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Effect of entomopathogenic fungi on feeding and oviposition of oil palm pest *Coelaenomenodera lameensis* (Coleoptera: Chrysomelidae)

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Abstract

Oil palm, the main source of vegetable oil, is under attack from the leafminer *Coelaenomenodera lameensis* Berti et Mariau (Coleopera: Chrysomelidae). The adults of this insect feed on the leaflets, causing the palms to dry out. The aim of this study was to evaluate the effect of the entomopathogenic fungi *Metarhizium anisopliae* (Met 358 and Met 359) and *Beauveria bassiana* (Bb 11) on the feeding and oviposition activity of *C. lameensis*. The work was carried out under controlled infestation on an oil palm plot at the University Jean Lorougnon Guédé in Daloa. Male and female adults were collected and placed on leaflets covered with muslin sleeves. These leaflets served as a substrate for *C. lameensis* feeding and oviposition. Two feeding scenarios were considered. In the first case, the adults were placed on treated leaflets; in the second the entire setup (insects and leaflets) was treated. With regard to oviposition activity, pairs of *C. lameensis* surviving treatments with different entomopathogenic fungi were placed in sleeves. The length of the furrows dug when adults were placed on treated leaflets was reduced in males and females to 10.83 cm (Met 358) and 8.42 cm (Met 359) respectively, compared with the control which was 22.60 cm (males) and 30.65 cm (females). When both insects and leaflets were treated, furrow length was significantly reduced in males and females, with 7.46 cm (Met 359) and 0 cm (Met 359) respectively. The number of eggs laid by *C. lameensis* females surviving the treatments was reduced from 40 (Bb 11), 32 (Met 358) and 25 (Met 359) on day 5 to 0 for all fungi on day 40. This study demonstrates the potential of these entomopathogenic fungi as effective biocontrol agents as part of an integrated pest management strategy for sustainable oil palm cultivation.

Keywords: Oil Palm; *Coelaenomenodera lameensis*; Metarhizium Anisopliae; Beauveria Bassiana; Leaf Miner

1. Introduction

Palm oil and palm kernel oil are essential ingredients in many food products. They are also used in the manufacture of many cosmetic products and biofuels [1]. However, this crop is threatened by several harmful insect pests. *Coelaenomenodera lameensis* has been identified as the main insect pest affecting oil palm cultivation [2,3,4,5]. The adults of this insect feed on the leaflets by scraping grooves through the entire thickness of the leaflet. The action of *C. lameensis* on the leaflets therefore leads to a reduced photosynthetic activity and drying of the palms, which leads to a reduction in yield. Given the extent of the damage caused by these leaf beetles, control methods have been used. Chemical control has proven to be very effective. Unfortunately, the use of synthetic insecticides leads to environmental pollution and human poisoning [6]. Due to their harmful effects, it is therefore essential to research effective control methods that do not harm the environment or human health. In Côte d'Ivoire, no studies have been conducted on the control of *C. lameensis* through the use of entomopathogenic fungi. The use of entomopathogenic fungi for crop protection as an alternative to synthetic insecticides would have many advantages. Several trials using entomopathogenic fungi have yielded good results on many insect pests [7,8,9,10]. Studies have shown that

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entomopathogenic fungi eliminate insects through a multi-step process, leading to the death of the host depending on its stage of development or immune status [11]. These stages include the adhesion of fungal conidia to the surface of the insect's integument, followed by their germination. The fungus then breaks down the cuticle to penetrate it, transforming its hyphae into blastospores inside the host. These blastospores exploit the nutrients present in the hemocoel and release toxins into the hemolymph. Finally, the fungus emerges from the host through openings in the cuticle to produce spores on the surface of the carcass [12]. To the best of our knowledge, no studies have been conducted in Côte d'Ivoire on the use of entomopathogenic fungi against *C. lameensis*. In this study, we propose to evaluate the effect of two entomopathogenic fungi, *Metarhizium anisopliae* (Met 358 and Met 359) and *Beauveria bassiana* (Bb 11), on the feeding and egg-laying activity of *C. lameensis* adults.

2. Material and methods

2.1. Study area

The study was conducted at the University Jean Lorougnon Guédé, located northeast of Daloa, capital of the Haut-Sassandra region between 6°54' north latitude and 6°26' west longitude. It is influenced by a humid tropical climate, with rainfall ranging from 1,200 to 1,600 millimeters per year [13]. Temperatures vary from 25 to 28°C, with a mean of 26.62 ± 1.02°C. Relative humidity vary from 73 to 84%, with a mean of 79.83 ± 4.12% [13].

2.2. Rearing of *C. lameensis*

C. lameensis was reared in large muslin sleeves (300 cm x 80 cm). These sleeves were featured an opening sealed with adhesive tape to prevent the emergence of insects placed on the leaflets. Metal hoops were placed inside the sleeves to give them a cylindrical shape. Using 8 cm-diameter, 10 cm-high cylindrical boxes fitted with lids, adult pairs of *C. lameensis*, whose females were ovipositing, were collected and transferred to leaflets covered with muslin sleeves. The pairs were monitored for 120 days, during which time new individuals were obtained for testing.

2.3. Production of entomopathogenic fungi

The *Metarhizium anisopliae* (Met 358 and Met 359) and *Beauveria bassiana* (Bb 11) isolates used in the present study were obtained from the fungal culture collection of the International Institute of Tropical Agriculture of Bénin (IITA-Benin).

For fungal production, 39 g of Potato Dextrose Agar (PDA) powder was dissolved in 1 liter of distilled water in a beaker. After homogenization in a water bath for 5-10 minutes, the mixture was autoclaved for 15 minutes at a temperature of 120°C and a pressure of 15 PSI for sterilization. Next, the medium was poured into sterile Petri dishes (diameter= 9cm, height= 1.5 cm) under a laminar flow hood. After the medium had cooled and solidified, a small quantity of conidia from the fungal isolates was removed using a sterilized bacteriological needle and spread evently over the surface of the PDA medium. Petri dishes were covered with parafilm. Each plate was marked with the name of the isolate and the date of subculturing.

These Petri dishes were incubated in a photo period of 12 h light and 12 h dark for 21 days.

2.4. Application of entomopathogenic fungi

2.4.1. Impact of entomopathogenic fungi on the feeding of C. lameensis in a controlled environment

Two tests were conducted on male and female adults oh *C. lameensis* using a concentration of 10^{10} spores/ml for each of three entomopathogenic fungal isolates (Bb 11, Met 358 and Met 359).

2.5. Spraying fungi before insect introduction

Palm leaflets were trimmed to a length of 25 cm the rachis. A sleeve, covering 8 leaflets (4 per side of the rachis), was placed on each of 40 leaflets per insect batch. The fungal isolates were sprayed once onto the leaflets within the sleeves. Immediately after spraying, one *C. lameensis* adult was introduced into each corresponding sleeve. Four batches of 40 insects each were used: batch 1a (test males), 1b (control males), 2a (test females), and 2b (control females).

2.6. Spraying fungi after insect introduction.

Four additional batches of 40 insects each were created (batch 3a: test males, 3b: control males, 4a: test females, 4b: control females). One insect was introduced into each sleeve, which was set up as described in Test 1. After a 24-hour acclimatization period, the fungal isolates were sprayed once directly onto the insects and the leaftets inside the sleeves.

For both tests, the insects remained in the sleeves for 5 days. After this period, the average length (Ls) of the feeding furrows dug by the adults was measured in centimeters.

$$\text{Ls (cm)} = \frac{(\sum \text{(pi x ri)})}{(\sum \text{ri})}$$

Pi = length of furrows in leaflets; ri = number of insects

The rate of reduction (Tr) in the length of furrows dug by insects to feed was calculated as a function of the control.

$$\text{Tr (\%)} = \frac{\text{Furrows of control insects} - \text{Furrows of treated insects}}{\text{Furrows of control insects}} \times 100 \qquad (2)$$

2.6.1. Impact of entomopathogenic fungi on oviposition activity of C. lameensis in infestation

- **Test on surviving insects:** The test used pairs of *C. lameensis* that had survived prior treatment with the entomopathogenic fungal isolates (Bb 11, Met 358, or Met 359) at a concentration of 10^8 spores/ml.
- **Experimental setup:** Four batches of 40 insect pairs each were established:
  - Batch C1: Pairs treated with a fungal isolate.
  - Batch C2: Pairs treated with a different fungal isolate.
  - Batch C3: Pairs treated with another fungal isolate different from the first two.
  - Batch C4: Control pairs (untreated). As in previous tests, 40 sleeves were placed on the distal ends of palm leaves. The insect pairs from each batch were introduced into the sleeves.

2.6.2. Egg-laying assessment

Female egg-laying was monitored for 40 days. The pairs were transferred to new sleeves every 5 days. The eggs, which were visible on the upper leaf surface, were counted using a hand-held magnifying glass. The average number of eggs laid per female was calculated for each batch.

2.7. Statistical analysis

Data were processed using Statistica software (version 7.1). We used analysis of variance (ANOVA) to identify significant differences, followed by the Newmans-Keuls test (at the 5% significance level) for post-hoc comparison of means into homogeneous groups.

3. Results

3.1. Spraying entomopathogenic fungi (Bb 11, Met 358 and Met 359) on palm leaflets followed by the introduction of *C. lameensis* adults into the sleeves.

This experiment exposed male and female insects to three entomopathogenic fungal strains (Bb 11, Met 358 and Met 359) for five days.

The results showed that all fungal treatments caused mortality, with Met 358 being the most lethal. Furthermore, all treatments significantly reduced the insects tunneling activity (furrow length) compared to the untreated control group. Met 359 was the most effective at inhibiting this behavior. Statistical analysis confirmed that the differences in furrow length across the treatments were highly significant for both sexes. The specific mortality and furrow length data are detailed in table 1.

Table 1 Efficacy of Met 358, Met 359 and Bb 11 on the feeding of *C. lameensis* adults after leaflet spraying followed by insect introduction

	Furrow length (cm)		**Train path length reduction rate (%)**	
Products	**Males**	**Females**	**Males**	**Females**
Bb 11	14.78 ± 4.19 b	13.7 ± 3.91 b	34.60 %	58.46 %
Met 358	10.83 ± 3.51 b	9.43 ± 3.23 bc	52.08 %	69.23 %
Met 359	11.65 ± 3.01 b	8.42 ± 3.50 c	48.45 %	72.53 %
Witnes	22.60 ± 4.96 a	30.65 ± 5.10 a	-	-
F	17.66	66.94		
ddl	3	3		
p	0.000	0.000		

The lengths followed by the same letters in the same column were not significantly different.

3.2. Introduction of *C. lameensis* adults into the sleeves followed by spraying with entomopathogenic fungi (Bb 11, Met 358 and Met 359)

The experimental demonstrated a strong lethal and sublethal impact of entomopathogenic fungi on insects over a 5-day period.

3.2.1. Mortality

All fungal strains caused significant mortality, with Met 359 being the most virulent, killing all 40 female insects and 36 males.

3.2.2. Behavioral impact (furrow digging)

The fungi severely reduced the insects' tunneling activity. While untreated females were more active than males in the control group, all treatments drastically reduced furrow length. The effect was most severe in females treated with Met 359, which were completely unable to dig furrows.

3.2.3. Statistical significance

The differences in furrow length between the treated groups and the control were found to be highly significant for both males and females.

The specific numerical data for mortality and furrow length are detailed in table 2.

Table 2 Efficacy of Met 358, Met 359 and Bb 11 on the feeding of *C. lameensis* adults after insect introduction followed by leaflet spraying

	Furrow length (cm)		**Train path length reduction rate (%)**	
Products	**Males**	**Females**	**Males**	**Females**
Bb 11	10.38 ± 4.47 b	9.61 ± 2 b	54.07 %	68.65 %
Met 358	9.21 ± 2.92 b	7.78 ± 1.62 b	59.25 %	74.62 %
Met 359	7.46 ± 1.05 b	-	66.99 %	-
Witness	22.60 ± 4.96 a	30.65 ± 5.10 a	-	-
F	24.32	85.43		
ddl	3	3		
p	0.000	0.000		

The lengths of the grooves of the same letters in the same column were not significantly different.

3.3. Impact of entomopathogenic fungi on the oviposition activity of C. lameensis

The application of entomopathogenic fungi significantly reduced the oviposition activity of female *C. lameensis* over a 40-day period compared to the untreated control group. The Met 359 strain had the most severe impact, completely suppressing egg-laying by day 40.

A pronounced and sustained reduction in the number of eggs laid was observed for all fungal treatments (Bb 11, Met 358, Met 359) over time.

Statistical analysis confirmed that the differences in egg production between the treated and control groups were highly significant. The specific numerical data are presented in figure 1.

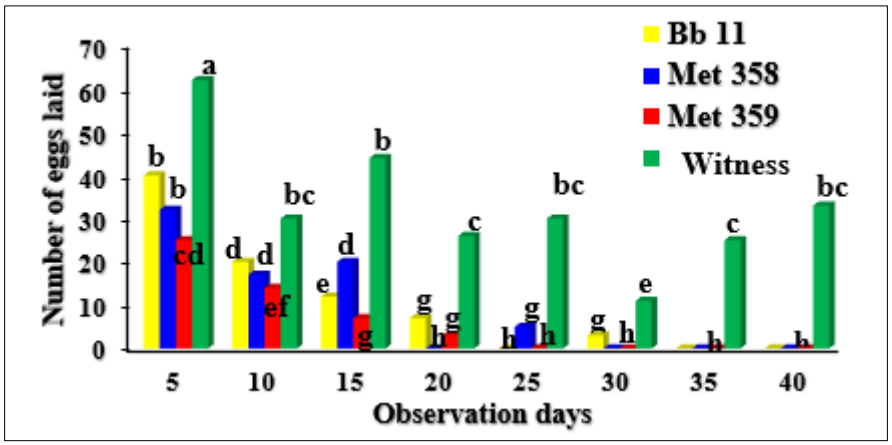

Figure 1 Number of eggs laid by *C. lameensis* females surviving entomopathogenic fungus sprays

Newmans-Keuls test at 5% threshold (F = 14.44; ddl= 31; P = 0.000). Means followed by the same letters are not significantly different.

4. Discussion

4.1. Impact of entomopathogenic fungi on food intake

Entomopathogenic fungi such as *M. anisopliae* and *B. bassiana*, unlike chemical insecticides, do not have a rapid effect on insect pests.

The results show that the entomopathogenic fungi used in this study reduce the food intake of *C. lameensis*. This would be due to the production of toxins that affect the nervous system or other physiological functions of the insects, which can lead to a reduction in their appetite and ability to feed. [14], in their work after testing *Bacillus thuringiensis* toxins against a wide range of insects, including Lepidoptera, Diptera, and Coleoptera, mention that the reduction in growth observed in infected individuals is thought to be due to either an inhibition of food intake under the effect of toxins on the nervous system. Our results concur with those of [15], who found that the use of INRS-IP and INRS-CFL isolates at a rate of 10^{13} conidia/ha affected the feeding behaviour of *L. lineolaris*. The reduction in food intake by *C. lameensis* is also thought to be due to a bitter taste or a reduction in the appetite of treated insects. This bitter taste would cause the insects to refuse to feed. This argument was raised by [16] when studying the effect of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* var acridum, on the feeding behaviour of *Schistocerca gregaria*. These authors reported that the reduction in food intake noted in *S. gregaria* larvae infected by the two fungi may be linked to a reduction in appetite. They also suggested that when the fungal biomass is high, the insects can no longer absorb enough nutrients. The harmful effect of *Beauveria bassiana* and *Metarhizium anisopliae* on the feeding behaviour of crop pests has been demonstrated by several authors including [16], who showed that all three products tested (*M. anisopliae*, Triflumuron and henna) in his work inhibited the consumption of *L. migratoria* L5 larvae treated by contact. [17] also reported that third instar nymphs of *Uvarovistia zebra* treated with *Beauveria bassiana* and *Metarhizium anisopliae* at 10^6 spores/ml resulted in a 60 and 63% reduction in mean food intake/insect, respectively. Similarly, [18] showed that mixing *B. bassiana* spores in the diet of soldier flies led to a reduction in food intake, resulting in a reduction in the weight of larvae and adults. [19], also reported that the effect of fungal infection with the entomopathogenic fungus *Metarhizium anisopliae* resulted in reduced feeding by the pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae) on different host plants.

4.2. Impact of entomopathogenic fungi on oviposition activity

The entomopathogenic fungi (Bb 11, Met 358 and Met 359) used in this study on *C. lameensis* had variable effects on the number of eggs laid by females caged every 5 days for 40 days. The considerable reduction in the number of eggs laid by females treated with these entomopathogenic fungi is thought to be linked to their ovicidal effect. The results obtained are in line with those of [20] during their work on the larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in Benin. These authors reported that L3 stage larvae exposed to *M. anisopliae* (Met 31) at concentrations different did not lay any eggs, whereas controls laid 13 eggs. In the same work, the same authors also stated that the number of eggs laid by L4 stage larvae treated with Met 31 and Bb 11 respectively was low.

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[23] also reported an adverse effect of *Bacillus subtilis* and *Bacillus thuringiensis* on the oviposition rate of the migratory locust *Locusta migratoria* (Linnaeus, 1758) (Oedipodinae, Acrididae). Our results are also in agreement with those of [24] who showed that *M. anisopliae* significantly reduced the number of eggs laid by *Plutella xylostella* females, 101.55 eggs/female treated and 192.55 eggs/female control. [25] in their work on *Tetranychus ludeni* showed that *B. bassiana* has the power to suppress future generations of mites while reducing the rate of egg laying. Furthermore, [26] reported in their studies that *B. bassiana* (GZGY-1-3) has toxic and sublethal effects by reducing the reproductive success of *Frankliniella occidentalis* Pergande (Thysanotera: Thripidae). [27] in their work on Frankliniella occidentalis, claimed that thrips that survived B. bassiana action had less reproductive success. The effectiveness of entomopathogenic fungi on insect reproduction is also thought to be due to proteins derived from these pathogens. This observation was made by [28] who reported that proteins derived from B. bassiana had an impact on the reproduction of Bemisia tabaci.

5. Conclusion

In conclusion, the use of the entomopathogenic fungi *M. anisopliae* (Met 358 and Met 359) significantly reduced food intake by *C. lameensis*. These fungi also significantly reduced the number of eggs laid by female *C. lameensis* on the first day and suppressed egg laying from the 35th to the 40th day. These fungi could be used as an alternative to chemical insecticides to control the population of *C. lameensis*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest with respect to this article.

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