J. Biol. Control, 23(3): 277–283, 2009

Effect of mass multiplication media on sporulation, field efficacy and shelf life of *Beauveria bassiana* against rhizome and pseudostem weevils of banana

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ABSTRACT: The pseudostem weevil, *Odoiporus longicollis* (Olivier) and rhizome weevil *Cosmopolites sordidus* (Germar) are considered as major insect pests of banana in all the countries of the world. Studies were made on the virulence of the fungal pathogen *Beauveria bassiana* against these pests in relation to the mass production media and also the method of application under field conditions. Among the various methods, spraying the conidia spore suspension of *B. bassiana* 17-6 and immersion of the weevils in spore suspension recorded 100% adult mortality of both pseudostem and rhizome weevils in 6 days. Use of rice chaffy grains recorded maximum spore production (6×10^{10} CFU g^{-1}) and significant mortality. Of the different methods of application, traps swabbed with rice chaffy grain formulation registered higher degree of weevil catch reduction in infestation levels and yield of banana than the maize formulation. Our study showed that the use of 17-6 isolate of *B.bassiana* has potential as a biological control agent of rhizome and pseudostem weevils.

KEY WORDS: Banana, *Beauveria bassiana*, biological control, *Cosmopolites sordidus*, mass production, *Odoiporus longicollis*, shelf life

INTRODUCTION

India is the largest producer of banana in the world with an annual production of 16.17 million tons. Among the economically important insect pests, banana rhizome weevil, *Cosmopolites sordidus* (Germar) and pseudostem weevil, *Odoiporus longicollis* (Olivier) have been the most destructive and contribute to yield losses up to 90% and sometimes total crop failure results in farms where the weevils are not managed efficiently (Padmanaban and Sathiamoorthy, 2001; Padmanaban *et al*., 2002). Besides India, these pests pose great threat to banana production in leading banana producing countries like China, Thailand, Indonesia, Malaysia, Latin America and Hawaii (Valmayor *et al*., 1994; Anitha *et al*., 1999. Padmanaban and Sathiamoorthy, 2001). Traditional control methods such as cultural practices and chemical controls are not only unsatisfactory at many times, but result in resistance build up of insects, environmental pollution and pesticide residue in products (Padmanaban and Sathiamoorthy, 2001; Padmanaban *et al*., 2002). Hence, there has been a greater emphasis on biological control as an alternative to chemical control in recent years (Collins *et al*., 1991; Treverrow *et al*., 1991; Anitha *et al*., 1998; Yue and Wen, 2004).

Earlier attempts on biological control have not reduced the weevil populations in the areas of their introduction. Factors such as low inoculum levels of introduction, inadequate methods of application and dissemination, and cost are attributed to their non–efficacy (Nankinga and Latigo, 1996). A review of literature also suggests that use of entomopathogenic fungi for the management of banana rhizome weevil has been widely evaluated (Nankinga, 1994; Nankinga *et al*., 1994; Nankinga and Latigo, 1996 and Nankinga *et al*., 1996, 1999), but the information on stem weevil is very limited (Anitha *et al*., 1998; Yue and Wen, 2004, Yue *et al*., 2003). In the present research, we aimed at developing a cost–effective method of production and evaluation of efficient endemic strains of the entomopathogenic fungus, *Beauveria bassiana* against both rhizome and pseudostem weevils.

MATERIALS AND METHODS

Test insects

The banana pseudostem and rghizome weevils were collected from endemic areas in Lower Pulney hills in Dindigul district of Tamil Nadu. Longitudinal split pseudostem traps (30cm) were used to capture the weevils. Collected weevils were identified in the laboratory and

maintained on banana stem and moistened rhizome pieces in 20 litre capacity containers, specially designed for weevil rearing. Before inoculation, the weevils were washed in tap water and surface sterilized with 1.0% sodium hypochlorite solution and finally thrice with sterile distilled water as per the method described by Nankinga and Latigo (1996).

Efficacy in relation to method of application

The potential of *B. bassiana* 17–6 strain against rhizome and pseudostem weevils was evaluated by three different methods: (i) spraying the spore suspension (1×10^8 spores ml^{-1}) over the weevils (ii) immersing the weevils in spore suspension (1×10^8 spores ml^{-1}) for 5min. (iii) allowing the weevils to feed on the split pseudostem trap sprayed with spore suspension (1×10^8 spores ml^{-1}) for a week under laboratory conditions. Weevils sprayed with sterile distilled water were kept as controls. Twenty adult weevils were considered as one replicate and 10 replicates were organized for each test. These experiments were carried out in plastic containers (20L capacity) at 24 ± 2°C. Mortality was assessed every day till end point was achieved.

Mass production of strain 17–6 of *B. bassiana* in different organic substrates and its shelf life

Five different substrates were selected for mass production of B. *bassiana* 17–6 such as ragi flour, maize flour, sorghum flour, rice chaffy grains and wheat bran. Hundred grams of each substrate was mixed with 1% jaggery solution to sufficiently moisten the substrate for mycelial growth (Thangavelu *et al*., 2004). The substrates were then taken in polypropylene bags separately and their tops were plugged with cotton and then sterilized at 121°C for 2 h. Sterilization of bags was repeated the following day to rule out the presence of any microbes in the substrate. After sterilization, the organic substrates were inoculated individually with an 8 mm mycelial disc of *B. bassiana* 17–6 obtained from 7–day–old culture under aseptic conditions. Ten replicates were set up for each treatment. A standard commercially available talc powder formulation of *B. bassiana* was included for comparison. The bags were incubated at room temperature for up to 90 days and samples were drawn from each treatment at 30 days interval. Population of *B. bassiana* 17–6 isolate on different organic substrates was estimated by serial dilution technique using potato dextrose agar (Thangavelu *et al*., 2004).

Field evaluation of *B. bassiana* 17–6 under high land production system

The field trials were conducted in a farmer's field at Thadiyankudisai, in Lower Pulney hills (MSL 1200ft) of Dindigul district in Tamil Nadu which is an endemic area for both rhizome and pseudostem weevil on cv.Virupakshi (Pome–AAB). Four fields were selected for this study with a distance of 120 meters from each other containing 100 banana plants (approximately seven months old) in each field. Since maize flour and rice chaffy grain based formulations supported high density of *B .bassiana* spores for up to 90 days, these two formulations were selected for these studies. In addition, the normal recommended practice of monocrotophos 36 EC injection was also included for comparison (Padmanaban and Sathiamoorthy, 2001). A field with 100 plants without any treatment served as a control. About 15 g each of maize flour and rice chaffy grain formulation of *B. bassiana* 17–6 isolate was swabbed over the full length of cut surface of the split pseudostem trap. Seven months after planting, up to 20 such traps were kept 5 meters apart over the soil surface, approximately covering the entire trial area equally (Padmanaban *et al*., 2002). The dried split pseudostem trap was replaced weekly with a new trap applied with respective formulations for up to eight weeks. In case of monocrotophos treatments, two injections on 7^{th} and 8^{th} months after planting were given at four weeks interval. The trapped weevils were brought to laboratory in sterile containers (one litre capacity) and mortality rate was recorded. The percentage infestation data for pseudostem stem weevils was subjected to a scale of 0–6 (where, 0: no hole /feeding damage on the pseudostem, 1: 1–10% infestation, 2: 11–25% infestation, 3: 26–50% infestation, 4: 51–75% infestation, 5: 76–100% infestation, 6: dead plant). The infestation data for rhizome weevil could not be taken up, as it necessitated destruction of banana plants. The average bunch weight (kg/plant) was also recorded.

Statistical analysis

All the experiments were conducted in a completely randomized block design (CRD) and repeated at least twice except the field experiments. The results were subjected to analysis of variance and the mean values were compared using Fisher's unprotected least significant difference test ($P \leq 0.05$). The data on percentage of mortality of weevils were transformed into arcsine square root values to normalize distributions before analysis of variance. The data on the population of *B. bassiana* in different organic substrates (CFU g^{-1}) were log–transformed to improve the homogeneity of variances.

RESULTS AND DISCUSSION

Mortality of weevils in relation to method of application

Among the three methods of application studied, spraying the conidial spore suspension of *B. bassiana* 17–6 and immersion of weevils in spore suspension recorded 100% mortality of both species of weevils in six days, whereas 90% mortality of both species was recorded when the weevils were allowed to feed on fungus sprayed pseudostem

(Table 1). Pathogenicity due to *B. bassiana* was confirmed in 100% of the dead weevils isolated from all three treatments. Our results are in conformity with the earlier studies in relation to pathogenicity and efficacy in relation to method of application (Mesquita, 1988, Nankinga, 1994, Nankinga and Latigo, 1996). This study established that strains of *B. bassiana* isolated from *C. sordidus* were most pathogenic when applied directly on the insects, causing 70 to 100% mortality within 36 days. However, compared to the earlier isolates as repeated by Nankinga (1994) and Nankinga and Latigo (1996), the present isolate was observed to be more virulent, taking into consideration the mortality, dosage and time taken for infection.

Mass production of potential strain of *B.bassiana* 17–6 on different organic substrates and their shelf life

Among the five different substrates tested, rice chaffy grain and maize flour formulations recorded significantly higher spore production of 6×10^{10} and 1×10^{9} CFU g^{-1} of materials, respectively. Moreover, both these substrates maintained significantly higher spore density of *B.bassiana* 17–6 isolates (1×10^{8} CFU g^{-1}) even after 90 days of incubation (Table 2). Pandey and Kanaujia (2005) reported highest spore production of 5. 39 x 10^{7} spores ml^{-1} and spore viability of 96.6% on finger millet. Rice chaffy grain seems to be a superior medium. It is a well established fact that addition of sucrose or other carbohydrates to the organic substrates increases the spore load of fungal pathogens (Pandey and Kanaujia 2005; Thangavelu *et al*., 2004; Maglhas *et al*., 1994). Our present study is in conformity with these earlier published results in that the addition of 1% jaggery significantly increased the spore production in all the substrates tested.

Table 1. Intensive screening of *Beauveria bassiana* 17-6 against rhizome and pseudostem weevils of banana under *in vitro* conditions

Treatments	% mortality [a]	
	Rhizome weevil	Pseudostem weevil
Spraying spore suspension of *B. bassiana* @10^{8} spores ml^{-1}	100[ba]	100[ba]
Immersion of weevil in spore suspension of *B. bassiana* containing 10^{8} spores ml^{-1}	100[ba]	100[ba]
Pseudostem trap sprayed with spore suspension of *B. bassiana*	90.0[cb]	90.0[cb]
Control (water)	0[c]	0[c]
CD ($P \leq 0.05$)	5.45	1.37
CV%	9.32	2.91

[a] Mean of ten replications; [b] End point mortality achieved at 6 days; [c] End point achieved at 8 days; means followed by the same letter in a column are not significantly different according to Fisher's unprotected least significant difference test ($P \leq 0.05$)

Field evaluation of *B. bassiana* 17-6 isolate

The field trials involving the maize and chaffy grain formulations swabbed in split pseudostem trap resulted in the trapping of both rhizome and pseudostem weevils in large numbers (Table 3). Significantly higher numbers of both weevils were trapped in traps swabbed with rice chaffy grain formulation than the maize formulation. Similarly the percentage mortality of both the weevils was higher in rice chaffy grain formulation (77.23%) than the maize flour formulation (72.47%). When the infestation levels of the pseudostem weevil were compared, there was a significantly higher reduction in infestation level (62.5%) with rice chaffy grain formulation than that observed with maize flour formulation (55%). However, the maximum reduction of infestation level of 81.25% was achieved in the standard treatment where the plants were injected with monocrotophos 36 EC (Table 3). With regard to yield, monocrotophos injection recorded an increase of 155.78% over control, whereas, the rice chaffy grain and maize flour formulations recorded 114.73 and 85.26% increase over control, respectively (Fig. 1). In Brazil, Batista *et al*. (1987) infected field collected *C. sordidus* with *B. bassiana* and *Metarhizium anisopliae* cultured on rice and beans. The

Table 2. Sporulation and shelf life of strain 17-6 of *Beauveria bassiana* in different solid media

Treatments	CFU g^{-1} of material			
	Days of storage			
	0+	30	60	90
Ragi flour + 1% jaggery	$1x10^8$ e (8.00)	$1.5x10^9$ c (9.17)	$5x10^7$ d (7.69)	$2x10^6$ c (6.30)
Maize flour + 1% jaggery	$1x10^9$ b (9.00)	$2.5x10^9$ b (9.39)	$3x10^8$ b (8.47)	$1x10^8$ a (8.00)
Sorghum flour + 1% jaggery	$2.5x10^8$ d (8.39)	$1.5x10^8$ d (8.17)	$8x10^7$ c (7.90)	$1x10^7$ b (7.00)
Rice chaffy grain + 1% jaggery	$6x10^{10}$ a (10.77)	$1x10^{10}$ a (10.00)	$2x10^9$ a (9.30)	$1x8^8$ a (8.00)
Wheat bran + 1% jaggery	$4x10^8$ c (8.60)	$10x10^7$ e (8.00)	$1.5x10^7$ e (7.17)	$9x10^7$ a (7.95)
Commercial grade	$1x10^6$ f (6.00)	$1x10^5$ f (5.00)	$1x10^4$ f (4.00)	$1x10^4$ d (4.00)

+ Full coverage of the substrate was observed in 10 days post–inoculation; *mean of 10 replications; figures in parentheses are $\log_{10}$ values; in a column means followed by a common letter are not significantly different according Fisher's unprotected least significant difference test ($P \leq 0.05$); the LSD for interaction of substrates and days of storage is 0.10 ($P \leq 0.05$)

Table 3. Field evaluation of *B. bassiana* 17-6 against banana weevils *Cosmopolites sordidus* and *Odoiporus longicollis* in cv. Virupakshi (Pome –AAB) under highland banana production system

Treatment	No. of weevils trapped[a]			Weevil mortality[a]			% infestation (0 - 6 scales) for pseudostem weevil			
	BPW	BRW	Total	BPW	BRW	Total % mortality	Initial	Final	Actual	% reduction over control
B. bassiana maize flour formulation	224^a	132^a	356^a	158^a (70.53)	100^a (75.75)	72.47^a	0.70^b	2.5^b	1.80^c	55.00^a
B. bassiana chaffy grain formulation	262^b	144^b	406^b	196^b (74.8)	118^b (81.94)	77.33^b	0.70^b	2.2^b	1.50^b	62.50^b
Control	-	-	-	-	-	-	0.50^a	3.80^c	4.00^d	-
Standard check-monocrotophos stem injection	-	-	-	-	-	-	0.75^{bc}	1.50^a	0.75^a	81.25^c

BPW = Banana pseudostem weevil, BRW-Banana rhizome weevil; stem injection of pseudostem was performed by injecting 4 ml of Monocrotophos 36 EC (diluted as 150 ml in 350 ml water) per plant twice; 0-6 Scale: 0- No infestation, 1-1 to 10 %, 2-11 to 25% infestation, 3- 26 to 50% infestation, 4- 51-75% infestation, 5- 76-100% infestation, 6- Plant dead; *mean from 20 replications, [b]n=100; in a column means followed by a common letter are not significantly different according to Fisher's unprotected least significant difference test ($P \leq 0.05$)

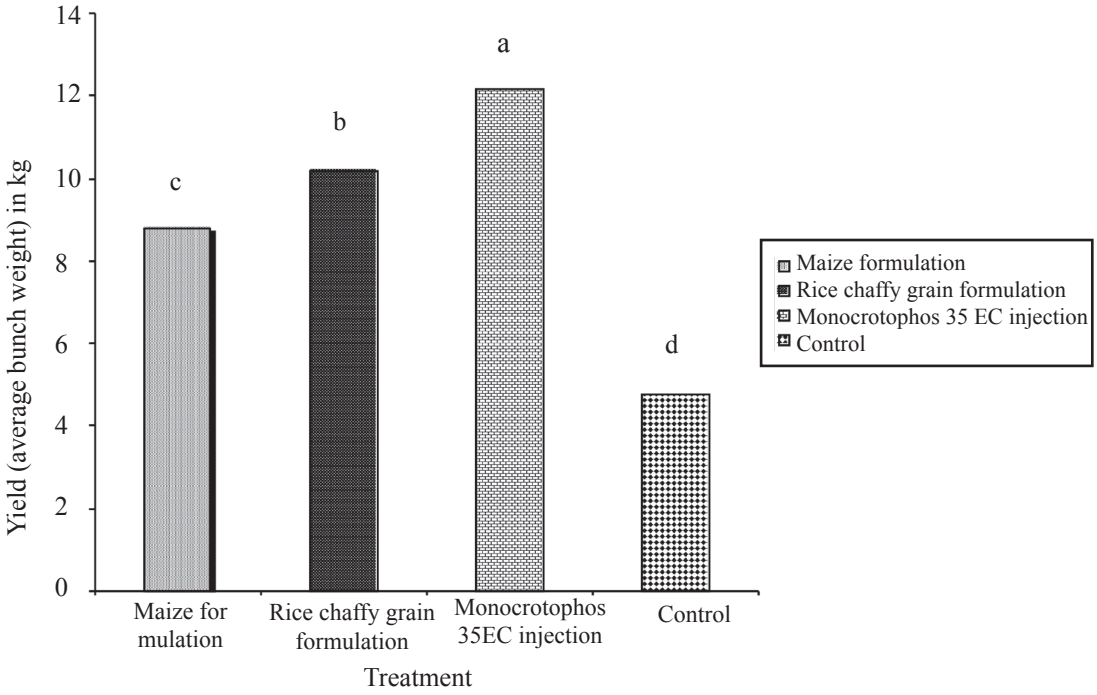

Fig. 1. Effect of *B. bassiana* treatment on the yield of banana, in relation to mass multiplication media (In a column means followed by a common letter are not significantly different according to Fisher's unprotected least significant difference test (P≤ 0.05). Error bar represents standard deviation from the mean

two fungal cultures mass produced on rice caused rhizome weevil mortality between 85 and 97% respectively, while, *M. anisopliae* cultured on beans caused only 56% mortality. Nankinga and Latigo (1996) also reported that complete mortality was attained within three weeks post–inoculation of *B. bassiana* cultured on maize and rice. However, when spore suspension was applied on to the soil and only to covered pseudostem traps, mortalities in the range of 18.6 – 54.3% were attained within three weeks.

In the present study, full growth of white mycelial mat was observed in all the dead weevils collected from the trap. Profuse fungal growth was also recorded on the split trap and below the trap on the soil, which according to our view would have further provided increased inoculum for spread of infection to the visiting weevils as well as in the soil. Therefore, though the per cent mortality of weevils was less compared to that recorded *in vitro*, under field conditions the infection and subsequent death of insects could lead to production of conidia which in turn provide fresh inoculum on a sustainable basis. Moreover, earlier studies by Nankinga and Latigo (1996) reported that infected weevils can transmit the pathogen to eggs and larvae within the traps and thereby maintain the weevil populations below the economic threshold level.

This is the first report of field evaluation of biocontrol agent *B. bassiana* against pseudostem weevil, *O. longicollis*. We conclude that this strain of *B. bassiana* 17–6 has potential as a biocontrol agent to manage both rhizome and pseudostem weevils of banana.

ACKNOWLEDGEMENTS

The authors wish to thank the Director (NRCB), Head, Crop Protection Division, NRCB, Tiruchirapalli, for providing necessary facilities, Mr. S. Palanichamy (Lab Technician) for technical assistance and Dr. Manoj Kumar Nayak, Senior Research Scientist, DPI & F, Indooroopilly, Brisbane for his valuable suggestion on an earlier draft of the manuscript.

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(Received: 07.01.2009; Revised: 26.05.2009; Accepted: 06.07.2009)