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Efficacy of *Beauveria bassiana* substrates and formulations for the control of banana weevil

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Abstract

For effective use of the entomopathogen *Beauveria bassiana* in the management of the banana weevil, high inoculum levels and appropriate formulations are mandatory. Evaluations of *B. bassiana* formulations were carried out under laboratory conditions at Kawanda, Uganda with specific objectives of determining conidial yield, efficacy of different formulations against the banana weevil, and their persistance over time. The entomopathogen was produced using a diphasic method. Substrates evaluated for conidial yield were: cracked maize, maize bran, "machicha" bagasse, cotton husks, maize bran + bagasse, maize bran + cotton husks, and bagasse + spent yeast, formulated with clay or loam soils. Data on conidia counts was done using the improved haemocytometer. Cracked maize grains and maize bran were the best substrates with 3.2 x10^9 conidia per gram and 3.1 x10^9 conidia per gram, respectively. Evaluation of the *B. bassiana* formulations against the banana weevil again showed cracked maize to be best with over 80% weevil mortality in 30 days. Following laboratory storage conditions for three months, recorded weevil mortality after exposure to the cracked maize grains formulation was > 85% in 30 days, but this declined to 20% following 180 days of storage. Further studies are being undertaken on fungal formulation and storage, to improve fungus viability and infectivity over longer periods.

Key words: Banana weevil, *Beauveria bassiana*, conidia, formulations, substrates.

Introduction

The banana weevil *Cosmopolites sordidus* (Germar; Coleoptera: Curculionidae) is an important insect pest on highland banana and plantain in Africa (Gold *et al.* 2001). The adult weevils are free living in the soil, feeding on rotten materials of banana and plantain and visiting the plant for oviposition (Cuille, 1950; cited by Godonou, 1999). This behaviour makes the weevil an easy target for entomopathogens that can be applied under moist pseudo stem traps or under the mulch around the mat. Strains of the entomopathogen *Beauveria bassiana*, isolated from *C. sordidus*, other lepidopteran insect hosts and galleria-bait in soils of banana stands, have been shown to infect and kill the banana weevil under laboratory and field conditions (Batista *et al.*, 1987, Kaaya *et al.*, 1993, Nankinga, 1999, Nankinga and Ogenga -Latigo, 1996, Nankinga and Moore, 2000). Laboratory pathogenicity tests using different strains of *B. bassiana* in Uganda (Nankinga, 1994) and in West Africa (Traore, 1995, Godonou, 1999) produced 50-100% mortality in banana weevil adults in 14 days. In Kenya, Kaaya *et al.* (1993) found that four local isolates of *B. bassiana* were pathogenic to the third instar of *C. sordidus* causing 98-100% mortality, nine days post exposure to the dry fungal spores. Godonou (1999) attained mortalities between 53-76 % in 28 days for weevils exposed to the fungal conidia under field conditions. It is evident from the forgoing that *B. bassiana* is highly infective under laboratory than under field conditions.

Beauveria bassiana occurs naturally in *C. sordidus* affected areas and survives as parasite or as saprophyte in absence of the host (Charles, 1941). The saprophytic nature of the fungus suggests that the fungus can readily establish on a variety of organic substrates. Indeed, both nutritive solid substrates such as cracked maize, maize bran, rice and non-nutritive ones such as vermiculite, sponge, cloth, solid substrates have been reported to variably support the growth and sporulation of *B. bassiana* (Jenkins and Goettel, 1997). High inoculum levels are required for effective pest control. This will require that appropriate substrates be identified and used for large-scale production of *B. bassiana*. A substrate that supports high conidia yield, cheap, readily available and easy to handle (culture), is most desirable. It is therefore necessary to evaluate and quantify conidia yield of *B. bassiana* cultured on selected substrates.

Equally important is the development of a suitable formulation that will persistent within the target environment, in this case the field soil ecosystem. This necessitates that the active ingredient (fungal conidia) be

formulated or mixed with other materials (Lomer, 1997). This formulation enables *B. bassiana* to withstand the biotic and abiotic stresses within the environment; the soil antagonists, ultraviolet light, temperature and humidity extremes commonly encountered in the field. Photo inactivation has emerged as one of the major environmental factors affecting efficacy and thus persistence of entomogenous fungi. Indeed, formulation has been reported to greatly enhance the efficacy and persistence of entomopathogens under field conditions (Navon and Ascher, 2000). Besides, appropriate formulations ensure safer and improved effectiveness of the entomopathogen (Lomer, 1997). However, appropriate formulation continues to be a challenging and often success-limiting step (Paau, 1998). Similarly, for *B. bassiana* to be effective in the applied environment, it must be formulated with the right materials. Elsewhere, *B. bassiana* has been formulated with a number of materials, but with varying performance against the target insect (Lomer, 1997).

Unfortunately, limited information is available on such studies and thus the objectives of this study were two fold;
1) To evaluate selected substrates for *B. bassiana* conidia production,
2) To evaluate efficacy of selected *B. bassiana* formulations and the infectivity of cracked maize *B. bassiana* formulation over time

Materials and methods

Conidia yield of selected B. bassiana formulation

The highly infective strain of *B. bassiana* (code G41), characterised by high pathenogenicity to *C. sordidus*, superior growth and sporulation (Nankinga, 1999) was used in the study. It was cultured at Kawanda Agricultural Research Institute (KARI) Insect pathology laboratory. Eight substrates were assessed for conidia production: cracked maize, maize bran, bagasse, "machicha", cotton husks, maize bran + bagasse, maize bran + cotton husks and bagasse + spent yeast. The substrates were cultured following the modified diphasic method (Nankinga, 1999), which entails preparation of sucrose yeast, fungal inoculation and incubation of the substrates.

The amount of conidia produced in each gram of substrate was determined using the improved Neuber Hemacytometer counting chamber (0.100mm deep, Hausser, made in USA), following the method as described by Lomer (1997). One gram of fungal substrate was weighed using a sensitive balance, and placed into a test tube, and mixed with 10 ml of distilled water. Two drops of liquid soap to act as a surfactant, were added to facilitate wetting of conidia surfaces and minimise clustering; and the solution was left to settle for 10 minutes, and shaken vigorously. One ml of solution was measured off and mixed with 9 mls of distilled water (= 10^{-1} dilution). One drop was introduced into the counting chamber using a dropper. Spores were counted from the 5 diagonal big squares, in 2 grids. This was repeated three times for each substrate. The conidia counts were analysed using ANOVA models on SAS package (SAS institute Inc. 1990). Mean separation was carried out on the treatments using the least significant different (LSD).

Pathogenicity of B. bassiana formulations

Eleven *B. bassiana* formulations were evaluated for their efficacy under laboratory conditions; maize bran alone, maize bran + loam soil, maize bran + clay soil, "Machicha" ("bussa") alone, "machicha" + loam soil, "machicha" + clay soil, Cracked maize alone, cracked maize + loam soil, cracked maze + clay soil, Loam soil alone, clay soil alone, and control (nothing added).

The loam soil was collected from the banana field at KARI with the physical characteristics of estimate levels of sand (52%), silts (28-50%), clay (7-28%), and high water holding capacity (23%). The clay soil was the grey type, mined from a waterlogged swamp near Kawanda, with particle size of approximately 0.002 mm.

The *B. bassiana* grown on cracked maize substrate was used as the standard because of its determined high conidial yield. For cracked maize, one gram was used in each replicate and mixed with one gram of either clay or loam soil (1:1 ratio). The amounts of other substrates used depended on the amount of conidia determined in a unit gram, but they were also in the ratio of 1:1(substrate: formulation combination). The formulated substrates were weighed into plastic petri dishes and replicated three times.

The banana weevils used were of same age (0-4 weeks old) and mixed sex. The weevils were sexed on the basis of punctuations (pits) patterns on the rostrum after Longoria (1968; cited and used by Rukazambuga, 1996) and angle of inclination of 9th abdominal segment after Roth and Wills (1963; cited and used by Rukazambuga, 1996). In the females, the punctuations on the rostrum were found towards the head end and they did not extend beyond the middle of the rostrum length, while in the males the punctuations extended beyond the middle of the length of the rostrum towards the tip of the mouth. On the other hand, the 9th abdominal segment of males was sharply curved, while in the females the bend was not sharp. The weevils were obtained from the stock reared on corms in metallic drums under a shade, following Nankinga's method (1994). This method entailed use of clean, freshly paired banana corms, to which adult weevils were introduced and left to oviposit for seven days. There after, the corms were kept in metallic drums for 60 days under shaded and moist conditions. The corms were later poured on the ground from which the newly emerging weevils were trapped. The weevils were quickly rinsed in distilled water to remove any surface contamination. Ten weevils of mixed sex (1:1 ratio) were introduced into each plastic petri dish to which the formulations were added, and then left over night to ensure complete coverage with fungal conidia. The weevils were introduced in the different substrates at the same point in time. There after, the weevils were transferred into petri

dishes lined with moistened tissue paper, but covered with the top lid to prevent weevil escape. Each treatment was replicated three times and kept. The treatments were assigned in a completely randomized design (CRD). The number of dead and live weevils was recorded at five-day intervals for a period of 30 days. Dead weevils were removed, and put into moistened tissue paper where they were observed for any fungal growth. Only the counts of weevils that showed mycosis were subjected to analysis using the ANOVA models on SAS package (SAS Institute Inc. 1990). Treatment means were separated using the Least Significant Difference (LSD).

Persistance of B. bassiana under laboratory conditions

An important characteristic of a fungal pathogen is its ability to remain viable and infective after a period of storage (Pereira and Roberts, 1990; cited by Godonou, 1999). Therefore, the *B. bassiana* was kept in the laboratory shelves at 26^{o}C, 79% R.H and with 11.5% moisture content (M.C). Several 100g lots of the fungus packed in autoclavable bags were kept in a plastic bucket, placed on top of the cupboard in the laboratory, to mimic the farmer's storage conditions. The samples were routinely monitored for infectivity against *C. sordidus*. At 30-day intervals, 2g samples were picked at random from the bags, weighed and placed into petri dishes. Six samples were picked at each sampling time. Ten weevils of mixed sex and age (2-4 weeks old), were introduced into each dish and left to stand over night. The weevils were then removed and put into other sets of petri dishes with moistened tissue paper where they were observed for mortality at 5-day intervals for 30 days. Dead weevils were removed, placed into clean moistened tissue and observed for mycosis. The number of weevils that showed mycosis was subjected to analysis using the ANOVA on SAS package (SAS Institute Inc. 1990). Treatment means were separated using the Least Significant Difference (LSD).

Results

Results of conidia yield are presented in Table 1. Both cracked maize and maize bran produced significantly higher conidia than the other substrates. Cracked maize yielded the highest amount of conidia per unit gram of substrate ($3.2x10^9$ conidia per gram), while cotton husks had the lowest ($2.6 x10^8$ conidia per gram). However, the conidial yield from cracked maize and maize bran was not significantly different ($P>0.05$). It was generally observed that *Beauveria* on cracked maize sporulated more profusely than that on other substrates.

Data for the weevil mortality caused by different *B. bassiana* formulations is presented in Table 2. Again, weevil mortality was significantly different ($p<0.05$) as observed between the different formulations. Cracked maize-based *B. bassiana* formulations caused the highest weevil mortality (90%), while maize bran + loam soil, caused the lowest. It was also observed that soil formulations of "machicha" and maize bran reduced its infectivity except perhaps the clay based formulation of cracked maize (Table 2).

Results for persistance of *B. bassiana* over time are presented in Table 3. For the first three consecutive months (90 days), *B. bassiana* caused weevil mortality over 85% in 30 days; this was not significantly different ($P>0.05$) from mortalities observed at 0 days or 60 days. Thereafter, the fungus started loosing its efficacy and by 180 days, it could cause mortality of only 20%. The freshly prepared cracked maize formulation was characteristically white, however, as storage duration increased, the formulation lost its colour due to attack by mites (Plate 1). The dead cadavers also expressed a lot of *Beauveria* sporulation on their surfaces (Plate 2).

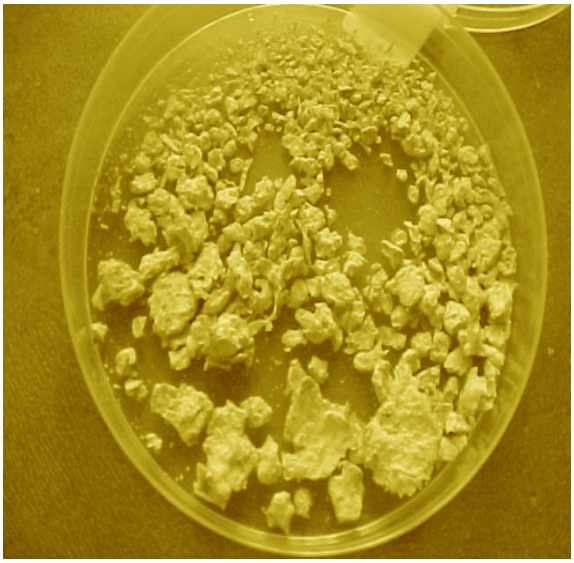
Discussions

The results above portray variability in *B. bassiana* conidia production on the different substrates evaluated; cracked maize was the best substrate. As compared to other substrates, cracked maize is readily prepared and hence experiences less chances of substrate caking during *B. bassiana* culturing and incubation. Godonou (1999), working with Rice achieved a lower yield of $5x10^8$-$6x10^8$ conidia per gram. Whereas Feron and Antia-London *et al*., (1992; cited by Godonou, 1999) working with rice achieved a higher yield of $6x10^9$ and $7.7x10^9$ conidia per gram respectively. *B. bassiana* is a saprophyte which requires a carbon source for its growth. The fungal substrates that provide sufficient quantities of carbon will most likely support fungal growth and sporulation. In the present study, cotton husks, "machicha" and bagasse are insufficient in the *B. bassiana* requirement, and thus, did not facilitate its establishment. It is therefore most likely that *B. bassiana* conidia yield is influenced by both the substrate and culturing condition. Cotton husks, bagasse, and "machicha" were comparatively deficient in conidia yield and therefore are not good substrates for the fungus production.

Maize is available and cheap in Uganda, especially in the harvest season; it can therefore be used for production of *B. bassiana*. However, its use as human tfood poses a huge challenge to its utilzation in biological control programmes.

Results further demonstrated the role of formulation in increasing infectivity of *B. bassiana*. Clay seemed to have some synergistic effect in all the formulations as indicated by higher mortality in most of the formulations where it was incorporated. Contrastingly, loam-based formulations reduced weevil infectivity. Not withstanding its recorded high conidial yield, maize bran was not as effective against the weevil. However, this is an area for further investigation. The influences of clay, although not positively quantified, appear to be indirect. Clay is known to be adhesive and absorb water (Prasad and Power, 1997), and it is most likely that it reduced the moisture content of the *B. bassiana*

a. Mite infested formulation after 180 days of storage



b. Newly cultured formulation.

Plate 2: Dead, *B. bassiana* infected weevils from the fresh cracked maize formulation.

Table 1: *Beauveria bassiana* conidia yield on selected substrates

Substrate	Mean conidia count/gram of substrate
Cracked maize	$3.27x10^9$
Maize bran	$3.07x10^9$
Maize bran + bagasse	$6.5x10^8$
" Machicha"	$5.0x10^8$
Bagasse	$3.8x10^8$
Cotton husks + Maize bran	$3.7x10^8$
Bagasse + spent yeast	$2.9x10^8$
Cotton husks	$2.6x10^8$
LSD (P=0.05)	$3.6x10^8$
CV (%)	18.8

Table 2: Banana weevil mortality caused by different *B. bassiana* formulations

Formulation	Mean weevil mortality* (%)
Cracked maize + clay	90.0
Cracked maize alone	80.0
Cracked maize + loam	70.0
" Machicha"	63.3
"Machicha" + clay	46.7
Maize bran + clay	30.0
"Machicha" + loam	26.7
Maize bran alone	13.3
Maize bran + loam	3.3
LSD (P=0.05)	2.8
CV (%)	34.9

*Weevil mortality assessed for a period of 30 days.

Table3: Persistence of *B. bassiana* towards banana weevil control over time (days)

Storage Period (Days)	Mean weevil mortality (%)*
0	100.0
30	98.3
60	100.0
90	86.7
120	58.3
150	60.0
180	20.0
LSD (P=0.05)	11.03
CV (%)	12.58

Weevil assessment for each period done at 5-day intervals for 30 days

substrates, and hence increased their infectivity (Fargues *et al*., 1993, Studdert *et al*., 1990). Clay could as well have facilitated the adherence of the spores onto the banana weevils. Indeed, earlier studies established that entomopathogenic conidia maintained at low moisture content, are highly pathogenic (Lingg & Donaldson, 1981). The relatively high mortality in the clay-based formulations is testimony to this fact. Elsewhere, clay based formulations are being advocated for in pest management (Fargues *et al*., 1993, Studdert *et a*l., 1990).

The laboratory infectivity studies indicated that *B. bassiana* conidia remained effective for 150 days (>50% mortality); however, it is known that the spores of entomopathogenic fungi can remain viable for two or more years depending on the storage conditions (Smith, 1990; cited by Nankinga, 1994). Nankinga (1994) showed that *B. bassiana* conidia could remain viable in the soil under laboratory conditions for at least two years. These findings are in sharp contrast with the present study. It is most likely that unfavourable conditions prevailed in the storage area. In fact mites (Arachnida) had infested the formulated fungus in the bags by 120 days of storage (Plate 1(a). This could have led to the fast degradation of the maize substrate, and subsequently the fungus.

Basing on these results, it is recommended that the fungus should not be stored longer than 150 days under such conditions postproduction. Apparently, this is a limited shelf life, which has also been pointed out in earlier studies as one of the limitations of using entomopathogens in pest control (Tanada and Kaya, 1993). This study has shown that there is a need to improve the production system of the fungus with emphasis on proper drying, packaging and storage methods.

Conclusion

Overall, cracked maize grains proved the most ideal substrate for *B. bassiana* production. The clay based formulation of the same medium was most effective against the banana weevil and clay seemed to have a synergistic effect on the entomopathogen. There is still limited shelf life for the cracked maize *B. bassiana* formulation, under the current production, drying and packaging techniques.

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References

Batista, F.A., Camago, L.M., Nyazaki, I., Cruz, B.P.B. and Oliveira, D.A., 1997. Controle biologico do 'Molaque' da bananeira (*Cosmoplolites sordidus*, Germar, 1824) Pelo uso de fungos entomogenous no loboratorio. Biologico Sao Paulo 53:1-6.

Charles, V.K., 1941. Preliminary checklist of the entomogenous fungi of North America. *Bulletin Insect Pest Survey* Bull. 22, 707-785.

Fagues, J., Reisinger, O., Roberts, P.H. and Aubart, C., 1993. Biodegradation of Entomopathogenic hyphomycetes: influence of clay Coating on *B. bassiana* blastospores survival in the soil. *Journal of Invertebrate pathology* 41:131-142.

Godonou Ignace. 1999. *The potential of B. bassiana for the management of C. sordidus (Germar, 1824) on plantain (Musa, AAB),* PhD Thesis, University of Ghana, Legon, 161pp.

Gold , C.S., Pena ,J , E., and Karamura, E. B., 2001. Biology and Integrated management of the banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curcurionidae*). Integrated Pest Management Reviews* 6:79-155.

Jenkins, N.E, and Goettel, M.S., 1997. *Mass production of fungal Pathogens for Insect Control.* Insect Pathology Manual. Lower C.H. and Lower CJ., Eds, pp 201-228. IITA, Cotonou, Benin.

Kaaya G.P., K.V. Seshu Reddy, E.D. Kakwano and D.M. Munyinyi., 1993. Pathenogenicity of *B. bassiana*, *Metarrhizium anisopliae* and *Serratia marcesceus* to the banana weevil, *C. Sordidus. Biological Science and Technology* 3: 177-187.

Lomer C. J, and Lomer C. H., 1997. *Insect Pathology Manual*, 241pp.

Lingg, A. L. and Donaldson M.D., 1981. Biotic and abiotic factors affecting stability of *B. bassiana* conidia in the soil. *Journal of invertebrate Pathology* 38:191-200.

Nankinga Calorine., 1994. *Potential of indigenous fungal pathogens for the biological control of the Banana weevil Cosmopolites sordidus* Germar, *in U g a n d a* , MSc.Thesis, Makerere University, 97pp.

Nankinga Calorine., 1999. *Characterisation of entomopathogenic fungi and evaluation of delivery systems of B. bassiana for biological control of banana weevil*, (*Cosmopolites sordidus*), PhD Thesis, University of Reading, 277pp.

Nankinga, C. and Ogenga-latigo; Pathogenicity of indigenous isolates of *B. bassiana* and *M. anisopliae* to the Banana weevil, C*. sordidus. African Journal of Plant protection*, **6**: 1-2.

Nankinga, C. and Moore, D. 2000. Reduction of banana weevil populations using different formulations of the entomopathogenic fungus, *B. bassisana. Biocontrol Science and Technology, 10, 645-657.*

Navon, A. and Ascher K. R.S. 2000. *Bioassays of*

Entomopathogenic Microbes and Nematodes, CABI Publishing, CABI International, 324pp.

Paau, A. S., 1998. Formulation of beneficial organisms to soil. In: *Formulation of microbial biopesticides* (*Ed*. Burges, H.D Ed). Kluwer Academic Publishers, Dordrecht, pp235-254.

Rukazambuga, N. D. T. M., (1996). *The effects of banana weevil (Cosmopolites sordidus* Germar) on the growth and productivity of bananas (Musa AAA-*EA) and the influence of host vigour on attack*. Ph.D. Thesis, University of Reading United Kingdom, 249pp.

SAS Institute, Inc., (1990). *SAS/STAT User's Guide* Version 6, 4th Edition Vol 1, SAS Institute, Inc. Cary, NC. USA, 785pp.

Studdert J.P., Kaya , H.K., and Duniway J. M., 1990. Effect of water potential, temperature and clay-coating on survival of *B. bassiana* conidia in a loam and peat soil. *Journal of Invertebrate Pathology* 55: 417-425.

Tanada, Y. and Kaya, H.K., (1993). *Insect Pathology*. Academic Press, Inc. New York, 666pp.

Traore, L., 1995. *Facteurs biologiques de mortalite de curcurionidae en mileux tempere et tropical*. Ph.D. Dissertation. McGill University, Montreal, Canada.192pp.