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Efficacy of Entomopathogenic Fungus *Beauveria bassiana* Isolates against *Callosobruchus maculatus* (*Coleoptera: Chrysomelidae*) under Controlled Conditions

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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.

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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 and has been demonstrated to have potential in control of *C. maculatus* in cowpea during storage. 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

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against *C. maculatus* were assessed. Initial assessment involved determination of the most effective concentration among the isolates following dilution. The isolates showed significant differences on the mortality of cowpea bruchids at the different concentrations. 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. The current study shows the pathogenic effectiveness of *B bassiana* on *C. maculatus*.

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

1. INTRODUCTION

Callosobruchus maculatus, is a major field-to-store post-harvest pest of economically important legumes [1] in the tropics and worldwide. The egg and adult stages and larval and pupal stages are found on the grain and inside the grain respectively. The eggs are small and translucent grey, inconspicuous domed structures with oval, flat bases. Female bruchids lay individual eggs to the seed testa which hatch within 5-6 days of oviposition [2]. Larvae hatch in about 5 days and penetrate through seed coat by chewing into the seed to complete their development. Bruchids emerge within 25-30 days after oviposition and mature after 24-36 hours. An emerging female shortly finds a suitable mate and produces ovipositions within an hour giving rise to about 100 offspring when well nourished. Adult bruchids are small (2 to 5.4 mm long), orange brown with dark markings with a triangular shape. Adult bruchids lay eggs on pods in the field or seeds in storage.

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 [3]. 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 [4].

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 [4]. *Beauveria bassiana* and *M. anisopliae* have been found to be effective on stored products pests like *Sitophillus oryzae* (L.), *Rhyzopertha dominica* (F.), *Acanthoscelides obtectus* Say and *C. maculatus*. Several products based on *B bassiana* have been developed and made commercially available for control of a range of agricultural pests [5]. A considerable amount of literature, patents and techniques have been developed and applied by commercial entities [6]. 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, which in future may be up scaled to field trials in view of commercial product development.

2. MATERIAL 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 %. However, it was expected that at high conidial densities also inhibited growth of neighboring mycelia and therefore the counts were done on earliest possible data collection periods.

2.2 Mortality by Immersion

Five ml of the isolate product was serial diluted and applied at four different concentrations 10^3 to

10^6. 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 [7].

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.

2.3 Statistical Analysis

Analysis of variance (ANOVA) was carried out on the data using SAS Version 9.1 statistical software and tested for significance at 99% level of confidence to determine differences between the mortality of *Callosobruchus maculatus* after treatment with *B. bassiana* isolates. The treatment means were then compared using the Fishers LSD test to determine the differences between the means of the mortality of *c. maculatus*.

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3. RESULTS AND DISCUSSION

The *B. bassiana* isolates indicated significant differences (P≤0.01) in mortality of the cowpea bruchids at different concentrations during the observed days 3 to 15. There were significant differences (P≤0.01) observed among the isolates' effect on the mortality of the cowpea bruchids at different concentrations for both assays at day 3 (Table 1). The highest mortality at day 3 across all concentrations was observed on isolate J36 at concentration 4.86 X 10^6 at 31.7% during assay one. This was followed closely by isolate J35 and J36 at concentration 4.86 X 10^7 with 30 % and 26.7% mortality records, though not significantly different from the highest record. Isolates BBC, BVT, J59 and J35 recorded the highest mortalities of 18.3%, 15.0%, 13.3% and 11.7% respectively at 4.86 X 10^5 concentration. These isolates were significantly different (P≤0.01) from the rest of the isolates at the same concentration. BVT and J35 isolates recorded the lowest mortality at 6.7% at concentration 4.86 X 10^6, although not significantly different (P≤0.01) from J59 at 8.3% and J39 at 10 %. Isolate J57 recorded the lowest mortality at 1.6% at concentration 4.86 X 10^7. BBC recorded the highest mortality at 4.86 X 10^5 concentration of 18.3%. BVT recorded the highest mortality of 15% at 4.86 X 10^4 concentration, which was significantly different (P≤0.01) from the rest of the isolates (Table 1).

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

	Assay 1				Assay 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	1.6c	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	<0.001				<0.001			
L.S.D	10.6				11.7			
CV%	5.9				4.1			

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^7, 10^{-4}= 4.86 X 10^6, 10^{-5}= 4.86 X 10^5, 10^{-6}= 4.86 X 10^4

The mortality recorded at assay two had similar trends as compared to assay one. The highest mortality was recorded by isolates J36 and J35 with 29.2% at 4.86 X 10^7 and 29.1% at 4.86 X 10^7 concentrations respectively. J57 recorded the lowest mortality at 1.6%, a reading that was not significantly different (P≤0.01) from J59 (9.1%), J29 (9.5%), and BBC (9.6%) mortalities at concentration 4.86 X 10^7. Isolate J36 recorded the highest mortality at concentration 4.86 X 10^6 at 29.2% while BVT recorded the lowest mortality at this concentration at 6.1% though not significantly different(P≤0.01) from isolates J35, J59, J39 and J29 which recorded 6.2%,8.0% 9.9% and 10.6% mortalities respectively. Isolates J39, BVT, BBC, J59 and J35 recorded the highest mortalities of 17.9%, 17.5%, 13.1%, 12.8% and 11.4% respectively at 4.86 X 10^5 concentration without significant differences (P≤0.01). These isolates were however significantly different (P≤0.01) from the rest of the isolates at the same concentration. BVT recorded the highest mortality of 15% at 4.86 X 10^4 concentration, which was significantly different (P≤0.01) from the rest of the isolates (Table 2).

The mortality of cowpea bruchids recorded significant differences (P≤0.01) at day 5 at different concentrations in the two assays (Table 2). At 4.86 X 10^7 concentration, J35 had the highest mortality (66.6%) which was however not significantly different (P≤0.01) from J36 (63.1%). The lowest mortality was recorded at isolate J57 at 12.3%, which was not significantly different from isolate J59 (14%). J36 recorded the highest mortality of 64.9% at 4.86 X 10^6 concentration which was significantly different from the next isolate, BBC with mortality at 45.6 %. The lowest mortalities at 4.86 X 10^6 concentration were recorded by isolate BVT at 8.8% followed by J35 (15%) and J39 (15.8%) with non-significant differences (P≤0.01). These were however significantly different (P≤0.01) from isolates J29 (21.1%), RI (21.1%), J59 (22.8%) and J57 (24.6%) mortalities under the same concentration. Isolates BBC and BVT recorded the highest mortality at concentration 4.86 X 10^5 at 47.4% and 45.6% respectively. This was significantly different (P≤0.01) from J29 at 29.8% and J59 at 26.3% mortality records with no significant differences (P≤0.01). Isolate J36 recorded the lowest mortality of 7.0% at the same concentration.

Assay two recorded a similar trend in mortalities recorded comparing to assay one. At concentration 4.86 X ^{107}, isolates J35 and J36 recorded the highest mortalities with no significant differences (P≤0.01) at 66.3% and 58.1% respectively. At concentration 4.86 X 106, isolate J36 recorded the highest mortality at 59.6% which was significantly different from the rest of the isolate mortalities. Concentration 4.86 X 10^5 showed a similar trend during assay 2 where BBC recorded 43.1% and BVT 42.2% mortalities respectively without significant differences (P≤0.01). A significant difference (P≤0.01) in mortality was recorded between the highest mortality records the rest of the isolates. At concentration 4.86 X 10^4, BVT and BBC recorded the highest mortalities at 29.9% and 23.1% mortalities which were not significantly different(P≤0.01) from each other but significantly different (P≤0.01) from isolates J29, J36, J57, RI and J35 which recorded 9.9%, 9.9%, 9.9%, 9.8% and 8.3% respectively. The lowest mortalities were recorded by isolates J39 (1.7%).

At day 7, during assay one, isolate J35 recorded the overall highest mortality at 86.3%. At concentration 4.86 X 10^7, isolates J35, BBC and J36 recorded the highest non-significant (P≤0.01) mortalities of 86.3%, 74.5% and 76.5 % respectively. These were significantly different (P≤0.01) from isolate J29 which recorded a mortality of 54.9% (Table 3). At the same concentration, J57 recorded the lowest mortality at 11.8%, which were not significantly different from J59 at 15.7%. BVT recorded mortality of 80.4% at concentration 4.86 X 10^6 followed closely by J36 at 74.5 % with no significant differences (P≤0.01) but significantly differed (P≤0.01) from isolate BBC at 62.5%. BVT recorded the highest mortality at 4.86 X 10^5 at 80.4% followed closely and non-significantly (P≤0.01) by BBC at 76.5% and significantly by isolate J29 at 51%. Isolates BVT recorded the highest mortalities of 49% at concentration 4.86 X 10^4 followed by 47.1%. The lowest mortality was recorded by isolate J29 at 2.0% (Table 3).

There was a similar trend in mortalities recorded during assay two as compared to assay one. Isolates J35 and J36 recorded the highest non-significant (P≤0.01) mortalities at 82.8% and 70.8% respectively. These were however significantly different (P≤0.01) from the next highest mortalities recorded by isolates BBC (66.9%) and J29 (52%). The lowest mortalities were observed at isolates J57 and J59 at 11.3% and 15.2% respectively. Isolates BVT and J36 recorded the highest but non-significant mortalities at 74.5% and 70.6% respectively with significant differences (P≤0.01) with isolate BBC at 60.1% mortality at 4.86 X 10^6 concentration. Isolate BVT recorded the highest mortality (72.7%) at concentration4.86 X 10^5 which was significantly different (P≤0.01) from isolate BBC at 69.9% mortality. The lowest mortality was recorded by isolate J39 at 1.8%.

On the 9th day, Isolate BBC recorded the highest mortality on overall during assay one and two at 100% and 94.7% respectively. Isolates BVT, BBC and J36 recorded the highest mortalities at concentration 4.86 X 10^6 at 97.9%, 87.5% and 81.2% respectively without significant differences (P≤0.01). The lowest mortality was recorded by isolate J59 at 39.6% at the same concentration. Isolates BBC and BVT recorded the highest mortalities without significant differences (P≤0.01) at concentration 4.86 X 10^5 at 95.8 % and 89.6% respectively. These were however significantly different (P≤0.01) from isolates J29 and J59 which both recorded mortality of 72.9% The highest mortality was recorded by BVT at 83.3% which was significantly different (P≤0.01) from the rest of the isolates. The lowest mortality was recorded by isolate J39 at 4.2% (Table 4).

The highest mortality was recorded by BBC (94.7%) and lowest by J57 (27.5%) at 4.86 X 10^7 concentration. Isolates BVT, BBC and J36 recorded the highest mortalities at 90.3%, 84.9%

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

	Assay 1				Assay 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	11.5c	24.2b	8.3c	9.9c
J59	14.0d	22.8c	26.3c	3.5d	12.6c	21.5c	23.6c	3.3d
RI	24.6c	21.1c	21.1c	10.5d	23.1b	19.7c	19.8c	9.8c
P-Value			<0.001				<0.001	
L.S.D			15.9				15.1	
CV%			4.3				1.5	

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^7, 10^{-4}= 4.86 X 10^6, 10^{-5}= 4.86 X 10^5, 10^{-6}= 4.86 X 10^4

Table 3. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 5 during season one and two

	Assay 1				Assay 2			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC	76.5a	62.7b	76.5a	47.1b	66.9b	60.1b	69.9b	45.2c
BVT	35.3c	80.4a	80.4a	49.0b	33.9d	74.5a	72.7a	47.1c
J29	54.9b	37.3c	51.0b	15.7d	52.0b	35.8d	46.3c	15.1e
J35	86.3a	17.6d	23.5d	7.8d	82.8a	16.9e	23.2e	7.5e
J36	74.5a	74.5a	33.3c	11.8d	70.8a	70.6a	32.4d	11.3e
J39	33.3c	25.5d	39.2c	2.0e	31.4d	24.3d	36.2d	1.8e
J57	11.8d	35.3c	23.5d	9.8d	11.3e	33.9d	22.6e	9.4e
J59	15.7d	23.5d	45.1c	4.5e	15.2e	22.6e	43.3c	5.6e
RI	35.3c	21.6d	25.5d	13.7d	32.8d	20.7e	25.6e	13.2e
P-Value			<0.001				<0.001	
L.S.D			20.2				18.2	
CV%			3				0.5	

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^7, 10^{-4}= 4.86 X 10^6, 10^{-5}= 4.86 X 10^5, 10^{-6}= 4.86 X 10^4

Table 4. Percentage mortality of adult bruchids ate different concentrations under *Beauveria bassiana* isolates at day 7 during season one and two

	Assay 1				Assay 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	39.6d	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	<0.001				<0.001			
L.S.D	23.5				20.6			
CV%	2.9				1.9			

Treatments with different letters in the same column are significantly different at 1% probability
CFU/ml are as follows; 10^{-3}= 4.86 X 10^{7}, 10^{-4}= 4.86 X 10^{6}, 10^{-5}= 4.86 X 10^{5}, 10^{-6}= 4.86 X 10^{4}

and 75.9 % that were not significantly different from each other. They however were significantly different (P≤0.01) from isolate J29 which recorded at 53.7% mortality. Isolate BBC maintained lead at concentration 4.86 X 10^{5} with 88.3% mortality while isolate J39 recorded 38.4% mortality at the same concentration. BVT recorded a mortality of 76.8% which was significantly different (P≤0.01) from isolate BBC at 64.7 % at concentration 4.86 X 10^{4} (Table 4).

At day 11 in the first assay, the 4.86 X 10^{7}, 4.86 X 10^{6} and 4.86 X 10^{5} concentrations showed 100% cumulative mortality on the isolate BBC Isolate BVT showed 100% cumulative mortality at 4.86 X 10^{7}, 4.86 X 10^{6} concentrations. Isolates J59 recorded the lowest mortality under the 4.86 X 10^{6} concentration which was significantly different (P≤0.01) from the rest of the isolates. In the second assay, isolates BBC, BVT, J29, J35 and J36 showed the highest cumulative mortality at over 80% that were not significantly different (P≤0.01) from each other at the 4.86 X 10^{7} concentration. The highest mortalities at concentrations 4.86 X 10^{5} were recorded by isolates BVT (97.7%), BBC (95.3%) and J35 (82.8%).

At day 13, isolates BBC, BVT, J29, J35, J36 and J39 had more than 90% cumulative mortality of the cowpea bruchids at concentration 4.86 X 10^{7} in assay one. These mortalities were significantly different (P≤0.01) from isolates RI, J57 and J59, which recorded 80.9%, 73.8% and 71.4% respectively. Isolate J29 recorded the lowest cumulative mortality at 85.7%, which were significantly different (P≤0.01) from the rest of the isolates at concentration 4.86 X 10^{6}. Isolate J36 and J39 recorded the lowest cumulative mortalities at 73.8% and 80.9% respectively at concentration 4.86 X 10^{5} which were significantly different (P≤0.01) from the rest of the isolates. At concentration 4.86 X $10^{4,}$ the highest mortality was recorded by isolate BBC at 100% and the lowest by J59 at 19.1% (Table 5).

A similar trend was observed during assay two. Mortalities at concentration 4.86 X 10^{6} were higher than 80% with non-significant differences (P≤0.01). The lowest mortalities were recorded by isolates J36 (75.6%) and J39 (70%) which were significantly different (P≤0.01) from the rest of the mortality readings at the same concentration. There were significant differences between isolate mortalities at concentration 4.86 X 10^{4} with BBC recording 98% mortality and J59 18.6% as the lowest (Table 6).

At day 15, all the bruchids had died at 4.86 X 10^{7}, 4.86 X 10^{6} and 4.86 X 10^{5} concentrations during assay 1.). There were significant differences (P≤0.01) between the isolates at concentration 4.86 X 10^{4} with BBC recording the highest at 100% and J59 the lowest at 23% (Table 7). During the second assay, the isolates did not differ significantly (P≤0.01) at 4.86 X 10^{7} and 4.86 X $10^{6,}$ 4.86 X 10^{5} concentrations where they recorded the highest cumulative mortality (99%). Concentration 4.86 X 10^{4} recorded significant differences (P≤0.01) in mortality where BBC recorded the highest mortality at 99% while J59 the lowest at 23%.

There was significant variation in virulence among the isolates of *B. bassiana* in control of *C. maculatus* confirming the findings by Cherry et al. (2005) that the use of *B bassiana* significantly reduced *C. maculatus* adults population. Across all the days the highest mortality was observed under concentration 4.86 X 10^6 for BBC and BVT. Concentration 4.86 X 10^7 was most effective giving the highest mortality for the isolates J29, J35, J36, J39, J57, J59 and RI. The *B bassiana* isolates at different time intervals within the fifteen days under observation indicated that the mortality was highest between days 5 and 7.

The results demonstrate that all the tested *B. bassiana* isolates were infective to *C. maculatus* adults especially at high concentrations especially 4.86 X 10^6 and 4.86 X 10^7 where 100% mortality was recorded on all isolates in the mortality by immersion experiment by day 15. This further supports the findings by Cherry et al. [8] 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.

Mohamed and Maher [9] explains that faster hyphal growth was shown in all highly virulent isolates than low virulent ones with the speed of hyphal growth being one of the factors affecting the variation in virulence among tested isolates.

Table 5. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 9 during season one and two

	Assay 1				Assay 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	56.6c	41.4c
J39	75.6b	64.4b	62.2b	17.8d	72.3b	61.0b	61.0b	17.4d
J57	62.2b	68.9b	80.0a	37.8c	56.0b	67.5b	78.4b	35.6c
J59	64.4b	48.9c	82.2a	15.6d	63.2b	47.9c	80.6a	15.2d
RI	64.4b	75.6b	73.3b	24.4d	63.2b	74.0b	71.9b	24.0d
P-Value	0.006				<0.001			
L.S.D	21.1				19.9			
CV%	18.5				0.3			

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^7, 10^{-4}= 4.86 X 10^6, 10^{-5}= 4.86 X 10^5, 10^{-6}= 4.86 X 10^4 probability

Table 6. Percentage mortality of adult bruchids under *Beauveria bassiana* isolates at different concentrations at day 11 during season one and two

	Assay 1				Assay 2			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC	100.0a	100.0a	100.0a	100.0a	98.0a	98.0a	98.0a	98.0a
BVT	100.0a	100.0a	100.0a	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	<0.001				<0.001			
L.S.D	13.9				13.9			
CV%	2.3				2.4			

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^7, 10^{-4}= 4.86 X 10^6, 10^{-5}= 4.86 X 10^5, 10^{-6}= 4.86 X 10^4

Table 7. Percentage mortality of bruchids under *Beauveria bassiana* isolates at different concentrations at day 13 during season one and two

	Assay 1				Assay 2			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC	100.0a	100.0a	100.0a	100.0a	99.0a	99.0a	99.0a	99.0a
BVT	100.0a	100.0a	100.0a	94.8a	99.0a	99.0a	99.0a	94.0a
J29	100.0a	100.0a	100.0a	33.3b	99.0a	99.0a	99.0a	38.0c
J35	100.0a	100.0a	100.0a	41.0b	99.0a	99.0a	99.0a	39.0c
J36	100.0a	100.0a	100.0a	48.7b	99.0a	99.0a	99.0a	48.0b
J39	100.0a	100.0a	100.0a	25.6c	99.0a	99.0a	99.0a	30.0c
J57	100.0a	100.0a	100.0a	46.1b	99.0a	99.0a	99.0a	53.0b
J59	100.0a	100.0a	100.0a	23.0c	99.0a	99.0a	99.0a	23.0d
RI	100.0a	100.0a	100.0a	35.9b	99.0a	99.0a	99.0a	36.0c
P-Value	<0.001				<0.001			
L.S.D	9.1				7.7			
CV%	1.2				0.7			

Treatments with different letters in the same column are significantly different at 1% probability, CFU/ml are as follows; 10^{-3}= 4.86 X 10^{7}, 10^{-4}= 4.86 X 10^{6}, 10^{-5}= 4.86 X 10^{5}, 10^{-6}= 4.86 X 10^{4}

He further explains that faster colonization of the infected insects could be as a result of faster hyphal growth thereby leading to increased virulence. Lower concentrations gave lower mortalities as observed across the experiments at concentration 4.86 X 10^{4}. Wraight et al. [10] and Ansari et al. [11] found out that insect susceptibility to fungal infection is dose dependent thereby depending on the concentration of conidial suspension. On the same thought, Lui et al. [12] 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.

4. CONCLUSION

The current study further proves the pathogenic effectiveness of *B bassiana* on *C. maculatus* albeit variations in virulence recorded with the various isolates at varied concentrations, doses and formulations. Overall, the study is a representation of an important initial step in developing a biopesticide based on *B. bassiana* for use against *C. maculatus* in cowpea.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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