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The effects of spore concentrations of entomogenous fungi on larval mortality and development of the maize stem borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae).

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ABSTRACT

Objective: The use of chemical insecticides to control insect pests has detrimental effects on the environment. There is therefore the need to look for alternative means to manage pests which can achieve substantial pest control with no negative effects on the environment. This study was conducted to determine the effects of spore concentrations of entomogenous fungi on the mortality of *Eldana saccharina*. *Methodology and Results*: Entomogenous fungi were isolated from dead *E. saccharina* larvae and screened for pathogenicity. Different spore concentrations of the fungal species were tested against 2nd instar *E. saccharina*. Median lethal concentration (LC_{50}) for each fungus was determined using probit transformed dose-response graphs. Fungal species identified were *Aspergillus flavus, Verticillium albo-atrum, Trichothecium spp., Fusarium oxysporum* and *Alternaria brassicicola. A. flavus* had the least LC_{50} whilst *F oxysporum* recorded the largest LC_{50}. Median lethal concentration of *A. brassicicola* was not determined because the pathogenicity test recorded only 22.5 % mortality.
Conclusions and applications of findings: The effectiveness in the use of a fungus as a biological control agent for the control of insect pest depends on both the pathogenicity of the fungus and the number of infective spores that the insect is exposed to. The use of entomogenous fungi is a viable alternative in the control of insect pests. They have an added advantage because they kill only the target pests and are therefore environmentally friendly. The study indicated that mortality is influenced by dosage and therefore higher doses produced higher mortalities. These fungi occur naturally and can therefore be conserved and used as a component in an integrated approach to the management of maize stem borers.

INTRODUCTION

Maize is an important cereal crop cultivated by many small-scale farmers in Africa. The increased production of maize in Africa is however constrained by a number of factors, the most important of which is the incidence of lepidopteran stem borers (Polaszek, 1998; Bosque-Perez and Schulthess, 1998; Buadu *et al*., 2003). The most important stem borers of maize in Africa are *Sesamia calamistis* Hampson, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) and *Eldana saccharina* (Walker) (Lepidoptera: Pyralidae) which have evolved with native grasses, sedges or other wild hosts (Schulthess *et al*., 1997). Stem borers have been the subject of many studies because of their economic importance. Stem borers seriously limit potentially attainable maize

yields by infesting the crop throughout its growth, from seedling stage to maturity (Youdeowi, 1989). The damage that stem borers cause to maize plants is enormous. Thus farmers, in an attempt to increase production apply synthetic chemical insecticides to control them.

Control of stem borers with chemical insecticides is neither affordable nor sustainable for the mostly resource- poor African farmers. In recent years however, the overuse of the conventional chemical insecticides has resulted in insect resistance to insecticides (Kim *et al*., 2001). Additionally, the problem of pesticide residues in crops has engaged the attention of many countries. Due to the harmful effects of chemical insecticides on non target organisms and the problem of environmental contamination, attempts are being made on the use of other methods of control which are environmentally friendly (Baidoo and Botchey, 2006). Biological control of insect pests appears to be the alternative to the use of chemical insecticides. This includes the use of entomopathogenic fungi (Latge and Papierok, 1988; Hajek and St. Leger, 1994).

Biological control is the action of natural enemies (parasites, predators, parasitoids and microbial agents) including naturally occurring agents and agents which are introduced and managed by humans for pest control (Dent, 1991). Fungi are potentially the most versatile entomopathogens. Some produce toxins with the potential for causing mortality in insects, but they are slow in their action (Fuxa, 1987). Most insect pests are susceptible to fungal pathogens. Some fungi such as *Entomophthora* and related species are fairly specific with regard to the groups of insects affected; others such as *Beauveria* sp have a wider host range (Hoffmann and Frodsham, 1993).Transmission of fungal toxins is by environmental contamination and often causes natural epizootics that devastate natural populations. More than 750 fungal species representing approximately 100 genera have been reported to infect insects. Nearly all major fungal groups are represented, and virtually every type of insect is represented. Although the potential of fungi for control of insect pests is underexploited, a few isolates are being developed. Fungal pathogens currently being used as insect control agents are, *Beauveria bassiana*, *Hirsutella thompsonii* and *Metarrhizium anisopliae* (Fuxa, 1987).

Insect pathogens play important roles in the population dynamics of many insect species (Caruthers and Soper, 1987; Gerg, 1992). Thus insect pathogens if properly harnessed, can serve as an environmentally friendly alternative to chemical insecticides. This study therefore assessed the potential of local isolates of fungal pathogens and how different spore concentrations affected mortality and development of surviving larvae.

MATERIALS AND METHODS

Dead and live *E. saccharina* larvae were collected from abandoned maize farms at the end of the minor growing season of 2008. Dead larvae were incubated on moist filter paper in a Petri-dish at 30 ± 1.0 ^{o}C. Dead larvae on which fungal growth were observed were further studied. Fungal spores were examined in lactophenol mounts under the microscope at x 400 magnification. Spores were cultured by plating onto Potato Dextrose Agar (PDA) (Merck Ltd, Darmstadt, Germany) until sporulation. Single colonies were re-isolated onto PDA slants and incubated until sporulation. Fungal species were identified using the Lubilosa Technical Bulletin (Lomer and Lomer, 1996). Spores from the identified fungal species were used for the pathogenicity test. Fungal species which recorded mortality of 50% and above were used for median lethal concentration determination.

Spore production and counting: Groundnut oil without added antioxidants was added to the fungal spores in the 25ml universal bottles and a spatula was used to scrape the spore off the media into the oil. The suspension was then transferred into another bottle. An electronic shaker was used to separate spores from one another. Spore counting was done under the light microscope with an improved Neubea haemacytometer.

Determination of LC_{50} (Median Lethal Concentration): A series of fungal spore suspensions, obtained through serial dilutions, were used to determine the LC_{50} for each fungal pathogen. For each

species, four different concentrations ($5x10^0$, $5x10^2$, $5x10^4$ and $5x10^6$ cfu/ml) were used and 20 neonates 2nd instar *E. saccharina* larvae were each sprayed with 50ul of each concentration. A control was set up with 20 larvae each of which was sprayed with 50ul of groundnut oil. Each set-up was replicated 3 times for each fungal species. Each larva was kept in a separate bottle and fed with a piece of maize stem which was changed every 48hrs. At the time of changing the food the numbers of dead larvae were counted and recorded. The LC_{50} of each fungal species was determined using probit analysis. Larvae were observed until they pupated. The numbers of pupae for each spore concentration were counted and recorded. Pupae were put singly in bottles and observed daily until adult emergence. Percent adult emergence for each spore concentration was calculated.

Data analysis: Data collected were subjected to analysis of variance (ANOVA). Where the difference was significant the different means were separated using the Student Neumann Keul's (SNK) test using the SAS programme (Version 9) (SAS, 2005). Significant difference was set at $P< 0.05$. Regression analysis was used to determine the median lethal concentrations of the fungal isolates using probit analysis. Correlation analysis was performed to study the effect of spore concentration on mortality.

RESULTS

Fungal species that were isolated from *E. saccharina* and used for the pathogenicity test were *Aspergillus flavus, Verticillium albo-atrum, Trichothecium* sp., *Fusarium oxysporum* and *Alternaria brassicicola*. However, no further studies were performed with *A. brassicola* because pathogenicity test recorded mortality well below 50% and therefore the median lethal concentration was not determined.

Median lethal concentration (LC_{50}) of fungal species

Aspergillus flavus: With *A. flavus*, the least spore concentration recorded the least larval mortality, whilst the largest concentration recorded the highest mortality. With the exception of the highest concentration, surviving larvae that pupated recorded 100% adult emergence (Table 1). LC_{50} determined from the probit transformed dose- response graph was log inverse of 3.00 ($1x10^3$ cfu/ml) (Fig. 1). Mortality produced by the four different concentrations of *A. flavus* spores was significantly different ($P=0.004$). With the exception of the highest spore concentration, all the other concentrations recorded 100% adult emergence (Table 1). The correlation coefficient of 0.576 suggests that increase of concentration of *A. flavus* spores is positively correlated to mortality.

Table 1: Effect of *A. flavus* spore concentration on larval mortality, number of pupae and adult emergence of *E. saccharina*

Spore concentration (Conidia/ml)	% larval mortality	Number of pupae	% adult emergence
5×10^0	16.7[a]	16.5[a]	100[a]
5×10^2	46.6[b]	13.0[a]	100[a]
5×10^4	83.3[c]	6.5[b]	100[a]
5×10^6	86.6[c]	4.4[b]	80[b]

Within the same column means with the same letter are not significantly different ($P> 0.05$)

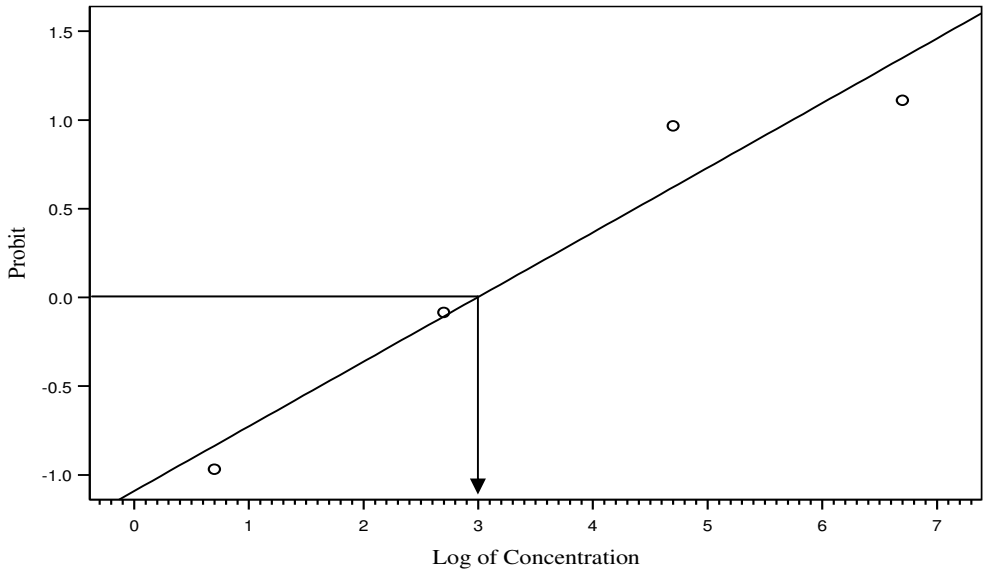

Figure 1: Probit transformed dose-response graph indicating the LC_{50} of A. *flavus* against *E.saccharina*

Verticillium albo-atrum: *Verticillium albo-atrum* spores, at the least concentration produced the least mortality of 9.9%, whilst the largest mortality of 76.6% was recorded by the highest concentration (Table 2). LC_{50} determined from the probit transformed dose-response graph was log inverse of 4.31 ($2.04x10^4$ cfu./ml) (Fig. 2). Increasing spore concentration resulted in fewer surviving larvae, pupae and reduction in percent adult emergence (Table 2). There was a high positive correlation (0.78) between the concentration of spore and percent mortality.

Table 2: Effect of *V. albo-atrum* spore concentration on larval mortality, number of pupae and adult emergence of *E. saccharina*

Spore concentration (Conidia/ml)	% larval mortality	Number of pupae	% adult emergence
5×10^0	9.9[a]	17.5[a]	100.0 [a]
5×10^2	36.6[bc]	15.0[a]	94.7 [a]
5×10^4	53.3[c]	7.5[b]	85.7 [a]
5×10^6	76.6[d]	3.4[b]	57.1 [b]

Within the same column means with the same letter are not significantly different (P> 0.05)

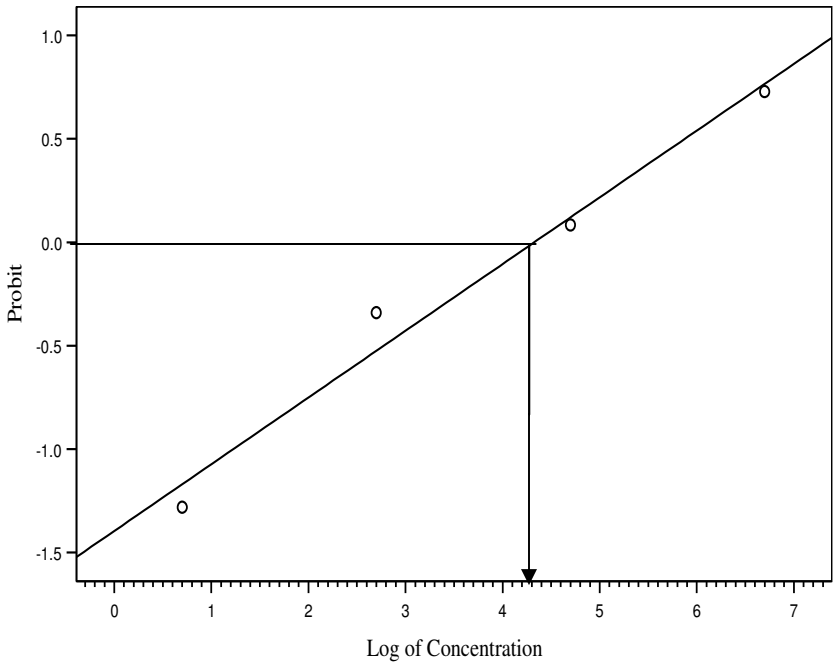

Figure.2: Probit transformed dose-response graph indicating the LC_{50} of *V. albo-atrum* against *E. saccharina*

Trichothecium spp.: At the concentration of $5x10^0$ cfu /ml, mortality was least (13.3%), whilst the largest mortality of 59.9% was recorded for the highest concentration (Table 3). It can be seen that increasing spore concentration of *Trichothecium spp*., increased mortality. LC_{50} determined from probit transformed dose-response graph was log inverse of 5.71 ($5.12x10^5$ cfu/ml) (Fig.3). With the exception of the highest concentration, 100% adult emergence was recorded for the other concentrations. When spore concentration was compared to mortality by *Trichothecium spp.*, a correlation coefficient of 0.847 was obtained.

Table 3: Effect of *Trichothecium* sp. spore concentration on larval mortality, number of pupae and adult emergence of *E. saccharina*

Spore concentration (Conidia/ml)	% larval mortality	Number of pupae	% adult emergence
5×10^0	13.3[a]	17.5[a]	100.0[a]
5×10^2	23.3[bc]	15.5[a]	100.0[a]
5×10^4	40.2[c]	9.5[b]	100.0[a]
5×10^6	59.9[d]	6.4[b]	66.7[b]

Within the same column means with the same letter are not significantly different (P> 0.05)

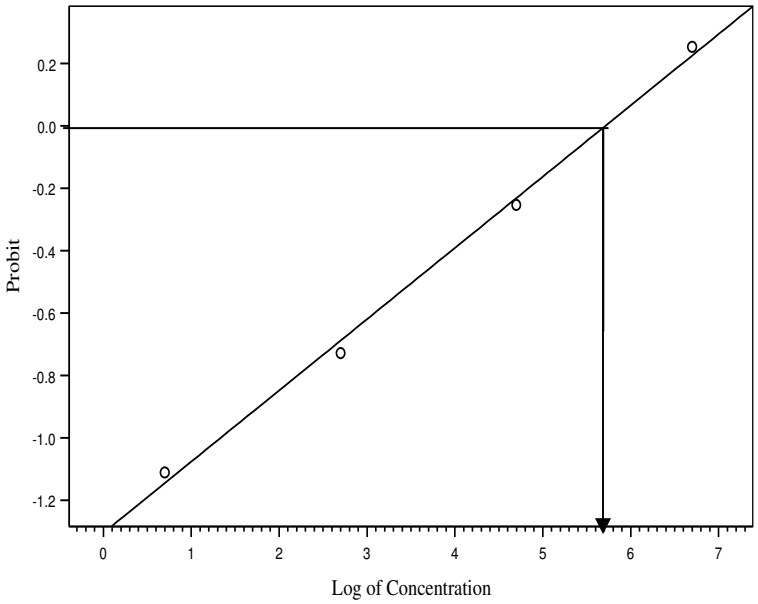

Figure3: Probit transformed dose-response graph indicating the LC_{50} of *Trichothecium spp.* against *E. saccharina*.

Fusarium oxysporum: There was no mortality (0.0%) at the concentration of $5x10^0$ cfu /ml. The least mortality of 3.5 was produced by the concentration of $5x10^2$, whilst the largest mortality of 53.3% was produced by the largest concentration (Table 4). Adult emergence from pupae decreased with increasing spore concentration. LC_{50} determined from probit transformed dose-response graph was log inverse of 6.03 ($1071.5x10^3$ cfu/ml) (Fig. 4). A correlation coefficient of 0.666 was obtained when concentration increase was compared to mortality

Table 4: Effect of *F. oxysporum* spore concentration on larval mortality, number of pupae and adult emergence of *E. saccharina*

Spore concentration (Conidia/ml)	% larval mortality	Number of pupae	% adult emergence
5×10^0	0.0^a	20.0^a	100.0^a
5×10^2	23.3^{bc}	14.5^b	100.0^a
5×10^4	43.3^c	11.5^b	94.1^a
5×10^6	53.3^d	8.4^{bc}	71.4^b

Within the same column means with the same letter are not significantly different (P> 0.05)

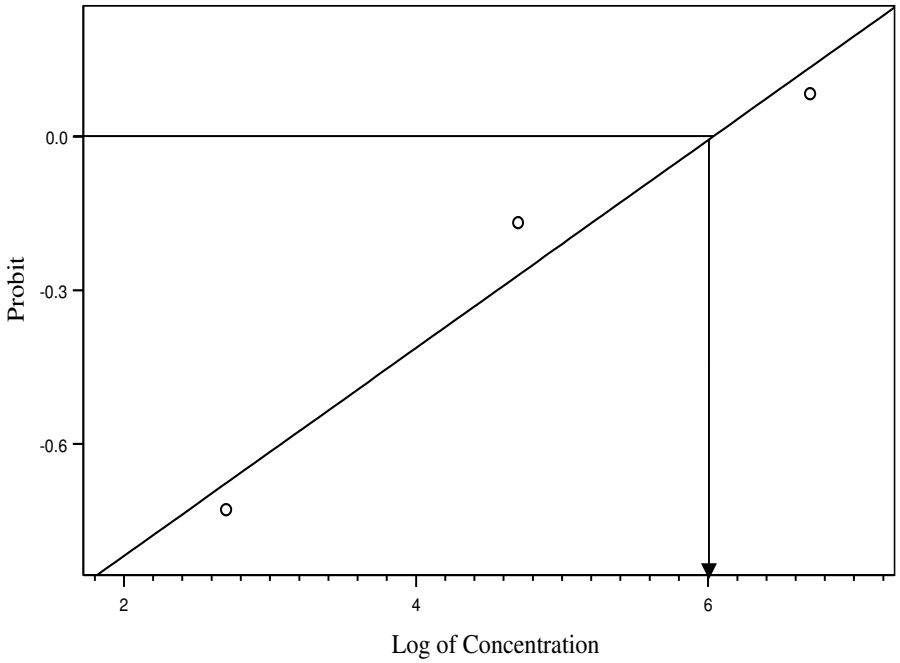

Figure 4: Probit transformed dose-response graph indicating the LC_{50} of *F. oxysporum* against *E. saccharina*

DISCUSSION

The exposure of a chemical to an insect pest and the spectrum of effects produced are referred to as the dose–response relationship. This relationship is presented graphically to produce the median lethal concentration of that chemical which is the quantity of that chemical which is able to cause death in 50% of a test population during a bioassay. This is done by administering a series of different doses of a single chemical to equal numbers of the test animals.

The estimation of the LC_{50} varies according to the fungal strain, insect species and the modes of contamination. Contamination can be by topical application of the innoculum; by spraying a spore suspension directly on the insect or by treating a plant or an inert substance, upon which the insects are placed; by free or forced ingestion; by contamination of rearing substrate or by immersion of insects in a titrated suspension of spores.

Median lethal concentration values obtained from this study showed that *A. flavus* required a lower dose ($1x10^3$ cfu/ml) than the other three isolates to cause 50% mortality of the test larvae and could therefore be said to be more potent than the other isolates. *Verticillium albo-atrum* also produced 50% mortality at a concentration of $2.04x10^4$ cfu/ml. This concentration was however higher than that of *A. flavus* making it less potent and therefore less pathogenic to *E. saccharina* compared to *A. flavus.* Vestergaard *et al*. (1995) found that *Verticillium lecanii* isolated from thrips was only weakly pathogenic to *Frankiniella occidentalis* (Pergande). LC_{50} determined for *Trichothecium spp*. was higher than that of *A. flavus* and *V. albo-atrum* suggesting that a very high dose is required to obtain the desired effects. *F. oxysporum* had LC_{50} value greater than *A. flavus*, *V. albo-atrum* and *Trichothecium spp.* This makes *F. oxysporum* the least potent of all the isolates since a much higher concentration was required to produce similar effects. According to Lomer and Lomer (1996) *F. oxysporum* is a common laboratory contaminant and also naturally occurring and could be a saprophyte rather than a pathogen.

There were obvious differences in the potency of the spores of the different fungal species. These reflected in the different LC_{50} values recorded during the study. Differences in the rate of kill among different fungal species have been reported for different insect species

(Moorhouse *et al*., 1993., Poprawski *et al*., 1985). Results of the median lethal concentration determination indicated that *A. flavus* was the most potent of the fungal species whilst *F. oxysporum* was the least potent. In all of the fungal species tested, low concentrations of conidia recorded relatively lower mortalities, whilst higher concentrations recorded relatively higher mortalities. This was particularly true of *A. flavus*, which recorded 86.6% mortality with the highest concentration. Similar work done by Kim *et al*. (2001) in Korea indicated that higher concentrations of 1x 10^9 and 1x10^8 conidia/mL gave almost 100% mortality after 7 and 9 days respectively. This was an indication that to reduce insect pests below the economic injury levels, relatively higher conidial doses of these fungal species must be applied.

Median lethal concentration of *Alternaria brassicicola* was not determined because when its spores were tested against larvae of *E. saccharina*, mortality was well below the 50% threshold required for a microorganism to be used as a pest control agent. This suggests that *A. brassicicola* could be a saprophyte rather than a pathogen (Pritchard and Muir, 1987).

It appears that the effects of the conidia on surviving larvae and subsequent development were not pronounced. It was observed that larvae that survived infection were able to completely recover from the effects of the pathogen. This was because these surviving larvae successfully pupated, with high adult emergence. In some cases, as high as 100% adult emergence was recorded. Only a few of the surviving larvae passed through supernumerary moult and thus could not go through the moulting process successfully. The high positive correlation coefficients recorded for all the fungal species suggests that concentration is positively correlated with an increase in mortality. Thus increasing spore concentration had a direct and positive effect on the test insects.

Small scale field tests have indicated that entomogenous fungi such as *B. bassiana, B. brongniartii*, and *M. anisopliae* have good potential for control of soil inhabiting insects such as wireworms and cockchafers, and of others such as lepidoptereous larvae (NAS, 1979). In Russia, *B. bassiana* is being used for the control of the Colorado potato beetle, *Leptinotarsa decemlineata* (NAS, 1979). The use of entomopathogens for the control of other insect pests such as the control of *Plutella xylostella* by *Beauveria bassiana* has been reported (Yoon *et al*., 1999). Even though entomopathogens are being exploited as alternative to chemical insecticides, one drawback to their utilization in insect pest control is that they are slow in action and have low persistence. Even though they are slow in action their importance under field conditions has been reported (Miller, 1997; Poprawski *et al*., 1999) and are being exploited in the control of many pests in the developed countries.

CONCLUSION

A. flavus requires a lesser dose to produce 50% mortality. It is therefore more pathogenic to *E. saccharina* than the other three fungal species. All the fungal species also demonstrated that pathogenicity is influenced by dose and therefore higher doses produced higher significant mortalities. Different species of fungi are present on insects and interact with them in biological relationships other than being pathogens and therefore not all fungi present on insects are pathogenic to their insect hosts. Due to their slow action, pathogenic fungi on their own may not be able to reduce insect pests below the economic injury level, but can be incorporated into an integrated pest management programme.

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