EVALUATION OF GROWTH CHARACTERISTICS AND EFFICACY OF *Beauveria bassiana* ISOLATES AGAINST COWPEA BRUCHID (*Callosobruchus maculatus*) UNDER *IN-VITRO* CONDITIONS

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DECLARATION

This thesis is my original work and it has not been presented for an award of a degree in any other university.

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To my beloved son Joe for giving me the first chance to be called mother and my beautiful daughter Joelle, for being a mirror of my life.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BVT®	Beauvitech
CFU	Colony Forming Unit
KEPHIS	Kenya Plant Health Inspectorate Service
PDA	Potato Dextrose Agar
SDAY	Sabourdad Dextrose Agar amended with 1% wt./v yeast extract

GENERAL ABSTRACT

The cowpea bruchid, *Callosobruchus maculatus* is a major pest of stored cowpea (*Vigna unguiculata*) in Kenya that has been reported to cause damage reaching 100% of stored cowpea grain. The cowpea bruchid is also known to decrease germination potential and reduce the commercial, nutritional and aesthetic value of the grain due to physical contamination by insects, eggs and excrement. There are several control methods for the cowpea bruchid including the use of the safe entomopathogenic fungi, *Beauveria bassiana*. Experiments were conducted at the KEPHIS laboratory to evaluate the morphological, growth characteristics of nine *B. bassiana* isolates. Evaluation of the spore concentration, viability, relative hyphal growth, rate of sporulation, and rate of conidial growth as well as virulence to the cowpea bruchid, *C. maculatus* was conducted for each isolate. The white to cream white colonies were characterized to be those of *B. bassiana* with conidia appearing round with single or branched conidiophores. The highest spore count and viability was recorded for isolate J59 and J57, respectively. The highest rate of sporulation was recorded for isolate BVT® while isolate BBC® recorded the highest rate of conidial growth. There were positive and negative correlation between the tested growth characteristic variables. Mortality of the cowpea bruchids was evaluated through mortality by immersion, effective dose, optimal dose and farmer storage simulation tests. Mortality rates increased by increasing concentration of *B. bassiana* conidia. The isolates J35, BBC®, J29, J39 and J59 recorded higher percentage mortality of *C. maculatus* at 4.86×10^7 cfu/ml concentration while J36, BVT® , J57, RI performed best at 4.86×10^6 cfu/ml concentration. The half and double rates recorded highest and lowest mortality of *C. maculatus* respectively for effective dose test. Isolate

BVT® and J59 recorded the highest grain damage followed by control in assay one and two, respectively, while isolate J29 recorded the lowest grain damage in both assays when the isolates were used in suspension form. Isolate J57 recorded the highest grain damage followed by control in both assays, while isolate J59 recorded the lowest grain damage in both assays when the isolates were used in powder form. There were positive and negative correlations recorded between the growth characteristics and mortality of the cowpea bruchids. These findings provide preliminary pathogenicity status of *B. bassiana* isolates against *C. maculatus* in cowpea grain during storage and may in future be upscaled to field trials in view of commercial product development.

CHAPTER ONE

INTRODUCTION

1.1. Background

Post-harvest losses threaten food, nutrition and income security in many households. According to the World Bank, 2011 report, huge volumes of food estimated at USD 4 billion are lost during post-harvest. In Africa, these losses have been estimated to range between 20% and 40%, and sometimes reach 80%, a value highly significant considering the low agricultural productivity in several regions of Africa (Abbas *et al.,* 2014).

Cowpea (*Vigna unguiculata*) is one of the most versatile annual food legumes in the tropical and subtropical regions of the world (Jackai and Adalla, 1997). Cowpea plant has many uses ranging from human food, fodder for livestock and improving soil fertility among other uses. Cowpea is drought tolerant, well adapted to the arid and semi-arid areas and grows best in clay, sandy or loamy soils (CPC, 2018). Cowpea production and marketing in Sub Saharan Africa is affected adversely by non-availability of market preferred varieties, pest and disease problems, low yield, lack of improve tools, high cost of farmland preparation, high cost and lack of labour, high cost of pesticides, poor marketing and pricing channels. However, the major problem is the menace by insect pests (Sabo *et al.,* 2013). The cowpea bruchid (*Callosobruchus maculatus*) is a major storage pest of leguminous crops especially cowpea causing both quantitative and qualitative damages in the tropics (Abd-El-Aziz, 2011). Qualitative damage results in product alteration and lowering of grain market value, which leads to loss of nutritional and aesthetic value, loss of seed viability (Oluwafemi, 2012). The presence of webbing

and dead insects in the product renders it difficult to consume infested grains (Radha, 2014). The cowpea bruchid infests the pods in the fields and seeds in storage and multiplies very fast in storage causing a damage of up to 60%. *Callosobruchus maculatus* is mainly controlled using pirimiphos and permethrin (Swella and Mushobozy, 2007). However, these chemicals pose health hazards such as toxicity to users, environmental pollution, are expensive, leave residues on grain and increases resistance of pests (Boyer *et al.,* 2012). Strict regulation in the judicious use of chemical pesticides for control of storage pests due to continued food contamination and insecticide resistance has left farmers with very few options. Among the identified safe options are entomopathogenic fungi, which are able to infect through the hosts integument by contact and direct penetration (Potrich *et al.,* 2006). *Beauveria bassiana* (Bals- Criv) Vuill. is a naturally occurring, widely distributed, entomopathogenic fungus with a very wide host range, causing white muscardine disease of many insect species. It is the most extensively studied entomopathogenic fungus and the most widely used and commercially available biological control agent against important pests in agriculture and forestry (Freed *et al.,* 2011b).

1.2. Problem statement

Cowpea has a relatively low production ranging from 50 to 350 kg/ha in traditional cropping systems (Takim and Uddin, 2010). In tropical countries, efficient storage of grains is mainly constrained by insect and vertebrate pests with insect pests accounting for 20-50% of post-harvest losses (Anankware *et al.,*, 2013; Akinkurolere *et al.,* 2006). In Kenya, there has been a significant reduction in cowpea yield potential which has reduced from 1500 kg/ha to 239 kg/ha due to a number of biotic and abiotic factors

including low soil fertility, insufficient farm inputs, weeds, drought, parasitic weeds, pests and diseases, low plant population and mixed cropping (Kimiti *et al.,* 2009). Cowpeas are more suitable for *C. maculatus* oviposition than other pulses causing much damage with substantial grain losses (Radha, 2014). Umeozor (2005) reported that bruchid damage can reach 100% of stored cowpea seeds with quantitative weight losses of up to 60%. Maina (2011) reported that cowpea infestation can lead to upto 100% losses after three to six months of storage.

The cowpea bruchid has been controlled using chemical, cultural, physical, biological, varietal and genetic control among other methods (Lacey and Solter, 2012). In Kenya, no chemical has been registered exclusively to control *C. maculatus* in cowpea during storage making farmers rely on physical and cultural methods and general pest control chemicals. A number of food safety challenges have also been recorded following the use of chemical pest control methods, especially during storage, leading to efforts in search of safer pest control methods. Despite this, there is limited information on the use of biopesticides for control of cowpea bruchids in tropical regions. There is limited literature on the efficacy of *B. bassiana* as a biological control agent for cowpea bruchid and since it takes long before the impact of control is felt, biological control products are given least attention. There is scanty information demonstrating the efficacy of *B. bassiana* for control of *C. maculatus* in stored cowpea grain in Kenya. Tests to determine efficacy of local *B. bassiana* isolates against *C. maculatus* in stored cowpea grain in Kenya have not been carried out.

1.3. Justification

Chemical, cultural, physical, biological, varietal and genetic control are among the many methods employed worldwide in the management of the cowpea bruchid, *C. maculatus*, which has caused high losses in cowpea grain. *Callosobruchus maculatus* has been effectively controlled using chemical insecticides and fumigants, such as pirimiphos methyl, permethrin, carbon disulphide and phosphine to protect bulk-stored grain. There are challenges of high persistence, genetic resistance, poor knowledge of application, high cost and health hazards of chemical insecticides necessitating the search for safer solutions to the environment and humans. The use of entomopathogenic fungi to control storage insect pests has been employed worldwide. Entomopathogenic fungi are natural, easy to formulate, effective, environmentally safe with no residual activity, with low resistance development and do not cause damage to the grains. Some products based on entomopathogenic fungi are already commercially available and can be applied directly to stored grain and food commodities as they are regarded as generally safe. Even though *B. bassiana* has been used to control various pests, there is limited information on the use of this fungus against the cowpea bruchid. The use of entomopathogenic fungi including *B. bassiana* present an alternative to synthetic pesticides for control of stored product pests. Furthermore, use of Kenyan local isolates among the test isolates would provide an important position on the possibility of employing locally adapted *B. bassiana* in the control of *C. maculatus* in storage for grain products.

The findings of the current study will generate knowledge on the potential of using *B. bassiana* as a safe option that can be part of integrated management of the cowpea bruchid. Based on these considerations, this study assessed nine *B. bassiana* isolates for

their comparative growth characteristics and their effectiveness in controlling the cowpea storage pest, *C. maculatus* under laboratory conditions.

1.4. Objectives

1.4.1. General objective

To reduce post-harvest losses in cowpea grain using a safe approach in the management of *Callosobruchus maculatus.*

1.4.2. Specific objectives

i. To evaluate the growth characteristics of selected *Beauveria bassiana* isolates under laboratory conditions;

ii. To determine the efficacy of selected *B. bassiana* isolates against *C. maculatus* in cowpea grains under storage conditions.

1.5. Hypotheses

i. There are no variations in the growth characteristics of selected *B. bassiana* isolates under laboratory conditions

ii. *B. bassiana* isolates are not effective in the management of *C. maculatus* that infests cowpea grain in storage

CHAPTER TWO

LITERATURE REVIEW

2.1. Cowpea plant (*Vigna unguiculata*)

Cowpea, (*Vigna unguiculata*) is an annual food legume in the tropical and subtropical regions of the world, belonging to the family Fabaceae (Jackai and Adalla, 1997). It is an important grain legume, grown worldwide on an estimated area of 12.5 million hectares annually with an annual production of more than 3 million metric tons (Egho, 2011; Takim and Uddin, 2010). Cowpea is an important part of traditional cropping systems in Kenya (Kimiti *et al.,* 2009).

2.2. Economic importance of cowpea

Cowpea is a multipurpose crop where the green pods, leaves and dry grains are consumed (Hallensleben *et al*., 2009). It provides cost effective nutrition to the diet with high plant protein and digestible carbohydrates for many African people (Okosun and Adedire, 2010). Cowpea grain contains approximately 24 to 30% protein and several minerals and vitamins (Owolabi *et al.,* 2012).

Cowpea generates income for farmers and traders (Oyerinde *et al*., 2013). It is a preferred crop to fight nutritional and food security challenges as it flourishes under water stressed conditions in the arid and semi-arid areas (Hallensleben *et al*., 2009). Cowpea has deep root systems that help in improving soil structure, and provide a canopy cover that saves moisture and prevents soil erosion (Timko and Singh, 2008). Cowpea is an efficient nitrogen-fixing crop, which is used during crop rotation regimes and as a green manure crop to improve soil fertility for subsequent crops (Ojiem *et al.,* 2007). Cowpea leaves

and stems (stover) can be dried and stored as high quality hay for stock feed. Leaves can also be dried and preserved for human consumption.

2.3. Cowpea production in Kenya

Cowpea is considered an important part of traditional cropping systems in Kenya with a total hectarage of 24,431 ha and a production of 65,096 MT (AFFA, 2014). Eastern province accounts for 85% of this area with the Coast, Western and Central provinces combined taking 15% (Kimiti *et al.,* 2009). In Kenya, cowpea is either grown alone or intercropped with cereals (maize, sorghum), pulses (beans, pigeon peas), leafy vegetables, and roots and tubers (cassava and sweet potatoes) and has a short growing season (Hallensleben *et al*., 2009). In traditional cropping systems, cowpea production ranges from 50 to 350 kg/ha (Takim and Uddin, 2010). A number of biotic factors (such as insect pests, nematodes, diseases and parasitic weeds) and abiotic factors (such as drought, high temperature, flooding, low soil fertility, aluminum toxicity and low pH) among others affect the yield potential of cowpea in Kenya (Kimiti *et al.,* 2009).

2.4. Cowpea production constraints in Kenya

Cowpea production in Kenya is at subsistence level and the crop faces constraints in production. These constraints include poor soil, insect pests, diseases and drought. A wide range of pests and diseases attack the cowpea crop in the field and during storage causing both quantitative and qualitative losses (Makoi *et al.,* 2010). Every stage of cowpea growth is attacked by pests sometimes in high densities that often lead to total grain loss if no interventions are taken (Asiwe *et al.,* 2005). In the field, damping off (*Pythium aphanidermatum*), anthracnose of bean (*Colletotrichum lindemuthianum)*, basal

rot (*Fusarium oxysporum)*, bean blight (*Xanthomonas axonopodis pv. phaseoli*) and soybean bacterial pustule (*Xanthomonas axonopodis* pv. *glycines*) are among the bacterial diseases affecting cowpea. Viral diseases affecting cowpea in Kenya include blackeye cowpea virus (BLCMV), cowpea mild mottle virus (CPMMV), Alfalfa mosaic virus (AMV), Peanut mottle virus (PeMoV) while nematodes include root knot nematode (*Meloidogyne* spp.), dagger nematode (*Xiphinema* spp.) and root lesion nematode (*Pratylenchus* spp*)* (CPC, 2017). The foliage beetle, *Ootheca mutabilis* Sahl, the cowpea aphid, *Aphis craccivora* Koch, the flower bud thrips, *Megalurothrips sjostedti* Tryomb, the legume pod borer, *Maruca vitrata* Fab and a host of pod sucking bugs are known to affect the cowpea plant in the field from seedling to harvest (Egho, 2010). The Bruchidae family hosts the most storage insect pests with *Callosobruchus maculatus, C. chinensis* and *C. analis* causing the most damage in stored cowpea (Bressani, 1985).

2.5. *Callosobruchus maculatus* biology

Callosobruchus maculatus is a major field-to-store post-harvest pest of economic importance in legumes, especially the cowpea in the tropics and worldwide (Rahman and Taluker, 2006). It is cosmopolitan and polyphagous affecting cowpea, lentils, green grams and black gram (Park *et al.*, 2003). Eggs are laid on the pods when the cowpea is still in the field, at maturity, harvest time or in the store where infestation rate can be low or sometimes undetectable. The egg and adult stages are found on the grain while the larval and pupal stages are found inside the grain. The eggs are small, translucent grey and inconspicuous. Eggs are domed structures with oval, flat bases. Female cowpea bruchids lay individual eggs onto the seed testa, which hatch within 5-6 days of oviposition (Credland, 1987).

Larvae emerge after about 5 days and penetrate through the seedcoat by chewing into the seed to complete their development. At about 27℃, adult eclosion occurs within the seed. The adult cowpea bruchid emerges within 25-30 days after oviposition and matures after 24-36 hours. An emerging female quickly finds a suitable mate and oviposits within an hour giving rise to about 100 offspring under favorable nutritional conditions. Adult bruchids are small (2 to 5.4 mm long), orange brown with dark markings with a triangular shape. They require neither food nor water to reproduce but may feed on pollen and nectar on flowers. Morphological differences can be used to distinguish the adult sexes with an unaided eye. Females possess dark stripes on each side of the posterior dorsal, which males lack. Adults die 10-12 days after emergence (Credland, 1987).

2.6. Losses due to cowpea bruchid

Adult bruchids lay eggs on pods in the field or seeds in storage. The larvae bore into and feed within seeds creating galleries. They cause weight loss, decreased germination potential and reduction in commercial, nutritional and aesthetic value due to physical contamination of grain by insects, eggs and excrement (Oluwafemi, 2012).

Caswell (1981) reported approximately 50% loss of cowpeas in storage due to cowpea bruchid in 3 to 4 months. Redden and McGuire (1983) reported up to 100% infestation of the seed in 3 to 5 months under storage conditions, while Singh *et al.* (1983) reported that the number of exit holes are directly proportional to weight loss, which translates to yield losses. The cowpea bruchid can attain three to four generations even in low initial infestations each taking about a month to cause severe losses approximating 60% grain weight loss (Kaita *et al.*, 2000). During storage, re-infestation occurs where 100%

infestation can occur after three to six months' storage leading to about 60% loss of weight (Maina, 2011). Losses of between 20 to 50% have been recorded on stored cowpea as a result of attack by cowpea bruchid. Sometimes, the loss can reach 100% making it necessary to control this pest on stored grains (Udo and Harry, 2013).

2.7. Management of cowpea bruchid

There are numerous control methods for managing cowpea bruchids. They include chemical, cultural, physical, biological and genetic control (Lacey and Solter, 2012). *Callosobruchus maculatus* has been effectively controlled using residual chemical insecticides and fumigants, such as pirimiphos methyl, permethrin, carbon disulphide and phosphine (Swella and Mushobozy, 2007). There are, however, challenges of high persistence, genetic resistance, poor knowledge of application, high cost and health hazards necessitating the search for safer solutions to the environment and humans (Akinkurolere *et al.*, 2006).

In many African countries, breeding programmes targeting the reduction of postharvest losses due to bruchid infestation have been limited (Keneni *et al.*, 2011). Cultural techniques that have been employed include timely and frequent harvesting, good crop and store hygiene, planting legumes away from granaries, storage of legumes within pods, use of light and sound, use of ashes, sand and plant leaves, ozonation, and intercropping maize with cowpeas (Lacey and Solter, 2012).

The use of irradiation, controlled atmospheres of carbon dioxide, vacuum heating, cooling and sterilization have been demonstrated to control bruchids but the need for specialized equipment restricts their use (Mbata *et al.*, 1996). Solarization through sun drying and heating have been reported to control the cowpea bruchid under different

regimes but its effectiveness is dependent on the thinness of the layer of the grains when spread under the sun and duration of exposure (Kitch *et al.,* 1992). Semiochemicals and repellants have also been effectively employed against cowpea bruchids (Abd-El-Aziz, 2011). The use of entomopathogenic fungi as biopesticides is greatly encouraged as a safe strategy to control insect pests (Panda *et al.,* 2014).

2.8. Entomopathogenic fungi as alternatives to control cowpea pests

Entomopathogenic fungi are useful in regulation of insect populations and have been known to cause mortality in various insect pests (Freed *et al.,* 2012). They 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 (Haas-Costa *et al.,* 2011). 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 (Freed *et al.,* 2012). The genera *Lecanicillium, Beauveria, Isaria, Metarhizium* and *Hirsutella* are best known as biological control agents of insect pests (Grent, 2011). Some products are already commercially available and can be applied directly to stored grain and food commodities as they are regarded as generally safe (Lacey *et al.,* 2008). Their uniqueness in control is as a result of them not being limited to controlling sucking and biting insects as they infect through the hosts` surface by contact, thereafter penetrating the cuticle (Potrich *et al*., 2006). The insect mouthparts, at inter-segmented folds and spiracles are suitable sites of invasion since there is high humidity preferred for germination of spores and conidia (Clarkson and Charnely, 1996). Vilas Boas *et al.* (1996) and Cherry *et al*. (2005) tested

the entomopathogenic fungi *B. bassiana* and *Metarhizium anisopliae* against *C. maculatus* adults and recorded significant reduction in the pest population.

Success in control of insect species in stored products has been recorded for both laboratory and field tests (Sabbour and Abd El-Aziz, 2010). 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 (Steenberg, 2005). Recently, research has focused on the use of *B. bassiana* isolates in biological control of many important insect pests (Akello *et al.,* 2009).

2.9. Use of *Beauveria bassiana* as a biocontrol agent

Beauveria bassiana is the most studied biological control agent that is commercially available for use in agriculture (Khan *et al.,* 2012). *B. bassiana* colonies are white in colour and have aerial mycelium. Conidia are globose to oval shape, which are usually larger than 3.5 cm in diameter (1.5-5.5. X 1-3 mm). Conidiophores are single or branched, oblong, cylindrical, or flask shaped bearing laterally or at extremity, vesicles giving rise to porogeneous cells (phialides). Phialides generally are globose, sometimes cylindrical, flash-like and curved or straight. It is a naturally occurring entomopathogenic fungus that can easily be isolated from host insects, mites, soil and vegetation (Freed *et al.,* 2011b). It causes white muscardine disease and has a very wide host range worldwide (Tanada and Kaya, 1993).

Beauveria bassiana conidia germinate when in contact with the host external integument to produce a germ tube, which penetrates the host into the hollow body cavity. In the

haemocoel, the tissues are colonized forming blastophores, which are yeast like hyphal bodies. All cells are damaged causing the host`s death soon after the hyphae emerge from the cadaver. Under appropriate conditions of humidity and temperature, they produce conidia on the host's exterior whose infective spores are transmitted to nearby larvae either by wind or water (Lacey and Solter, 2012).

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 (Darbro *et al.,* 2011) across the world. *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* (Sabbour and SingerWraigt, 2014; Abdel-Raheem and Zakai, 2013). Vilas Boas *et al.* (1996) and Cherry *et al*. (2005) tested *B. bassiana* and *Metarhizium anisopliae* against *C. maculatus* adults and recorded significant reduction in population. A considerable amount of literature, patents and techniques developed and applied by commercial entities for control of insect pests and *B. bassiana* products are available (Lacey and Solter, 2012).

2.10. Identification and growth characteristics of *Beauveria bassiana*

Many entomopathogenic fungi are facultative pathogens, which are easily grown in pure culture. Identification of entomopathogenic Hypocreales relies fully on observation of conidia and conidiogenous cells. Direct counts of inoculum for quantification for bioassays or field applications are among the main methods applied. A haematocytometer quantifies numbers of propagules per unit volume or weight. Turbidimetric methods which rely on transmittance of light through the propagule suspension have also been

used to provide an estimate of propagule populations. Haematocytometer and turbidimetric methods however do not provide details on spore viability. Viability of conidia can be measured through plating techniques, which take approximately 3-7 days before data collection. Germination aspects can be assessed within 24 hours where the time required to germinate a percentage of the propagules is measured. Spore viability provide details on spore concentrations to support accurate dose determination in view of bioassays and field tests. Propagules are considered viable if germ tube lengths are more than two times the propagule diameter. Viable propagules per unit volume can be calculated through multiplication of the total counts estimated with the haematocytometer multiplied by the germination percentage (Lacey and Solter, 2012).

Conidial viability has been relied upon by manufacturers of entomopathogenic fungi based biopesticides to determine the quality of the products whether formulated or non-formulated (Faria and Wraight, 2007). Substrates mainly from agricultural products or porous inorganic carriers have been employed successfully. When hydrated and sterilized, they readily absorb nutrients from liquid medium resulting in healthy fungal growths thereby allowing mass production. Grains like rice, barley, rye, wheat, groats bulgur are commonly used as substrate as they are cheap and readily available. Rice is however the most suitable for *B. bassiana* mass culture as it produces high quantities of conidia making it economically viable during mass production (Sharma *et al.,* 2002).

Virulence, which is the ability of entomopathogenic fungus to cause death of organisms, is of great importance as it determines the success of the EPF as biological control agents (Asghar, 2013). It is also important to identify strains active at low doses to guarantee control success of microbial agents (St. Leger and Wang, 2010).

CHAPTER TWO

EVALUATION OF GROWTH CHARACTERISTICS OF *BEAUVERIA BASSIANA* ISOLATES UNDER LABORATORY CONDITIONS

Abstract

An evaluation of growth characteristics of nine *Beauveria bassiana* isolates was conducted under laboratory conditions. The growth characteristics were evaluated to provide information on growth characteristics of various *B. bassiana* isolates. The isolates were plated on Potato Dextrose Agar (PDA) for colony observation and slides prepared for microscopic examination. Growth characteristics were evaluated by assessing the spore concentration, viability, relative hyphal growth, rate of sporulation and rate of conidial germination of the isolates. The isolates were characterized as *B. bassiana* as the colony colors varied between white and cream white and had flat and cottony consistence with aerial mycelium. The conidia were rounded bearing hyphae and conidiophores. The growth characteristics varied significantly. The highest spore count and viability was recorded for isolate J59 and J57 respectively. All isolates recorded a viability of above 78%. Isolate RI registered the highest relative hyphal growth at day 2, 4 and 6 thereafter it was overtaken by isolate J57 at 8^{th} and 10^{th} day of observation. Isolate BVT® recorded the highest rate of sporulation while isolate BBC® recorded the highest rate of conidial germination for the hours of observation. There were positive and negative correlation between the tested growth variables. The rate of conidial germination was positively correlated to the rate of sporulation and spore concentration and negatively correlated to the viability (r=-0.183) and relative hyphal growth (r=-0.779). Determination of growth characteristics and their correlation is important when screening

for the efficacy of the isolates in view of employing them as biological control agents. Identified effective isolates can be formulated and made available to farmers for use in controlling the pests of concern.

3.1. Introduction

Beauveria bassiana has been widely studied and is commercially available for use as a biological control agent in agriculture (Khan *et al.*, 2012). *B. bassiana* colonies vary between white and cream white in colour and have aerial mycelium. Conidia are globose, and are usually larger than 3.5 µm in diameter to oval shape (1.5-5.5. X 1-3µm). Conidiophores are single or branched, oblong, cylindrical, or flask shaped bearing laterally or at extremity, vesicles giving rise to porogeneous cells (phialides). Phialides generally are globose, sometimes cylindrical, flash like and curved or straight.

Beauveria bassiana is a naturally occurring entomopathogenic fungus (EPF) that can easily be isolated from insects, mites, soil and vegetation (Freed *et al.*, 2011b). Photographic documentation of microscopic images is now universally accepted as a preferred method of illustration as compared to free hand drawings (Lacey and Solter, 2012). The current study was carried out to determine the growth characteristics of the selected *B. bassiana* isolates under laboratory conditions. Growth characteristics like viability, hyphal growth and sporulation rates are important in determining the virulence of fungal organisms to their hosts. The findings of the study would be used to support viability of employing successfully virulent isolates as biological control agents for cowpea bruchid during storage.

3.2. Materials and methods

3.2.1. Fungal isolates

Beauveria bassiana isolates J29, J35, J36, J39, J57, J59 and RI were sourced as pure plated cultures from biopesticide production companies in Kenya, namely Dudutech Limited and Real IPM Limited. Two products BBC® ® and Beauvitech® (BVT®) were obtained from Genetics Technologies International Limited and Dudutech Limited companies in Kenya, respectively.

3.2.2. Development and maintenance of stock culture of *Beauveria bassiana*

3.2.2.1. Inoculum preparation

Development and maintenance of stock culture was conducted following a modification of the Lacey and Solter (2012) procedure. *B. bassiana* was plated on Sabourdaud Dextrose Agar amended with 1% wt./v yeast extract (SDAY) on 9 cm Petri dishes and incubated for 10 to 15 days until sporulation. BBC® and BVT® products were sub-cultured on SDAY until pure *B. bassiana* cultures were obtained. The nine pure isolates were then cultured as monoculture for 10-15 days until sporulation (Lacey and Solter, 2012).

3.2.2.2. Spore harvesting and drying

Spore harvesting and drying was conducted following a modification of the Lacey and Solter (2012) procedure. The spores were scraped from one agar plate surface using a sterile blade into 100ml sterile distilled water in a flask and then incubated for 72 - 96 hrs on a 120 to 150 r.p.m rotary shaker at 24 to 28℃. One hundred grams of rice was put in

25 cm by 30 cm autoclave bags that were closed using a thermal impulse bag sealer. The rice was autoclaved at 121℃ for 21 min and allowed to cool. Under sterile conditions, an opening was created at a corner of the bag using a sterile blade thereafter 10 ml/100 g of the inoculum was introduced into the bags using a sterile syringe. The bags were then held at the opening and manually massaged to evenly mix and disperse the inoculum through the rice substrate. The openings were then secured with sterile cotton wool balls to allow air circulation and placed for incubation by laying them on their sides, flattened to 3 to 4 cm thick beds. The solid substrate bags were incubated at room temperature in the dark for 15 days. Checks for contamination were conducted every day to ensure production of clean cultures. These were identified through observation of wet spots or poor mycelial growth in the substrate. Any contaminated bags were carefully removed, autoclaved and disposed-off immediately upon detection. After 5 days of incubation, the bags were hand-mixed further to separate the rice particles well enough to maximize surface area for good growth and conidiation. Growth was allowed for 15 days before harvesting of the conidia impregnated rice. The conidial substrate was transferred to sterile plastic plates, crumbled gently and aseptically by hand and allowed to air dry for 5 days in a dehumidified room. The substrate was aseptically turned daily so that cultures dried evenly. The substrate was then blended to provide a powder that would be used as stock for the experiments. This was stored in air tight and water impermeable containers under room temperature.

3.3. Identification of *Beauveria bassiana* isolates

Beauveria bassiana fungus was cultured on SDAY media for growth optimization in 9 cm Petri dishes and incubated at 25 ± 2°C in complete darkness for 10-15 days. Isolates were then macroscopically and microscopically identified based on their morphological characteristics on SDAY. Spore shape and colony morphology of the fungal strains were used in the identification with a light microscope according to the key described by Humber (1997). Three replicate plates of each isolate were used and arranged in a completely randomized design.

3.3.1. Evaluation of quality characteristics of *Beauveria bassiana* isolates

3.3.1.1. Spore concentrations

Beauveria bassiana isolates were maintained for 14 days until sporulation on SDAY media. The number of conidia per unit weight of each product was determined by suspending 0.1 g samples taken at random in 10 ml sterile water containing 0.05% sterile Tween 20 in clear glass vials with lids. This was vortexed for 30 seconds to produce a homogeneous suspension. The number of conidia or spore concentrations for each isolate was determined using a haematocytometer at 10^2 dilution to determine spore concentration. Each vial served as replicate with 3 vials for each isolate.

3.3.1.2. Viability test

The viability of the conidia was determined by adding 200 µl aliquot of each conidial suspension to 20 ml of water agar in 9 cm Petri dishes. A sterile microscope cover slip was placed on each plate and the plates incubated in complete darkness at 25 ± 2°C for 20 hours. Percentage germination was determined by assessing the number of germ tubes

formed among 100 randomly selected conidia on the surface area covered by each cover slip under the light microscope (400X). Germination was considered to have occurred when the germ tube was twice the diameter of the conidium. The treatments were arranged in a completely randomized design with three replications. To calculate viability of the cells in propagules per ml, the total haematocytometer counts obtained from the spore concentration experiment above were multiplied by germination percentage.

3.3.1.3. Virulence tests

Relative hyphal growth

The conidial suspension (0.2ml) of each isolate was inoculated on SDAY plate and incubated at 22°C for 48 hours. Mycelium discs of 6mm diameter were removed using a sterile cork borer and placed in the center of freshly prepared SDAY plates. The diameter of the growing colony (radial growth exceeding 6mm diameter of the discs) was measured every 2 days for 10 days on a premarked line with a clear ruler. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

Rate of sporulation

The conidial suspension (0.2 ml) of each isolate was inoculated on water agar and incubated at 22°C for 14 days. Using a sterile cork borer, 5 discs (4 mm diameter) were randomly removed from the culture and placed in 10ml sterile distilled water with 0.01 Tween 80 in test tubes. The discs were agitated for 3 hours in a rotary shaker to suspend the conidia. Conidial concentrations in the aliquots of 0.1 ml of 10-fold serial dilutions of the aqueous suspensions were determined using a haematocytometer. The mean conidial

counts per volume of conidial suspension was calculated for each isolate. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

Rate of conidial germination

The conidial suspension (0.2 ml) of each isolate was inoculated on SDAY broth and incubated at 22°C for 48 hours. Germination was assessed thereafter bi-hourly for 10 hours. Germination was considered to have occurred when the germ tube was twice the diameter of the conidium. The treatments were arranged in a completely randomized design replicated three times with each plate acting as a replicate.

3.4. Data Analysis

Analysis of variance (ANOVA) was carried out on the quantitative data using SAS Version 9.1 statistical software and tested for significance at 99% level of confidence to determine the means of the *B. bassiana* isolates` growth characteristics. The treatment means were then separated using the Fishers Protected LSD to determine the differences between *B. bassiana* growth characteristics. Spearman's correlation analysis was conducted to measure the relationship between the growth characteristic and mortality variables.

3.5. RESULTS

3.5.1. Culturing and morphological identification of *Beauveria bassiana* isolates

3.5.1.1. Macroscopic observation of *Beauveria bassiana* isolates

The isolates were characterized as *B. bassiana*, according to the following description; macroscopic characteristics; flat and cottony consistence with aerial mycelium, colors varied between white and cream white as shown in the Figure 3.1.

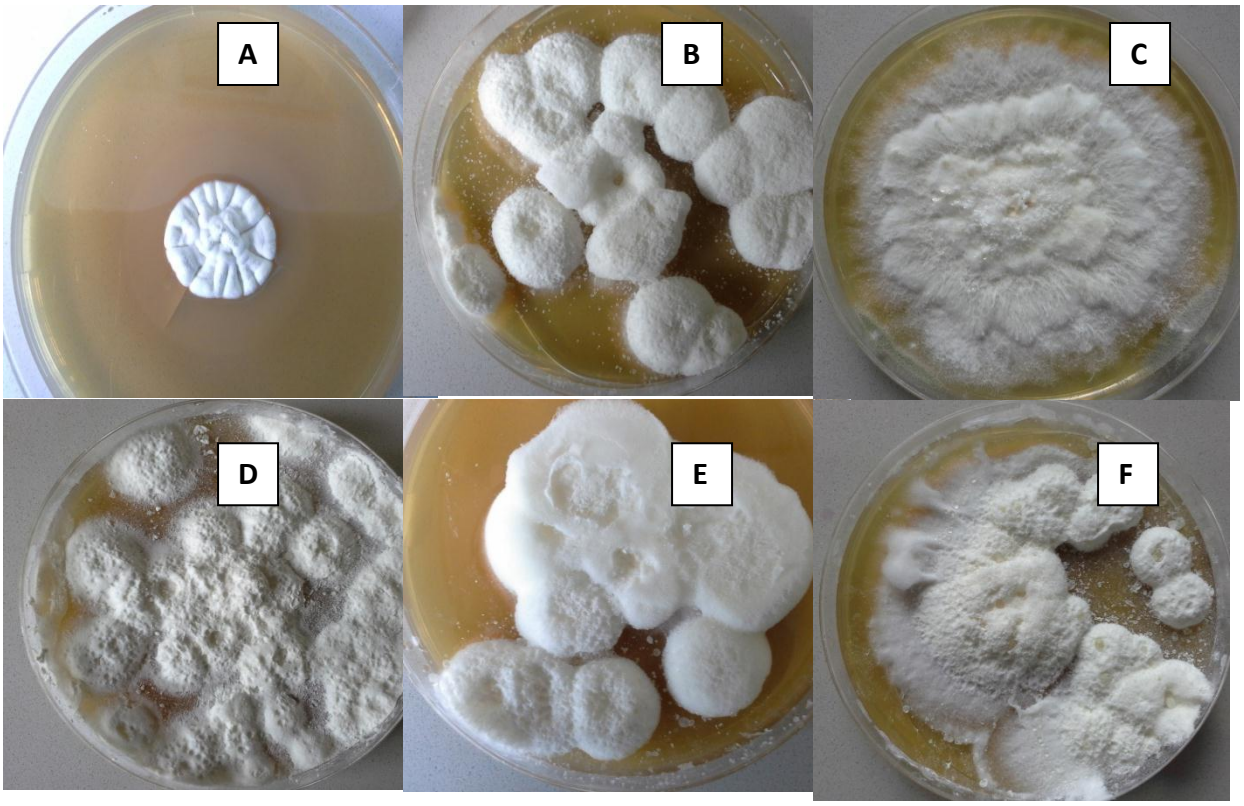

Figure 3.1: Growth of 14-day-old *Beauveria bassiana* isolates (A-BBC® , B-J39, C-J29, D-J36, E-J59, F-J35 isolates) on PDA media

3.5.1.2. Microscopic observation of *Beauveria bassiana* isolates

Observations made on *B. bassiana* conidia showed that they were globose to oval shape as shown in Figure 3.2. The conidia were round with either single or branched conidiophores.

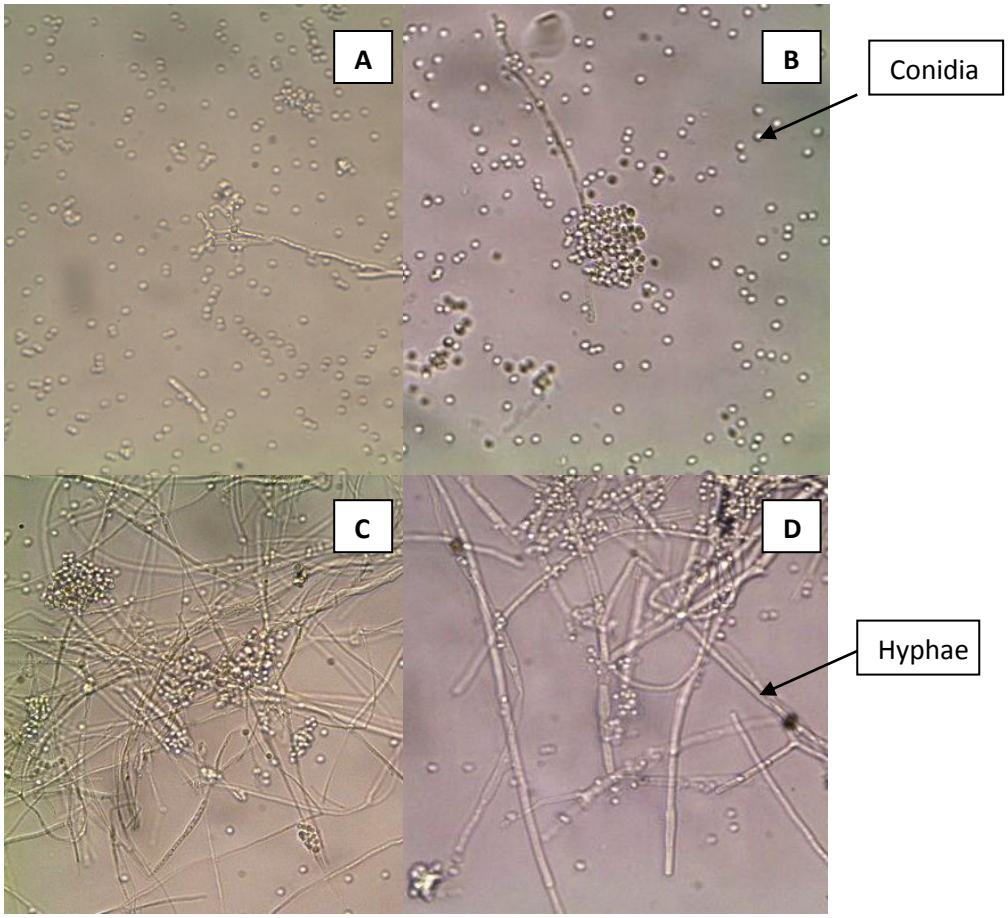

Figure 3.2: Microscopic examination of *Beauveria bassiana* grown for 14 days showing abundant conidiophores (X40 magnification) (Isolates; A-BBC®, B-J39, C-J36, D-J39)

3.5.2. Spore concentration of *Beauveria bassiana* isolates

Beauveria bassiana isolates significantly differed on the spore concentration for both assays (Table 3.1). The highest spore count was recorded for isolate J59, which was

significantly different from the rest of the isolates in assay 1, except BBC® and J35. Isolate J29 recorded the lowest spore count, and was significantly different from isolate J39. A similar trend was observed during assay two where isolate J59 recorded the highest spore count. The lowest spore count was recorded for isolate J29, and was significantly different from the spore counts recorded for the rest of the isolates (Table 3.1).

Table 3.1: Mean spore concentration (spores/ml) of *Beauveria bassiana* isolates

Isolate	Spore counts (spores/ml)
BBC®	110b
BVT®	90d
J29	68g
J35	110b
J36	90d
J39	87e
J57	80f
J59	120a
RI	100c
P-Value	<0.001
L.S.D	0.116
CV%	0.8

Treatments with different letters within the same column are significantly different at 5% probability

3.5.3. Viability of *Beauveria bassiana* isolates

The viability of the isolates differed significantly during assay one. All isolates recorded viability above 80 cfu/ml except for isolate BBC® in assay one and BBC®, J36 and J59 in assay 2. The highest and lowest viability was recorded for isolates J57 and BBC®, respectively in assay one. A similar trend was observed during assay two where isolate J57 recorded the highest viability, which was not different from all isolates except

BBC®, J36 and J59. Isolate BBC® recorded the lowest viability but was not different from isolate J36 and J59 (Table 3.2.).

Table 3.2: Mean viability (cfu/ml) of *Beauveria bassiana* isolates

Isolate	Viability (cfu/ml)
BBC®	79.81h
BVT®	83.19d
J29	83.12d
J35	84.81c
J36	80.18g
J39	82.07e
J57	86.37a
J59	81.13f
RI	86.07b
P-Value	<0.001
L.S.D	0.103
CV%	1.2

Treatments with different letters within the same column are significantly different at 5% probability

3.5.4. Relative hyphal growth of *Beauveria bassiana* isolates

There were significant differences in the relative hyphal growth recorded every two days up to ten days for the isolates tested. Isolate RI recorded the highest relative hyphal growth, which was significantly different from that of isolates J29, J35, J36, J39, J57 and J59 at day two of observation. Isolate RI recorded the highest relative hyphal growth for the second, fourth and sixth day of observation while isolate J57 recorded the highest relative hyphal growth on day eight and ten of observation. Isolate BVT® recorded the lowest relative hyphal growth throughout the ten days of observation. There were significant differences between hyphal growth rates for BBC® and BVT® except for day

two of observation. Isolates J29, J35, J36 and J39 were not different on all days of observation except day four (Fig.3.3).

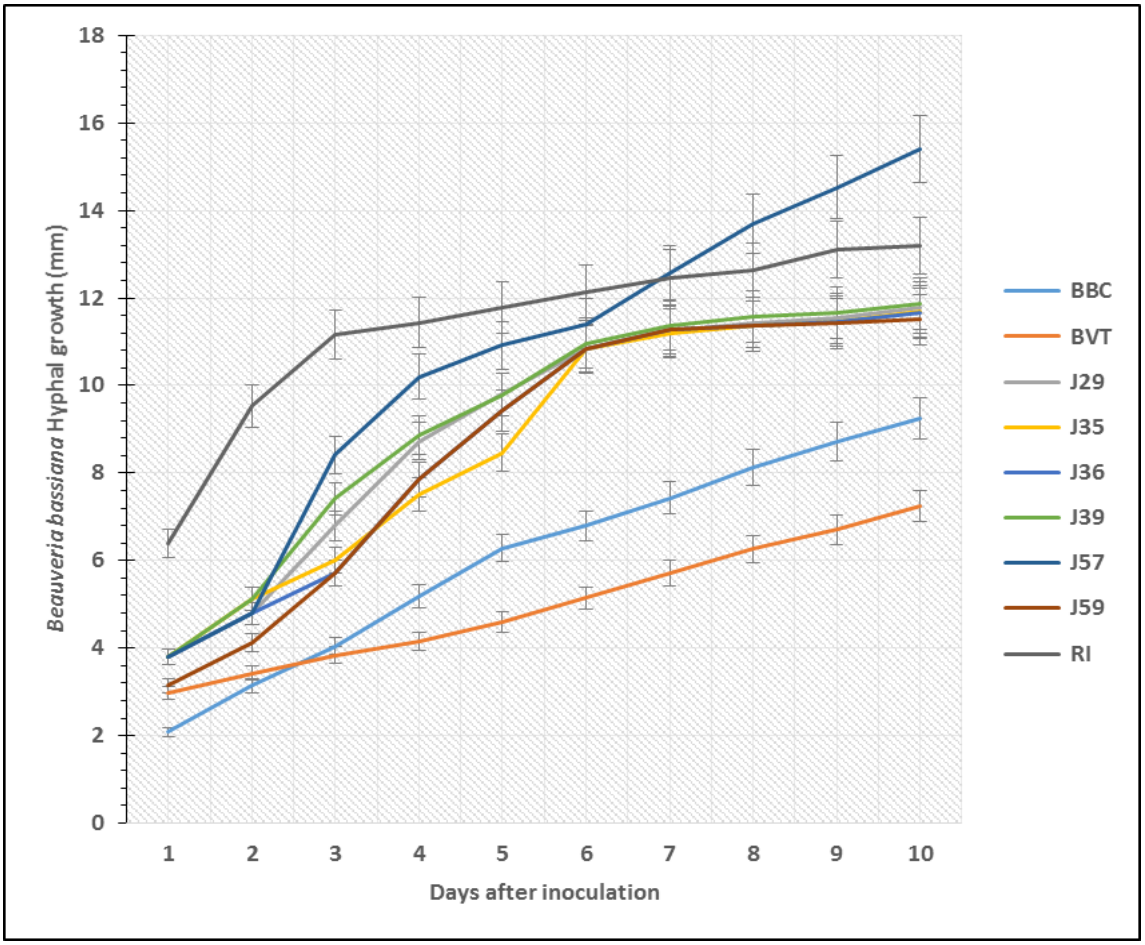

Figure 3.3: Relative hyphal growth of *Beauveria bassiana* isolates

A similar trend was recorded during assay two where isolate RI recorded the highest relative hyphal growth throughout the ten days of observation. There were significant differences in the relative hyphal growth recorded every two days up to ten days for the isolates. Isolate BBC® recorded the lowest hyphal growth from day 4 to 10. There were

no differences in relative hyphal growths recorded for isolates J29, J35, J36, J39 and J57 in day two, eight and ten (Fig 3.4.).

3.5.5. Rate of sporulation of *Beauveria bassiana* isolates

In assay one, isolate BVT® recorded the highest rate of sporulation, which was significantly different from isolates BBC® , J29, J35, J57, J59 and RI. Isolate J36 recorded the lowest rate of sporulation although not different from isolate J39 (Fig 3.5.).

A similar trend was recorded during assay two where isolate BVT® recorded the highest rate of sporulation, which was significantly different from the rest of the isolates. Isolate J36 recorded the lowest rate of sporulation but was not different from isolate J39 (Fig.3.5).

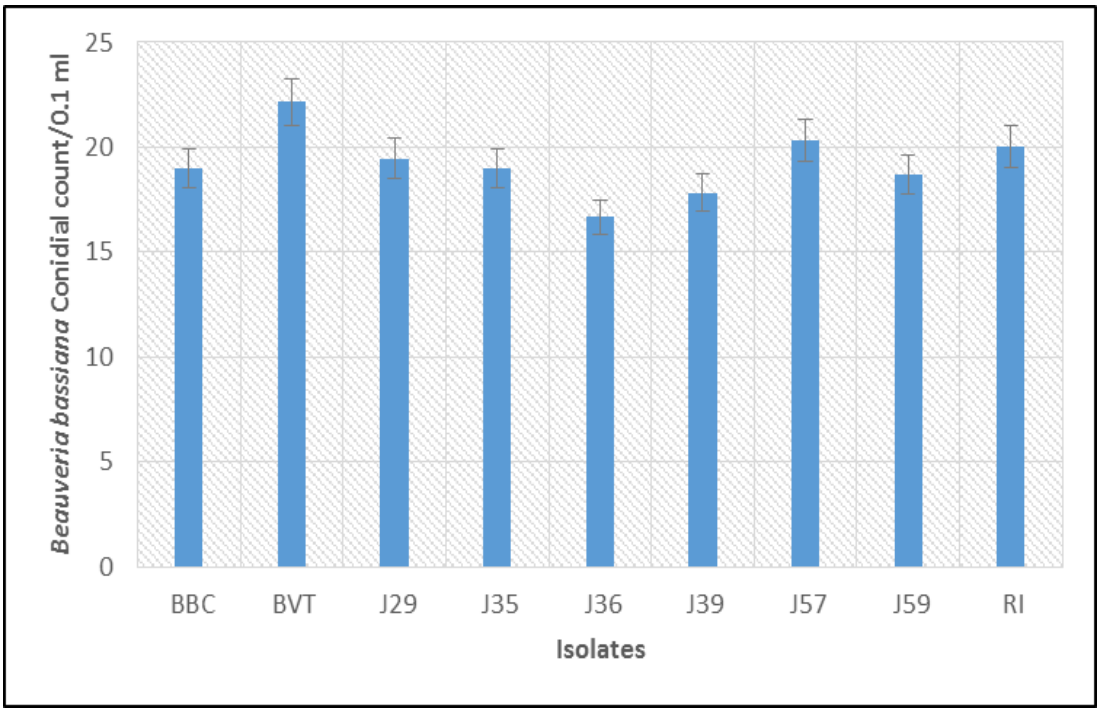

Figure 3.4: Rate of sporulation of *Beauveria bassiana* isolates (conidia count per 0.1 ml)

3.5.6. Rate of conidial germination of *Beauveria bassiana* isolates

The rate of conidial germination between the isolates was significantly different through the observation period, during the first and second assay. Isolate BBC® recorded the highest rate of conidial germination from the second through to the tenth hour of observation (Fig.3.6.).

There were no differences between the rate of conidial germination recorded for isolates BBC® and BVT® except for the second hour after inoculation. Isolate J29 recorded the least rate of conidial germination throughout the observation period (Fig.3.6.).

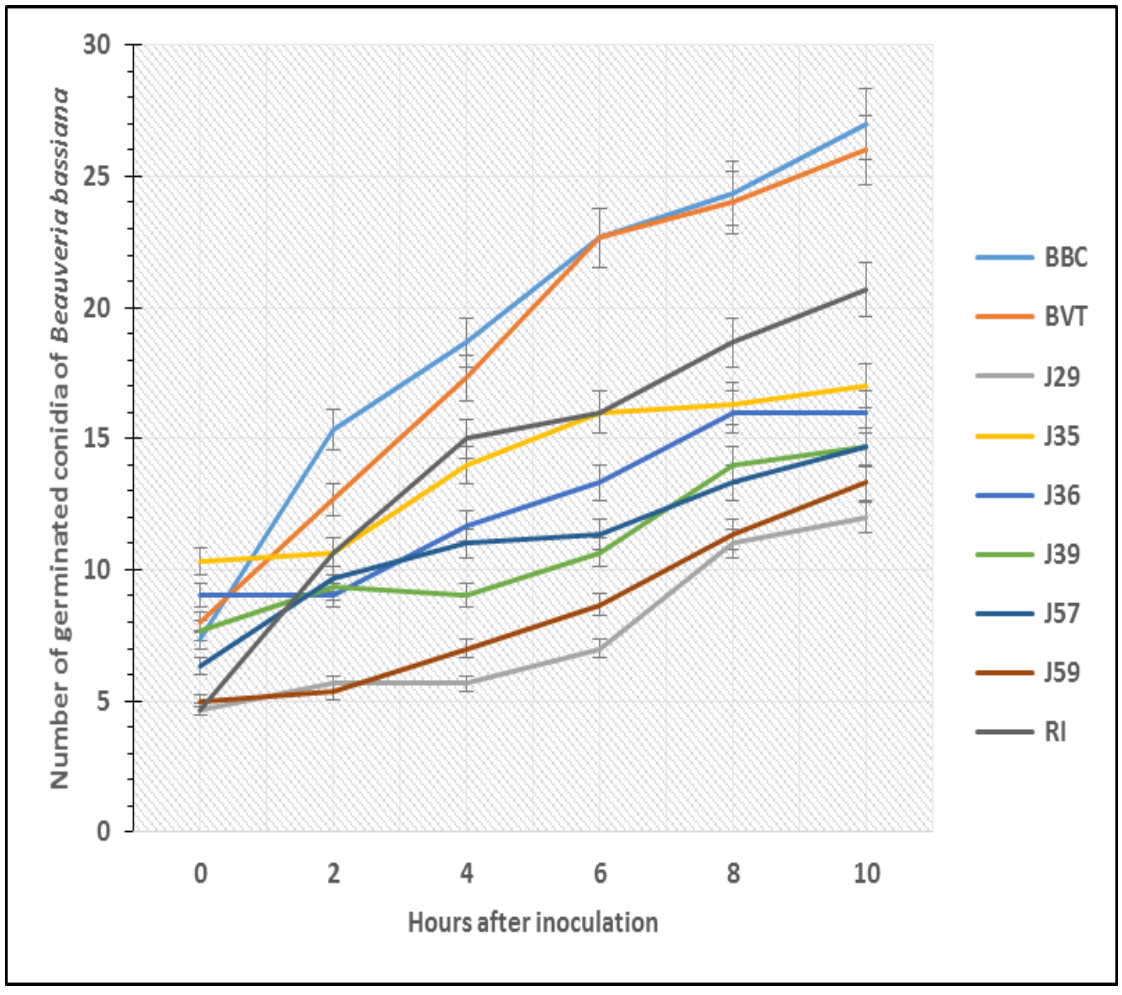

Figure 3.5: Rate of conidial germination of *Beauveria bassiana* isolates

3.6. Correlation analysis of growth variables of *Beauveria bassiana* isolates

The rate of conidial germination was negatively but significantly correlated to the relative hyphal growth (r=-0.779) and viability (r=-0.183) characteristics of the isolates. There was a positive correlation between the rate of conidial germination and the rate of sporulation (r=0.513). The rate of conidial germination positively correlated to the spore concentration (r=0.401). The hyphal growth negatively but significantly correlated with the rate of sporulation (r=-0.544) and spore concentration (r=-0.112) but positively correlated with viability (r=0.414). The rate of sporulation negatively correlated with spore concentration (r=-0.042) but positively correlated with viability (r=0.601). Spore concentration negatively correlated with viability (r=-0.259).

Table 3.3: Spearman's correlation matrix for growth characteristics of *Beauveria bassiana* isolates

	Rate of Conidial germination	Hyphal Growth	Rate of Sporulation	Spore Concentration	Viability
Rate of Conidial germination	-				
Hyphal Growth	-0.779*	-			
Rate of Sporulation	0.513*	-0.544*	-		
Spore Concentration	0.401	-0.112*	-0.042*	-	
Viability	-0.183*	0.414*	0.601*	-0.259*	-

*=Significant at P=0.01

3.7. Discussion

Morphological characteristics are used as the mode of identification for most fungi. These results show major similarities in the macroscopic, microscopic appearance and structures confirming the characterization of the isolates as *B. bassiana.* The variance recorded in the spore counts among the isolates could be as a result of the *B. bassiana* isolates strain related vigor differences depicted by the amount of spores produced. Li *et al.* (2001) reported that *B. bassiana* isolates that produced higher number of spores were generally most virulent. This indicates that isolate J59 could be the most virulent while isolate J29 could be the least virulent since they provided the highest and lowest spore counts, respectively in both assays.

The viability of the isolates was based on conidial germination and spore counts. Alizadeh *et al.* (2007) underscores the importance of evaluating conidial germination of fungi when considering them for pest control purposes. Conidial germination determines the fungal capacity to germinate on the host (Alizadeh *et al.,* 2007).

The findings show that all isolates recorded a viability of above 78% possibly because of the presence of favorable germination environmental conditions like light, temperature and relative humidity. The viability tests were conducted at an optimum temperature range of 25 ± 2°C as suggested by Hall (2011) and Benz (2015), which led to high viability records. According to Oliveira *et al*. (2011) and Jin *et al.* (2013), viability of conidia is of great importance during the use of entomopathogenic fungi, because the conidia are the most infectious agents commonly used. The isolates J57 and RI had the highest conidia viability indicating their likelihood as the most infectious among the isolates evaluated. Isolate BBC® had the least viability, which may infer low infectious

ability. Low viability may be caused by poor environmental conditions as explained by Glare *et al.* (2012).

Isolate J57 recorded the highest relative hyphal growth probably because of high rate of cell multiplication, while the reverse was recorded for isolate BBC® . Liu *et al.* (2002) reported that relative hyphal growth is important for causing infection through direct penetration of the host cuticle as the faster the colonization of infected host the higher the virulence. Further, Hajek and St. Leger (1994), and Varela and Morales (1996) found that biological control agents with rapid germination and hyphal growth rates may infer quicker host infection. This finding suggests that isolates RI and J57 may be quick in causing infections among the isolates evaluated.

Isolate BBC® recorded the highest rate of conidial germination during the ten-hour observation period. This could possibly be due to faster response to host cues upon contact. Hajek and St. Leger (1994) and Varela and Morales (1996) reported that fungal pathogens which have rapid germination and hyphal growth rates can exhibit faster host infection exhibiting superior virulence. Isolate RI in this case could be a better candidate causing fast host infection exhibiting higher virulence. The rate of sporulation was positively correlated to the rate of conidial germination implying that the vigor of the isolate would likely cause it to sporulate and germinate faster. The spore concentration and viability were negatively correlated. This is probably because not all spores counted were viable thereby giving lower viability counts. The findings from this study in relation to previous studies indicate that various characteristics can influence the virulence of the isolates. These factors are important for successful infection to take place.

CHAPTER FOUR

EFFICACY OF *Beauveria bassiana* ISOLATES IN THE CONTROL OF *Callosobruchus maculatus* UNDER LABORATORY CONDITIONS

4.1. Abstract

Callosobruchus maculatus, is a major post-harvest pest of cowpea in the tropics and other parts of the world. *Beauveria bassiana* is a prospective entomopathogenic fungi for control of *C. maculatus* in cowpea grain during storage. This study evaluated nine *B. bassiana* isolates for effectiveness in controlling *C. maculatus* in cowpea grain in the laboratory. Mortality of the cowpea bruchids was evaluated through mortality by immersion, effective dose, optimal dose and farmer storage simulation tests. Mortality rates increased with increasing concentration of *B. bassiana* conidial inoculum. The isolates J35, BBC®, J29, J39 and J59 recorded high percentage mortality of *c. maculatus* at 4.86×10^7 cfu/ml concentration while J36, BVT®, J57, RI performed best at 4.86×10^6 cfu/ml concentration. The half and double rates recorded highest and lowest mortality of *c. maculatus* of cowpea bruchids, respectively during effective dose test. The highest mortality of *C. maculatus* during assay one and two of the optimal dose rate experiment were recorded for isolates J57 and J35, respectively. The highest grain damage after the control treatment was recorded for grain treated with isolates BVT® and J59 in assay one and two. respectively while isolate J29 recorded the lowest grain damage in both assays when the isolate was used in suspension form. Isolate J57 recorded the highest grain damage after the control in both assays, while isolate J59 recorded the lowest grain damage in both assays when the isolate was used in powder form. There was positive and negative correlation recorded between the growth characteristics and mortality of the

cowpea bruchids. The findings of this study will provide information on the potential *B. bassiana* isolates for the biological control of the cowpea bruchid as a safe option. The results demonstrate the effectiveness of *B. bassiana* isolates particularly J29 and J59, respectively, in the control of *C. maculatus* in cowpea grain during storage as a safe option to the farmer.

4.2 Introduction

Callosobruchus maculatus, is a major pest of cowpea in the tropics and worldwide, laying egges on cowpea in the field and later causing damage in the store (Rahman and Taluker, 2006). The eggs are small and translucent grey, inconspicuous domed structures with oval, flat bases and are laid on the grain. The larvae are found in the grain while adults are found among the grains. Individual eggs are laid on the seed testa by female bruchids which emerge within 5-6 days of oviposition (Credland, 1987). In about 5 days, larvae emerge and enter the grain by chewing into the seed and feed within the seed. Adult bruchids are small (2 to 5.4 mm long), orange brown with dark markings with a triangular shape. They emerge within 25-30 days after oviposition. The larvae bore into and feed within seeds creating galleries.

Bruchids cause reduction in commercial and aesthetic value as a result of physical damage and contamination of grain by adults, eggs, excrement, weight loss, decreased nutritional value and germination potential (Oluwafemi, 2012). There are many methods used for controlling cowpea bruchids including chemical, cultural, physical, biological, varietal and genetical. Entomopathogenic fungi are unique in control because they are not limited to

controlling sucking and feeding insects since they infect through the hosts surface by contact thereafter penetrating the cuticle (Potrich *et al*., 2006). *Beauveria bassiana* has been employed to control many important pests in various crops across the world (Darbro *et al.*, 2011). A number of scientists including Sabbour and Abd El-Aziz (2010) have examined the effect of entomopathogenic fungi, *Metarhizium anisopliae* and *B. bassiana* on a range of insect species. Vilas Boas *et al.* (1996) and Cherry *et al*. (2005) recorded significant reduction in population of *C. maculatus* adults and when using *B. bassiana* and *M. anisopliae* entomopathogenic fungi. There are several commercial products based on *B. bassiana* for the control of many agricultural pests (Faria and Wraight, 2007). After evaluating the growth characteristics of *B. bassiana* isolates, in chapter 3 of this thesis, there was need to determine their potential effectiveness in controlling *C. maculatus*. The study, therefore, focused on screening the isolates at different concentrations and levels to control *C. maculatus*.

4.3. Materials and methods

4.3.1. Establishment of *Callosobruchus maculatus* insect colony

A laboratory colony of *C. maculatus* was established from mixed age and sex bruchids sieved from samples of highly infested cowpea grain obtained from a local market in Machakos town- Kenya. Two hundred cowpea bruchids (5 - 10 days old) were placed for 10 days in a jar containing clean uninfested cowpea grains to allow oviposition. The cowpea with bruchid eggs were then separated from the bruchids by gently sieving. These were used to start the rearing process in a ventilated chamber. Mass cultures were maintained in 1.5 kg large plastic containers and sub-cultured in 100 g small plastic

containers with cowpea grain 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%. The cowpea bruchids used for these experiments as test insects were of mixed ages and sex.

4.3.2. Standardization of isolate concentration

One gram of the stock powder was drawn and serially diluted. Using the germination percentage and spore concentration counts, the viability of the stock powder was determined in every 1 g drawn. The lowest concentration was determined to be 4.86×10^{10} cfu/ml concentration. The quantities to be drawn for the rest of the isolates were determined to get a standard concentration of 4.86×10^{10} cfu/ml concentration across all isolates.

4.3.3. Screening of *Beauveria bassiana* isolates for comparative virulence on cowpea bruchids

Comparative virulence of the isolates was determined by bioassays as per experiments below:

4.3.3.1. Mortality of cowpea bruchids by immersion experiment

Five milliliters of 4.86×10^{7} cfu/ml of each isolate was serially diluted and applied at four different concentrations 10^{-3} to 10^{-6}. For each replication, 20 cowpea bruchids (5 - 10 days old) were treated by immersion for 5 sec in 5 ml of conidial suspensions at the different concentrations separately. The control bruchids were 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 24h at 28 ±2˚C and 60 ±5 % RH before 10 clean un-infested cowpea grain were introduced as food. Mortality was recorded at every two days ending at 15 days. The experiment was repeated twice. The number of dead bruchids counted were corrected using the Abbott formula (Abbott, 1925).

Dead insects were surface sterilized in sodium hypochlorite (2%), alcohol (70%) and then rinsed with sterile distilled water for 15 sec. 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.

4.3.3.2. Effective dose test experiment for *Beauveria bassiana* isolates

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. Thirty grams of clean, unbroken and uninfested cowpea grain were placed in 100g clean, dry plastic tins. The concentrations of each measuring 5 ml were separately sprayed using a spray atomizer to each set. The control was treated with sterile distilled water. Twenty cowpea bruchids (5 - 10 days old) 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 ending at 15 days. Each tin served as a replicate with three tins for each isolate in a completely randomized design. The experiment was repeated twice. The number of dead bruchids counted were corrected using the Abbott formula (Abbott, 1925).

Dead insects were surface sterilized in sodium hypochlorite (2%) and then rinsed with sterile distilled water for 15 sec. 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.

4.3.3.3. Optimal dose rate experiment for *Beauveria bassiana* isolates

From the effective dose rate experiment above, the dose rate with the highest mortality was used in this experiment. For each replicate, 30 g of clean, unbroken and uninfested cowpea grains were placed in clean, dry plastic tins. The concentrations, each measuring 5 ml, of the identified effective dose rate were sprayed on the cowpea grain. Twenty cowpea bruchids (5 - 10 days old) 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 of 28 ±2˚C and 60-±5 % RH for 14 days. Each tin served as replicate with three tins for each isolate in a completely randomized design. The experiment was repeated twice. The number of dead bruchids counted were corrected using the Abbott formula (Abbott, 1925).

Dead insects were surface sterilized in 2% sodium hypochlorite and then rinsed with sterile distilled water for 15 sec. 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.

4.3.3.4. Effect of *Beauveria bassiana* in suspension form on cowpea bruchids during storage

Two kilograms of clean, unbroken and uninfested cowpea grain were placed in 10kg gunny bags and sprayed with the *B. bassiana* isolate that recorded the highest mortality in the optimal dose test experiment above. One hundred cowpea bruchids (5 - 10 days old) were added to each set of treated grains. Each gunny bag served as replicate with three gunny bags for each isolate in a completely randomized design. The control treatment was treated with sterile distilled water. The gunny bags were carefully mixed and then fastened with a string. The experimental set up was kept at a temperature of 28 ± 2˚C and 60 ± 5 % RH to simulate the farmer's storage conditions. At two months after uninterrupted storage, data was collected on damaged grain based on 200 g sample of the stored grain. The experiment was repeated twice. The number of dead bruchids were counted and corrected using the Abbott formula (Abbott, 1925). Dead insects were surface sterilized in 2% sodium hypochlorite and rinsed with sterile distilled water for 15 sec. 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. The experiment was conducted twice.

4.3.3.5. Effect of *Beauveria bassiana* in powder form on cowpea bruchids during storage

Two kilograms of clean, unbroken and uninfested cowpea grain were placed in 10kg gunny bags and mixed manually with the *B. bassiana* isolate that recorded the highest mortality in the optimal dose test experiment. The control treatment was treated with dry

rice powder. The gunny bags were carefully mixed from the outside and fastened with a string. One hundred cowpea bruchids (5 - 10 days old) were added to each set of treated grains. Each gunny bag served as replicate with three gunny bags for each isolate in a completely randomized design. The experimental set up was kept at a temperature of 28 ± 2˚C and 60±5 % RH to simulate the farmer's storage conditions. At two months after uninterrupted storage, data was collected on damaged grain based on 200 g sample of the stored grain. The experiment was repeated twice. The number of dead bruchids were counted and corrected using the Abbott formula (Abbott, 1925). Dead insects were surface sterilized in 2% sodium hypochlorite and rinsed with sterile distilled water for 15 sec. 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.

4.4. Data 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*. Spearman's correlation analysis was conducted to identify the correlation between the growth characteristics and the mortality of the bruchids during the optimal dose rate experiment.

4.5. RESULTS

4.5.1. Mortality of cowpea bruchids by immersion

The *B. bassiana* isolates exhibited significant differences in percentage mortality of the cowpea bruchids at different concentrations during day three to fifteen of observation. The highest mortality was recorded for 4.86×10^7 cfu/ml concentration while the lowest was recorded for 4.86×10^4 cfu/ml concentration across all observation days. The highest percentage mortality of cowpea bruchids was recorded for isolate J35, which was significantly different from that recorded for isolates J57, J59, BVT® and BBC® at 4.86×10^7 cfu/ml concentration. The percentage mortality of *C. maculatus* treated with 4.86×10^6 cfu/ml and 4.86×10^5 cfu/ml concentrations did not differ during the first assay. The highest percentage mortality of *C. maculatus* was recorded for isolate BVT®, which was significantly different from mortality recorded for isolates J29, J35, J36, J39, J59 and RI. No mortality was recorded for isolates J39 and J59 (Table 4.1).

A similar trend was observed during assay two where isolate J35 recorded the highest percentage mortality of *C. maculatus* which was significantly different from that recorded for isolates BBC, J59, J29 and J57 at 4.86×10^7 cfu/ml concentration. The lowest mortality was recorded for isolate J57 at the same concentration. There were no differences between percentage mortalities caused by the isolates at 4.86×10^6 cfu/ml and 4.86×10^5 cfu/ml concentrations. The highest mortality of *C. maculatus* at 4.86×10^4 cfu/ml concentration was recorded for isolate BVT® which was significantly different from that recorded for isolates J29, J35, J36, J59 and RI. No mortality was recorded for isolates J39 and J59 (Table 4.1).

Table 4.1: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates` treatment at different concentrations on day 3

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	10.0b	21.7a	18.3a	6.6ab
BVT®	13.1b	6.7a	15.0a	15.0a
J29	10.0ab	11.7a	6.6a	5.0b
J35	30.0a	6.7a	11.7a	3.3b
J36	26.7a	31.7a	6.6a	1.6b
J39	18.3ab	10.0a	5.0a	0.0b
J57	1.6b	15.0a	6.6a	8.3ab
J59	10.0b	8.3a	13.3a	0.0b
RI	15.0ab	16.6a	5.0a	5.0b
P-Value	**0.004**	**0.06**	**0.02**	**0.006**
L.S.D	**16.9**	**26.7**	**14.6**	**9.3**
CV%	**19.6**	**2.2**	**17.3**	**40.1**

Treatments with different letters within the same column are significantly different at 5% probability

CFU/ml are as follows; $10^{-3}= 4.86 \times 10^{7}$, $10^{-4}= 4.86 \times 10^{6}$, $10^{-5}= 4.86 \times 10^{5}$, $10^{-6}= 4.86 \times 10^{4}$

Beauveria bassiana isolates across the four concentrations, at day five of observation, differed significantly in the cumulative percentage mortalities of cowpea bruchids. The highest cumulative percentage mortality of bruchids at day five of observation was recorded for isolate J35 but was not different from the cumulative percentage mortality caused by isolate J36 at 4.86×10^{7} cfu/ml concentration. These were however significantly different from the cumulative percentage mortality caused by the rest of the isolates at the same concentration. The highest cumulative percentage mortality was recorded for isolate J36 while the lowest was recorded for isolate BVT® at 4.86×10^{6} cfu/ml concentration. At 4.86×10^{4} cfu/ml concentration, isolate J39 had no cumulative percentage mortality of cowpea bruchids on the fifth day of observation while the highest mortality was recorded for isolate BVT® (Table 4.2.).

A similar trend was observed during assay two. Isolate J35 recorded the highest cumulative percentage mortality of *C. maculatus* which was not different from isolate J36, but significantly different from the cumulative percentage mortality recorded for the rest of the isolates at 4.86×10^7 cfu/ml concentration. Isolate J36 recorded the highest cumulative percentage mortality of *C. maculatus* while isolate BVT® recorded the least cumulative percentage mortality at 4.86×10^6 cfu/ml concentration. The highest cumulative percentage mortality was recorded for isolate BBC® which was not different from that recorded for isolate BVT® at 4.86×10^5 cfu/ml concentration. The lowest cumulative percentage mortality was recorded for isolate J36 under the same concentration. The highest cumulative percentage mortality of *C. maculatus* was recorded for isolate BVT® which was not different from isolate BBC®, but both were significantly different from the cumulative percentage mortality recorded for the rest of the isolates at 4.86×10^4 cfu/ml concentration. Isolates J35 and J39 displayed a reduction in the cumulative percentage mortality with decrease in concentration of spores per milliliter in both assays (Table 4.2).

Table 4.2: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates` treatment at different concentrations on day 5

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	33.3b	45.6ab	47.4a	24.6ab
BVT®	28.1b	8.8c	45.6a	31.6a
J29	29.8b	21.1bc	29.8b	10.5b
J35	66.6a	15.0bc	12.3c	8.8b
J36	63.1a	64.9a	7.0c	10.5b
J39	26.3b	15.8bc	12.3c	0.0b
J57	12.3b	24.6bc	8.8c	10.5b
J59	14.0b	22.8bc	26.3b	3.5b
RI	24.6b	21.1bc	21.1bc	10.5b
P-Value	**<0.001**	**<0.001**	**<0.001**	**0.002**
L.S.D	**29.2**	**26.3**	**13.8**	**16.9**
CV%	**4.3**	**15.4**	**8.7**	**21.8**

Treatments with different letters within the same column are significantly different at 5% probability

CFU/ml are as follows; $10^{-3}= 4.86 \times 10^{7}$, $10^{-4}= 4.86 \times 10^{6}$, $10^{-5}= 4.86 \times 10^{5}$, $10^{-6}= 4.86 \times 10^{4}$

The cumulative percentage mortality of *C. maculatus* recorded for isolates J35, BBC® and J36 were significantly different from those obtained for isolates BVT®, J39 and RI at 4.86×10^{7} cfu/ml concentration. The highest mortality of *C. maculatus* was recorded for isolate J35 while the least was in isolate J57 at the same concentration. Isolate BVT® recorded the highest and similar cumulative percentage mortality at 4.86×10^{6} cfu/ml and 4.86×10^{6} cfu/ml concentrations. The cumulative percentage mortality of *C. maculatus* of same isolate was, however, not different from that recorded for isolate J36 at 4.86×10^{6} cfu/ml concentration. The two isolates were, however, significantly different from the rest of the isolates at 4.86×10^{6} cfu/ml concentration. Isolates BVT® recorded the highest cumulative percentage mortality of *C. maculatus* at 4.86×10^{4} cfu/ml concentration but was not different from that of isolate BBC® . The two isolates, however, recorded

cumulative percentage mortality of *C. maculatus* that was significantly different from the rest of the isolates (Table 4.3).

A similar trend was observed during assay two with significant differences in the cumulative percentage mortalities of *C. maculatus* being recorded across the isolates under different concentrations. The highest cumulative percentage mortality was recorded for isolate J35 which was not different from that recorded for isolates J36 and BBC® at 4.86×10^7 cfu/ml concentration (Table 4.3).

Table 4.3: Cumulative percentage mortality of bruchids at different concentrations caused by *Beauveria bassiana* isolates` treatment on day 7

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	76.5a	62.7ab	76.5ab	47.1a
BVT®	35.3bc	80.4a	80.4a	49.0a
J29	54.9ab	37.3bc	51.0bc	15.7b
J35	86.3a	17.6c	23.5d	7.8b
J36	74.5a	74.5a	33.3cd	11.8b
J39	33.3bc	25.5c	39.2cd	2.0b
J57	11.8c	35.3bc	23.5d	9.8b
J59	15.7c	23.5c	45.1cd	4.5b
RI	35.3bc	21.6c	25.5cd	13.7b
P-Value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
L.S.D	**35.6**	**32.4**	**25.7**	**26.2**
CV%	**8.4**	**5.5**	**1.7**	**11.5**

Treatments with different letters within the same column are significantly different at 5% probability

cfu/ml are as follows; $10^{-3}= 4.86 \times 10^7$, $10^{-4}= 4.86 \times 10^6$, $10^{-5}= 4.86 \times 10^5$, $10^{-6}= 4.86 \times 10^4$

These isolates, however, recorded *C. maculatus* mortality that was significantly different from the percentage cumulative mortality recorded for the rest of the isolates under the

same concentration. The highest cumulative percentage mortality of *C. maculatus* was recorded for isolate BVT® followed closely by isolate BBC® and both were significantly different from the rest of the isolates at 4.86×10^4 cfu/ml concentration (Table 4.3).

Isolates BBC® and BVT® interchangeably recorded the highest cumulative percentage mortality of *C. maculatus* during assay one and two across all concentrations except for 4.86×10^7 cfu/ml concentration at day nine of observation. Isolate BBC® had recorded the highest cumulative percentage mortality of 100% at 4.86×10^7 cfu/ml concentration followed closely by isolates J35, J36 and J29. These however, recorded C. *maculatus* mortality that was significantly different from that of isolates J57 and J59 under the same concentration. Isolate J39 recorded the lowest cumulative percentage mortality of *C. maculatus* compared to all the recorded cumulative percentage mortalities at 4.86×10^4 cfu/ml and 4.86×10^4 cfu/ml concentration sin both assays (Table 4.4).

A similar trend was observed during assay two where isolate BBC® had recorded the highest cumulative percentage mortality of *C. maculatus* of 100% at 4.86×10^7 cfu/ml concentration followed closely by isolates J35, J36 and J29. This cumulative percentage mortality of *C. maculatus* was however significantly different from that of isolates J57 and J59 under the same concentration. Isolate BVT® recorded the highest cumulative percentage mortality of *C. maculatus* which was not different from that recorded for BBC® at 4.86×10^4 cfu/ml concentration. The lowest cumulative percentage mortality of *C. maculatus* was recorded for isolate J39, under the same concentration (Table 4.4).

Table 4.4: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates' treatments at different concentrations on day 9

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	100.0a	87.5ab	95.8a	66.7a
BVT®	58.3bc	97.9a	89.6ab	83.3a
J29	81.2ab	56.2bc	72.9ab	22.9bc
J35	87.4ab	47.9c	50.0b	16.7bc
J36	85.4ab	81.2ab	50.0b	31.2b
J39	56.2bc	45.8c	39.6b	4.2c
J57	31.2c	54.2bc	54.2b	31.2b
J59	39.6c	39.6c	72.9ab	8.3bc
RI	52.1bc	45.8c	50.0b	20.8bc
P-Value	**0.001**	**0.002**	**0.003**	**<0.001**
L.S.D	**39.7**	**38.1**	**35.7**	**24.7**
CV%	**8.4**	**8.1**	**4.7**	**3.3**

Treatments with different letters within the same column are significantly different at 5% probability

cfu/ml are as follows; $10^{-3}= 4.86 \times 10^7$, $10^{-4}= 4.86 \times 10^6$, $10^{-5}= 4.86 \times 10^5$, $10^{-6}= 4.86 \times 10^4$

No differences were observed between the cumulative percentage mortalities of *C. maculatus* recorded at 4.86×10^7 cfu/ml and 4.86×10^6 cfu/ml concentrations recorded for all isolates at day eleven. The highest cumulative percentage mortality of *C. maculatus* was recorded for isolates BBC® and BVT® at both concentrations. The cumulative percentage mortality of *C. maculatus* recorded for the two isolates was significantly different from that observed in all other isolates, except J29 at 4.86×10^5 cfu/ml concentration. The highest mortality was recorded for isolate BVT®, followed closely by isolates BBC®, J29, J35, J36 and J39 at 4.86×10^7 cfu/ml concentration. The cumulative percentage mortality recorded for these isolates was however not different from each other, but significantly different from that recorded for isolates J57, J59 and RI under the same concentration. The two were, however, not different from cumulative

percentage mortality recorded for isolate J57 under the same concentration. There were significant differences in cumulative percentage mortalities recorded at 4.86×10^5 cfu/ml and 4.86×10^5 cfu/ml concentrations across all isolates at day eleven. Isolates J35 and J39 displayed a reduction in the cumulative percentage mortality with decrease in concentration of spores per milliliter in both assays (Table 4.5).

Table 4.5: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates' treatments at different concentrations on day 11

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	100.0a	100.0a	100.0a	97.8a
BVT®	100.0a	97.8a	100.0a	86.7a
J29	93.3a	75.6a	84.4ab	24.4bc
J35	91.1a	82.2a	75.6bc	22.2bc
J36	86.7a	82.2a	57.8c	42.2b
J39	75.6a	64.4a	62.2c	17.8c
J57	62.2a	68.9a	80.0b	37.8bc
J59	64.4a	48.9a	82.2b	15.6c
RI	64.4a	75.6a	73.3bc	24.4bc
P-Value	**0.021**	**0.047**	**<0.001**	**<0.001**
L.S.D	**45.5**	**53.7**	**16.9**	**23.2**
CV%	**4.5**	**6.8**	**6.6**	**4.5**

Treatments with different letters within the same column are significantly different at 5% probability

CFU/ml are as follows; $10^{-3}= 4.86 \times 10^7$, $10^{-4}= 4.86 \times 10^6$, $10^{-5}= 4.86 \times 10^5$, $10^{-6}= 4.86 \times 10^4$

The highest cumulative percentage mortality of *C. maculatus* was 100% were recorded for isolates BBC® and BVT® in all concentrations except at 4.86×10^4 cfu/ml concentration where isolate BVT® recorded a lower concentration at day 13 of observation in both assays. The 3 isolates BBC®, BVT® and J36 did not differ with J29, J35 and J39 in the cumulative mortality of *C. maculatus* recorded at the same

concentration. There were no differences in cumulative percentage mortality of *C. maculatus* recorded for isolates at 4.86×10^6 cfu/ml concentration for all isolates in both assays. The lowest cumulative percentage mortality of *C. maculatus* was recorded for isolate J59, which was not different from that recorded for all other isolates except BBC® and BVT® at 4.86×10^4 cfu/ml concentration during both assays. Isolate J36 displayed a reduction in the cumulative percentage mortality with decrease in concentration of spores per milliliter in both assays (Table 4.6).

Table 4.6: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates at different concentrations on day 13

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	100.0a	100.0a	100.0a	100.0a
BVT®	100.0a	100.0a	100.0a	90.5a
J29	93.9ab	85.7a	90.5ab	35.7b
J35	95.2ab	92.8a	95.2a	23.4b
J36	100.0a	95.2a	73.8c	43.5b
J39	92.8ab	88.1a	80.9c	26.2b
J57	73.8b	95.2a	88.1ab	42.8b
J59	71.4b	92.8a	97.6a	19.1b
RI	80.9ab	90.5a	92.9ab	28.5b
P-Value	**0.005**	**0.267**	**<0.001**	**<0.001**
L.S.D	**22.1**	**16.9**	**13.6**	**26.8**
CV%	**3.2**	**3**	**4.5**	**3.7**

Treatments with different letters within the same column are significantly different at 5% probability

CFU/ml are as follows; $10^{-3} = 4.86 \times 10^7$, $10^{-4} = 4.86 \times 10^6$, $10^{-5} = 4.86 \times 10^5$, $10^{-6} = 4.86 \times 10^4$

All the isolates tested at concentrations 4.86×10^7 cfu/ml, 4.86×10^6 cfu/ml and 4.86×10^5 cfu/ml concentrations recorded 100% cumulative percentage mortality of *C. maculatus* during assay one. Isolate BBC® was the only one that recorded 100%

cumulative percentage mortality of *C. maculatus*. The cumulative mortality of *C. maculatus* did not differ from that of isolate BVT® at 4.86×10^4 cfu/ml concentration. The two were however significantly different from the percentage cumulative mortality of *C. maculatus* recorded for the rest of the isolates under the same concentration. A similar trend was observed during assay two at day fifteen of observation. All the isolates at concentrations 4.86×10^7 cfu/ml, 4.86×10^6 cfu/ml and 4.86×10^5 cfu/ml achieved 99% cumulative percentage mortality of *C. maculatus*. Isolate BBC® was the only isolate that recorded 99% cumulative percentage mortality of *C. maculatus*, which was not different from isolate BVT® at 4.86×10^4 cfu/ml. The lowest cumulative mortality of *C. maculatus* was recorded for isolate J59 in both assays (Table 4.7.).

Table 4.7: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates' treatments at three selected dose rates on day 15

Isolate	10^{-3}	10^{-4}	10^{-5}	10^{-6}
BBC®	100.0a	100.0a	100.0a	100.0a
BVT®	100.0a	100.0a	100.0a	94.8a
J29	100.0a	100.0a	100.0a	33.3b
J35	100.0a	100.0a	100.0a	41.0b
J36	100.0a	100.0a	100.0a	48.7b
J39	100.0a	100.0a	100.0a	25.6b
J57	100.0a	100.0a	100.0a	46.1b
J59	100.0a	100.0a	100.0a	23.0b
RI	100.0a	100.0a	100.0a	35.9b
P-Value	*****	*	*	**<0.001**
L.S.D	*****	*	*	**26.1**
CV%	*****	*****	*****	**8.6**

Treatments with different letters within the same column are significantly different at 5% probability

CFU/ml are as follows; $10^{-3}= 4.86 \times 10^7$, $10^{-4}= 4.86 \times 10^6$, $10^{-5}= 4.86 \times 10^5$, $10^{-6}= 4.86 \times 10^4$

4.5.2. Optimal dose rate experiment for *Beauveria bassiana* isolates

The findings of the mortality by immersion experiment were used to determine the viable isolate concentrations to be used during the effective dose rate experiment. Isolates J35, BBC®, J29, J59 and RI were selected at concentration 4.86×10^7 while isolates J36, BVT® and J57 were selected at 4.86×10^6.

There were significant differences between the mortality percentage recorded during the days of observation across the half and full rates at day three of observation. The highest percentage mortality was recorded for isolate BVT® which was not different from that recorded for isolate J29 which was significantly different from that recorded for the rest of the isolates at full rate during assay one. The percentage mortality of *C. maculatus* recorded for isolate BBC® and BVT® was not different from that recorded for isolates J36, J35 and RI but were significantly different $P \leq 0.01$) from that recorded for isolates J57 and J59 at half rate (Table 4.8).

There were no differences in percentage mortality of *C. maculatus* recorded at double rate for all the isolates in both assays. Isolates BVT®, J57 and J59 displayed a reduction in the cumulative percentage mortalities with decrease in concentration of spores per milliliter in both assays. A similar trend was observed during assay two where the highest percentage mortality was recorded for isolate BVT® that was significantly different from isolate J29 but not different from that recorded for the rest of the isolates at full rate. The percentage mortality recorded for isolate BBC® was not different from that recorded for isolates J36, J35, J39 and RI but significantly differed from that recorded for isolates J57 and J59 at half rate (Table 4.8).

Table 4.8: Percentage mortality of bruchids caused by *Beauveria bassiana* isolates' treatments at three selected dose rates on day 3

Isolate	Full Rate	Half Rate	Double Rate
BBC®	51.7ab	85.0a	20.0a
BVT®	65.0a	46.7bc	13.3a
J29	31.7c	41.7bc	5.0a
J35	33.3bc	58.3ab	5.0a
J36	40.0bc	71.7ab	13.3a
J39	35.0bc	50.0bc	5.0a
J57	36.7bc	20.0c	5.0a
J59	43.3bc	25.0c	6.7a
RI	50.0ab	58.3ab	8.3a
P-Value	**0.002**	**<0.001**	**0.271**
L.S.D	**18.7**	**31.1**	**18.7**
CV%	**8.6**	**9.9**	**15.4**

Treatments with different letters within the same column are significantly different at 5% probability

Full Rate: Isolates J35, BBC® , J29, J59 and RI = 4.86×10^7; Isolates J36, BVT® and J57 = 4.86×10^6

Half Rate: Isolates J35, BBC® , J29, J59 and RI = 2.43×10^7; Isolates J36, BVT® and J57 = 2.43×10^6

Double Rate: Isolates J35, BBC® , J29, J59 and RI = 9.72×10^7; Isolates J36, BVT® and J57 = 9.72×10^6

No differences in cumulative percentage mortality of *C. maculatus* was recorded for the isolates under full, half and double rates during assay one and two on day five. The half and double rates generally recorded highest and lowest cumulative percentage mortality of *C. maculatus* respectively in all the isolates during both assays. Two isolates, BBC® and BVT® recorded 100% cumulative percentage mortality of *C. maculatus* at half rate on day five of observation during assay one. Isolate J29 recorded the lowest cumulative percentage mortality of *C. maculatus* at half rate in both assays (Table 4.9).

Table 4.9: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates at three-selected dose rates on day 5

Isolate	Full Rate	Half Rate	Double Rate
BBC®	82.5a	100.0a	28.1a
BVT®	87.7a	100.0a	40.4a
J29	70.2a	70.2a	19.3a
J35	77.2a	98.2a	8.8a
J36	73.7a	89.5a	19.3a
J39	80.7a	91.2a	17.5a
J57	77.2a	93.0a	14.0a
J59	78.9a	93.0a	7.0a
RI	86.0a	96.5a	8.8a
P-Value	**0.636**	**0.036**	**0.292**
L.S.D	**19.2**	**22.4**	**34.6**
CV%	**4.9**	**1.1**	**11.8**

Treatments with different letters within the same column are significantly different at 5% probability

Full Rate: Isolates J35, BBC® , J29, J59 and RI = 4.86×10^7; Isolates J36, BVT® and J57 = 4.86×10^6

Half Rate: Isolates J35, BBC® , J29, J59 and RI = 2.43×10^7; Isolates J36, BVT® and J57 = 2.43×10^6

Double Rate: Isolates J35, BBC® , J29, J59 and RI = 9.72×10^7; Isolates J36, BVT® and J57 = 9.72×10^6

No differences in cumulative percentage mortality of *C. maculatus* were recorded for the isolates at all tested concentrations at day seven of observation. The half and double rates generally recorded highest and lowest cumulative percentage mortality of *C. maculatus* respectively for all isolates during both assays. In both assays, at the full and half rates tested, the cumulative percentage mortality was above 70%. Isolate J35 recorded the lowest cumulative percentage mortality followed closely by that recorded for isolate J57 during assay one at double rate of application. Isolate J59 on the other hand recorded the

lowest cumulative percentage mortality followed closely by isolate RI with no significant differences during assay two at double rate of application (Table 4.10.).

Table 4.10: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates at three selected dose rates on day 7

Isolate	Full Rate	Half Rate	Double Rate
BBC®	100.0a	100.0a	33.3a
BVT®	92.6a	100.0a	40.7a
J29	90.7a	88.9a	25.9a
J35	92.6a	100.0a	11.9a
J36	85.2a	100.0a	33.3a
J39	94.4a	98.1a	29.6a
J57	92.6a	100.0a	14.8a
J59	94.4a	100.0a	24.1a
RI	96.3a	100.0a	22.2a
P-Value	**0.432**	**0.174**	**0.411**
L.S.D	**16.1**	**8.5**	**32.8**
CV%	**3.5**	**2**	**14.4**

Treatments with different letters within the same column are significantly different at 5% probability

Full Rate: Isolates J35, BBC® , J29, J59 and RI = 4.86×10^7; Isolates J36, BVT® and J57 = 4.86×10^6

Half Rate: Isolates J35, BBC® , J29, J59 and RI = 2.43×10^7; Isolates J36, BVT® and J57 = 2.43×10^6

Double Rate: Isolates J35, BBC® , J29, J59 and RI = 9.72×10^7; Isolates J36, BVT® and J57 = 9.72×10^6

In assays one and two, on day nine of observation, the cumulative percentage mortality of *C. maculatus* was above 95% at full and half rates of application. No differences in cumulative percentage mortalities were recorded among the isolates for all rates of application at day nine. Isolate J35 recorded the least cumulative percentage mortality of *C. maculatus* at double rate in both assays. The half rate recorded a higher cumulative percentage mortality of *C. maculatus* as compared to the full rate during assay two except

for BVT® and RI isolates. The double rates in both assays one and two had low cumulative percentage mortality of *C. maculatus* ranging from 21- 46.5% in assay one and 16-41.9% in assay two (Table 4.11).

Table 4.11: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates at three selected dose rates on day 9

Isolate	Full Rate	Half Rate	Double Rate
BBC®	100.0a	100.0a	34.0a
BVT®	100.0a	100.0a	46.5a
J29	97.9a	100.0a	31.9a
J35	97.9a	100.0a	20.3a
J36	97.9a	100.0a	38.2a
J39	100.0a	100.0a	34.0a
J57	100.0a	100.0a	21.5a
J59	100.0a	100.0a	29.8a
RI	100.0a	100.0a	25.7a
P-Value	**NS**	*****	**NS**
L.S.D	**12.4**	*****	**38.1**
CV%	**5.4**	*****	**6.9**

Treatments with different letters within the same column are significantly different at 5% probability

Full Rate: Isolates J35, BBC® , J29, J59 and RI = 4.86×10^7; Isolates J36, BVT® and J57 = 4.86×10^6

Half Rate: Isolates J35, BBC® , J29, J59 and RI = 2.43×10^7; Isolates J36, BVT® and J57 = 2.43×10^6

Double Rate: Isolates J35, BBC® , J29, J59 and RI = 9.72×10^7; Isolates J36, BVT® and J57 = 9.72×10^6

On day eleven of observation, the cumulative percentage mortality of *C. maculatus* was above 99% at full and half rates of application in both assays. No differences in cumulative percentage mortality of *C. maculatus* were recorded among the isolates for all rates of application. At double rate, isolate BVT® had the highest cumulative percentage mortality of *C. maculatus* at 69%. There were no differences in the cumulative mortality of bruchids

caused by *Beauveria bassiana* isolates for all the three dose rates on day 9. In assay one, isolates J59 and RI had the least cumulative percentage mortality of *C. maculatus* while isolate J35 had the least cumulative percentage mortality of *C. maculatus* in assay two at double rate of application (Table 4.12.).

Table 4.12: Cumulative percentage mortality of bruchids caused by *Beauveria bassiana* isolates at three-selected dose rates on day 11

Isolate	Full Rate	Half Rate	Double Rate
BBC®	100.0a	100.0a	35.7a
BVT®	100.0a	100.0a	69.0a
J29	100.0a	100.0a	35.7a
J35	100.0a	100.0a	51.1a
J36	100.0a	100.0a	43.4a
J39	100.0a	100.0a	35.7a
J57	100.0a	100.0a	35.7a
J59	100.0a	100.0a	30.6a
RI	100.0a	100.0a	30.6a
P-Value	*****	*	**0.411**
L.S.D	*****	*	**60.8**
CV%	*****	*****	**14.4**

Treatments with different letters within the same column are significantly different at 5% probability

Full Rate: Isolates J35, BBC® , J29, J59 and RI = 4.86×10^7; Isolates J36, BVT® and J57 = 4.86×10^6

Half Rate: Isolates J35, BBC® , J29, J59 and RI = 2.43×10^7; Isolates J36, BVT® and J57 = 2.43×10^6, Double Rate: Isolates J35, BBC® , J29, J59 and RI = 9.72×10^7; Isolates J36, BVT® and J57 = 9.72×10^6

4.5.3. Effect of optimal dose rate of *Beauveria bassiana* isolates on cowpea bruchids

All isolates were selected at half rate for the optimal dose rate experiment. Mortality of the cowpea bruchids at day 14 after application of *B. bassiana* isolates showed significant differences in both assays (Fig.4.1). A high cumulative mortality of *C. maculatus* was

recorded during the first assay compared to the second assay. The highest mortality was recorded for isolate J57, and was significantly different from isolates J36, BVT®, J39 and RI (Fig.4.1). No differences in mortality of *C. maculatus* were recorded for isolates J35 and J29 in assay one. The lowest mortality of *C. maculatus* was recorded for isolate J59, which was significantly different from isolates BBC®, J29 and J35 (Fig.4.1). A similar trend was observed during assay two where the highest mortality was recorded for isolate J35, which was not different from isolate J57 and RI. The mortality of *C. maculatus* caused by the three isolates was however significantly different from that caused by isolates BBC®, BVT® and J39. The lowest mortality of *C. maculatus* was recorded for isolate J59 in assay two (Fig.4.1).

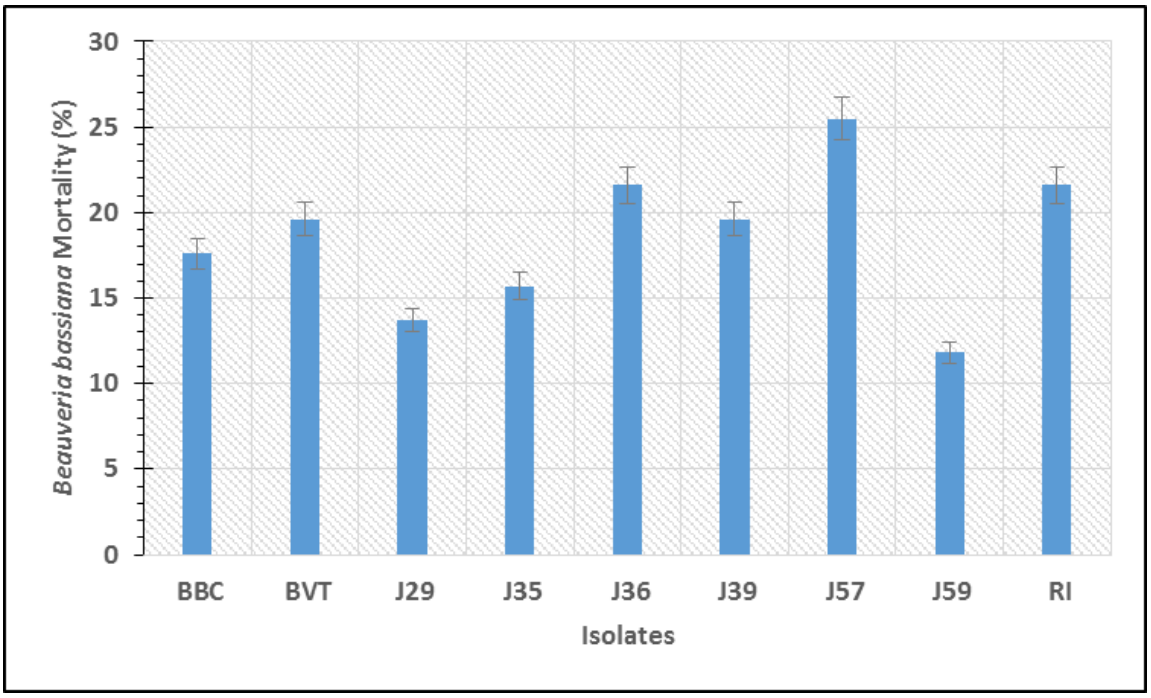

Figure 4.1: Mortality of cowpea bruchids caused by *Beauveria bassiana*

4.5.4. Effect of *Beauveria bassiana* on cowpea bruchids during two months of storage

Beauveria bassiana in suspension form significantly affected the damage on cowpea grain caused by the cowpea bruchid in the farmer simulated experiment. The damaged grains of the cowpea under the farmer-simulated experiment using the suspension form of *B. bassiana* isolates showed significant differences in both assays. The control, where only distilled water was applied, had the highest grain damage in both assays. During the first assay, the treatment with no form of *B. bassiana* (control) applied, had the highest grain damage, which was significantly different from that recorded for isolate BVT®. The least grain damage was recorded for isolate J29, which was not significantly different from that recorded for isolate J36. A similar trend was observed during assay two of the experiment where the treatment with no form of *B. bassiana* (control) was applied, recorded the highest grain damage, and was significantly different from that recorded for isolate J59. The lowest grain damage was recorded for isolate J29, which was significantly different from that recorded for isolate J36 (Fig. 4.2).

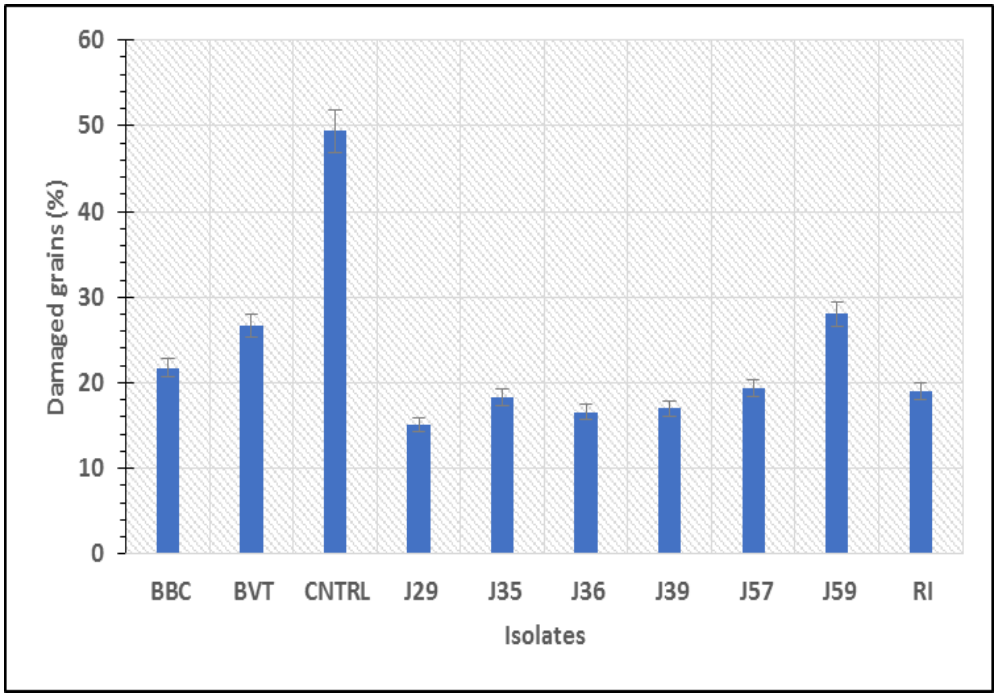

Figure 4.2: Cowpea grain damage after two months of storage for *Beauveria bassiana* isolates in suspension form during assay one and two

The damage on cowpea grains under the farmer-simulated experiment using the powder form of *B. bassiana* isolates was significantly affected in both assays. During the first assay, the control, where only plain ground rice was applied and no form of *B. bassiana* was applied, recorded the highest grain damage, and significantly differed from that of isolate J57. The least grain damage was recorded for isolate J59 and was not different from that recorded for isolates BBC®, BVT®, J36 and J39. A similar trend was observed during assay two where the treatment with no form of *B. bassiana* (control) was applied, recorded the highest grain damage and was significantly different from the grain damage recorded for isolate J57. The lowest grain damage was recorded for isolate J59 which, was significantly different from the grain damage recorded for isolate J39, BVT®, J36 and

BBC®. A higher damage was recorded in the control compared to all other *B. bassiana* treatments (Figure 4.3).

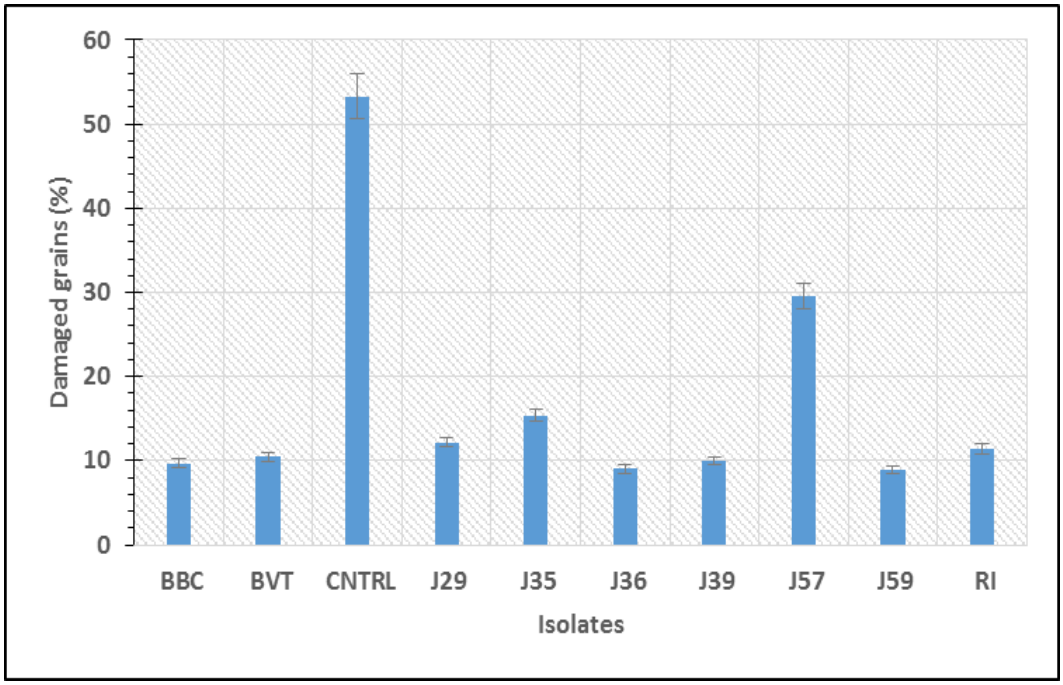

Figure 4.3: Cowpea grain damage after two months of storage under *Beauveria bassiana* isolates in powder form during assay one and two

High damage of cowpea grains was observed in the experimental set up where the control had the inert rice powder compared to the distilled water set up. However, all the tested *B. bassiana* isolates except J57 recorded lower grain damage when *B. bassiana* isolates were used in powder form compared to the liquid suspension (Fig. 4.4).

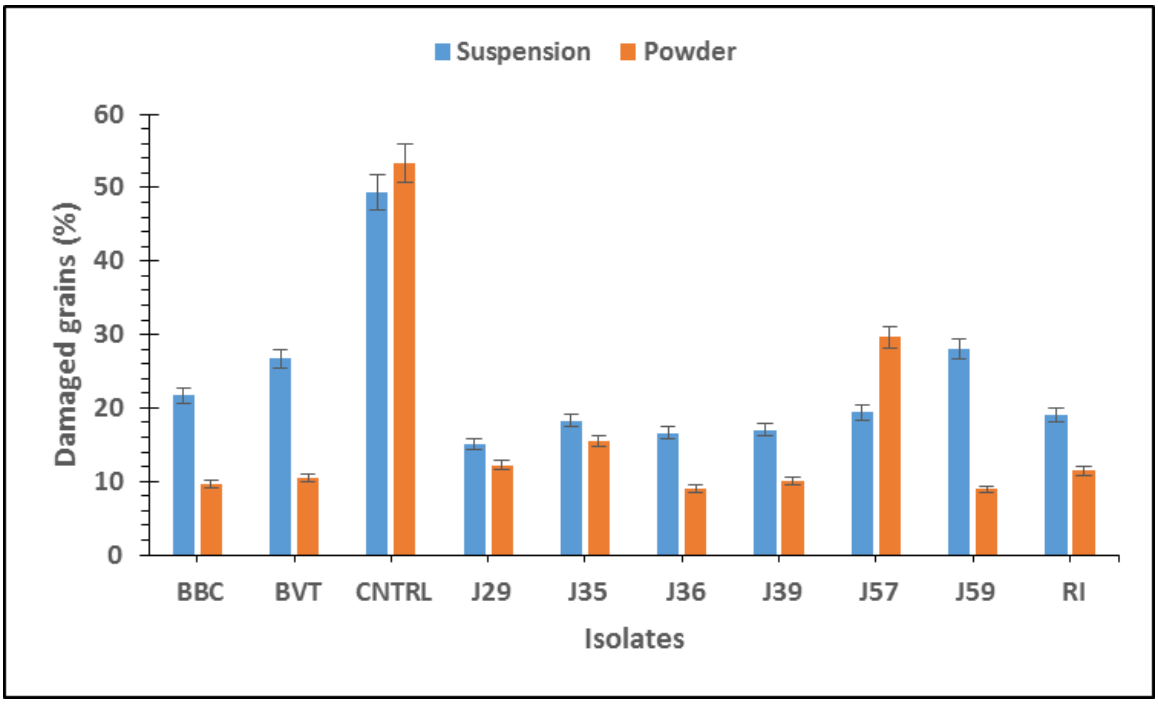

Figure 4.4: Comparison between grain damage after two months of storage using *Beauveria bassiana* isolates in suspension and powder form

A similar trend was observed in assay two where the control with rice recorded a higher grain damage compared to the distilled water. All the isolates tested except J57, recorded a lower grain damage when isolates were used in powder form compared to liquid suspension (Fig. 4.4).

4.6. Correlation analysis of *Beauveria bassiana* growth characteristics, optimal dose rate mortality and grain damage in suspension and powder form

There was a positive correlation between the rate of conidial germination of isolates and the optimal dose rate mortality of cowpea bruchids with a correlation coefficient of r=0.356. The rate of conidial germination recorded a positive correlation with grain

damage in suspension form (r= 0.513), rate of sporulation (r=0.501) and spore concentration (r= 0.303) (Table 4.13).

The optimal dose rate mortality was negatively correlated to the grain damage where *B. bassiana* was used in suspension (r=-0.431) and in powder form (r=-0.813). There was a positive correlation between rate of conidial germination and cowpea grain damage (r=0.513) when the isolates were applied in suspension form and a negative correlation (r=-0.538) when the same isolates were applied in powder form. A positive correlation was recorded between the relative hyphal growth and the mortality of the cowpea bruchids (r=0.428). The viability of spores had a positive correlation with the mortality of bruchids (r=0.751) (Table 4.13).

Table 4.13: Spearman's correlation (P<0.05) between *Beauveria bassiana* growth characteristics, optimal dose rate mortality and grain damage in suspension and powder form matrix

	Rate of Conidial germination	Mortality %- ODR	Hyphal Growth	Rate of Sporulation	Spore Concentration	Viability	Grain damage (S)	Grain damage (P)
Rate of Conidial germination	-							
Mortality % - ODR	0.356*	-						
Hyphal Growth	-0.707*	0.428*	-					
Rate of Sporulation	0.501*	0.244*	-0.564*	-				
Spore Concentration	0.303*	-0.222*	-0.101*	-0.035*	-			
Viability	-0.155*	0.751*	0.363*	0.556*	-0.281			
Grain damage (S)	0.513*	-0.431*	-0.710*	0.613	0.453*	-0.242*	-	
Grain damage (P)	-0.538*	-0.813*	0.165	-0.194	-0.317	-0.172*	-0.144	-0.119

ODR - Optimal Dose Rate, S-Suspension, P-Powder

*= Significant at p=0.01

4.7. Discussion

This study showed the potential of *B. bassiana* in controlling *C. maculatus* in cowpea during storage. This could be attributed to the ability of the entomopathogenic fungi, *B. bassiana,* to germinate and penetrate the *C. maculatus* cuticle causing infection and eventual death. These results are in agreement with many previous works. Akbar *et al.* (2004) recorded that *B. bassiana* was highly effective against the major storage grain insect pests. Cherry *et al.* (2005) reported that the use of *B. bassiana* significantly reduced *C. maculatus* population when used as grain treatment for stored cowpea. Equally, Khashaveh *et al.* (2011) reported that pests injurious to stored wheat could effectively be controlled using *B. bassiana.*

All the *B. bassiana* isolates caused variable mortality when applied at different rates to *C. maculatus* in various experiments during this study. While testing for mortality by immersion, isolate BBC® killed the bruchids fastest compared to isolate J57, which was the slowest based on the mortality records obtained during this study. The variation in mortality could be due to the varied virulence of the isolates against the cowpea bruchid. Differences in the mortality rates as caused by the isolates may have been related to differences in the enzymes produced by the various *B. bassiana* isolates, conidial attachments onto the insect cuticle, suppression of the hosts immune system or modes of germination as earlier reported by Chandler *et al*. (1993).

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 Zahra *et al.,* (2011) 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. On fifteenth day, 99-100% mortality was recorded at 4.86×10^6 cfu/ml and 4.86×10^7 cfu/ml concentrations of the fungus using the immersion technique. Shopiya *et al.* (2014) recorded 100% mortality of the test pest at 2.0×10^4, 2.0×10^5 and 2.0×10^6 concentrations by eleventh day of treatment. Cherry *et al.* (2005) on the other hand showed that different *B. bassiana* isolates can provide good *C. maculatus* control by immersion bioassay at 12 days. Isolates BBC® and BVT® had recorded 100% mortality of bruchids by day 13 except for BVT® at 10^4 concentration. This demonstrates that the isolates can provide good control of cowpea bruchid.

Cowpea bruchid percentage mortality increased with time. Day three had the least mortality while the highest was recorded after 15 days during the mortality by immersion experiment. Similar observations were made while testing the isolates for the optimal dose of application.

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. The different concentrations of *B. bassiana* gave significant population reduction that recorded upto 100% and 99% during assay one and two respectively at day 15 for all isolates and concentrations except 4.86×10^4 cfu/ml when applied directly in the mortality by immersion experiment. The increase in mortality was caused by the increase in the viable spores in higher concentrated inoculum for colonization. Shopiya *et al.* (2014) also

tested *B. bassiana* fungi against *Pericallia ricini* and recorded pathogenicity at different conidial concentrations with 2.0 x 10^4, 2.0 x 10^5 and 2.0 x 10^6 spores/ml causing 100% mortality under laboratory conditions demonstrating that mortality depended on inoculum concentration.

All isolates provided the highest mortality at half dose rates compared to full and double dose rates except isolate J29 on day 7 of observation. The lowest mortality was recorded at double dose rate across all isolates during all days of observation. The reduction of mortality when the dose was doubled could be attributed to toxicity of the isolates when used in higher concentration. The increase in mortality when the isolates were used at half concentration demonstrated that lower inoculum could achieve a similar control. Wraight and Ramos (2005) and Ansari *et al.* (2004) reported that insect susceptibility to fungal infection is dose dependent thereby depending on the concentration of conidial suspension.

The damaged grain obtained after use of the isolates in suspension form was higher compared to the one obtained following use of isolates in powder form in both assays. This finding indicates that it is not very necessary to introduce humidity for effectiveness of the fungi in control of cowpea bruchid during storage. It is widely expected that entomopathogenic fungi require high moisture conditions for increased efficacy and that they may not be effective with low moisture. Boucias and Pendland (2008) indicate that there is need for higher humidity for effective fungal germination and conidial activation. Akbar *et al.* (2004) attributed favorable microclimates surrounding the hosts to have the

ability to cause the fungi to work at lower humidity levels. Further, Jeffs *et al.* (1999) reported that the dry conidia of both *M. anisopliae* and *B. bassiana* are hydrophobic where hydrophobic interactions are responsible for adherence of the spore to the cuticle and in response to stimuli the conidia germinates, eventually penetrating the cuticle. In the same regard, the finding that application of the isolate in suspension formulation is not necessary is very important as moisture increase during storage of grains supports growth of molds. Athanassiou and Steenberg, (2007) observed that lower grain and atmospheric moisture, could increase the efficacy of entomopathogenic fungi especially *B. bassiana* in storage facilities.

Secondly, this finding sheds light on the effect of the formulation during delivery of the isolate to the target pest. Moore and Prior (1993) indicates that conidial viability can be affected by formulations in turn affecting their shelf life and virulence of the isolate. Lower grain damage was recorded for all isolates except J57 when the isolates were used in powder form as compared to the suspension form.

The increased mortality of cowpea bruchids was associated with decreasing grain damage demonstrating the potential effects of *B. bassiana* in control of cowpea bruchid in cowpea grain during storage. This indicates that *B. bassiana* is a good choice for control of *C. maculatus*. The lowest grain damage was recorded for isolate J29 and J59 when the isolates were used as a suspension and powder respectively.

Concerning the method of application of *B. bassiana*, higher and faster bruchid mortality was recorded for immersion method compared to contact of insects with isolate spores when mixed with the grain during the optimal dose rate and storage simulation. The

differences could be attributed to the intensity of exposure of the bruchids to the inoculum. Although the fungal inoculum is standardized before application, there may be variation in the inocula that reaches the insects thereby interfering with the extent of colonization, germination, rate of mycelial growth and conidia resulting in variation in cowpea bruchid mortality.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. General Discussion

The study demonstrates that there are variations in growth characteristics of *B. bassiana* isolates. Growth characteristics of entomopathogenic fungi (EPF), concentration of conidial inoculum and the type of formulation used are crucial for an understanding of the considerable differences in the virulent activity of EPF. The spore counts, rate of conidial germination, hyphal growth, rate of sporulation and viability records varied among the isolates. Although *B. bassiana* is known to infect and control a number of pests, little is known on its virulence of the different isolates against the economically important cowpea bruchid during storage.

The study demonstrates that the growth attributes affect the virulence of *B. bassiana* on *C. maculatus.* The conidial viability positively correlated with the optimal dose rate mortality of cowpea bruchids indicating that *B. bassiana* successfully germinated and penetrated the insect cuticle, infecting and causing mortality. Isolates J57 and J35 had the highest mortality dose rate of cowpea bruchids in the two assays the because they had a higher number of viable spores. This demonstrates that viability of the spores define the virulence of entomopathogenic fungi, in this case, *B. bassiana* making it necessary to determine viability of spores when exploring the use of the entomopathogenic fungi in control of cowpea bruchids or other insect pests (De Olivera and Neves, 2004).

Other factors directly and positively correlated to optimal dose rate mortality included hyphal growth, rate of sporulation and rate of conidial germination. These factors are

necessary for successful colonization of the insects by *B. bassiana*. Mohammad and Maher (2014) observed that faster hyphal growth was exhibited in all highly virulent isolates causing faster colonization of the infected insects. Lopes *et al.* (2013) and Faria *et al.* (2015) indicated that conidia that are relatively slow to germinate may have low vigor.

The use of half dose to achieve similar control compared to the full dose and double dose is an important economic aspect in production. Liu *et al.* (2002) emphasized the need to economically evaluate the optimal concentration of conidia to lower the cost of pest control while achieving high effectiveness in control. The study has demonstrated that application in form of suspension recorded lower mortality of bruchids and higher cowpea grain damage compared to the powder form. It is important to consider other forms of formulation beyond the scope of this study to determine the efficacy and shelf life among other stability attributes of the product.

Beauveria bassiana isolates had variable effectiveness in the management of *C. maculatus* infesting cowpea in storage. From the results, *B. bassiana* gave effective control of *C. maculatus* at concentrations 2.0×10^6, 2.0×10^7 and 2.0×10^6 cfu/ml. The study emphasizes the need for higher conidial population for maximum mortality. A reduction in grain damage was recorded when the isolates were used with both powder and suspension formulations although higher reduction was recorded for the powder form. These results demonstrate that the isolates were able to cause mortality to the cowpea bruchids, thereby reducing the damage to the cowpea grain. Isolate J29 and J59 recorded the lowest grain damage when the isolates were used in suspension and powder

form, respectively emerging to be the most effective against the bruchids. Isolates BVT® and BBC® closely followed in reduction of damage in cowpea grain when used both in powder and suspension formulation.

The characteristics of *B. bassiana* isolates in this study with respect to the virulence and pathogenicity indicates that they could potentially be good candidates as an alternative control method of cowpea bruchids during storage. The use of biopesticides such as entomopathogenic fungi as a pest control option is perceived to be ecologically preferable. Farmers will benefit from this storage technology, which is regarded as safe and sustainable and will allow them to store cowpea grain a little longer for consumption or for sale.

5.2. Conclusion

- The findings of this study have demonstrated that *B. bassiana* isolates vary in growth characteristics that influence their pathogenic effectiveness to insect pests. The current study serves as a preliminary screening bioassay procedure to identify the most effective *B. bassiana* isolates against the cowpea bruchid.
- Isolate J29 and J59 were the most effective isolates which recorded the lowest grain damage when the isolates were used in suspension and powder form respectively. Isolates BVT® and BBC® closely followed in reduction of damage in cowpea grain when used both in powder and suspension formulation.
- Results from the current study indicates the pathogenic effectiveness of *B. bassiana* on *C. maculatus* albeit with variations in virulence recorded for the various isolates at varied concentrations, doses and under suspension and powder

formulations. This study represents an important initial step in evaluating the potential of *B. bassiana* in controlling *C. maculatus* in view of future use as a biopesticide.

5.3. Recommendations

- Further studies to determine the growth parameters and mortality values to be used as standards when evaluating the efficacy of *B. bassiana* isolates against cowpea bruchids during storage for regulatory purposes.
- Further experiments to determine the efficacy of the identified efficacious *B. bassiana* isolates under field conditions and for upscaling as commercial products to be used by the farmers. In addition, further experiments should be conducted to determine the appropriate formulations and delivery methods of *B. bassiana* for effective control.

REFERENCES

Abbas A. B., Ndunguru, G., Mamiro, P., Alenkhe, B., Mlingi, N., and Bekunda, M. (2014). Post-harvest food losses in a maize-based farming system of semi-arid savannah area of Tanzania. *J. Stored Prod. Res*., 2014, 57, 49–57.

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*, *18*: 265-267.

Abd-El-Aziz, M. F (2011). Bioactivities and biochemical effects of marjoram essential oil used against potato tuber moth *Phthorimaea operculella* zeller (lepidoptera: Gelechiidae). J.of Life Science (1):288-297.

Abdel-Raheem, M. A., and Zakia, R. A. (2013). Using Entomopathogenic Fungi to Control the Cotton Aphid, *Aphis gossypii* Glover on Sugar Beet. *Bulletin National Research Centre*, 35(1):57-64.

AFFA. (2014). Horticulture validated report 2014.www.agriculture authority.go.ke. Accessed 24-07-2017.

Akbar W., Lord J.C. Nechols J.R., and Howard R.W. (2004). Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. *J. Econ. Entomol*. 97 (2): 273–280

Akello, J., Dubois, T., Coyne, D., and Kyamanya, S. (2009). The effects of *Beauveria bassiana* dose and exposure duration on colonization and growth of tissue cultured banana (*Musa sp*.) plants. *Biol Control*, 49: 6-10.

Akinkurolere, R. O., Adedire, C. O., and Odeyemi, O. O. (2006). Laboratory evaluation of the toxic properties of forest *anchomanes*, *Anhomanes difformis*, against pulse beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Insect Science*, 13, 25-29.

Alizadeh A, Samih MA, Khezri M, Riseh RS. (2007) Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. *Int J Agric Biol*.9: 31-34.

Anankware, P. J., Obeng-Ofori, D., and Osekre, E. (2013). Neglected and Underutilized Insect Species for Nutrition and Health. In*: International Conference on Neglected and Underutilized Species for a Food-Secure Africa*. Accra, Ghana, 27-29 September 2013.

Ansari, M.A., Tirry, L., Vestergaard, S., Moens, M. (2004). Selection of a highly virulent fungal isolate, *Metarhizium anisopliae* CLO 53, for controlling *Hoplia philanthus .J. Invertebr. Pathol*. 85, 89–96.

Asghar, Mohammadbeigi (2013). Virulence of *Beauveria bassiana* and *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae) passed through artificial media and an

insect host *Uvarovistia zebra* (Orthoptera: Tettigoniodae). *International Journal of Agriculture and Crop Sciences*. 6-16; 1147-1152

Asiwe, J. A. N. (2005). Does varying cowpea spacing provide better protection against cowpea pests *Crop Protection*. 24 :465-471.

Athanassiou, C.G. and Steenberg T. (2007) Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). BiolControl 40:411–416

Benz, G. (2015) Environment, In: J.R. Fuuxa and Y.Tanada. (Eds). Epizootiology of Insect Diseases. Wiley and Sons, New York, pp. 177-214.

Boucias, D. G and Pendland, J. C. (2008). Principles of Insect Pathology. Kluwer Academic Publisher, Boston Massachutes.

Boyer, S., Zhang, H. and Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored- product insects. *Bulletin of Entomological Research*, **102**, 213-229.

Bressani, R. (1985). Nutritive value of cowpea. In: Singh SR, Rachie KO (eds). *Cowpea Research, Production and Utilization*. John Wiley and Sons, Ltd., Chichester, NY, pp. 353–359.

Caswell G. H. (1981). Damage to stored cowpeas in Northern Nigeria. *Samaru Journal of Agricultural Research*. 1: 11- 19.

Centre for Agriculture and Bioscience International (CPC) (2018) *Crop Protection Compendium* http://www.cabi.org/cpc/. Accessed 02-2-2017.

Chandler, D., Heale, J.K. and Gillespie, A.T. (1993) Competitive Interaction between Strains of *Verticillium lecanii* on Two Insect Hosts. *Annals of Applied Biology*, 122, 435-440.

Cherry, A. J., Abalob, P. and Hella, K. (2005). A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) *Vuillemin* and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. *Journal of Stored Product Research*, 41: 295–309.

Clarkson, J. M. and Charnley, A. K. (1996). New insights into the mechanisms of fungal pathogenesis in insects. *Trends Microbiol*. 4: 197-203.

Credland, P. F. (1987). Effects of host change on the fecundity and development of an unusual strain of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Journal of Stored Products Research* 23: 91–98

Darbro, J. M., Graham, R. I., Kay, B. H., Ryan, P. A. and Thomas, M. B. (2011). Evaluation of entomopathogenic fungi as potential biological control agents of the dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). *Biocontrol. Sci. Technol*. 21, 1027– 1047.

De Olivera, R.c and Neves, P.M.O.J. (2004). Biological Control compatibility of *Beauveria bassiana* with acaricides. *Neotropical Entomol.,* 33: 353-8

Egho, E. O. (2011). Effects of two agro-ecological zones on insect species of cowpea (*Vigna unguiculata L*.) Walp during the late cropping season, Delta State, Southern Nigeria. Agriculture and Biology. *Journal of North America 2*(3), 448.

Faria, M. R. and Wraight, S. P. (2007). Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Control,* 43: 237-256

Faria, M.R., Lopes, R.B., Souza, D.A., Wraight, S.P. (2015). Conidial vigor vs. viability as predictors of virulence of entomopathogenic fungi. J.Invertebr.Pathol.125,68-72.

Freed, S., Jin, F. L. and Ren, S. X. (2011b). Phylogenetics of entomopathogenic fungi isolated from the soils of different ecosystems. *Pakistan Journal of Zoology*, 43:417-425. 24

Freed, S., Jin, F. L., Naeem, M., Ren, S. X. and Hussian, M. (2012). Toxicity of Proteins Secreted by Entomopathogenic Fungi Against *Plutella Xylostella* (Lepidoptera: Plutellidae). *Int. J. Agric. Biol.,* 14: 291- 295.

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Ko¨hl, J., Marrone, P., Morin, L., and Stewart, A. (2012), 'Have Biopesticides Come of Age', *Trends in Biotechnology*, 30, 250-258.

Grent T. N. H. (2011) *Plant Protection – New Style*. Hoofddorp: Bio-Collection.

Haas-Costa J, Alves LFA, Daros AA. (2010). Safety of *Beauveria bassiana* (Bals.) Vuill to *Gallus domesticus* L. *Brazilian Archives of Biology and Technology*; 53(2):465-471

Hajek, A.E., St. Leger, R.J., 1994. Interaction between fungal pathogens and insect hosts. Annu. Rev. Entomol. 39, 293–322

Hall, R.A. (2011). A new insecticide against greenhouse aphids and whitefly; the fungus *Verticilium lecanii*. *Ohio Florists`Association* Bulletin, 626: 3-4

Hallensleben, M., Polreich, S., Heller, J. and Maass, B. L. (2009). Assessment of the importance and utilization of cowpea (*Vigna unguiculata* L. Walp.) as leafy vegetable in small-scale farm households in Tanzania-East Africa. *Paper presented at the Conference on international research on food security, natural resource management and rural development*. University of Hamburg, Tropentag. October 6-8th, 2009.

Humber, RA. (1997). Fungi: Identification. In: Lacey LA, editor. Manual of techniques in insect pathology. San Diego, CA: Academic Press, 153-185.

Jackai, L. E. N. and Adalla, C. B. (1997). Pest management practices in cowpea. A review. In: Singh, B. B., D. R. Morgan Rai, K. E. Dashiel and L. E. N. Jackai (Eds). *Advances in Cowpea Research.*Co-publication of IITA. pp 240 – 258.

Jeffs, L. B., Xavier, I. J., Matai, R. E. and Khachatourians, G. G. (1999). Relationships between fungal spore morphologies and surface properties for entomopathogenic members of the genera Beauveria, Metarhizium, Paecilomyces, Tolypocladium, and Verticillium. *Can J Microbiol* 45, 936–948.

Jin, X. X., Huang, Y. B., Thomson, S. J. and Elliott, R. B. (2013). Effects of conidial densities and spray volume of *Metarhizium anisopliae* and *Beauveria bassiana* fungal suspensions on conidial viability, droplet size and deposition coverage in bioassay using a novel bioassay spray system. *Biocontrol Sci Technol* 23, 362–366.

Kaita, S. M., Vincent, C., Schmit, J.B., Ramaswamy, S. and Belanger, S. (2000). Effects of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stored Products Res*., 36: 355-364.

Keneni, G., Bekele, E., Getu, E., Imtiaz, M., Damte, T., Mulatu, B. and Dagne, K. (2011). Breeding food legumes for resistance to storage insect pests: potential and limitations. *Sustainability*, 3, 1399-1415.

Khan *et al.* (2012). Entomopathogenic Fungi as Microbial Biocontrol Agent, *Molecular Plant Breeding*, Vol.3, No.7 63-79

Khashaveh A, Ghosta M, Safaralizadeh MH, Ziaee M (2011). The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. J. Agr. Sci. Tech., 13: 35-43.

Kitch, L.W., Ntoukam, G., Shade, R.E., Wolfson, J.L., Murdock, L.L., 1992. A solar heater for disinfesting stored cowpeas on subsistence farms. *J. Stored Prod. Res.* 28, 261–267.

Kimiti, J. M., Odee D. W. and Vanlauwe, B. (2009). Area under grain legume cultivation and the problems faced by smallholder farmers in legume production in the semiarid eastern Kenya. *Journal of Sustainable Development in Africa*, 11:305-315.

Lacey, L. A., Wraight, S. P. and Kirk, A. A. (2008). Entomopathogenic fungi for control of *Bemisia spp*.: foreign exploration, research and implementation, In: Gould, J.K., Hoelmer, K., Goolsby, J. (Eds.), *and Classical Biological Control of Bemisia tabaci in the USA*: A Review of Interagency Research and Implementation. H. Hokkanen, Dordrecht, Progress in Biological Control, series ed. vol. 4. Springer, The Netherlands, pp. 33–70.

Lacey, L. A. and Solter, L. F. (2012). 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.

Li, Z., C. Li, B. Huang and N. Nan. (2001). Discovery and demonstration of the teleomorph of

Beauveria bassiana (Bals) Vuill., an important entomogenous fungus. *Chinese Science Bulletin*, 46: 751– 753.

Liu, H.M., Skinner, M., Parker, B.L., Brownbridge, M. (2002). Pathogenicity of *Beauveria bassiana, Metarhizium anisopliae,* (Deuteromycotina: Hyphomycetes), and other entomopathogenic fungi against *Lygus lineolaris* (Hemiptera: Miridae). *Journal of Economic Entomology* 95, 675–681.

Lopes, R.B., Martins,I., Souza,D.A., Faria, M.R. (2013) Influence of some parameters on the germination assessment of mycopesticides. *J.Invertebr.Patholo*.112, 236-242.

Maina, Y. T. (2011). Effects of different levels of infestation and storage durations on the development of *Callosobruchus maculatus* (Fab.) in stored cowpea *Vigna unguiculata* (L.) Walpers. *Production Agriculture and Technology*, 7 (1): 49 –54.

Makoi JHJR, Belane AK, Chimphango SBM, Dakora FD (2010). Seed flavonoids and anthocyanins as markers of enhanced plant defence innodulated cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Res*. 118: 21-27.

Mbata, G., Reichmuth, C. and Ofuya, T. (1996). A comparative study on the toxicity of carbon dioxide to the developmental stages of *Callosobruchus maculatus* (Fab.) and *Callosobruchus subinnotatus* (Pic.). P*ostharvest Biology and Technology*, 7(3):271-276

Mohammad S. Al-alawi and Maher Obeidat (2014) Selection of *Beauveri*a *bassiana* (Balsamo) Vuillemin isolates for management of *Myzus persicae* (Sultzar) (Hom.: Aphidae) based on virulence and growth related characteristics. *American Journal of Agricultural and Biological Sciences* 9 (1): 94-100

Moore, D and Prior, C. (1993). The potential of mycoinsectides. *Biocontrol News Info.* 14,31N-40N

Ojiem, J. O., Vanlauwe, B., Ridder, N. and Giller, K. E. (2007). Niche-Based Assessment of Contributions of Legumes to the Nitrogen Economy of Western Kenya Smallholder farms. *Plant Soil Journal*. 292: 119-135.

Okosun, O. O. and Adedire, C. O. (2010). Potency of cowpea seed bruchid, *Callosobruchus maculatus* (Fabricius) [Coleoptera: Bruchidae], of African Nutmeg seed [*Monodora myristica* (Gaertn.) Dunal] extracted with different solvents. *Niger. Journal Entomology,* 27: 89-95

Oliveira, I., Pereira, J.A., and Bento, A. (2011) Viability of *Beauveria bassiana* isolates after storage under several preservation methods. *Ann Microbiol* 61: 339.

Oluwafemi, A. R. (2012). Comparative effects of three-plant powders and pirimiphosmethyl against the infestation of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in cowpea seeds. *SOAJ Entomol. 1*, 87–99.

Owolabi, A. O., Ndidi, U. S., James, B. D. and Amune, F. A. (2012). Proximate, Antinutrient and Mineral composition of five varieties (improved and local) of cowpea, *Vigna unguiculata,* commonly consumed in Samaru community, Zaria-Nigeria. *Asian Journal of Food Science and Technology*, 4(2):70-72.

Oyerinde, A. A., Chuwang, P. Z. and Oyerinde, G. T. (2013). Evaluation of the effects of climate change on increased incidence of cowpea pests in Nigeria. *The Journal of Plant Protection Sciences*, *5*(1), 10-16.

Panda, S.S, Sahoo, K, Muduli, S.D, Sahoo,G , Ahemad, M.D.J, Nayak, B.B and Dhal, N.K (2014). Chromium tolerant indigenous fungal strains from industrial effluents of Anugul district, odisha, india. *Biolife*. 2(2):634-640.

Park, C., Kim, S. I. and Ahn, Y. J. (2003). Insecticidal activity of asarones identified in *Acorus gramineus* rhizome against three coleopteran stored-product insects. *Journal of Stored Products Research* 2003; 39(3):333–342.

Potrich, M.; Alves, L.F.A.; Mertz, N.R.; Silva, E.R.L. 2006 Avaliação de *Beauveria bassiana* (Bals.) Vuill. *Metarhizium anisopliae* (Metsch.) Sorok. para controle de *Sitophilus zeamais* (Coleoptera: Curculionidae). *BioAssay,* vol. 12, pp. 1-9.

Radha, R. (2014). Toxicity of three plant extracts against bean weevil, *Callosobruchus maculatus* (F.) and maize weevil, *Sitophilus zeamais* motsch. I*nt. J. Curr. Res*., 6: 6105-6109.

Rahman, A. and Taluker, F. A. (2006). Bioefficacy of some plant derivatives that protect grain against the pulse beetle (*Callosobruchus maculatus). Journal of Stored Products Research*; 6 (3): 1 – 10.

Redden RJ, McGuire J (1983). The genetic evaluation of bruchid resistance in seed of cowpea. *Australian Journal of Agricultural Research* 34:707-715.

Sabbour, M. M. and Abd-El-Aziz, Sh. E. (2010). Efficacy of some bioinsecticides against *Bruchidius incarnates* (Boh.) (Coleoptera: Bruchidae) infestation during storage. *J. Plant Prot. Res*. 50 (1): 25–31.

Sabbour M. M. and Singer, S. M. (2014). Evaluations of Two Metarhizium varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. *International Journal of Science and Research* (IJSR) ISSN (Online): 2319-7064.

Sabo, Elizabeth, Bashir, R. M., Gidado, A. S., Sani, R. M. and Adeniji, O. T (2013) Investigation on production constraints and adoption of inorganic insecticides and spraying regime in management of cowpea (*Vigna uncuiculata* L. Walp) insects in Mubi zone, Nigeria. *Journal of Agricultural Extension and Rural Development* Vol.6 (1), pp.11-20.

Sharma, H. C., Crouch, J. H., Sharma, K. K., Seetharama, N. and Hash, C. T. (2002) Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sci*, 163:381–395

Singh, S.R., B.B. Singh, L.E.N. Jackai and B.R. Ntare, 1983. Cowpea research at IITA: Information series No. 14. International Institute Tropical Agriculture, Ibadan, Nigeria, pp: 20.

St. Leger RJ, and Wang C (2010) Genetic engineering of fungal biocontrol agents to achieve greater efficacy against insect pests. *Appl Microbiol Biotechnol* 85:901–907

Shophiya J. Nancy, K. Sahayaraj, J.M.V. Kalaiarasi and Jebitta M Shirlin (2014) Biocontrol Potential of Entomopathogenic Fungus *Beauveria Bassiana* (Balsamo) Against *Pericallia Ricini* (Fab.) (Lepidoptera: Arctiidae) Larvae. Biolife , 2 (3) 813-824

Steenberg, T. (2005). 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*. 10-11 June, 2005, Locorotondo, Italy

Swella, G. B. and Mushobozy, D. M. K. (2007). Evaluation of the efficacy of protectants against cowpea bruchids (*Callosobruchus maculatus* (F.) on cowpea seeds (*Vigna unguiculata* (L.) Walp.). *Plant Protection Science* 43:68–72

Takim, F. O. and Uddin, R.O. (2010). Effect of weed removal on insect populations and yield of cowpea (*Vigna unguiculata*) (L.) Walp. *Australian Journal of Agricultural Engineering (AJAE),*1 (5):194-199.

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

Timko, M. P. and Singh, B. B. (2008). Cowpea, a multifunctional legume. In: Moore PH, Ming R (eds). *Genomics of tropical crop plants*. Springer, New York, pp 227-257.

Udo, I. O. and Harry, G. I. (2013). Effect of groundnut oil in protecting stored cowpea; *Vigna unguiculata* from attack by cowpea weevil, *Callosobruchus maculatus. J Biol Agric Health Care,* 3(1):89-92.

Umeozor, O.C. (2005). Effect of the infection of *Callosobruchus maculatus* (Fab.) on the weight loss of stored cowpea (*Vigna unguiculata* (L.) Walp). *Journal of Applied Science and Environmental Management,* 9 (1): 169-172.

Varela, A. and Morales, E. (1996) Characterization of some *Beauveria bassiana* isolates and their virulence toward the coffee berry borer (*Hypothenemus hampei)*. *Journal of Invertebrate Pathology,* 67, 147–152

Vilas Boas, A. M., Oliveira, J. V., Campos, A. L., Andrade, R.M., Silva, R. L. X., 1996. (Pathogenicity of wild strain and mutants of *M. anisopliae* and *Beauveria bassiana* to
Callosobruchus maculatus (Coleoptera: Bruchidae). Arquivos de Biologia e Tecnologia, 39: 99–104.

World Bank, NRI and FAO. (2011). *Missing Food:* The case of postharvest grain Losses in Sub-Saharan Africa. Report No. 60371-AFR

Wraight, S.P and Ramos M. E. (2005). Synergistic interaction between *Beauveria bassiana* and *Bacillus thuringiensis tenebrionis* based biopesticides applied against field populations of Colorado potato beetle larvae. *Journal of Invertebrate Pathology*. 90. 139-150.

Zahra Mahdneshin, Samad Vojoudi, Youbert Ghosta, Mohammad Hassan Safaralizadae and Moosa Saber (2011) 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* 5(29), 5215-5220.