International Journal of Plant & Soil Science

33(8): 36-46, 2021; Article no.IJPSS.67898
ISSN: 2320-7035

Effectiveness of Entomopathogenic Fungus *Beauveria bassiana* Isolate on *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) Mortality under Different Concentrations in Kenya

Mellon Kabole[1,2*], Dora Kilalo[2], Maina Muiru[2] and Danny Coyne[3]

[1]*Kenya Plant Health Inspectorate Services, P.O.Box 49592-00100, Nairobi, Kenya.*
[2]*Department of Plant Science and Crop Protection, University of Nairobi P.O Box 30197-00100, Nairobi, Kenya.*
[3]*International Institute of Tropical Agriculture. P.O.Box 30772-00100, Nairobi, Kenya.*

Authors' contributions

This work was carried out in collaboration between the authors. Author MK designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MK, DK, MM and DC reviewed the study design and all drafts of the manuscript. Authors MK and MM managed the analyses of the study and performed the statistical analysis. Author MK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i830456
Editor(s):
(1) Prof. Alejandro Hurtado Salazar, Universidad de Caldas, Colombia.
Reviewers:
(1) M. Pramila, Pavendar Bharathidasan College of Arts and Science, India.
(2) J.B. Narendra Kumar, India,
Complete Peer review History: http://www.sdiarticle4.com/review-history/67898

Original Research Article

Received 25 February 2021
Accepted 04 May 2021
Published 10 May 2021

ABSTRACT

Callosobruchus maculatus Fab. (*Coleoptera: Bruchidae*), is a major field-to-store post-harvest pest of in the tropics and worldwide. They cause weight loss, decreased germination potential and reduction in commercial and aesthetic value as a result of physical contamination of grain by insects, eggs and excrement, decreased nutritional value. Entomopathogenic fungi have been employed in control of a number of storage pests. Nine *B. bassiana* isolates were evaluated for effectiveness in controlling *C. maculatus* in cowpea grain under controlled laboratory conditions. Mortality of the bruchids was evaluated stepwise where the most effective isolate concentrations against *C. maculatus* were assessed. Initial assessment involved determination of the most

**Corresponding author: E-mail: mellonkabole@gmail.com;*

effective concentration among the isolates following dilution. The most effective isolate concentrations were then doubled and halved to identify the most effective dose rate. The isolates showed significant differences on the mortality of cowpea bruchids at the different concentrations. The halved rate from the most effective rate with BBC recording 85% and 76.7 % cumulative mortality at day 3 during the first and second season respectively. Isolate J35 had 98.2% mortality at 5 days after application during the first season and 88.3% during the second season, which was only lower to the formulated isolates BBC and BVT. At the optimal dose rate experiment, isolate J57 presented the highest cumulative mortality (25.5%) at 14 days after application while J59 recorded the lowest cumulative mortality at 11.8% at the same time. The combination of isolate J39 and RI showed the highest cumulative mortality of 40.4% and 44.4% during the first and second season respectively. Results obtained from this study support the importance of exploring the use of *B. bassiana* in control of *C. maculatus* during storage.

Keywords: Callosobruchus maculates; entomopathogenic fungi; mortality; effective dose rate.

1. INTRODUCTION

Entomopathogenic agents are natural, non-toxic to humans, environmentally safe, easy to formulate, with no residual activity, the fungi do not damage grain mass, can be mass produced and are less likely to develop resistance [1]. Different species and strains of entomopathogenic fungi have been tested using different formulations and application methods and have been observed to cause mortality in various insect pests [2]. Success in control of insect species in stored products has been recorded in both laboratory and field tests [3]. Entomopathogenic fungi have also been used to treat empty stores to remove residual pests before a new harvest is brought in, or applied as a mixture with grain as curative or preventive treatment [4]. Recently, research has focused on the use of *B. bassiana* isolates in biological control of many important insect pests [5].

Callosobruchus maculatus, is a major field-to-store post-harvest pest of economically important legumes in the tropics and worldwide [6]. Many methods including chemical, cultural, physical, biological, varietal and genetic have been employed to control cowpea bruchids. Until recently, biological control had been considered as minimally important in pest management [7]. Entomopathogenic fungi has uniqueness in control which is as a result of them not being limited to controlling sucking and feeding insects as they infect through the hosts surface by contact thereafter penetration of cuticle. *Beauveria bassiana* has been used to control many important pests in various crops and has been tested on various target insects' pathogens, blood feeding insects and vectors of disease across the world [8].

Several products based on *B. bassiana* have been developed and made commercially available for control of a range of agricultural pests [9]. A considerable amount of literature, patents and techniques have been developed and applied by commercial entities [10]. The study therefore focuses on screening the isolates at different concentrations and levels to control *C. maculatus*. The findings are expected to provide preliminary pathogenicity status of the isolates on different concentrations which will advise recommended rates for application on the control of the insect pest.

2. MATERIALS AND METHODS

2.1 *Callosobruchus maculatus* Insect Colony

A laboratory colony of *C. maculatus* was established from adult bruchids sieved from samples of highly infested cowpea grain obtained from a local market in Machakos town- Kenya. The cowpea bruchids used for these experiments as test insects were of mixed ages and sex. Two hundred adults of cowpea bruchid mixed sex and age were placed for 10 days in a jar containing clean cowpea grains to allow oviposition. The bruchids in the cowpea were then sieved out, harvested and used to infest the clean grain to start the rearing process on uninfested cowpea grains in a ventilated chamber. Mass cultures were maintained in 1.5 kg large plastic containers and subcultures in 100 g small plastic containers with cowpea seed as food medium. Each container was covered with 10 mm mesh sieve to allow free air circulation and also prevent insects from escaping. Temperature in rearing room was maintained at 28 ± 2°C and relative humidity of 60 ± 5 %.

2.2 Bioassay on Screening of the Fungal Isolates

2.2.1 Mortality by immersion

Five ml of the isolate product was serial diluted and applied at four different concentrations 103 to 106. For each replication, 20 individuals of mixed sex and age were treated by immersion for 5 sec in 5 ml of conidial suspensions at the different concentrations separately. The control was immersed in sterile distilled water. Treated insects and 1 ml of the suspension were subsequently poured onto a plate containing a sterile filter paper. Filter paper helped absorb the excess moisture and increase conidial load on each insect allowing a secondary spore pick up.

Treated insects were kept without food for 24 hours at 28 ±2°C and 60 ±5 % RH before 10 clean un-infested cowpea seeds being introduced as food. Mortality was recorded at every two days ending at 15 days. The number of dead bruchids counted were corrected using the Abbott formula [11]. Abbott's Formula is used to calculate insecticide efficiency with a correction involving natural deaths whereby it subtracts the natural deaths of insects using results from a control plot.

Dead insects were surface sterilized in sodium hypochlorite (2%), alcohol (70%) and then rinsed with sterile distilled water for 15 seconds. They were then placed in clean petri dishes with moist filter papers. Observation of mycosis on the dead insects was made and recorded for two weeks. Only dead insects which had fungal growth were considered to be killed by *B bassiana* fungus.

2.2.2 Effective dose test

From the bioassay by immersion experiment above, the concentration with the highest mortality was considered as the full dose rate. The full dose rate was doubled and halved to make three dose rates, which were used in this experiment. For each tin, 10 clean un-infested, unbroken cowpea grains were placed in clean, 100 g dry plastic tins. These concentrations were separately sprayed using a spray atomizer to each set. The control was treated with sterile distilled water. Twenty bruchids of mixed sex and age were added to each set of treated grains. Each container lid was punctured to create small holes to allow free air circulation. The set up was kept at a temperature 28 ± 2°C and 60 ± 5 % RH. Mortality of bruchids was recorded every two days and ending at 15 days. Each tin served as replicate with three tins for each isolate in a complete randomized design. The number of dead bruchids counted were corrected using the Abbott formula [11].

Dead insects were surface sterilized in sodium hypochlorite (2%), alcohol (70%) and then rinsed with sterile distilled water for 15 seconds. They were then placed in clean petri dishes with moist filter papers. Observation of mycosis on the dead insects was made and recorded for two weeks. Only dead insects which had fungal growth were considered to be killed by *B. bassiana* fungus.

2.2.3 Optimal dose test

From the effective dose rate experiment above, the dose rate with the highest mortality was used in this experiment. For each replicate, 30 grams of clean, unbroken cowpea grains were placed in clean, dry plastic tins. 5 ml of the identified effective dose rate was sprayed on the cowpea seeds. Twenty mixed sex and age bruchids were added to each set of treated grains. Each container was perforated with small holes to allow free air circulation. The control was treated with sterile distilled water. The set up was kept at a temperature 28 ±2°C and 60 ±5 % RH for 14 days. Each tin served as replicate with three tins for each isolate in a complete randomized design. The number of dead bruchids counted were corrected using the Abbott formula [11].

Dead insects were surface sterilized in 2% sodium hypochlorite and 70% alcohol and then rinsed with sterile distilled water for 15 seconds. They were then placed in clean petri dishes with moist filter papers. Observation of mycosis on the dead insects was made and recorded for two weeks. Only dead insects which had fungal growth were considered to be killed by *B. bassiana* fungus.

2.2.4 Isolate-combination optimal dose test

Combinations of the dose rates with highest mortality from the effective dose rate experiment above were used. This was done for each isolate at the ratio of 1:1. The mixed two-isolate solutions were separately sprayed using a spray atomizer on clean cowpea seeds.

For each replicate, 30 grams of clean, unbroken cowpea grains were placed in clean, dry plastic bowls. Two 2.5 ml of the identified effective dose rate combinations were done and was sprayed on the cowpea seeds. Twenty mixed sex and age bruchids were added to each set of treated grains. Each container was perforated with small holes to allow free air circulation. The control was treated with sterile distilled water. The set up was kept at a temperature 28 ±2°C and 60 ±5 % RH for 14 days. Each tin served as replicate with three tins for each isolate in a complete randomized design. The number of dead bruchids counted were corrected using the Abbott formula [11].

Dead insects were surface sterilized in 2% sodium hypochlorite and 70% alcohol and then rinsed with sterile distilled water for 15 seconds. They were then placed in clean petri dishes with moist filter papers. Observation of mycosis on the dead insects was made and recorded for two weeks. Only dead insects which had fungal growth were considered to be killed by *B. bassiana* fungus.

2.3 Statistical Analysis

Analysis of variance (ANOVA) was carried out on the quantative data after cleaning and tabulation using SAS Version 9.1 statistical software and tested for significance at 99% level of confidence. The treatment means were then separated using the Fishers LSD test where significance was found at P≤0.01.

3. RESULTS AND DISCUSSION

3.1 Mortality by Immersion

The *B. bassiana* isolates generally indicated significant differences (P≤0.01) in mortality of the cowpea bruchids at different concentration levels during the observed days 3 to 15. Significant differences were observed among the isolates' effect on the mortality of the cowpea bruchids for both seasons at day 3 (Table 1). Isolates J35 and J36 showed the highest mortality at 10^3 concentrations with 30% and 26.75% respectively while isolate J36 showed the highest mortality of 31.7% at 10^4 concentration during the first season. The concentration of 10^6 showed the lowest mortality under isolates J39 and J59 although almost the entire isolates did not differ significantly between their mortalities at 10^6 during season one. During the second season, isolate J35 had the highest mortality (29.1%) at 10^3 concentration which was although not significantly different from that of J36 (29.2%) at 10^4 concentration. There were no bruchids mortality under the 10^6 concentration for J39 and J59 isolates (Table 1).

At day 5, significant differences on the mortality of the cowpea bruchids were recorded between the *B. bassiana* isolates at the different concentrations (Table 2). At 10^3 concentration, J35 had the highest mortality (66.6%) which was however insignificantly different from J36 (64.9%) at 10^4 concentration. The lowest mortality was recorded on the 10^6 concentration in J36 and J59 isolates. A similar trend was observed in season two where isolate J35 had the highest mortality (66.3%) followed by J36 at 63.1% at 10^3 concentration while J59 and J39 had mortality of 3.3% and 1.7% respectively at 10^6 concentration (Table 2).

Table 1. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrates at day 3 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	10.0c	21.7a	18.3b	6.6c	9.6c	20.7ab	17.5b	6.4c
BVT	13.3b	6.7c	15.0b	15.0b	12.8b	6.1c	13.1b	14.3b
J29	10.0c	11.7b	6.6c	5.0c	9.5c	10.6c	6.2c	4.9c
J35	30.0a	6.7c	11.7b	3.3c	29.1a	6.2c	11.4b	3.2c
J36	26.7a	31.7a	6.6c	1.6c	25.6a	29.2a	6.2c	1.7c
J39	18.3b	10.0c	5.0c	0.0c	17.6b	9.8c	17.9b	0.0c
J57	1.67c	15.0b	6.6c	8.3c	1.6c	14.4b	6.4c	8.0c
J59	10.0c	8.3c	13.3b	0.0c	9.1c	8.0c	12.8b	0.0c
RI	15.0b	16.6b	5.0c	5.0c	14.4b	15.7b	4.8c	4.8c
P-Value	<.001				<.001			
L.S.D	10.586				11.706			
CV%	5.9				4.1			

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

Table 2. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 5 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	33.3c	45.6b	47.4b	24.6c	31.3b	39.8b	43.1b	23.1b
BVT	28.1c	8.8d	45.6b	31.6c	26.4b	8.2c	42.2b	29.9b
J29	29.8c	21.1c	29.8c	10.5d	28.0b	19.5c	28.0b	9.9c
J35	66.6a	15.0d	12.3d	8.8d	66.3a	14.6c	11.2c	8.3c
J36	63.1a	64.9a	7.0d	10.5d	58.1a	59.6a	6.6c	9.9c
J39	26.3c	15.8d	12.3d	0.0d	24.5b	14.3c	10.9c	1.7d
J57	12.3d	24.6c	8.8d	10.5d	11.5	24.2b	8.3c	9.9c
J59	14.0d	22.8c	26.3c	3.5d	12.6	21.5c	23.6c	3.3d
RI	24.6c	21.1c	21.1c	10.5d	23.1b	19.79	19.8c	9.8c
P-Value			<.001				<.001	
L.S.D			15.88				15.12	
CV%			4.3				1.5	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

At day 7, during the first and second season the highest cumulative mortality was recorded on isolate J35 with 86.3% and 82.8% at 10^3 concentration respectively (Table 3). This was followed closely by Isolate BBC at 76.5% during season one and J36 at 70.8% during season two. Many other isolates had a cumulative mortality of over 70% at different concentrations for both seasons while isolate J39 had the lowest cumulative mortality of 2.0% during the first season and 1.8% during the second season at the 10^6 concentration.

On the 9th day, the isolate BBC showed 100% cumulative mortality of the cowpea bruchids at the 10^3 concentration during the first season while the isolate BVT had 97.9% mortality at 10^4 concentration during the same period of time as shown in Table 4. The lowest cumulative mortality was recorded at 10^6 concentration and specifically on J39 and J59 isolates at 4.2% and 8.3% respectively. A similar trend was observed during season two where, a 94.7% cumulative mortality of the cowpea bruchids was recorded under the isolate BBC at the 10^3 concentration and 90.3% on the isolate BVT at 10^4 concentration. Similarly, J39 and J59 had the lowest cumulative mortalities during this season.

At day 11 in the first season, the 10^3, 10^4 and 10^5 concentrations showed 100% cumulative mortality on the isolates BBC (Table 5). Isolate BVT showed 100% cumulative mortality at 103, 10^4 concentrations. Isolates J29 and J35 had

Table 3. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 7 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	33.3c	45.6b	47.4b	24.6c	31.3b	39.8b	43.1b	23.1b
BVT	28.1c	8.8d	45.6b	31.6c	26.4b	8.2c	42.2b	29.9b
J29	29.8c	21.1c	29.8c	10.5d	28.0b	19.5c	28.0b	9.9c
J35	66.6a	15.0d	12.3d	8.8d	66.3a	14.6c	11.2c	8.3c
J36	63.1a	64.9a	7.0d	10.5d	58.1a	59.6a	6.6c	9.9c
J39	26.3c	15.8d	12.3d	0.0d	24.5b	14.3c	10.9c	1.7d
J57	12.3d	24.6c	8.8d	10.5d	11.5	24.2b	8.3c	9.9c
J59	14.0d	22.8c	26.3c	3.5d	12.6	21.5c	23.6c	3.3d
RI	24.6c	21.1c	21.1c	10.5d	23.1b	19.79	19.8c	9.8c
P-Value			<.001				<.001	
L.S.D			15.88				15.12	
CV%			4.3				1.5	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

more than 90% cumulative mortality at 10^3 concentration during the same season with low mortality recorded under the 106 concentration for J59 and J39 isolates. In the second season, isolates BBC, BVT, J29, J35 and J36 showed the highest cumulative mortality at over 80% that were not significantly different from each other at the 10^3 concentration. Similar cumulative mortalities were noted at 10^4 concentrations with significant differences between J29 and BBC, BVT, J35 and J36. BBC and BVT had 95.3% and 97.7% at 10^5 concentration respectively while BBC recorded 95.0% at 10^6 concentration (Table 5).

At day 13, isolates BBC, BVT, J29 and J35 had more than 90% cumulative mortality of the cowpea bruchids which were significantly higher than the other isolates at 10^3 concentration during the first season (Table 6). In the second season, the 10^4 concentration showed that the isolates had more than 80% cumulative mortality with the lowest recorded under the 10^6 concentration (Table 6).

3.2 Effective Dose Rate Experiment

Significant differences were observed between the isolates on the cumulative mortality of cowpea bruchids after 3 days for both seasons under the most effective rate from the mortality by immersion experiment, halved rate and doubled rate (Table 7). In the first season, the commercial isolate BBC at the halved rate

Table 4. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 9 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	100a	87.5a	95.8a	66.7b	94.7a	84.9a	88.3a	64.7b
BVT	58.3c	97.9a	89.6a	83.3a	56.6b	90.3a	81.9a	76.8a
J29	81.2a	56.2c	72.9b	22.9d	75.2a	53.7b	70.7a	22.2d
J35	87.4a	47.9c	50.0c	16.7d	82.0a	46.5c	46.6c	16.2d
J36	85.4a	81.2a	50.0c	31.2d	80.5a	75.9a	47.0c	30.3c
J39	56.2c	45.8c	39.6	4.2e	54.4b	40.8c	38.4c	3.7d
J57	31.2d	54.2c	54.2c	31.2d	27.5d	49.0c	52.5b	30.3c
J59	39.6d	39.6d	72.9b	8.3e	38.4c	38.4c	70.7a	8.7d
RI	52.1c	45.8c	50.0c	20.8d	50.5b	44.5c	48.5c	20.2d
P-Value			<.001				<.001	
L.S.D			23.47				20.62	
CV%			2.9				1.9	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

Table 5. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 11 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	100a	100a	100a	97.8a	93.9a	96.0a	95.3a	95.0a
BVT	100a	97.8a	100a	86.7a	97.0a	95.2a	97.7a	84.9a
J29	93.3a	75.6b	84.4a	24.4d	86.5a	74.0b	82.8a	24.0d
J35	91.1a	82.2a	75.6b	22.2d	87.6a	80.6a	74.0b	21.8d
J36	86.7a	82.2a	57.8b	42.2c	83.9a	80.6a	56.6b	41.4c
J39	75.6b	64.4b	62.2b	17.8d	72.3	61.0b	61.0b	17.4d
J57	62.2b	68.9b	80.0a	37.8c	56.0	67.5b	78.4b	35.6c
J59	64.4b	48.9c	82.2a	15.6d	63.2	47.9c	80.6c	15.2d
RI	64.4b	75.6b	73.3b	24.4d	63.2	74.0b	71.9b	24.0d
P-Value			0.006				<.001	
L.S.D			21.12				19.96	
CV%			18.5				0.3	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

showed the highest cumulative mortality (85%) while the lowest mortality was observed under the doubled rate with as low as 5% mortality. During the second season, the commercial isolate BBC recorded the highest cumulative mortality (76.7%) at the halved rate of the most effective rate from the first experiment on mortality by immersion. The lowest cumulative mortality was also observed in the doubled rate as indicated in the first season.

At day 5, significant differences were observed on the isolates on the cumulative mortality in the different dosages for both seasons (Table 8). During the first season, 100% cumulative mortality was recorded on isolates BBC and BVT on the halved rate from the full rate from the first experiment on mortality by immersion. For the second season, 92.3% and 90.0% cumulative mortality on the BBC and BVT isolates were recorded respectively while the lowest mortality was under the doubled rate where isolate J35 had as low as 7.4% mortality.

During the 7th day, more than 88.9% cumulative mortality was recorded under all the isolates at the halved rate from the full dose rate from the mortality by immersion experiment (Table 9). At the doubled rate, the lowest cumulative mortality of cowpea bruchids was observed with the highest only indicating 40.7% under the BVT isolate. During the second season, only isolate J29 showed 70.2% cumulative mortality whereas all the other isolates had more than 89.5% at the

Table 6. Percentage mortality of bruchids under *Beauveria bassiana* isolates at different concentrations at day 13 during season one and two

	Season 1				Season 2			
	10^3	10^4	10^5	10^6	10^3	10^4	10^5	10^6
BBC	100a	100a	100a	100a	98.0a	98.0a	98.0a	98.0a
BVT	100a	100a	100a	90.5a	98.0a	98.0a	98.0a	89.6a
J29	93.9a	85.7b	90.5a	35.7b	90.0a	81.7a	89.6a	35.0c
J35	95.2a	92.8a	95.2a	23.4c	89.3a	91.0a	92.7a	21.0d
J36	100a	95.2a	73.8b	43.5b	98.0a	93.0a	70.0b	39.6c
J39	92.8a	88.1a	80.9b	26.2c	91.0a	83.3a	75.6b	25.6c
J57	73.8b	95.2a	88.1a	42.8b	72.3b	93.0a	86.3a	42.0c
J59	71.4b	92.8a	97.6a	19.1c	70.0b	90.0a	95.6a	18.6d
RI	80.9b	90.5a	92.9a	28.5b	77.7b	88.3a	90.6a	28.6c
P-Value	<.001				<.001			
L.S.D	13.95				13.904			
CV%	2.3				2.4			

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

Table 7. Mortality percentage of adult bruchids under *Beauveria bassiana* isolates at different rates at day 3 during season one and two

	Season 1			Season 2		
	Full rate	Halved rate	Doubled rate	Effective rate	Halved rate	Doubled rate
BBC	51.7b	85.0a	20.0d	49.1c	76.7a	19.0e
BVT	65.0b	46.7c	13.3e	60.0b	43.7c	12.7e
J29	31.7c	41.7c	5.0e	29.0d	38.7c	4.8f
J35	33.3c	58.3b	5.0e	31.7d	54.0b	4.8f
J36	40.0c	71.7a	13.3e	38.0c	61.7b	12.7e
J39	35.0c	50.0b	5.0e	32.0d	47.0c	4.5f
J57	36.7c	20.0d	5.0e	34.8d	19.0e	4.6f
J59	43.3c	25.0d	6.7e	38.3c	23.8d	6.3f
RI	50.0b	58.3b	8.3e	46.8c	55.4b	7.7f
P-Value	<0.001			<0.001		
L.S.D	16.36			15.79		
CV%	2.2			6.6		

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

Table 8. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different rates at day 5 during season one and two

	Season 1			Season 2		
	Full rate	**Halved rate**	**Doubled rate**	**Effective rate**	**Halved rate**	**Doubled rate**
BBC	82.5a	100a	28.1c	79.2b	92.3a	26.9c
BVT	87.7a	100a	40.4c	80.5a	90.0a	35.6c
J29	70.2b	70.2b	19.3d	67.4b	63.5b	18.5c
J35	77.2b	98.2a	8.8d	74.1b	88.3a	7.4d
J36	73.7b	89.5a	19.3d	70.7b	82.2a	18.5c
J39	80.7a	91.2a	17.5d	72.6b	84.3a	16.8c
J57	77.2b	93.0a	14.0d	74.1b	86.3a	15.2c
J59	78.9b	93.0a	7.0d	75.8b	85.3a	8.4d
RI	86.0a	96.5a	8.8d	80.3a	89.0a	8.4d
P-Value		0.005			0.034	
L.S.D		20.82			18.28	
CV%		2.2			2.9	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

Table 9. Mortality of adult bruchids under *Beauveria bassiana* isolates at different rates at day 7 during season one and two

	Season 1			Season 2		
	Effective rate	**Halved rate**	**Doubled rate**	**Effective rate**	**Halved rate**	**Doubled rate**
BBC	100a	100a	33.3b	82.5a	100a	28.1c
BVT	92.6a	100a	40.7b	87.7a	100a	40.4c
J29	90.7a	88.9a	25.9b	70.2b	70.2b	19.3d
J35	92.6a	100a	11.9c	77.2b	98.2a	10.8d
J36	85.2a	100a	33.3b	73.7b	89.5a	19.3d
J39	94.4a	98.1a	29.6b	80.7a	91.2a	17.5d
J57	92.6a	100a	14.8c	77.2b	93.0a	14.0d
J59	94.4a	100a	24.1b	78.9b	93.0a	7.0d
RI	96.3a	100a	22.2b	86.0a	96.5a	8.8d
P-Value		0.001			0.005	
L.S.D		19.34			17.79	
CV%		1.3			1.8	

Treatments with different letters in the same column are significantly different at 1% probability. BVT-Beauvitech® WP; BBC-Dudutech® WP

halved rate while the lowest cumulative mortality was observed on isolate J59 with only 7.0% in the doubled rate.

3.3 Optimal Dose Rate Experiment

Mortality of the cowpea bruchids at day 14 after application of the *B. bassiana* isolates showed significant differences for both seasons (Fig. 1). A higher cumulative mortality was recorded during the first season as compared to the second season where isolate J57 had the highest cumulative mortality (25.5%) followed by J36 and RI isolates with 21.6% each. The lowest cumulative mortality (11.8%) was recorded on the J59 isolate. During the second season, isolate J35 had the highest cumulative mortality (21.0%) and closely followed by J57 and RI isolates at (20.6%)) and (19.1%) respectively. The lowest cumulative mortality was observed in the J59 isolate with 9.3%.

3.4 Isolate-Combination Experiment at Optimal dose Rate

The mortality of the cowpea bruchids differed significantly ($P \leq 0.01$) between the isolate combinations at day 14 after application for both seasons (Fig. 2). During the first season, the combination of isolate J39 and RI led to the highest mortality at 40.4% after 14 days from application with the combination of J35 and BVT, and J35 and J39 indicating 37.7% and 35.39% respectively. The combination of isolates J36 and BVT had the lowest mortality of 9.26% (Fig. 2). In the second season lower mortality of the cowpea bruchids was observed 14 days after application of isolates where the highest (44.4%) was under the combination of isolates J39 and RI. The lowest mortality was recorded in the combination of isolates J29 and J35 with 9.2%.

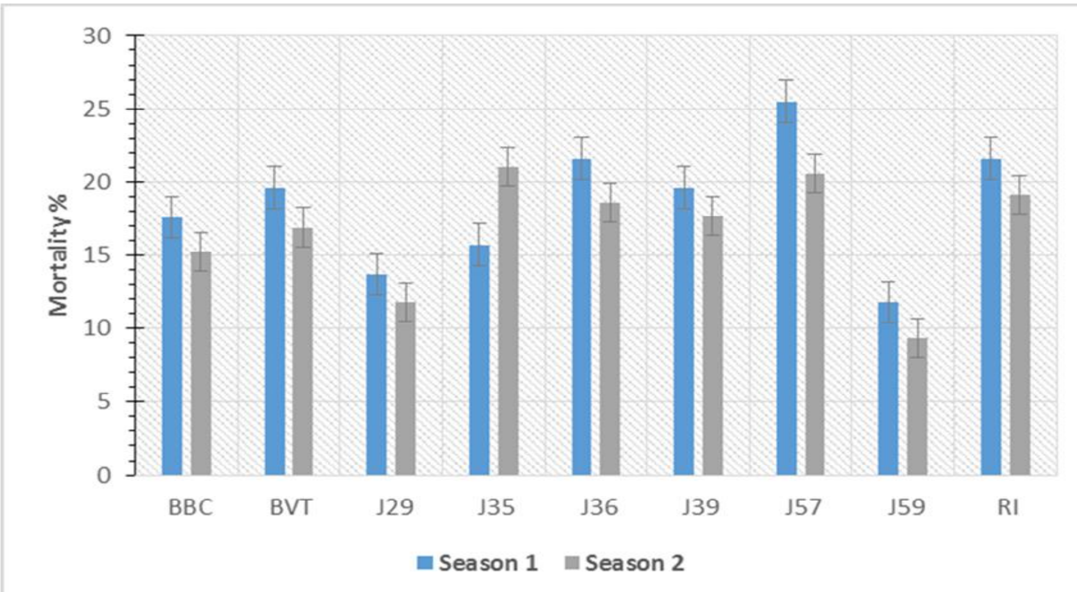

Fig. 1. Mortality of adult bruchids using obtained *Beauveria bassiana* optimal dose rate

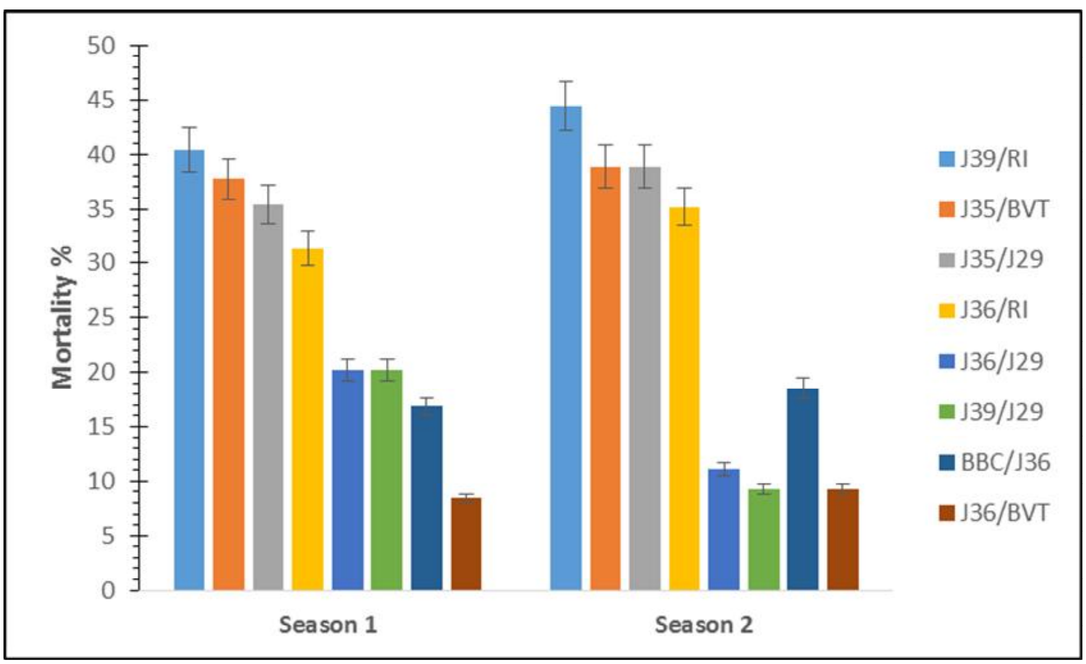

Fig. 2. Highest and lowest recorded mortality of adult bruchids using combined *Beauveria bassiana* isolates

4. DISCUSSION

The results demonstrate that all the tested *B. bassiana* isolates were infective to *C. maculatus* adults especially at high concentrations 10^3 and 10^4 where 100% mortality was recorded on all isolates in the mortality by immersion experiment by day 15. This further supports the findings by [12] who also showed that different *B. bassiana* isolates can provide good *C. maculatus* control by immersion bioassay at 12 days. Lower concentrations generally gave lower mortalities as observed through the experiments at concentration 10^6. Equally, [13] reported that pests injurious to stored wheat could be effectively be controlled using *B. bassiana.*

Beauveria bassiana at high concentration caused over 70% mortality of cowpea bruchids after day 13 of exposure during this experiment. This work closely compares with that of [14] who recorded over 80% mortality in cowpea bruchids after 13 days of exposure while testing the efficacy of *B. bassiana* on cowpea bruchid during storage. Generally, the halved dose rate in the effective dose rate experiment provided the best mortality as compared to the full dose rate. The highest mortality of BBC was observed on the halved dose rate which was insignificantly different from J35, J36 and RI during day 3 after infection. J57 indicated the highest mortality at the same halved dose rate during day 5 which was closely followed by J59 at the same dose rate. The greatest and significant mortality of the cowpea weevil were observed on these two intervals thereafter recording a low and insignificant mortality difference until day 11. This experiment indicated that the use of halved rate gave insignificant differences compared to using full rate which is economically important for the farmer. Studies by [15] found out that insect susceptibility to fungal infection is dose dependent thereby depending on the concentration of conidial suspension. On the same thought, [16] emphasizes on the need to economically evaluate the optimal concentration of conidia for spray so as to lower the cost of pest control while achieving high control effectiveness.

Cowpea bruchid mortality increased with increasing concentration of *B. bassiana* conidial inoculum. All the tested *B. bassiana* isolates were highly infective to *C. maculatus* at 4.86×10^6 cfu/ml and 4.86×10^7 cfu/ml. Shopiya et al. (2014) further recorded higher pathogenicity at concentration 2.0×10^6 spores/ml compared to other spore concentrations comparing well to the findings in this study.

5. CONCLUSION

The current study shows the pathogenic effectiveness of B. bassiana on *C. maculatus*. The results further demonstrate that although *B. bassiana* caused mortality on *C. maculatus* adults, the different isolates used in this study varied in their growth characteristics and virulence. Although there is indication of the effectiveness of the *B. bassiana* to control *C. maculatus* in farmer simulated conditions, further research is necessary evaluate the efficacy of the identified isolates under field conditions.

ACKNOWLEDGEMENT

The research was supported by International Institute of Tropical Agriculture (IITA), COMPRO II project by Bill and Melinda Gates Foundation. The facilitation by IITA, the Managing Director-KEPHIS and all those who participated in making the research successful are greatly acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Haas-Costa J, Alves LFA, Daros AA. Safety of *Beauveria bassiana* (Bals.) Vuill to *Gallus domesticus* L. Brazilian Archives of Biology and Technology 2010;53(2):465-471.
2. Freed S, Jin FL, Naeem M, Ren SX, Hussian M. Toxicity of Proteins Secreted By Entomopathogenic Fungi Against *Plutella xylostella* (Lepidoptera: Plutellidae). Int. J. Agric. Biol. 2012;14:291- 295.
3. Sabbour MM, Singer SM. Evaluations of Two Metarhizium varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. International Journal of Science and Research (IJSR) ISSN (Online). 2014;2319-7064.
4. Steenberg T. Safety Aspects Related to the Use of Entomopathogenic Fungi in Stored Products. In Proceedings of the 6th meeting of COST Action 842 Working Group IV Biocontrol of Arthropod Pests in Stored Products. Locorotondo, Italy. 2005;10-11.

5. Akello,J. Dubois,T. Coyne,D. Kyamanya,S. The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (Musa sp.) plants. Biol Control. 2009;49:6-10.
6. Rahman A, Taluker FA. Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus.* Journal of Stored Products Research. 2006;6(3):1 – 10.
7. Tamo M, Ekesi S, Maniania NK, Cherry A. Biological control, a non-obvious component of IPM for cowpea. In Biological control in IPM systems in Africa, edited by *P. Neuenschwander, C. Borgemeister*, and *J. Langewald*. CAB International, Wallingford, Oxon. 2003;256–309.
8. Darbro JM, Graham RI, Kay BH, Ryan PA, Thomas MB. Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). Biocontrol. Sci. Technol. 2011;21:1027– 1047.
9. Faria MR, Wraight SP. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biol Control. 2007;43:237-256.
10. Lacey LA, Solter LF. Initial handling and diagnosis of diseased invertebrates, pp. 1Ð14. In L. A. Lacey (ed.), Manual of techniques in invertebrate pathology, 2nd ed. Elsevier Ltd., London, United Kingdom; 2012.
11. Abbott WS. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 1925;18:265-267.
12. Wraight SP, Ugine TA, Ramos ME, Sanderson JP. Efficacy of spray applications of entomopathogenic fungi against western flower thrips infesting greenhouse impatiens under variable moisture conditions, Biological Control. 2016;97:31-47.
DOI: 10.1016/j.biocontrol.2016.02.016
13. Khashaveh A, Ghosta M, Safaralizadeh MH, Ziaee M. The use of entomopathogenic fungus, Beauveria bassiana (Bals.) Vuill. in assays with storage grain beetles. J. Agr. Sci. Tech. 2011;13:35-43.
14. Zahra Mahdneshin, Samad Vojoudi, Youbert Ghosta, Mohammad Hassan Safaralizadae, Moosa Saber. Laboratory evaluation of the entomopathogenic fungi, Iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch) Sorokin against the control of the cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae). African Journal of Microbiology Research 2011;5(29):5215-5220.
15. Ansari MA, Tariq MB. Influence of the application methods and doses on the susceptibility of black vine weevil larvae Otiorhynchus sulcatus to Metarhizium anisopliae in field-grown strawberries, Bio Control. 2004;58(2):257-267.
DOI: 10.1007/s10526-012-9491-x
16. Liu H, Skinner M., Parker BL, Brownbridge M. Pathogenicity of *Beauveria bassiana, Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae). Journal of Economic Entomology. 2002;95:675–681.

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