# The Pathogenicity of *Beauveria bassiana* on *Diatraea centrella* Larva of Sugarcane in Guyana

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**ABSTRACT---** ***Sugarcane is a highly beneficial crop grown worldwide and is greatly influenced by numerous insect pests. These insect pests have adapted to the diversity of conditions under which sugarcane is grown thus causing a great degree of damage to the crop. These insect pests feed on different parts of the sugarcane. For instance, the early shoot borer attacks the cane before internode formation, while the internode borer feeds after internode formation, the foliar pest feeds on the leaves and some would feed on the rootstalks.***

***Guyana has large areas of land which are free of agricultural chemicals and thus are suitable for organic farming. Organic farming is practiced in Guyana on a small scale. However, crops such as cocoa, pineapple and heart of palm have already been grown organically for export. Similarly, like organic farming, biological control of pests and diseases is also encouraged worldwide. Biological control in agriculture is a method of controlling pests such as insects, weeds and diseases by using natural enemies such as predators, parasites, herbivores, or any other natural mechanisms. In addition, like organic farming biological controls are also ecologically friendly and are very effective. Both approaches (organic farming and biological control) are significant since the use of pesticides and chemical fertilizers would be eliminated, thus reducing health issues in the environment and of consumers.***

***This research work is on the pathogenicity of Beauveria bassiana on the insect pest Diatraea centrella larva of sugarcane in Guyana. The entomopathogenic fungus, Beauveria bassiana, is a promising control agent against many insect pests [1]. Beauveria bassiana is commonly found in soils worldwide and has a host range of over 700 insect species, including insects from the orders Lepidoptera, Coleoptera and Hymenoptera. The research carried out focuses on the effect of Beauveria bassiana spores on Diatraea centrella larva and the rate at which these spores are effective, as well as the target site of infection of these spores on the larva.***

***The study conclusively proves that a higher number of Beauveria bassiana spores are more effective on Diatraea centrella larva in a short period of time and the dorsal, ventral and intestinal tissues of the Diatraea centrella larva are more susceptible to the spores of Beauveria bassiana.***

**Keywords---** Pathogenicity, Beauveria bassiana, Diatraea centrella, Sugarcane, Pathogenicity

## 1. INTRODUCTION

Agriculture involves the growing of crops and is considered to be one of the leading economic activities in Guyana. In Guyana, agriculture represents a substantial amount of Guyana's Gross Domestic Product (GDP), which is about 25 percent. In 2004, agricultural exports were about a third of Guyana's total exports. In 2005, approximately 90 percent of Guyana's agricultural exports were rice and sugar products. With adequate investments, Guyana could indeed become the 'breadbasket of the Caribbean' and at the same time increase its exports in North America and Europe. Numerous crops are grown in Guyana; in the nineteenth century sugar and rice were the primary agricultural products where sugar was primarily produced for export while rice was mostly produced for domestic consumption. Other crops that are grown in Guyana includes bananas, coconuts, coffee, cocoa, citrus fruits and small quantities of vegetables and tobacco. In addition, some farmers were successful in growing and expanding on specialty products, which include heart-of-palm and asparagus, which were exported to Europe.

Sugarcane, which is one of the main economic crops grown in Guyana, is currently estimated to be cultivated on 50, 000 hectares of land in Guyana. Approximately 3.5 million tons of canes are produced each year from eight sugar estates belonging to the Guyana Sugar Corporation [2]. In recent years, it is estimated that 3.3 million tons of sugar is the average annual production of which 90% was exported mainly to the United States and the European Community. The production of sugar in Guyana accounts for nearly 12% of Guyana's Gross Domestic Product (GDP), over 20% of Guyana's export, approximately 15% of Guyana's Gross National Product (GNP) and 40% of its agricultural output. The sugarcane industry in Guyana, which was established three centuries ago, is the biggest income generator, the main corporate contributor to public revenue and the chief source of foreign exchange. The by-product sugar and molasses of sugarcane is used for the fermentation of alcohol, beverage- making and in the brewing industry. By-products of sugarcane are also used in the confectionary department to produce sweets and other tasty products. Furthermore, this industry is prospective to produce and process agricultural goods, which can be used for energy purposes that is, the by-product bagasse (crushed cane fibers) is used to generate electricity. It is estimated that 30.8 million liters of ethanol per annum can be obtained from the sugarcane industry in Guyana which can be used as a source of energy. Guyana's sugarcane industry provides direct employment to 20 000 people [3].

The control of insect pests is a major problem in every sugar industry. Some of these pests are common to several countries while others are specific to one area. In Guyana, there are numerous species of insect pests affecting the yield of cane and the production of sugar. Some of these insect pests include the following: the Small Moth Borer (*Diatraea sp*.) it tunnels through the cane and also causes red rot, which is very common; the Giant Moth Borer (*Castnia licoides*) (it also tunnels through the stalks, leaving bored portions thus yielding virtually no sugar); species of the Hard Back Beetles (*Dyscenetus bidentatus, D. geminatus*) and other insect species which cause great destruction to the plant. Pest management is therefore required for sugarcane in Guyana. Presently in Guyana no chemical control is used on insect pests of sugarcane. However, when a variety of sugarcane is infested with pests, it is usually abolished or sometimes it may be replanted after about three growing seasons.

*Diatraea centrella* is basically a pest of Northern South America, specifically in Guyana where it is the dominant species and a major pest of the sugarcane. The *Diatraea centrella* larva can be distinguished from the larva of the *Diatraea saccharalis* by its brown forehead. *Diatraea centrella* lay their eggs in masses under the green cane leaves. The newly emergent larvae move up and down the leaves feeding on its surface tissues and later moving behind the leaf sheath feeding on its tender tissues. In addition, when the larvae become older they bore into the stalks of the sugarcane forming a tunnel. However, these older larvae may abandon their tunnel and move into the harder, mature internodes where feeding usually occurs in the top internodes. Near the edge of the stalks these mature larvae hollow a pupation chamber and chew a hole large enough for the adults to escape. The adults have a short lifespan with few living more than three days after emergence, during which mating and egg-laying occur. The rate at which *D. centrella* larvae develop is regulated by many factors including temperature and food quality. Extreme climatic conditions such as cold weather, would reduce *Diatraea* species to a single stage, thus only a very narrow range of life stages would be present for several subsequent generations [4] [5].

*Beauveria bassiana* is a highly beneficial fungus, which kills its host (insect pest) by infection when the insect is in contact with the fungal spores. When the spores of this fungus come in contact with the cuticle of susceptible insects, they germinate producing structures called hyphae that penetrate directly through the cuticle to the inner body of the insect. The fungus inside its host proliferates, producing toxins and draining the insect of nutrients. This fungus causes white muscardine disease in the insects it contaminates and may take 3-5 days for the insects to die [6]. Once the fungus has killed its host, it grows back out through the softer portions of the cuticle, covering the insect with a layer of white mold (hence the name white muscardine disease). Contaminated insect can spread the fungus through mating, while infected cadavers may serve as a source of spores for secondary spread of the fungus. In addition, the fungal spores infect best in cool to moderate temperatures and are readily killed by solar radiation [7] [8].

A research was conducted in Yaounde to determine the pathogenicity of native isolates of *Beauveria bassiana* (obtained from a stem borer in a maize farm) to control malaria vectors, *Anopheles gambiae*. Results from this study showed that an increased in time and density of dry conidia of *Beauveria bassiana* increases mortality of both male and female *A. gambiae*. Treated *A. gambiae* had an average mortality of 80% compared to the 10% in the untreated group [9].

In a laboratory study, three strains of *Beauveria bassiana* were tested for pathogenicity against adult cactus weevil (*Metamasius spinolae*). *M. spinolae* were inoculated with the *B. bassiana* strains which resulted in mortality of the weevils at conidia concentration of $1\times10^8$. The study also reveals that the mortality rate of female weevils was higher than the male weevils for all isolates (with isolate Bb88 causing considerably higher mortality (82%) in females while male mortality was the same for all the isolates) [10].

A study was conducted using six strains (obtained from dead insect larvae in a farmer's field from Andhra Pradesh, India) of the fungus *Beauveria bassiana* for controlling termites. Results of the research work showed that isolates 4-23 and 4-10 had 65% and 52% mortality rate respectively (with worker communities of the termites more susceptible to the fungus) which was due to cuticular infection and ingestion of the inoculum [11].

## 2. MATERIALS AND METHOD

The preparation and storage of *Beauveria bassiana* and other laboratory work which include treatments set up were conducted at the LBI laboratory of the Guyana Sugar Corporation (GUYSUCO). Guysuco. *Beauveria bassiana* was isolated from termite nest and culture on different growth media such as Potato Dextrose Agar (PDA), Potato Dextrose Yeast Extract Agar (PDYEA) and Oatmeal Agar (OMA), temperatures ($25^0$C, $28^0$C, and $30^0$C) and pH (5.5, 6.5 and 7.5). The best growth media, temperature and pH were used to culture *Beauveria bassiana*. However, the treatments set up were conducted under normal conditions. The *Diatraea centrella* larvae were collected from the Enmore sugarcane estate. Randomized blocked design was used. Treatments were done in replicates of four to avoid bias in report under standard conditions. Three treatments were conducted: **Treatment 1(control):** contains uniform pieces of sugarcane with 10 *Diatraea centrella* larvae. **Treatment 2:** contains uniform pieces of sugarcane with 10 *Diatraea centrella* larva and 5ml liquid culture *Beauveria bassiana*. **Treatment 3:** contains uniform pieces of sugarcane with 10 *Diatraea centrella* larva and 10ml liquid culture *Beauveria bassiana.*

Treatments prepared were observed on day 3 and day 5 and the numbers of alive and dead larvae were recorded

**Testing soil samples for *Beauveria bassiana***

Soil type 16 from Uitvlugt estate (ICBU)

**Testing termites nest for *Beauveria bassiana***

Termite nests was crushed into a powder. PDA medium was melted and placed in a sterilized chamber. Antibiotics were added to the medium and were swirled to dissolve. The PDA medium was then poured into petri plates and left until solidified. Small quantities of the crushed termite nest were sprinkled onto the center of the media in the petri plates. Inoculated media were placed in the inoculation chamber for about three days and observations were made (Plate 1).

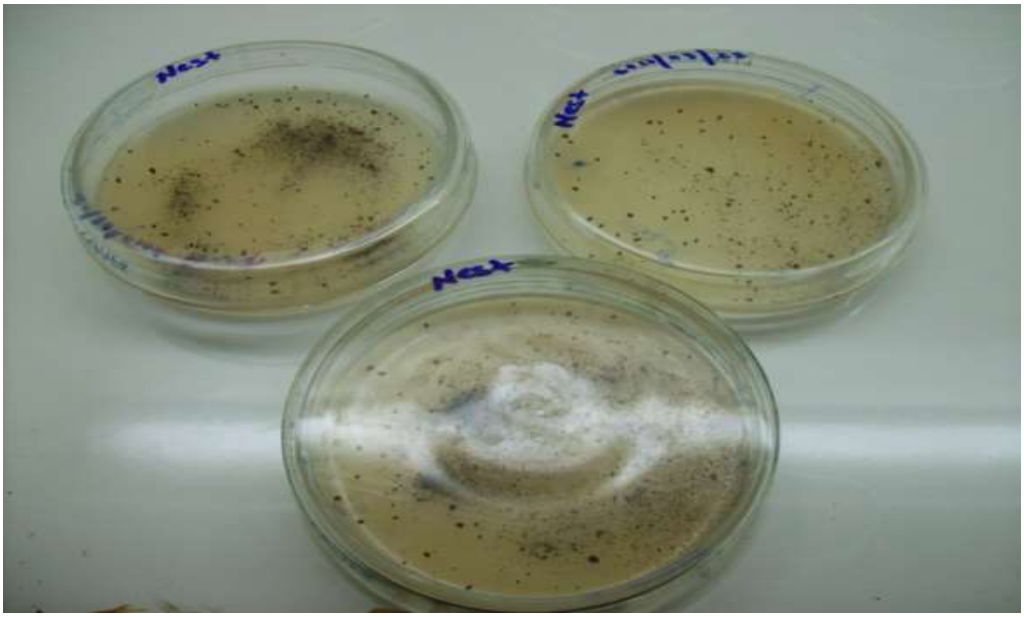


**Plate 1:** Showing inoculation of termite nest on growth media

### Culturing of *Beauveria bassiana*

Potato dextrose agar was melted at 100°C and placed in the sterilized inoculation chamber. Antibiotic capsules were added to the medium and swirled until dissolved. The medium was then poured into petri plates and left to be for solidified. Samples of *Beauveria bassiana* were sub cultured by using an inoculation needle which was heat- sterilized each time before the *Beauveria bassiana* was transferred onto the PDA growth medium. The sub cultures prepared were placed into the incubation chamber. Observations were made at two-day intervals for twelve days. Steps 1 – 6 were repeated for PDYEA and OMA.

### Storage of *Beauveria bassiana*

Potato dextrose agar medium has melted and poured into test tubes which were left in a slantwise position to cool, thus forming an agar slope. *Beauveria bassiana* were inoculated onto the agar slope by using a heat-sterilized needle. The

mouths of the test tubes were capped with cotton wool and left in the incubation chamber for growth. Inoculated test tubes with *Beauveria bassiana* were placed in the refrigerator for storage.

### Calculating number of *Beauveria bassiana* spores

One micro liter (1µl) of the liquid *Beauveria bassiana* was measured using a micro pipette and placed on a hemocytometer which was observed under a compound light microscope. Spores were counted and an estimation of the number of spores per one micro liter was made. Three other slides were prepared and counted in the same manner and the average number of spores for one micro liter was calculated

### Inoculation of *Beauveria bassiana* with *Diatraea centrella* larva

Three large petri plates were labeled C, $T_1$ and $T_2$ which represents control, treatment 1 and treatment 2 respectively. Sugarcane were collected and cut into small uniform pieces. Approximately 25 pieces of sugarcane were placed in each petri plates. Ten *Diatraea centrella* larvae were also placed into each petri plates. Under sterilized conditions 5 ml and 10 ml *Beauveria bassiana* were measured and placed in $T_1$ and $T_2$ respectively. Clear plastics were taped over the surface of the petri plates and a pin was used to make vents on the clear plastics. All treatments were left under normal conditions and observations were made on day 3 and day 5 (Picture 5). Steps 1-7 were repeated three times. The dead larve were collected and kept in formalin for histological preparation.

### Histological Preparation

Histological preparation was done based on Ghose, K. C. and Manna, B. 2007[12] methods.

## 3. RESULTS AND DISCUSSION

Previous studies have shown that *Beauveria bassiana* was isolated either soil from samples or from dead insect hosts. However, in this study both soil samples and termite nests were used to find isolates of *Beauveria bassiana*. In soil type 16, *Beauveria bassiana* were not identified; however, other microorganisms were found in a great abundance in the less diluted soil sample compared to the more diluted soil samples inoculated. On the other hand, *Beauveria bassiana* was identified from the inoculated termite nest which was subcultured to obtain a pure culture of the fungus.

| | Days | PDA (mm) | PDYEA(mm) | OMA(mm) |
|---|---|---|---|---|
| **Culture *Beauveria bassiana*** | 0 | 0 | 0 | 0 |
| | 2 | 2.6 | 2.3 | 2.0 |
| | 4 | 3.3 | 2.9 | 2.4 |
| | 6 | 4.2 | 3.5 | 3.0 |
| | 8 | 5.4 | 4.0 | 3.3 |
| | 10 | 6.9 | 4.8 | 4.0 |
| | 12 | Plate Fully covered | 5.6 | 5.0 |

**Table 1:** Showing growth of *Beauveria bassiana* on different media.

*Beauveria bassiana* was cultured on three different growth media (potato dextrose agar (PDA), potato dextrose yeast extract agar (PDYEA) and oatmeal agar (OMA). Based on observations the PDA medium was selected for culturing the *Beauveria bassiana* since the pure culture of the fungus was obtained from this medium in abundance (Table1).

| | Days | $25^0$C(mm) | $28^0$C(mm) | $30^0$C(mm) |
|---|---|---|---|---|
| **Culture *Beauveria bassiana*** | 0 | 0 | 0 | 0 |
| | 2 | 2.6 | 3.3 | 2.5 |
| | 4 | 3.0 | 4.9 | 3.4 |
| | 6 | 3.9 | 5.5 | 3.6 |
| | 8 | 4.4 | 6.0 | 3.9 |
| | 10 | 5.9 | 7.8 | 4.0 |
| | 12 | 6.7 | Plate Fully covered | 5.0 |

**Table 2:** Showing growth of *Beauveria bassiana* at different temperatures.

*Beauveria bassiana* was cultured at different temperatures ($25^0$C, $28^0$C and $30^0$C) for 12 days and observations were recorded at every two-day interval. The growth of the fungus was measured and compared for the different temperatures. Results obtained showed that *Beauveria bassiana* grows best at $28^0$C since the plate was fully covered with the fungus on day 12, compared to the results obtained for the other temperatures ($25^0$C and $30^0$C) on day 12 (Table 2). Previous studies conducted show that isolates of *Beauveria bassiana* were placed in incubators at 28, 32, 35 and 37 °C. Results obtained showed that isolates did not grow at 35°C and 37°C [13].

| | Days | 5.5 pH (mm) | 6.5pH(mm) | 7.5pH(mm) |
|---|---|---|---|---|
| **Culture *Beauveria bassiana*** | 0 | 0 | 0 | 0 |
| | 2 | 2.4 | 3.3 | 3.0 |
| | 4 | 2.9 | 4.9 | 4.6 |
| | 6 | 3.0 | 5.5 | 5.7 |
| | 8 | 3.6 | 6.0 | 6.9 |
| | 10 | 4.0 | 6.6 | 7.4 |
| | 12 | 5.0 | 7.0 | Plate Fully covered |

**Table 3:** Showing growth of *Beauveria bassiana* at different pH values.

*Beauveria bassiana* was cultured under different pH conditions (5.5, 6.5and 7.5) for 12 days and observations were taken at every two-day interval. The growth of the fungus was measured and compared for the different pH values. Results obtained showed that *Beauveria bassiana* grows best at the more alkaline pH (7.5) since the plate was fully covered with the fungus on day 12, compared to the results obtained for the other pH values (5.5 and 6.5) on day 12 (Table 3).

| **Slides** | **Total Spores/1ul** Mean ± SD | **# Spores/ 5ml** | **# Spores/10ml** |
|---|---|---|---|
| **1** | 14± 4.58 | 70000 | 140000 |
| **2** | 17±6.56 | 85000 | 170000 |
| **3** | 12±1.00 | 60000 | 120000 |
| **4** | 15±1.00 | 75000 | 150000 |
| **Total # spores** | | 72500 | 145000 |

**Table 4:** Showing calculated number of *Beauveria bassiana* spores

The spores of one micro liter of the liquid fungus were observed under a compound light microscope and the total numbers of spores were calculated for 5ml and 10 ml of the liquid fungus (Table 4).

| **Treatments** | ***Diatraea centrella* larvae (Mean ± SD)** | | | | | |
|---|---|---|---|---|---|---|
| | **Alive** | | | **Dead** | | **Mortality rate (%)** |
| | **Day 1** | **Day 3** | **Day 5** | **Day 3** | **Day 5** | |
| Control | 10 ± 0 | 10 ± 0 | 9.5 ± 0.58 | 0 | 0.5 ±0.5 | 12 |
| 5 ml *Beauveria bassiana* | 10 ± 0 | 8.7±1.26 | 9.5 ± 0.57 | 1.3 ±1.3 | 0.5 ±0.6 | 30 |
| 10 ml *Beauveria bassiana* | 10 ± 0 | 8.7 ± 0.5 | 9.0 ± 0.82 | 1.3 ±0.5 | 1± 0.8 | 38 |

**Table 5:** Showing the rate of mortality among treatments

The mortality rate is greater in the treatment with the 10 ml *Beauveria bassiana* (38%) and lowest in the control (12%) and 30% in 5 ml treatment. This can be a result of the number of *Beauveria bassiana* spores present in the 10 ml treatment (145000) compared to the 5 ml treatment with 72500 spores (Table 5).

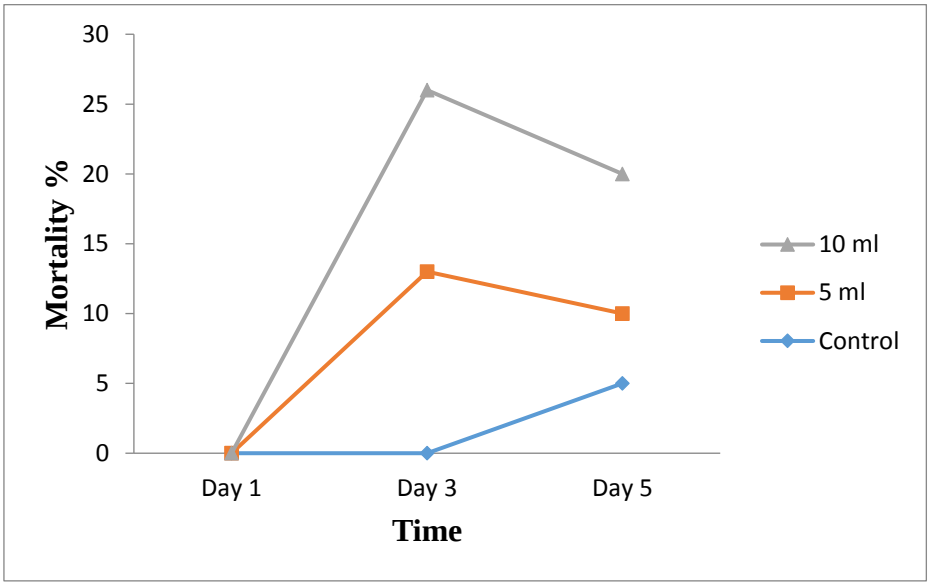
Based on previous research the pathogenicity of native isolates of *Beauveria bassiana* was used to control malaria vectors, *Anopheles gambiae*. Results from this study showed that an increase in time and density of dry conidia of *Beauveria bassiana* increases mortality for *A. gambiae*[9].

*Beauveria bassiana* isolated from a termites' nest showed some pathogenicity to the *Diatraea centrella* larve which were exposed to 72500 and 145000 fungal spores in treatments 1 and 2 respectively. Based on these two treatments conducted, treatment 2 (145000 spores) showed more pathogenicity to the *D. centrella* larve compared to treatment 1 (72500 spores) which shows less pathogenicity to the larve (Table 5). This difference in pathogenicity between treatments 1 and 2 is related to the difference in number of the fungal spores applied. This can be supported from an earlier research conducted which shows that a lower dosage of spores that was applied for one strain of *Beauveria bassiana* resulted in a low virulence in cactus weevil (*Metamasius spinolae*) compared to the other strains which were applied with a higher dosage of spores[10].

| **Treatments** | **$t_{calculated}$ / $t_{critical}$** | **Probability level** | **Significant (S) / not significant (NS)** |
|---|---|---|---|
| Control | 1.73< 3.18 | P≤ 0.05 | NS |
| 5 ml *Beauveria bassiana* | 3.65<3.18 | P≤ 0.05 | S |
| 10 ml *Beauveria bassiana* | 9.00<3.18 | P≤ 0.05 | S |

**Table 6:** Showing significances of treatments

Treatments 1 and 2 showed significance in the rate of mortality (since the value for $t_{calculated}$ is greater than the $t_{critical}$ value) while there was no significance in the rate of mortality for the control (Table 6). The results presented in this study demonstrate a pathogenic effect of *Beauveria bassiana* on *Diatraea centrella* larva under laboratory conditions.




**Graph 1 Percentage Mortality**

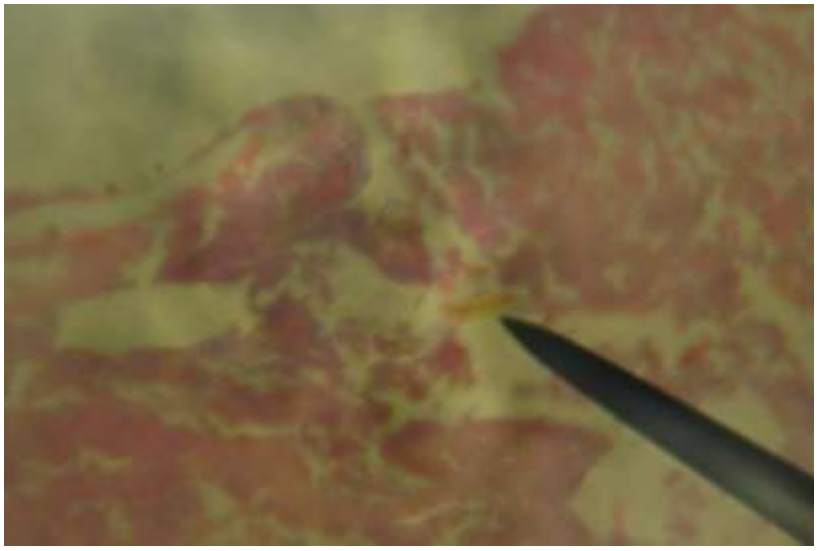
This *Beauveria bassiana* fungus causes white muscardine disease in the insects it contaminates and may take 3-5 days for the insects to die [6]. This experiment was extended for five days. During day's 1-3 mortality of *Diatraea centrella* larve were observed for treatments 1 and 2. However, during days 3-5 a decrease in mortality rate was observed (Graph 1). The change in mortality rate after day3 can be a result of the larve becoming resistant to the fungus and unsuitable conditions for the growing of the fungal spores. *Beauveria bassiana* spores can be readily killed in high temperatures but infects its host best in cool to moderate temperatures [7][8].

In the control, it was observed that after day 3 the rate of mortality increased. This could have resulted from contamination with microorganisms since the treatments were disturbed on day 3 to make observation of dead and alive larve. During the treatments an infestation of ants was seen, which were attracted to the sweetness of the sugarcane. This however, was also a major disturbance to the treatments carried out. In addition, the pathogenicity of *Beauveria bassiana* on treatments 1 and 2 was poor. This could have been a result of unsuitable temperature for growth of the fungal spores since treatments were done under normal conditions. Previous studies conducted show that isolates of *Beauveria bassiana* were placed in incubators at 28°C, 32°C, 35°C and 37 °C. Results obtained showed that isolates did not grow at 35°C and 37°C [12].

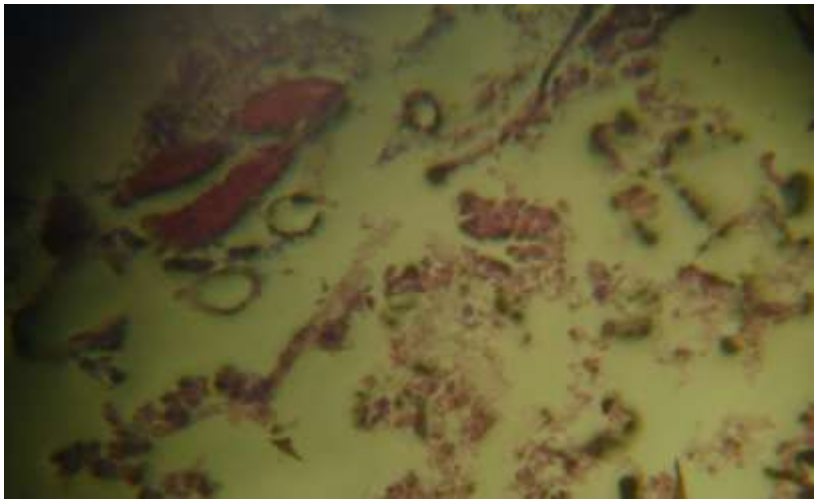
## Histological Observations

Tissues of treated *Diatraea centrella* larve were mounted and observed under the microscope. Results obtained showed that the dorsal, ventral and intestinal tissues of the *Diatraea centrella* larve were infected with *Beauveria bassiana* spores (mounted tissues 1, 2 and 3). Therefore, the dorsal, ventral and intestinal tissues are more vulnerable to the spores of *Beauveria bassiana*. *Beauveria bassiana* infects its host (insect) mainly through its integument, although they can also be ingested and enter the organism through the digestive tract, or through the trachea, or wounds [13].

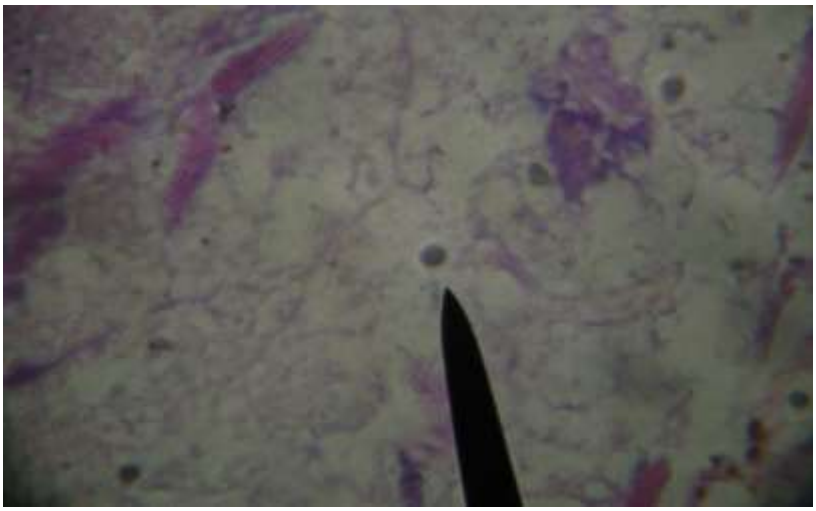
**Mounted tissues 1:** Showing infection of *Beauveria bassiana* spores on the dorsal tissues



**Mounted tissues 2:** Showing infection of *Beauveria bassiana* spores on the ventral tissues



**Mounted tissues 3:** showing infection of *Beauveria bassiana* spores on the intestinal tissues



## 4. CONCLUSIONS

The spores of the *Beauveria bassiana* were more effective in the higher quantity since mortality was higher in treatment two which had more spores compared to treatment one which had a lower quantity of *Beauveria bassiana* spores. During days 1-3 the mortality rate of treatments 1 and 2 increases and then decreases between day 3 and day 5. Hence, the *Beauveria bassiana* spores are more effective in the early stage of the treatments. The intestinal, dorsal and ventral tissues of the *Diatraea centrella* larvae were more susceptible to the spores of *Beauveria bassiana.*

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