Potential of *Beauveria bassiana* for the Control of Maize Weevil and Bean Beetle in the Laboratory

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Abstract

The susceptibility of the maize weevil, *Sitophilus zeamais* Motsch., and the bean beetle, *Callsobruchus chinensis* L., to ten isolates of *Beauveria bassiana* (Balsamo) Vuillemin was studied in the laboratory. Adult insects were exposed to spore suspensions of *B. bassiana* isolates at a concentration of $1X10^8$ conidia ml^{-1}. Controls were sprayed with 0.01% Tween 80 in distilled water. All isolates tested were capable of infecting both test insects but their virulence, determined by adult mortalities and median lethal time (MLT), varied. A total of five (I89-481, I90-520, I89-477, I90-533 and I90-907) most virulent (MLT = 2.8-4.2 days), three (I92-736, I93-906, and I92-761A) intermediate (MLT = 4.2-6.03 days) and two (I93-868 and I93-870) weakly (MLT $\geq$7.5 days) virulent isolates were identified against *S. zeamais*. On the other hand, based on four days cumulative mortality data, five (I90-520, I90-533, I92-736, I94-907 and I89-477) virulent (MLT = 1.9-2.4 days); three (I89-481, I92-761A and I93-906) intermediate (MLT = 1.3-2.5 days) and two (I93-870 and I93-868) weakly virulent (MLT = 3.1-3.5 days) isolates were identified on *C. chinensis*. The study considered *B. bassiana* as a potentially valuable mycopathogen for the microbial control of storage pests.

Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the bean beetle *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), are destructive pests inflicting heavy damage and losses to cereals (maize, sorghum, wheat and barley) and pulse (beans and peas) crops, respectively, (Hill 1983, Dobie et al. 1984). Significant reduction in the viability of the grain is also a common effect of infestation by storage pests (Okiwelu et al. 1987). There is a considerable scope for better use of conventional pesticides to control storage pests. However, because of problems associated with chemical control such as environmental pollution, hazard to the users, resistance development and pest resurgence, in recent years research is focused on the use of alternative methods of control (Morallo-Rejesus 1987, Dick 1988). Recent developments in microbial control of insect pests indicated that entomopathogenic fungi show promise for insect control in a variety of environments and some are used commercially. Species of *Beauveria* and *Metarhizium* are well studied in this context (Ferron 1978, Burges 1981, Hall & Papierok 1982, Federici 1990). In the past few years, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has been extensively studied and used for the control of many important insect pests on various crops. Some of the tested target pests included Colorado potato beetle, *Leptinotarsa decemlineata* (Say),

(Gillespie & Norman 1989); banana weevil, *Cosmopolites sordidus* Germar, (Busoli et al. 1989); African bollworm, *Heliocovarpa armigera* (Gopalakrishnan & Narayanan 1990); sweet potato weevil, *Cylas formicarius* (Khader et al. 1990); diamondback moth, *Plutella xylostella* (L.) (Ibrahim & Low 1993, Williamson-Mora 1993). However, the possibilities for microbial control of storage pests have been accorded little attention. Khan and Selman (1984, 1987, 1988) examined the effect of *Nosema whitei* alone or in combination with insecticides on *Tribolium castaneum.* Frydocva et al. (1989) also studied the susceptibility of *Sitophilus granarius, Oryzaephilus surinamensis* and *Tribolium confusum* to the two Czech Bio-preparations of *Beauveria bassiana*, *viz*. *Boverol* and *Boverosil* and found them effective. Rodrigues and Pratissoli (1990) worked with *Metarhizium anisopliae* (Mots.) and *Beauveria brongniartii* (Sacc.) against *S. zeamais* and *Acanthoscelides obtectus* and produced promising results which are likely to be an important consideration in storage pest management. Several studies investigated the effect of *Beauveria bassiana* on Coleoptera but few of them have dealt with *Sitophilus zeamais* and *Callosobruchus chinensis*.

The objective of the present study was to investigate the virulence of ten isolates of *B. bassiana* against *S. zeamais* and *C. chinensis* in the laboratory.

Materials and Methods

Sources and Rearing of Test Insects

The initial stock culture of *S. zeamais* and *C. chinensis* was obtained from Imperial College insectary and maintained at 30°C and 70% relative humidity. Maize and bean seeds were cleaned and disinfected by storing at -20°C for four days. Cultures were set up by dispensing 500 g of the medium (maize and beans) in glass jar (2.5 l capacity) and in plastic box (2 l capacity) for *S. zeamais* and *C. chinensis*, respectively. Each medium was then infested with 300 unsexed adult *S. zeamais* and *C. chinensis*. The inside top 5 cm (plastic box) and 10 cm (glass jars) was coated with thin film of fluorocarbon resin, 'fluon', to prevent the insect climbing out. A total of four cultures was set out for each pest and incubated for 15 days at 30°C and 70% relative humidity. On the 15th day the original adults were removed by sieving and the seeds were kept for about two months for collection of new progeny. Once new progeny started to emerge, each culture was checked daily to collect the progeny which were kept in separate jars, according to their age group, until required.

Source of *B. bassiana* isolates

All ten isolates of *B. bassiana* were obtained from the International Institute of Biological Control (IIBC) and each isolate has different host and origin. The isolates were cultured at 25°C and maintained at 4°C on Sabouraud Dextrose Agar (SDA).

Preparation of conidial suspension

Cultures of all ten isolates were grown on SDA, pH of 6.8, at 25°C. A 7 day-old culture of each isolate was suspended in Tween 80 (0.01% v/v aqueous solution) in distilled water, vortexed and ultrasonicated for approximately 3 min, to break up the conidial balls (confirmed by microscopic examination). The suspension was then filtered through a 75μm sieve to remove debris and hyphal fragments. Number of conidia were estimated with a haemocytometer and adjusted to $1X10^8$ conidia ml^{-1}. The suspension was then stored at 4°C for 3-4 days before use. Viability of the conidia was checked by germination test prior to the experiment and found to be > 90% for all isolates.

Virulence of isolates

Sitophilus zeamais and *C. chinensis* adults were collected from the culture and placed separately in a clear plastic box and mixed thoroughly to facilitate random selection of the insects. Thereafter, 44 and 33 petri-dishes containing filter paper (9 cm) were assembled separately for *S. zeamais* and *C. chinensis*, respectively. Twenty *S. zeamais* and ten *C. chinensis* (each 1-3 days old) were placed in each petri dish. The treatments were assigned randomly to each petri dish for both pests. Using a perfume sprayer each replicate of *S. zeamais* was sprayed directly with 1 ml of conidial suspension

Table 1. Mean corrected percentage cumulative mortality by day and median lethal time of adult *Sitophilus zeamais* treated with various isolates of *Beauveria bassiana* in the laboratory (values are means of four replications).

Isolates tested	Percent mortality (corrected)[@]	Median lethal time (days)
I89-481	100.00 (89.36)a	2.80 d
I90-520	98.22 (85.66)a	3.32 d
I94-907	95.17 (78.69)a	3.55 d
I89-477	93.87 (79.43)a	4.22 cd
I90-533	93.56 (79.17)a	3.41 d
I92-736	84.59 (73.85)ab	4.19 cd
I93-906	65.13 (54.30)ab	6.03 bc
I92-761A	62.37 (53.36)ab	5.86 bc
I93-868	43.17 (40.00) b	8.29 a
I93-870	37.16 (38.18) b	7.50 ab
SE (±)	10.69 (8.52)	0.46
CV(%)	27.50 (25.36)	18.71

[@]*Values in parenthesis are angular transformed values of corrected cumulative mortality used for analysis; values followed by the same letter are not significantly different from each other at P<0.001 (Duncan's multiple range test [DMRT]).*

Table 2. Mean corrected percentage cumulative mortality by day 4 and median lethal time of adult *C. chinensis* treated with various isolates of *Beauveria bassiana* in the laboratory (values are means of three replications).

Isolates tested	Percent mortality (corrected)[@]	Median lethal time (days)
I90-520	100.00 (90.00)a	1.91bc
I89-477	100.00 (90.00)a	1.97 abc
I94-907	95.79 (83.06)ab	2.41abc
I90-533	95.79 (83.06)ab	2.21abc
I92-736	95.79 (83.06)ab	2.29 abc
I89-481	86.09 (72.18)abc	2.45 abc
I92-761A	74.77 (60.36)bcd	2.97 ab
I93-906	62.15 (52.88)cd	1.32 c
I93-868	46.26 (42.83) d	3.45 a
I93-870	44.35 (41.67) d	3.13 ab
SE (±)	6.89 (6.32)	0.31
CV(%)	14.92 (15.65)	22.48

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Table 2. Mean corrected percentage cumulative mortality by day 4 and median lethal time of adult *C. chinensis* treated with various isolates of *Beauveria bassiana* in the laboratory (values are means of three replications).

Isolates tested	Percent mortality (corrected)[@]	Median lethal time (days)
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containing $1X10^8$ conidia ml^{-1} in 0.01% Tween 80 in distilled water. Similarly, *C. chinensis* was sprayed with 0.5 ml of the same concentration. Controls were sprayed with 0.01% Tween 80 in distilled water alone. Each treated replicate of the test insects was then kept in its petri dish for 24 hr without food to avoid possible physical loss of conidia caused by the insects crawling between grains. After spraying, each petri dish was tightly sealed to prevent insects from escaping and placed in room conditions of 27°C and 70% relative humidity. After 24 hr the test insects from each replicate were transferred into clean plastic cups (250 ml capacity) containing 20 and 10 undamaged maize and bean seeds, respectively, and each cup was sealed by muslin cloth and perforated lid. All test replicates were maintained at 27°C and 70% relative humidity.

At 24 hr intervals, dead insects were removed from their replicates and placed on damp filter paper to determine how many produced sporulating hyphae. Data for cumulative percentage mortality were corrected for the corresponding level of control mortality (Abbott 1925) and subjected to angular transformation for analysis. Analysis was made using two-way analysis of variance and means were separated by Duncan's multiple range test using RANGE procedure in MSTAT-C programme (Michigan State University 1985). Data for cumulative daily mortality were used to estimate the median lethal time using MELTIMOR programme (R.P. Bateman personal communication).

Results and Discussion

All isolates tested were able to infect both *S. zeamais* and *C. chinensis*, confirming their pathogenicity. Percentage cumulative mortality and the resulting median lethal time of each isolate against adult *S. zeamais* and *C. chinensis* are presented (Tables 1 & 2). Under the specific condition of the present work, there were highly significant differences ($P < 0.001$) among isolates with respect to mortality of both test insects. Among the tested isolates, those derived from *Hylobious abietis*,I93-870 and I93-868 (both temperate climate isolates), caused the lowest mortalities of *S. zeamais*, possibly because the bioassay temperature of 27°C was too high for their optimal activity. These two isolates did not differ significantly from each other but they were significantly different from the most virulent isolates (I89-481, I90-520, I89-477, I90-533, and I94-907) which showed mortalities in the range of 93 to 100% within the test period. There were no significant differences between these highly virulent isolates. The isolates I92-736, I93-906 and I92-761A showed intermediate virulence with 84.6, 65.1 and 62.4% mortality of *S. zeamais*, in that order.

Unlike *S. zeamais*, *C. chinensis* is a short-lived insect, usually not exceeding three weeks and adults do not feed on the grain. Assessment on adult mortality was carried out for eight days but for this study the analysis was done based on the four days mortality data. Similarly, on *C. chinensis*, the isolates I93-868 and I93-870 caused the lowest mortality (42 and 43%, respectively) and they were significantly different from the most virulent isolates (I90-520, I89-477, I94-907, I90-533.and I92-736) which showed mortalities ranging from 96 to 100% within four days. There were no significant differences among these highly virulent isolates. Three isolates (I93-906, I92-761A and I89-481) showed intermediate virulence with 62.2, 74.8 and 86.1% mortality.

The time at which the treated insect died of infection by *B. bassiana* varied considerably among the tested isolates. On *S. zeamais*, the earliest death occurred on day 3 with isolates I89-481, I90-520, and I90-533, I94-907 (Fig. 1a); however, I89-481 caused 68% mortality at this stage. One of the most virulent isolates, I89-477, caused 53% mortality at the fourth day and by the seventh day mortality in all highly virulent isolates ranged from 92 to 100%. With the intermediate and less virulent isolates the first mortality was recorded at day 4 and 71% mortality was recorded with isolate I92-736 (Fig. 1b). Isolates I93-868, I93-870, I93-906 and I92-761A caused 3 to 10% mortality at this stage.

Unlike *S. zeamais*, the earliest death in *C. chinensis* commenced after 24 hr of inoculation

and by this time 17 to 27% mortality was recorded in all isolates (Fig. 2a,b). On the third day mortality for the most virulent isolates reached 61-79% (Fig. 2a). As the experiment progressed, cumulative mortality increased for all isolates capable of infecting *S. zeamais* and *C. chinensis*. Death increased more rapidly with more virulent isolates (Fig. 1a & 2a) while it was slower for the less virulent isolates (Fig. 1b & 2b).

Highly significant ($P<0.001$) differences in median lethal time were recorded for the different isolates in both test insects. For *S. zeamais*, the lowest time (2.8-3.55 days) was recorded for isolates I89-481, I90-520, I94-907, and I90-533 with no significant differences among these four isolates although they were significantly different from I92-761A, I90-906, I93-870, I93-868 which showed median lethal time in the range of 5.86-8.29 days. Two further isolates (I89-477 and I92-736) showed a median lethal time of 4.2 days (Table 1). For *C. chinensis* the lowest median lethal time (1.32 days) was recorded with isolate I93-906; six further isolates showed a median lethal time ranging from 2 to 2.41 days while with the other three isolates (I92-761A, I93-870 and I93-868) the median lethal time ranged from 3 to 3.45 days (Table 2). The median lethal time for *C. chinensis* was lower than from that of *S. zeamais*, indicating the increased susceptibility of the species to all *B. bassiana* isolates.

Observations showed a decrease in the activity of infected adults of *S. zeamais* from approximately 3 days after inoculation. In contrast to the controls, the treated insects showed uncoordinated movement, often appearing to stagger and sometimes unresponsive to external stimuli. Moribund insects do not feed actively on the grain. It was not possible to observe these events on *C. chinensis* as the beetles started to die a day after inoculation. External white mycelial growth from all cadavers was generally evident within 24-48 hr of death. The exact length of time was strain and insect dependent: for *S. zeamais* that were infected with isolates I93-868 and I93-906, the mycelia started to appear after 48 hr and were not prolific in growth. On the other hand, *S. zeamais* that were infected with the other isolates showed mycelial growth after 24 hr; these grew very fast, covering most part of the cadavers in 3 days. In contrast, on *C. chinensis*, external white mycelial growth was first observed 24 hr after death for all isolates and was prolific with isolates I92-736, I89-481, I90-520 and I90-533. Characteristic white sporulation covering the insect body was first observed 3 days after development of external mycelia, becoming intensive at day four both on *S. zeamais* and *C. chinensis*.

In general, the study demonstrated the potential for mycopathogen use to control storage pests. A range of nonspecific isolates of *B. bassiana* showed pathogenicity against *S. zeamais* and *C. chinensis*; however, their virulence varied greatly. Isolates I89-481, I90-520, I89-477, I90-533, and I94-907 were most virulent to *S. zeamais*. Isolates I93-868 and I93-870 were least virulent. Isolates, I92-736, I93-906, and I92-761A were intermediate in their virulence to *S. zeamais*. On the other hand, based on the 4 days cumulative mortality data, five (I90-520, I90-533, I92-736, I94-907 and I89-477) virulent, three (I89-481, I92-761A and I93-906) intermediate, and two (I93-870 and I93-868) least virulent isolates were identified against C. chinensis.

There are many factors that influence the pathogenicity of *B. bassiana* isolates to a given insect species. Some of these include the production and activity of cuticle-degrading enzymes (lipolytic, proteolytic, and chitinolytic activity), the speed of induction of cuticle-degrading enzymes and production of toxins (Charnley 1982). High or low level of cuticle-degrading enzyme and toxins may increase or decrease the virulence of a given strain. In this study one difference we observed was that the colour of the fungal colony viewed through the SDA varied from lemon yellow (I90-520, I90-533 and I90-481) to yellowish (all other isolates). Different colouration is believed to be correlated with the production of different levels of toxins (Roberts 1981). In this study, the intensely pigmented isolates were more

pathogenic to both *S. zeamais* and *C. chinensis*. However, it is not clear that the differences in pathogenicity were related to toxin production and it needs further investigation.

All ten isolates examined, none of which had been obtained from *S. zeamais* and *C. chinensis*, were found to be pathogenic. More virulent isolates may be found from systematic searching, including isolates specific to various storage pests. With the present condition of this study, however, microbial control of *C. chinensis* should not be targeted only at the adults as they have high rate of mortality and short survival. The egg masses which are laid on the surface of the seed should also be considered as one of the targets for biological control using entomopathogens.

In conclusion, recent studies (Rodrigues & Pratissoli 1990) indicate that *B. brongniartii* and *M. anisopliae* have a potential as biological control agents for various storage pests. This study considered *B. bassiana* as a potentially valuable mycopathogen for the microbial control of *S. zeamais* and *C. chinensis*. Further necessary work includes obtaining a selection of more virulent isolates against a range of stored product pests, establishing standard assay techniques for stored product pests, finding cost effective and easy ways for the mass production of *B. bassiana* and establishing standard rates and application techniques.

Although this is only preliminary work, the results are interesting. Non-specific isolates were shown to be effective, indicating the susceptibility of *S. zeamais* and *C. chinensis* to water formulated mycopathogens. However, under normal circumstances, grains in stores should be dry to avoid mould and other grain diseases. Development of mycoinsecticide for storage pests control should aim to kill the maximum number of target pests by direct contact, as is the case with other insecticides rather than to establish epizootics. The work of Ferron (1978) indicated that infection following topical application of deuteromycete conidia to some insects may proceed independently of ambient relative humidity. This indicated that epizootics in insect population are limited by the inhibiting effect of low relative humidity on sporulation than on infection. Thus, if conidia can be delivered to the target insect pests, they may infect even under ambient conditions too dry for their *in situ* production. Therefore, future work should investigate the use of dry formulations (granules) of *B. bassiana* against a range of storage pest complex.

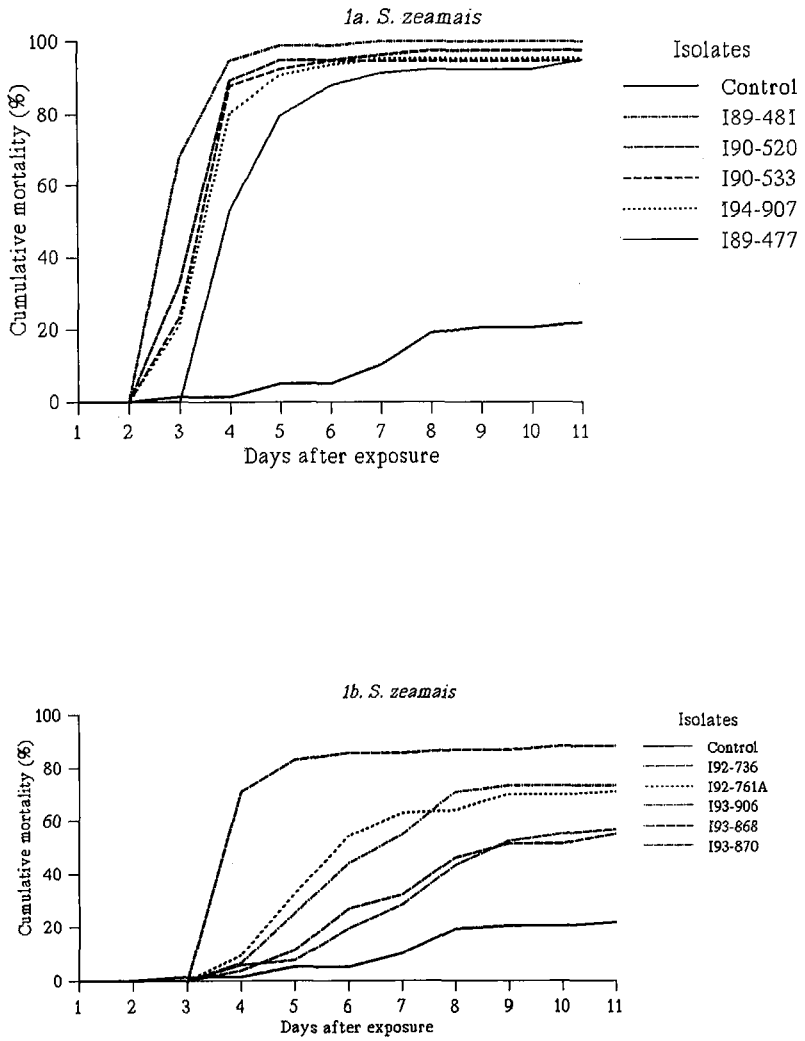
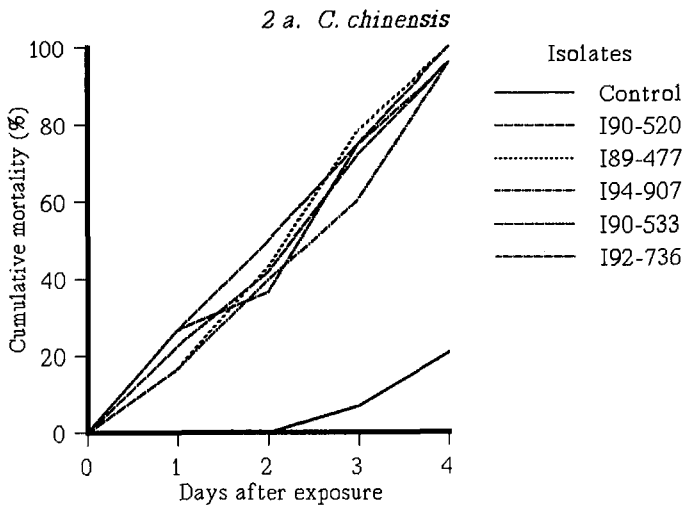


Figure 1. Percent cumulative mortality of adult *S. zeamais* following exposure to different *B. bassiana* isolates. (a) virulent and (b) intermediate and least virulent isolates.



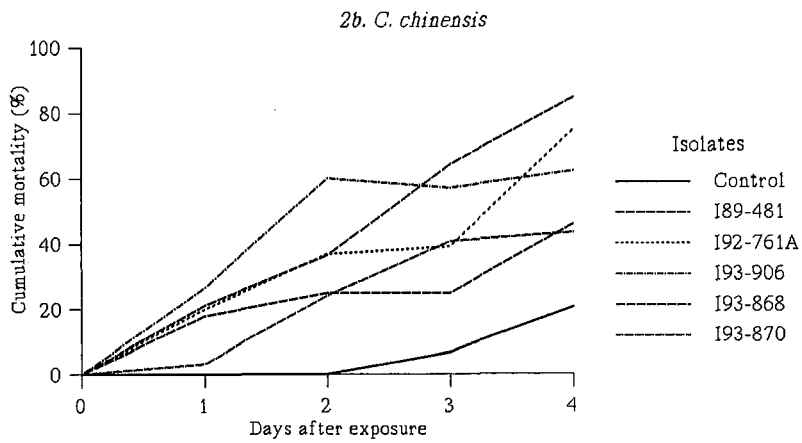


Figure 2. Percent cumulative mortality of adult *C. chinensis* following exposure to different *B. bassiana* isolates. (a) Virulent and (b) intermediate and least virulent isolates.

Acknowledgements

The authors would like to thank Dr. R.P. Bateman of the International Institute of Biological Control (IIBC) and the late Gemetchu Gedano of the Institute of Agricultural Research (Ethiopia) for their provision of statistical packages; the IIBC insect pathology group for their material support and useful suggestions and the weed pathology group of IIBC in providing a sprayer for the study. Our special thanks to J. Morley Davies of IIBC for her full assistance during fungus culture establishment and mass production processes, Malcolm Lane of Imperial college (IC) in providing the initial insect culture and Mr J. Culverhouse of IC for his assistance. This study was financially supported by the Ethiopian Institute of Agricultural Research.

References

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

Burges HD. 1981. *Microbial control of pests and plant diseases: 1970-1980.* Academic Press, London. 949 pp.

Busoli AC, Fernandes OA, Tayra O. 1989. Control of the banana weevil borer, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) by the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metschn.) Sorok. Hyphomycetes. An. Soc. Entomol. Brazil **18**:33-37.

Charnley AK. 1982. Physiological aspects of destructive pathogenesis in insects by fungi: a speculative review. In *Invertebrate-Microbial Interaction*, eds. JM Anderson, ADM Rayner, DWH Walton, pp.229-268. London: Academic Press.

Dick K. 1988 A review of insect infestation of maize in farm storage in Africa with special reference to ecology and control of *Prostephanus truncatus*. Natural Resource Institute Bulletin Number 18. 42 pp+v.

Dobie P, Haines CP, Hodges RJ, Prevett PF. 1984. Insects and arachnids of tropical stored products: Their biology and identification, a training manual. London: Tropical Development and Research Institute, 273 pp.

Federici BA. 1990. Bright horizons for invertebrate pathology. In *5th International colloquium on invertebrate pathology and microbial control: proceedings and abstracts*, ed. DE Pinnock, pp. 5-9. Adelaide. 548 pp.

Ferron P. 1978. Biological control of insect pests by entomopathogenic fungi. Annual Review of Entomology 23:409-442.

Frydocva B, Verner PH, Bartos J. 1989. The comparative effectiveness of the two bio-preparations based on entomogenous fungus *Beauveria bassiana* especially their effect on storage pests. Stornik-Vysoke-skoly-Zemedeiske-v-praze-Fakulta-Agronomika, Roda-A, Rostlinna-Vyroba Number 50. pp. 243-264.

Gillespie AT, Norman C. 1989. The use of entomopathogenous fungi for pest control and role of toxins in pathogenesis. Pesticide Science 27:203-215.

Gopalakrishnan C, Narayanan K. 1990. Studies on the dose-mortality relationship between the entomopathogenic pathogen *Beauveria bassiana* (Bals.) Vuillemin and *Heliothis armigera* Hubner (Lepidoptera: Noctuidae). Journal of Biological Control 4(2):112-115.

Hall RA, Papierok B. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology 84:205-240.

Hill DS. 1983. *Agricultural insect pests of the tropics and their control* (2nd ed.). Cambridge University Press: Cambridge. 746 pp.

Ibrahim YB, Low W. 1993. Potential of mass production and field efficacy of isolates of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Plutella xylostella*. International Journal of Pest Management 39:288-292.

Khader KH, Jayaraj S, Rabindra RJ. 1990. Evaluation of mycopathogens against the sweet potato weevils, *Cylas formicarius* (F.). Journal of Biological Control 4(2):109-111.

Khan AR, Selman BJ. 1984. Effect of insecticide, microsporidian, and insecticide-microsporidian doses on the growth of *Tribolium castaneum* larvae. Journal of Invertebrate Pathology 44:230-232.

Khan AR, Selman BJ. 1987. Effect of pirimiphos-methyl, *Nosema whitei*, pirimiphos-methyl-*Nosema whitei* doses on the growth of *Tribolium castaneum* adults. Journal of Invertebrate Pathology 49:336-338.

Khan AR, Selman BJ. 1988. On the mortality of *Tribolium castaneum* adults treated sub-lethally as larvae with pirimiphos-methyl, *Nosema whitei* and pirimiphos-methyl-*Nosema whitei* doses. Entomophaga 33:377-380.

Michigan State University. 1985. MSTAT-C Statistical Program Version 1.42. Russell Freed D and Eisensmith Scott P. Michigan State University: East Lansing.

Morallo-Rejesus B. 1987. Botanical pest control research in the Philippines. Philippine Entomologist 7 (1):1-30.

Okiwelu SN, Adu OO, Okankwo VN. 1987. The effect of *Sitophilus zeamais* (Most.) (Coleoptera: Curculionidae) on the quality and viability of stored

maize in Nigeria. Insect Science and Its Application 8(3):379-384.

Roberts DW. 1981. Toxins of entomopathogenic fungi, In *Microbial control of pests and plant diseases 1970-1980*, ed. HD Burges, pp. 441-464. London: Academic Press.

Rodrigues C, Pratissoli D. 1990. Pathogenicity of *Beauveria brongniartii* (Sacc.) Petch. and *Metarhizium anisopliae* (Mols.) Sorok. and its effect on corn weevil and bean beetle. Ans. Soc. Entomol. Brazil 19 (2):302-306.

Williamson-Mora S. 1993. *Feasibility studies on the use of entomopathogenic fungus Beauveria bassiana to control diamondback moth on cabbage in Nicaragua.* Masters thesis. London: Imperial College. 94 pp.