In vitro pathogenicity evaluation of South African and exotic strains of Beauveria bassiana against two Coleopteran storage pests: Sitophilus zeamais (Motschulsky) and Lasioderma serricorne (Fabricius)

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A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters of Science.

Johannesburg, 2007
DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science to the University of the Witwatersrand, Johannesburg. It has not been submitted to any other university for any other purposes or examination.

Masiyiwa Ngoni Sakupwanya

_____ day of _________ 2007
ABSTRACT

Entomopathogenic fungi are ubiquitous, soil borne microorganisms of scientific importance owing to their potential use as biological control agents of a wide range of arthropod pests of agricultural produce. The aims of this study included an assessment of this potential against two prominent insect pests of stored agro-commodities under controlled laboratory conditions, which presented the opportunity to isolate strains indigenous to South Africa. Three local strains of the entomopathogen, *Beauveria bassiana*, were successfully isolated from soils in the Northern Province and in comparative bioassays with five exotic strains of the same fungus evaluated to:

1. Determine the effects of different temperature on vegetative growth of the fungus.
2. Determine the effects of *B. bassiana* against the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera) using water formulations and dry spores to infect the insect.
3. Determine the effects of *B. bassiana* against the tobacco beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera) using water formulations at inundative rates of $10^8$ conidia ml$^{-1}$ to infect the pest.

Results indicated that the growth of local isolates of *B. bassiana* at different temperature was comparable with that of exotic isolates. Most isolates were found to grow optimally at 25 °C, with two isolates growing optimally at 20 °C and one at 28 °C. *In vitro* evaluations of the effects of *B. bassiana* against the maize weevil results included inundative rate inoculations, dose response assessments, effects of various temperatures on fungal virulence and dry spore assessments. In preliminary bioassays assessing the pathogenicity of *B. bassiana* against the maize weevil, insects were infected using water formulations of the fungus at inundative rates of $10^8$ conidia ml$^{-1}$. The insect was highly susceptible to infection, with an average of 92% mortality obtained for the eight isolates tested. Local isolates conferred mortality levels that were equal to and better than exotic isolates, highlighting that native strains of *B. bassiana* can be used to equal effect against the maize weevil as exotic strains. Tests to confirm the occurrence of an entomopathogenic infection through the stimulation of mycosis resulted in all fungus-inoculated insects being mummified by fungal growth and scanning electron micrographs are presented showing this growth through insect appendages. For evaluation of fungal
virulence at different conidial concentrations, a local isolate (PPRI 04307) was selected and mortality levels of between 10 and 100% were obtained using conidial concentrations that ranged from $10^3$ to $10^8$ conidia ml$^{-1}$. Results indicated that insect mortality levels were dependent on conidial concentration, and rose with an increase in conidia within the inoculum. The lethal dose of \textit{B. bassiana} to kill 50% of inoculated maize weevils was established to be $10^6$ conidia ml$^{-1}$. To evaluate fungal pathogenicity under different environmental conditions using temperature as the main parameter, a local isolate (PPRI 04306) was used. Fungal virulence was ultimately retarded at 15 °C and 37 °C, however results indicate a clear pattern in both mortality and mycosis of an increase from 15°C to a maximum at 25 °C followed by a decrease at 30 °C and 37 °C. Further assessments used \textit{Beauveria bassiana}-mycosed cadavers as carriers to deliver or transmit infectious dry fungal spores to live insects with no added moisture. Mortality levels were significantly higher in fungus-treated plots compared with fungus-free plots, and ranged from between 73 to 90% for the three isolates evaluated which included one exotic (IMI386 701) and two local (PPRI 04306 and PPRI 04307) isolates. Inundative conidial concentrations ($10^8$ conidia ml$^{-1}$) of \textit{B. bassiana} water formulations were also used to infect the tobacco beetle and results showed that the insect was highly susceptible to infection by the fungus with mortality levels of between 85 and 100% obtained using both local and exotic isolates. This is a first account reporting this insect-pathogen interaction \textit{in vitro}. Light and scanning electron microscopy images are presented of the resultant mycosis from fungus-treated cadavers showing the emergence of spores and mycelia from within the insect. This study establishes that the entomopathogen \textit{B. bassiana} can be harnessed locally for use in the development of bioinsecticides, provides base-line data of its effects against the two afore mentioned pests and provides further evidence of the wide host range and ubiquitous nature of \textit{B. bassiana}. 
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LIST OF ABBREVIATIONS

BCA - biological control agent
*Bt* - *Bacillus thuringiensis*
BV - baculoviruses
DSW - distilled sterile water
EPF - entomopathogenic fungi
GAP - good agricultural practices
NPV - nuclear polyhedrosis virus
OMA - oatmeal agar
PDA - potato dextrose agar
SDA - Sabouraud dextrose agar
SE - standard error
SEM - scanning electron microscopy