Biological control of larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) with entomopathogenic fungi -* Beauveria bassiana* (Balsamo) vaillemin (Hypocreales: Cordycipitaceae)

Popoola AO¹, Oshipitan AA², Afolabi CG³, Oke OA⁴

¹, ², ³Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, P. M. B. 2240, Abeokuta, Ogun State, Nigeria.
⁴Department of Biological Science, College of Natural Science, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria.
*Corresponding author e-mail: osipitan1@yahoo.com, Tel: (+234)8033930581

The use of synthetic insecticide has been faced with challenges of resistance among other drawbacks. This has necessitated the search for bio-pesticide that are environmentally friendly, non-toxic to humans and have a residual effect. This study evaluated the entomopathogenic fungi, *Beauveria bassiana* for biological control of larger grain borer (LGB) *Prostephanus truncatus* in maize grains. Pathogenicity examination of dead adult LGB in maize grains treated with conidia of *B. bassiana* was done to confirm the source of LGB mortality in *B. bassiana* treated maize grains. Adult dead LGB were subjected to high humidity and observed for the growth of white mould (*Muscadine disease*), which was cultured on Potato Dextrose Agar and identified. Eighty six percent of the dead insects from treated maize grains showed fungal growth *B. bassiana*. Mortality of LGB generally increased with the concentration and the exposure time of the treatments. The “weight of grain dust”, “percentage of grain damaged” and “percentage of grain weight loss” were significantly (p<0.05) higher in the untreated maize kernels. *Beauvaria bassiana* formulation was effective in controlling LGB and is recommended for maize storage. Further studies should be conducted to test the formulation under farmer situations in order to deal with practical challenges.

Key words: *Beauveria bassiana*, fungal growth, pathogenicity, potato dextrose agar, mortality

INTRODUCTION

Maize is a major staple food crop in Africa and it contributes significantly to the agricultural sector; a substantial part of the outputs however, is lost to insect pests on the field and in storage. This constitutes a major constraint to food security and income generation in Sub-Saharan Africa (Abebe et al., 2009; Oshipitan et al., 2011).

Larger grain borer (LGB) – *(Prostephanus truncatus)* is a native of Mexico, Central America and exotic to Africa. The insect has since been introduced into Africa through Tanzania in 1981 (McFarlane, 1988; Pike et al., 1992; Boxall, 2002), where it is currently a more destructive pest of stored maize and cassava than in its native Central America (Dick et al., 1988). LGB is currently
established in most parts of Africa threatening maize production due to its aggressive nature and extensive damage it causes within a short period of time. The insect represents the main storage pest in maize stock with destructive effects over long periods of time (Maboudou et al., 2004). Storage losses in LGB-infested maize vary between 15% and 30% depending on regions (ADA, 2010).

Entomopathogenic fungi were among the first organisms to be used for the biological control of pests and more than 700 species from around 90 genera are pathogenic to insects (Florez, 2002). They are generally safe in terms of low risks as compared to chemical pesticide and bear a considerable potential for the control of different stored product pests (Anonymous, 2000; Cox et al., 2004). *Beauveria bassiana* is one of the mostly used entomopathogenic fungi because of its proven efficiency. It has been used to control stored grains pests such as *Acanthoscelides obtectus*, *Rhizopertha dominica*, *Sitophilus oryzae*, *S. granarius*, *S. zeamays*, *Tribolium castaneum* and other pests of economic importance (Padin et al., 2002; Smith et al., 2006; Shams et al., 2011; Mahdneshin et al., 2011). It is a common soil borne saprophyte fungus that occurs worldwide and attacks range of both immature and adult insects such as silkworms, whiteflies, aphids, grasshopper or lady beetles. Commercial formulations of the fungus are available and registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control applications (Lord, 2001).

In the last 50 years, synthetic chemicals pesticides have been the mainstay of insect pest control. However, insecticide resistance, pest resurgence and concern over environmental impact of agricultural inputs have led to search for alternative biologically based control measure that is relatively safer, cost effective and environmentally friendly (Francisco et al., 2012). Gerg (1992) opined that pathogens play important roles in the population dynamics of many insect species. Insect pathogens such as *B. bassiana* if properly harnessed could serve as an environmentally friendly alternative to chemical insecticides (Pawar and Borikar, 2005). This study therefore, evaluates an isolate of *B. bassiana* for pathogenicity on larger grain borer (*P. truncatus*) in maize grains.

**MATERIALS AND METHODS**

**Assessment of the isolate of Beauveria bassiana for pathogenicity to Prostephanus truncatus**

**Source of Beauveria bassiana and larger grain borer (LGB)**

Commercially formulated conidia of *B. bassiana* strain GHA (Botanigard 22 WP) was sourced from Lawn and Garden Product Inc., Fresno California, United States of America. Adult LGB were obtained from the Entomological Laboratory of the Department of Crop Protection, Federal University of Agriculture, Ogun State, Abeokuta, Nigeria.

**Mortality Bioassay**

The isolate of *B. bassiana* was used at the rate of 0.00, 0.35, 0.70, 1.05 and 1.40g per 40 ml of water per 100 g maize kernels. One hundred grams (100g) samples of the maize grains were measured into 500 cm³ glass jars covered with netted lid that provide sufficient aeration. Ten ml each of the treatments was introduced into the kernels with the aid of micro syringe, mixed thoroughly and air dried in the laboratory. Twenty adults of *P. truncatus* of 1-5 days were introduced into each of the glass jars. The control was 100 g maize grains in 500 cm³ glass jars covered with netted lid, infested with *P. truncatus* of 1-5 days, but not treated with conidia of *B. bassiana*. The treatments and control were replicated four times and arranged on work tables in the laboratory in a Completely Randomized Design. The mortality of the introduced LGB was recorded at 5 days interval for 15 days to determine the effectiveness of the treatments with exposure time.

The treated maize grains were left for two months to assess the residual effects of the treatments on at least two filial generations (progenies) of the introduced LGB. At two months post-treatment of the maize grains, the insects were sieved out of the grains and separated into dead and living. The grains were separated into damaged and undamaged and each category counted and weighed. The number of dead LGB and weight of grain dust (g) were also recorded. Percentage grain damage and weight loss were calculated as follows:

(i) % Grain damage= \[
\frac{\text{Weight of damaged grains}}{\text{Total weight of grains}} \times 100
\]

(ii) % Grain weight loss= \[
\frac{\text{Initial weight- Final weight}}{\text{Initial weight}} \times 100
\]

**Pathogenicity Examination of Dead Insects**

Ten dead LGB from *B. bassiana* treated maize grains were washed in 70% ethanol, rinsed three times in sterile distilled water to remove any surface contaminants and kept separately in Petri dishes (Adel and Chelav, 2013). These plates were then incubated in a plastic box with high relative humidity (approximately 100%) to observe the growth of fungus (Khashaveh, et al., 2011; Guarana et al., 2012; Adel and Chelav, 2013). The same treatment was done to ten dead insects from the untreated maize kernels. The white mould from the cadavers was isolated.
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Table 1. Weight of grain dust, percentage grain damage and grain weight loss of maize kernels treated with *Beauveria bassiana*

<table>
<thead>
<tr>
<th>Concentration (g/40ml water)</th>
<th>Weight of Grain Dust (g)</th>
<th>% Grain Damage</th>
<th>% Grain Weight Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>4.90±2.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.30±2.26&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6.78±0.35&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.05</td>
<td>8.10±0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.80±1.36&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.70±1.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7</td>
<td>12.50±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.80±1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.80±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.35</td>
<td>13.22±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.10±1.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.37±1.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>18.10±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.00±1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.25±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within the same row with the same letter are not significantly different according to Duncan’s New Multiple Range Test (n = 20, P ≤ 0.05)

on Potato Dextrose Agar (PDA) medium with antibiotics (Streptomycin) to limit the growth of bacteria. Subsequent subculturing was grown on PDA. The fungal growth was identified under the microscope with the assistance of a pathologist.

Statistical Analysis

Mortality in the control was corrected by using Abbott’s (1925) formula. Data on insect mortality and percentage mortality were normalized using arcsine transformation. Data collected were subjected to analysis of variance (ANOVA) and significant means were separated by Duncan’s New Multiple Range Test P ≤ 0.05. Probit analysis was used to estimate both LC50 and LC95 of the isolates with 95% Confidence Limits.

RESULTS

Mortality of LGB in maize kernels treated with *Beauveria bassiana* at five, ten and fifteen days after treatment

Mortality of LGB generally increased with concentration and exposure time of the treatments. At 0.35 g/40ml H<sub>2</sub>O, 10 insects died at 5 days After Application Treatment (AAT), 44.38 died at 10 days AAT and 47.60 at 15 days AAT. At 0.70 g/40ml H<sub>2</sub>O, 27.57 insects died at 5 days AAT, 47.07 died at 10 days AAT and 52.40 died at 15 days AAT. At 1.05 g/40ml H<sub>2</sub>O, 33.55 insects died at 5 days AAT, 48.42 died at 10 days AAT and 65.17 died at 15 days AAT. At 1.4 g/40ml H<sub>2</sub>O, 41.18 insects died at 5 days AAT, 94.80 died at 10 and 15 days AAT. None of the LGB in the untreated maize kernels which serve as control died at 5, 10 and 15 days (Fig.1).

Percentage grain damage and weight loss of maize kernels treated with *Beauveria bassiana*

There were significant differences (P < 0.05) in the percentage of the grains damaged and weight loss of maize kernels treated with different concentration of *B. bassiana* (Table 1). The Percent Grain Damaged (PGD) and Percent Grain Weight Loss (PGWL) were high in the control. PGD and PGWL varied directly with the concentration of the treatments. The PGD (63.10) in maize treated with 0.35g of the treatment was significantly (P < 0.05) higher than the PGD (22.30) in maize kernels treated with 1.4 g (Table 1). Similarly, the PGWL (16.37) in maize kernels treated with lowest conidia mixture (0.35 g/40ml H<sub>2</sub>O) of *B. bassiana* was significantly (P < 0.05) higher than the PGWL in maize kernels treated with higher conidia mixture (1.4 g /40ml H<sub>2</sub>O) of *B. bassiana* (Table 1).

Weight of grain dust in LGB-infested maize grains treated with *B. bassiana*

The Weight of Grain Dust (WGD) in maize grains treated with 0.35 g/40ml H<sub>2</sub>O conidia mixture was significantly (P < 0.05) higher than the WGD from maize grains treated with higher concentration (1.4 g /40ml H<sub>2</sub>O) of conidia mixture (Table 1). Significantly (P < 0.05) higher grain dust (18.10 g) was obtained from untreated maize grains compared to all treated maize grains (Table 1).
Mortality and reproductive success of LGB in maize kernels treated with *Beauveria bassiana* at sixty days after application of treatments

The mean number of dead and living LGB at sixty days after application of treatments is shown on Table 2. The total (dead and living) number (69.00) of the insect in the untreated maize kernels was significantly (P < 0.05) higher than the number in all the treated maize grains. The total number of LGB in maize grains treated with 1.4 g/40ml H_{2}O of conidia mixture was significantly (P < 0.05) higher than the number of the insect in maize grains treated with other conidia mixtures (Table 2).

The mean number (68.00) of living LGB was significantly (P < 0.05) higher in the untreated maize grains compared to the treated ones. The number of the insect in other treated maize grains were not significantly (P > 0.05) different from each other. At the highest conidia mixture (1.4 g/40ml H_{2}O), the mean number of living LGB was 0.06 and it was 6.75 at the lowest (0.35 g/40ml H_{2}O) conidia mixture (Table 2).

Significantly (P < 0.05) higher number (20.00) of LGB died in maize grains treated with conidia mixture of 1.4 g/40ml H_{2}O compared to other treated and untreated maize grains. Similarly, significantly (P < 0.05) higher percent (99.70) of the insect died in the maize grains treated with the conidia mixture at highest rate of 1.4 g/40ml H_{2}O. The mean number (1.50) and percent (2.17) of dead LGB in the untreated maize grains (control) was significantly (P < 0.05) lower compared to the number in all the treated maize grains (Table 2).
Pathogenicity examination of the dead LGB and identification of fungal growth

In the pathogenicity examination of the dead insects, 86.0% of the cadavers subjected to the examination grew the white moulds (muscadine disease) associated with *Beauveria bassiana* (Plate 1). Growth of *B. bassiana* hyphae from culture of moulds from dead LGB examined microscopically is shown in Plate 2.

**DISCUSSION**

In this study, the spores of *Beauveria bassiana* strain GHA caused 86.0 % mortality of LGB in treated maize kernels. This is similar to the results of Bourassa et al. (2001) which reported that *B. bassiana* IMI330194 led to 100 % mortality of *P. truncatus* larvae. Similarly, Hussein et al. (2013) in a trial of the insecticidal efficacy of *Trichoderma album on Rhyzopertha dominica* reported...
that *T. album* caused 20% mortality within seven days post spraying at the lowest concentration and 100% mortality at the highest concentration of the spores. The authors reported that the spores of the fungi germinated on the host cuticle, penetrated them and spread through the body. Hluchi and Samsinakova (1989) reported that the formulation of entomopathogenic fungi Boverosil® caused 90% mortality of insect at 5.92 x 10^5 conidia/ml. Similarly, Héraux et al. (2005) reported that lytic enzymes secreted by the fungus may likely play a role in the process of damage. Askary *et al.* (1998) in a laboratory bioassays of *B. bassiana* against several life stages of the pine beauty moth, *Panolis flammea* reported high mortality of the fifth instar larvae and spraying the spores of the pathogen against the field populations of the grasshopper, *Melanoplus sanguinipes* resulted in high rates of population decline. Weiguo et al. (2005) reported that entomopathogenic fungi produce proteases, chitinases and lipases which can degrade insect cuticle. In the pathogenicity examination of the dead LGB, 86.0% of the cadavers showed symptoms of infection suggesting that most of the insects died as a result of infection by *B. bassiana*. Mul et al. (2009) and Kaoud (2010) suggested that after killing the insect the fungus could grow out of the insect cadaver and produce more spores thus increasing the chance for other individuals to be killed. Butt *et al.* (2001) and Uma Devi et al. (2008) indicated that the fungal isolates particularly *B. bassiana* are found naturally in soils, parasitizing insects, killing or disabling as it has rapid germination and sporulation, with a high virulence and good discharge of conidia which makes it an efficient control agent. Lingappa et al. (2005) reported that fungal pathogen particularly, *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests.

The results of this study indicated higher efficacy of the biopesticide and mortality of LGB at higher concentration suggesting that the mortality was directly related to dose. This is in agreement with the findings of Caston and Makaka (2008) which indicated that an increase in the concentration of spores generally increases the mortality and might generate a faster result. Malavaranan et al. (2010) studied the effect of *B. bassiana* on *Spodoptera litura* at four different concentrations (2.4 x 10^7, 2.4 x 10^6, 2.4 x 10^5, 2.4 x 10^4 conidia/ml) and reported that the least pupation (43.33%) was observed in larvae treated with the highest spore concentration (2.4 x 10^7) of the fungi and the healthy moth emergence was least in (2.4 x 10^4) spore concentration of the treatment, while the fecundity was completely arrested in the highest.

**CONCLUSION**

This study concluded that the entomopathogenic fungus, *B. bassiana* was effective in the control of larger grain borer in stored maize grains. A long term use of this formulation of entomopathogenic fungi is therefore recommended for maize storage. However, further investigation should be conducted to identify other isolates of entomopathogenic fungi that have potential as biopesticide against LGB and other important storage insect pests. These studies were conducted under laboratory environments which differ from the actual farmer environments. Further studies should therefore be conducted to test the formulation under farmer situations in order to deal with practical challenges.

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Accepted 20 January, 2015


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