia.

Table 2.1 Origin of fungal strains of *B. 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

2.2.5 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 $1x10^8$ conidia ml^{-1}. 30 less than 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. 1996). After 24 hour the treated insects were transferred into 250 ml glass jars with 50g of rice and kept at 28±2^{o}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 for three minutes, rinsed in sterile distilled water three times and kept in Petri dishes with wet filter paper at 28±^{o}C to observe fungus outgrowth. The experiment was a 22 x 2 factorial arranged in 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 1925). Data was subjected to analysis of variance (ANOVA) using GenStat for Windows, 17th edition (Payne et al. 2014). Means were compared using the Fisher's Least Significant Difference at a 5.0% level of significance.

2.2.6 Production of dry conidia

A concentration of 10^8 conidia ml^{-1} was determined using a Neubauer Improved Hemocytometer. 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^{o}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 five days. Conidia were harvested by scraping them off from the surface of the dried medium using a sterile scalpel blade. The fungal harvested 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^{o}C for further use.

2.2.7 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 m^-

^{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, 30 less than 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 Hemocytometer, 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, 30 less than 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 250 ml glass jars with 50g of rice grains and kept at 28±2^{o}C and 65±5.0% RH for 10 days. Mortality was counted every two days for 10 days. Glass jars were placed as per a 6 x 6 x 2 factorial experiment arranged in randomized complete block design (RCBD). This bioassay was performed twice. Mortality was monitored every two days for 10 days, and corrected on the basis of natural mortality observed in the control treatment using Abbott's formula (Abbott 1925). Data was subjected to analysis of variance (ANOVA). Means were compared using the Fisher's Least Significant Difference 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. 2014).

2.3 Results

2.3.1 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 (Table 2.2).

All *B. bassiana* strains were pathogenic and mycelia growth demonstrated that most of the tested insects died due to the fungus (Fig. 2.1). *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 levels ranged from 14.29 to 96.4% and 10.71 to 82.20%, for *L. serricorne* and *S. zeamais*, respectively (Fig. 2.2). 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. The remaining seven strains caused mortality < 50.0% (Fig. 2.2).

In the case of *S. zeamais* 20 strains of the *B. bassiana* caused mortality levels less than 51.0%, indicating that *S. zeamais* was more resistant than *L. serricorne* following exposure to the same strains. However, Strain 7769 was pathogenic against this insect and caused 82.2% mortality when applied at dose of $1x10^8$ conidia ml^{-1}. Although the commercial Strain R444 used in this bioassay only caused <50.0% mortality against *S. zeamais*, it was highly virulent on *L. serricorne* and caused a 92.9% mortality level (Fig. 2.2).

Table 2.2 ANOVA table for main effects and interaction effects for the pathogenicity of 21 *B. bassiana* strains against two insects

Source of variation	d.f.	s.s.	m.s.	F values	P values
Strains	20	57068.1	2853.41	64.59	<.001
Insects	1	32581	32581	737.51	<.001
Strains x insects	20	10027.5	501.37	11.35	<.001
Residual	82	3622.51	44.18		
Total	125	103479			
LSD	10.796				
Mean ± SE	46.99 ± 5.427				
CV%	14.1				

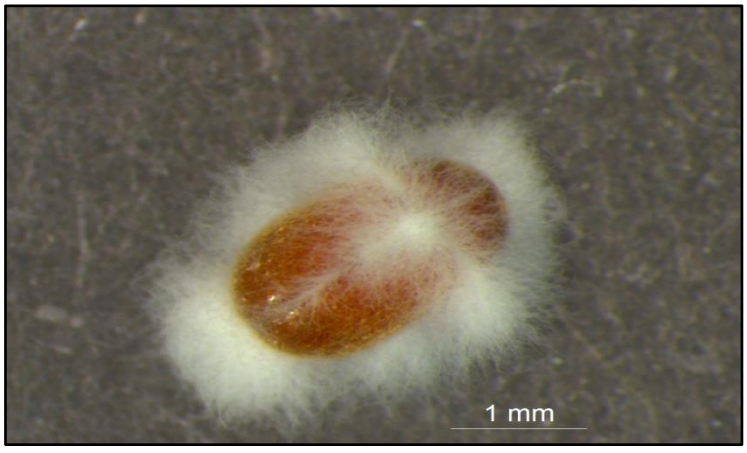

Fig. 2.1 Mycelial growth emerging from an adult of *Lasioderma serricorne* infected by *Beauveria bassiana* Strain 7284

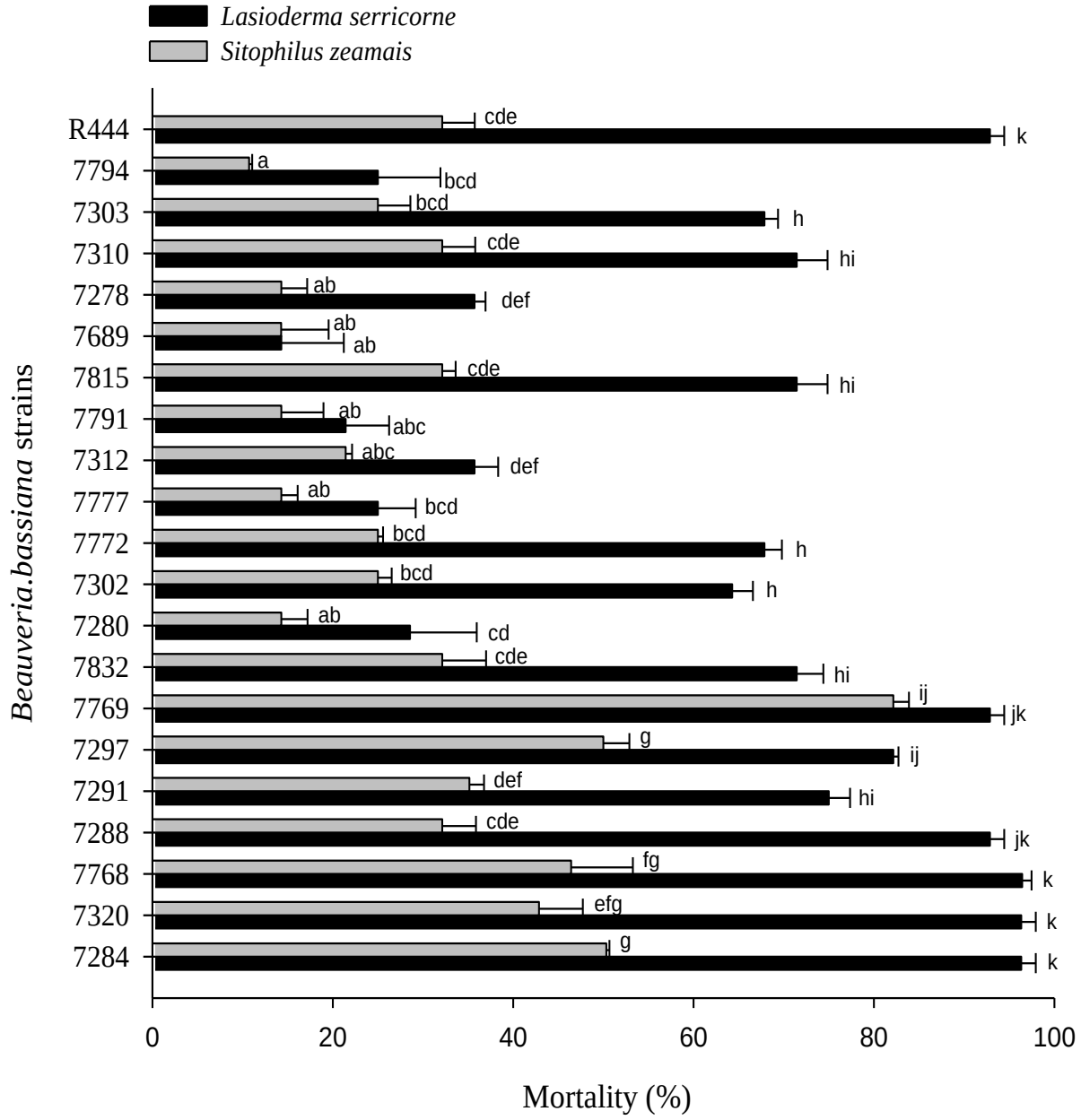

Fig. 2.2 Corrected mortality (Abbott's correction) of cigarette beetle, *Lasioderma serricorne* and maize weevil, *Sitophilus zeamais* adults following 10 days exposure to 21 strains of *Beauveria bassiana* at $1x10^8$ conidia ml^{-1}. Bars are standard error.

2.3.2 Multiple bioassays using water suspensions and powder formulations

All main effects as well as related interactions were highly significant at $p < 0.001$ (Table 2.3). 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 $R^2 > 0.95$ (Fig. 2.3). Dose responses always reach a plateau where increased doses do not increase mortality. The plateau was reached at a dose of $2x10^8$ conidia using either the water suspensions per ml or the powder formulations per g. Therefore a useful practical range is $2x10^4$ – $2x10^8$, which may be achievable in practice.

Water suspensions of the six selected strains in the first bioassay against the adults of *L. serricorne* consistently revealed higher mortality levels at all doses compared to the powder formulations. 100% mortality was achieved with 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 (Fig. 2.3).

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} (Fig. 2.3).

Probit analyses were performed separately for each strain formulated in a water suspension or powder formulation, and the results are presented in Fig. 2.4 and Fig. 2.5. *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 there were few differences between strains. The six strains performed relatively well and even the least virulent caused considerable mortality levels.

Table 2.3 ANOVA table for main effects and associated interactions for the effects of water suspension and powder formulation in multiple dose bioassays against the adults of *L. serricorne*

Source of variation	d.f.	s.s.	m.s.	F values	P values
Strains	5	5824.796	1164.959	134.83	<.001
Formulations	1	38564.69	38564.69	4463.4	<.001
Doses	4	109023	27255.76	3154.53	<.001
Strains x Formulations	5	375.983	75.197	8.7	<.001
Strains x Doses	20	1757.292	87.865	10.17	<.001
Formulations x Doses	4	846.557	211.639	24.49	<.001
Strains x Formulations x Doses	20	1521.31	76.065	8.8	<.001
Residual	118	1019.543	8.64		
Total	179	158952.3			
LSD	4.753				
Mean SE	42.47				
CV%	6.9				

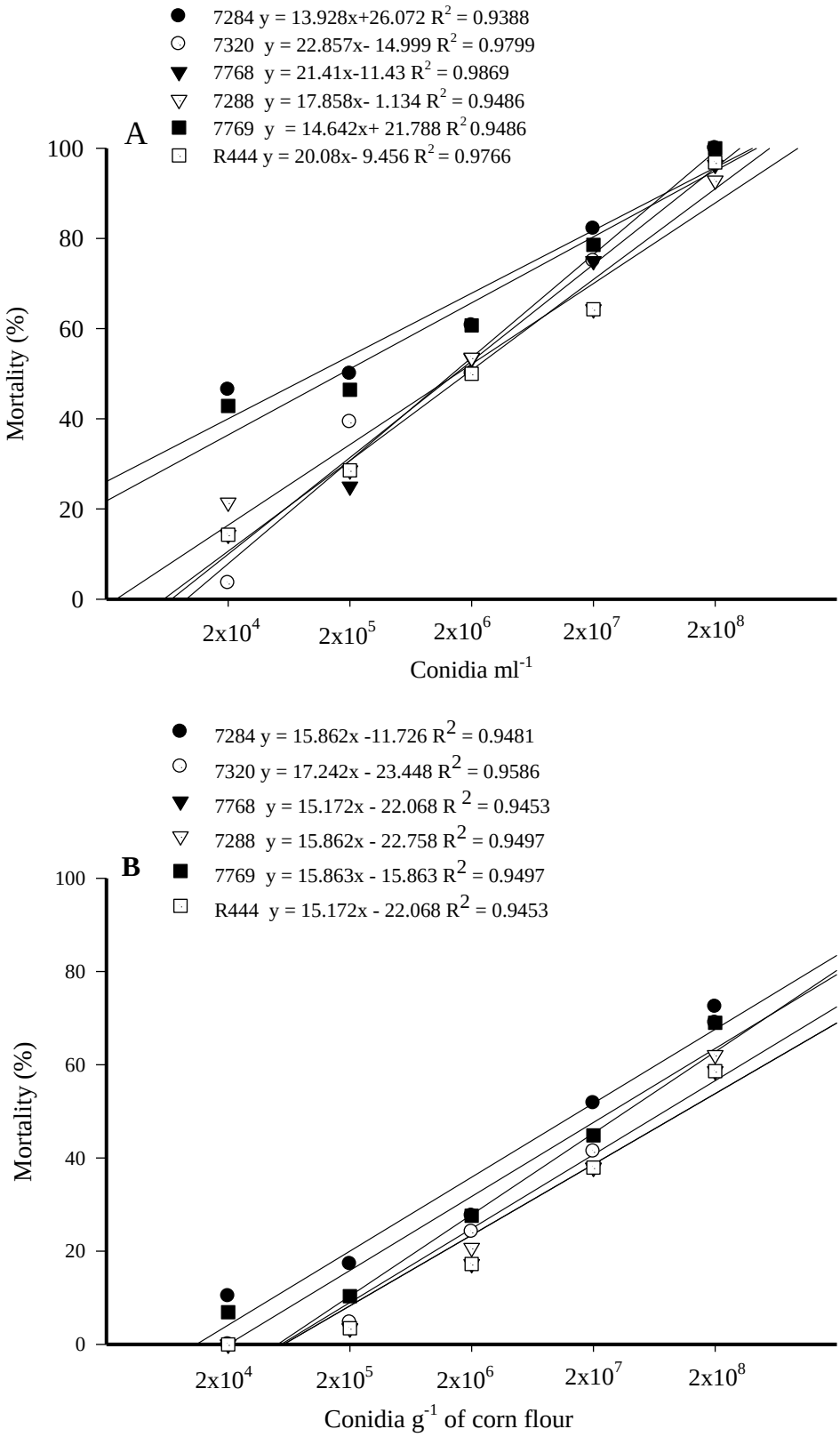

Fig. 2.3 Corrected mortality (Abbott's correction) of cigarette beetle, *Lasioderma serricorne* adults after exposure to different doses of selected *Beauveria bassiana* strains using **A** = water suspension and **B** = powder formulation

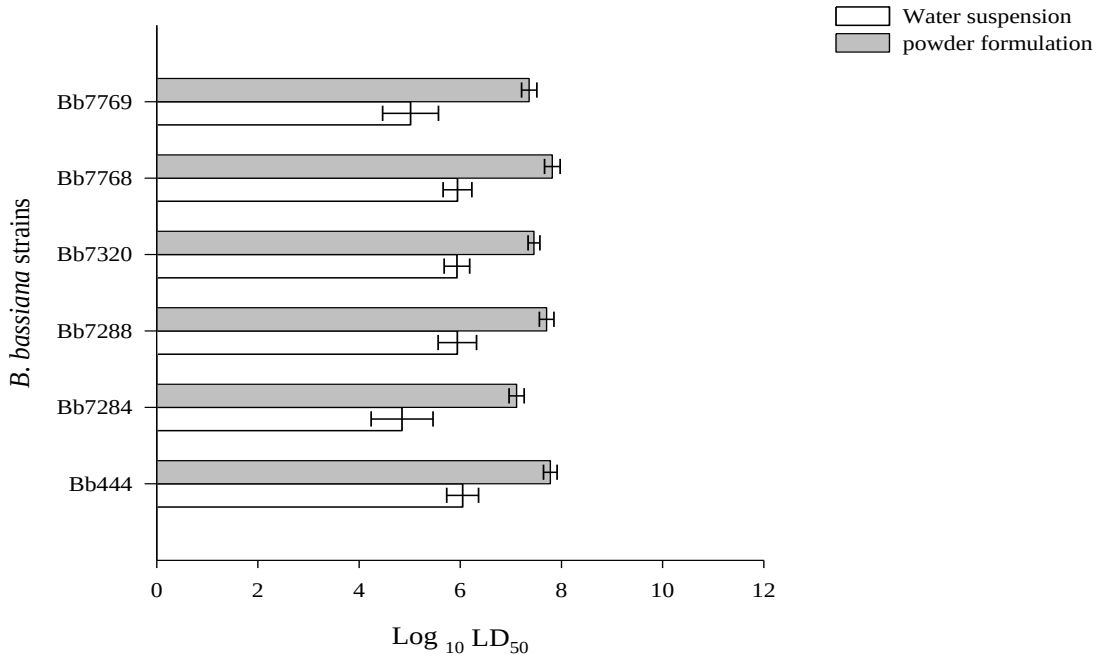

Fig. 2.4 Lethal dose (LD_{50}) of cigarette beetle, *Lasioderma serricorne* adults upon exposure to water suspension and powder formulation of six selected *Beauveria bassiana* strains at five different doses. Bars are standard error.

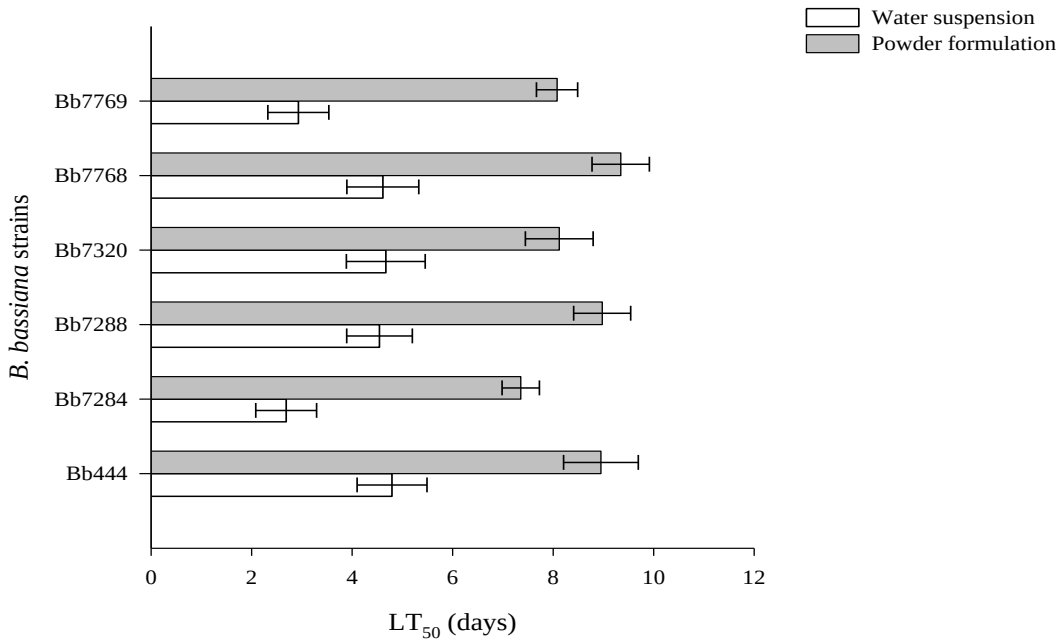

Fig. 2.5 Lethal time (LT_{50}) of cigarette beetle, *Lasioderma serricorne* adults upon exposure to water suspension and powder formulation of six selected *Beauveria bassiana* strains at a highest dose. Bars are standard error.

2.4 Discussion

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

L. serricorne was more susceptible than *S. zeamais*. Among the tested strains, 14 strains achieved more than 50.0% mortality levels against *L. serricorne*, with only seven recording less than50.0% mortality. These results are in agreement with Cherry et al. (2005) who found that 100% mortality of *C. maculatus* resulted from exposure to the aqueous suspension of various isolates 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, with 20 strains achieving mortality levels less than 51.0%. Only one strain caused 82.2% mortality against this insect, indicating the higher resistance of this insect toward the tested strains of *B. bassiana* used in this study. Shams et al. (2011) observed similar patterns with *C. maculatus*, which was more susceptible than *S. granarius* following exposure to five different concentrations of *B. bassiana* under laboratory conditions. In the same context Rondelli et al. (2012) 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%. Similar to the findings of this study, Rondelli et al. (2012) found that only three of the twelve isolates caused mortality levels > 50.0% against *S. zeamais*. Golshan et al. (2013) 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 by water suspension, and by powder formulation using cornflour as a 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. 2005). The higher mortality levels and lower LT_{50} achieved by Strains 7284 and 7769 at the lower doses are important because biocontrol of the storage pests in granaries will need to be achieved at such 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 suspension of *B. bassiana* and other entomopathogenic fungi increased the efficacy of these fungi toward target insects and resulted in high mortality levels (Teshome & Tefera 2009; Mahdneshin et al. 2011; Faraji et al. 2013). 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 provide 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. These results indicate that less mortality was caused with pwoder formulations. Nevertheless, use of powder formulations is more acceptable and practical in storage of grains than water formulation. These results stand in agreements with Khashaveh et al. (2011) 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 *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. Abdel-Raheem et al. (2015) observed mortalities of 50.3, 54 and 51.0% for *R. dominica*, *S. oryzae* and *O. surinamensis*, respectively. Following exposure to *B. bassiana* formulated in talc powder at $0.42x10^6$ conidia g^{-1}, this dose was equivalent to ten times less than the conidial concentration used in this study.

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 remains a need to screen more virulent isolates against maize weevil, *S. zeamais* for use in the management of this pest.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Economic Entomology 18: 265-267.

Abdel-Raheem M, Ismail I, Rahman RA, Farag N, Abdel I. 2015. Entomopathogenic fungi, *Beauveria bassian*a (Bals.) and *Metarhizium anisopliae* (Metsch.), as biological control agents on some stored product insects. Journal of Entomology and Zoology Studies 3: 316-320.

Adane K, Moore D, and Archer S. 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. Journal of Stored Products Research 32: 105-113.

AiYing L, Li L, Xiao Z, HaiYan H, XiaoMin Z, Hui Y, RangXin W. 2009. Pathogenicity of *Beauveria bassiana* to *Ephesia elutella* and *Lasioderma serricorne*. Guizhou Agricultural Sciences 28: 84-85.

Cherry AJ, Banito A, Djegui D, Lomer C. 2004. Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. International Journal of Pest Management 50: 67-73.

Cherry A, Abalo P, Hell K. 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Products Research 41: 295-309.

Cosmas P, Christopher G, Charles K, Friday K, Ronald M. 2012. *Tagetes minuta* formulation effect *Sitophilus zeamais* (weevils) control in stored maize grain. International Journal of Plant Research 2: 65-68.

da Paz Júnior F B, Guaraná CFR, da Paz E, de Queiroz C, Lima E, de Azevedo JL. 2012. Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. isolates to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in cowpea grains. African Journal of Microbiology Research 6: 7365-7369.

Devi KU, Sridevi V, Mohan CM, Padmavathi J. 2005. Effect of high temperature and water stress on in vitro germination and growth in isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. Journal of Invertebrate Pathology 88: 181-189.

Faraji S, Mehrvar A, Derakhshan Shadmehri A. 2013. Studies on the virulence of different isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin against Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). African Journal of Agricultural Research 8: 4157-4161.

Giga D, Mutemerewa S, Moyo G, Neeley D. 1991. Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. Crop Protection 10: 287-292.

Golshan H, Saber M, Majidi-Shilsar F, Karimi F. 2013. Efficacy of nine isolates of *Beauveria bassiana* against adults of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Acta Entomologica Sinica 56: 201-206.

Golshan H, Saber M, Majidi Shilsar F, Karimi F, Ebadi A. 2014. Laboratory evaluation of *Beauveria bassiana* isolates on red flour beetle *Tribolium castaneum* and their characterization by random amplified polymorphic DNA. Journal of Agricultural Science and Technology 16: 747-758.

Jyothi P, Rao N, Lakshmipathy R. 2014. Laboratory evaluation of entomopathogenic fungus alone or in combination with edible oils on progeny adult build-up of lesser grain borer on stored paddy. Asian Journal of Biological Science 9: 139-145.

Kavallieratos NG, Athanassiou CG, Aountala MM, Kontodimas DC. 2014. Evaluation of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* for control of *Sitophilus oryzae*. Journal of Food Protection 77: 87-93.

Khashaveh A, Ghosta Y, Safaralizadeh MH, Ziaee M. 2011. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. Journal of Agricultural Science and Technology 13: 35-43.

Mahdneshin Z, Vojoudi S, Ghosta Y, Safaralizadae MH, Saber M. 2011. Laboratory evaluation of the entomopathogenic fungi, Iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin, for the control of the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). African Journal of Microbiology Research 5: 5215-5220.

Mahroof RM, and Phillips TW. 2008. Life history parameters of *Lasioderma serricorne* (F.) as influenced by food sources. Journal of Stored Products Research 44: 219-226.

Meikle WG, Cherry AJ, Holst N, Hounna B, Markham RH. 2001. The Effects of an Entomopathogenic Fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), *Sitophilus zeamais* Motschulsky (Col.: Curculionidae), and Grain Losses in Stored Maize in the Benin Republic. Journal of Invertebrate Pathology 77: 198-205.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. GenStat for Windows, 17th Edition. VSN International, Hemel Hempstead, UK.

Rondelli VM, de Carvalho JR, Pratissoli D, Polanczyk RA, de Alencar JRDC, Zinger FD, Pereira SMA. 2012. Selection of *Beauveria bassiana* (Bals.) Vuill. isolates for

controlling *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae). Idesia (Arica) 30: 97-102.

Sabbour M, Abd-El-Aziz S, Sherief M. 2012. Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modifications for the control of three pyralid moths in stored grains. Journal of Plant Protection Research 52: 359-363.

Shams G, Safaralizadeh MH, Imani S, Shojai M, Aramideh S. 2011. A laboratory assessment of the potential of the entomopathogenic fungi *Beauveria bassiana* (Beauvarin) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). African Journal of Microbiology Research 5: 1192-1196.

Tefera T, Pringle K. 2007. Biological control of the spotted stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) with the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*. SINET: Ethiopian Journal of Science 30: 65-70.

Teshome A, Tefera T. 2009. Susceptibility of *Sitophilus zeamais* (Mostch.) (Coleoptera: curculionidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. SINET: Ethiopian Journal of Science 32: 21-28.

Throne JE. 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. Environmental Entomology 23: 1459-1471.

Yuan Z, Li F, Bin C, ZhengYue L. 2009. Virulence of *Beauveria bassiana* against the instar-larvae of *Lasioderma serricorne* (Fabricius). Journal of Yunnan Agricultural University 24: 42-46.

Chapter Three

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)

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 $2x10^8$ 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 $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia g^{-1}. Strain MS-8 consistently caused higher mortality levels on the three insects than the other two strains. The same strain achieved the lowest LD_{50} and LT_{50} 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 phylogenetic 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.

3.1 Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a pest of economic importance for storage grains in the tropics. Infestation of grain by this pest typically takes place in the field, but it causes the most damage during storage (Abebe et al. 2009). In tropical countries, 20% to 90% grain losses have been reported as a result of the maize weevil (Giga et al. 1991). The almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae), is another major storage pest. It occurs both in tropical and temperate regions and attacks grains, nuts, dried fruits and a great variety of other stored products (Horak 1994). The cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), is cosmopolitan and also occurs in both tropical and subtropical areas. This insect is usually found in stored food products such as dry fruit, grains, cereal flour and animal food (Papadopoulou & Buchelos 2002).

Use of fumigants and synthetic insecticides is one of the means for preventing crop loss to insects during the storage of grains. However, there is opposition to their use because of insecticide resistance, chemical residues in food stuffs, increasing costs, and environmental pollution Pereira et al. (2009). Therefore, it is essential to find out alternative control strategies such as the use of entomopathogenic fungi (EPF) against stored product insect pests (Wang et al. 2003). *Beauveria bassiana* (Balsamo) Vuillemin is one such fungal biopesticide that is especially valuable due to its non-toxic nature towards non-target animals and humans. It is a fungus that grows naturally in soil throughout the world and acts as a potent parasite on various insect species, causing white muscardine disease. Many isolates of *B. bassiana* have been formulated and registered as commercial products against a wide range of insect's pests (Feng et al. 1994). However, no commercial products of EPF have been registered for the biocontrol of stored-grain insects (Batta 2016).

There is much genetic variation among isolates of *B. bassiana*. Pathogenicity and virulence to different arthropods as well as enzymatic and DNA characteristics vary among different isolates (Almeida et al. 1997; Moino et al. 1998). The selection of virulent isolates adapted to local components of the agroecosystem is one of the most important aspects in the development of mycoinsecticides (Cortez-Madrigal et al. 2003).

Exact identification of fungal species can be difficult and sometimes impossible by means of morphological characteristics alone. Morphological criteria are generally used to identify and classify *Beauveria* spp. Distinctions between *Beauveria* species have always been

problematic, due to the heterogenicity of *B. bassiana* (Glare & Inwood 1998). Because of these difficulties with identification, a number of molecular techniques have been developed to assist in the identification of *Beauveria* isolates (Kosir et al. 1991; St Leger et al. 1992; Hegedus & Khachatourians 1993, 1996).

Numerous studies have shown that isolates of *B. bassiana* are potential microbial control agents for use against stored product pests (Hidalgo et al. 1998; Crespo et al. 2002; Khashaveh 2011; Sedehi et al2014). Therefore the objective of this study was to isolate and identify native entomopathogenic fungi from various soil sources; and to evaluate their virulence against *S. zeamais, E. cautella* L3 larvae and *L. serricorne* L3 larvae and adults.

3.2 Material and methods

3.2.1 Soil samples

Soil samples were collected in the winter season at the beginning of May 2014 at Ukulinga Research Farm, Pietermaritzburg (KwaZulu-Natal Province) (latitude 29. 67 S, longitude 30.41 E, 809 meters above sea level). The lands were previously used for maize, sorghum, beans and other field crops, or for livestock and poultry. Thirteen sampling points at ≥ 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 4^{o}C; (iv) the samples were processed within two weeks of collection.

3.2.2 Isolation of fungi

Entomopathogenic fungi were isolated using a bait trap method using larvae of the wax moth (*Galleria mellonella* L.) (Lepidoptera: Pyralidae) (Zimmermann 1986), which were reared on artificial media according to Metwally et al. (2012).

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 200g were placed into glass jars[1] with a capacity of 250 ml, and were moistened with distilled water. Five larvae of

[1] Victoria Packaging c.c., Agricultural Packaging Equipment and Safety Wear, 298 Victoria Road, Pietermaritzburg 3201, South Africa.

third or fourth instars (approximately four weeks old larvae) were released into each glass jar with sterile forceps, followed by covering them with insect nets[2] to facilitate air circulations.

The glass jars were incubated in the dark at 25^{o}C in the incubator for two weeks. Checking was done every three days and dead larvae were removed from the soil and placed in a 1% sodium hypochlorite solution for 3 min to surface sterilize the larvae, after which they were washed in sterile distilled water for 4 min. The dead larvae were then placed individually in 9-cm Petri dishes[3] on humid filter paper. The Petri dishes were sealed with Parafilm, and then incubated at 25^{o}C and checked every Two days until the presence of a fungus was observed. Observation of fungal mycelium on an infected wax moth larva was considered to result from an EPF. After sporulation, conidia were removed from the insect surface with a sterile loop and were streaked onto potato dextrose agar plates containing 0.05 g/L streptomycin and ampicillin[4]. The fungi were identified according to Humber (2012).

3.2.3. 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. *S. zeamais* and *E. cautella* were reared on yellow maize grains[5]. *L. serricorne* was reared on rice grains[6]. The grains were initially stored at -20^{o}C for one week to eliminate unwanted natural infestation. Approximately ten adults of each insect of mixed sexes were placed in glass jars of 250 ml, each containing 100g of respective grains. The jars were covered with insect nets to facilitate air circulation and kept under experimental conditions of 28±2^{o}C and 65±5.0% RH. The adults were removed from the jars after one week of infestation. *S. zeamais* adults, *E. cautella* L3 larvae and *L. serricorne* L3 larvae and adults were used in the experiments.

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[2] Film Flex Plastic Natal cc, Unit 10, Shepstone Park, Cnr. Shepstone and Blasé Roads, New Germany, South Africa.
[3] Prestige Laboratory Supplies CC, 9 Marshall Dr, Durban, 4302, South Africa.
[4] Inqaba Biotechnical Industries (Pty) Ltd, 525 Walker St, Pretoria, 0002, South Africa.
[5] Smith Animal Feed, Unit 8 Davlen Pk, 11 Halsted Rd, M'Kondeni, Pietermaritzburg, 3201. The seed morphology was intermediate between flint and dent and the source was Brazil but the variety was unknown.
[6] Milled white rice, Osman's Taj Mahal, 180 Sirdar Road, Clairwood, Durban, South Africa.

3.2.4 First screening bioassay

All 28 *B. bassiana* strains were used in the first screening bioassay. The dry conidia of these strains were prepared as described in Chapter Two, Section 2.2.6. Each strain was formulated in kaolin as the carrier at a dose of $2x10^8$ conidia g^{-1}, which was determined using a Neubauer Improved Hemocytometer[7]. Thirty adults of *S. zeamais* of less than two days old were introduced into 250 ml glass jars with 100g maize grain. Three replicates were used for each strain. The Control was treated with carrier only. Insect mortality was checked every two days for 10 days. Dead insects were examined for fungal outgrowth, to confirm the cause of insect death was fungal infection. The experiment was arranged in a randomized complete blocks design (RCBD).

3.2.5 Multiple screening bioassays

The best three *B. bassiana* strains that caused mortality levels >70.0% against *S. zeamais* in the first bioassay were then used at various doses in the second bioassay. For each strain, conidia were prepared from an initial stock of $2x10^9$ conidia g^{-1} in kaolin, which was measured using a Neubauer Improved Hemocytometer and then diluted with kaolin to $2x10^8$, $2x10^7$, $2x10^6$, $2x10^5$ conidia g^{-1}. Each dose was replicated three times. The Control was the carrier only. For each replicate, 30 adults of *S. zeamais*, 30 L3 larvae of *E. cautella* and 30 L3 larvae and adults of *L. serricorne* were each placed into glass jars with 100g rice grain, after applying the respective treatment. The experiment was a 3 x 4 x 5 factorial experiment arranged in a randomized complete blocks design (RCBD).

3.2.6 Identification of fungal strains by sequence analysis

The three best *B. bassiana* strains, MS-3, MS-4 and MS-8, were sent to Inqaba Biotech (South Africa) for forward and reverse strand sequencing of the ITS1 and ITS2 region. Sequences were checked in FinchTV for quality and sections of suitable quality were uploaded to BioEdit (Hall 1999). Consensus sequences were produced (~470 bp) and used to perform BLAST searches on the Genbank database against type material for tentative identification. A total of 11 ITS-aligned sequences from type material for *Beauveria*/*Cordyceps* species were downloaded from Genbank. Along with the consensus sequences of the three strains, these sequences were aligned in Bioedit using ClustalW. The

[7] Hirschmann®, Pietermaritzburg, South Africa.

aligned sequences were uploaded to Mega7 (Kumar et al. 2016). The best DNA model for a Maximum Likelihood was determined and the ML tree was produced by testing phylogeny with 1000 bootstrap replications.

3.2.7 Statistical analysis

Mortalities of insects were corrected relative to the control using Abbott's formula (Abbott 1925). 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 performed to calculate the lethal dose (LD_{50}) and lethal time (LT_{50}). The analyses were conducted using GenStat for Windows, 17^{th} edition (Payne et al. 2014).

3.3 Results

3.3.1 Soil samples

The fungus *B. bassiana* was more abundant than *Metarhizium anisopliae* in the tested soil samples. Maize field soil was the richest source of *B. bassiana* (33.34%) followed by a grass land (annual burn 65 years) (GAB) (26.67%) (Fig. 3.1). The poorest source *B. bassiana* 6.67% were soils from the Horses Pasture1, the Chicken Farm and the Cattle Pasture 1 (Fig. 3.1).

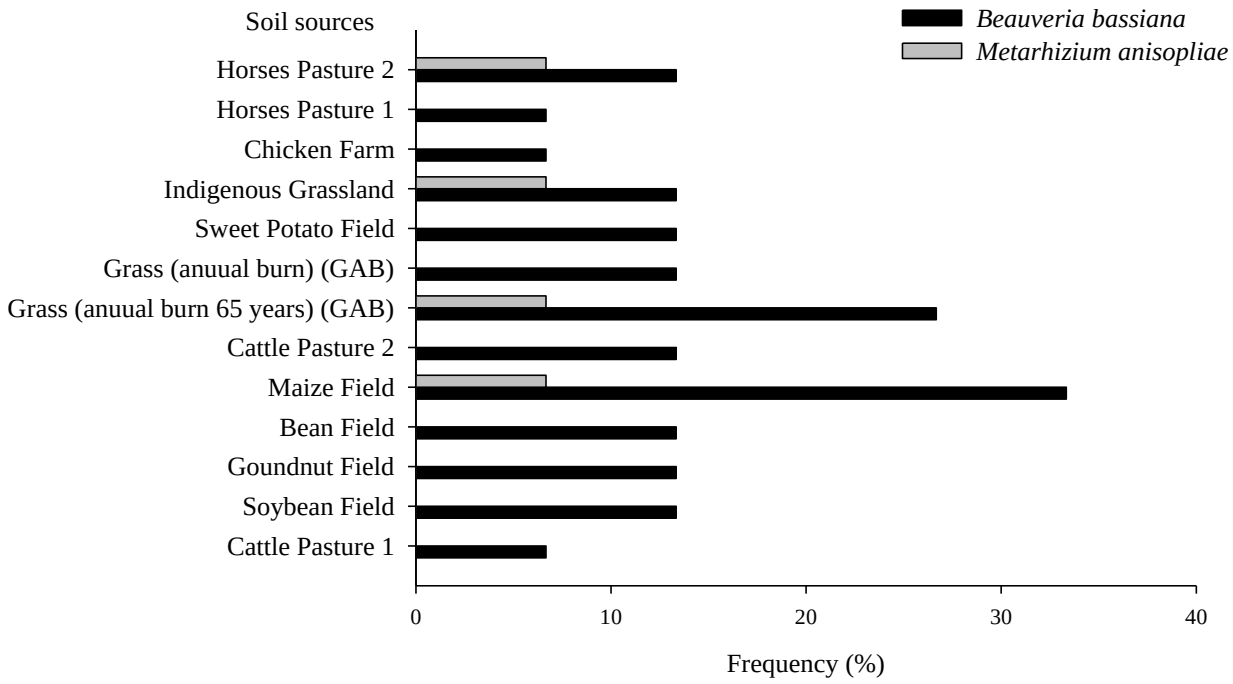

Fig. 3.1 Frequency of isolation of *Beauveria bassiana* and *Metarhizium anisopliae* from soil samples

3.3.2 First Screening bioassay

There were highly significant differences between fungal strains in their pathogenicity on *S. zeamais* ($F = 106.06$; $P < 0.001$) (Table 3.1).

All tested *B. bassiana* strains were pathogenic to *S. zeamais*. Mortality levels range from 10.0 to 80.0%. Among the 28 tested strains, three strains (MS-3, MS-4 and MS-8) caused mortality levels >70.0%. Four strains (MS-22, MS-27, MS-25 and MS-28) caused mortality levels of 50.0 to 63.0%. The remaining 21 strains caused mortality levels <50.0% (Fig. 3.2).

B. bassiana Strain MS-8 caused the highest mortality level of 80.0% against *S. zeamais* followed by Strains MS-3 and MS-4, which caused mortality levels of 73.0% and 70%, respectively (Fig. 3.2). Observations made by scanning electron microscope[8] (SEM) demonstrated that Strain MS-8 has ability to penetrate the cuticle of *S. zeamais* (Fig. 3.3).

Table 3.1 ANOVA table for the pathogenicity effects of 28 *B. bassiana* strains on *S. zeamais*

[8] ZEISS, EVO LS 15, Germany.

Source of variation	d.f.	s.s.	m.s.	F value	P value
Strains	27	32379.75	1199.25	106.06	<.001
Residual	54	610.57	11.31		
Total	83	33011.75			
CV%	9.4				
Mean ± SE	35.75 ± 2.746				
LSD	5.504				

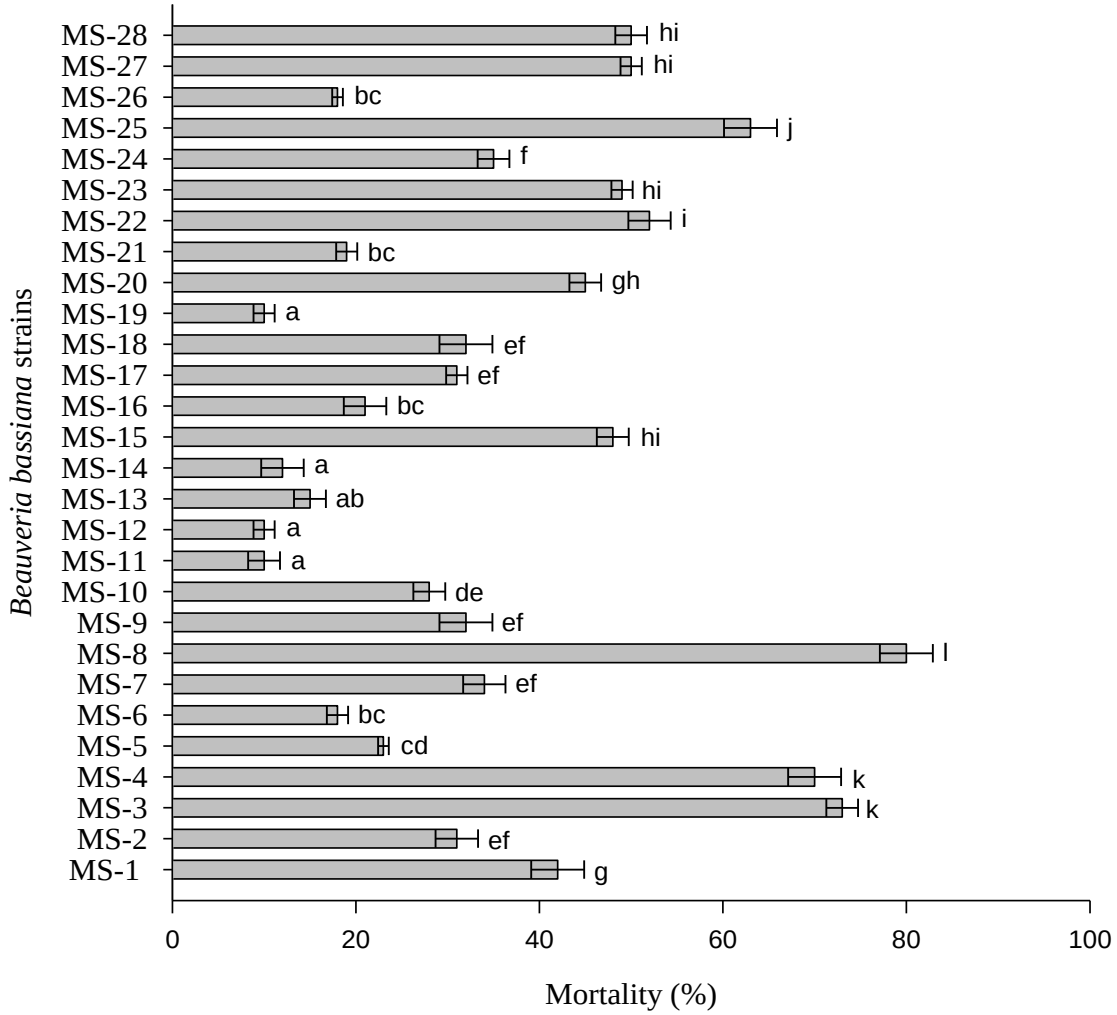

Fig. 3.2 Corrected mortality (Abbott's correction) of maize weevil, *Sitophilus zeamais*, following 10 days of exposure to 28 Strains of *Beauveria bassiana* at $2x10^8$ conidia g^{-1} in a kaolin carrier. Bars represent the standard error.

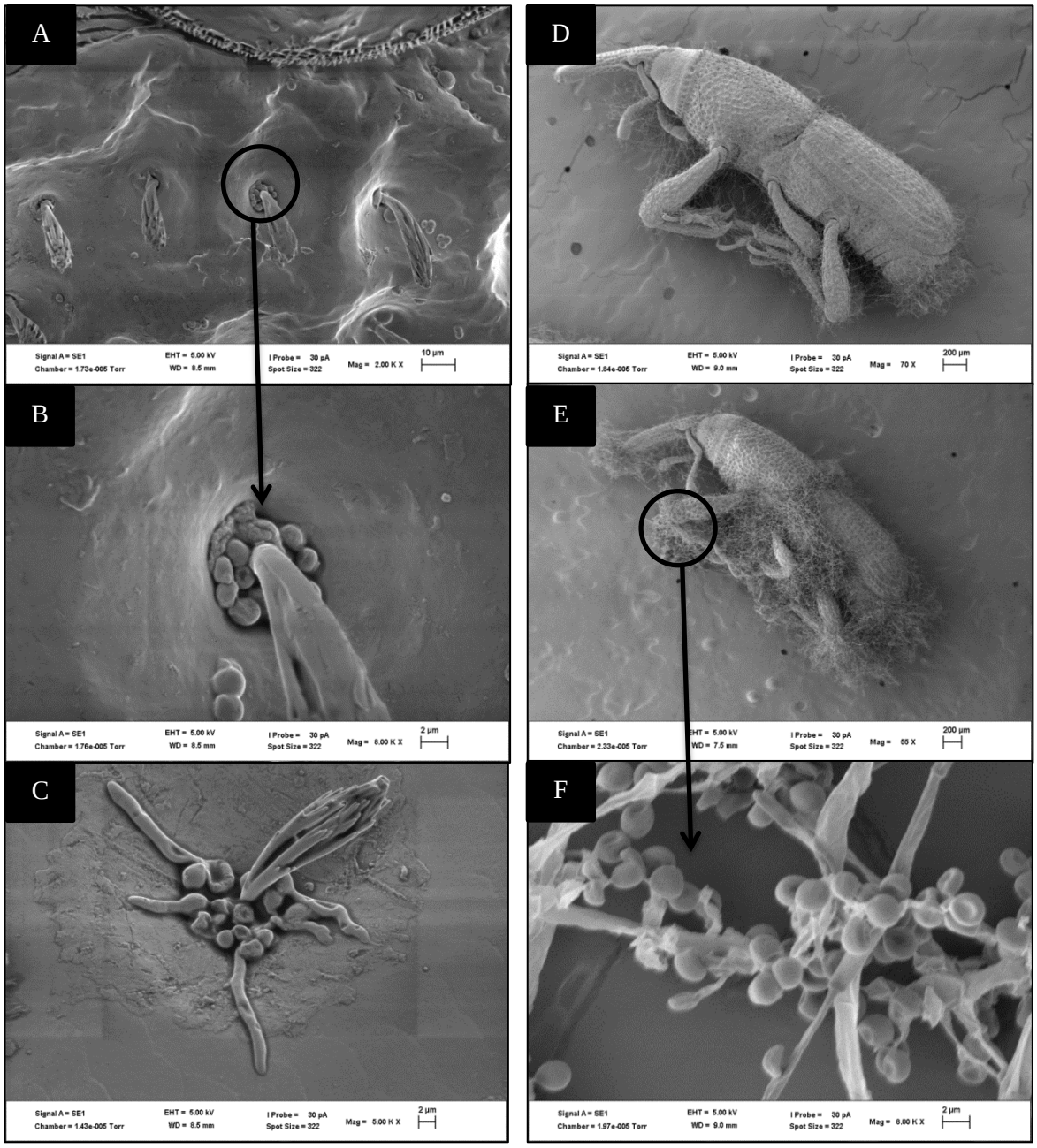

Fig. 3.3 Scanning electron micrographs of *Beauveria bassiana* Strain MS-8 on *Sitophilus zeamais* (A-F). On Day 1 no germination of conidia was found (A and B); germination of conidia and penetration of germ tube were seen on Day 2 (C); mycelia emerged from the insect body on Day 5 (D); E and F show intense sporulation on insect legs by Day 10.

3.3.3 Multiple Screening bioassays

Highly significant differences were observed between strains (F = 92.29; P < 0.001), doses (F = 673.76; P < 0.001) and mortality of tested insects (F = 88.97; P < 0.001). The interaction between doses and insects (F = 4.33; P < 0.001) was also significant. However, no significant interaction was found between strains and insects (F = 1.41; P = 0.217) because Strain MS-8 was better against all tested insects and better than Strains MS-3 and MS-4. The three way interaction between strains and doses and insects (F = 0.46; P = 0.969) was also non-significant, because although *S. zeamais* and *L. serricorne* L3 larvae were more resistant, application of higher doses overcome their resistance. Therefore, increasing the chances of contact with conidia compensated for the short time of exposure in the weevil lifecycle, and the limited mobility of *L. serricorne* L3 larvae (Table 3.2).

The entomopathogenic activity of the Strain MS-8 was confirmed by the presence of fungal mycelia on the body of dead insects. Approximately five days after death, saprophytic growth of mycelium was observed emerging from the intersegmental parts and subsequently, the fungus emerged from all parts of the body of tested insects (Fig. 3.4). *L. serricorne* L3 larvae and *E. cautella* L3 larvae treated with Strain MS-8 displayed a reddish coloration after death (Fig. 3. 4).

A linear response to dose was observed with all tested insects and strains with $R^2 > 0.94$. Mortality increased as the result of dose increase. *B. bassiana* Strain MS-8 generated a higher mortality levels than the other strains at all doses against the three tested insects (Fig. 3.5). Application of Strain MS-8 at the highest dose of $2x10^8$ conidia g^{-1} caused mortality levels of 90.0%, 76.05, 75.0% and 65% for *E. cautella* L3 larvae, *L. serricorne* adults, *S. zeamais* and *L. serricorne* L3 larvae, respectively (Fig. 3.5). These results indicates that *E. cautella* L3 larvae was the most susceptible probably because of the highly mobility of the larvae, which would have increased the chances of their contact with conidia. In contrast, *L. serricorne* L3 larvae were the least affected, probably due to their lack of movement. *L. serricorne* adults and *S. zeamais* were affected in the same way. However, *L. serricorne* adults were more susceptible to the lower doses of conidia than *S. zeamais* (Fig. 3.5).

Table 3.2 ANOVA of the effects of three *B.bassiana* strains on three insects at dose of 0.03g conidia kg^{-1} of grain formulated in various doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Strains	2	5234.38	2617.19	92.29	<.001
Doses	3	57318.75	19106.25	673.76	<.001
Insects	3	7568.75	2522.92	88.97	<.001
Strains x Doses	6	290.62	48.44	1.71	0.128
Strains x Insects	6	240.62	40.1	1.41	0.217
Doses x Insects	9	1106.25	122.92	4.33	<.001
Strains x Doses x Insects	18	234.38	13.02	0.46	0.969
Residual	94	2665.62	28.36		
Total	143	74943.75			
CV%	12.6				
Mean + SE	42.29+ 4.348				
LSD	8.633				

Probit analysis was performed separately for each tested insects to determine the lethal dose (LD_{50}) values and lethal time (LT_{50}) values (Fig. 3.6 and Fig. 3.7).

Constant ranking of *B. bassiana* strains were observed with both LD_{50} and LT_{50} values. Strain MS-8 caused the lowest LD_{50} values and LT_{50} values against the three tested insects, followed by Strain MS-3 and Strain MS-4. In contrast, the lowest LD_{50} values and LT_{50} values were observed with the same order for *E. cautella* L3 larvae, *L. serricorne* adults, *S. zeamais* and *L. serricorne* L3 larvae (Fig. 3.6 and Fig. 3.7).

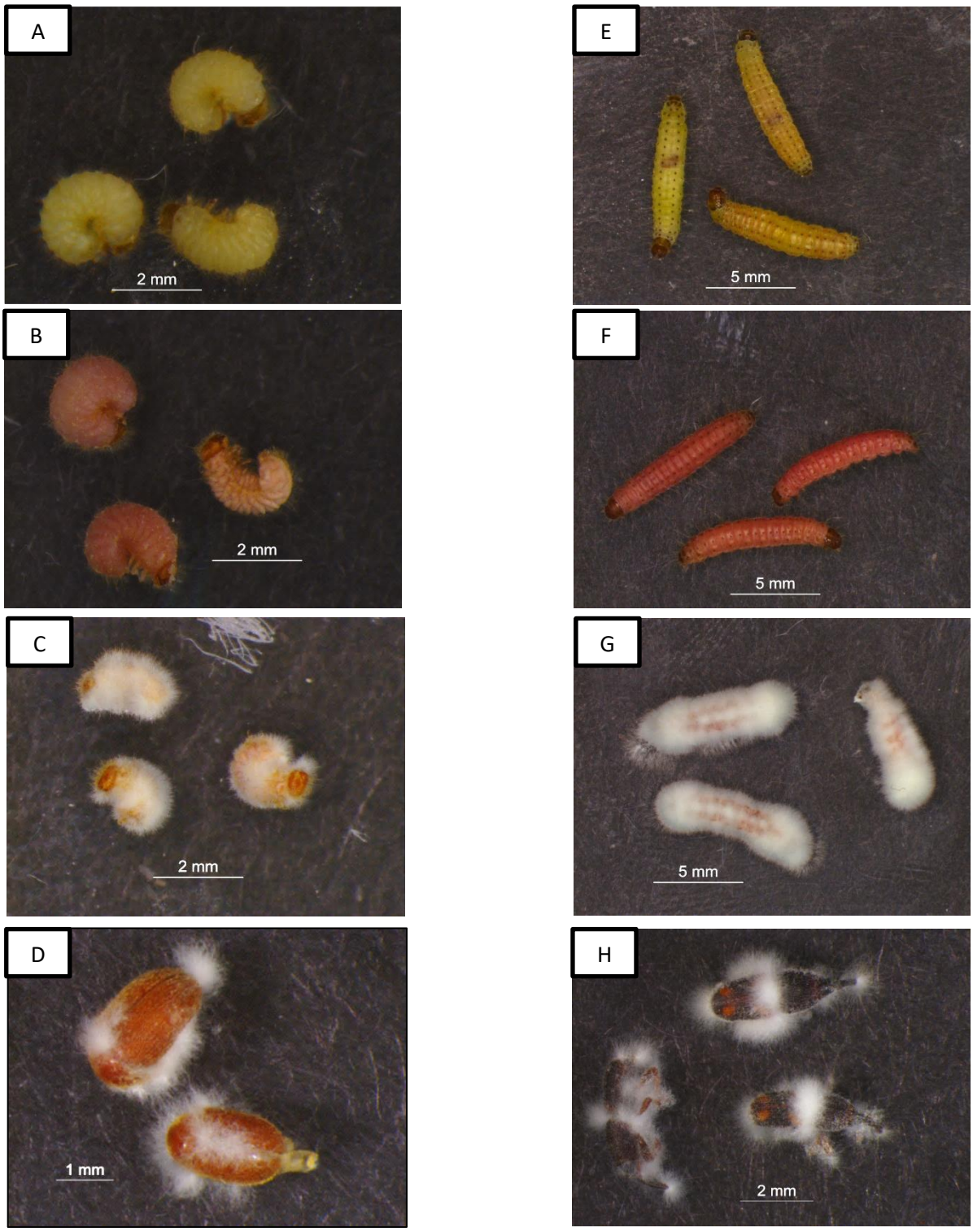

Fig. 3.4 Stereo microscope micrographs of *Beauveria bassiana* Strain MS-8 on *Lasioderma serricorne* L3 larvae (**A** = Control; **B** = red coloration typical of *B. bassiana* infection; **C** = mycelial growth); *L. serricorne* adults (**D** = mycelial growth); *Ephestia cautella* L3 larvae (**E** = Control; **F** = red coloration; **G** = mycelial growth); and *Sitophilus zeamais* (**H** = mycelial growth)

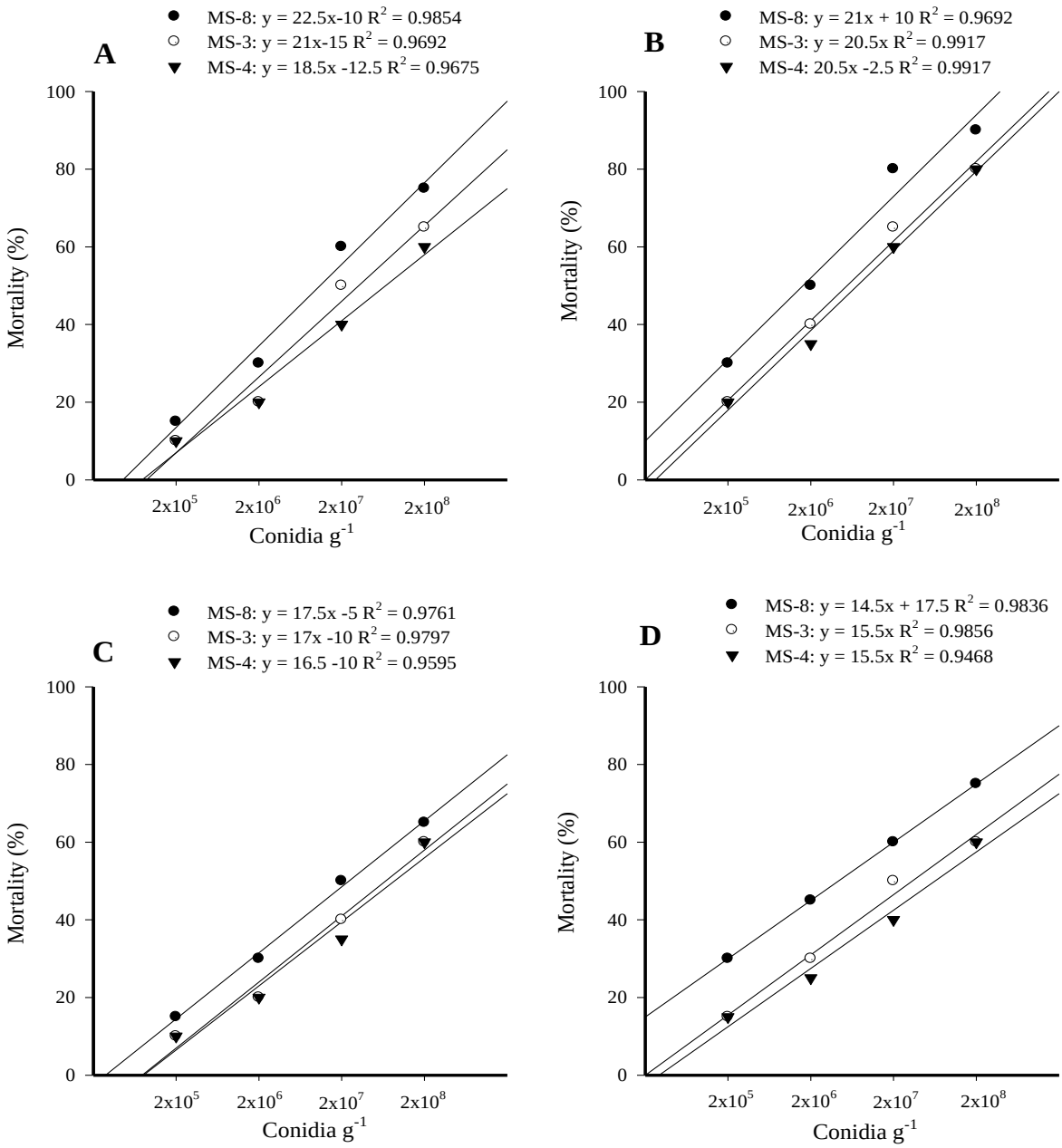

Fig. 3.5 Corrected mortality (Abbott's correction) of **A** = *Sitophilus zeamais* adults, **B** = *Ephestia cautella* L3 larvae, **C** = *Lasioderma serricorne* L3 larvae and **D** = *Lasioderma serricorne* adults, upon exposure to three *Beauveria bassiana* strains applied at doses of $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia g^{-1} formulated in a kaolin carrier, observed 10 days after treatment.

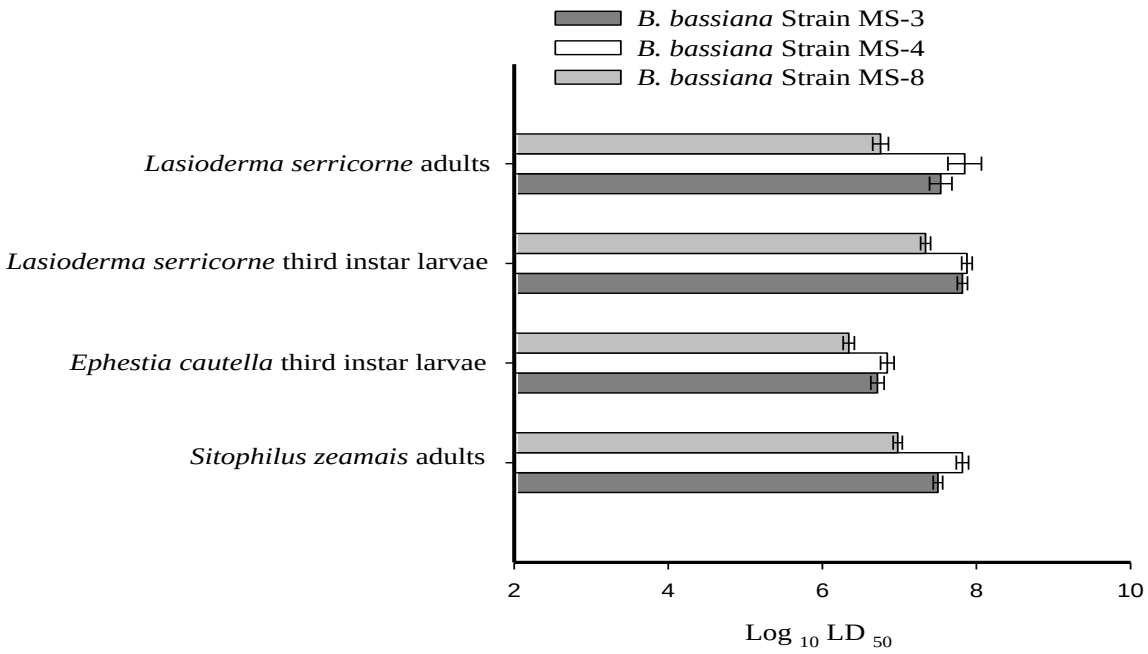

Fig 3.6 Lethal dose (LD_{50}) of the three tested insects upon exposure to three *B. bassiana* strains formulated in kaolin at doses of $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia g^{-1}.

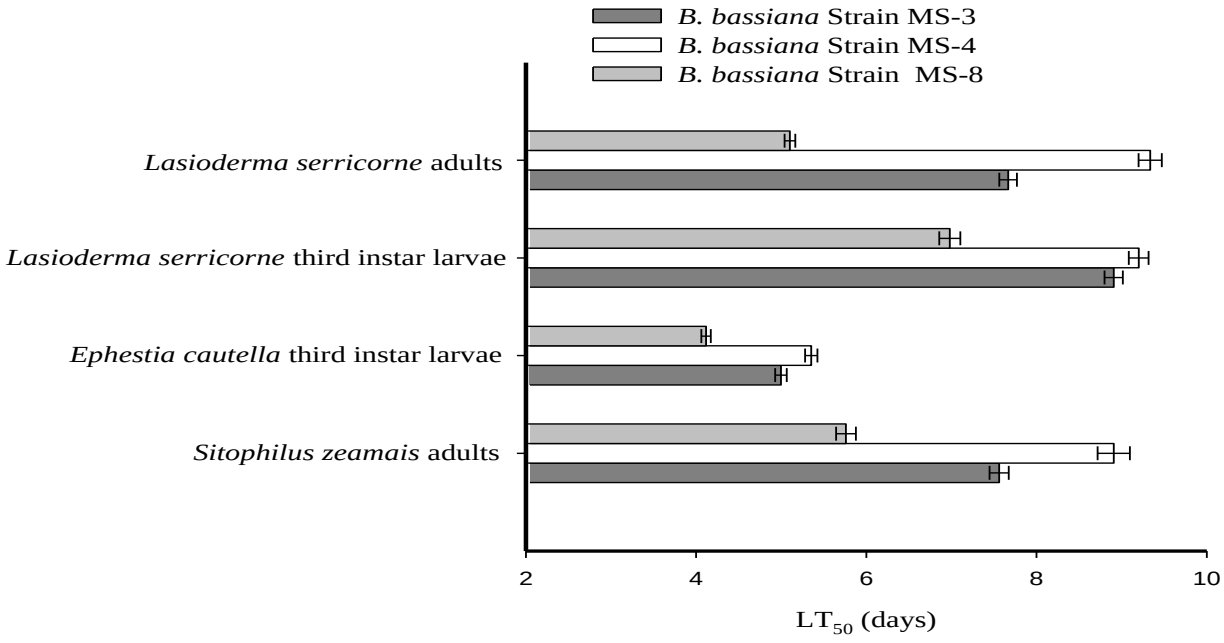

Fig. 3.7 Lethal time (LT_{50}) of the three tested insects upon exposure to three *B. bassiana* strains formulated in kaolin at a dose of $2x10^8$ conidia g^{-1}.

3.3.4 Identification of fungal strains by sequence analysis

BioEdit analysis showed that strains MS3 and MS4 were identical and were therefore grouped together for the BLAST results for all three strains shown in Table 3.3 MS-3/MS-4 had a 99.1% sequence identity match with MS-8. The BLAST search revealed a greater than 97% match to several species within the genus *Beauveria*. All three strains showed the highest matching identity to *B. bassiana*; however percentage identity to other species differed between MS-3, MS-4 and MS-8. Note that *Cordyceps* is the teleomorphic form of *Beauveria*.

Table 3.3 Selected Genbank sequence matches for the three strains. Matches were only selected from type material that had 100% coverage of the query sequence and no less than 97% identity.

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

An ML tree was produced and the tree with the highest log likelihood (-949.1464) is shown in Fig. 3.8. The evolutionary history was inferred from the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993) with a discrete Gamma distribution to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.0500)). The analysis involved 14 nucleotide sequences (11 from Genbank and the three strains). All positions containing gaps and missing data were eliminated. The final dataset included a total of 452 positions.

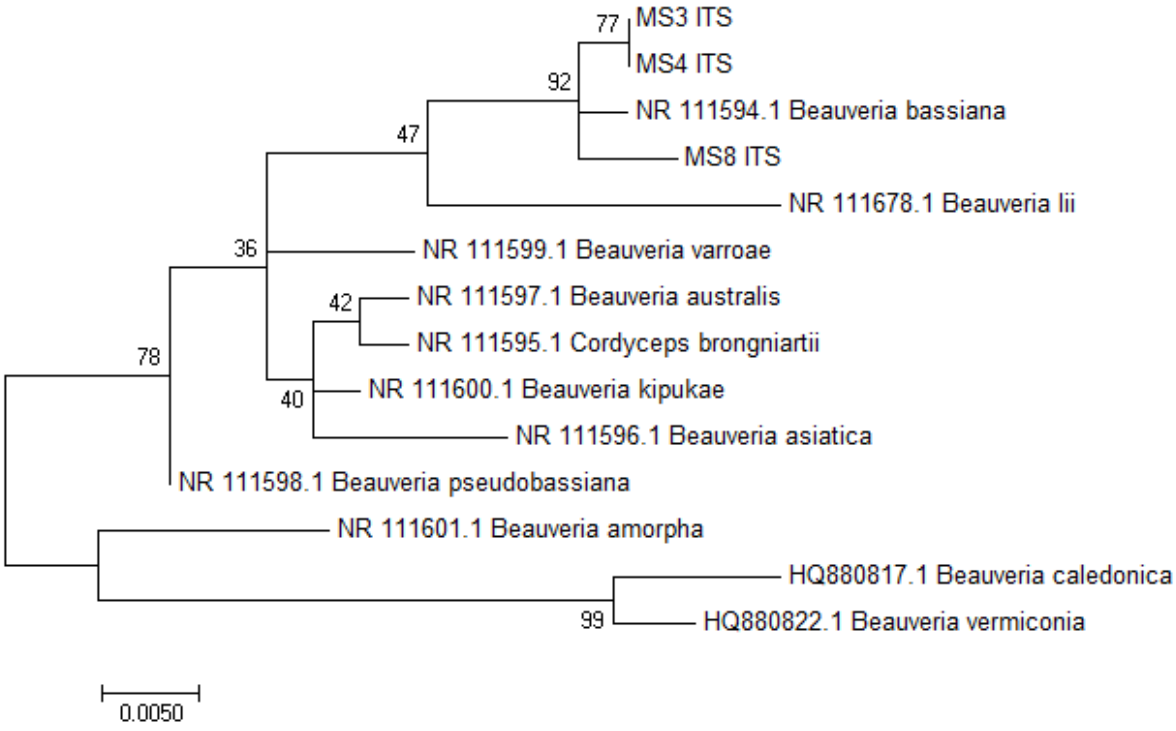

Fig. 3.8 ML tree of MS-3, MS-4 and MS-8 with 11 other type-material sequence accessions from the NVCBI Genbank database. Accession numbers are shown for Genbank serquences. The scale for branch length indicates the number of substitutions per site and bootstrap values are shown at the nodes.

3.4 Discussion

The use of *G. mellonella* larvae as abait produced many isolates of entomopathogenic fungi, verifying that the bait method with *G. mellonella* (Zimmermann 1986) is a very sensitive method for the detection of entomopathogenic fungi in soil samples (Keller et al. 2003). Accordingly, in this study the entomopathogeinc fungi were isolated from soil samples baited with the larvae of *G. mellonella.* The entomopathogenic fungus *B. bassiana* was found more

frequently than *M. anisopilae*. Over 80% of our collected isolates were *B. bassiana*. This confirm the results of Hassan et al. (2012) who found that *B. bassiana* was the dominant entomopathogenic fungus in their study when they targeted specific sites there. Similarly, Asensio et al. (2003) observed that *B. bassiana* was the most frequent found entomopathogenic fungi in the Alicante Province in Spain, being found in 32.8% of tested soils.

The insecticidal efficacy of *B. bassiana* is highly influenced by several factors such as insect behavior, population density, age, nutrition and genetic information. The physiology and morphology of the host affect its sensitivity to biological control agents such as entomopathogenic fungi (Fargues et al. 1996). In general, host physiology and fungal physiology (activity enzymes and toxins) have a great impact on the virulence of entomopathogenic fungi. In this study mortality levels ranged from 10.0 to 80.0% on *S. zeamais* following exposure to 28 *B. bassiana* strains applied at a dose of $2x10^8$ conidia g^{-1}. Among these strains, MS-8, MS-3 and MS-4 caused the highest mortality levels of 80.0% 73.0% and 70.0% on *S. zeamais*, respectively. Adane (2003) also reported that *B. bassiana* strains appeared virulent on *S. zeamais* but only at doses higher than $1x10^7$ conidia ml^{-1}; and that variability within the different *B. bassiana* strains was apparent.

The dose response assay indicated that *B. bassiana* Strain MS-8 caused higher mortality levels at all doses against the three tested insect than Strain MS-3 and Strain MS-4. Application of the same strain at a dose of $2x10^8$ conidia g^{-1} caused mortality levels of 90.0, 76.0, 75.0 and 65% for *E. cautella* L3 larvae, *L. serricorne* adults, *S. zeamais* and *L. serricorne* L3 larvae, respectively. The same strain caused the lowest LD_{50} values and LT_{50} values against the three insects, followed by Strains MS-3 and MS-4. These results indicated that *E. cautella* was the most susceptible to Strain MS-8. This susceptibility might have been due to the high mortality of this insect which would increase the chances of the larvae making contact with conidia. This result contradicts the finding of Kaur et al. (2014) who observed mortality less than 50.0% on the Indian meal moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), after application with *B. bassiana* at a dose of $2.2x10^8$ spore ml^{-1}. In contrast, *L. serricorne* L3 larvae were the most tolerant to application of Strain MS-8. According to Zhu et al. (2009), the application of a conidial suspension of *B. bassiana* (Bb050722) at a dose of 1.46 x 10^8 spores ml^{-1} caused 100% mortality of *L. serricorne* L2 larvae. Contrary to this finding, the present study found that *L. serricorne* L3 larvae were the

most tolerate to Strain MS-8. This might be due to the limited movement of these larvae, which would reduce their chances of contacting conidia.

In the case of *S. zeamais* and *L. serricorne* adults, Strain MS-8 caused moderate mortality with LD_{50} values of $9x10^6$ and $5x10^6$ conidia g^{-1} and LT_{50} of 5.8 and 5.2 days, respectively. Considering formulation type Eshome & Tefera (2009) observed a similar LT_{50} of 4.0 days for *S. zeamais* after treatment with *B. bassiana* PPRC-GC at a dose of $1x10^8$ conidia ml^{-1}. Kassa et al. (2002) observed that *B. bassiana* PPRC-HH generated an LD_{50} of $2.04x10^6$ conidia ml^{-1} on *S. zeamais*, which was in the same range as found in this study.

The BLAST search suggested that the most likely identity of all the strains was *B. bassiana*. The slight difference in sequence similarity between MS-8 and MS-3/MS-4 prompted further phylogenetic analysis of the strains. While Nilsson et al. (2012) suggest that BLAST identity of 97–98% with 90% coverage is suitable for species identification in many cases, they also warned of this 'oversimplification' of fungal taxonomic assignment. However, the phylogenetic tree elucidated further on the BLAST results, showed that the strains fell within the clade that included *B. bassiana;* and since Nilsson et al. (2008) suggested a 3% threshold for intraspecific variation, it is most likely that these represent strains of *B. bassiana*.

Meikle et al. (2001) found that the application of *B. bassiana* conidia formulated in oil was able to reduce a population of the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), but did not prevent grain losses entirely. This finding suggests that further development of an effective stored-product biocontrol agent should concentrate on finding effective isolates and appropriate formulation (Meikle et al. 2001).

In conclusion, *B. bassiana* Strain MS-8 emerged as the best candidate for further research in order to develop a mycoinsecticide for stored grain pests. Further aspects, such as mass production, formulation, storage, spectrum of activity to broad range of stored grain pests and safety to non-target organisms are essential steps for biocontrol product development.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Economic Entomology 18: 265-267.

Abebe F, Tefera T, Mugo S, Beyene Y, Vidal S. 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). African Journal of Biotechnology 8: 5937-5943.

Adane K. 2003. Development and Testing of Mycoinsecticides Based on Submerged Spores and Aerial Conidia of the Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) For Control of Locusts and Grasshoppers. PhD Thesis, Georg-August-Univesity, Gottingen, Germany.

Almeida JD, Alves S, Pereira R. 1997. Selection of *Beauveria* spp. isolates for control of the termite *Heterotermes tenuis* (Hagen, 1858). Journal of Applied Entomology 121: 539-543.

Asensio L, Carbonell T, López-Jiménez J, Lopez-Llorca L. 2003. Entomopathogenic fungi in soils from Alicante province [Spain]. Spanish Journal of Agricultural Research 1: 37-45.

Batta YA. 2016. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology 26: 1171-1183.

Cortez-Madrigal H, Alatorre-Rosas R, Mora-Aguilera G, Bravo-Mojica H, Ortiz-Garcia C, Aceves-Navarro L. 2003. Characterization of multisporic and monosporic isolates of *Lecanicillium* (= *Verticillium*) *lecanii* for the management of *Toxoptera aurantii* in cocoa. BioControl 48: 321-334.

Crespo R, Júrez M, Dal Bello G, Padin S, Fernández GC, and Pedrini N. 2002. Increased mortality of *Acanthoscelides obtectus* by alkane-grown *Beauveria bassiana*. BioControl 47: 685-696.

Fargues J, Goettel M, Smits N, Ouedraogo A, Vidal C, Lacey L, Lomer C, Rougier M. 1996. Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic Hyphomycetes. Mycopathologia 135: 171-181.

Feng M, Poprawski T, Khachatourians GG. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology 4: 3-34.

Giga D, Mutemerewa S, Moyo G, Neeley D. 1991. Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. Crop Protection 10: 287-292.

Glare TR, Inwood AJ. 1998. Morphological and genetic characterisation of *Beauveria* spp. from New Zealand. Mycological Research 102: 250-256.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41: 95-98.

Hassan W, Assaf LH, Abdullah SK. 2012. Occurrence of entomopathogenic and other opportunistic fungi in soil collected from insect hibernation sites and evaluation of their entomopathogenic potential. Bulletin Iraq Natural History Museum 12: 19-27.

Hegedus DD, Khachatourians GG. 1993. Construction of cloned DNA probes for the specific detection of the entomopathogenic fungus *Beauveria bassiana* in grasshoppers. Journal of Invertebrate Pathology 62: 233-240.

Hegedus DD, Khachatourians GG. 1996. Identification and differentiation of the entomopathogenic fungus *Beauveria bassiana* using polymerase chain reaction and single-strand conformation polymorphism analysis. Journal of Invertebrate Pathology 67: 289-299.

Hidalgo E, Moore D, Le Patourel G. 1998. The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize. Journal of Stored Products Research 34: 171-179.

Horak M. 1994. A review of Cadra Walker in Australia: five new native species and the two introduced pest species (Lepidoptera: Pyralidae: Phycitinae). Australian Journal of Entomology 33: 245-262.

Humber R. 2012. Identification of entomopathogenic fungi, *In* Lacey LA [ed.], Manual of Techniques in Invertebrate Pathology. Elsevier, Waltham, USA.

Kassa A, Zimmermann G, Stephan D, Vidal S. 2002. Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to entomopathogenic fungi from Ethiopia. Biocontrol Science and Technology 12: 727-736.

Kaur S, Thakur A, Rajput M. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Journal of Stored Products Research 59: 185-189.

Keller S, Kessler P, Schweizer C. 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metarhizium anisopliae*. BioControl 48: 307-319.

Khashaveh A. 2011. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill., in assays with storage grain beetles. Journal of Agricultural Science and Technology 13: 35-43.

Kosir JM, MacPherson JM, Khachatourians GG. 1991. Genomic analysis of a virulent and a less virulent strain of the entomopathogenic fungus *Beauveria bassiana*, using restriction fragment length polymorphisms. Canadian Journal of Microbiology 37: 534-541.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870-1874.

Meikle WG, Cherry AJ, Holst N, Hounna B, Markham RH. 2001. The effects of an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), *Sitophilus zeamais* motschulsky (Col.: Curculionidae), and grain Losses in stored maize in the Benin Republic. Journal of Invertebrate Pathology 77: 198-205.

Metwally HM, Hafez GA, Hussein MA, Hussein M, Salem H, Saleh M. 2012. Low cost artificial diet for rearing the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), as a host for entomopathogenic nematodes. Egyptian Journal of Biological Pest Control 22, 15-17.

Moino A, Alves S, Pereira R. 1998. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored-grain pests. Journal of Applied Entomology 122: 301-305.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson KH. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evolutionary Bioinformatics Online 4: 193-201.

Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JA, Bergsten J, Porter TM. 2012. Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys 4: 37-63.

Papadopoulou SC, Buchelos CT. 2002. Identification of female adult *Lasioderma serricorne* (F.) by simple external observation of the abdomen. Journal of Stored Products Research 38: 315-318.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. Genstat for Windows, 17th Edition. VSN International, Hemel Hempstead, UK.

Pereira C, Pereira E, Cordeiro E, Della Lucia T, Tótola M, Guedes R. 2009. Organophosphate resistance in the maize weevil *Sitophilus zeamais*: magnitude and behavior. Crop Protection 28: 168-173.

Sedehi A, Sedaghatfar E, Modarres Najafabadi S. 2014. Studies on effect of the *Beauveria bassiana* on eggs and larvae of *Plodia interpunctella*. Candian Journal of Basic and Applied Sciences 2: 40-45.

St Leger R, Allee L, May B, Staples R, Roberts D. 1992. World-wide distribution of genetic variation among isolates of *Beauveria* spp. Mycological Research 96: 1007-1015.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

Teshome A, Tefera T. 2009. Susceptibility of *Sitophilus zeamais* (Mostch.) (Coleoptera: Curculionidae) to *Beauveria bassiana* and *Metarhizium anisopliae*. *SINET:* Ethiopian Journal of Science 32: 21-28.

Wang C, Shah FA, Patel N, Li Z, Butt TM. 2003. Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. Environmental Microbiology 5: 908-915.

Zhu Y, Fang L, Chen B, Li ZY. 2009. Virulence of *Beauveria bassiana* against the Instar-Larvae of *Lasioderma serricorne* (Fabricius). Journal of Yunnan Agricultural University 24: 42-46.

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

Chapter Four

Evaluating carriers for the formulation of conidia of *Beauveria bassiana* (Balsamo) Vuillemin Strain MS-8, testing kaolin versus cornflour against three species of storage pests in rice grains

Abstract

The effects of kaolin alone at doses of 1, 2, 3, 4, 6 and 10 g kg^{-1} of grain was evaluated against *Sitophilus zeamais, Ephestia cautella* L3 larvae and *Lasioderma serricorne* L3 larvae and adults. Kaolin applied at a dose of 3g kg^{-1} of grain caused mortality levels less than 50.0% for the three insects. At a dose of 10g kg^{-1} 100% mortality occurred for two insects, but only 20.0% for *S. zeamais*. The effects of kaolin and cornflour used as carriers for conidia of *Beauveria bassiana* Strain MS-8 were evaluated at doses of $2x10^6$, $2x10^7$, $2x10^8$ and $2x10^9$ conidia g^{-1}, against the same tested insects. Kaolin was a consistently better carrier than cornflour at all doses against the three insects, except for *E. cautella* L3 larvae, for which both carriers had similar effects on mortality. The lowest LD_{50} and LT_{50} were observed with kaolin for the three insects. Observations made with scanning electron microscope showed kaolin abraded the waxy epidermis of *S. zeamais* and *L. serricorne* adults while insects were not affected when the carrier was cornflour. The best conidial dose ($2x10^9$ conidia g^{-1}= 0.03g conidia kg^{-1} of grain) was formulated in both carriers at doses of 1, 2, 3, and 4g kg^{-1} of grain. A 100% mortality of insects was observed with Strain MS-8 at kaolin doses of 3g and 4g kg^{-1} of grain against the tested insects, except *S. zeamais. S. zeamais* and *E. cautella* L3 larvae did not show a dose response to *B. bassiana* formulated in cornflour at any dose. In contrast, *L. serricorne* responded to the same doses and developed mortality levels of between 60-75.7% for L3 larvae and 76.0-95.0% for adults. The present study showed that *B. bassiana* MS-8 at a dose of $2x10^9$ conidia g^{-1}= 0.03g conidia kg^{-1} of grain, formulated in kaolin provided good control of the tested insects in rice.

4.1 Introduction

Fumigants and conventional insecticides have been used for the management of storage pests in grain stores. These practises have problems (Michalaki et al. 2006). There is an increasing awareness of quality and environmental issues, such as pesticide contamination, emergence of pesticide tolerant insects and pesticide hazards to the environment and especially to

consumers. It is clear that global agriculture needs alternative strategies to control stored grain pests.

Entomopathogenic fungi (EPF) have been tested in several studies against various stored grain pests under both laboratory and field conditions (Akbar et al. 2004; Batta 2004; Kavallieratos et al. 2006; Samodra & Ibrahim 2006a; Sabbour 2010). *Beauveria bassiana* (Balsamo) Vuillemin has been one of the most widely researched EPF and has displayed potential in controlling storage grain pests (Akbar et al. 2004; Lord 2001, 2005).

Formulation of *B. bassiana* is an essential aspect in biocontrol product development because it may improve the efficacy of the fungal conidia, and may allow a product to be stored over a long period of time. The performance of fungi against target pests can be improved by suitable formulation and application technologies (de Faria & Wraight 2007). Materials such as talc, kaolin, tapioca flour and cornflour have been used as carriers to enhance the shelf life, delivery, persistence and efficacy of various mycoinsecticides.

Kaolin is a naturally occurring, chemically inert clay mineral. It has been used to decrease the adverse effects of environmental stresses on crop plants, to suppress diseases, and to protect crops from insect pests (Glenn & Puterka 2005; Kahn & Damicone 2008). Complete mortality was achieved in one week after exposure of the adults of *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) and *Tribolium confusum* Duval (Coleoptera: Tenebrionidae) in wheat grains treated with kaolin alone at dose of 8% w/w (El-Sayed et al. 2010). Kaolin has also been effective against the Mexican bean weevil, *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae), after exposing the insect to beans treated with kaolin at doses of 2, 4 and 8g kg^{-1}. Complete mortality was recorded even at the lowest dose of 2g kg^{-1}, in seven and six days, for male and female insects, respectively (Mikami et al. 2010). In contrast, the use of acid-activated kaolin at 75.0% w/w against the adult populations of four storage grain insects infesting paddy rice showed that the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), was the most tolerant (Permual & Le Patourel 1990).

Kaolin is also effective in controlling insects when used in combination with other components. Samodra & Ibrahim (2006a) studied the admixture of entomopathogenic fungi *B. bassiana* isolates BbGc and BbPs with either kaolin, talc or tapioca flour (20.0% w/w a.i.). Applied at the lowest rate of 0.05g a.i. in 50g of long grain rice caused in excess of 80.0% mortality of *S. oryzae* adults by the 7th day of exposure. On the other hand, the use of

cornflour as the carrier for *B. bassiana* Strain GHA (BotaniGard® 22WP-Canada) at a dose of $1x10^8$ conidia g^{-1} to control varroa mites in honey bee hives was not effective (Sinia 2013).

The insecticidal activity of kaolin toward many storage grain pests has been reported. However, there is no report on the insecticidal effect of kaolin alone or in combination with *B. bassiana* on *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae). As for the insecticidal activity of kaolin against *S. zeamais* and the almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae) it is limited. In addition, cornflour has not been investigated as a carrier of *B. bassiana* conidia against these test insects. Therefore, the objectives of this study were: (i) to evaluate the effects of kaolin alone at various doses against *S. zeamais, E. cautella* L3 larvae, and *L. serricorne* L3 larvae and adults; (ii) to compare kaolin versus cornflour as the carrier for *B. bassiana* Strain MS-8 against the three insects; and (iii) to evaluate the effects of increasing the volume of carriers applied with a fixed dose of *B. bassiana* Strain MS-8.

4.2 Material and methods

4.2.1 Insects 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. The tested insects were reared as described in Chapter Three, Section 3.2.3, and were then used under experimental conditions of 28±2°C and 65±5.0% RH.

4.2.2 Fungal strain and dried conidia preparations

The strain of *B. bassiana*, MS-8, used in this study was isolated from soil using *Galleria mellonella* L (Lepidoptera: Pyralidae) as a live insect bait (Zimmermann 1986). The soil sample was collected from the Ukulinga Research Farm, Pietermaritzburg (KwaZulu-Natal Province), situated at the geographical coordinates of latitude 29.67S, longitude 30.41E and altitude of 809 meters above sea level. The fungus was grown for 15 days at 25±1°C in the dark on potato dextrose agar[1] (PDA) (Biolab Merck (Pty) Ltd. The PDA was prepared using 4g potato extract, 20g dextrose and 15g agar in 1 L of distilled water as directed by the manufacturer. Ampicillin and streptomycin[2] at 0.05g L^{-1} were added to the medium to inhibit

[1] Merck (Pty) Ltd 1, Friesland Drive, Longmeadow Business Estate , Modderfontein, 1645, South Africa
[2] Inqaba Biotechnical Industries (Pty) Ltd, 525 Walker St, Pretoria, 0002, South Africa.

contaminant bacteria and to obtain a pure culture of the fungus. Mycelium and conidia were removed from agar surface by scraping the surface with a sterile scalpel before placing them into a 10ml McCartney bottle with 5ml of (30.0% v/v) glycerol, after which the bottles were then stored at -20^{o}C as a stock suspension for further use (Oliveira et al. 2011).

A 1ml conidial suspension from the stock was suspended in 10 ml of sterile distilled water with 0.01% Tween 80 in a sterile 20 ml bottle. The suspension was filtered through a mesh cloth to remove mycelial debris. A concentration of 10^8 conidia ml^{-1} was determined using a Neubauer Improved Hemocytometer[3]. The dried conidia were prepared as described in Chapter Two, Section 2.2.6.

4.2.3 Carriers

Kaolin was provided by Plant Health Products[4] (PHP) (Pty) Ltd. Cornflour was obtained from a local supermarket (Premier[5] (Pty) Ltd). The moisture content of the cornflour was not quantitatively estimated but a visual and tactile assessment was that it was dry with no perceptible moisture levels.

4.2.4 Effects of kaolin on tested insects

Kaolin was applied to rice grains at doses of 1, 2, 3, 4, 6 and 10 g kg^{-1} of grain. Kaolin powder and rice grains were mixed by manual shaking to obtain an even distribution of powders. For each dose, three glass jars with 100g kaolin treated rice were used as replicates. For the control, rice grains were kept untreated. Thirty adults of *S. zeamais* (two days old), 30 L3 larvae of *E. cautella*, and 30 *L. serricorne* L3 larvae and adults (one day old) were introduced independently into each jar. Mortality was recorded daily for 10 days for all the tested insects.

4.2.5 Evaluation of kaolin versus cornflour as carriers for *B. bassiana* Strain MS-8 in multiple dose assays

For each carrier, the conidial doses were prepared from an initial stock of $2x10^{10}$ conidia g^{-1}(kaolin or cornflour), which was prepared by using a Neubauer Improved Hemocytometer,

[3] Hirschmann®, Pietermaritzburg, South Africa.
[4] Plant Health Products (Pty) Ltd., P.O. Box 207, Nottingham Road, South Africa.
[5] Premier (Pty) Ltd, Building 5, Maxwell Office Park, Magwa Crescent West, Waterfall City, 2090, South Africa.

and diluted with their respective carrier to $2x10^9$, $2x10^8$, $2x10^7$ and $2x10^6$ conidia g^{-1}. Thirty adults of *S. zeamais*, 30 L3 larvae of *E. cautella*, and 30 *L. serricorne* L3 larvae and adults were placed separately into glass jars with 100g rice treated with the respective doses. The control contained carrier only. Each treatment was replicated three times. Mortality was monitored dialy for 10 days. Dead insects were removed and fungal growth was checked. Scanning electron microscope[6] (SEM) observations were made to evaluate the effects that each carrier alone had on *S. zeamais* and *L. serricorne* adults.

4.2.6 Effect of increasing the volume of carriers on mortalities of tested insects

Kaolin and cornflour were used at doses of 1, 2, 3, 4 g kg^{-1} of grain. Treatments were prepared by mixing *B. bassiana* MS-8 at fixed dose of 0.03g conidia kg^{-1} of grain with kaolin and cornflour at each dose. Thirty adults of *S. zeamais*, 30 L3 larvae of *E. cautella* and 30 L3 larvae and adults of *L. serricorne* were each introduced into glass jars containing 100g rice after applying the respective treatment. The treatments were replicated three times for each dose, with an untreated control (UTC). The experiment was carried out for 10 days and the daily mortality counts were taken. To establish whether mortality in the tested insects had been caused by the fungus, all cadavers were checked under a light microscope, and the presence or absence of hyphae and conidia on cadaver surfaces were determined.

4.2.7 Statistical analysis

For evaluation of kaolin versus cornflour as carriers for *B. bassiana* Strain MS-8 mortality counts of insects were corrected relative to the control using Abbott's formula (Abbott 1925). Mortality was zero in the control treatment for the other experiments. The number of insects with mycosis was estimated as a percentage of dead insects. The data were subjected to analysis of variance (ANOVA) using GenStat for Windows, 17th edition (Payne et al. 2014). Means of mortality were separated using Fisher's Least Significant Difference test at the $P < 0.05$ level of significance. Probit analysis was used to estimate the lethal dose (LD_{50}) and lethal time (LT_{50}) causing 50.0% mortality of *B. bassiana* MS-8 formulated in kaolin or cornflour against the three insects.

[6] ZEISS, EVO LS 15, Germany.

4.3 Results

4.3.1 Effects of kaolin on tested insects

All main effects as well as the associated interaction were highly significant at $p < 0.001$ (Table 4.1). *S. zeamais* adults were not affected by the kaolin treatment on rice even at the maximum dose of 10g kg^{-1} of grain, which resulted in 20.0% mortality. However, in *E. cautella* L3 larvae 100% mortality was observed at the same dose of 10g kg^{-1} of grain. At 3g and 4g kg^{-1} of grain, 41.0% and 43.0% mortality occurred, respectively. At 6g kg^{-1} of grain the mortality level was doubled to 87.0% for *E. cautella* L3 larvae (Fig. 4. 1).

L. serricorne L3 larvae were little affected by kaolin treatments at doses of 1g to 3g kg^{-1} of grain, with the resulting mortalities of 4.0% to 23.33%, respectively. Mortality almost doubled to 50.0% and 90.0% for the same insect at doses of 4g and 6g kg^{-1} of grain, respectively. 100% mortality on *L. serricorne* L3 larvae was observed at a kaolin dose of 10g kg^{-1} of grain. The adults and L3 larvae of *L. serricorne* were affected in the same way except at 3g kg^{-1} of grain where the adults were twice as susceptible as L3 larvae (Fig. 4.1).

Table 4.1 ANOVA table for main effects and associated interaction for kaolin effects against the test insects

source of variation	d.f.	s.s.	m.s.	F values	P values
Insects	3	26949.6	8983.2	633	<.001
Doses	5	58202.9	11640.58	820.25	<.001
Insects x Doses	15	12646.82	843.12	59.41	<.001
Residual	46	652.81	14.19		
Total	71	98557.99			
CV%	9.8				
Mean ± SE	38.26 ± 2.18				
LSD	6.191				

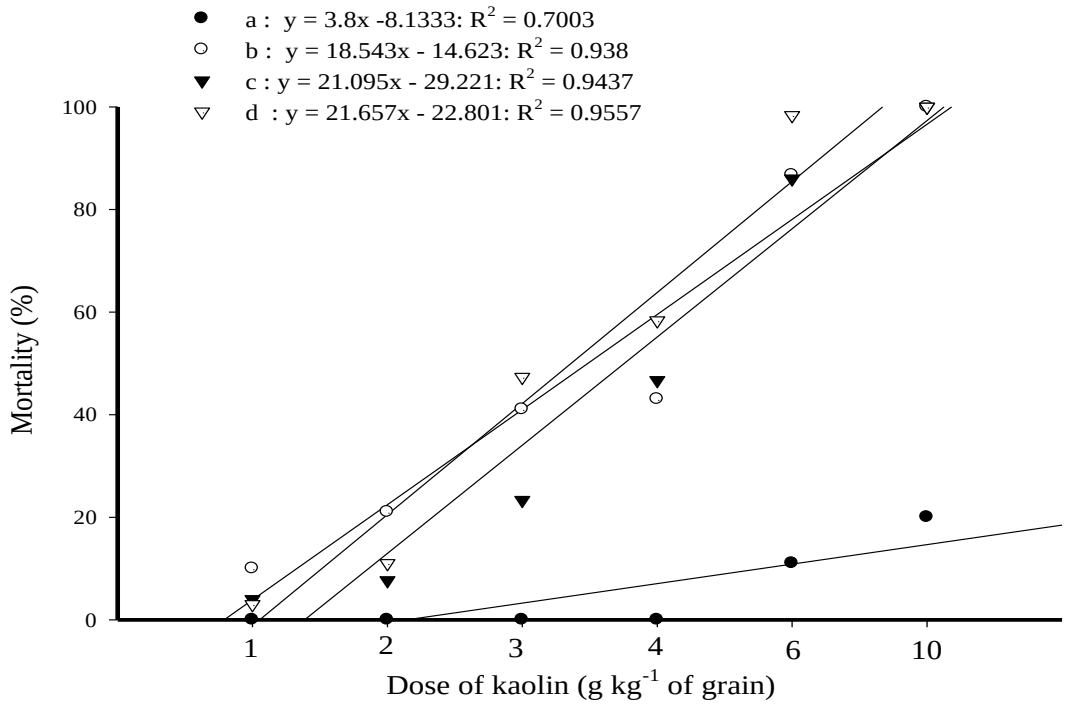

Fig. 4.1 Mortality of **a** = *Sitophilus zeamais* adults, **b** = *Ephestia cautella* third instar larvae and **c** = *Lasioderma serricorne* third instar larvae and **d** = *Lasioderma serricorne* adults, after exposure to kaolin treated rice at different doses, observed 10 days after treatment. Control = zero mortality

4.3.2 Evaluation of kaolin versus cornflour as carriers for *B. bassiana* Strain MS-8 in multiple dose assays

All main effects, as well as the associated interactions, were highly significant at $p < 0.001$ except for the interaction of carriers x dose, and the three way interaction, which were significant at $P \leq 0.05$ (Table 4.2). Mortality increased with increased dosage levels for all insects treated with *B. bassiana* strain MS-8 formulated in kaolin or cornflour.

As a carrier of *B. bassiana* MS-8 kaolin was consistently better than cornflour at all doses against three insects, except in *E. cautella* L3 larvae where both carriers affected the insect in the same way (Fig. 4.2). The highest mortality levels were observed after exposure of the insects to *B. bassiana* MS-8 formulated in kaolin at a dose of $2x10^9$ conidia g^{-1}. *E. cautella* L3 larvae (95.0%) was the most affected followed by *L. serricorne* adults (91.0%), while *S. zeamais* and *L. serricorne* L3 larvae 80.0%, respectively, were affected in the same way (Fig. 4.2). *S. zeamais* and *L. serricorne* L3 larvae were less affected by *B. bassiana* MS-8

formulated in cornflour, even at the highest dose of $2x10^9$ conidia g^{-1}, where mortality levels were 62.0% and 64.0%, respectively (Fig. 4.2). Observation by SEM showed kaolin abraded the waxy epidermis of *S. zeamais* and *L. serricorne* adults. Cornflour did not abrade the insects (Fig. 4.3).

Table 4.2 ANOVA parameters for the effects of *B. bassiana* MS-8 formulated in kaolin and cornflour at various doses against three insects

source of variation	d.f.	s.s.	m.s.	F values	P values
Carriers	1	2081.34	2081.34	103.46	<.001
Doses	3	34812.5	11604.5	576.84	<.001
Insects	3	4812.11	1604.04	79.74	<.001
Carriers x Doses	3	239.61	79.87	3.97	0.012
Carriers x Insects	3	1214.95	404.98	20.13	<.001
Doses x Insects	9	775.09	86.12	4.28	<.001
Carriers x Doses x Insects	9	369.93	41.1	2.04	0.049
Residual	62	1247.23	20.12		
Total	95	46403.49			
CV%	7.9				
Mean ± SE	56.57 ± 2.59				
LSD	7.32				

Probit analyses were performed separately for each insect to determine the LD_{50} and LT_{50} values. Results are presented in Fig. 4.4 and Fig. 4.5. Lower LD_{50} and LT_{50} values were achieved for three insects by using kaolin, compared to cornflour, with the exception of *E. cautella* L3 larvae.

In the case of *E. cautella* L3 larvae, there were no significant differences in LD_{50} and LT_{50} values resulting from both carriers. The lowest LD_{50} and LT_{50} values were recorded for *S. zeamais* adults and *E. cautella* L3 larvae when using kaolin as carrier (Fig. 4.4 and Fig. 4.5).

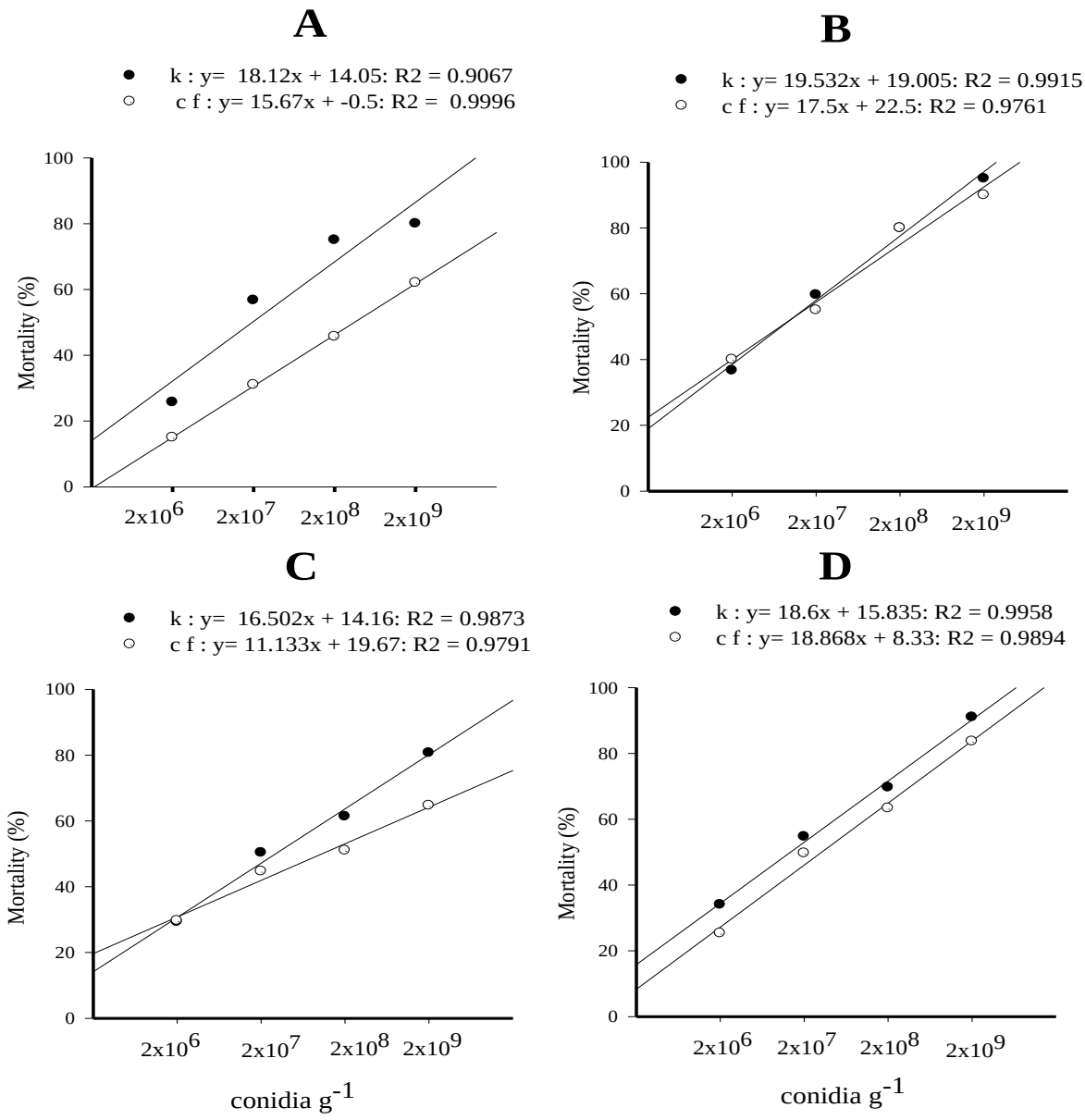

Fig. 4.2 Corrected mortality (Abbott's correction) of **A** = *Sitophilus zeamais,* **B** = *Ephestia cautella* third instar larvae, **C** = *Lasioderma serricorne* third instar larvae and **D** = *Lasioderma serricorne* adults, upon exposure to rice treated with *Beauveria bassiana* MS-8 formulated in **k** = kaolin and **c f** = cornflour at doses of $2x10^6$, $2x10^7$, $2x10^8$ and $2x10^9$ conidia g^{-1} in kaolin and cornflour, observed 10 days after treatment.

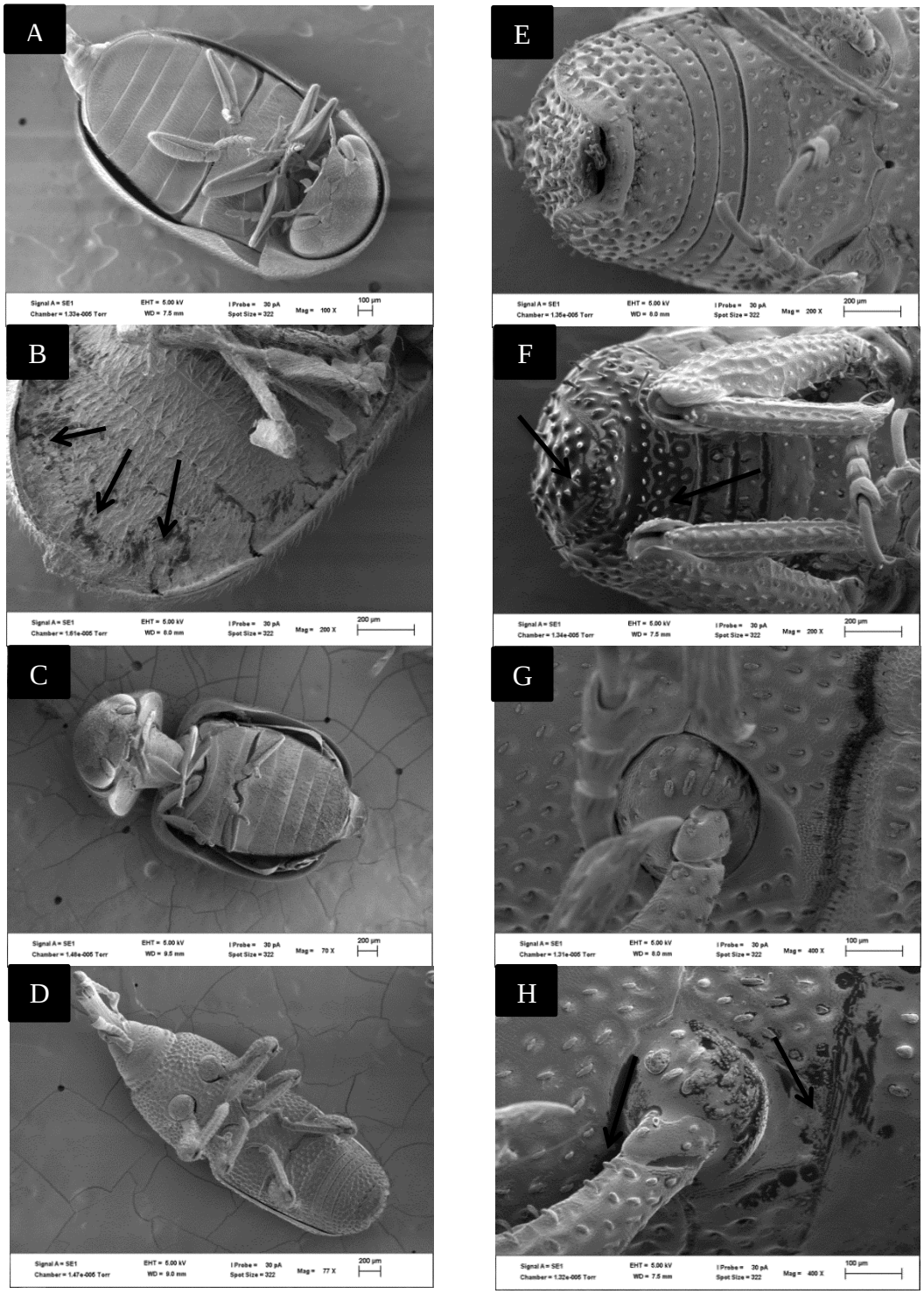

Fig. 4.3 Scanning electron micrographs of kaolin and cornflour effects applied at a dose of 1g kg^{-1} of grain on *Lasioderma serricorne*. **A** = Control; **B** = arrows show kaolin effects on the posterior end; **C** = cornflour effect on *Sitophilus zeamais*; **D** = cornflour effect; **E** = Control; **F** = arrows show kaolin effects on the posterior end; **G** = Control; **H** = arrows show kaolin effects on the forelegs coxa.

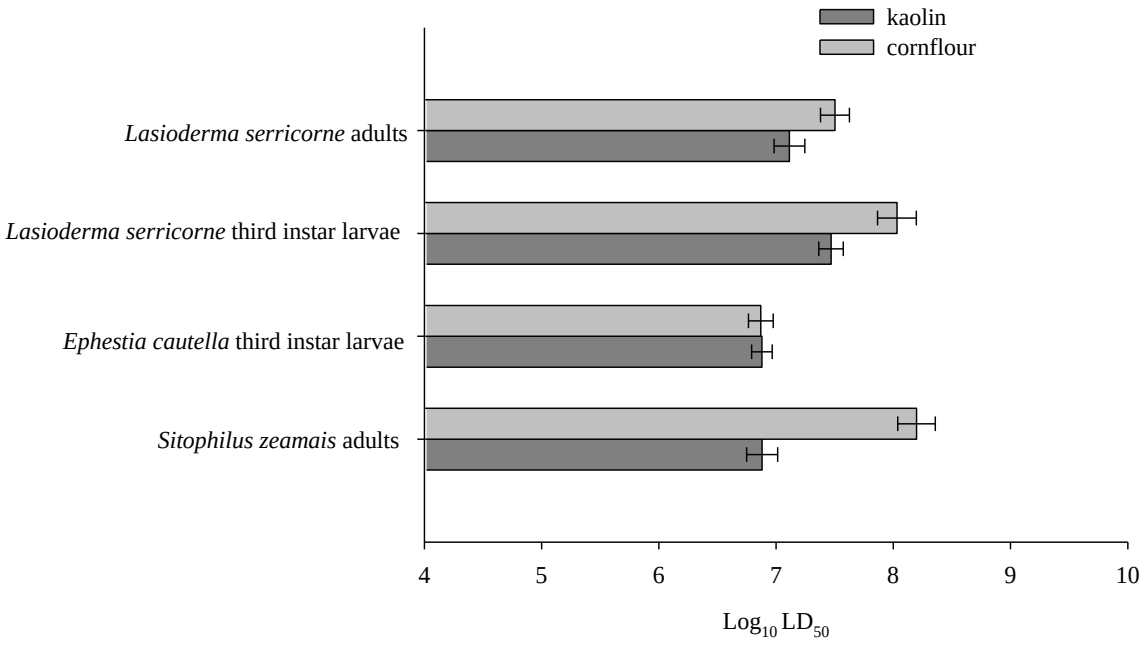

Fig. 4.4 Lethal dose for the 50.0% (LD_{50}) of the tested insects, upon exposure to rice treated with *Beauveria bassiana* MS-8 formulated in kaolin or cornflour at doses of $2x10^6$, $2x10^7$, $2x10^8$ and $2x10^9$ conidia g^{-1}

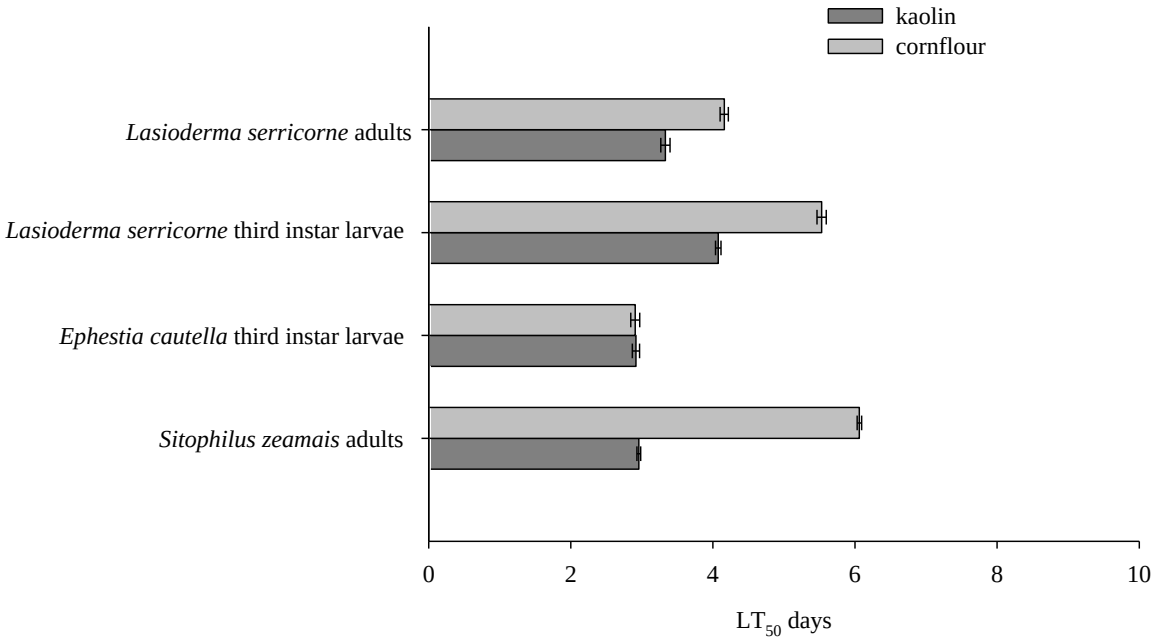

Fig. 4.5 Lethal time for 50.0% of the tested insects (LT_{50}), upon exposure to rice treated with *Beauveria bassiana* MS-8 formulated in kaolin or cornflour at a dose of $2x10^9$ conidia g^{-1}.

4.3.3 Effect of carriers doses on mortalities of tested insects

All main effects, as well as the associated interactions, were significant at the $p < 0.001$ level, except for the carriers x doses interaction, which was not significant and the three way interaction that was significant at $P = 0.012$ (Table 4.3).

After increasing carriers doses, kaolin generated a higher mortality than cornflour at all doses for the three insects except for *E. cautella* L3 larvae, where both carriers affected the insect in similar way and mortality levels were not significantly different. *B. bassiana* MS-8 formulated in kaolin at doses of 3g or 4g kg^{-1} of grain caused 100% mortality against two insects, but with *S. zeamais*, the highest mortality level was 94.50% at a kaolin dose of 4g kg^{-1} (Fig. 4.6). *B. bassiana* Strain MS-8, formulated in cornflour at doses of 1g to 4g kg^{-1} of grain, caused mortalities ranging from 62.0 – 65.0% and 90.0 – 95.0%, for *S. zeamais* and *E. cautella* L3 larvae, respectively (Fig. 4.6).

In contrast, *L. serricorne* L3 larvae and adults responded to the same doses and developed mortality levels of 60.0 – 75.0% and 76.0 – 95.0%, respectively. *S. zeamais* was the most tolerant insect after being treated with *B. bassiana* MS-8 formulated in kaolin or cornflour. *E. cautella* L3 larvae were more susceptible compared with the target pests. With *L. serricorne* the adults were more susceptible than the L3 larvae (Fig. 4.6).

Table 4.3 ANOVA table for main effects and associated interactions for mortality of tested insects

Source of variation	d.f.	s.s.	m.s.	F values	P values
Carriers	1	4473.65	4473.65	210.63	<.001
Doses	3	2233.7	744.57	35.06	<.001
Insects	3	6939.05	2313.02	108.9	<.001
Carriers x Doses	3	101.79	33.93	1.6	0.199
Carriers x Insects	3	1130.17	376.72	17.74	<.001
Doses x Insects	9	537.71	59.75	2.81	0.008
Carriers x Doses x Insects	9	503.42	55.94	2.63	0.012
Residual	62	1316.86	21.24		
CV%	5.5				
Mean ± SE	84.06 ± 2.661				
LSD	7.522				

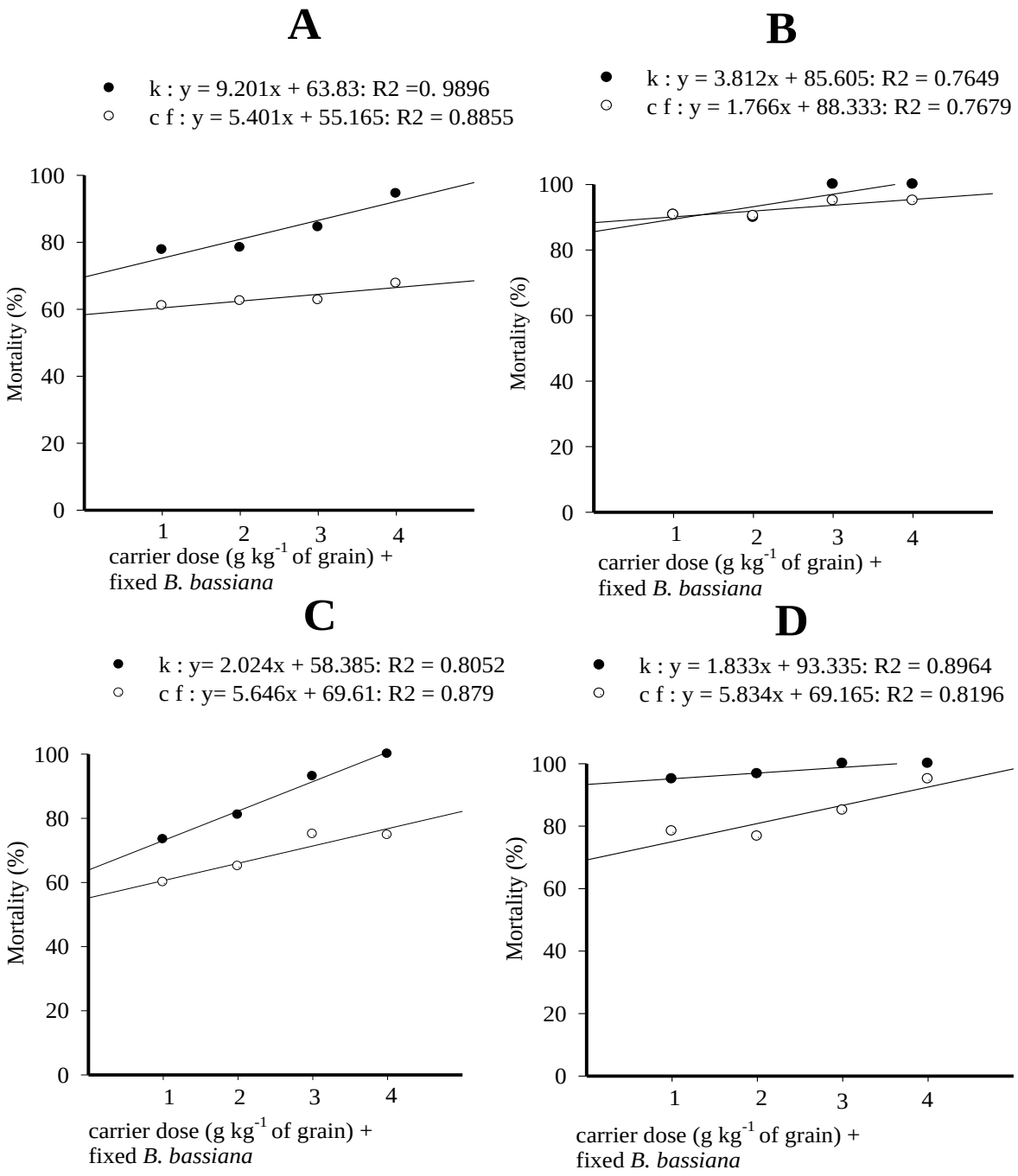

Fig. 4.6 Mortality of **A** = *Sitophilus zeamais,* **B** = *Ephestia cautella* third instar larvae*,* **C** = *Lasioderma serricorne* third instar larvae and **D** = *Lasioderma serricorne* adults, upon exposure to rice treated with a fixed dose of *Beauveria bassiana* MS-8 at 0.03g conidia kg^{-1} of grain formulated in **k** = kaolin and **cf** = cornflour at 1, 2, 3 and 4g kg^{-1} of grain, observed for 10 days after introduction. Control = zero mortality

4.4 Discussion

The effects of kaolin powder at doses of 1, 2, 3, 4, 6, and 10 g kg^{-1} of grain as a control measure against *S. zeamais*, *E. cautella* L3 larvae and *L. serricorne* L3 larvae and adults were studied, aiming to discover novel treatments to manage these insects. With kaolin treated rice after 10 days *S. zeamais* was found to be the most tolerant, even at a high dose of 10g $kg^{-1}$of grain. Only 20.0% mortality was recorded as compared to 100% mortality for the other insects. This may be due to the hard cuticle of this insect. This result is in agreement with Permual & Le Patourel (1990) who reported that *S. zeamais* was relatively tolerant after it was treated with acid-activated kaolin at 75.0% w/w against the adult populations of four storage insects on paddy rice. If kaolin insecticidal effects are consistant acrros species, then our result would apply to *S. oryzae* and the closely related species *R. dominica* and *T. confusum*. In that case, our results differ from those of El-Sayed et al. (2010) who observed complete mortality after one week of exposure of the adults of *S. oryzae*, *R. dominica* and *T. confusum* to kaolin treated wheat at a dose of 8.0% w/w. This might have been due to the application of a higher dose which was at least eight times more than the doses in this study.

In contrast, *E. cautella* L3 larvae and *L. serricorne* L3 larvae and adults were relatively susceptible following exposure to kaolin treated rice at doses of 6g and 10g kg^{-1} of grain, and suffered mortality levels of 86.0% and 100%, respectively. Similar results were reported by Mahmoud et al. (2010) who observed that 100% protection was achieved against adults of both *Callosobruchus maculatus* Fabricius and *Callosobruchus chinensis* Linnaeus (Coleoptera: Bruchidae) following exposure to broad bean seeds treated with kaolin powder at doses of 1.0 and 2.0 w/w (kaolin / seeds).

Kaolin alone was not able to control *S. zeamais* even at high doses. Mixtures of carriers such as kaolin and/or cornflour with entomopathogenic fungi are needed for practical use to achieve maximum protection of stored grains. In this particular study, maximum protection was achieved using carriers in combinations with conidia of entomopathogenic fungi such as *B. bassiana* MS-8. The enhanced effect might be due to the synergy between the carrier and the fungal conidia. This has a practical importance in reducing the amount of carrier needed.

B. bassiana MS-8 formulated in kaolin caused a higher mortality than when formulated in cornflour for the tested insects, except for *E. cautella* L3 larvae. *E. cautella* L3 larvae showed non-significant results for both carriers. The highest mortality level was observed on *E. cautella* L3 larvae followed by *L. serricorne* adults. Kaolin as a carrier caused a lower LD_{50}

and LT_{50} values for the three tested insects than cornflour, with the exception of *E. cautella* L3 larvae where the insect responding to both carriers similarly. Samodra & Ibrahim (2006a) reported that admixtures of the isolates BbGc and BbPs with either kaolin, talc or tapioca flour (20.0% w/w a.i.) applied at the lowest rate of 0.05 g a.i. in 50 g rice resulted in excess of 80.05% mortality to the adults of *S. oryzae* by the 7^{th} day of exposure. In general, fungal formulations in kaolin and talc provided better results than formulations in tapioca flour or an unformulated control. In this study it was observed that whenever the insects moved in the grains treated with kaolin, parts of the waxy layer of the integuments were abraded. This would make it easier for fungus to attach and infect through the exoskeleton.

S. zeamais and *L. serricorne* L3 larvae were highly tolerant of *B. bassiana* conidia formulated in cornflour even at the highest doses. This result is in agreement with the findings of Sinia (2013) who reported that cornflour as a carrier for *B. bassiana* GHA at a dose of $1x10^8$ conidia g^{-1} caused no significant mortality in varroa mite in honey bee hives.

Better mortality was obtained after formulating *B. bassiana* MS-8 at fixed conidial dose (0.03g conidia kg^{-1} of grain = $2x10^9$ conidia g^{-1}) in kaolin or cornflour at a doses of 1, 2, 3, and 4g kg^{-1} of grain. Kaolin was consistently better than cornflour at all doses against the three tested insects after 10 days of exposure, except for *E. cautella* L3 larvae. High mortality levels were achieved with kaolin as the carrier at a dose of 3g or 4g kg^{-1} of grain against the tested insects, except *S. zeamais*. The results are in agreement with the findings of Samodra & Ibrahim (2006a) who found that *B. bassiana* isolates BbGc and BbPs formulated in kaolin and talc caused more mortality of *S. oryzae* than those formulated in tapioca flour.

S. zeamais adults and *E. cautella* L3 larvae developed mortality levels of 62.0-65.0% and 90.0-95.0%, respectively, with *B. bassiana* MS-8 formulated in cornflour and applied in doses of 1g to 4g kg^{-1} of grain. In contrast, *L. serricorne* L3 larvae and adults developed mortality levels of 60.0-74.0% and 76.0-95.0%, respectively. This might have been due to the lack of abrasive activity against insects by cornflour. However, for the other tested insects, fungal conidia can penetrate without abrasive activity. These results are in agreement with Samodra & Ibrahim (2006b) who reported that formulations of *B. bassiana* in either kaolin, talc or tapioca flour (20.0% w/w a.i.) provide complete mortality of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) larvae by the 12^{th} day of treatment.

In conclusion, *B. bassiana* MS-8 conidia formulated in kaolin can provide for effective protection of stored grains. To assess the practicality of these findings, further large scale

experiments will be required to evaluate the efficacy of formulated fungal treatments against grain pests in long term trials.

References

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Economic Entomology 18: 265-267.

Akbar W, Lord, JC, Nechols JR. Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Batta Y. 2004. Control of rice weevil *Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. Crop Protection 23: 103-108.

de Faria MR, Wraight SP. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43: 237-256.

El-Sayed F, El-Zun H, El-latif AA, Nasr M. 2010. Insecticidal effect of some inert dusts against three of stored grain insects at Kafr El-Sheikh governorate. Journal of Plant Protection and Pathology 1: 959-972.

Glenn DM, Puterka GJ. 2005. Particle films: a new technology for agriculture. Horticultural Reviews 31: 1-44.

Kahn BA, Damicone JP. 2008. Kaolin particle film product applications before harvest begins may not improve marketable yields of fresh tomatoes. HortTechnology 18: 144-147.

Kavallieratos N, Athanassiou C, Michalaki M, Batta Y, Rigatos H, Pashalidou F, Balotis G, Tomanović Ž, Vayias B. 2006. Effect of the combined use of *Metarhizium anisopliae* (Metschinkoff) Sorokin and diatomaceous earth for the control of three stored-product beetle species. Crop Protection 25: 1087-1094.

Lord JC. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94: 367-372.

Lord JC. 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biological Control 34: 180-186.

Mahmoud AG, El-Sebai O, Shahen A, Marzouk A. 2010. Impact of kaolin-based particle film dusts on *Callosobruchus maculatus* (F.) and *C. chinenesis* (L.) after different storage periods of treated broad bean seeds. Julius Kühn-Institut, 425: 638-646.

Michalaki M, Athanassiou C, Kavallieratos N, Batta Y, Balotis G. 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) (Deuteromycotina: Hyphomycetes) applied alone or in combination with diatomaceous earth against *Trilobium confusum* larvae (DuVal) (Coleoptera: Tenebrionidae): Influence of temperature, relative humidity and type of commodity. Crop Protection 25: 418-425.

Mikami AY, Pissinati A, Fagotti D, Júnior M, de Oliveira A, Ventura MU. 2010. Control of the Mexican bean weevil *Zabrotes subfasciatus* with kaolin. Ciência Rural 40: 1497-1501.

Muzemu S, Chitamba J, Goto S. 2013. Screening of stored maize (*Zea mays* L.) varieties grain for tolerance against maize weevil, *Sitophilus zeamais* (Motsch.). International Journal of Plant Research 3: 17-22.

Oliveira I, Pereira JA, Bento A, Baptista P. 2011. Viability of *Beauveria bassiana* isolates after storage under several preservation methods. Annals of Microbiology 61: 339-344.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. GenStat for Windows, 17th Edition. VSN International, Hemel Hempstead. UK

Permual D. Le Patourel G. 1990. Laboratory evaluation of acid-activated kaolin to protect stored paddy against infestation by stored product insects. Journal of Stored Products Research 26: 149-153.

Sabbour M. 2010. Efficacy of some bioinsecticides against *Bruchidius incarnatus* (Boh.) (Coleoptera: Bruchidae) infestation during storage. Journal of Plant Protection Research 50: 28-34.

Samodra H. Ibrahim Y. 2006a. Effects of dust formulations of three entomopathogenic fungal isolates against *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice grain. Journal of Biosciences 17: 1-7.

Samodra H. Ibrahim YB. 2006b. Effectiveness of selected entomopathogenic fungi in packed rice grain at room temperature against *Corcyra cephalonica* Stainton. ASEAN Journal on Science and Technology for Development 23: 183-192.

Sinia, A. (2013). Evaluation of the Fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Clonostachys rosea* as Bio-control Agents Against the Honey Bee Parasitic Mite, *Varroa destructor*. PhD Thesis, The University of Guelph, Ontario, Canada.

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

Chapter Five

Ovicidal, larvicidal and insecticidal activity of strains of *Beauveria bassiana* (Balsamo) Vuillemin against the cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), on rice grain

Abstract

Seven strains of *Beauveria bassiana* were evaluated for their effects on the eggs of *Lasioderma serricorne* under laboratory conditions. Strains were applied at a single dose of 0.03g conidia kg^{-1} of grain = $2x10^9$ conidia g^{-1} of kaolin kg^{-1} of grain. All strains were pathogenic on the eggs of *L. serricorne*. Two strains, MS-8, and 7284, caused the highest levels of egg mortality. These two strains were then formulated at a fixed dose of 0.03g conidia, with kaolin as an active carrier, at doses of 0.0, 0.5g, 1g, 2g and 3g of kaolin kg^{-1} of grain. These powder formulations were evaluated for their effects on adult mortality, larval mortality, number of adults emerged, level of grain damaged and level of weight loss on rice grains. The highest levels of adult mortality and larval mortality were caused by Strain MS-8 at a kaolin dose of 3g kg^{-1}. It also caused the lowest levels of adult emerged (10%), grain damaged (1.07%) and weight loss (0.55%). When unformulated, the same dose of conidia performed poorly for the same parameters (64%; 20.2%; 3.37%). The highest levels of grain damaged (27%) and weight loss (6.04%) were observed in the untreated control treatment (UTC). Observations made with a scanning electron microscope (SEM) showed that unformulated conidia clumped together whereas conidia formulated in kaolin remained as discrete conidia.

5.1 Introduction

The cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), is a widespread and destructive pest that feeds on a wide variety of products, such as grains, dry yeast, cotton seeds, chilli powder, ginger, turmeric, saffron, dates, raisins, dried figs, leather, cocoa, herbs, dried vegetables and even pyrethrum powder (Ashworth 1993; Mahroof & Phillips 2008). In addition, this pest has been recorded in dehydrated bee pollen (Poderoso et al. 2013). The greatest damage caused to commodities is due to larval feeding. Adults do not feed in their short lifespan, where they emerge, mate, and lay eggs (Minor 1979). Various methods have been used for the management of this pest, such as using low temperatures

(Imai & Harada 2006; Collins & Conyers 2010), or elevated temperatures (Yu et al. 2011). Today its control is largely dependent on the use of phosphine (Vincent & Lindgren 1977; Rajendran et al. 2004; Allahvaisi 2013). However, this pest has developed a significant level of resistance to phosphine (Rajendran & Narasimhan 1994; Zettler & Keever 1994; Hori & Kasaishi 2005; Sağlam et al. 2015). As a result, other control strategies are being investigated, including the use of bio-pesticide products. Entomopathogenic fungi (EPF) have been used successfully in studies against many stored product insect pests under both laboratory and field conditions (Adane et al. 1996; Hidalgo et al. 1998; Lord 2001; Athanassiou & Steenberg 2007; Nboyine et al. 2015). Among the EPFs, *Beauveria bassiana* (Balsamo) Vuillemin has often been used. Recently, several studies have been done by formulation of this fungus for the management of stored grain pests (Akbar et al. 2005; Radha et al. 2014; Shafighi et al. 2014).

Thus far, little is known about the susceptibility of *L. serricorne* life stages to entomopathogenic fungi (EPF). The insecticidal effects of some entomopathogenic nematode (EPN) strains have been investigated under laboratory conditions against *L. serricorne* (Rumbos & Athanassiou 2012). That study indicated that *Steinernema carpocapsae* (Weiser) strain outperformed other strains and caused mortality of 58.9% after eight days of exposure, whereas larval mortality did not exceed 19% with all tested EPN strains. The effects of Japanese *Bacillus thuringiensis* (Berliner) isolates against *L. serricorne* have also been evaluated. The ß-exotoxin related ingredients of these isolates were responsible for toxicity to the cigarette beetle, demonstrating that the cigarette beetle is vulnerable to the effects of these entomopathogenic bacteria (Tsuchiya et al. 2002).

To date, most of the studies conducted on the management of *L. serricorne* were focused on the management of this pest on stored tobacco (Blanc et al. 2002; Tsuchiya et al. 2002; Blanc et al. 2004; Imai 2010), although this pest has the ability to feed on other commodities in storage. Recently, Mokhtar et al. (2016) observed that the damage caused by larvae of this insect extends to humans by causing canthariasis in a 1-year old infant. That study postulated that the larvae acquired from contaminated food were responsible for the gastrointestinal symptoms in the patient. This finding indicates the importance of controlling this pest in the foods consumed by humans. This is the first study we are aware of to evaluate the insecticidal activity of *B. bassiana* powder formulations for the control of the cigarette beetle life stages in stored rice grains.

5.2 Material and methods

5.2.1 Insects rearing

The initial culture of *L. serricorne* was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Sciences (SAEES), University of KwaZulu-Natal, Pietermaritzburg, South Africa. Adult insects were reared on rice grain at 28±2^{o}C and 65±5% relative humidity. The grains were initially stored at -20^{o}C for one week to remove prior natural infestations of pests. Ten adult *L. serricorne* of mixed sexes were placed in glass jars[1] of 250 ml each with 100g of rice grain. The jars were covered with insect nets[2] to enable air circulation. The adults were removed from the jars after one week of infestation. These jars were kept for 45 days and newly (one day old) emerged adults were then used in the subsequent experiments.

5.2.2 Fungi

Seven *B. bassiana* strains were used in this study. Five strains were obtained from the Plant Protection Research Institute, Agricultural Research Council[3] (PPRI-ARC, South Africa), and one commercial strain (R444) was provided by Plant Health Products[4] (PHP) (Pty) Ltd. One Strain was isolated using *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) as a live insect bait (Zimmermann 1986).

5.2.3 Productions of dried conidia

Conidia obtained from re-infected cadaver of *L. serricorne* larvae were maintained at 28^{o}C on PDA medium supplemented with 0.05 g L^{-1} ampicillin and streptomycin[5]. Sporulating conidia (15 days old) were harvested and suspended in Tween 80 (0.1 ml L^{-1}) in sterile distilled water and vortexed for 3 min to produce a homogenous suspension. The suspension was filtered through five layers of gauze to remove mycelia and debris. Using a Neubauer Improved Hemocytometer[6] spore concentrations were determined and adjusted to $1x10^{8}$ conidia ml^{-1}.

[1] Victoria Packaging c.c. Agricultural Packaging Equipment and Safety Wear, 298 Victoria Road, Pietermaritzburg 3201, South Africa.
[2] Film flex Plastisc Natal cc, Unit 10, Shepstone Park, Cnr. Shepstone and Blasé Roads, New Germany, South Africa.
[3] Agricultural Research Council, 1134 Park Street, P.O. Box 8783, Hatfield, Pretoria 0001.
[4] Plant Health Products (Pty) Ltd., P.O. Box 207, Nottingham Road, South Africa.
[5] Whitehead Scientific (Pty) Ltd, Unit 9, Van Biljon Industrial Park, Winelands Close, Strickland, 7530.
[6] Hirschmann®, Pietermaritzburg, South Africa.

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 25ºC 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 five 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 4ºC for further use.

5.2.4 Effect of powder formulations of *B. bassiana* strains on the eggs of *L. serricorne*

Seven strains of *B. bassiana* were evaluated in a single dose bioassay under laboratory conditions for their effects on the eggs of *L. serricorne*. For each strain, a fixed dose of 0.03g conidia = $2x10^9$ conidia g^{-1} was formulated in 1g of kaolin. Three Petri dishes with 0.01g of formulated conidia were used as replicates. Ten pairs of newly emerged adults (one day old) of mixed sexes were introduced into the Petri dishes. The control consisted of the carrier only. Adult insects were left over night for oviposition. After nine days of incubation, the number of dead eggs on the formulated conidia and control plates was counted daily for five days. Dead eggs were examined for outgrowths of fungi under a Scanning Electron Microscope[7] (SEM). Eggs with fungal mycelia were assumed to have died because of fungal infection. Treatments were arranged in a randomized complete block design (RCBD). Egg mortality was corrected using Abbott's formula (Abbott 1925). Data was subjected to analysis of variance (ANOVA), using GenStat for Windows, 17th edition (Payne et al. 2014). Means were compared using Tukey at a 5% level of significance.

5.2.5 Effects of *B. bassiana* powder formulations on adults and larvae of *L. serricorne* and rice grain quantity and quality

The two most virulent strains identified in the previous test were used in this study. For each strain, kaolin was used at doses of 0.0, 0.5, 1, 2 and 3g kg^{-1} of grain. Treatments were prepared by mixing a fixed dose of 0.03g conidia of *B. bassiana* strains with kaolin at each concentration. Ten pairs from newly emerged adults (one day old) were placed into glass jars containing 100g rice treated with the respective treatment. The control rice grains were left

[7] ZEISS, EVO LS 15, Germany.

untreated. The treatments were replicated three times. Adult mortality was checked every two days for 10 days. After hatching, larvae were also checked for mortality every three days fo1 18 days. After 45 days rice grains were sieved and the clean grains were weighed and expressed as a percentage weight loss of the original weight (Tefera et al. 2011). 10g of rice grains were randomly selected to assess the levels of grain damage, and then the level of grain damaged was expressed as a proportion of original weight of grain. Adult emergence was recorded at 45 days.

$$\text{Weight loss } (\%) = \ (\text{original weight} - \text{weight after 45 days})/(\text{original weight}) \ \text{x } 100$$

The treatments were arranged in randomized complete designs (RCBD). Data was subjected to analysis of variance (ANOVA), for each strain using GenStat for Windows, 17th edition (Payne et al. 2014). Means were compared using Tukey at a 5% level of significance.

5.3 Results

5.3.1 Effect of powder formulations of *B. bassiana* strains on the eggs of *L. serricorne*

There were highly significant differences among the fungal strains ($F = 92.59; P < 0.001; d.f. = 6$), time ($F = 88.42; P < 0.001; d.f. = 4$) and the th interation between strains and time ($F = 3.51; P < 0.001; d.f. = 24$) in their effects on the eggs of *L. serricorne*. All *B. bassiana* strains were pathogenic on the eggs of *L. serricorne* (Fig. 5.1). The infection process of the fungus observed by SEM confirmed that most of the eggs died due to the fungus (Fig 5.2). These results indicated that *B. bassiana* could be employed to treat empty stores to control eggs of this pest that were laid before a new harvest was brought in. At a dose of $2x10^9$ conidia g^{-1} Strain MS-8 caused the highest egg mortality level of 35%, followed by Strain 7284, which caused an egg mortality level of 25% (Fig. 5.1). A mortality level of less than 5% was observed in the UTC. The commercial Strain R444 caused the lowest level of egg mortality of less than 10% together with Strain 7768, despite excellent activity versus other target insects such as whitefly (PHP 2016), housefly (Mwamburi et al. 2010) and two spotted mite (Gatarayiha et al. 2012).

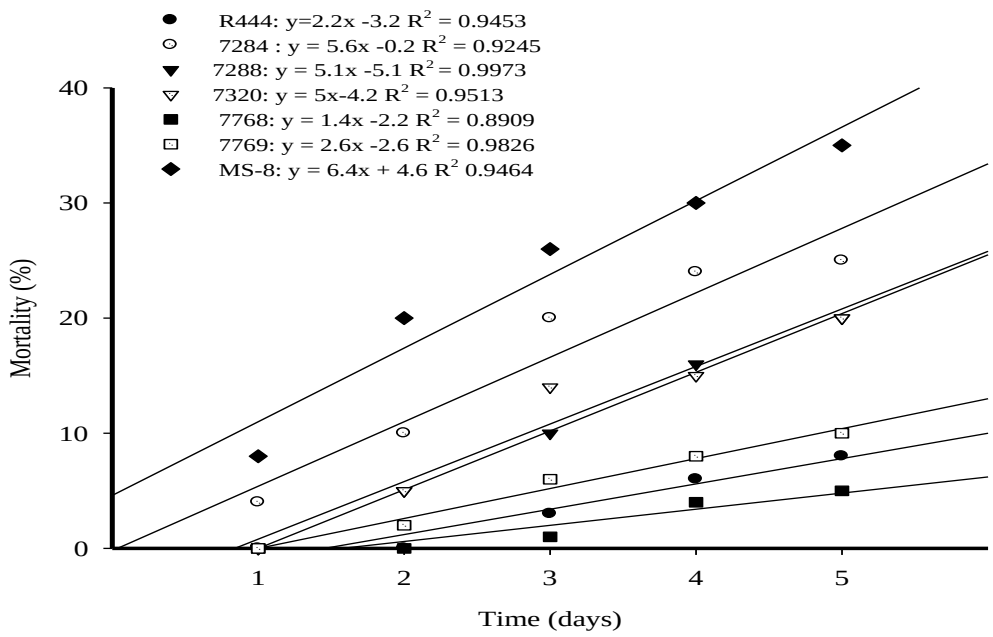

Fig. 5.1 Corrected mortality (Abbott's correction) of the eggs of *Lasioderma serricorne* upon exposure to a fixed dose of $2x10^9$ conidia g^{-1} of seven *Beauveria bassiana* strains, observed for five days after an incubation period of nine days.

5.3.2 Effects of *B. bassiana* powder formulations on adults and larvae of *L. serricorne* and rice grain quantity and quality

Highly significant differences were observed among the fungal stains (*F = 4704; P < 0.001; d.f. =1*) and doses (*F = 115.18; P < 0.001; d.f. = 4*) and time (*F = 290.59; P < 0.001; d.f. = 4)* for their pathogenicity against the adults of *L. serricorne*. The interaction between dose and time (*F = 7.54; P < 0.001; d. f. =16*) was also significant. However, no significant interactions effects were found between strains and doses (*F = 1.55; P = 0.194; d.f. = 4*), strains and time (*F = 1.06; P = 0.382; d.f. =4*) and strains, doses and time (*F = 0.39; P = 0.982; d.f. =16*). The dose response curve was linear for both strains ($R^2 > 0.84$) (Fig 5.3). Strain MS-8 and 7284 caused higher mortality levels on the adults of *L. serricorne* at all doses of kaolin than the UTC. However, before death the adults deposited their eggs on rice grains. Strain MS-8 caused the highest levels of mortality at all doses of kaolin, followed by Strain 7284. At the highest kaolin dose of 3g kg^{-1} a mortality level of 100% was observed with Strain MS-8 compared to the highest mortality of only 85% with Strain 7284. There were no interaction effect between strains and doses because both strains had a similar dose response (Fig. 5.3). Unformulated conidia of both strains caused poor levels of adult mortality of less

than 50%. Observations made with an SEM showed unformulated conidia clumping together while formulated conidia in kaolin remained independent from other conidia (Fig. 5.4).

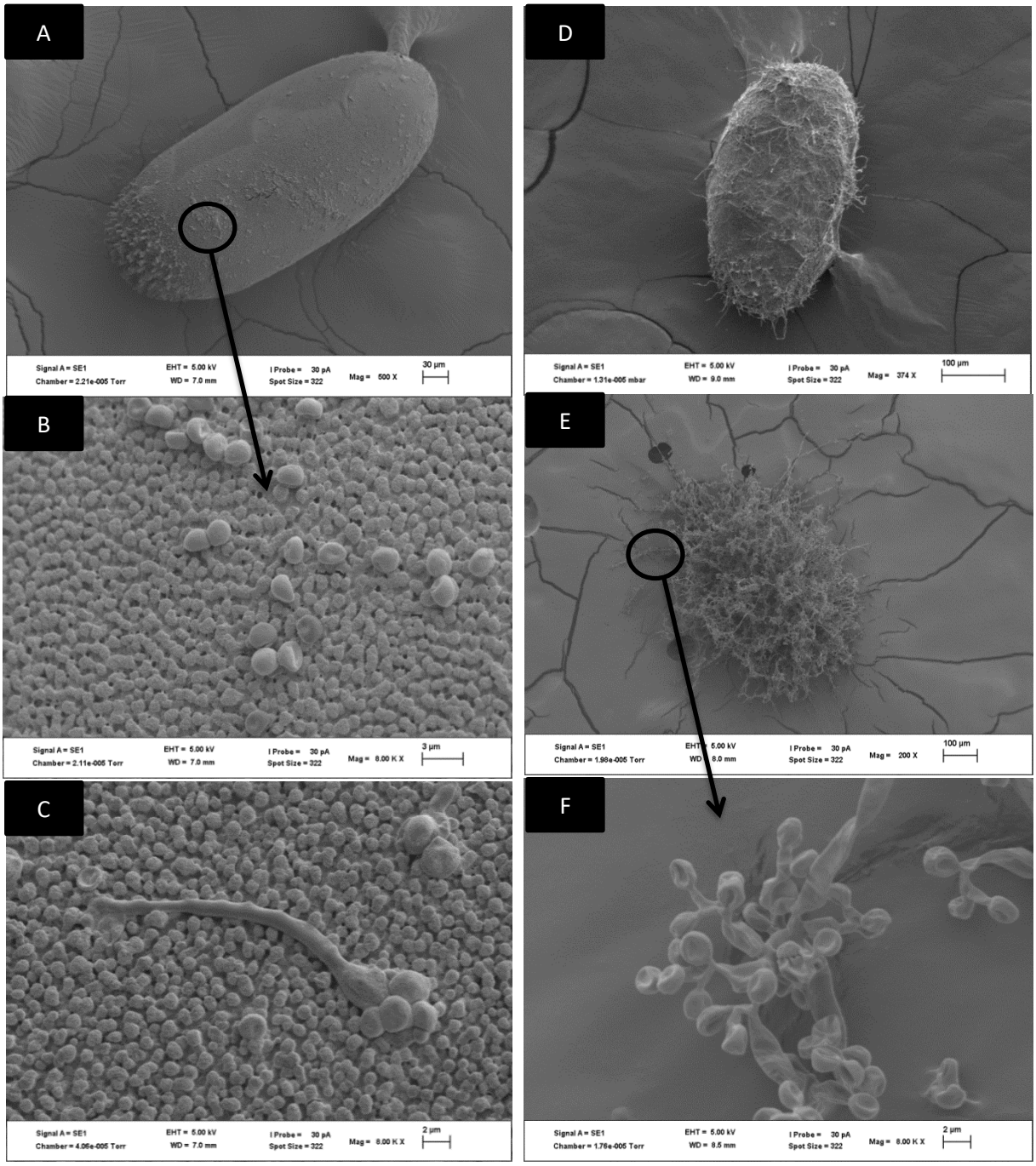

Fig. 5.2 Scanning electron micrographs of *Beauveria bassiana* Strain MS-8 on eggs of *Lasioderma serricorne* (A-F). On Day 1 no germination of conidia was found (A and B); germination of conidia and penetration of germ tube were seen on Day 2 (C); mycelia emerged from the eggs on Day 4 (D); E and F show intense sporulation on eggs by Day 8.

Three main factors caused significant differences in the mortality of *L. serricorne* larvae, namely the strains (*F = 121.93; P < 0.001; d.f. = 1*), doses (*F = 400.76; P < 0.001; d.f. = 4*) and time ((*F = 1141.86; P < 0.001; d.f. = 5*) 18 days after treatment). The interactions effects between strains and time (*F = 5.6; P < 0.001; d.f. = 5*) and doses and time (*F = 16.69; P < 0.001; d.f. = 20*) were also significant. However, no significant interactions effect was found between strains and doses (*F = 1.05; P = 0.387; d.f. = 4*) and strains, doses and time (*F = 1.48; P = 0.103; d.f. = 20*). The dose response curve was linear for both strains ($R^2 > 0.96$) (Fig. 5. 5). Strain MS-8 caused a higher level of larval mortality at all doses of kaolin than Strain 7284. At a kaolin dose of 3g kg^{-1} Strain MS-8 caused larval mortality of 87% compared to larval mortality of only 75% with Strain 7284 (Fig. 5.5). For both strains larval mortality increased when dose and time increased. However, after 18 days of exposure Strain 7284 caused the same mortality at doses of 1g, 2g and 3g kg^{-1}. More time and higher doses were required with Strain 7284 to achieve the same performance as Strain MS-8. After 18 days of exposure, unformulated conidia of both strains caused mortality levels of less tahn 50% (Fig. 5.5).

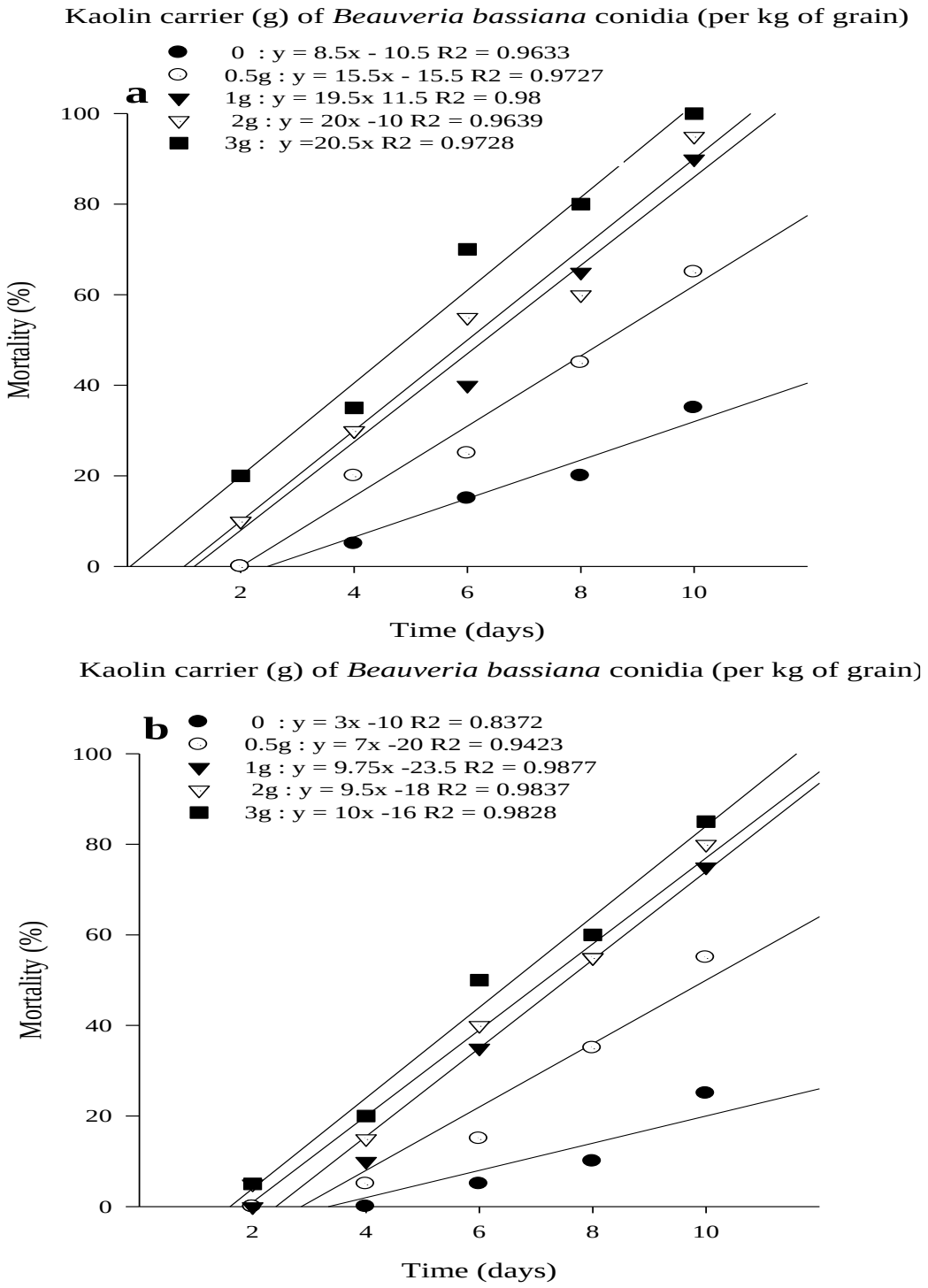

Fig. 5.3 Corrected mortality (Abbott's correction) of *Lasioderma serricorne* adults after exposure to a fixed dose of 0.03g conidia kg^{-1} of grain of *B. bassiana*; **a** = Strain MS-8 and **b** = Strain 7284formulated in kaolin at 0. 05, 1, 2 and 3g kg^{-1} of grain, observed for 10 days.

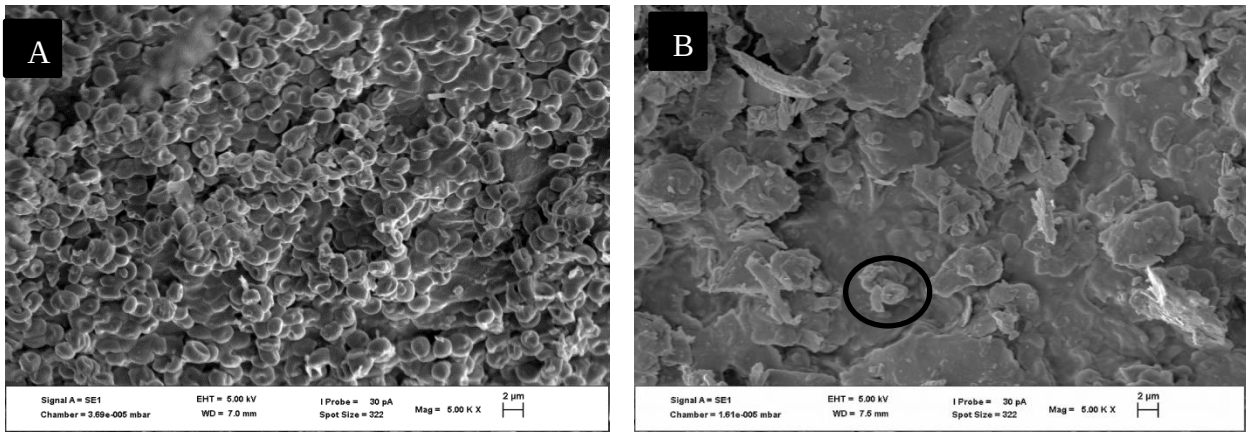

Fig. 5.4 Scanning electron microscope micrographs showing: **A** = unformulated conidia clumping; and **B** = highlights areas showing conidia remaining independent when formulated with kaolin at a dose of 1g kg^{-1}, on the surface of rice grains.

Strain MS-8 was consistently more efficacy than Strain 7284 for all parameters measured (adult emergence, grain damage and weight loss) and at each dose for kaolin carrier. However, these differences were significant for some doses of the kaolin carrier, except for kaolin dose of 3g kg^{-1} where the differences were significant for all three parameters (Table 1).

As the discrete analysis of kaolin doses x strains, there was a highly significant dose response for both strains. Whilst kaolin at 3g kg^{-1} was the best dose, it was not significantly better than 2g and 1g kg^{-1} for all three parameters measured Fig. 5.6. The unformulated conidia with no carrier performed poorly, and were not significantly better than the UTC for strain 7284 for grain damage and weight loss. By contrast, unformulated conidia of Strain MS-8 was still significantly effective more relative to the UTC (Fig. 5.6).

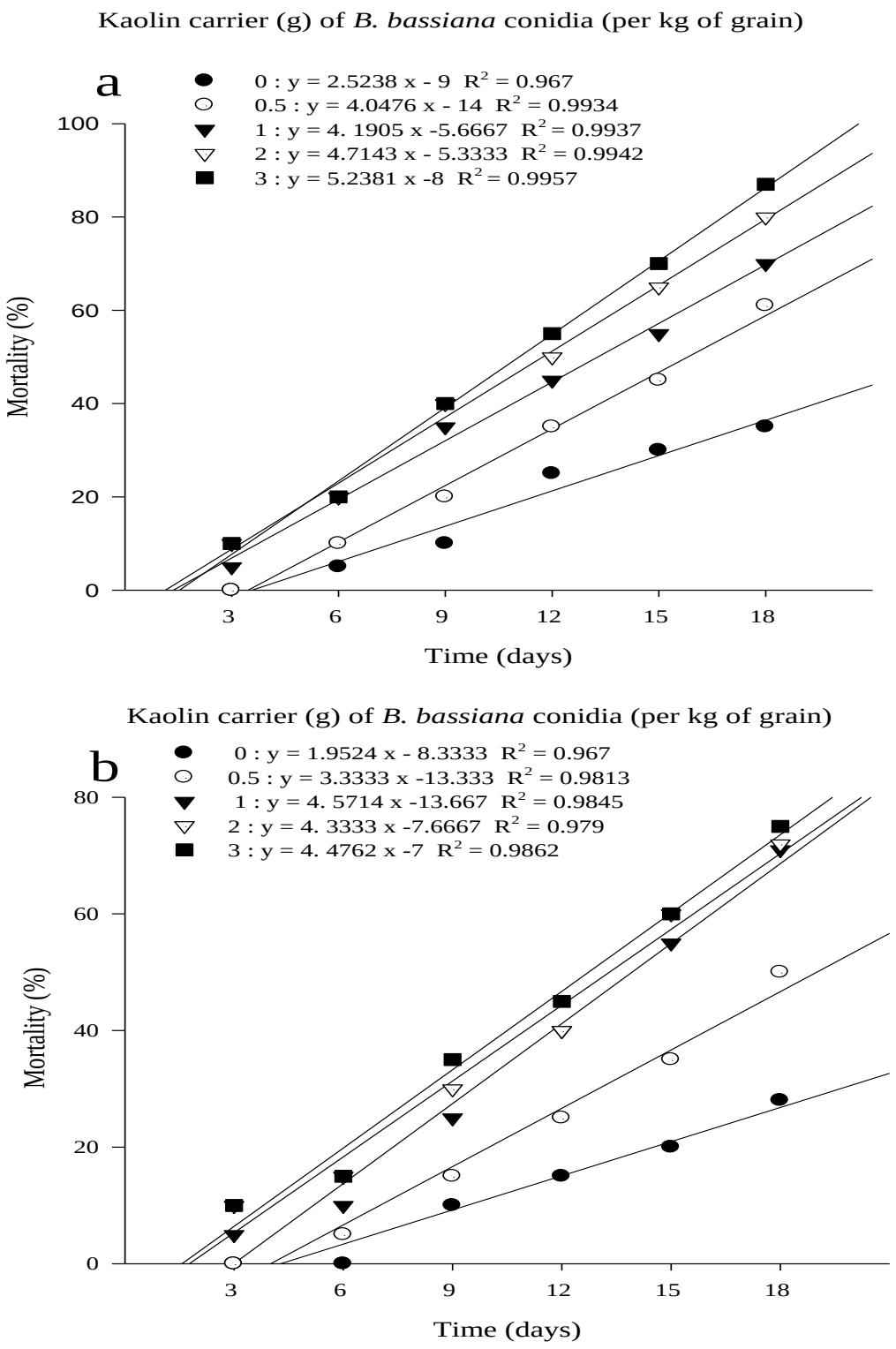

Fig. 5.5 Corrected mortality (Abbott's correction) of *Lasioderma serricorne* larvae after exposure to a fixed dose of 0.03g conidia kg^{-1} of grain of *B. bassiana*; **a** = Strain MS-8 and **b** = Strain 7284, formulated in kaolin at 0. 05, 1, 2 and 3g kg^{-1} of grain, observed for 18 days after hatching.

Table 5. 1 ANOVA table comparing the efficacy of two strains of *Beauveria bassiana* in five doses of a kaolin carrier

Parameters	Strains	Kaolin carrier (g) *B. bassiana* conidia (per kg of grain)					
		3g	2g	1g	0.5g	0g	Control
Adult emergence (%)	MS-8	10	19	22	38	64	100
	7284	24	27	28	52	71	100
	LSD	4.303	22.54	7.452	12.91	13.83	NA
	P <	0.005	0.163	0.074	0.043	0.161	NA
	F value	196	4.68	12	21.78	4.74	NA
	CV%	7.2	30.1	8.5	8.2	5.8	NA
Grain damage (%)	MS-8	1.07	2.2	7	12.04	20.2	26.7
	7284	7.22	12.24	12.4	20	21.5	27
	LSD	2.13	2.166	9.81	2.44	17.39	12.5
	P <	0.007	0.003	0.139	0.005	0.778	0.919
	F value	145.02	397.9	5.73	197.92	0.1	0.01
	CV%	14.2	8.5	28.8	4.3	23.7	13.3
Weight loss (%)	MS-8	0.55	0.79	1.33	2.33	3.37	6.04
	7284	1.6	2.52	2.69	3.17	4.45	6.04
	LSD	0.7204	0.3975	1.366	0.997	2.512	4.303
	P<	0.024	0.003	0.05	0.068	0.613	1
	F value	39.33	350.73	18.34	13.13	0.35	0
	CV%	19.1	6.8	19.3	10.3	20.2	20.3

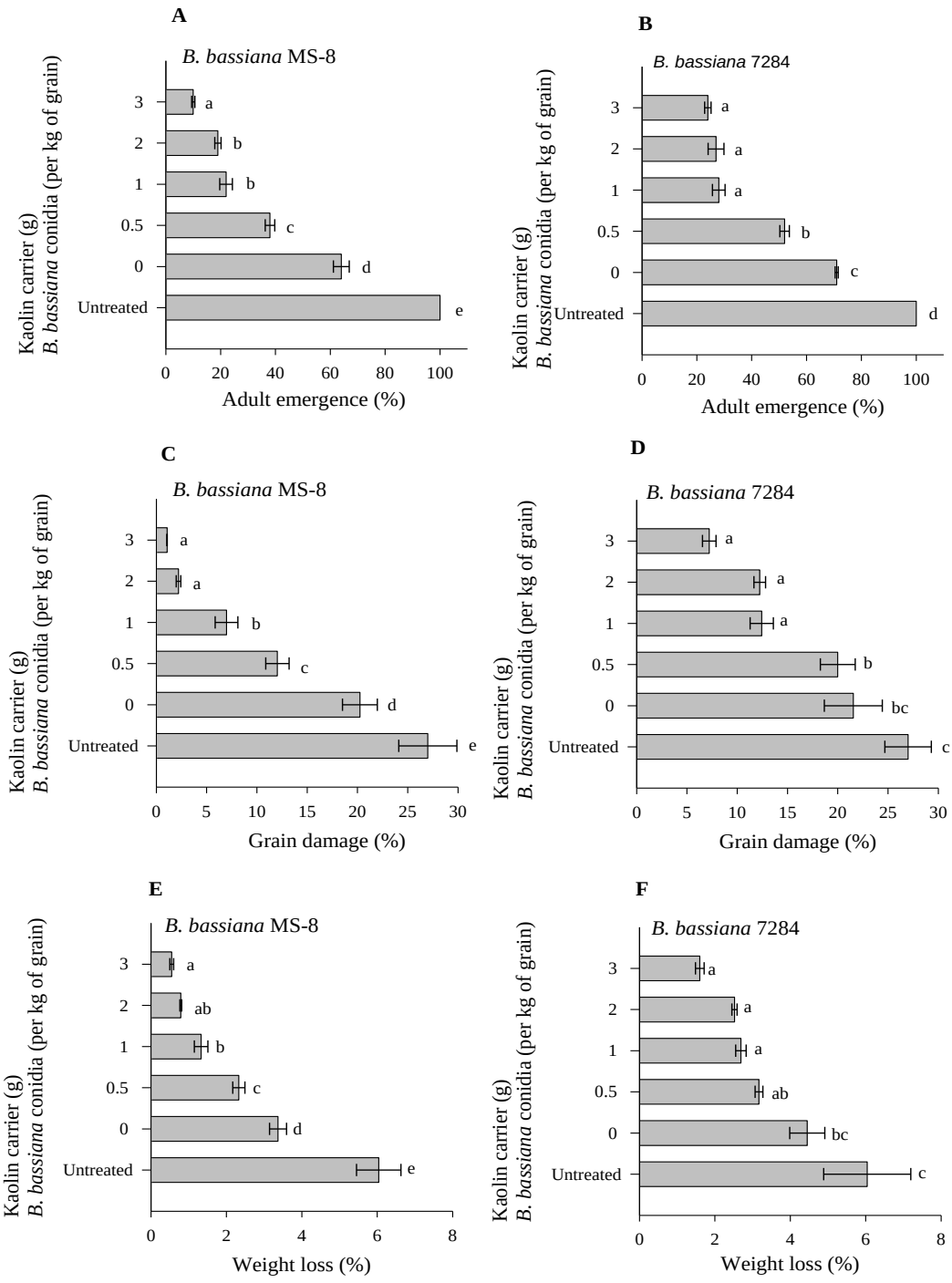

Fig 5.6 *Lasioderma serricorne* adult emergence (A ; B), grain damage (C ; D) and weight loss (E ; F) from rice grain exposed to two strains of *Beauveria bassiana* (Strain MS-8 and 7284) at 0.03g conidia kg^{-1} of grain ($2x10^9$ conidia g^{-1} of kaolin) formulated in various doses of kaolin carrier. P values were < 0.001 for all three treatments and both strains of *B. bassiana*

5.4 Discussion

Among the seven tested strains of *B. bassiana*, the highest level of egg mortality of 35% was observed as a result from Strain MS-8 at a dose of $2x10^9$ conidia g^{-1}. This demonstrated that the fungus could be applied to rice grains to reduce the number of emerging adults of the subsequent generation, or can be employed to treat empty stores to control eggs of grain-stored insects that remain before the introduction of a new crop. This is similar to observations of Acheampong et al. (2016) who observed that the least number of larger grain borer (LGB) *Prostephanus truncates* Horn (Coleoptera: Bostrichidae) adults emerged from the kernels infested with the (LGB) eggs after treatment with *B. bassiana* at a dose of 3.16 x 10^9 cfu kg^{-1}.

The two best strains had highly significant effects toward *L. serricorne* in rice grains compared to the UTC. Interestingly, 100% and 85% mortality was observed in the adults of *L. serricorne* using Strains MS-8 and 7284 at a dose of 3g kg^{-1} of grain, respectively. This indicates that adults were susceptible to both tested strains. However eggs were observed on treated rice grains. Adults had laid the eggs soon after being released on the rice grains, and before infection with *B. bassiana* took place. These results are in agreement with those of (Samodra & Ibrahim 2006a) who observed that *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) laid eggs in wheat grains before *B. bassiana* could kill them, resulting in multible cycles of weevil reproduction, but with much reduced population per generation. The juveniles were not infected by the fungus. But in this study the opposite was true and the tested Strains MS-8 and 7284 achieved levels of 87% and 75% larval mortality at the highest dose of 3g kg^{-1}, respectively, after 18 days of exposure, compared to zero mortality in the UTC. This would significantly reduce the numbers of survivors in subsequent generations of the insect. Samodra & Ibrahim (2006b) observed 100% larval mortality of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) after 15 days of exposure in rice grains treated with *B. bassiana* isolates formulated in kaolin or tapioca flour. Similarly Kaur et al. (2014) reported significantly higher larval mortality on *C. cephalonica* after treating the sorghum grains with *B. bassiana* at a dose of $2.02x10^8$ spores ml^{-1}.

After 18 days of storage most of the remaining larvae grouped on two or more rice grains together and disappeared inside. This behaviour led to less movement of these larvae, which reduced their chances of exposure to fungal infections. In this study the percentages of *L. serricorne* adults emerged from rice grains treated with *B bassiana* strains MS-8 and 7284

were greatly reduced than in the UTC. The fewest adults emerged after treatment with Strain MS-8 at the highest dose of 3g kg^{-1}. Khashaveh et al. (2011) suggested that the ability of *B. bassiana* to reduce progeny emergence from treated grains was as important as parental mortality. In contrast, with unformulated conidia both Stains performed poorly in their control of the adults and larvae of *L. serricorne*. Accordingly, the greatest number of *L. serricorne* adults was observed in the unformulated conidia treatments. The poor performance of unformulated conidia can be explained by the observations made with scanning electron microscopy that was showed clumping of the unformulated conidia. Clumping makes the conidia less evenly distributed through over the treated rice grains and this would reduce the chances of the target insects encountering free conidia. This result is in agreement with research by (Akbar et al. 2004; Athanassiou & Steenberg 2007).

Treatment of rice grains with either Strains MS-8 or 7284 significantly reduced the level of grain damaged and weight loss caused by *L. serricorne*. However, Strain MS-8 outperformed Strain 7284 at all doses except with unformulated conidia where both strains performed similarly. The least grain damage of 1.07% was observed on rice grains treated with MS-8 at a dose of 3g kg^{-1}, compared to 27% grain damage in the UTC. This result is in agreement with that of Padin et al. (2002) who observed that wheat grains infested with *S. oryzae* without *B. bassiana* conidia were significantly more damaged by weevils than grains treated with *B. bassiana*. Batta (2004) observed that formulating *Metarhizium anisopliae* (Metchnikoff) conidia with oven ash at a ratio of 1:4 (w/w) reduced the level of damage of wheat grains to 0.5% compared to the control (6.0%).

The same pattern of performance was observed with regards to the level of weight loss in rice grains infested with *L. serricorne* after treatment with Strain MS-8 and 7284. Both Strains caused significantly lower levels of weight loss than the UCT. The least weight loss of 0.55% was achieved using Strain MS-8 at 3g kg^{-1}, compared to the highest weight loss of 6.04% weight loss that occurred in the UCT. In addition, at a lower dose of 1g kg^{-1} Strain MS-8 significantly reduced the level of weight loss in rice grains infested with *L. serricorne*. Hafez (2011) also observed a significant decrease in weight loss of treated wheat flour treated with *B. bassiana*.

In conclusion, our results indicated that *L. serricorne* has the ability to infest stored rice grains and cause considerable damage. The powder formulation of *B. bassiana* Strain MS-8 can be used successfully to control of *L. serricorne* in stored rice. Further experiments will be

undertaken to evaluate the performance of the powder formulation of Strain MS-8 in stored rice in long term trials.

References

Abbott W. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265-267.

Acheampong MA, Cornelius EW, Eziah VY, Fening KO, Luke B, Moore D, Clottey, VA, Storm C, Potin O. 2016. *Beauveria bassiana* affects immature stage development of *Prostephanus truncatus* (Coleoptera: Bostrichidae) in stored maize. Biocontrol Science and Technology 26: 1516-1525

Adane K, Moore D, Archer S. 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. Journal of Stored Products Research 32: 105-113.

Akbar W, Lord JC, Nechols JR, Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Akbar W, Lord JC, Nechols JR, Loughin, TM. 2005. Efficacy of *Beauveria bassiana* for red flour beetle when applied with plant essential oils or in mineral oil and organosilicone carriers. Journal of Economic Entomology 98: 683-688.

Allahvaisi S. 2013. Controlling *Lasioderma serricorne* F.(Col.: Anobiidae) by Fumigation and packaging. World Applied Sciences Journal 28: 1983-1988.

Ashworth JR. 1993. The biology of *Lasioderma serricorne*. Journal of Stored Products Research 29: 291-303.

Athanassiou C, Steenberg T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40: 411-416.

Batta Y. 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae.* Crop Protection 23: 103-108.

Blanc M, Kaelin P, Gadani F. 2002. *Bacillus thuringiensis* (Bt) for the control of insect pests in stored tobacco: a review. Contributions to Tobacco Research 20: 15-22.

Blanc MP, Panighini C, Gadani F, Rossi L. 2004. Activity of spinosad on stored-tobacco insects and persistence on cured tobacco strips. Pest Management Science 60: 1091-1098.

Collins D, Conyers S. 2010. The effect of sub-zero temperatures on different lifestages of *Lasioderma serricorne* (F.) and *Ephestia elutella* (Hübner). Journal of Stored Products Research 46: 234-241.

Gatarayiha M, Laing M, Miller R. 2012. Selection of *Beauveria bassiana* strains against the two spotted spider mite, *Tetranychus urticae* Koch in laboratory and greenhouse trials. African Journal of Microbiology Research 6: 2694-2703.

Hafez S. 2011. Efficacy of the entomopathogenic fungus *Beauveria bassiana*, Balsamo against *Tribolium confusum*, duval on stored wheat flour. *Mansoura University* Journal of Plant Protection and Pathology 2: 203-211.

Hidalgo E, Moore D, Le Patourel G. 1998. The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize. Journal of Stored Products Research 34: 171-179.

Hori M, Kasaishi Y. 2005. Development of a new assay method for quickly evaluating phosphine resistance of the cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), based on knockdown of the adult beetles. Applied Entomology and Zoology 40: 99-104.

Imai T, Harada H. 2006. Low-temperature as an alternative to fumigation to disinfest stored tobacco of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). Applied Entomology and Zoology 41: 87-91.

Imai T. 2010. Suppression of the population of *Lasioderma serricorne* in stored tobacco by relocation of warehouses to cooler areas. Julius Kühn-Institut, 425: 668-670.

Kaur S, Thakur A, Rajput M. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Journal of Stored Products Research 59: 185-189.

Khashaveh A, Ghosta Y, Safaralizadeh MH, Ziaee M. 2011. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. Journal of Agricultural Science and Technology 13: 35-43.

Lord JC. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94: 367-372.

Mahroof RM, Phillips TW. 2008. Life history parameters of *Lasioderma serricorne* (F.) as influenced by food sources. Journal of Stored Products Research 44: 219-226.

Minor MF. 1979. Do adult cigarette beetle feed? Tobacco Science 23: 61-64.

Mokhtar AS, Sridhar GS, Mahmud R, Jeffery J, Lau YL, Wilson JJ, Abdul-Aziz NM. 2016. First Case Report of Canthariasis in an Infant Caused by the Larvae of *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Medical Entomology 53**:** 1234-1237

Mwamburi L, Laing M, Miller R. 2010. Laboratory screening of insecticidal activities of *Beauveria bassiana* and *Paecilomyces lilacinus* against larval and adult house fly (Musca domestica L.). African Entomology 18: 38-46.

Nboyine JA, Asante SK, Nutsugah SK, Abudulai M, Ansaah-Agyapong F, Luke B, Clottey V. 2015. Biological control of the larger grain borer, *Prostephanus truncatus* (Horn) in stored maize using the fungal pathogen, *Beauveria bassiana* and the predator *Teretrius nigrescens* Lewis. Journal of Stored Products and Postharvest Research 6: 30-37.

Padin S, Dal Bello G, Fabrizio M. 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research 38: 69-74.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. Genstat for Windows, 17th Edition. VSN International, Hemel Hempstead, UK.

PHP 2016. http://plant-health.co.za. Accessed 20/10/2016.

Poderoso JCM, Correia-Oliveira ME, Vieira JM, Ribeiro GT, Ribeiro RC, Zanuncio JC. 2013. *Lasioderma serricorne* (Coleoptera: Anobiidae): first record in dehydrated bee pollen in Sergipe State, Brazil. Florida Entomologist 96: 682-685.

Radha R, Murugan K, Wei H, Amerasan D, Chen PMF, Kovendan K, Nataraj T, Nareshkumar A, Hwang JS, Govindarajan M. 2014. Insecticidal Activity of Essential Oils and Entomopathogenic Fungi Against Cowpea Bruchid, *Callosobruchus maculatus* (f.) (Insecta: Coleoptera: bruchidae). Journal of Current Innovation Research 1: 11-19.

Rajendran S, Narasimhan K. 1994. Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. International Journal of Pest Management 40: 207-210.

Rajendran S, Parveen H, Begum K, Chethana R. 2004. Influence of phosphine on hatching of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae), *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Pest management science 60: 1114-1118.

Rumbos CI, Athanassiou CG. 2012. Insecticidal effect of six entomopathogenic nematode strains against *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) and *Tribolium*

confusum (Coleoptera: Tenebrionidae). Journal of Stored Products Research 50: 21-26.

Sağlam Ö, Edde PA, Phillips TW. 2015. Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with Phosphine. Journal of Economic Entomology 108: 2489-2495.

Samodra H, Ibrahim Y. 2006a. Effects of dust formulations of three entomopathogenic fungal isolates against *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice grain. Journal of Bioscience 17: 1-7.

Samodra H, Ibrahim YB. 2006b. Effectiveness of selected entomopathogenic fungi in packed rice grain at room temperature against *Corcyra cephalonica* Stainton. Asean Journal on Science and Technology for Development 23: 183-192.

Shafighi Y, Ziaee M, Ghosta Y. 2014. Diatomaceous earth used against insect pests, applied alone or in combination with *Metarhizium anisopliae* and *Beauveria bassiana*. Journal of Plant Protection Research 54: 62-66.

Tefera T, Mugo S, Tende R, Likhayo P. 2011. Methods of Screening Maize for Resistance to Stem Borers and Post-harvest Insect Pests. CIMMY. Nairobi, Kenya.

Tsuchiya S, Kasaishi Y, Harada H, Ichimatsu T, Saitoh H, Mizuki E, Ohba M. 2002. Assessment of the efficacy of Japanese *Bacillus thuringiensis* isolates against the cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Invertebrate Pathology 81: 122-126.

Vincent L, Lindgren D. 1977. Toxicity of methyl bromide and phosphine at various temperatures and exposure periods to the metamorphic stages of *Lasioderma serricorne*. Journal of Economic Entomology 70: 497-500.

Yu C, Subramanyam B, Flinn PW, Gwirtz JA. 2011. Susceptibility of *Lasioderma serricorne* (Coleoptera: Anobiidae) life stages to elevated temperatures used during structural heat treatments. Journal of Economic Entomology 104: 317-324.

Zettler LJ, Keever DW. 1994. Phosphine resistance in cigarette beetle, *Lasioderma serricorne* (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. Journal of Economic Entomology 87: 546-550.

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

Chapter Six

Short and long term evaluation of a fixed dose of *Beauveria bassiana* Strain MS-8 formulated in various doses of kaolin as a powder formulation applied to rice grains to control almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae)

Abstract

Short term and long term evaluation studies were conducted against *Ephestia cautella* on rice grains, using *Beauveria bassiana* Strain MS-8 formulated in various doses of kaolin as an active carrier. In the short term study (45 days) a fixed dose of 0.03g conidia kg^{-1} grain of Strain MS-8 was formulated in kaolin at doses of 0.3, 0.5, 1, and 2g kg^{-1} of grain. These formulations were evaluated for their effects on larval mortality and number of adults emerged. The highest level of larval mortality (90.0%) and the lowest numbers of adults emerged (1.6 insect/ 100g of rice grain) were caused by Strain MS-8 in a kaolin dose of 2g kg^{-1}. However, Strain MS-8 in a kaolin dose of 1g kg^{-1} performed well for the same parameters. In the long term evaluation study (180 days) the same dose of Strain MS-8 was formulated in kaolin at doses of 0.5, 1, 2, 3g kg^{-1} of grain. These formulations were then evaluated against the levels of webbed grain, grain damage and weight loss. The lowest levels of webbed grain (2.0%), grain damage (3.0%) and weight loss (1.8%) was caused by Strain MS-8 in kaolin at a dose of 3g kg^{-1}, although Strain MS-8 in kaolin doses of 1g and 2g kg^{-1} also performed well for the same parameters. The highest levels of webbed grain (15.0%), grain damage (30.0%) and weight loss (9.0%) were observed in the untreated control treatment (UTC).

6.1 Introduction

Insect pests cause global losses to stored grains, quantitatively and qualitatively, particularly in tropical and subtropical countries (Madrid et al. 1990; Tripathi et al. 2009). It is estimated that each year insects destroy between 10.0% and 30.0% of all food produced in Africa (Oerke 2006; Dhaliwal et al. 2010). The assessments of rice yield losses due to insects in Africa range between 10.0% and 15.0% (IRRI 2010). This loss differs regionally, by country and by rice variety, and in some years may exceed 90.0% (Youdeowei 2004).

The almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae), is a pest of a wide range of commodities including cereals and cereal products, cocoa and oilseeds. It is found

throughout the tropics and subtropics (Arbogast et al. 2005). The larvae are the damaging form of this insect as they feed on the whole grains and the seed germ (Ofuya & Lale 2001). The webbing and the frass produced by the larvae of this pest affects the aesthetics and handling of infested produce. The insect also causes physical damage to grain, enhancing infection by *Aspergillus* species which releases mycotoxins (Semple et al. 1991).

Fumigation and application of neurotoxic contact insecticides have been widely used to protect stored grains. In the case of fumigants, the most commonly used product phosphine has lost its efficacy in some areas. Several species of stored-grain insects have developed a significant level of resistance to this compound, making its application ineffective in many parts of the world (Benhalima et al. 2004; Pimentel et al. 2009; Jittanun & Chongrattanameteekul 2014; Nguyen et al. 2015). Several stored-grain insects have developed resistance to commonly used pyrethroid and organophosphate insecticides (Guedes et al. 1995; Bughio & Wilkins 2004; Pereira et al. 2009; Araújo et al. 2011). The development of resistance to chemical insecticides, and concerns over the adverse effects of chemicals on the environment and human health, have provided the stimulus for developing microbial control agents to control of pests of stored grains.

Entomopathogenic fungi (EPF) represent one of the most promising alternatives to chemical control against stored-grain insect (Schöller et al. 1997; Wakil & Ghazanfar 2010). *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) is the most widely tested EPF for the control of stored-grain insects (Crespo et al. 2002; Cherry et al. 2005; Sabbour et al. 2012; Kaur et al. 2014). Dried conidia of EPFs may be formulated with various natural dusts such as diatomaceous earth, charcoal, oven ash and chalk powder (Akbar et al. 2004; Batta 2004; Athanassiou & Steenberg 2007; Riasat et al. 2011). However, little is known about the use of other natural dusts such as kaolin as carriers of the dried conidia of EPF to control *E. cautella* in rice. Therefore, the objective of this study is to evaluate the effects of a fixed dose of *B. bassiana* Strain MS-8 with various doses of kaolin as powder formulations applied to rice grains to control almond moth, *E. cautella*, in a one-generation, 45 days trial, and in a 180 days trial.

6.2 Material and methods

6.2.1 Insect rearing

The initial stock culture of *E. cautella* used in this study was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Science (SAEES), University of KwaZulu-Natal. 1 kg of yellow maize grain[8] was introduced into 6 L plastic boxes[9]. Ten couples of insects (one day old) were released into the boxes. Each box was covered with insect nets[10]. This was to allow for adequate aeration and to prevent moth escape. The culture was maintained under the experimental conditions (28±2^{o}C and 65±5% RH) until a new generation of adult insects emerged. Emerged insects were used for the experiments.

6.2.2 Fungus and dry conidia preparations

The fungus *B. bassiana* Strain MS-8 used in this study was isolated from soil using *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a live insect bait (Zimmermann 1986).

A procedure documented by Gouli et al. (2013), with modifications, was used to prepare the dry conida. Initial production of the fungus was conducted on potato dextrose agar (PDA) for a period of 14 days at 28^{o}C. Mature conidia were collected from the surface of the medium. A conidial suspension was adjusted to 1x10^{8} conidia ml^{-1} by using a Neubauer Improved Hemocytometer[11]. Rice grains were washed well (1.5 kg) and soaked overnight. The rice grains were dispensed into three sterile autoclavable bags[12] (305 x 660 mm). Each bag was filled with 500 g of grain. The bags and rice grains were then sterilized at 121^{o}C for 15 min. followed by a 24 h cooling period at 22-25^{o}C. 50 ml of fungal suspension was used to inoculate the sterilized rice grain in the plastic bags using a medical syringe. After inoculation, the contents of the bags were mixed to assure an even distribution of the fungal suspension among the grains and kept for 15 days at 25^{o}C. Each bag was closed using a special stopper, having a diameter of 10 cm and length of 6 cm, which formed the aeration mouth. The mouth of the bag was covered by a special stopper consisting of three layers

[8]Smith Animal Feed, Unit 8 Davlen Pk, 11 Halsted Rd, M'Kondeni, Pietermaritzburg, 3201. The seed morphology was intermediate between flint and dent and the source was Brazil but the variety was unknown.
[9] Basix Plastics, 400 Victoria Road, Pietermaritzburg, South Africa.
[10] Film Flex Plastic Natal cc, Unit 10, Shepstone Park, Cnr. Shepstone and Blasé Roads, New Germany, South Africa
[11] Hirschmann®, Pietermaritzburg, South Africa.
[12] Whitehead Scientific (Pty) Ltd, Unit 9, Van Biljon Industrial Park, Winelands Close, Strickland, 7530.

including paper, tissue and aluminium foil, which faced the exterior of the bag. The first two layers protected the nutrient substrate from contamination and the third layer prevented the premature drying of the cultivated material for the first four days. Four days after of inoculation the upper parts of the aluminium foil were removed to facilitate air circulation. The bag stoppers were opened after 10 days to expedite fungal sporulation. After 15 days, the fungal biomass was spread evenly into paper towels in a layer of 1 cm deep and air-dried for four to seven days on a laminar flow bench. The dried conidia were harvested by sieving the infested grains through a 100 mm diameter sieve (150 µm pore size), and kept at 4^{o}C for further use.

6.2.3 Grain preparations

White rice grains[13] were obtained from a local supermarket. In order to remove any hidden infestation by insects, the grains were kept in freezer at -20^{o}C for one week.

6.2.4 Effect of *B. bassiana* powder formulations on larval mortality and adults emerged in the short term trial

B. bassiana Strain MS-8 at a dose of 0.03g conidia kg^{-1} of grain was formulated in kaolin at doses of 0.3, 0.5, 1, and 2g kg^{-1} of grain. These powder formulations were tested for their effects on *E. cautella* larvae. For each treatment, three glass jars[14] of 250 ml each, containing 100g rice grain were used as replicates of each treatment. A Control treatment was kept untreated. Subsequently, a male and female of newly emerged adults were introduced into each glass jar, which were then covered with insect nets. Adult insects were removed after the oviposition period of one day. After eggs hatching, larvae were examined for mortality every three days for a period of 21 days. The number of adults emerged after 45 days of storage was recorded. To confirm the fungus as the cause of larval death, the dead larvae were surface sterilized with 70% sodium hypochlorite, followed by rinsing three times in distilled water, then placed in Petri dishes lined with filter paper. The Petri dishes were kept in an incubator at 28^{o}C. The larval counts showing fungal growth were only considered for larval mortality due to fungus.

[13] Milled white rice, Osman's Taj Mahal, 180 Sirdar Road, Clairwood, Durban, South Africa.
[14] Victoria Packaging c.c. Agricultural Packaging Equipment and Safety Wear, 298 Victoria Road, Pietermaritzburg 3201, South Africa.

6.2.5 Persistence of *B. bassiana* powder formulations against *E. cautella* in the long term trial

B. bassiana Strain MS-8 at a dose of 0.03g conidia kg^{-1} was formulated in kaolin at doses of 0.5, 1, 2, and 3 g kg^{-1} of grain. These powder formulations were evaluated for their activity against *E. cautella* for 180 days in stored rice grain. For each formulation, three glass jars of 3 liter capacity, each containing 1 kg of rice grains were used as replicates. Formulated conidia and rice grain were mixed manually for three minutes to achieve even distribution in the powder. Two pairs of newly emerged adults (one day old) were released in each jar, which were then covered with insect nets for air circulation. Adults were left to oviposition, and died within seven to nine days. Pairs of newly emerged adults were added every 45 days (one generation) for all the treatments including the UTC until the end of the experiment, to ensure that every treatment had a new infestation, particularly those that has strong effects against the larvae of *E. cautella*. This would mimic the arrived new moths to infest rice grains.

6.2.6 Data collection

Every 45 days for a period of 180 days subsamples of 100g of rice grain were taken from each treatment to measure specific parameters. The webbed grains were collected and weighed, and then the webbed grains were expressed as a proportion of the total weight of grains. The rice grains were then sieved and the clean grains were weighed to calculate the weight loss from the original weight (Tefera 2011). 10g of rice grain from each sub sample was taken and the number of grains damaged by insects feeding was expressed as a percentage of the total number of grains. The grain samples and insects (larvae and adults) were returned to the respective treatments after evaluations.

$$\text{Weight loss (\%)} = \text{(original weight} - \text{weight after 45 days)/(original weight)} \times 100$$

6.2.7 Statistical analysis

The experiments were arranged in a randomized complete blocks design. Mortalities were corrected relative to the Control using Abbott's formula (Abbott 1925). Data was subjected to analysis of variance (ANOVA) using GenStat for Windows, 17th edition (Payne et al. 2014). Means were separated using Fisher's Least Significant Differences at the 5% level of significance.

Correlation analysis was undertaken to determine the relationships between mortality and other insect parameters, and between these secondary parameters using GenStat for Windows, 17th edition (Payne et al. 2014.

6.3 Results

6.3.1 Effect of *B. bassiana* powder formulations on larval mortality

Highly significant differences were observed among the main effects and their interactions on larval mortality (Table 6.1).

Linear regressions were observed between time and doses on the mortality of *E. cautella* larvae with $R^2 > 0.98$ (Fig. 6.1). Application of a fixed dose of 0.03g conidia kg^{-1} of *B. bassiana* Strain MS-8 formulated in kaolin at 0.3, 0.5, 1 and 2g kg^{-1} of grain caused mortality levels of 50.0, 75.0, 80.0, and 90.0% on *E. cautella* larvae, respectively, after 21 days of exposure. These results indicated that mortality increased when dose and exposure time increased. Increasing kaolin doses would improve distribution of the fungal conidia and therefore it would have increased the frequency of contact between the larvae and conidia, and hence infestation and death (Fig. 6.1).

Table 6.1 ANOVA table for the response of *E. cautella* larvae to treatment with *B. bassiana* Strain MS-8 at a dose of 0.03g conidia kg^{-1}, formulated in four doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Doses	3	9267.857	3089.286	333.31	<.001
Time	6	35437.5	5906.25	637.24	<.001
Doses x Time	18	819.643	45.536	4.91	<.001
Residual	54	500.5	9.269		
Total	83	46107			
CV%	7.2				
Mean ± SE	42.50 ± 2.486				
LSD	4.984				

6.3.2 Effect of of *B. bassiana* powder formulations on number of adults emerged in the short term trial

Highly significant differences were observed between the tested doses (F = 40.45*; P* < 0.001) (Table 6.2). Treatment of rice grain with *B. bassiana* Strain MS-8 at all doses of kaolin significantly reduced the number of adults that emerged relative to the UTC (Fig. 6.2). Strain MS-8 at a kaolin doses of 2g and 1g kg^{-1} caused the lowest number of adults emerged (1.6 and 2 adults/ 100g of rice grain) compared to adults emerged of 12 adults/ 100g of rice grain on the UTC (Fig. 6.2).

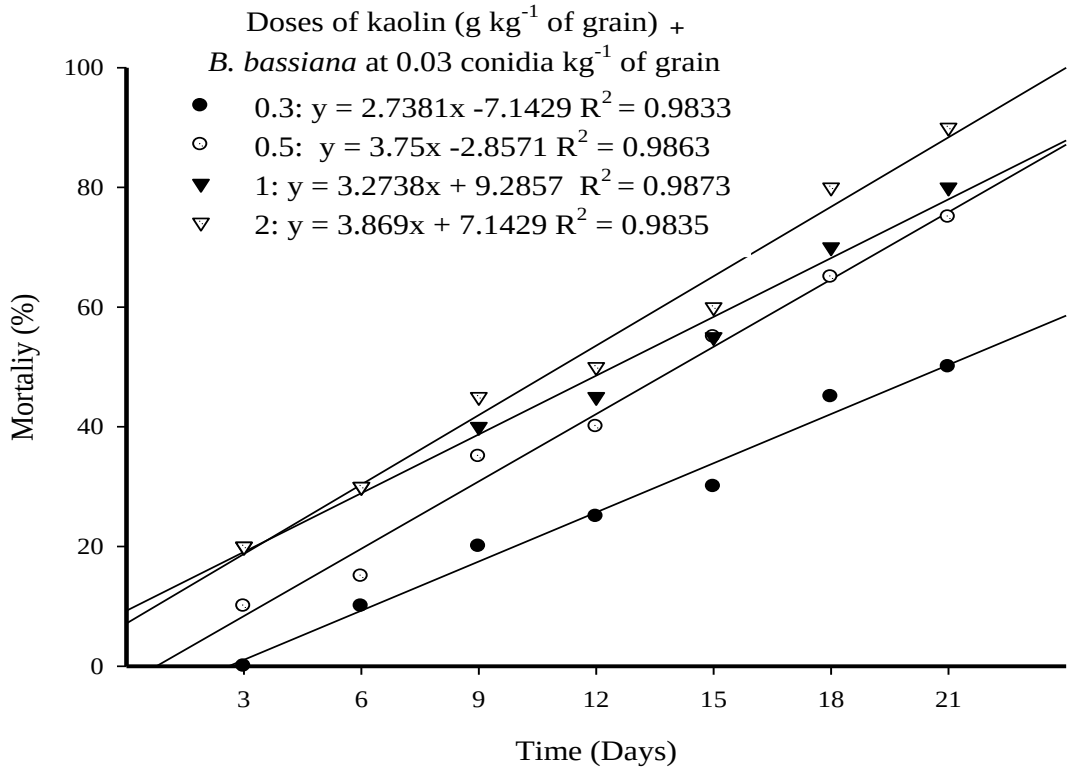

Fig. 6.1 Corrected mortality (Abbott's correction) of *E. cautella* larvae following exposure to *B. bassiana* Strain MS-8 at a fixed dose of 0.03 conidia g kg^{-1} formulated in kaolin at doses of 0.3, 0.5, 1 and 2g kg^{-1} of grain.

It was observed that a small number of *E. cautella* larvae avoided contact with the fungal conidia because they created a web cocoon extremely early, into which they migrated (Fig. 6.3). This may be a survival strategy by this pest to avoid hostile conditions, including natural incidence of entomopathogens. This behaviour limited their period of feeding and

subsequently the adults that emerged were small compared to UTC adults (Fig. 6.4). These small adults laid relatively few eggs, which slowed population growth.

Table 6.2 ANOVA table for the *E. cautella* adults that emerged from rice grain after exposure to a fixed dose of *B. bassiana* Strain MS-8 formulated in various doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F value	P value
Doses	4	234.6	58.65	40.45	<.001
Residual	8	11.6	1.45		
Total	14	275.1			
CV%	23.6				
Mean	5.1 ± 0.983				
LSD	2.267				

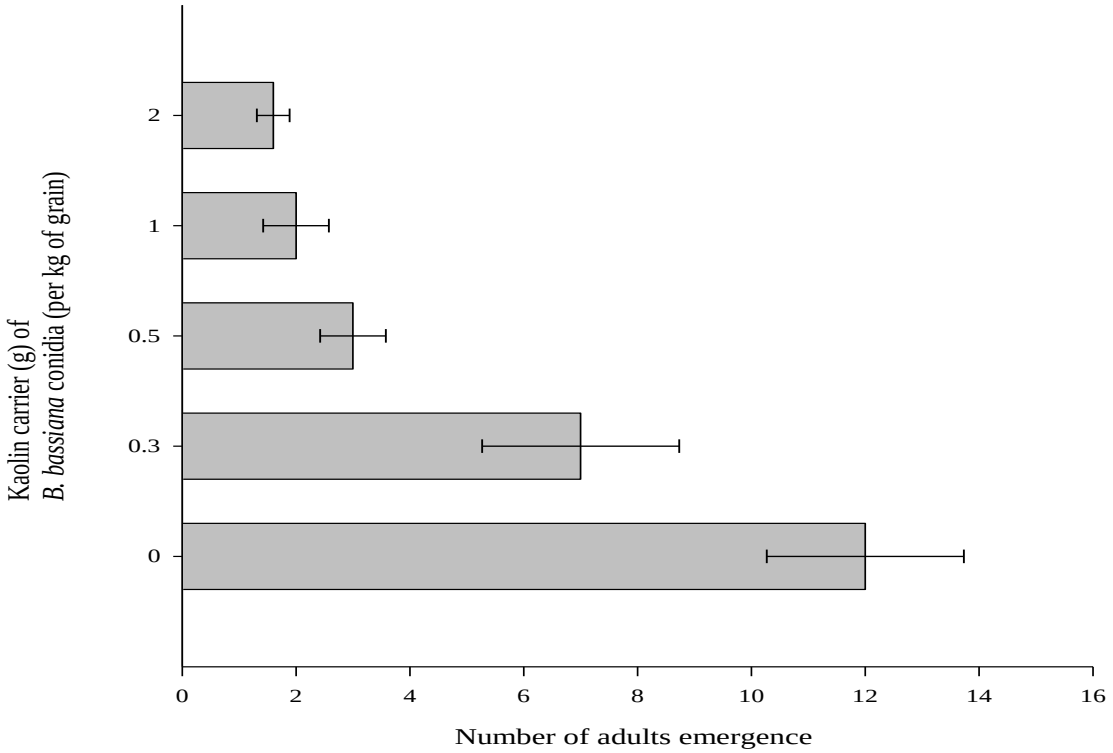

Fig. 6.2 Mean number of *E. cautella* adults that emerged from rice grain after 45 days of infestation following treatment with *B. bassiana* Strain MS-8 at 0.03g conidia kg^{-1} formulated in various doses of kaolin

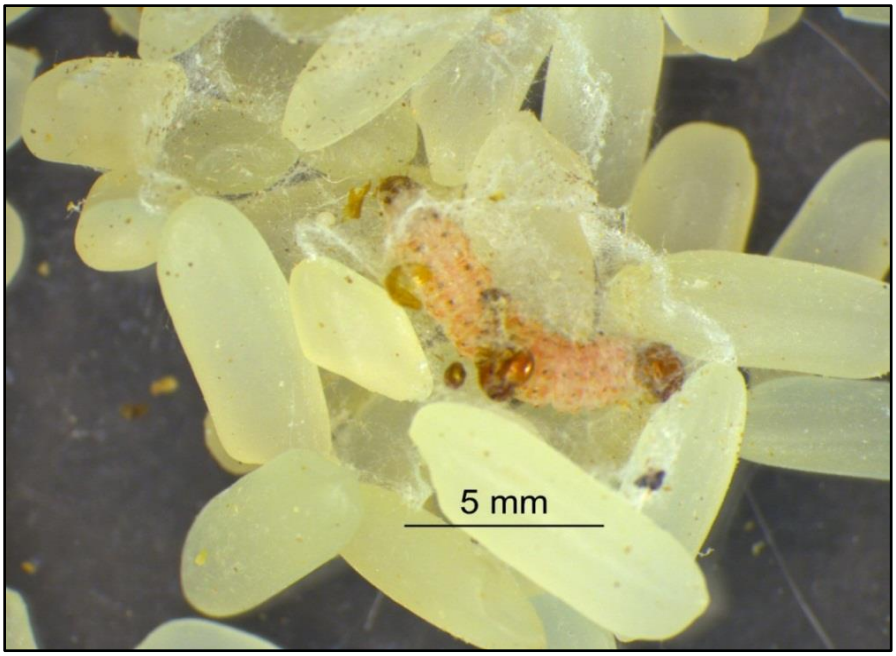

Fig. 6.3 Webs made by *E. cautella* larvae to avoid hostile conditions, following treatment of rice grain with *B. bassiana* MS-8 at a kaolin dose of 2g kg^{-1} of grain

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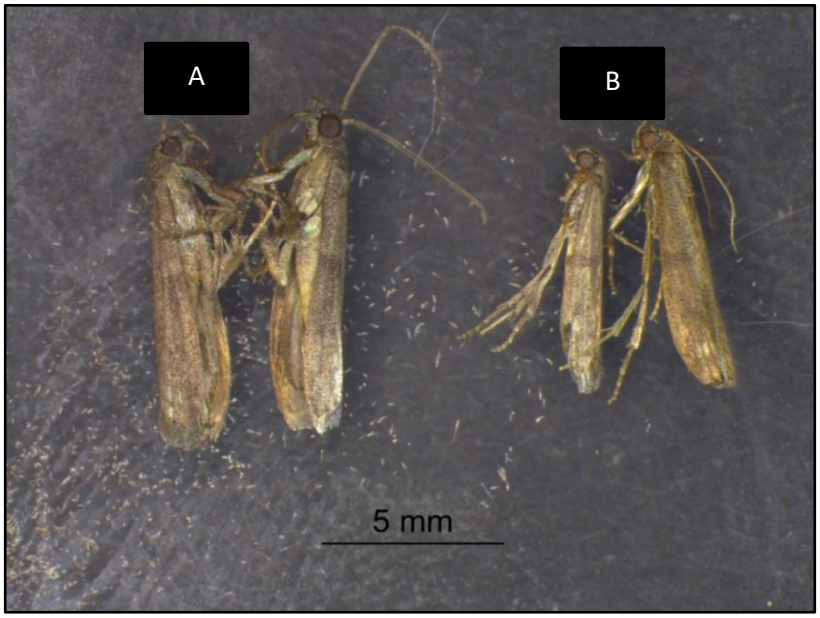

Fig. 6.4 **A**= *E. cautella* adults that emerged from untreated rice grain; **B** = adults that emerged from rice grains, following treatment with *B. bassiana* MS-8 at a kaolin dose of 2g $kg^{-1}$of grain, showing the difference in size.

6.3.3 Webbed grain (%) in the long term trial

There were highly significant differences between the doses (F = 53.51*; P* < 0.001) and time (F = 23.77*; P* < 0.001) for their effects on the level of webbed grain, after 180 days of storage. The interaction between doses and time (F = 2.64*; P* < 0.011) was also significant (Table 6.3).

A linear relationship was observed between dose and time for the level of webbed grain, with an $R^2 > 0.89$ (Fig. 6.5). Treatment of rice grain with Strain MS-8 at all doses of kaolin caused significantly lower levels of webbed grain after 180 days of storage. After the first 45 days of storage (one generation of *E. cautella*) no webbed grain was observed with Strain MS-8 at a kaolin dose of 3g kg^{-1}. However, webbed grain of 5.0% was observed with the UTC. These results were the inverse of mortality (Fig. 6.5).

After 180 days of storage (four generations of *E. cautella*) Strain MS-8 at a kaolin dose of 3g kg^{-1} caused the lowest level of webbed grain of 2.0% compared to a level of 15.0% for the UTC. No significant differences in the levels of webbed grain were observed for kaolin doses of 1g and 2g kg^{-1} by the end of the experiment (Fig. 6.5).

The coefficient of variance CV% was high at 34.7%, probably because webbing of grain is a derivative of the insect population. For instance, larvae could make a small web and then die of fungal infection.

Table 6.3 ANOVA table for the webbing of rice grains by *E. cautella* larvae after treatments with a fixed dose of *B. bassiana* Strain MS-8 formulated in various doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Dose	4	520.5	130.125	53.51	<.001
Time	3	173.4	57.8	23.77	<.001
Dose x Time	12	77.1	6.425	2.64	0.011
Residual	38	92.4	2.432		
Total	59	931			
CV%	34.7				
Mean ± SE	4.5 ± 1.273				
LSD	2.577				

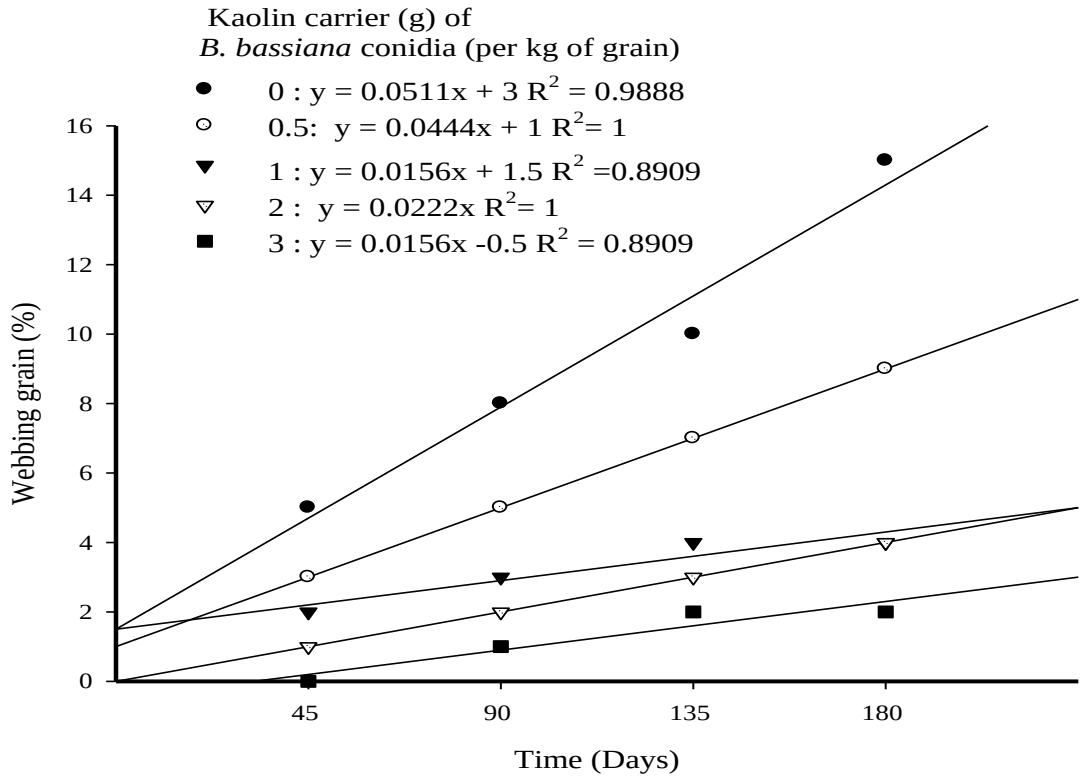

Fig. 6.5 Webbed grains over 180 days of *E. cautella* larvae infestation of rice following treatments with *B. bassiana* Strain MS-8 at 0.03g conidia kg^{-1} formulated in various doses of kaolin

6.3.4 Grain damage (%) in the ling term trial

Highly significant differences were observed between the main effects (doses and time) and their interaction dose x time (Table 6.4). The grain damage levels were reduced by all *B. bassiana* Strain MS-8 treatments.

Application of *B. bassiana* Strain MS-8 at all doses of kaolin to rice grain caused significant reductions in the levels of grain damaged by *E. cautella* larvae (Fig. 6.6). After 180 days of storage, the lowest level of grain damage (3.0%) was caused by Strain MS-8 at a kaolin dose of $3g^{-1}$, compared to a grain damage level of 30.0% in the UTC. In contrast, highly significant differences were observed with Strain MS-8 at kaolin doses of 0.5 and 1g kg^{-1} which reduced grain damage levels to 20.0% and 7.0%, respectively. These results indicate that kaolin improved the distribution of the fungal conidia and therefore more larvae were infected and killed. No significant differences were observed between the doses of kaolin 1, 2 and 3g kg^{-1} after the first 90 days of storage (Fig. 6.6).

Table 6.4 ANOVA table of rice grain damage caused by *E. cautella* larvae after treatments with a fixed dose of *B. bassiana* Strain MS-8 formulated in various doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Doses	4	2364.264	591.066	162.92	<.001
Time	3	R 771	256.853	70.8	<.001
Doses x time	12	504.192	42.016	11.58	<.001
Residual	38	137.859	3.628		
Total	59	3873.596			
CV%	24.4				
Mean ± SE	7.82 ± 1.555				
LSD	3.148				

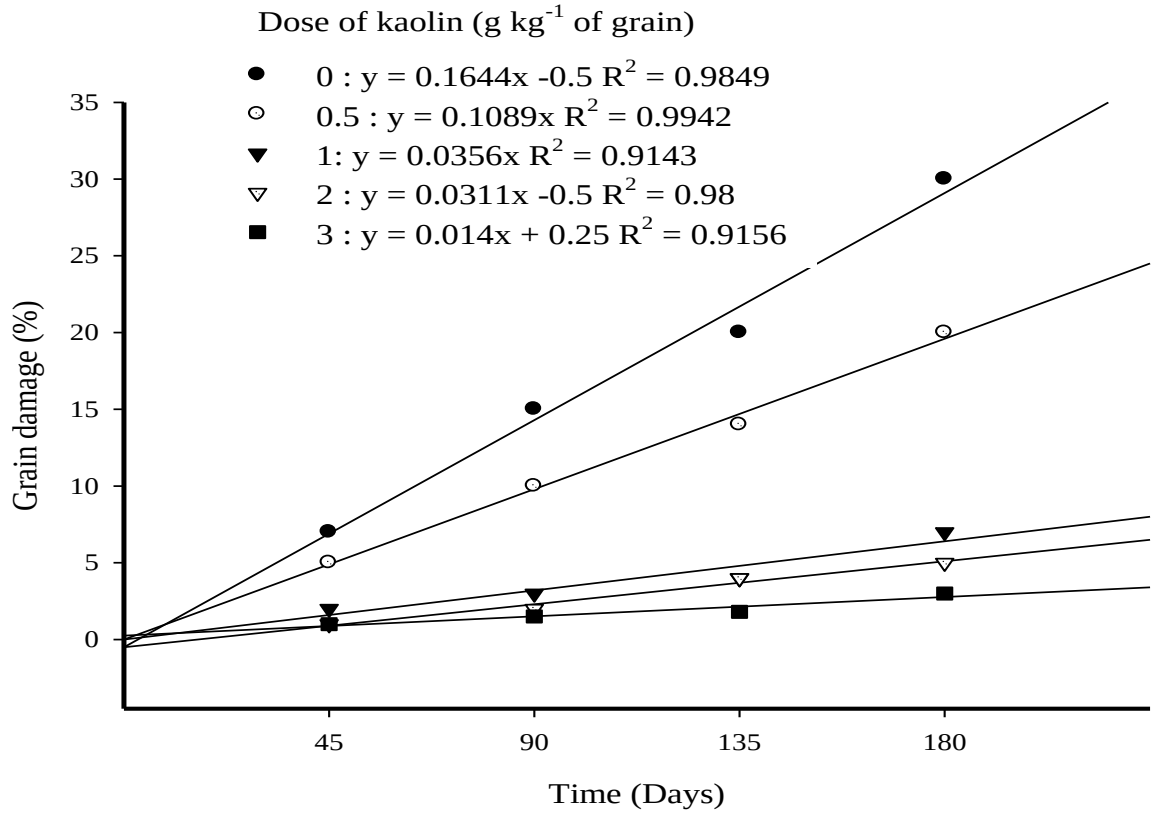

Fig. 6.6 Grain damage over 180 days after *E. cautella* larvae infestation on rice grain, following exposure to *B. bassiana* Strain MS-8 at 0.03g conidia kg^{-1} of grain formulated in various doses of kaolin

6.3.5 Grain weight loss (%) in the long term trial

Both main factors caused significant differences in the levels of weight loss, namely dose (F = 73.22*; P*< 0.001), and time (F = 36.35*; P*< 0.001). The interaction effect between dose and time (F = 3.83*; P*< 0.001) was also significant. Coefficient of variance, CV%, was high, probably because feeding initially would have caused weight loss for all treatments (Table 6.5).

Over the experimental period, a similar efficacy was observed for Strain MS-8 at kaolin doses of 2g and 3g on the levels of weight loss. However, at 180 days, treatment with Strain MS-8 at a kaolin dose of 3g kg^{-1} caused the lowest weight loss of 1.8%, relative to weight loss of 9.0% in the UTC. More than double of the weight loss was observed over the experimental period after application with Strain MS-8 at kaolin doses of 0.5g and 1g, except at the first 45 days during which there were no significant difference between treatments (Fig. 6.7).

Table 6.5 ANOVA table of weight loss of rice grain after *E. cautella* larvae infestation following treatments with a fixed dose of *B. bassiana* Strain MS-8 formulated in various doses of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Doses	4	203.916	50.979	73.22	<.001
Time	3	75.9405	25.3135	36.35	<.001
Doses x Time	12	31.992	2.666	3.83	<.001
Residual	38	26.459	0.6963		
Total	59	338.4285			
CV%	30.4				
Mean ± SE	2.74 ± 0.681				
LSD	1.379				

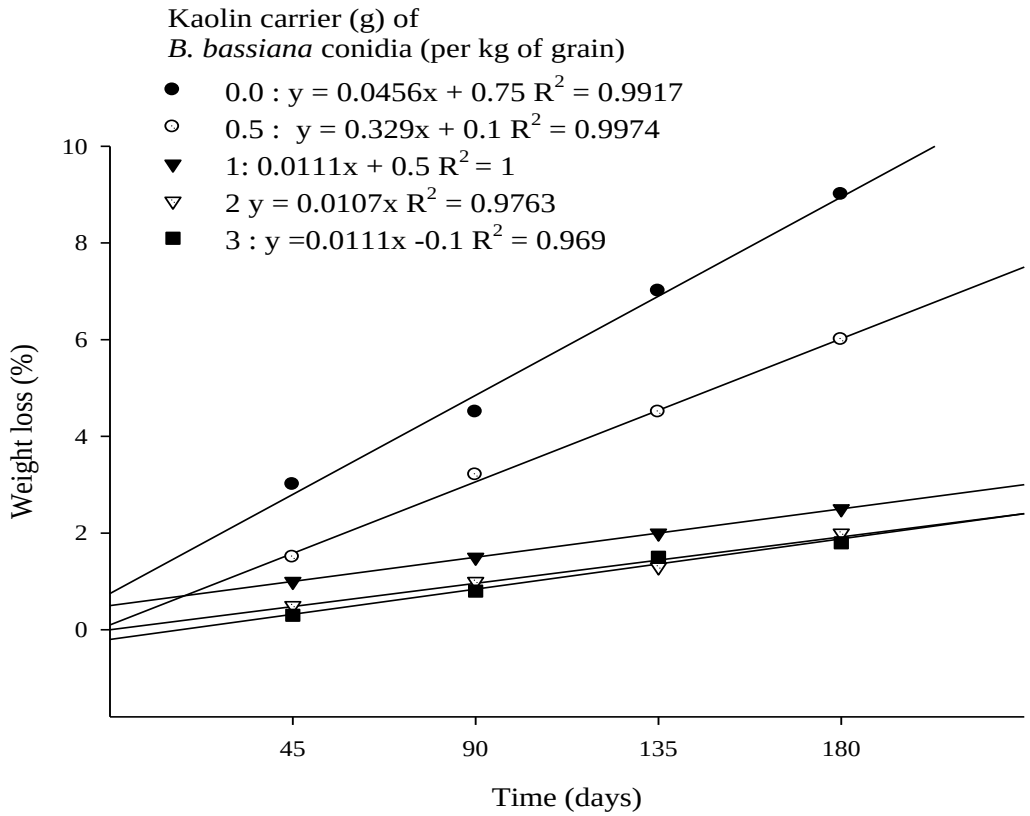

Fig. 6.7 Grain weight loss over 180 days of *Ephestia cautella* larvae infestation of rice grain following treatment with *Beauveria bassiana* Strain MS-8 at 0.03g conidia kg^{-1} of grain formulated in various doses of kaolin

The result of the correlation analysis between mortality and secondary parameters are presented in Table 6.6.

Table 6.6 Correlation coefficients between the measured secondary parameters and mortality of insects

	Adult emerged (%)	Grain damage (%)	Webbed grain (%)	Weight loss (%)	Mortality (%)
Adult emerged (%)	-				
Grain damage (%)	0.993***	-			
Webbed grain (%)	0.994***	0.989**	-		
Weight loss (%)	0.999***	0.996***	0.991***	-	
Mortality (%)	-0.996***	-0.981**	-0.999***	-0.995***	-

* Significant at P = 0.05; ** significant at P = 0.01; *** significant at P< 0.00

6.4 Discussion

Formulations of a fixed dose of *B. bassiana* Strain MS-8 in various doses of kaolin generated a range of mortality levels in *E. cautella*. For example, treatment of rice grains with Strain MS-8 at a kaolin dose of 0.3g and 2g kg^{-1} of grain caused mortality levels of 50.0 and 90.0% after 21 days of exposure, respectively. These results indicated that kaolin improved the efficacy of *B. bassiana* conidia either by increasing the distribution of fungal conidia or by abrasion of the waxy epidermis of target insects. These results are in agreement with Samodra & Ibrahim (2006a) who observed that the waxy layer of insect integuments was abraded by the kaolin, which allowed for greater conidial attachment and fungal penetration through the insect exoskeleton. Samodra & Ibrahim (2006b) evaluated the dried conidia of eight isolates of entomopathogenic fungi on *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in rice grains. Two isolates of *B. bassiana* (BbGc and BbPs) and one isolate of *Metarhizium anisopliae* (MaPs) caused high mortality levels on *C. cephalonica* larvae. Isolates of BbGc and BbPs formulated in kaolin and BbPs formulated in tapioca flour caused 100% larval mortality by the 15 days of exposure. More than 90% mortality was recorded when rice grains were stored for 4 months.

In the present study, twelve adults emerged in the UTC. However, application of Strain MS-8 at all doses of kaolin significantly reduced the number of adults that emerged from rice grains after 45 days of storage. Kaur et al. (2014) observed that exposure of *C. cephalonica* larvae to *B. bassiana* treated sorghum grains resulted in a significant reduction of adult emergence. Similarly, Cherry et al. (2005) found that *B. bassiana* 0362 at $1x\ 10^7$ and $1x10^8$ conidia g^{-1} grains led to significant adult mortality and reduced F1 emergence relative to an untreated population of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). The present study indicated that application of a fixed dose *B. bassiana* Strain MS-8 formulated in various doses of kaolin not only caused mortality in *E. cautella* but also produced small adults, which will ultimately lead to the suppression of the pest population. Kaur et al. (2014) observed morphological deformities in the adults of *C. cephalonica* following exposure to *B. bassiana* treatments.

Apart from direct infestation, the faeces and webbing produced by the larvae of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) spoil the product (Ghribi et al. 2012). In this study a significant reduction in the levels of webbed grain occurred after application of MS-8 at all doses of kaolin after 180 days of storage. The lowest level of webbed grain (2.0%) occurred

after treatment with Strain MS-8 in a kaolin dose of 3g kg^{-1}. This finding indicated that either kaolin interfered with the web formation or improved the fungal distribution of conidia and therefore increased the chances of larvae contacting conidia, becoming infected and dying.

The levels of grain damage and weight loss were the result of the higher or lower levels of pest populations that remained after treatment of rice grains with Strain MS-8 at all kaolin doses. For instance, the lowest levels of grain damage (3.0%) and weigh loss (1.80%) resulted from treatment with Strain MS-8 at a kaolin dose of 3g kg^{-1}, which also caused the highest level of larval mortality (90.0%) when applied at a dose of 2g kg^{-1} of grain. In contrast, the highest levels of grain damage and weight loss were both observed in the UTC. These results are in agreement with findings of Samodra & Ibrahim (2006a) who observed a direct relationship between levels of weight loss and the pest population after four month of *S. oryzae* infestation on rice grain, following treatment with *B. bassiana* isolates (BbGc and BbPs) formulated in kaolin. Padin et al. (2002) documented that damage to wheat grains by *S. oryzae* was reduced by treatments with *B. bassiana*.

The correlation analysis showed clearly that the secondary trials measured were highly inversely correlated with the primary parameter, namely mortality of insects. The secondary parameters were closely correlated with each other (99.3% - 99.9%).

In conclusions, *B. bassiana* Strain MS-8 formulated in kaolin was effective in the control of *E. cautella* in rice grain after 180 days of storage. Application of this strain could be used as a model to control other rice grains insects such as the rice moth, *C. cephalonica*. This study was conducted under controlled conditions, which differ from on farm conditions. Therefore further studies should be conducted to test the performance of this strain under on farm conditions in order to deal with the practical challenges of providing protection to stored grain in the field.

Refernces

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Economic Entomology 18: 265-267.

Akbar W, Lord JC, Nechols JR, Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Araújo RA, Williamson MS, Bass C, Field LM, Duce IR. 2011. Pyrethroid resistance in *Sitophilus zeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel. Insect Molecular Biology 20: 437-445.

Arbogast R, Chini S, Kendra P. 2005. Infestation of stored saw palmetto berries by *Cadra cautella* (Lepidoptera: Pyralidae) and the host paradox in stored-product insects. Florida Entomologist 88: 314-320.

Athanassiou C, Steenberg T. 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control 40: 411-416.

Batta Y. 2004. Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. Crop Protection 23: 103-108.

Benhalima H, Chaudhry M, Mills K, Price N. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research 40: 241-249.

Bughio F, Wilkins R. 2004. Influence of malathion resistance status on survival and growth of *Tribolium castaneum* (Coleoptera: Tenebrionidae), when fed on flour from insect-resistant and susceptible grain rice cultivars. Journal of Stored Products Research 40: 65-75.

Cherry A, Abalo P, Hell K. 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Products Research 41: 295-309.

Crespo R, Júrez M, Dal Bello G, Padin S, Fernández GC, Pedrini N. 2002. Increased mortality of *Acanthoscelides obtectus* by alkane-grown *Beauveria bassiana*. BioControl 47: 685-696.

Dhaliwal G, Jindal V, Dhawan A. 2010. Insect pest problems and crop losses: changing trends. Indian Journal of Ecology 37: 1-7.

Ghribi D, Elleuch M, Abdelkefi L, Ellouze-Chaabouni S. 2012. Evaluation of larvicidal potency of *Bacillus subtilis* SPB1 biosurfactant against *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae and influence of abiotic factors on its insecticidal activity. Journal of Stored Products Research 48: 68-72.

Gouli V, Provost C, Gouli S, Parker BL, Skinner M. 2013. Productivity of different species of entomopathogenic fungi based on one type of technology. Journal of Agricultural Technology 9: 571-580.

Guedes RNC, Lima JG, Santos JP, Cruz, CD. 1995. Resistance to DDT and pyrethroids in Brazilian populations of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Journal of Stored Products Research 31: 145-150.

IRRI, 2010. Global Rice Science Partnership (GRiSP). International Rice Research Institute, Los Baños, Philippines; Africa Rice Center, Cotonou, Benin; and International Center for Tropical Agriculture, Cali, Colombia.

Jittanun C, Chongrattanameteekul W. 2014. Phosphine resistance in Thai local strains of *Tribolium castaneum* (Herbst) and their response to synthetic pheromone. *Kasetsart* Journal (Natural Science) 48: 9-16.

Kaur S, Thakur A, Rajput M. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Journal of Stored Products Research 59: 185-189.

Madrid F, White N, Loschiavo S. 1990. Insects in stored cereals, and their association with farming practices in southern Manitoba. The Canadian Entomologist 122: 515-523.

Nguyen TT, Collins PJ, Ebert PR. 2015. Inheritance and characterization of strong resistance to phosphine in *Sitophilus oryzae* (L.). PloS one. Published 2015 April. Doi 10.1371/journal. pone.0124335.

Oerke EC. 2006. Crop losses to pests. The Journal of Agricultural Science 144: 31-43.

Ofuya TI, Lale NES. 2001. Pests of Stored Cereals and Pulses in Nigeria. Biology, Ecology and Control.. Dave Collins Publishers, Akure, Nigeria.

Padin S, Dal Bello G, Fabrizio M. 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research 38: 69-74.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. Genstat for Windows, 17th Edition. VSN International, Hemel Hempstead, UK.

Pereira C, Pereira E, Cordeiro E, Della Lucia T, Tótola M, Guedes R. 2009. Organophosphate resistance in the maize weevil *Sitophilus zeamais*: magnitude and behavior. Crop Protection 28: 168-173.

Pimentel M, Faroni LDA, Guedes R, Sousa A, Tótola M. 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Journal of Stored Products Research 45: 71-74.

Riasat T, Wakil W, Ashfaq M, Sahi ST. 2011. Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhyzopertha dominica* on stored wheat. Phytoparasitica 39: 325-331.

Sabbour M, Abd-El-Aziz S, Sherief M. 2012. Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modifications for the control of three pyralid moths in stored grains. Journal of Plant Protection Research 52: 359-363.

Samodra H, Ibrahim Y. 2006a. Effects of dust formulations of three entomopathogenic fungal isolates against *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice grain. Journal of Biosciences 17: 1-7.

Samodra H, Ibrahim YB. 2006b. Effectiveness of selected entomopathogenic fungi in packed rice grain at room temperature against *Corcyra cephalonica* Stainton. ASEAN Journal on Science and Technology for Development 23: 183-192.

Schöller M, Prozell S, Al-Kirshi AG, Reichmuth C. 1997. Towards biological control as a major component of integrated pest management in stored product protection. Journal of Stored Products Research 33: 81-97.

Semple R, Frio A, Hicks P, Lozare J. 1991. Mycotoxin prevention and control in food grains: A collaborative publication of the UNDP/FAO Regional Network Inter-Country Cooperation on Preharvest Technology and Quality Control of Food grains (REGNET) and the ASEAN Grain Postharvest Programme, Bankok, Thailand.

Tefera T, Mugo S, Tende R, Likhayo P. 2011. Methods of Screening Maize for Resistance to Stem Borers and Post-harvest Insect Pests. CIMMY. Nairobi, Kenya.

Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya P. 2009. A review on prospects of essential oils as biopesticide in insect-pest management. Journal of Pharmacognosy and Phytotherapy 1: 052-063.

Wakil W, Ghazanfar MU. 2010. Entomopathogenic fungus as a biological control agent against *Rhyzopertha dominica* F. (Coleoptera: Bostrychidae) on stored wheat. Archives of Phytopathology and Plant Protection 43: 1236-1242.

Youdeowei A. 2004. Fighting Hunger and Poverty: IPM Contributions in Africa. Plenary Lecture, 15th International Plant Protection Congress, Beijing, China. May.

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

Chapter Seven

Long term evaluation of a fixed dose of *Beauveria bassiana* (Balsamo) Vuillemin Strain MS-8, formulated in various doses of kaolin applied to maize grains against maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

Abstract

A long term evaluation of *Beauveria bassiana* Strain MS-8 at dose of 0.03g conidia kg^{-1} of grain formulated in kaolin at doses of 1, 2, 3, and 4g kg^{-1} of grain was conducted against *Sitophilus zeamais*. After six months, application of Strain MS-8 at all doses of kaolin significantly reduced the maize weevil population compared to the untreated control treatment (UTC). The best control of maize weevil was observed in the first four months of application. A similar performance of MS-8 at all doses of kaolin was observed over the six months. However, Strain MS-8 in a kaolin dose of 1g kg^{-1} outperformed the other doses and caused the lowest numbers of live weevils (36 insects/500g of maize grain), the lowest levels of grain damage (14.0%) and the least weight loss (7.0%) compared to number of live insects of (340 insects/500g of maize grain), grain damage of (68.0%) and weight loss of (51.0%) in the UTC.

7.1 Introduction

Maize (*Zea mays* L) is one of the most important cereal crops worldwide. In Sub-Saharan Africa (SSA) maize is the staple food crop, cash crop, and the major source of calories (Jones et al. 2011). In developing countries, maize is the main source of energy and provides up to 30% and 60% of the protein and energy, respectively (Mlyneková & Čerešňáková 2013).

In Africa, maize is grown by small-holder farmers for use as both human food and animal feed. Maize grain storage is crucial, in order to maintain a constant food supply throughout the year. For small-holder farmers in Africa, the main purpose of storage is to ensure household food supplies and seed for planting (Adetunji 2007; Owusu et al. 2007). Maize storage is challenged by a number of biotic constraints, the most important being insects, which destroy approximately 20 to 50% of stored maize in most African countries (Meikle et al. 2001; Boxall 2002). In addition to damage of grains by feeding insects also cause an increase in grain temperature and moisture content, which leads to increases in seed respiration and consequent losses in the quality and germinability of the grain (Suleiman et al.

2015). Insect damage also pre-disposes the grain to secondary attack by fungal pathogens such as *Aspergillus flavus* Link, leading to the production of mycotoxins (Sinha 1991; Beti et al. 1995; Birck et al. 2006).

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a serious pest of maize grain in Africa. The post-harvest losses due to the maize weevil have been recognized as an important constraint to grain storage and food security. In severe infestations, maize weevils can cause losses of 90.0% (Giga et al. 1991).

Chemical control with synthetic insecticides and fumigants has been the mainstay of control of stored-grain insects and have been highly effective (Zettler & Arthur 2000; Rajendran et al. 2004; Small 2007). However, grain contamination with chemical residues, development of resistance to the chemicals being used by key pests, and concerns over the environmental impact of agricultural inputs have led to a search for novel biologically-based control measures with few or no environmental hazards (da Paz Junior et al. 2012; Edde 2012; Popoola et al. 2015).

The use of entomopathogenic fungi (EPF) and other microbial control agents is seen to be a promising strategy with the potential to minimize the adverse effects of insecticides (Padin et al. 1997). These have considerable potential for the control of stored product pests (Nboyine et al. 2015). *Beauveria bassiana* (Balsamo) Vuillemin is one of the most widely used EPF because of its proven efficiency. It has been used to control stored grain pests such as the larger grain borer, *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae) (Nboyine et al. 2015), the rice moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (Kaur et al. 2014), the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Curculionidae) (Zamani et al. 2013) and the granary weevil, *Sitophilus granarius* Linnaeus (Coleoptera: Curculionidae) (Sabbour & Abdel-Raheem 2015).

Commercial formulations of *B. bassiana* are available and registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control applications (Lord 2001). However, no commercial products of EPF are registered for biocontrol of stored-grain insects (Batta 2016). The long term evaluation of the formulated form of EPF was previously carried out against various stored grain pests with various formulations (Meikle et al. 2001). However, using a powder formulation of *B. bassiana* with an inert dry carrier such as kaolin against *S. zeamais* on stored maize in long term trials has not been investigated. Therefore the present study aimed to evaluate the efficacy of the *B. bassiana* MS-8 using kaolin as the

carrier against *S. zeamais* in stored maize in an extended trial to reflect the storage period of maize by small scale farmers in the off season.

7.2 Material and methods

7.2.1 Insect rearing

The initial population of *S. zeamais* was obtained from the Department of Plant Pathology, School of Agricultural, Earth and Environmental Science (SAEES), University of KwaZulu-Natal. For each plot, five hundred grams of yellow maize grains[15] were placed into a 1 liter glass jar[16]. Approximately 200 unsexed adult insects were released into the jar, which was then covered with insect nets[17] to facilitate air circulation. After 10 days of ovipositioning all insects were removed through sieving. The sieved grains were introduced into a clean jar and kept for a period of 35 days for progeny emergence. Emerging progenies of *S. zeamais* were removed daily, and transferred to fresh maize grain in a glass jar covered with insect nets, and were kept under experimental conditions (28±2^{o}C and 50±5% RH) until sufficient numbers of *S. zeamais* were collected.

7.2.2 Grain preparation

Untreated yellow maize grain was used in this study. Grain was sieved to remove any dirt, dust or broken grain. Any hidden infestation in the grain was removed by putting the grain in an oven at 40^{o}C for 4 hours (Bekele 2002). Disinfested grain was kept in a freezer at approximately -1^{o}C to protect it from new further infestations. For experimental purposes, the grain was removed from the freezer and allowed to acclimatize at ambient temperature and relative humidity, and then sun dried.

7.2.3 Fungus and dry conidia preparations

The fungus, *B. bassiana* Strain MS-8, used in this study was isolated from soil from Ukulinga Research Farm, Pietermaritzburg (KwaZulu-Natal Province), using *Galleria mellonella* L.

[15] Smith Animal Feed, Unit 8 Davlen Pk, 11 Halsted Rd, M'Kondeni, Pietermaritzburg, 3201. The seed morphology was intermediate between flint and dent, the source was Brazil, but the variety was unknown.
[16] Victoria Packaging c.c. Agricultural Packaging Equipment and Safety Wear, 298 Victoria Road, Pietermaritzburg 3201, South Africa.
[17] Film Flex Plastic Natal cc, Unit 10, Shepstone Park, Cnr. Shepstone and Blasé Roads, New Germany, South Africa.

(Lepidoptera: Pyralidae) as a live insect bait (Zimmermann 1986). The powder formulation of this fungus was prepared as described in Chapter Six, Section 6.2.2.

7.2.4 Grain treatments

Across all five treatments 5 kg samples of yellow maize grain were used. Treatments were prepared by diluting *B. bassiana* MS-8 at dose of 0.03g conidia kg^{-1} of grain with kaolin at doses of 1, 2, 3 and 4 g kg^{-1} (i.e. 5, 10, 15, and 20 g $5kg^{-1}$ in the samples, respectively. Powder formulations and maize grain were mixed using a wooden spoon to ensure even distribution. To provide an initial infestation, 20 randomly selected adult insects of both sexes were released into 17.5 L plastic boxes[18] each containing 5 kg samples of treated yellow maize grain. A fifth treatment was untreated control (UTC) the treatments were replicated three times.

7.2.5 Data collection

Every 35 days for a period of 180 days 500g sub-samples from each treatment were weighed and sieved through a 300 mm diameter x 2.0 mm aperture sieve[19]. The number of live insects, number of damaged grains, weight of damaged grain, number of undamaged grains and weight of undamaged grain were recorded. Grain samples and insects were returned to the respective boxes after assessments. Grain damaged was expressed as a proportion of the total numbers of grain. Grain weight loss percentage was assessed using the method described by Gwinner (Gwinner et al. 1996).

$$\text{Weight loss (\%)} = (\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu}) \times 100) / (\text{Wu} \times (\text{Nd} + \text{Nu})$$

Where, Wu = Weight of undamaged grain, Nu = Number of undamaged grain, Wd = Weight of damaged grain, and Nd = Number of damaged grain. Experiments were arranged in a complete randomized blocks design. Data was subjected to analysis of variance (ANOVA) using GenStat for Windows, 17th edition (Payne et al. 2014). Means were separated using Fisher's Least Significant Difference test at a 5.0% level of significance.

[18] Basix Plastics, 400 Victoria Road, Pietermaritzburg, South Africa.
[19] Shalom Laboratory Supplies Cc, Office F8, International Plaza, 132 Dr A B Xuma St, Durban, 4000, South Africa.

7.2.6 Evaluation of remaining components

After six months a final evaluation was made of the remaining components: grain, grit, dust and weevils. From these measurements, grain loss (available food) was calculated.

7.3 Results

7.3.1 Number of live insects

All main effects were highly significant at the $P < 0.001$ level. However, the interaction between time and dose was not significant (Table 7.1).

Application of Strain MS-8 at all doses of kaolin caused significantly reduced numbers of live insects over six months (Fig. 7.1 and Fig. 7.2). The lowest number of live insects was observed in the first four months after treatments with Strain MS-8 at all doses of kaolin. However, a slight increase in insect numbers was observed by Month Five and Six (Fig. 7.2). A similar performance was observed among MS-8 treatments in the number of live insects over six months. After six months, Strain MS-8 at a kaolin dose of 1g kg^{-1} of grain caused the lowest number of live insects of 36 insects/500g of maize grain. The largest number of live insects was 340 insects/500g of maize grain in the UTC.

Table 7.1 ANOVA table for number of live weevils (Log_{10} transformed)

Source of variation	d.f.	s.s.	m.s.	F values	P values
Time	5	15.94017	3.18803	72.51	<.001
Dose	4	10.09695	2.52424	57.41	<.001
Time x Doses	20	0.77209	0.0386	0.88	0.614
Residual	58	2.55007	0.04397		
Total	89	29.52108			
CV%	18.7				
Mean +SE	1.118 ± 0.1712				
LSD	0.3427				

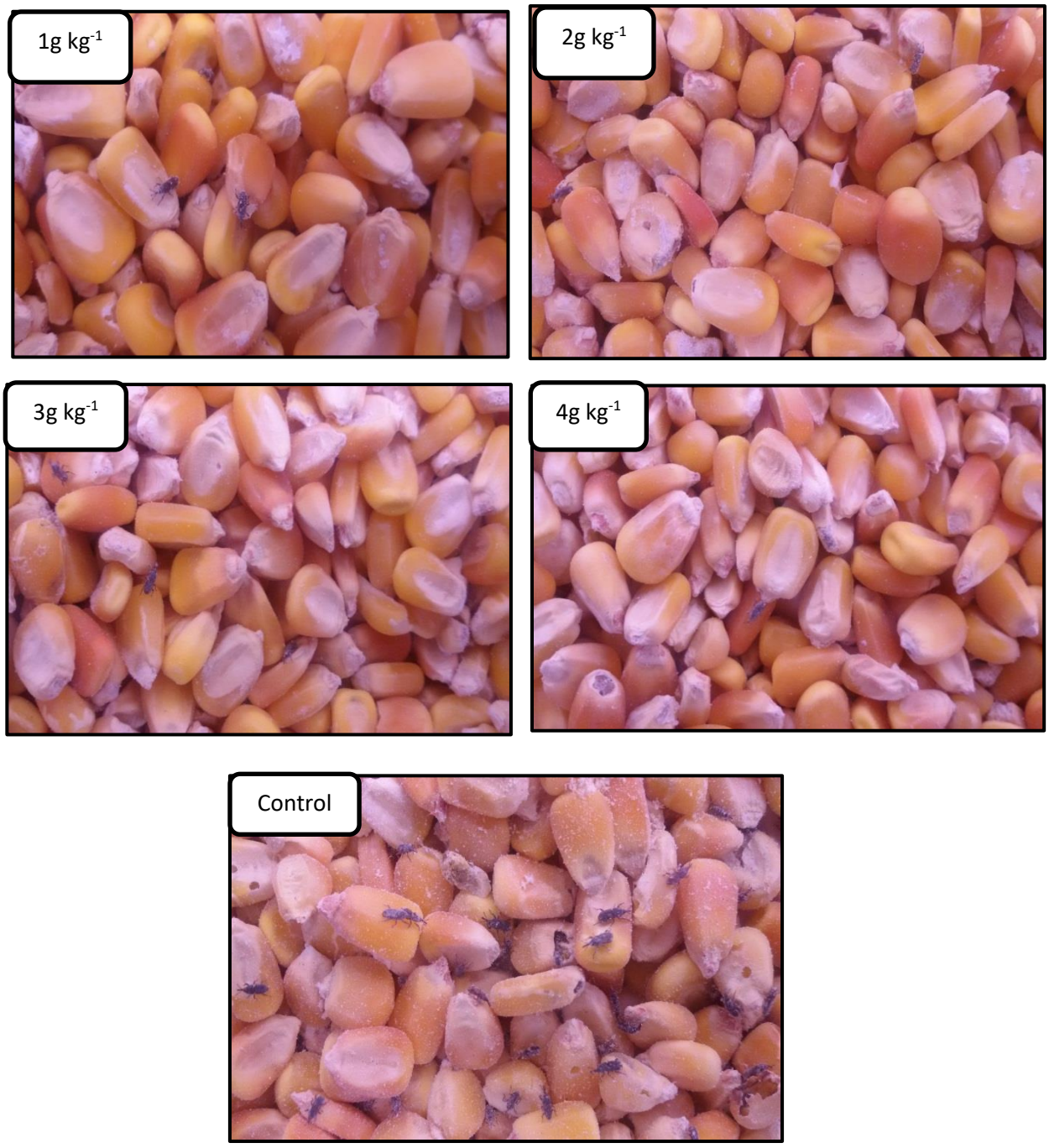

Fig. 7.1 The visual condition of maize grain after six months of *S. zeamais* infestation following treatment with a fixed dose of *B. bassiana* Strain MS-8 (0.03g conidia kg^{-1} of grain) formulated in kaolin at doses of 1, 2, 3, and 4g kg^{-1} of grain.

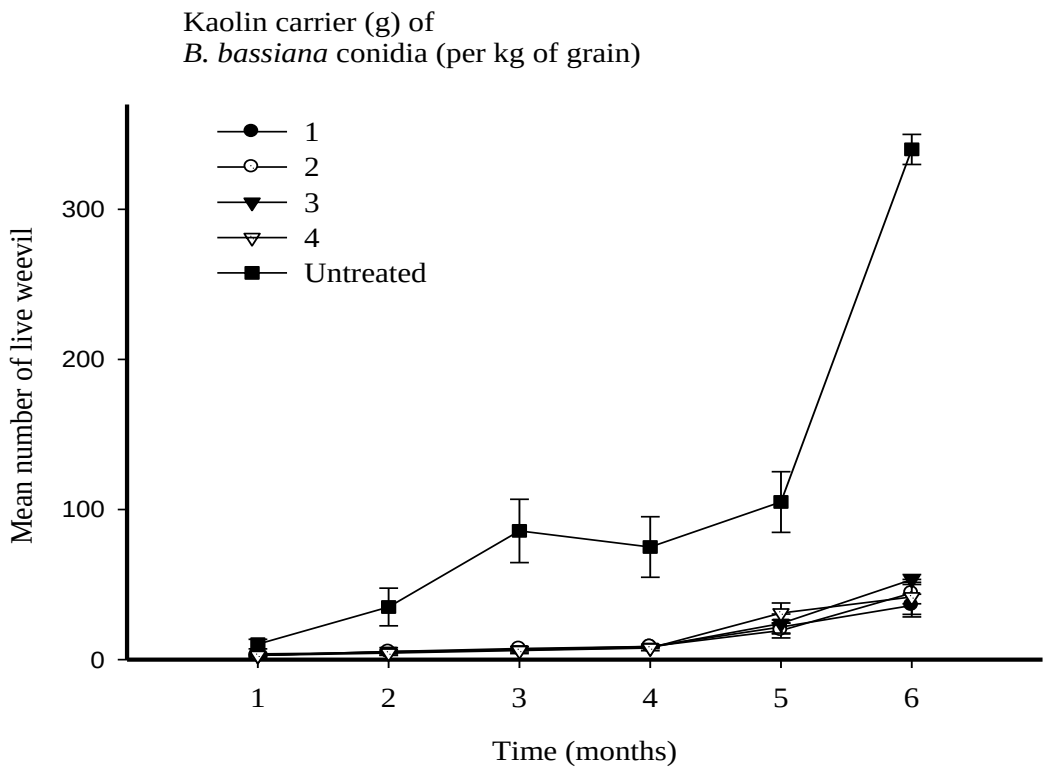

Fig. 7.2 Mean number of a live *S. zeamais* after six monthes of infestation of maize grain following treatment with a fixed dose of *B. bassiana* Strain MS-8 (0.03g conidia kg^{-1} of grain) formulated in kaolin at doses of 1, 2, 3 and 4g kg^{-1} of grain.

The pooled data for insect numbers per month after treatments with Strain MS-8 at all doses of kaolin (1, 2, 3, and 4g kg^{-1} of grain), relative to the UTC presented in Fig. 7.3. More live insects (28.50%) were observed in the first month. The relative numbers of live weevils decreased to 10.0% in Months Two to Four. A slight increase to 23% was observed in Month Five, but it decreased to 12% in Month Six. This indicates that the MS-8 treatments were still active after six months and that approximately 90% of emerging weevils were killed in most months (Fig. 7.3).

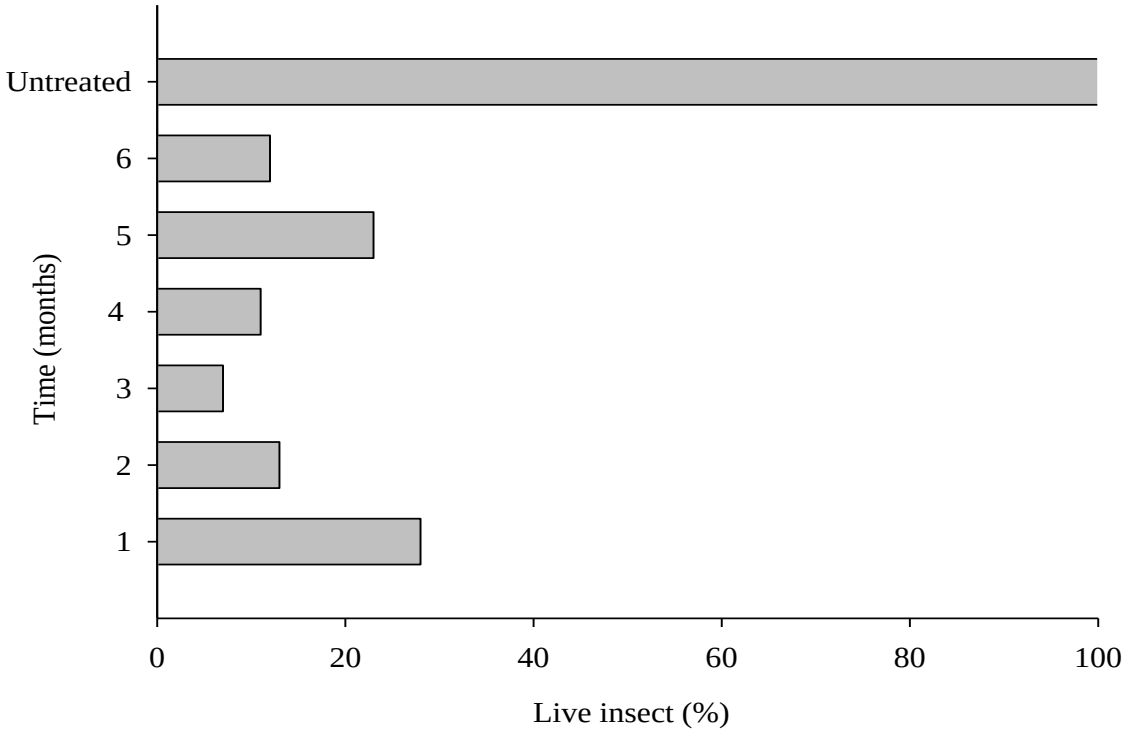

Fig. 7.3 Percent age of live weevils relative to the number in the untreated control treatment (pooled data of treatments with *B. bassiana* Strain MS-8 in 1, 2, 3 and 4g of kaolin kg^{-1} of grain after six month of weevil infestation

7.3.2 Grain damage (%)

All main effects were highly significant at the $P < 0.001$ level, and the interaction of dose × time was significant at $P = 0.015$ (Table 7.2).

Treatments of maize grain with Strain MS-8 at all doses of kaolin significantly reduced the level of grain damage caused by *S. zeamais*. In the first four months, maize treated with Strain MS-8 at all doses of kaolin_developed a very low level of grain damage. However, a slight increase was observed after five and six months of storage. After Month Six, Strain MS-8 in a kaolin dose of 1g kg^{-1} caused the lowest level of grain damage of 14.0% compared to grain damage of 68.0% in the UTC. Similar performances in reducing the levels of grain damage were observed at all doses after six months of treatment. The high level of grain damage in the UTC reduced the quality of the grain, and pre-disposed the grain to secondary attack by pathogens such as *Aspergillus flavus*, which would lead to the production of mycotoxins (Fig. 7.4).

Table 7.2 ANOVA table for the level of grain damage (angular transformed)

Source of variation	d.f.	s.s.	m.s.	F values	P values
Time	5	1.208144	0.241629	50.29	<.001
Doses	4	1.494933	0.373733	77.79	<.001
Time x Doses	20	0.200813	0.010041	2.09	0.015
Residual	58	0.278655	0.004804		
Total	89	3.210206			
CV %	23.3				
Mean ± SE	0.297 ± 0.05659				
LSD	0.11329				

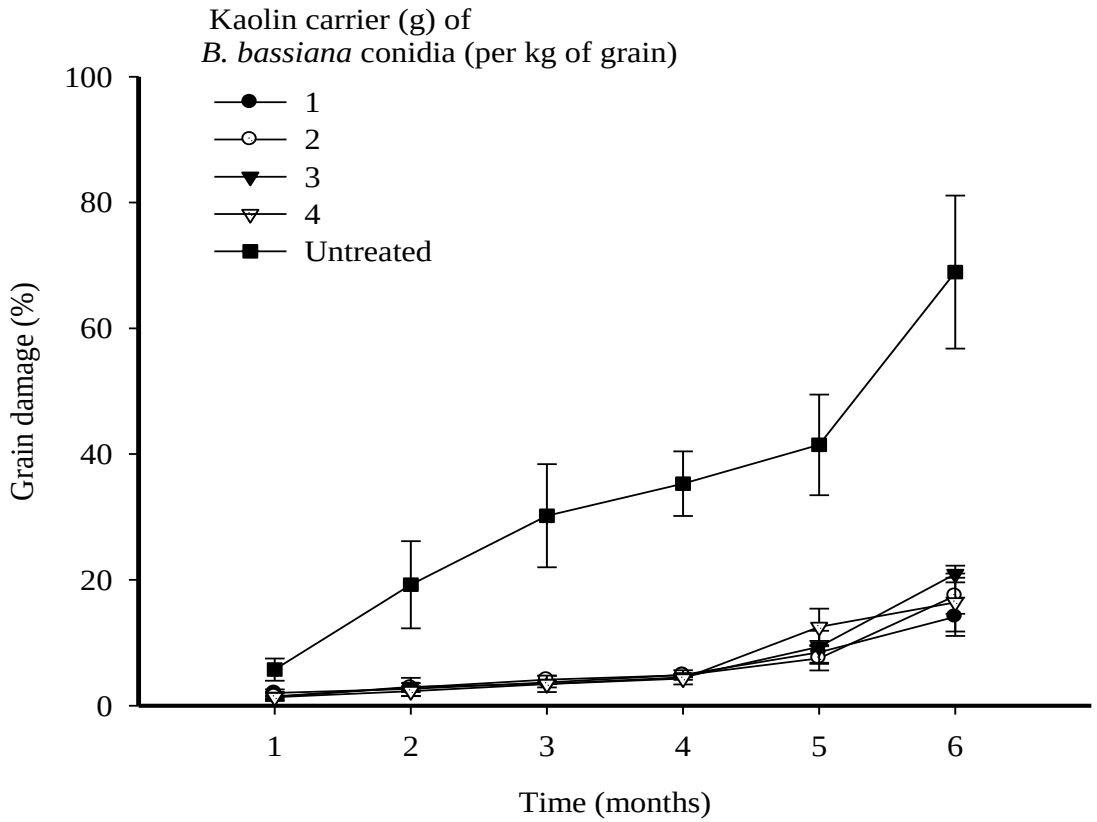

Fig. 7.4 Grain damage over six months of *S. zeamais* infestation of maize grains following treatment with *B. bassiana* Strain MS-8 at a fixed dose of 0.03g conidia kg^{-1} formulated in kaolin at doses of 1, 2, 3 and 4g kg^{-1} of grain.

7.3.3 Grain weight loss (%)

Highly significant differences were observed for time (F= 41.97*; $P < 0.001$*) and doses (F = 60.15*; $P < 0.001$*) for their effects on weight loss caused by *S. zeamais*. The interaction between time and doses (F = 2.31*; P* < 0.007) was also significant (Table 7.3).

A lower level of weight loss was observed after treatments with Strain MS-8 at all doses of kaolin than the UTC. In the first four months, application of Strain MS-8 at all doses of kaolin resulted in a weight loss of < 2.0% compared to a weight loss of 21% in the UTC. After six months, a low weight loss of ≤ 10.0% was observed as result from the MS-8 treatments compared to a weight loss of 51.0% in the UTC. A doubling of weight loss from 27.0% to 51.0% was observed in the UTC in the spring (August/ September).The four doses of kaolin (1, 2, 3, 4 g kg^1 of grain) were equally effective as carriers of the MS-8 conidia, with no significant differences in grain weight loss as a result of these treatments. However, the lowest level of weight loss was observed at a dose of 1g kg^{-1} (Fig. 7.5).

Table 7.3 ANOVA table of the level of weight loss (Angular transformation)

Source of variation	d.f.	s.s.	m.s.	F values	P values
Time	5	0.869047	0.173809	41.97	<.001
Doses	4	0.996517	0.249129	60.15	<.001
Time x Doses	20	0.191699	0.009585	2.31	0.007
Residual	58	0.240214	0.004142		
Total	89	2.317317			
CV%	35.5				
Mean ± SE	0.2042 ± 0.05255				
LSD	0.10518				

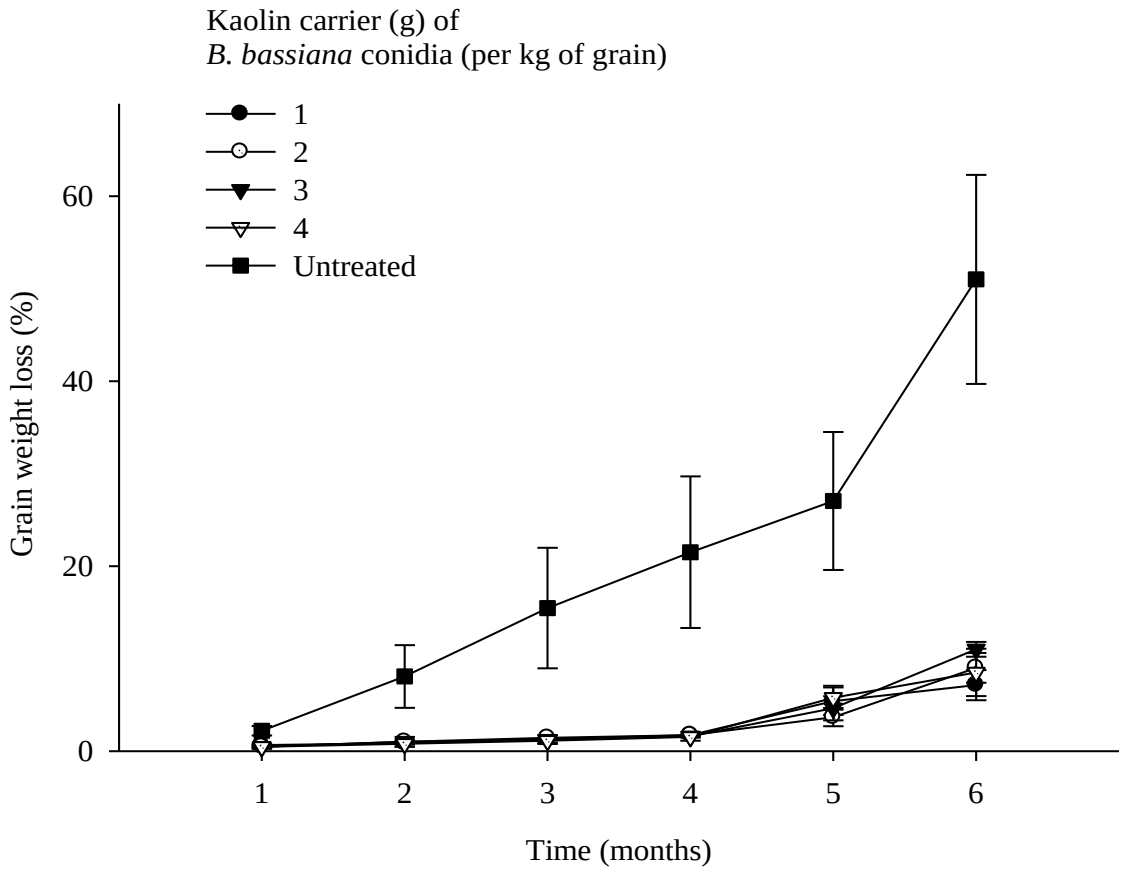

Fig. 7.5 Grain weight loss after six months of *S. zeamais* infestation of maize grains following treatment with a fixed dose of *B. bassiana* Strain MS-8 (0.03g conidia kg^{1} grain) formulated in kaolin at doses of 1, 2, 3 and 4g kg^{-1} of grain.

7.3.4 Evaluation of remaining components

After six months of weevil infestation, more edible grain remained after all biocontrol doses than the UTC. The greatest quantity of edible grain (4700g) was observed to result from the treatment of 1g kg^{-1}, compared to 2900g in the UTC (Table 7.4). Significantly ($P < 0.001$) more grain dust (90g) was obtained from untreated maize grain compared to all treated maize grain. Losses of >2000g were estimated from the weights of dust plus small grain particles in the UTC. The most insect biomass (33g) was measured in the UTC compared to an insect biomass of 8g in MS-8 treatments, a fourfold reduction (Fig. 7.6). Edible grain remaining (%) of 58% was estimated in maize grain when left untreated compared to 94% remaining after application of Strain MS-8 treatments (1g kaolin). Hence, for a farmer, 36% more grain would be available to eat or to sell. The alternative perspective is that his postharvest crop loss to maize weevil would diminish from 42% to 6%, a 700% reduction.

Table 7.4 Grain fractions after six months of *Sitophilus zeamais* infestation of maize grains

Doses	Initial grain	After six months of storage				
		Edible grain g^{-1}	Edible grain remaining (%)	Highly damaged grain + small fractions g^{-1}	Dust g^{-1}	Insect biomass
1g	5000g	4700 b	94 b	278 a	15 b	7 b
2g	5000g	4600 b	92 b	375 b	17 b	8 b
3g	5000g	4500 b	90 b	471 c	20 b	9 b
4g	5000g	4650 b	93 b	323 ab	18 b	9 b
Control	5000g	2900 a	58 a	1977d	90 a	33a
F		34.05	50.92	1424.10	73.40	171.91
P<		0.001	0.001	0.001	0.001	0.001
LSD		430.0	7.032	62.73	12.36	2.761
CV%		5.3	25.6	4.9	20.5	11.1

LSD = least significant difference; CV% = coefficient of variance. Means followed by the same letter do not differ significantly at $P < 0.05$ according to the Duncan's multiple range test.

Fig. 7.6 Insect biomass of *Sitophilus zeamais* after six months of infestation on maize grain

7.4 Discussion

A significant reduction ≥ 10.0% in the level of live *S. zeamais* adults was observed upon treatment of maize grain with *B. bassiana* MS-8 at all doses of kaolin. This indicates that MS-8 remained active over six months and that ≥ 90.0% of weevils were killed in most months. This is similar to the results of Bourassa et al. (2001) who observed that *B. bassiana* IMI330194 caused a mortality level of 100% in *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) larvae. Mul et al. (2009) observed that after killing insects, the fungus could grow out of the insect cadaver and produce more spores, thus increasing the chances of other individuals becoming infected. On the other hand, fungal infections also cause a decrease in the rate of oviposition and fertilisation, as a result of a decline in the female physiological state related to fungal colonisation of tissue, such as the fat body and the ovaries (Blay & Yuval 1999). In contrast, an exponential increase in the population of live weevils was observed in the untreated maize grain because under such conducive conditions, maize weevils require less development time, thus they produce more generations over shorter storage periods, and this compounds the damage caused to the maize grain.

Strain MS-8 at a kaolin dose of 1g kg^{-1} of grain caused the lowest level of grain damage (14.0%) compared to grain damage of 68.0% in the untreated maize. The high levels of grain damage in the UTC, together with the reproduction of the weevils, would have caused an increase in grain temperature and moisture content. This would have pre-disposed the damaged grains to secondary attack by pathogens such as *Aspergillus flavus* Link, which produce mycotoxins (Beti et al. 1995; Birck et al. 2006; Sinha & Sinha 1991). The lower levels of grain damage of 14.0% that was observed in our study would result in less infection and lead to lower mycotoxin levels. Setamou et al. (1997) detected lower levels of mycotoxin in less damaged maize than in more damaged maize. Bruns (2003) observed a direct association between insect feeding activity, fungal growth and mycotoxin production.

After six months, application of Strain MS-8 at a kaolin dose of 1g kg^{-1} caused the lowest level of weight loss (7.0%) compared to a weight loss of 51.0% in the UTC. The evaluation of other grain components indicated that treatment with Strain MS-8 at all doses of kaolin resulted in a higher level of edible grain remaining, lower levels of dust production, small fractions and lower levels of insect biomass. Padin et al. (2002) reported that wheat grain infested with *S. oryzae* without *B. bassiana* were significantly more damaged by weevils than grains treated with the fungus, and that weight loss decreased by 81.5% after four month of storage. They applied twenty-five grams of formulation into 500g of grain which is equivalent

to 50 times more than the dose used in this study. This result indicates that our study offers a practical level of application that could be adopted by small-scale farmers.

In conclusion, *B. bassiana* Strain MS-8 induced a high level of mortality in *S. zeamais*, which subsequently reduced the levels of grain damage and grain weight loss. These results suggest that *B. bassiana* Strain MS-8 could be used as an alternative to chemical control for the management of maize weevil infestations. However, this study needs to be supported with field studies before registration and commercialisation can be undertaken.

References

Adetunji M. 2007. Economics of maize storage techniques by farmers in Kwara State, Nigeria. Pakistan Journal of Social Sciences 4: 442-450.

Batta YA. 2016. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology 26: 1171–1183.

Bekele J. 2002. Evaluation of the toxicity potential of *Milletia ferruginea* (Hochest) Baker against *Sitophilus zeamais* (Motsch.). International Journal of Pest Management 48: 29-32.

Beti JA, Phillips TW, Smalley EB. 1995. Effects of maize weevils (Coleoptera: Curculionidae) on production of aflatoxin B1 by *Aspergillus flavus* in stored corn. Journal of Economic Entomology 88: 1776-1782.

Birck N, Lorini I, Scussel V. 2006. Fungus and mycotoxins in wheat grain at post harvest, *In* Proceedings of the 9th International Working Conference on Stored Product Protection. Campinas, São Paulo, ABRAPOS, Brazil.

Blay S, Yuval B. 1999. Oviposition and fertility in the Mediterranean fruit fly (Diptera: Tephritidae): effects of male and female body size and the availability of sperm. Annals of the Entomological Society of America 92: 278-284.

Bourassa C, Vincent C, Lomer C, Borgemeister C, Mauffette Y. 2001. Effects of entomopathogenic Hyphomycetes against the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), and its predator, *Teretriosoma nigrescens* Lewis (Coleoptera: Histeridae). Journal of Invertebrate Pathology 77: 75-77.

Boxall R. 2002. Damage and loss caused by the larger grain borer *Prostephanus truncatus*. Integrated Pest Management Reviews 7: 105-121.

Bruns HA. 2003. Controlling aflatoxin and fumonisin in maize by crop management. Journal of Toxicology: Toxin Reviews 22: 153-173.

da Paz Junior FB, da Paz ESL, Florecirc C, Lima EALA. 2012. Laboratory evaluation of *Beauveria bassiana* (Bals.) Vuill. isolates to control the *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in cowpea grains. African Journal of Microbiology Research 6: 7365-7369.

Edde PA. 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. Journal of Stored Products Research 48: 1-18.

Giga D, Mutemerewa S, Moyo G, Neeley D. 1991. Assessment and control of losses caused by insect pests in small farmers' stores in Zimbabwe. Crop Protection 10: 287-292.

Gwinner J, Harnisch R, Muck O. 1996. Manual on the prevention of post harvest seed losses, post harvest project. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Bonn and Eschborn, Germany.

Jones M, Alexander C, Lowenberg-DeBoer J. 2011. An initial investigation of the potential for hermetic Purdue improved crop storage (PICS) bags to improve incomes for maize producers in Sub-Saharan Africa. Working Paper, Department of Agriculture Economics, Purdue University, Indiana.

Kaur S, Thakur A, Rajput M. 2014. A laboratory assessment of the potential of *Beauveria bassiana* (Balsamo) Vuillemin as a biocontrol agent of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae). Journal of Stored Products Research 59: 185-189.

Lord JC. 2001. Desiccant dusts synergize the effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored-grain beetles. Journal of Economic Entomology 94: 367-372.

Meikle W, Cherry A, Holst N, Hounna B, Markham R. 2001. The effects of an entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes), on *Prostephanus truncatus* (Horn) (Col.: Bostrichidae), *Sitophilus zeamais* Motschulsky (Col.: Curculionidae), and grain losses in stored maize in the Benin Republic. Journal of Invertebrate Pathology 77: 198-205.

Mlyneková Z, Čerešňáková Z. 2013. Degradation of starch and crude protein in dent and dent x flint maize hybrids in different stages of maturity. Slovak Journal of Animal Science 46: 61-67.

Mul M, van Niekerk T, Chirico J, Maurer V, Kilpinen O, Sparagano O, Thind B, Zoons J, Moore D, Bell B. 2009. Control methods for *Dermanyssus gallinae* in systems for laying hens: results of an international seminar. Poultry Science Journal 65: 589-600.

Nboyine JA, Asante SK, Nutsugah SK, Abudulai M, Ansaah-Agyapong F, Luke B, Clottey V. 2015. Biological control of the larger grain borer, *Prostephanus truncatus* (Horn) in stored maize using the fungal pathogen, *Beauveria bassiana* and the predator *Teretrius nigrescens* Lewis. Journal of Stored Products and Postharvest Research 6: 30-37.

Owusu E, Osafo W, Nutsukpui E. 2007. Bioactivities of candlewood, *Zanthoxylum xanthoxyloides* (Lam.) solvent extracts against two stored-product insect pests. *African Journal of Science and Technology* 8: 17-21.

Padin S, Dal Bello G, Vasicek A. 1997. Pathogenicity of *Beauveria bassiana* for adults of *Tribolium castaneum* (Col.: Tenebrionidae) in stored grains. Entomophaga 42: 569-574.

Padin S, Dal Bello G, Fabrizio M. 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Products Research 38: 69-74.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM. 2014. Genstat for Windows, 17th Edition. VSN International, Hemel Hempstead, UK.

Popoola AO, Osipitan AA, Afolabi CG, Oke OA. 2015. Biological control of larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) with entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vaillemin (Hypocreales: Cordycipitaceae). International Journal of Entomology and Nematology 2: 2-8.

Rajendran S, Parveen H, Begum K, Chethana R. 2004. Influence of phosphine on hatching of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae), *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Pest Management Science 60: 1114-1118.

Sabbour M, Abdel-Raheem M. 2015. Toxicity of the fungus *Beauveria bassiana* and three oils extract against *Sitophilus granaries* under laboratory and store conditions. American Journal of Innovative Research and Applied Sciences 1: 251-256.

Setamou M, CardwelL KF, Schulthess F, Hell K. 1997. *Aspergillus flavus* infection and aflatoxin contamination of preharvest maize in Benin. Plant Disease 81: 1323-1327.

Sinha KK, Sinha AK. 1991. Effect of *Sitophilus oryzae* infestation on *Aspergillus flavus* infection and aflatoxin contamination in stored wheat. Journal of Stored Products Research 27: 65-68.

Small GJ. 2007. A comparison between the impact of sulfuryl fluoride and methyl bromide fumigations on stored-product insect populations in UK flour mills. Journal of Stored Products Research 43: 410-416.

Suleiman R, Rosentrater K, Bern C. 2015. Evaluation of maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. Journal of Stored Products Research 64: 97-102.

Zamani Z, Aminaee MM, Khaniki GB. 2013. Introduction of *Beauveria bassiana* as a biological control agent for *Tribolium castaneum* in Kerman province. Archives of Phytopathology and Plant Protection 46: 2235-2243.

Zettler JL, Arthur FH. 2000. Chemical control of stored product insects with fumigants and residual treatments. Crop Protection 19: 577-582.

Zimmermann G. 1986. The Galleria bait method for detection of entomopathogenic fungi in soil. Journal of Applied Entomology 102: 213-215.

Chapter Eight

Thesis Overview

8.1 Introduction

Almost, all stored grains are exposed to be attacked by insect pests causing damage to stored (Haq et al. 2005). It has been estimated that during storage 10 – 25 % of the worldwide grain crops are lost yearly mostly because of pests infestation. Losses caused by insects contain not only the direct feeding damage resulting in loss of weight, but they also badly reduce nutrients, lowering percentage of seeds germination, reducing grade and dropping their marketing value due to accumulation of waste, webbing and insect cadavers. The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae) and the cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) are among the most destructive stored grains and grain products in tropics (Abebe et al. 2009; Mahroof & Phillips 2008; Shehu et al. 2010).

Fumigants and conventional insecticides have been used for the management of storage pests in grain stores. These practises have problems (Michalaki et al. 2006; Price & Mills 1988). Stored grain pests such as *Tribolium castaneum* (Herb.), *Rhyzopertha dominica* (Fabr.), *Sitophilus zeamais* (Motsch.) and *Lasioderma serricorne* (Fabr.) have been reported to develop resistance against these insecticides (Araújo et al. 2011; Champ & Campbell-Brown 1970; Guedes et al. 1996; Sağlam et al. 2015). Accordingly, alternative control strategies based on the use of entomopathogenic fungi are being evaluated.

Entomopathogenic fungi (EPF) were among the first organism to be used for the biological control of stored grain insects. *Beauveria bassiana* (Balsomo) Vuillemin is one of the most important EPF and has been widely used to control stored insects (Golshan et al. 2014; Jyothi et al. 2014; Rondelli et al. 2012). The performance of fungi against target pests can be improved by suitable formulation and application technologies (de Faria & Wraight 2007).

Formulation of *B. bassiana* is a crucial part in biocontrol product development because it may enhance the effectiveness of the fungal conidia, and may allow a product to be stored over a long period of time. The performance of fungi against the target insect pests can be enhanced by suitable formulation and application technologies (de Faria & Wraight 2007).

8.2 Summary of the major findings

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

A total of 21 South African strains of *Beauveria bassiana* were evaluated for their virulence against the adults of *Lasioderma serricorne* and *Sitophilus zeamais*, under laboratory conditions. The highlights of the study were:-

- All the tested *B. bassiana* strains were pathogenic and caused some mortality of the tested insects.
- *L. serricorne* was more susceptible than *S. zeamais* after 10 days of exposure.
- The water suspension consistently provided a higher mortality than the powder formulation.
- *B. bassiana* Strains 7284 and 7769 were the most effective for control of cigarette beetle *L. serricorne*.
- There was a need to screen more virulent isolates against *S. zeamais* for use in the management of this pest.

Isolation and identification of the entomopathogenic fungus *Beauveria bassiana* strain and their virulence against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae, *Ephestia cautella* Walker (Lepidoptera: Pyralidae) and *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae)

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. All *Beauveria bassiana* strains were evaluated for their pathogenicity on the adults of *Sitophilus zeamais*, under laboratory conditions, using kaolin as carrier. The best Strains which caused higher mortality against *S. zeamais* were evaluated in dose response assays against *Sitophilus zeamais, Ephestia cautella* L3 larvae and *Lasioderma serricorne* L3 larvae and adults. The thee best *B. bassiana* isolates were then sent to Inqaba Biotech (South Africa) for forward and reverse strand sequencing of the ITS1 and ITS2 region.

- The most copious species were *Beauveria bassiana* (28 isolates) and *Metarhizium anisopliae* (4 isolates).

- All the tested *B. bassiana* strains were pathogenic against the *S. zeamais*. Among these strains, *B. bassiana* Strains MS-3, MS-4 and MS-8 caused mortality levels > 70.0%.
- *B. bassiana* Strain MS-8 caused the highest mortality levels, lowest LD_{50} and LT_{50} values against the three insects.
- All three strains showed highest identity to *B. bassiana*.

Evaluating carriers for formulation of *Beauveria bassiana* (Balsamo) Vuillemin Strain MS-8 using kaolin versus cornflour against three species of storage pest in rice grains

The effects of kaolin alone and kaolin versus cornflour as carriers for *Beauveria bassiana* Strain MS-8 were evaluated under controlled conditions against *Sitophilus zeamais*, *Ephestia cautella* L3 larvae and *Lasioderma serricorne* L3 larvae and adults.

- Kaolin applied alone at a dose of 10g kg^{-1} of grain caused 100% mortality against all tested insects, nonetheless only 20% mortality for *S. zeamais*.
- Kaolin was regularly better carrier than cornflour against the three insects, except for *Ephestia cautella* L3 larvae, which displayed the same response for both carriers.
- Treatment of rice grains with *B. bassiana* MS-8 at 0.03g conidia kg^{-1} of grain formulated in kaolin at doses of 1, 2, 3, and 4g kg^{-1} of grain caused higher mortality on the tested insects than treatment with kaolin alone at these doses.
- Kaolin abraded the wax layer of the insect epidermis than to cornflour.

Ovicidal, larvicidal and insecticidal activity of strains of *Beauveria bassiana* (Balsamo) Vuillemin against the cigarette beetle, *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae), on rice grains

Seven strains of *Beauveria bassiana* were evaluated for their virulence on the eggs of *Lasioderma serricorne* under laboratory conditions. The two best strains (MS-8 and 7284) were then formulated at 0.03g conidia kg^{-1} of grain in kaolin at various doses. These powder formulations were assessed for their effects on adult mortality, larval mortality, number of adults emerged, level of grain damaged and level of weight loss on rice grains after 45 days of storage.

- Among the seven strains, Strains MS-8 and 7284 caused egg mortality > 25%.

- *B. bassiana* Strain MS-8 at a kaolin dose of 3g kg^{-1} of grain caused better performance than strain 7284 against the all tested parameters.
- Treatment with the unformulated, the same dose of conidia performed poorly against the same parameters.
- Scanning electron microscope (SEM) showed that unformulated conidia clumped together while conidia formulated in kaolin remained as separate conidia.

Short and long term evaluation of a fixed dose of *Beauveria bassiana* Strain MS-8 diluted in various doses of kaolin as a powder formulation applied to rice grains to control almond moth, *Ephestia cautella* Walker (Lepidoptera: Pyralidae)

Short term and long term evaluation studies were conducted against *Ephestia cautella* on rice grains, using *Beauveria bassiana* Strain MS-8 at dose of 0.03g conidia kg^{-1} of grain formulated in various doses of kaolin as an active carrier.

- *B. bassiana* strain MS-8 at all doses of kaolin displayed better control against *E. cautella* in rice grains than untreated control treatment UTC.
- On the short study 45 days, Strain MS-8 at the highest dose of kaolin 2g kg^{-1} of grain caused the highest level of larval mortality and the lowest number of adult emerged.
- Small size of adults was observed in treated rice grain compared to UTC.
- On the long term study 180 days, Strain MS-8 at the highest dose of kaolin 3g kg^{-1} of grain, caused significant reduction on the levels of webbed grain, grain damage and weigh loss.

Long term evaluation of a fixed dose of *Beauveria bassiana* (Balsamo) Vuillemin Strain MS-8, formulated in various doses of kaolin applied to Maize grains against maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae)

A long term evaluation of *Beauveria bassiana* Strain MS-8 at dose of 0.03g conidia kg^{-1} of grain formulated in kaolin at doses of 1, 2, 3, and 4g kg^{-1} of grain was conducted against *Sitophilus zeamais*.

- *B. bassiana* Strain MS-8 at all kaolin doses caused significant reduction on *S. zeamais* infestation on maize grains.

- After six months of storage, Strain MS-8 at a kaolin dose of 1g kg^{-1} of grain caused the lowest number of the live insects, lowest levels of grain damage and weight loss.
- Treatment with Strain MS-8 at all kaolin caused the highest level of edible grain remaining, lower levels of dust, and lower insect biomass.

8.3 Further research

- Screenings for more virulent isolates and evaluating more methods of bioassays need to be examined in order to deal with the tolerant pests such as *S. zeamais*.
- As these *B. bassiana* Strains (MS-3, MS-4 and MS-8) have shown an effect in laboratory condition against the three insects, field trials should be conducted to determine the efficacy of these strains under storage conditions against various stored grain pests.
- Kaolin has proven to be active carriers for *B. bassiana* Strain MS-8 under controlled conditions, but further large scale experiment need to be undertaken to study the associated effect (synergic) and to determine the efficacy of formulated fungal against grain pests in farmer conditions.
- The effects of *B. bassiana* Strain MS-8 formulated in kaolin against the eggs of other rice grain insects need to be studied to determine whether they are affected by the formulated form of this strain or not. On the other hand, long term experiment need to be run to determine the efficacy of this strain against *Lasioderma serricorne* in rice grains.
- The best formulations of *B. bassiana* Strain MS-8 need to be used as the model to control other Pyralidae pests on rice grains.
- The highly performance of the Strain MS-8 against *S. zeamais* needs to be supported with field studies before registration and commercialisation can be undertaken. Moreover, the same technique needs to be used against other maize grains pests, to determine the efficacy of the Strain MS-8 against abroad range of stored grain pests.

References

Abebe F, Tefera T, Mugo S, Beyene Y, Vidal S. 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae). African Journal of Biotechnology 8: 5937-5943.

Akbar W, Lord JC, Nechols JR, Howard RW. 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97: 273-280.

Araújo RA, Williamson MS, Bass C, Field LM, Duce IR. 2011. Pyrethroid resistance in *Sitophilus zeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel. Insect Molecular Biology 20: 437-445.

Champ B, Campbell-Brown M. 1970. Insecticide resistance in Australian *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae)—II: Malathion resistance in eastern Australia. Journal of Stored Products Research 6: 111-131.

de Faria MR, Wraight SP. 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological Control 43: 237-256.

Golshan H, Saber M, Majidi Shilsar F, Karimi F, Ebadi A. 2014. Laboratory evaluation of *Beauveria bassiana* isolates on red flour beetle *Tribolium castaneum* and their characterization by random amplified polymorphic DNA. Journal of Agricultural Science and Technology 16: 747-758.

Guedes RNC, Dover BA, Kambhampati S. 1996. Resistance to chlorpyrifos-methyl, pirimiphos-methyl, and malathion in Brazilian and US populations of *Rhyzopertha dominica* (Coleopera: Bostrichidae). Journal of Economic Entomology 89: 27-32.

Haq T, Usmani N, Abbas T. 2005. Screening of plant leaves as grain protectants against *Tribolium castaneum* during storage. Pakistan Journal of Botany 37: 149-153.

Jyothi P, Rao N, Lakshmipathy R. 2014. Laboratory evaluation of entomopathogenic fungus alone or in combination with edible oils on progeny adult build-up of lesser grain borer on stored paddy. Asian Journal of Bio Science 9: 139-145.

Mahroof RM, Phillips TW. 2008. Life history parameters of *Lasioderma serricorne* (F.) as influenced by food sources. Journal of Stored Products Research 44: 219-226.

Michalaki MP, Athanassiou CG, Kavallieratos NG, Batta YA, Balotis GN. 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* Du Val larvae: Influence of temperature, relative humidity and type of commodity. Crop Protection 25: 418-425.

Price L, Mills K. 1988. The toxicity of phosphine to the immature stages of resistant and susceptible strains of some common stored product beetles, and implications for their control. Journal of Stored Products Research 24: 51-59.

Rondelli VM, de Carvalho JR, Pratissoli D, Polanczyk RA, de Alencar JRDC, Zinger FD, Pereira SMA. 2012. Selection of *Beauveria bassiana* (Bals.) Vuill. isolates for controlling *Sitophilus zeamais* (Mots.) (Coleoptera: Curculionidae). Idesia (Arica) 30: 97-102.

Sağlam Ö, Edde PA, Phillips TW. 2015. Resistance of *Lasioderma serricorne* (Coleoptera: Anobiidae) to fumigation with Phosphine. Journal of Economic Entomology 108: 2489-2495.

Shehu A, Obeng-Ofori D, Eziah VY. 2010. Biological efficacy of Calneem TM oil against the tropical warehouse moth *Ephestia cautella* (Lepidoptera: Pyralidae) in stored maize. International Journal of Tropical Insect Science 30: 207-213.

Appendices

Chapter Two

Table 2.1 Corrected mortality (Abbott's correction) of the cigarette beetle, *Lasioderma serricorne,* adults after exposure to various doses of selected *Beauveria bassiana* strains using water suspensions and powder formulations

B. bassiana Strains	Formu-lation	Dose (conidia g^{-1} of cornflour)				
		$2x10^4$	$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$
R 444	Powder	0.0 a	3.45 ab	17.24 ef	37.93 i	58.62 o
7284	Powder	10.34 cd	17.24 ef	27.59 h	51.72 ln	72.41 rs
7288	powder	0.0 a	3.45 ab	20.69 fg	37.93 i	62.07 op
7320	Powder	0.0 a	4.68 ab	24.14 gh	41.38 ij	68.97 qr
7768	Powder	0.0 a	3.45 ab	17.24 ef	37.93 i	58.62 o
7769	Powder	6.9 bc	10.34 cd	27.56 h	44.83 jk	68.97 qr
		conidia doses ml^{-1}				
		$2x10^4$	$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$
R 444	Water	14.29 de	28.57 h	50 mn	64.29 pq	96.34 vw
7284	Water	46.43 jkl	50 mn	60.71 op	82.14 u	100 w
7288	Water	21.43 fg	28.57 h	53.57 n	64.29 pq	92.86 v
7320	Water	3.57 ab	39.29 i	50 mn	75 st	100 w
7768	Water	14.29 de	25 gh	53.37 n	75 st	96.34 vw
7769	Water	42.86 ij	46.43 lm	60.71 op	78.57 tu	100 w

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in Chapter Two, Table 2.3.

Chapter Three

Table 3. 1 Corrected mortality (Abbott's correction) of three insects upon exposure to three *Beauveria bassiana* strains applied at doses of $2x10^5$, $2x10^6$, $2x10^7$ and $2x10^8$ conidia g^{-1} formulated in a kaolin carrier, observed 10 days after treatment

Insects	*B. bassiana* Strains	Dose (Conidia g^{-1} of kaolin)			
		$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$
S. zeamais adults	MS-3	10 a	20 b	50 f	65 g
	MS-4	10 a	20 b	40 de	60 g
	MS-8	20 b	30 c	60 g	75 h
E. cautella third instar larvae	MS-3	20 b	40 de	65 g	80 h
	MS-4	20 b	35 cd	60 g	80 h
	MS-8	30 c	50 f	80 h	90 i
L. serricorne third instar larvae	MS-3	10 a	20 b	40 de	60 g
	MS-4	10 a	20 b	35 cd	60 g
	MS-8	20 b	30 c	50 f	65 g
L. serricorne adults	MS-3	15 ab	30 c	50 f	60 g
	MS-4	15 ab	20 b	40 de	60 g
	MS-8	30 c	45 ef	60 g	75 h

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in Chapter Three, Table 3.2.

Chapter Four

Table 4.1 Mortality of tested insects following treatment with kaolin only applied at various doses

Insects	Kaolin dose (g kg^{-1} of grain)					
	1	2	3	4	6	10
Sitophilus zeamais adults	0 a	0 a	0 a	0 a	11 d	20 e
Ephestia cautella L3 larvae	10 cd	21 e	41 f	43 f	86.67 h	100 i
Lasioderma serricorne L3 larvae	4 abc	7.67 bcd	23.33 e	46.67 f	86 h	100 i
Lasioderma serricorne adults	3 ab	11 d	47.33 f	58.33 g	98.33 i	100 i

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in Chapter Four, Table 4.1.

Table 4.2 Mortality of three insects after exposure to various doses of *B. bassiana* Strain MS-8 formulated in kaolin or cornflour.

Carrier	Dose (conidia g^{-1} in kaolin or cornflour)	Insects			
		Sitophilus zeamais adults	*Ephestia cautella* L3 larvae	*Lasioderma serricorne* L3 larvae	*Lasioderma serricorne* adults
Kaolin	$2x10^6$	25.67 b	36.67 cd	29.33 bc	34 cd
	$2x10^7$	56.67 ghi	59.67 hi	50.33 fg	54.67 gh
	$2x10^8$	75 kl	80 lm	61.33 hi	69.67 jk
	$2x10^9$	80 lm	95 o	80.67 lm	91 no
Cornflour	$2x10^6$	15 a	40 de	29.67 bc	25.33 b
	$2x10^7$	31 bc	55 gh	44.67 ef	49.67 fg
	$2x10^8$	45.67 ef	80 lm	51 fg	63.33 ij
	$2x10^9$	62 hij	90 no	64.67 ij	83.67 mn

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in Chapter Four, Table 4.2.

Table 4.3 Mortality of three insects following treatment with *B. bassiana* Strain MS-8 formulated in kaolin or cornflour at various doses

Carrier	Dose of the carrier (g kg^{-1} of grain) + *B. bassiana* conidia (fixed at 0.03g conidia kg^{-1} of grain)	Insects			
		Sitophilus zeamais adults	*Ephestia cautella* L3 larvae	*Lasioderma serricorne* L3 larvae	*Lasioderma serricorne* adults
Kaolin	1	77.67 bc	90.67 de	73.33 b	95 ef
	2	78.33 bc	89.67 de	81 bc	96.67 ef
	3	84.45 cd	100 f	93 def	100 f
	4	94.45	100 f	100 f	100 f
Cornflour	1	62.78 a	90.67 de	60 a	78.33 bc
	2	65.33 a	90.33 de	65 a	76.67 bc
	3	62 a	95 ef	75 b	85 cd
	4	65 a	95 ef	74.67 b	95 ef

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in Chapter Four, Table 4.3.

Chapter Five

Table 5.1 ANOVA table for the effects of seven *Beauveria bassiana* strains on the viability of eggs of *Lasioderma serricorne*

Source of variation	d.f.	s.s.	m.s.	F value	P value
Isolates	6	2190	365	61.57	<.001
Total	20	2298			
CV%	13.5				
Mean ± SE	18 ± 1.988				
LSD	4.332				

Table 5. 2 ANOVA table for the mortality of *Lasioderma serricorne* adults following exposure to two *Beauveria bassiana* strains formulated in kaolin at various doses

Source of variation	d.f.	s.s.	m.s.	F values	P values
Doses	4	16005	4001.25	351.67	<.001
Strains	1	1267.5	1267.5	111.4	<.001
Doses x Strains	4	45	11.25	0.99	0.439
Residual	18	204.8	11.38		
Total	29	17537.5			
CV%	4.8				
Mean ± SE	70.5 ± 2.754				
LSD	5.786				

Table 5.3 ANOVA table for the mortality of *Lasioderma serricorne* larvae treated with a fixed dose of two *Beauveria bassiana* strains formulated in kaolin at various doses

Source of variation	d.f.	s.s.	m.s.	P values	F values
Doses	4	20671.86	5167.96	400.76	<.001
Strains	1	1572.36	1572.36	121.93	<.001
Time	5	73623.78	14724.76	1141.86	<.001
Doses x Strains	4	53.92	13.48	1.05	0.387
Doses x Time	20	4304.28	215.21	16.69	<.001
Strains x Time	5	360.78	72.16	5.6	<.001
Doses x Strains x Time	20	380.61	19.03	1.48	0.103
Residual	118	1521.66	12.9		
Total	179	102510.2			
CV%	11.2				
Mean +SE	32.14 ± 2.932				
LSD	5.806				

Table 5. 4 Corrected mortality (Abbott's correction) of *Lasioderma serricorne* larvae after treatment with a fixed dose of two *Beauveria bassiana* strains at 0.03g conidia kg^{-1} grain of formulated in kaolin at various doses

B. bassiana strains	Kaolin carrier (g) of *B. bassiana* conidia (per kg of grain)	Time (days)					
		3	6	9	12	15	18
MS-8	0	0 a	5 ab	10 bc	25 ef	30 fg	35 gh
	0.5g	0 a	10 bc	20 de	35 gh	45 ij	61 lm
	1g	5 ab	20 de	35 gh	45 ij	55 kl	70 no
	2g	10 bc	20 de	40 hi	50 jk	65 mn	80 p
	3g	10 bc	20 de	40 hi	55 kl	70 no	87 q
7284	0	0 a	0 a	10 bc	15 cd	20 de	28 f
	0.5g	0 a	5 ab	15 cd	25 ef	35 gh	50 jk
	1g	5 ab	10 bc	25 ef	40 hi	55 kl	71 no
	2g	10 bc	15	30 fg	40 hi	60 lm	72 o
	3g	10 bc	15 cd	35 gh	45 ij	60 lm	75 op

Means followed by the same letter do not differ significantly at $P < 0.5$ according to the Duncan's multiple range test. The ANOVA details are reported in the appendices, Table 5.3.

Table 5.5 ANOVA table for the adults of *Lasioderma serricorne* emerged from rice grains after treatment with a fixed dose of two *Beauveria bassiana* Strains formulated in kaolin at various doses

Source of variation	d.f.	s.s.	m.s.	F values	P values
Doses	5	31139.25	6227.85	646.79	<.001
Strains	1	600.25	600.25	62.34	<.001
Doses x Strains	5	211.25	42.25	4.39	0.006
Residual	22	211.833	9.629		
Total	35	32170.75			
CV%	6.7				
Mean +SE	46.25 ± 2.534				
LSD	5.254				

Table 5.6 ANOVA table of grain damaged by *Lasioderma serricorne* on rice after exposure to two Strain of *Beauveria bassiana* applied at 0.03g conidia kg^{-1} formulated in various doses of kaolin.

Source of variation	d.f.	s.s.	m.s.	F values	P values
Strains	1	238.857	238.857	28.82	<.001
Doses	5	2292.114	458.423	55.31	<.001
Strains x Doses	5	111.374	22.275	2.69	0.048
Residual	22	182.344	8.288		
Total	35	2839.17			
CV%	20.3				
Mean + SE	14.17 + 2.351				
LSD	4.875				

Table 5.7 ANOVA table for weight losses caused by *Lasioderma serricorne* in rice grains after exposure to two Strains of *Beauveria bassiana* at 0.03g conidia kg^{-1} applied formulated in different dose of kaolin

Source of variation	d.f.	s.s.	m.s.	F values	P values
Strains	1	9.1809	9.1809	20.39	<.001
Doses	5	99.4481	19.8896	44.17	<.001
Strain x Doses	5	2.5446	0.5089	1.13	0.374
Residual	22	9.9064	0.4503		
Total	35	123.5174			
CV%	23.1				
Mean ± SE	2.91 ± 0.548				
LSD	1.136				

Chapter Six

Table 6.1 Corrected mortality (Abbott's correction) of *Ephestia cautella* larvae following exposure to *Beauveria bassiana* Strain MS-8 at a dose of 0.03g conidia kg^{-1}, formulated in various doses of kaolin.

Kaolin carrier (g) of *B. bassiana* conidia (per kg of grain)	Time (Days)						
	3	6	9	12	15	18	21
0.3	0.0 a	10 b	20 d	25 e	30 f	45 i	50 j
0.5	10 b	15 c	35 g	40 h	55 k	65 m	75 o
1	20 d	30 f	40 h	45 i	55 k	70 n	80 p
2	20 d	30 f	45 i	50 j	60 l	80 p	90 q

Means followed by the same letter do not differ significantly at P <0.05 according Duncan multiple range test The ANOVA details are reported in Chapter Six, Table 6.1.

Table 6. 2 Webbed grains over 180 days caused by *Ephestia cautella* larvae infestation of rice following treatments with *Beauveria bassiana* Strain MS-8 at a fixed dose formulated in various doses of kaolin

Kaolin carrier (g) of *B. bassiana* conidia (per kg of grain)	Time (Days)			
	45	90	135	180
Untreated	5 de	8 fg	10 g	15 h
0.5	3 bcd	5 de	7 ef	9 fg
1	2 abc	3 bcd	4 cd	4 cd
2	1 ab	2 abc	3 bcd	4 cd
3	0.0 a	1 ab	2 abc	2 abc

Means followed by the same letter do not differ significantly at P <0.05 according Duncan multiple range test. The ANOVA details are reported in Chapter Six, Table 6.3.

Table 6. 3 Grain damage over 180 days after *Ephestia cautella* larvae infestation on rice grain following treatment with *Beauveria bassiana* Strain MS-8 at a fixed dose formulated in various doses of kaolin

Kaolin carrier (g) of *B. bassiana* conidia (per kg of grain)	Time (Days)			
	45	90	135	180
Untreated	7 cd	15 e	20 f	30 g
0.5	5 bc	10 d	14 e	20 f
1	2 ab	3 ab	4 abc	7 cd
2	1 a	2 ab	4 abc	5 bc
3	1 a	1.5 ab	1.8 ab	3 ab

Means followed by the same letter do not differ significantly at P <0.05 according Duncan's multiple range test. The ANOVA details are reported in Chapter Six, Table 6.4.

Table 6.4 Grain weight loss over 180 days of *Ephestia cautella* larvae infestation of rice grain after treatment with *Beauveria bassiana* Strain MS-8 at a fixed dose formulated in various doses of kaolin

Kaolin carrier (g) of *B. bassiana* conidia (per kg of grain)	Time (Days)			
	45	90	135	180
Untreated	3 de	4.5 f	7 g	9 h
0.5	1.5 abcd	3.2 ef	4.5 f	6 g
1	1 abc	1.5 abcd	2 bcde	2.5 cde
2	0.5 ab	1 abc	1.3 abc	2 bcde
3	0.3 a	0.8 ab	1.5 abcd	1.8 abcde

Means followed by the same letter do not differ significantly at P <0.05 according Duncan's multiple range test. The ANOVA details are reported in Chapter Six, Table 6.5.