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Full Length Research Paper

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

Jerry A. Nboyine[1]*, Stephen K. Asante[1], Stephen K. Nutsugah[1], Mumuni Abudulai[1], Freda Ansaah-Agyapong[1], Belinda Luke[2] and Victor Clottey[3]

[1]CSIR-Savanna Agricultural Research Institute, P. O. Box 52, Tamale. Ghana.
[2]CABI West Africa, P. O. Box CT8630, Cantonments, Accra, Ghana.
[3]CABI, Bakeham Lane, Egham, Surrey. TW20 9TY, UK.

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The larger grain borer (LGB), *Prostephanus truncatus* (Horn) is a cosmopolitan and major storage pest of maize causing up to 48% dry weight loss. Chemical pesticides are available for the management of this pest but these have potential negative effects on the consumer and the environment if misused. Thus, the present study aimed at assessing the compatibility of two bio-control agents (*Teretrius nigrescens* and *Beauveria bassiana* isolate IMI389521) for management of this pest. Two doses (1×10^9 CFU/Kg maize and 1×10^{10} CFU/Kg/maize) of *B. bassiana,* with and without the predator, *T. nigrescens,* were applied to maize containing *P. truncatus*. Controls with no *B. bassiana* were also tested. The results showed that the control had the lowest grain weight ($P<0.05$) whereas maize protected with upper dose (1×10^{10} CFU/Kg maize) of *B. bassiana* product only had the highest grain weight. Similarly, *P. truncatus* mortality was lowest ($P<0.05$) in the control and highest in the maize treated with the upper dose of the *B. bassiana* product followed by maize treated with the lower dose (1×10^9 CFU/Kg maize) of the *B. bassiana* product. Also, the *B. bassiana* product killed *T. nigrescens* but its mortality was less than fourfold that of *P. truncatus* in maize treated with the upper dose of *B. bassiana* product. In maize treated with the lower dose of *B. bassiana* product, *T. nigrescens* mortality was less than eightfold that of *P. truncatus.* Thus, the lower dose of the *B. bassiana* product can be used in storage systems where *T. nigrescens* is already established.

Key words: *Beauveria bassiana, Teretrius nigrescens,* LGB, maize, storage, bio-control.

INTRODUCTION

The larger grain borer (LGB), *Prostephanus truncatus* (Horn) is a major storage pest of maize in Africa. Since its accidental introduction in the late 1970s from the Americas into Tanzania, this pest has spread dramatically

*Corresponding author. E-mail: nboyinejerry@yahoo.co.uk.

and is now a common problem in 16 African countries (Hodges, 1994; Bergvinson and García-Lara, 2011). It is believed that *P. truncatus* may establish in all countries in sub-Saharan Africa (Bergvinson and García-Lara, 2011).

Losses from *P. truncatus* are estimated to be on average around 9% but losses of up to 48% dry weight has also been recorded (Nang'ayo et al., 1993). Studies by Tefera et al. (2011) suggested that a very small initial population of *P. truncatus* at the beginning of the storage season was sufficient to cause significant damage by the end. In Tanzania, dry weight losses of maize increased in excess of 30% over a short storage season after the accidental introduction of *P. truncatus* (Hodges et al., 1983; Keil, 1988; Henckes, 1992). Similarly, the average dry weight losses of farm-stored maize were estimated to have risen from 7 to 30% over a 6 months storage period in Togo after the introduction of *P. truncatus* (Pantenius, 1987, 1988; Helbig, 1995; Richter et al., 1997).

There are a number of strategies for management of *P. truncatus* in storage. For instance, Athanassiou et al. (2006, 2007) proved the efficacy of diatomaceous earth formulations, such as a mixture of crystalline silica and abamectin, for management of *P. truncatus*. Bergvinson and Garcia-Lara (2011) also reported synergistic effects of *Teretrius nigrescens* and insect resistant maize varieties on reducing grain losses caused by *P. truncatus*. In some countries, a common control measure for infestation of *S. zeamais* and *P. truncatus* is to treat grain with a dust formulation containing a mixture of an organophosphate (OP) and a pyrethroid insecticide. However, *P. truncatus* is tolerant to OPs and is likely to develop resistance to pyrethroids, which are not particularly effective in controlling *Sitophilus* spp. (Barbosa et al., 1995; Golob et al., 1991, Haubruge, 1990). Besides, these chemicals leave residues that have potential negative effects on the consumer and the environment especially if misused (Randhawa et al., 2014). Hence, it is necessary to explore the potential of biological control methods for the management of this cosmopolitan pest.

P. truncatus can be attacked by a variety of natural enemies, including fungi, bacteria and insects (Murphy, 1990). *T. nigrescens* Lewis, was identified as a predator that is strongly attracted to the aggregation pheromone of *P. truncatus. T. nigrescens* and *P. truncatus* naturally coexist within grain stores in Central America where unlike in Africa, *P. truncatus* causes minor losses (Nang'ayo et al., 1993). *T. nigrescens* was first released into Togo in 1991 and later into Ghana, Kenya and Benin. It is active across West Africa for management of *P. truncatus* (Helbig, 1995). Also, *Beauveria bassiana* is a soil-derived entomopathogenic fungus which maybe found naturally infecting various insects. Surveys across Kenya isolated *B. bassiana* from a number of insect pests in cribs such as *S. zeamais, Tribolium* spp. And *Carpophilus* spp. Infected insects accounted for about1% of all insects collected which provides evidence that cribs do offer a suitable environment to support growth of this fungus (Oduor et al., 2000). A number of experiments have also confirmed the infectivity of a number of strains of this entomopathogenic fungus towards *P. truncatus* (Bourassa et al., 2001; Dhuyo and Selman, 2007).

An isolate of *B. bassiana* (IMI389521) from UK storage beetles, has been developed into a novel dry powder formulation and has shown high levels of efficacy against EU storage beetle pests (Cox et al., 2004; Wakefield et al., 2013). It is not known whether the isolate and formulation would be effective against *P. truncatus* under African climatic conditions or when applied in admixture to the grain. Also, *T. nigrescens* is being mass reared for additional releases into areas where *P. truncatus* is a major problem in Ghana. This study therefore aimed at testing the compatibility of the *B. bassiana* isolate IMI389521 applied in the dry powder formulation with *T. nigrescens* for management of *P. truncatus* in stored maize.

MATERIALS AND METHODS

Study site

The study was conducted at the Plant Pathology Laboratory of the CSIR-Savanna Agricultural Research Institute, Nyankpala from 10 December, 2013 to 4 February, 2014. The trial was a 3 × 2 factorial experiment and the treatments were randomly arranged on a laboratory bench in 5 replications. The treatments were:

1. LGB + *Beauveria bassiana* (*Bb*) product at upper dose (1×10^{10} Colony Forming Units (CFU)/kg maize)
2. LGB + *Bb* product at lower dose (1×10^{9} CFU/kg maize)
3. LGB + *Teretrius nigrescens* (*Tn*) + *Bb* product at upper dose (1×10^{10} CFU/kg maize)
4. LGB + *Tn* + *Bb* product at lower dose (1×10^{9} CFU/kg maize)
5. Untreated control (LGB + maize)
6. LGB + *Tn* + maize

Insect rearing

Adults of *P. truncatus* were reared under laboratory conditions (25 ± 1°C and 55 ± 5% RH) in glass jars (250 ml) covered with a piece of fine cloth. Maize grains were frozen at -4°C for 48 h followed by oven drying at 120°C for 12 h. The oven dried maize were allowed to cool overnight then put in glass jars covered with metal gauze lids at a rate of 150 g per jar. Several adult males and females of *P. truncatus* were placed in each jar to allow for mating and oviposition. The adults were removed after 2 weeks and the jars containing maize with eggs were incubated at 25 ± 1°C and 55 ± 5% RH. After 30 days, the young *P. truncatus* cohorts were sieved out and used for the trial.

Sterilization of maize and establishment of experiment

The maize *cv* obatampa was frozen for 2 days followed by oven drying for 24 h at 50°C to kill any insect that might be present in the maize. The maize was cooled overnight. The top 3 cm of all glass jars to be used for the experiment were painted with fluon to prevent possible escape of *P . truncatus*. Ten glass jars were filled

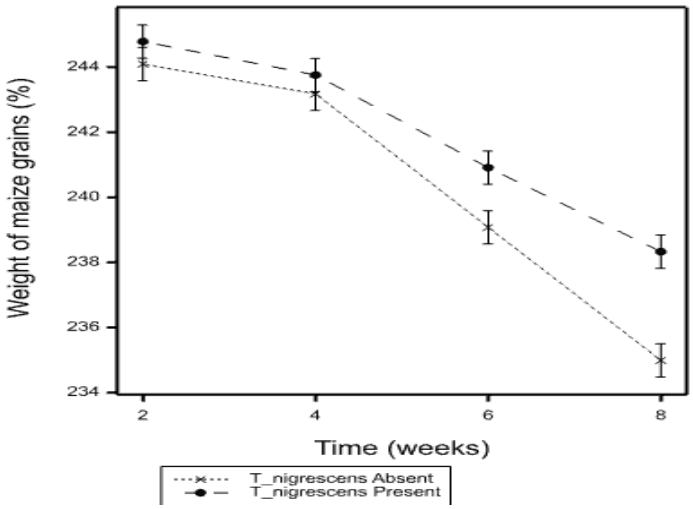

Figure 1. Bi-weekly effect of *T. nigrescens* on weight of *P. truncatus* infested maize. Data are mean grain weight (g) ± S.E of each treatment. Week 2=24th December, 2013; Week 4=7th January, 2014; Week 6=21st January, 2014; Week 8=4th February, 2014.

with 250 g of aliquots of maize for the treatments E and F. Treatments B and D, were prepared as follows; 2500 g of maize was treated with 5.775 g of the lower dose product (1×10^9 CFU/kg maize) in a large glass jar. The glass jar containing the product and maize were shaken and rotated through 360° for 1 min to ensure even coverage of the product on the maize. The maize was then divided into 10 glass jars with each glass jar containing 250 g aliquot. Similarly, treatments A and C were prepared by adding 6 g of the upper dose product (1×10^{10} CFU/kg maize) to 2500 g of maize and divided into 10 glass jars as described above. The treatments were left overnight and thereafter 50 unsexed *P. truncatus* were introduced into each of the glass jars for all the treatments. Also, 20 *T. nigrescens* were added to each sample in treatments C, D and F.

Data collection

Data were collected bi-weekly from 10th December, 2013 when the insects were introduced into the treatment jars. Thus, the data for the 1st, 2nd, 3rd and 4th assessments were collected on 24th December, 2013, 7th January, 2014, 21st January, 2014 and 4th February, 2014 respectively. The data collected were;

1. Weight of grains and powder
2. Number of live and dead *P. truncatus*
3. Number of live and dead *T. nigrescens* (*Tn*)
4. Percent mycosis

After data collection, the grains, live insects and powder were placed back into their respective glass jars. Daily minimum and maximum temperatures (°C) and relative humidity (%) of the laboratory were recorded during the study using a data logger.

Surface sterilization of dead insects

Dead insects were surface sterilized for 1 to 2 min in 10 ml of 2% sodium hypochlorite solution. The sterilized insects were transferred successively into two different Petri dishes each containing 10 ml sterile distilled water for 1 min. New solutions were used for each treatment. The sterilized insects were placed into Petri dishes containing moist filter paper. Insects from different treatments and replications were kept separate from one another. They were incubated at room temperature. The insects were checked for sporulation after 4 to 6 days and a subsample of the fungus was placed on a glass slide and examined under the microscope to confirm *B. bassiana* as the cause of death. At the termination of the experiment, the grains were dissected to retrieve both dead and live insects for counting.

Data analysis

Mortality data from the experiment were corrected to control mortality using Abbott's formula (Abbott, 1925). The data collected were subjected to Analysis of Variance using GenStat® Release 16.1 statistical software.

RESULTS

There was no significant interaction between *T. nigrescens* and *B. bassiana* product on grain weight and *P. truncatus* mortality (Table 1). However, the *B. bassiana* product dose had a significant (P<0.05) effect on both grain weight and *P. truncatus* mortality. *T. nigrescens* had a significant (P<0.05) effect on grain weight but not on *P. truncatus* mortality. Also, grain weight and *P. truncatus* mortality were significantly (P<0.05) affected by the different times of assessment (Table 1). *T. nigrescens* mortality was significantly (P<0.05) affected by the different doses of *B. bassiana* product (Table 2).

Effect of *T. nigrescens* and *B. bassiana* product on grain weight

Figure 1 shows the weight of maize grains during the bi-weekly assessments. There was no difference between the weight of maize protected with *T. nigrescens* and the untreated control maize (0 CFU/Kg maize) at weeks 2 and 4. At 6 and 8 weeks after infestation, there was a significant difference between the weight of *T. nigrescens* protected and the untreated control maize with the weight of the former being greater. In both *T. nigrescens* protected and untreated control maize, grain weight progressively reduced over time. Irrespective of the time of assessment, the untreated control maize lost significantly (P<0.05) more weight than *T. nigrescens* protected maize (Figure 2). Figure 3 shows the weight of maize grains protected with different doses of *B. bassiana* product. There was no diference between the weights of maize grains ptrotected with different doses of *B. basianna* product at weeks 2 and 4. Weights at weeks 2 and 4 were however significantly different from those at weeks 6 and 8. At weeks 6 and 8, maize protected with the upper dose of *B. basianna* product (1×10^{10} CFU/Kg

Table 1. Fpr values of the effects of measurement of time, *T. nigrescens,* and *B. bassiana* product doses on grain weight (g) and *P. truncatus* mortality.

Source of variation	Grain weight	*P. truncatus* (LGB)
Time (T)	<0.001	<0.001
T. nigrescens (*Tn*)	<0.001	0.324
B. bassiana product dose (*Bb*)	<0.001	<0.001
T × *Tn*	0.029	0.900
T × *Bb*	<0.001	<0.001
Tn × *Bb*	0.107	0.273
T × *Tn* × *Bb*	0.700	0.882

Table 2. Fpr values of the effects of measurement of time and *B. bassiana* product doses on *T. nigrescens* mortality.

Sources of variation	*T. nigrescens mortality*
Time (T)	<0.001
B. bassiana product dose (*Bb*)	<0.001
T × *Bb*	<0.001

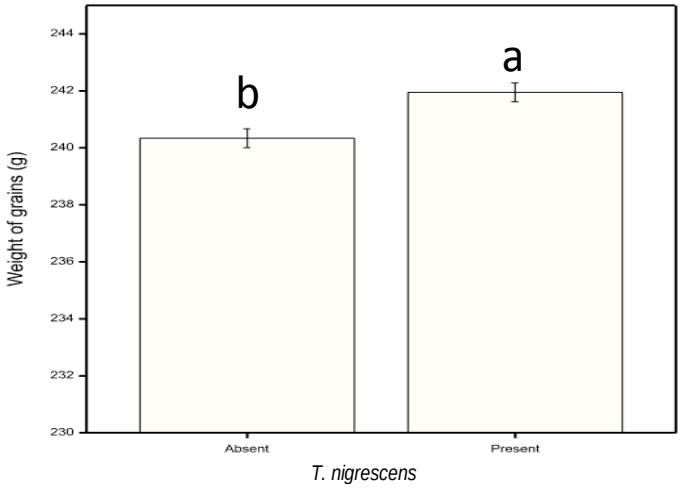

Figure 2. Effect of *T. nigrescens* on grain weight (g). Data are mean grain weight (g) ± S.E of each treatment. Means with different letters are significantly different at *P*<0.05.

of maize) had significantly higher weight than that protected with the lower dose of *B. basianna* product (1×10^9 CFU/Kg of maize). The untreated control maize (0 CFU/Kg of maize) recorded the lowest weights at weeks 6 and 8. In the untreated control maize, grain weight reduced significantly from the 4th to 8th week. Overall, grains protected with the upper dose of *B. basianna* product had the highest (*P*<0.05) weight in storage followed by the lower dose of the product. The lowest weight was recorded in the untreated control maize (Figure 4).

Effect of *B. bassiana* on *P. truncatus* and *T. nigrescens* mortality

Protecting *P. truncatus* infested maize with either the lower or upper dose of *B. basianna* product resulted in a significant (*P*<0.05) increase in *P. truncatus* mortality over time (Figure 5). *P. truncatus* mortality was significantly (*P*<0.05) higher in maize protected with the upper dose of *B. basianna* product than maize protected with either the lower dose of product or the untreated control maize. *P. truncatus* mortality in maize protected with the lower dose of *B. basianna* product was significantly higher than in the untreated control maize throughout the experiment. There was no significant difference between *P. truncatus* mortality in the untreated control maize from weeks 2 to 8 (Figure 7). Irrespective of the time of assesment, maize protected with the upper dose of *B. bassiana* product recorded the highest (*P*<0.05) *P. truncatus* mortality followed by maize protected with the lower dose of *B. bassiana* product. The untreated control had the lowest (*P*<0.05) *P. truncatus* mortality (Figure 6).

There was significant (*P*<0.05) *T. nigrescens* mortality when *P. truncatus* infested maize was protected with both *T. nigrescens* and either the upper or lower dose of *B. basianna* product (Figure 7). At weeks 2 and 4, *T. nigrescens* mortality was not significantly different among the treatments. However, *T. nigrescens* mortality in maize protected with the upper dose of *B. basianna* product was significantly higher than maize protected with either the lower dose or the untreated control maize at weeks 6 and 8. There was no difference between *T. nigrescens* mortality in maize protected with the lower dose of the

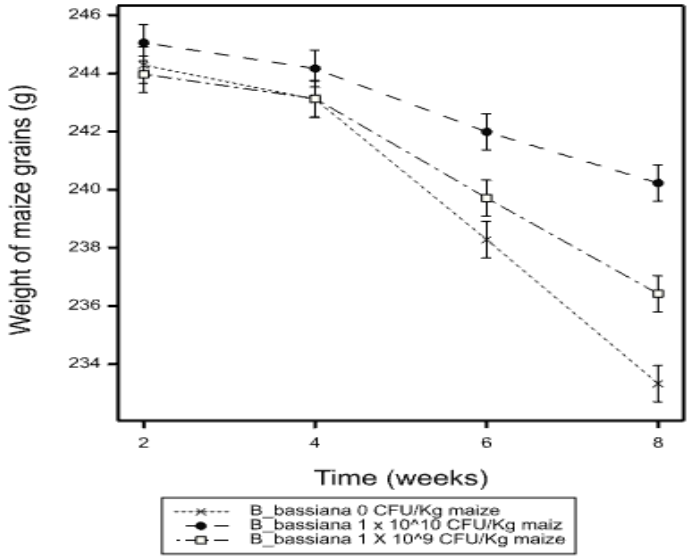

Figure 3. Bi-weekly efficacy of *B. bassiana* product on weight of *P. truncatus* infested maize. Data are mean grain weight ± S.E of each treatment. Week 2=24th December, 2013; Week 4=7th January, 2014; Week 6=21st January, 2014; Week 8=4th February, 2014.

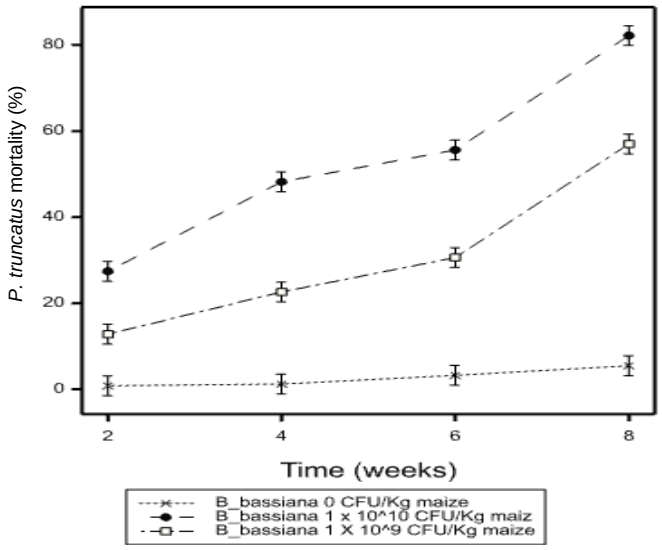

Figure 5. Bi-weekly effect of *B. bassiana* product doses on *P. truncatus* mortality. Data are mean *P. truncatus* mortality (%) ± S.E of each treatment. Week 2=24th December, 2013; Week 4=7th January, 2014; Week 6=21st January, 2014; Week 8=4th February, 2014

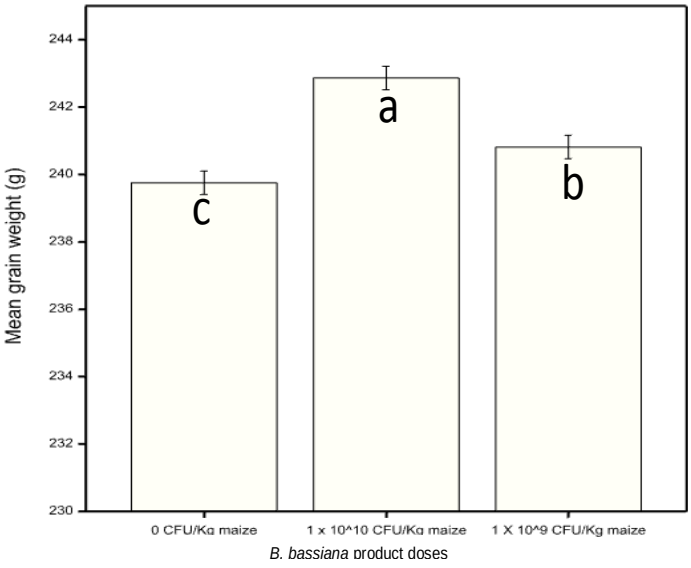

Figure 4. Effect of *B. bassiana* product doses on the mean grain weight (g). Data are mean grain weight ± S.E of each treatment. Means with different letters are significantly different at *P*<0.05.

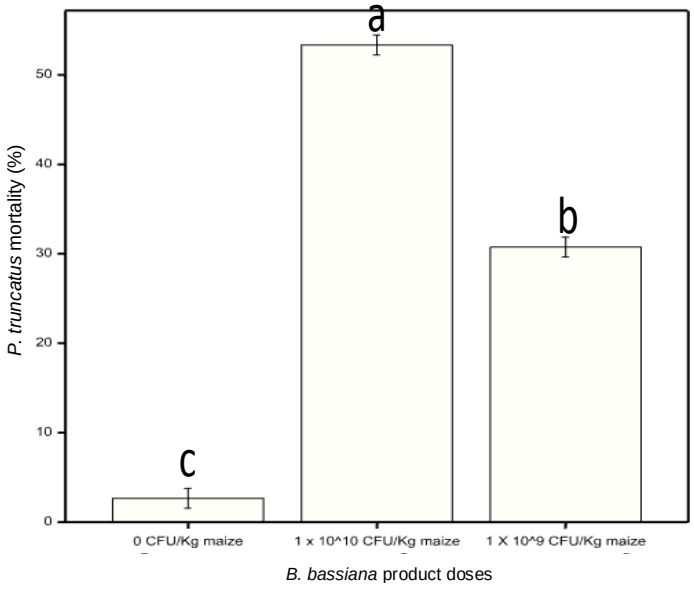

Figure 6. Effect of *B. bassiana* product doses on *P. truncatus* mortality. Data are mean *P. truncatus* mortality (%) ± S.E of each treatment. Means with different letters are significantly different at *P*<0.05.

product and the untreated control maize from weeks 2 to 6. At week 8, *T. nigrescens* mortality was significantly higher in maize protected with the lower dose of *B. bassiana* product than the untreated control maize. Irrespective of the time of assessment, *T. nigrescens* mortality was significantly higher (*P*<0.05) in maize protected with the upper dose of *B. bassiana* product followed by maize protected with the lower dose *B. bassiana* product. *T. nigrescens* mortality was lowest (*P*<0.05) in the untreated control (Figure 8).

Test carried out on the dead insects to ascertain the cause of mortality showed that all deaths apart from the untreated control treatments were due to *B. bassiana* infection. All insects from *B. bassiana* treated maize sporulated after four days (Plate 1). Observation of slides

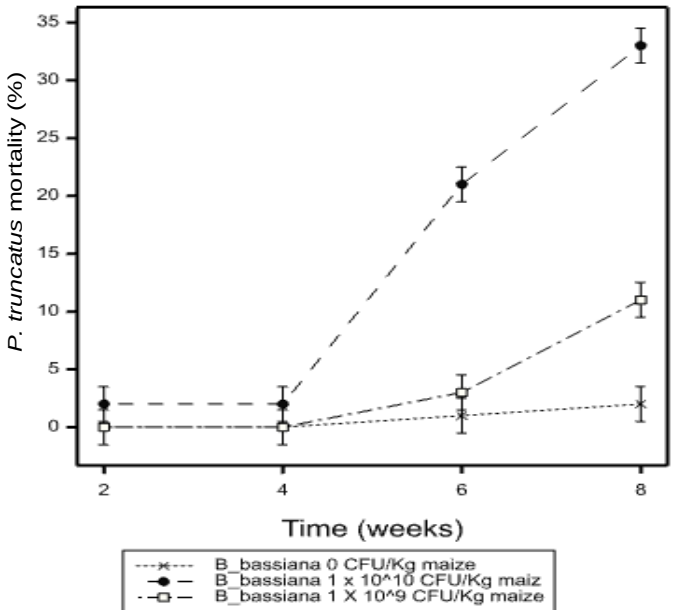

Figure 7. Effect of *B. bassiana* product on *T. nigrescens* mortality at bi-weekly intervals. Data are mean *T. nigrescens* mortality (%) ± S.E of each treatment. Week 2=24th December, 2013; Week 4=7th January, 2014; Week 6=21st January, 2014; Week 8=4th February, 2014.

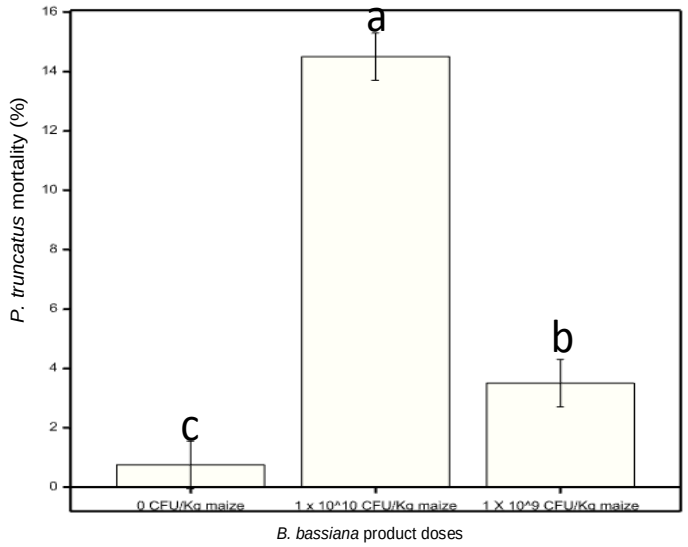

Figure 8. Effect of *B. bassiana* product doses on *T. nigrescens* mortality. Data are mean *T. nigrescens* mortality (%) ± S.E of each treatment. Means with different letters are significantly different at $P<0.05$.

under the microscope confirmed that *B. bassiana* was the cause of death.

DISCUSSION

The results from this study demonstrated the efficacy of the biological control agents, *T. nigrescens* and *B. bassiana,* to protect stored maize from *P. truncatus* damage. The weight loss in untreated control maize compared to *T. nigrescens* protected maize was due to damage by *P. truncatus*. Tefera et al. (2011) reported that the feeding activity of *P. truncatus* reduced maize grains into dust resulting in weight loss. The higher grain weight in protected maize was because *T. nigrescens* preyed on *P. truncatus* (Borgemeister et al., 2003). Although, *T. nigrescens* did not cause significant mortality to *P. truncatus* within the period of this study, its predatory activity was perhaps sufficient to reduce feeding damage by *P. truncatus*.

Plates 1. Cadaver of *P. truncatus* (LGB) infected with *B. bassiana.*

Similarly, protecting *P. truncatus* infested maize with either the lower or upper dose of *B. bassiana* product resulted in higher grain weight than the untreated control maize. The higher grain weight in the protected maize was due to *P. truncatus* mortality caused by the infection of *B. bassiana* (Bourassa et al., 2001; Dhuyo and Selman, 2007). As expected, the weight of grains was higher in maize protected with the upper dose of the product than grains protected with the lower dose of the product. This was because increasing the concentration of *B. bassiana* resulted in higher mortality (Sedehi et al., 2014) and consequently reduced damage to grains.

The susceptibility of *P. truncatus* to the *B. bassiana* product was due to the ability of the product to adhere to its body surface enabling the spores to germinate. Studies have shown that the susceptibility of storage beetles to *B. bassiana* isolates is determined by the presence of favourable conditions on the beetle's body for the spores to germinate (Wakefield, 2006). Additionally, the storage temperature (25°C) and relative humidity might have enhanced *B. bassina* infectivity of *P. truncatus* (Athanassiou and Steenberg, 2007; Lord, 2007). The cumulative *P. truncatus* mortalities in the lower and upper doses of *B. bassiana* product treatments

were 30 and 53% respectively. These results are consistent with studies by Sedehi et al. (2014) who reported that storage pest mortality increases with an increase in *B. bassiana* spore concentration.

Although *T. nigrescens* was susceptible to *B. bassiana* product, its cumulative mortality at the end of the 8 weeks period were 4 and 14% in the lower and upper dose of *B. bassiana* products respectively. The low *T. nigrescens* mortality relative to *P. truncatus* suggests that the former are more resistant to *B. bassiana* than the latter (Dhuyo and Selman, 2007). Bourassa et al. (2001) found that about two fold more *P. truncatus* are killed than *T. nigrescens* depending on the isolate of *B. bassiana* used. This notwithstanding, the results from this study suggests that the lower dose of *B. bassiana* can be used in grain storage systems where *T. nigrescens* is established to augment *P. truncatus* control. However, higher doses of *B. bassiana* may be used in storage systems where *T. nigrescens* is not used or fails to provide suitable control due to the use of insecticides (Golob et al., 1991).

In conclusion, this study showed the potential of the *B. bassiana* isolate IMI389521 applied in the dry powder formulation for the management of *P. truncatus*. The *B. bassiana* product kills both *P. truncatus* and *T. nigrescens* though mortality of the former is more than threefold that of the latter depending on the dose applied. Hence, it may be used at low doses with *T. nigrescens* for better protection of stored grains against *P. truncatus* damage.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

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