INVESTIGATION ON THE SHELF –LIFE (STORAGE TIME) AND VIABILITY OF *Beauveria bassiana* CONIDIA: UNDER VARIOUS TEMPERATURES USING SABOURAUD AND POTATO DEXTROSE MEDIUM.

Report submitted in partial fulfillment of the requirement for BIOL 3018/BL39C.

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May 2012

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ABSTRACT

The Coffee Industry Board (CIB) has undertaken a major *Beauveria bassiana* project since August 2011. The *Beauveria bassiana* is a biological control which is use to combat the Coffee Berry Borer, *Hypothenemus hampei* (Ferrari); this form of naturally occurring pest control is environmental friendly as compared to the use of chemical insecticides.

This research was conducted over a 25 days period using two different media: Sabouraud and Potato Dextrose Agar to aid in the investigation of the shelf-life (storage) and viability of the *Beauveria bassiana* conidia under four separate temperature range. These ranges are 0◦C, 4-8◦C, 10-14◦C and 24◦C; three replicates were used for each media equaling a total of 24 plates at the different temperature range. *Beauveria bassiana* germinated at temperatures of 10-14 and 24◦C, while there was no growth at 0 and 4-8◦C, as expected. Mycelial growth was measured by taking the reading from the centre of the inoculated spore (conidia) suspension on the medium. The measurement was taken at the three longest angles and the mean was determined for each plate. The findings showed respect to the growth of the fungal mycelium (which can be used to assess the viability of

the entamogenous fungus). This study was significantly different ($p < 0.001$) between storage temperature at 0 and 24◦C along with time over the 25 days duration. However, the plates that did not show growth were placed at 24◦C to test if freezing or low temperature had affected the viability; the discovery that it did not affect the viability of the *B. bassiana.*

The viability of four packages were examined, these packages had the same processing date but placed at separate temperatures of 0◦C, 4-8◦C, 10-14◦C and 24◦C respectively. At the end of the 25 days there were checked for viability on PDA medium. Growth was observed only at 0 and 24◦C. The findings indicated that there were no significant differences ($p = 0.056$) between shelf –life and the viability of *B. bassiana* at 0 and 24◦C. Statistical analysis used was the two paired t-Test. In concluding these result demonstrated that faster growth was observed on PDA, this PDA was detected with other microbial contamination. Hence, SDA medium would be more ideal due to the lack of contamination observed also growth on both medium was more rapid at 24◦C than 10-14◦C. The mean surface area at 24◦C was larger than 0◦C for mycelia growth.

Keywords:- *Beauveria bassiana*, *Hypothenemus hampei* (Ferrari); coffee berry borer, Jamaica, Mycelium, Sabouraud and Potato Dextrose agar, conidia, shelf-life, viability and temperature.

ACKNOWLEDGEMENT

First and foremost, I would like to thank the Lord for giving me health and strength in doing this research paper. Thanks to Mr. Jason Clarke from Coffee Industry Board who provided the packaged samples for the culturing of the fungus and for his knowledge in the area of packaging of *Beauveria bassiana* samples for field testing. Thanks extended to Dr. Paula Tennant and Stephanie Lyew who gave me some information regarding the biocontrol agent. Sincere gratitude goes out to Miss. Ann Marie Houssan and Mr. F. Boyd who provided the necessary materials and a laboratory to work in. Finally special thanks to my supervisor Dr. Dwight Robinson for the key role he played in guiding me in the write up of the research as well as analysis of the data.

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OBJECTIVE

Null Hypothesis (H_O):- There is no significant difference between shelf-life and viability of *Beauveria bassiana* conidia when assessed with temperature.

Alternative Hypothesis (H_A):- There is a significant difference between shelf-life and viability of *Beauveria bassiana* conidia when assessed with temperature.

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Introduction

Coffee is the second most traded commodity in the world. Hence, it generates major revenue both locally and abroad. In Jamaica the main regulatory body for the production and sustenance of coffee is the Coffee Industry Board (CIB). Coffee Industry Board was

established in 1950, the aim was to raise and maintain the quality of the Jamaican coffee being exported.

Jamaica produces what connoisseurs rank as one of the world's finest coffees, mostly grown on patches of a few acres between 2,000 to 5,000 feet (610 to 1,525 meters) above sea level. The moist, cool climate of the Blue Mountains lengthens the growing period from five to about 10 months, allowing sugars to develop in the beans that grow inside the berries. Many coffee lovers say the rich brew has a smooth, nutty flavor and a deep, intriguing after taste (*Coffee Industry Board, 2012).*

The roasted beans often sell for about $40 a pound in the United States, up to four times the price of other gourmet coffees. In Japan, the main market for Blue Mountain coffee, the beans fetch as much as $34 for a 100-gram (3.5-ounce) package.

Recently the Coffee Industry Board has been faced with numerous problems such as the global recession which results in higher or increasing prices for commodities: example, fertilizers and insecticides. Hence, the need for farmers to use cheaper and environmental friendly fertilizers and pesticides; the pesticides used is in this experiment is a natural enemy to the coffee berry borer (*Hypothenemus hamperi).* Recently the CIB announced that Jamaica and Japan are collaborating on a new programme for eco-friendly coffee production aimed at attacking pests now bedevilling crops chiefly marketed to the Asian country. The consumers of luxury products such as coffee are insisting that their coffee is free from pesticide residues. This is particularly critical in Japan. Japan is the strongest market for Jamaican coffee, purchasing an average of 85 per cent of Jamaican coffee for the last 10 years; according to Christopher Gentles the director of CIB (Avia Collinder*, 2010).*

Storage and viability can be an essential ingredient in development and sustainability; there is need for both storage and viability in the agriculture sector. This project clearly demonstrated how these two factors under various temperature affects the growth of *Beauveria bassiana* spores. Temperature is an all important factor that influences the growth and reproduction of all living organisms. Two types of media were used: Sabouraud and Potato Dextrose media, these media were ideal for the growth of entomogenous fungus.

Literature Review

Economic importance Coffee Berry Borer, *Hypothenemus hampei*

The coffee berry borer (Hypothenemus hampei) is considered the most important insect pest and the greatest economic threat to coffee (Soto-Pinto, Perfecto & Caballero-Nieto,

2002). Endemic in Central Africa, it has now spread to most coffee growing regions in the world. Plantations at too low altitude are more prone to that pest (Naturland, 2000). Coffee Berry Borer leads to substantial economical loss in the production of coffee production world wide, some countries reported high loss of up to 35% plus losses. The coffee berry borer (*Hypothenemus hampei*) (Ferrari) (Coleoptera: Curculionidae, Scolytinae), called *broca* in Spanish, is a bark beetle endemic to Central Africa that is now distributed throughout all coffee-producing countries in the world, with the exception of Nepal and Papua New Guinea. CBB is the most economically important coffee pest worldwide (Le Pelley, 1968 and Vega 2008). Beetles of the subfamily Scolytinae are among the most damaging insects in the world: Their life cycle inside the host plant makes these insects difficult to control (Rudinsky, 1962). The coffee berry borer female (1.4-1.78 mm) attacks immature and mature coffee berries from about eight weeks after flowering up to harvest season (>32 weeks). Females bore a hole into the coffee berry and then construct galleries in the seeds (beans) where the eggs are deposited, followed by larval feeding on the coffee seed (Bustillo et al. 1998, Barrera 2008). Three types of damage have been reported: 1) premature fall of young berries, 2) increased vulnerability of infested ripe berries to fungus or bacterial infection, and 3) reduction in both yield and quality of coffee, reducing the income of coffee growers (Damon 2000, Jaramillo et al. 2006). The coffee berry borer can cause yield losses of 30-35% with 100% of berries infested at harvest time. Damage may be greater if harvest is delayed (Barrera 2008).

Management of Coffee Berry Borer, *Hypothenemus hampei*

Several management practices are implemented to control the Coffee Berry Borer (CBB), these techniques can be referred to as Integrated Pest Management (IPM). IPM is an approach to pest management which aims to develop the right mix of control measures which are cost effective and safe for the farmer and consumer, but at the same time are ecologically sustainable. While IPM may include chemical control, it usually seeks to minimize or eliminate the use of pesticides because of their cost and the dangers they pose to the environment and human health (*Pesticide Action network United Kingdom*, 1998).

Beauveria bassiana **as a biological control**

The various biological control methods used to control this insect, the fungal entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) is highly promising. The fungus has shown to cause high mortality in various countries where the coffee berry borer is present (Barrios 1992, as cited by Posada, 2008).

As time progresses agriculturalist becomes more aware of the use of chemical insecticides and their environmental impact; this impact have a negative effect such as environmental pollution. The recognition of deleterious effects of pesticides has prompted the development of microbial control agents. Hence, the search for natural enemies to the pest has been paramount in all areas world wide (Dayakar and Kanaujia, 2003).

Beauveria bassiana are commonly used for several economic agriculture insects (pests) such as thrips, whiteflies, aphids, caterpillars, silk worm moths, weevils,

grasshoppers, ants, Colorado potato beetle, mealybugs, and the coffee berry borer (Pedigo, 2002).

Beauveria bassiana is widely used because of the compatibility with other pesticides; *Beauveria* products are compatible with a number of adjuvants and chemical and biological insecticides (*University of Connecticut, 1999*).

Classification of *Beauveria bassiana*

Kingdom:	*Fungi*
Phylum:	*Ascomycota*
Class:	*Sordariomycetes*
Order:	*Hypocreales*
Family:	*Cordycipitaceae*
Genus:	*Beauveria*
Species:	***B. bassiana***

Beauveria bassiana is an imperfect entomopathogenic fungus that attacks a wide range of agricultural pests (Feng et al., 1994) and also grows on soil as saprophyte (Bidochka et al., 1998). This fungus grows naturally in soils throughout the world and acts as a parasite on various arthropod species, causing white muscardine disease; it thus belongs to the entomopathogenic fungi. It is being used as a biological insecticide to control a

number of pests such as termites, thrips, whiteflies, aphids and different beetles. *Beauveria bassiana* can be used as a biological insecticide to control a number of pests such as termites, whiteflies, and many other insects. Its use as an insecticide, the spores are sprayed on affected crops as an emulsified suspension or wettable powder or applied to mosquito nets as a mosquito control agent.

Diversity of *Beauveria bassiana*

As a specie with great ability, *Beauveria bassiana* parasitizes a very wide range of arthropod hosts. However, different strains vary in their host ranges, some having rather narrow ranges, like strain Bba 5653 that is very virulent to the larvae of the Diamondback Moth and kills only few other types of caterpillars. Some strains do have a wide host range and should therefore be considered nonselective biological insecticides. These should not be applied to flowers visited by pollinating insects. This research zones in on the shelf life of *B. bassiana* in Jamaica, this entomogenous fungus agent is used as the main biological control of the coffee berry borer (*Hypothenemus hampei*). The viability of conidia was examined before carrying out the bioassay using the microculture method (De la Rosa *et al*., 1997).

Bassi himself recognized the potential to use organisms such as *Beauveria bassiana* to control insect pests (Bassi, 1836; cit. in Van Driesche & Bellows, 1996)

Description of *Beauveria bassiana*

Beauveria Bassiana causes a muscardine Disease, the word muscardine may be referring to as a type of diseases cause by a fungi. In such disease the fungus emerges from the body of insect, covering the animal with a characteristics kind of fungal mat resembling, in a way, a French bonbon or candy mint (French muscardin). *B. bassiana* was named after Bassi who, in the first half of the nineteenth century, this fungus is responsible for large number of disease in insect and as shown promise as a biological control with pest of economical significance in the field of Agriculture (Debach, 1964). *B. bassiana* usually appears as a loose white cottony or mealy growth, at times completely enveloping the insect. The conidiophores form closely packed conidia, creamy white in colour. The fungus grows more rapidly on artificial media where it produces a somewhat more fluffy type of growth, *B. bassiana* on the integument of the some insects and under conditions of appropriate temperature and humidity.

Application and Mode of Action of *Beauveria bassiana*

B. bassiana conidium sends out germination hyphae that penetrates the body wall of the insect. The insect will die in about three days (Debach Paul, 1964).

According to Cornell University Organic Source Guide *Beauveria bassiana* kills the pest by infection as a result of the insect coming into contact with fungal spores. An insect can come into contact with the fungal spores in several ways: by having the spray droplets land on its body, by moving on a treated surface, or by consuming plant tissue treated with the fungus (the latter is not a major method of uptake). Once the fungal spores attach to the insect's skin (cuticle), they germinate sending out structures (hyphae) that penetrate the insect's body and proliferate. It may take 3-5 days for insects to die, but

infected cadavers may serve as a source of spores for secondary spread of the fungus. Insects can also spread the fungus through mating (Long et al. 2000). High humidity and free water enhance activity of the conidia and the subsequent infection of the insect. Fungal spores are readily killed by solar radiation and infect best in cool to moderate temperatures (Goettel et al., 2000, Wraight and Ramos 2002). Because the spores may have a short life, it is important that the spray or spray deposit has sufficient opportunity to contact the insect. Therefore, good coverage is essential with a large number of droplets containing a high concentration of spores. Care should be taken to apply the material to the undersides of the leaves or wherever the pest species primarily occurs. For insects that bore into a plant (e.g. the European corn borer), control will be very difficult. For best results, applications should be made during the early growth stages of the insect before much damage has occurred, as it may take several days for the insect to die. *Beauveria Bassiana* is more effective on the early stages of insect development as compared to the older stages (Pedigo, 2002). Speed of kill depends on the number of spores contacting the insect, insect age, susceptibility and environmental conditions (*Cornell* University, 2003).

According to - Susan Mahr, University of Wisconsin- Madisons, *(*unknown date): with all insect-pathogenic fungi, *Beauveria* produces spores that are resistant to environmental extremes and are the infective stage of the fungal life cycle. The spores (called conidia in this case) infect directly through the outside of the insect's skin as mentioned above. Under favorable temperature and moisture conditions, a conidium (singular of "conidia") adhering to the host cuticle will germinate. The fungal hypha growing from the spore secretes enzymes which attack and dissolve the cuticle, allowing

it to penetrate the skin and grow into the insect body. Once inside the insect it produces a toxin called Beauvericin that weakens the host's immune system. After the insect dies, an antibiotic (oosporein) is produced that enables the fungus to outcompete intestinal bacteria. Eventually the entire body cavity is filled with fungal mass. When conditions are favorable the fungus will grow through the softer parts of the insect's body, producing the characteristic "white bloom" appearance. Relative humidity must be 92% or more for *B. bassiana* to grow outside the insect. These external hyphae produce conidia that ripen and are released into the environment, completing the cycle. In addition to infecting insects, *B. bassiana* can colonize corn plants, having the capability of living in the vascular tissue of certain corn cultivars as an endophyte. European corn borer tunnelling is reduced in corn plants with the fungus. Studies in Iowa, the fungus colonized the plant when applied as a granular formulation of conidia on foliage at whorl stage, moved internally in the plant, and persisted throughout the season to provide significant suppression of corn borers. *B. bassiana* is available commercially as a microbial insecticide since *Beauveria* can now be mass produced by a fermentation process and formulated to enable the fungus to withstand ultraviolet light, and temperature and humidity extremes commonly encountered in the field.

Influence of Environmental factors on *Beauveria bassiana*

Environmental factors that affect the efficacy of B. *bassiana* are climate; this fungus is likely to be more effective in farm microclimates with high relative humidity, such as valley bottoms (Lo et al. 1999). Entomopathogenic fungi such as *B. bassiana* are usually considered to be more effective at higher relative humidity, but reports differ on

the importance of ambient humidity for the infection of *B. bassiana* in various insects (Ferron 1977, Ramoska 1984, Searle and Doberski 1984, Marcandier and Khachatourians 1987, Luz and Fargues 1999, Haraprasad et al. 2001). Because Diatomaceous earth (DE) is a desiccant, it is most effective at low humidity under which insects transpire at a high rate (Mewis and Ulrich, 2001). Diatomaceous earth and *B. bassiana* seem to be complementary in their moisture optima.

The rate at which *Beauveria* spores kill their host is dependent on temperature. At a constant 72 ^{o}F, small potato beetle larvae are killed in 3-5 days. Under field conditions in Maine, it may take 7 to 10 days to kill larvae (University of Connecticut, *1999*).

The thermotolerance and cold activity of 60 entomopathogenic fungal isolates, including five species of Beauveria were examined as to tolerance of temperatures that might be encountered during field use. High variability in conidial thermotolerance was found among the Beauveria spp. isolates after exposure to 45 degrees C for 2h, as evidenced by low (0-20%), medium (20-60%), or high germination (60-80%). The thermal death point (0% germination) for three rather thermotolerant B. bassiana isolates (CG 138, GHA and ARSEF 252) was 46 degrees C for 6h. At low temperatures (5 degrees C), with few exceptions (e.g. CG 66, UFPE 479, CG 227, CG 02), most of the B. bassiana isolates germinated well and grow faster under low temperatures close to 5^{0}C (fernadez et al., 2007).

Laboratory Culturing of *Beauveria bassiana*

The culturing of the *Beauveria bassiana* starts with sterilised parboiled rice being inoculated with the *Beauveria bassania* fungus, and this is then placed in an incubator

where its growth is monitored over a set period. The rice is, thereafter, transferred to grow bags in a 'cool room', and when there is sufficient quantity it is removed from the bag and stored on sterilised brown paper (still in the cool room) and left to drain for 10 days (Christopher Serju, 2012).

Fungi are dependent on the medium or the substrate for all the elements and compounds they require or utilize, except molecular oxygen and carbon dioxide which they obtain from the atmosphere (Tauro *et al.,* 1984). Knowledge of the effects of theses nutrients may assist in understanding the population dynamics of the fungi under the influence of both biotic and abiotic factors in the soil and help in developing strategies for successful application of the fungi as biocontrol agents (Sharma *et al*., 2002).

For culture growing, the carbohydrate storage should be greatest with glucose as the carbohydrate that supplements the function of storage carbohydrate to supply the organism with an available carbon source under starvation conditions and in some fungi, to supply the spore with an utilizable carbon source (Hegedus et al., 1990)

The production of conidia and their viability dependent upon the culture medium and carbon source plays an important role in determining the quantity and the quality of conidia in terms of spore germination and infection (Pandey and Kanaujia , 2008).

Materials and Methods

Preparing Sabouraud and Potato Dextrose medium

19.5 grams of Potato Dextrose agar (PDA) was mixed in 500ml of distilled water. This solution was agitated for one minute to completely dissolve the medium then placed in an Autoclave at 121°C for 15 minutes.

32.5 grams of Sabouraud Dextrose agar (SDA) was mixed in 500ml of distilled water. This solution was agitated for one minute to completely dissolve the medium; Autoclaving was done at 121°C for 15 minutes.

Collection of samples and assessment of viability

Five (5) package samples were collected from the Coffee Industry Board (CIB), with five different dates. Dates were as follows: November 23, 2011, December 7, 20 2011, January 10 and February 02, 2012. 5 grams were taken from each samples, this 5 grams was mixed separately in different beakers with 100ml of distilled water; 1000 microliter (μ) was pippetted out and plated on SDA medium. All operations were done under the fume hood carrying out aseptic technique. These 5 plates (Petri dish) were eventually place in a room at 24^0C and observed. After growth was observed, the Petri dish which had the highest viability was use for the spore inoculation.

Spore inoculation/suspension

10ml of distilled water was placed in a single beaker. A sterile loop was used take up a surface of fungal mycelium. This surface was immersed in the beaker and mixed until the solution had a cloudy appearance. 100 microliter (μ) was eventually pipette out with a micropipette, and placed in the centre of the 12 PDA and 12 SDA medium.

Transfer and Incubation of Agar medium

The total of 24 medium on Petri dishes was divided in their respective temperature variables. Therefore, a total replicate of 3 plates were used for both SDA and PDA medium at different temperature interval, a total of 6 plates were incubated at 0^0C, 4-8^0C, 10^0C-14^0C, 24^0C, in 4 separate rooms; these temperatures were monitored on a daily basis. A 1 mm graph paper was graduated by a 30cm ruler for precision to measure the mycelium growth of the emerging fungus. Three (3) of the longest angles (radius) on the fungal mycelium were measured from the centre and recorded.

Viability Check of stored packages:

Four sealed package (all labeled February 02, 2012) from the storage were obtained from four separate sites containing different temperatures (0^0C, 4-8^0C, 10^0C-14^0C, 24^0C) respectively. 5g was taken from each package and slightly mixed with 100ml of sterile distilled water. 1000µ was pipette out with a micropipette, and placed in the centre of the 12 PDA medium. Three replicates were used for the four temperature variables to assess viability of *B. bassiana* spores stored at room temperature over a five day period. To determine viability of *B. bassiana* the surface area was precisely measured on a 1mm graph paper.

Result and Discussion

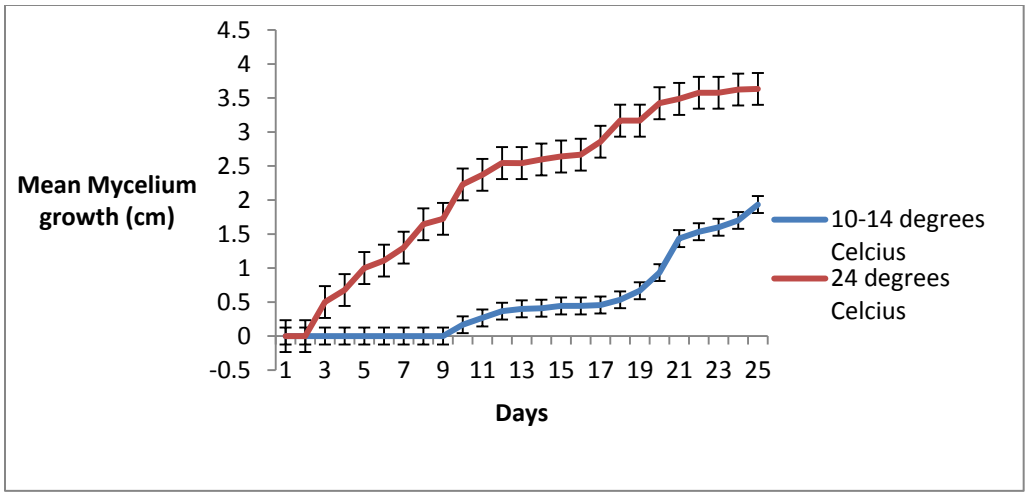

Figure 1(a). Graph of the mean mycelium growth over a 25 days period on SDA medium at two temperatures.

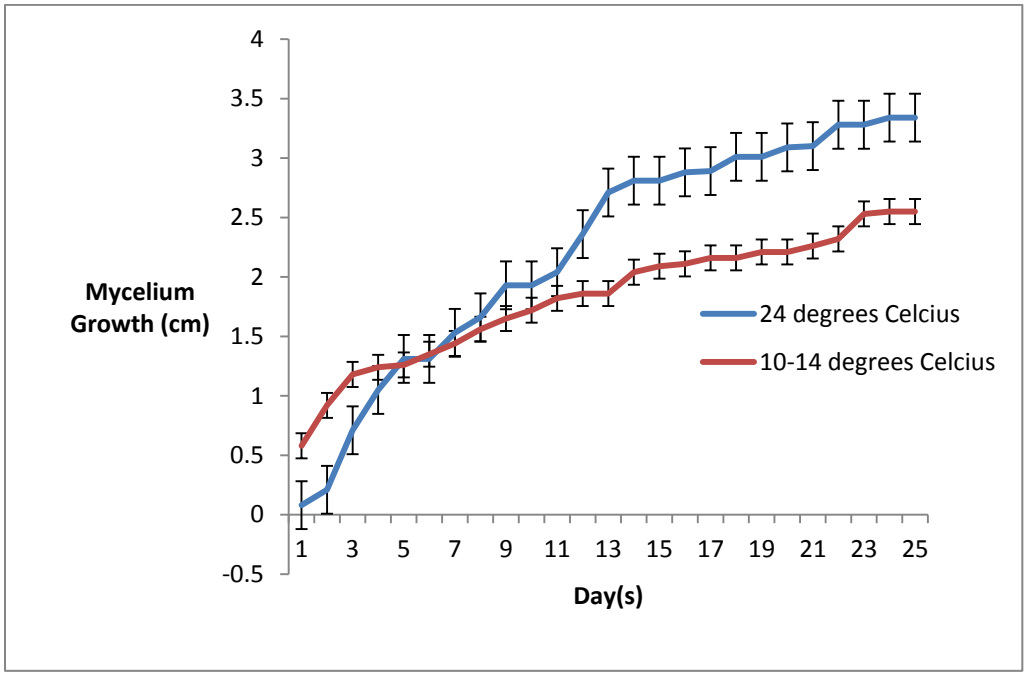

Figure 1(b). Graph of the mean mycelium growth over a 25 days period on PDA medium at two temperatures.

Figure 1(a), consist of two mean growth curves: 10-14◦C and 24◦C, the 24◦C curve had the highest mycelia growth; Mycelial growth (germination) began after 3 days as compared to curve 10-14◦C where growth began after 10 days, this curve had the lowest

germination rate. Both curves had steady progression of growth over the 25 days period, with curve 24◦C increases more rapidly than curve 10-14◦C. However, curve at 24◦C levels off during the latter stages of the experiment while 10-14◦C was still on the rise.

Similarly Figure1 (b), consist of two mean growth curves 10-14◦C and 24◦C, the 24◦C curve had the highest mycelia growth overall. Mycelial growth (germination) began after the first day for all curves. Curve 10-14◦C germination rate was greater, from day 1 to day 3, on day 4 both of the two curves had the same mean mycelium growth (germination level); at the 5^{th} day curve 24◦C had slightly increased over curve 10-14◦C, the 6^{th} day curve 10-14◦C had a slight increase in germination. However, day 7 it was distinct that curve 24◦C had higher germination through out as the other curve steadily increased.

Based on the findings, growth was recorded at two temperatures: 10-14◦C and 24◦C on both PDA and SDA medium. The growth on the PDA medium showed that there is a significant difference (the two-tailed paired test was done) between shelf-life of *Beauveria bassiana* spores and temperature (p-value less than 0.001). Over the 25 days period the mean growth curve increases on a daily basis, this may be inferred that the temperature of 24◦celcius being favourable for growth of *B. bassiana.* The growth rate influenced by temperature was significantly different (p-value less than 0.001).

The growth rate was also significantly influenced by the time, as day progresses length of the mycelium increases gradually (p-value is less than 0.001).

The ideal results would be to get no growth at temperatures 0◦C and 4-8◦C. Hence, the graph showed that no growth was observed during the 25 days of storage for

plates at 0◦C and 4-8◦C; since there is no growth curve. However, after plates were removed from storage area and placed at 24◦C growth began after 3 days for plates that were incubated (stored) at 4-8◦C while those at 0◦C had growth after 5 days. This result is corresponding to Clark, et al., (2009): low temperature does not disrupt or harm microbial cells; during this time the *B. bassiana* cellular metabolism were halted. Inside the *B. bassiana* the membrane begins to gel at low temperature; transportation process begins to slow down that growth cannot occur. This kind of storage or incubation technique is ideal for long term self life of the biological agent for future use in the field, in other words it could be said that this method is needed for preservation of conidia. When temperature is at 10-14◦C, it may be that the enzymes inside the cell are reacting at a constant rate. Therefore, cellular metabolism is at an optimal rate for the entamogenous fungus, while at 24◦C enzymatic reactions may be occurring at a maximal possible rate. At this temperature it could be concluded that the *B. bassiana* feeds on the available nutrients in the medium, simultaneously there is a possibility that the mycelium growth increases until there is depletion of nutrients on the PDA and SDA medium or the decrease in space on the petri dish with the media; this is depicted in Figure 1(a), at 24◦C the curve levels off at day 21 to 25 days, and Figure 1 (b) at 24◦C, the curve levels off at day 24. 10-14◦C the curve levels off at day 23.

Time significantly influenced the growth rate of the *B. bassiana* mycelium (p-value less than 0.001); most fungal specie develops or germinates as time progresses. Furthermore, temperature simultaneously influences the growth of mycelium positively: the curve of 24◦C constantly increases; this corresponds to the work of Croft B.A. et al. (1998) and Bugeme et al. (2008). Error bars were used to show the margin of error based

on the measurement of the growth rate (length); it can be deduced that the longer the growth of mycelium the larger the value for the standard error.

The germination of *B. bassiana* followed two characteristic forms; a relatively short hyphal growth followed by penetration or extensive mycelial growth and branching, was observed on the PDA and SDA media. However, the SDA media had less mycelium growth than the PDA as demonstrated by Figure 1(a), This is true because the PDA media is a rich nutrient media which can be used to culture a wide range of fungus, this media is not as complex as the SDA media which is a more specific media for fungus due to its acidity, this is acidity level is beneficial because it inhibits the growth of bacteria. Therefore, the SDA medium is a selective or restrictive (Anthony, 2012). Also, during the experiment there were no signs of contamination from any other organisms such as bacteria or fungi. It could be concluded that *B. bassiana* germinated faster than on the PDA medium as compared to the SDA as indicated by the both Figure 1 (a) and (b).

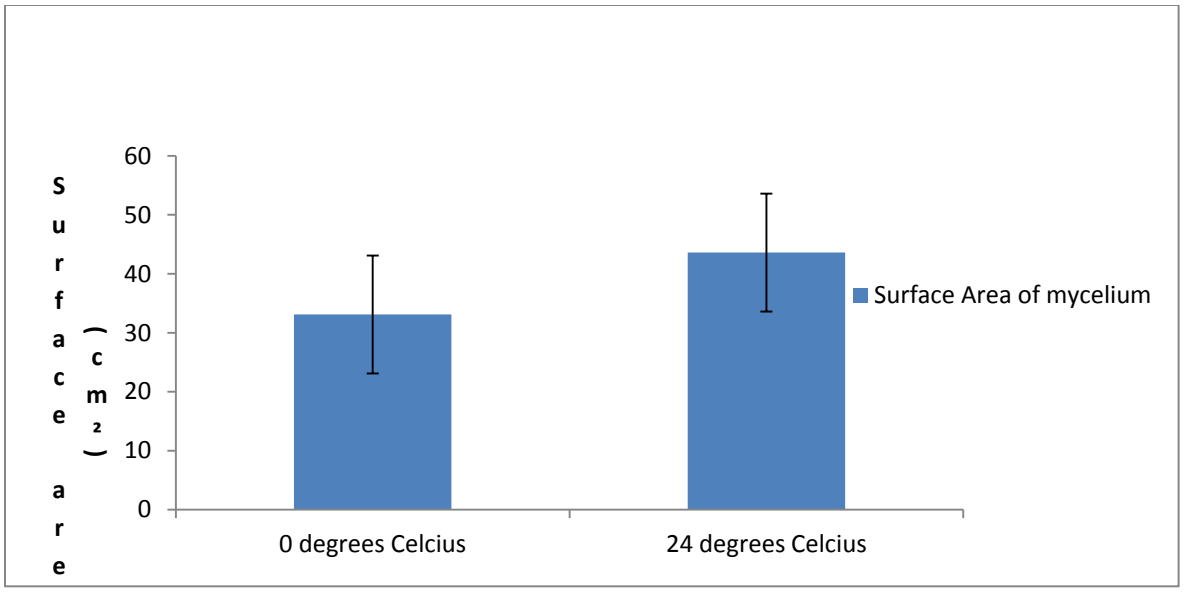

Figure 2. Bar graph of temperatures 0◦C and 24◦C and the mean surface area on PDA medium.

There is no significant difference (p= 0.056) between shelf life and viability of *B. bassiana.* Hence, there is no difference between the 25 days of storage with the package conidia (on rice) and the surface area of the mycelium growth under both 0◦C and 24◦C temperature. The α or *Significant* Two-tailed Result (value) is higher than 0.05 and therefore, using the decision rule, one should state there is no significant difference on the basis that there is not enough evidence to state otherwise. Therefore, it could be concluded that packaging and storing of *B. bassiana* conidia at 0◦C and 24◦C would lead to viable germination of fungus at similar rate on a daily basis.

During the observational period plates were observed that had contaminations from other unknown fungus which can leads to inhibition of the *B. bassiana* growth

creating an antagonistic relationship. One can hypothesis that these fungus were transferred to the medium by mites in the storage area as cited by Choi, et al. (1999).

Conclusion and Recommendations

1)**.** Base on the culturing of the *B. bassiana* on Sabouraud Dextrose Agar (SDA) medium could be recommended over Potato Dextrose Agar (PDA) medium due to its selective nature, which inhibits bacterial growth.

2). Packages containing *B. bassiana* conidia can be placed at temperatures 0◦C and 24◦C for storage by farmers, this will lead to germination.

3). The mean growth rate of *B. bassiana* at temperature 24 is significantly higher ($p \leq 0.001$) than the mean growth rate at 10-14◦C. Therefore, the mean mycelium growth was greater at 24◦C for the entamogenous fungus on both SDA and PDA medium. Further temperature assessment could be done at different temperature range overtime a longer time period.

Limitations

1. Duration of 25 days to complete the research is inadequate for the storage (shelf-life) of *Beauveria bassiana.* This research should span over a longer duration to get scientifically sound data. Therefore, continued research should be carried out to determine storage and viability.
2. Several PDA plates had sign of contamination from other fungus; this was due to difficulty of culturing fungi. Strict aseptic techniques should be adhered to at all times.
3. To test efficacy of viability on *Beauveria bassiana*, a conidial count should be carried out using a heamatocytometer.
4. Manual thermometer was used to check the temperature of each storage area; a computerized or automated temperature recorder should be used to regular monitor the temperature for any deviations. Due to the fact that automated would pick up fluctuations in temperature.
5. Continuous and thorough research is needed in the investigation of physiology, morphology, and molecular biology of *Beauveria bassiana* in order to effectively use it as a biocontrol against the coffee berry borer (*Hypothenemus hampei).* To improve storage and formulation of application of the biocontrol.
6. To identify the various strains of *B. bassiana* in Jamaica, this would be useful to test how the strains could be cultured, and stored for their effectiveness at different temperature.

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APPENDIX

Day 1: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	0.2	0.1	0	0	0	0
	0.1	0.1	0	0	0	0
	0.1	0.1	0	0	0	0
Average	0.13	0.1	0	0	0	0

Day 2: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	0.5	0.2	0	0	0	0
	0.5	0.2	0	0	0	0
	0.3	0.2	0	0	0	0
Average	0.43	0.2	0	0	0	0

Day 3: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	1.7	0.9	0	0.5	0.5	0.5
	1.2	0.9	0	0	0	0
	0.8	0.9	0	0	0	0
Average	1.23	0.9	0	0.5	0.5	0.5

Day 4: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	1.75	2.4	0	1	1.5	0.5
	1.2	1.4	0	0.5	0.5	0.9
	1.2	1.5	0	0.4	0.3	0.7

Average	1.38	1.77	0	0.633	0.7	0.7

Day 5: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	3.1	2.4	0	1	1.5	0.7
	1.6	1.4	0	1	0.5	0.7
	2.0	1.5	0	1	0.3	1.0
Average	2.23	1.77	0	1	1.2	0.8

Day 6: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	3.1	2.4	0	1	1.5	0.7
	1.6	1.4	0	1	0.5	0.7
	2.0	1.5	0	1	0.3	1.0
Average	2.23	1.77	0	1	1.2	0.8

Day 7: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	3.1	2.9	0	1.5	2.1	1.7
	1.6	2.0	0	1.4	1.0	0.8
	2.0	2.2	0	1.0	1.2	1.0
Average	2.23	2.37	0	1.3	1.43	1.17

Day 8: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	0.5	0.2	0.2	1.0	2.0	1.0
	0.5	0.2	0.2	1.0	0.6	1.0
	0.3	0.2	0.2	1.0	1.4	1.0
Average	2.37	2.40	0.2	1.0	1.33	1.0

Day 9: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	0.5	0.2	0.2	1.0	2.0	1.0
	0.5	0.2	0.2	1.0	0.6	1.0
	0.3	0.2	0.2	1.0	1.4	1.0
Average	2.37	2.40	0.2	1.0	1.33	1.0

Day 10: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	3.6	3.2	1.0	2	2.2	2.3
	2.3	2.2	0.4	1.8	1.4	1.6
	2.3	2.0	0.4	1.6	1.0	0.9
Average	2.73	2.47	0.6	1.8	1.53	1.6

Day 11: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	3.7	3.3	1.1	2.2	2.2	2.4
	2.3	2.3	0.5	1.8	1.5	1.7
	2.5	2.1	0.6	1.6	1.1	1.0
Average	2.83	2.57	0.73	1.87	1.6	1.7

Day 12: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.0	2.9	0.9	2.8	2.5	2.8
	2.9	3.5	1.0	1.7	2.3	1.9
	2.6	2.6	0.8	1.9	2.2	2.0
Average	3.17	3.0	0.9	2.13	2.33	2.23

Day 13: Three replica plates on both PDA and SDA plates at 24^0C.

PDA	SDA

	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.2	3.7	2.0	3.1	2.6	2.8
	3.2	3.1	1.0	2.0	2.4	2.1
	2.9	3.3	1.0	2.0	2.4	1.9
Average	3.43	3.37	1.33	2.37	2.47	2.27

Day 14: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.0	3.9	2.0	3.2	2.6	3.0
	3.5	3.3	1.0	2.4	2.5	2.1
	3.0	3.3	1.3	2.3	2.7	2.1
Average	3.50	3.50	1.43	2.63	2.6	2.4

Day 15: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.0	3.9	2.0	3.2	2.6	3.0
	3.5	3.3	1.0	2.4	2.5	2.1
	3.0	3.3	1.3	2.3	2.7	2.1
Average	3.50	3.50	1.43	2.63	2.6	2.4

Day 16: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.1	3.9	2.0	3.4	3.0	3.0
	3.5	3.7	1.0	2.5	2.5	2.0
	3.0	3.4	1.3	2.5	2.7	2.4
Average	3.53	3.67	1.43	2.8	2.73	2.47

Day 17: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.1	3.9	2.0	3.5	3.1	3.4

	3.5	3.7	1.0	2.6	2.5	2.5
	3.0	3.4	1.3	2.5	3.1	2.5
Average	3.53	3.67	1.43	2.87	2.9	2.8

Day 18: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.1	4.0	2.0	3.5	3.2	3.6
	3.6	4.0	1.0	3.0	3.4	2.7
	3.0	4.0	1.4	2.6	3.2	3.3
Average	3.57	4.0	1.47	3.03	3.27	3.2

Day 19: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.1	4.0	2.0	3.5	3.2	3.6
	3.6	4.0	1.0	3.0	3.4	2.7
	3.0	4.0	1.4	2.6	3.2	3.3
Average	3.57	4.0	1.47	3.03	3.27	3.2

Day 20: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.2	4.0	2.0	4.0	3.2	3.7
	4.0	4.0	1.2	3.3	3.4	3.5
	3.0	4.0	1.4	2.8	3.2	3.7
Average	3.73	4.0	1.53	3.37	3.27	3.63

Day 21: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.3	4.0	2.0	4.1	3.2	3.8
	4.0	4.0	1.2	3.4	3.5	3.7
	3.0	4.0	1.4	2.8	3.2	3.7

Average	3.77	4.0	1.53	3.43	3.30	3.73

Day 22: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.4	4.0	2.0	4.2	3.2	3.8
	4.0	4.0	1.2	3.4	3.5	3.7
	4.5	4.0	1.4	2.5	3.2	3.7
Average	4.3	4.0	1.53	3.70	3.30	3.73

Day 23: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.4	4.0	2.0	4.2	3.2	3.8
	4.0	4.0	1.2	3.4	3.5	3.7
	4.5	4.0	1.4	2.5	3.2	3.7
Average	4.3	4.0	1.53	3.70	3.30	3.73

Day 24: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.5	4.0	2.0	4.2	3.3	3.9
	4.5	4.0	1.2	3.4	3.5	3.7
	4.5	4.0	1.4	2.5	3.4	3.7
Average	4.5	4.0	1.53	3.70	3.40	3.77

Day 25: Three replica plates on both PDA and SDA plates at 24^0C.

PDA				SDA		
	Plate# 1	Plate# 2	Plate# 3	Plate# 1	Plate# 2	Plate# 3
	4.5	4.0	2.0	4.2	3.3	3.9
	4.5	4.0	1.2	3.4	3.5	3.7
	4.5	4.0	1.4	2.5	3.5	3.7
Average	4.5	4.0	1.53	3.70	3.43	3.77

Day 1: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.0	0	0.9
	0.5	0	0.9
	1.0	0	0.9
Average	0.83	0	0.9

Day 2: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.0	1.0	0.9
	0.6	1.0	0.9
	1.0	1.0	0.9
Average	0.87	1.0	0.9

Day 3: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.2	1.1	1.2
	1.5	1.2	1.2
	1.0	1.0	1.2
Average	1.23	1.1	1.2

Day 4: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	1.5	1.7
	1.0	1.2	1.0
	1.2	1.2	0.9
Average	1.23	1.3	1.2

Day 5: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.3	1.5	1.7
	1.0	1.2	1.0
	1.5	1.2	0.9
Average	1.27	1.3	1.2

Day 6: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.4	1.5	1.5
	1.0	1.3	1.4
	1.6	1.8	0.8
Average	1.3	1.53	1.23

Day 7: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	1.5	1.0
	1.4	1.3	2.0
	1.1	1.8	1.4
Average	1.33	1.53	1.47

Day 8: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	2.0	1.8
	1.5	1.0	1.5
	1.6	1.7	1.4
Average	1.53	1.57	1.57

Day 9: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3

	1.8	2.0	1.9
	1.6	1.1	1.4
	1.5	1.8	1.8
Average	1.63	1.63	1.70

Day 10: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.4	2.1	1.7
	2.0	1.2	1.6
	2.0	2.1	1.4
Average	1.80	1.8	1.57

Day 11: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	2.1	1.7
	2.0	1.2	1.7
	2.0	2.1	2.1
Average	1.83	1.8	1.83

Day 12: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	2.1	1.7
	2.0	1.3	1.8
	2.0	2.2	2.1
Average	1.83	1.87	1.87

Day 12: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.5	2.1	1.7
	2.0	1.3	1.8

	2.0	2.2	2.1
Average	1.83	1.87	1.87

Day 14: Three replica plates on both PDA and plates at 24^0C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.6	2.2	2.3
	2.0	2.3	2.0
	2.0	1.9	2.1
Average	1.87	2.13	2.13

Day 15: Three replica plates on both PDA and plates at 10-14^0C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.6	2.2	2.3
	2.0	1.9	2.1
	2.0	2.3	2.4
Average	1.87	2.13	2.27

Day 16: Three replica plates on both PDA and plates at 24^0C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	1.8	1.5	2.3
	2.0	2.6	2.1
	2.0	2.3	2.4
Average	1.93	2.13	2.27

Day 17: Three replica plates on both PDA and plates at 10-14^0C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.0	1.5	2.3
	2.0	2.6	2.1
	2.2	2.3	2.4
Average	2.07	2.13	2.27

Day 18: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.0	2.2	2.3
	2.0	1.9	2.1
	2.2	2.3	2.4
Average	2.07	2.13	2.27

Day 19: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.0	2.4	2.3
	2.0	1.9	2.2
	2.2	2.4	2.5
Average	2.07	2.23	2.33

Day 20: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.0	2.4	2.3
	2.0	1.9	2.2
	2.2	2.4	2.5
Average	2.07	2.23	2.33

Day 21: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.1	2.4	2.3
	2.0	1.9	2.2
	2.2	2.4	2.6
Average	2.1	2.30	2.37

Day 22: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.1	2.5	2.6
	2.0	1.8	2.3
	2.2	2.6	2.5
Average	2.1	2.3	2.47

Day 23: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.1	2.7	2.4
	2.0	2.7	2.7
	2.2	3.5	2.5
Average	2.1	2.97	2.53

Day 24: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.2	2.8	2.4
	2.0	2.7	2.7
	2.2	3.5	2.5
Average	2.13	3.0	2.53

Day 25: Three replica plates on both PDA and plates at 10-14^{0}C.

PDA			
	Plate# 1	Plate# 2	Plate# 3
	2.2	2.8	2.4
	2.0	2.7	2.7
	2.2	3.5	2.5
Average	2.13	3.0	2.53