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# Indigenous fungal entomopathogens associated with the oil palm leaf miner *Coelaenomenodera lameensis* Berti and Mariau in Ghana

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## ABSTRACT

*Background and Objective*: The oil palm leaf miner *Coelaenomenodera lameensis* (Coleoptera: Chrysomelidae) is the most devastating insect pest of the African oil palm *Elaeis guineensis* Jacquin. Like most insect control programmes, control in Ghana has been through the use synthetic insecticides. The over-dependence on chemical control has brought in its wake adverse effects such as toxicity to the user and non-targeted organism. Entomopathogens have proven to be effective in the management of many insect species and these are environmentally-friendly. In this study we investigated reports by field workers of Council of Scientific and Industrial Research (CSIR)-Oil Palm Research Institute of Ghana who observed the presence of mycelia on the cuticle of cadavers of *C. lameensis* in their daily phytosanitary surveillance.

*Methodology and Results*: Field surveys were conducted in three oil palm plantations viz: CSIR-Oil Palm Research Institute and a commercial oil palm farm, both at Kusi in the Eastern Region, and Twifo Oil Palm Plantation of Unilever Ghana Limited at Twifo Praso in the Central Region of Ghana to collect cadaver of *C. lameensis* infected with fungi. The cadavers were aseptically cultured in the laboratory on Potato Dextrose Agar and fungi isolated and identified. A total of 17 fungal species were isolated from cadavers of the leaf miner. These include *Aspergillus* sp, *Metarhizium* sp, *Paecilomyces* sp, *Penicillium* sp, *Pestalotia* sp, *Rhizoctonia* sp, and three unidentified species. Bioassays conducted to ascertain the pathogenicity of the fungi against *C. lameensis* adult showed an overall mortality ranging between 12.5% - 77.5% within 7 days. Growth of mycelia on treated dead insects ranged from 0% - 47.5%. The unidentified fungus coded BKFF was found to be the most lethal inducing about 77% mortality in the insect and thus more entomopathogenic, followed by *Paecilomyces* sp. (Ioprik31 and *Pestalotia* sp. (CKFF) (both 65%) whilst *Rhizoctonia* sp was found to be the least lethal.

*Conclusion and application of findings*: This study presents important naturally occurring fungal species associated with the oil palm leaf miner *C. lameensis* in the field which famers can utilize as a control option upon further field studies.

**Key words:** *Coelaenomenodera lameensis,* entomopathogenic fungi, isolate, pathogenicity, mycelia

## INTRODUCTION

Oil palm is an economically important crop providing income for peasant farmers and foreign exchange for countries where they are found. According to Carrere (2006) world production of oil palm yielded 17.5 million tons of palm oil and 2.1 million tons of palm kernel oil in 1997 and almost doubled to 30 million tons by 2005. In 2001, there were 200,000 hectares of oil palm plantations in Thailand and this increased to 280,000 hectares by 2005. A total area of three million hectares is covered by oil palm in Nigeria (Carrere, 2006). Malaysia and Indonesia, the world's leading producers of oil palm had a total of 4 million hectares and 5.3 million hectares covered by oil palm respectively in 2005. In Ghana, total land under the cultivation of oil palm has increased from 18,000 hectares in 1977 to 103,000 between 1970 and 1990 (Gyasi, 1992) and this has increased to about 304,000 hectares in 2002 (Carrere, 2006). The production of oil palm worldwide is increasing because palm oil is the world's best selling vegetable oil, representing 56% of the total global trade in edible oils (Carrere, 2006). Oil palm is high in oil content and has the highest potential of oil yield per acre when compared to other vegetable oils (Anyane, 1961). It is the only crop from which two kinds of oils can be obtained – palm oil and palm kernel oil. One major constraint to oil palm production is infestation by insect pests. The most important insect pest of the oil palm in West Africa is the oil palm leaf miner, *Coelaenomenodera lameensis* (Yawson, 2009). Damage is caused mostly by the larvae which mine the leaflets within which they live resulting in drying up of the fronds (Plates 1 and 2). The adults on the other hand cause a considerable damage only when their numbers exceed economic threshold of 1.5 adults per frond (Yawson *et al.*, 2006). The adult feeds on the underside of the leaflets leading to partial drying up of the fronds. Heavy infestations can cause severe defoliation which can reduce seed production by 30-50% (Lecoustre, 1998).

**Plate 1**. Larvae of *C. lameensis* on palm fronds

**Plate 2**. Damage caused to oil palm trees by *C. lameensis*

The control of *C. lameensis* has over the years been by synthetic insecticides through hot fogging, trunk injection, fluid air spraying, by phytosanitary surveillance, biological control and by planting resistant varieties of the oil palm. Currently, Evisect S ® is the only preferred synthetic insecticide available for the control of adult *C. lameensis in* Ghana (Yawson, 2007). However, due to the incidence of development of resistance to synthetic insecticides by insects from prolonged use, the harmful effects of these chemicals on the environment, and residues in the fruits produced, it has become critical to develop other alternative control methods (Obeng-Ofori, D. 1998). The use of biological control appears to be environmentally friendly and safe in curbing incidence of insect pests. In the search for new avenues in biological control, the importance of entomopathogens has been highlighted as an environmentally-friendly pest control method (Paray and Rajabalee, 1997). According to Scholte *et al*., 2004, fungal diseases in insects are common, widespread and can decimate pest populations in spectacular epizootics. Virtually all insect orders are susceptible to fungal diseases (Scholte *et al*., 2004). Large numbers of insect pathogenic organisms have been identified as possible biological control agents for grasshoppers (Bidochka and Khatchatourians, 1992). Commercial formulations of some entomopathogens such as Dipel 2x (*Bacillus thuringiensis* based-bio-product) have been made available for control of insect pests and these have proven to be efficient. At CSIR-OPRI field workers reported the presence of mycelia on cuticles of cadavers of *C. lameensis* during their daily phytosanitary surveillances suggesting that these fungal microbes may be exerting some control on the pest. Thus the search for these naturally occurring entomopathogens of the oil palm leaf miner became necessary. This study presents important naturally occurring fungal species associated with the oil palm leaf miner *C. lameensis* in the field.

## MATERIALS AND METHODS

Cadavers of *C. lameensis* were collected from three locations i.e. CSIR-OPRI plantation, a commercial oil palm farm both at Kusi in the Eastern Region and Twifo Oil Palm Plantation Ltd at Twifo Praso in the Central Region of Ghana. Cadavers collected were surface sterilized with 1% Sodium hypochloride and plated onto Potato Dextrose Agar (PDA) and this was observed for sporulation. The sporulated fungi were aseptically sub-cultured on PDA to obtain pure cultures. The various sporulated fungi were then coded based on the location of collection, mounted on slides and indentified using literature and identification keys (Smith, 1960; Barnett,

1962; Poinar and Thomas, 1978; Humber, 2005). The occurrence of the various fungi collected from the farms was then calculated.

**Bioassays:** The spores of the various fungi were harvested and suspended in a conical flask containing 10 mL of sterile distilled water and 0.05% Tween® 80 solution. The concentrations of the spores of the various inocula prepared were determined by direct counting using the Improved Neubauer Haemocytometer® (Weber Scientific International Ltd, London). The spore concentration was adjusted to a $10^7$ conidia/mL for each isolated fungus and used in bioassays. A volume of 1µL of each inoculum was topically applied to the notum of each adult *C. lameensis* and placed in sterilized petri dishes and kept in controlled laboratory conditions of 27 ± 2ºC and 70 ± 5% relative humidity. The control adult beetles were treated with sterilized distilled water containing 0.05% Tween® 80. There were ten insects per treatment and replicated four times. Mortality counts were recorded every 24 hours for 7 days. Dead insects included those showing emergence of mycelia on the cuticle and those which failed to respond when prodded with a blunt probe. Dead insect showing emergence of mycelia were then transferred aseptically onto PDA and incubated at 27 ± 2°C for 7-10 days. Sporulating fungi were re-isolated and re-identified.

**Data analysis:** The incidence of the various fungi was presented in a pie chart. Mortality data was subjected to Analysis of Variance (ANOVA) and means separated using Duncan Multiple Range Test (DMRT).

## RESULTS

**Isolated Fungi:** Seventeen fungal species in all were isolated from the cadavers of *C. lameensis* and coded as Aoprik31, Boprik31, Coprik31, Ioprik31, Joprik31, Koprik31, Oprik31, Poprik31, Qoprik31, Roprik31, AKFF, BKFF, CKFF, DKFF, A/Twifo, B/Twifo and C/Twifo (Table 1). These were identified to include six *Penicillium* species (35%), three *Pestalotia* species (17%), two *Rhizoctonia* species (12%), one *Aspergillus* species, one *Metarhizium* species, one *Paecilomyces* species (both 5%) and three other unidentified species (18%) (Figure 1).

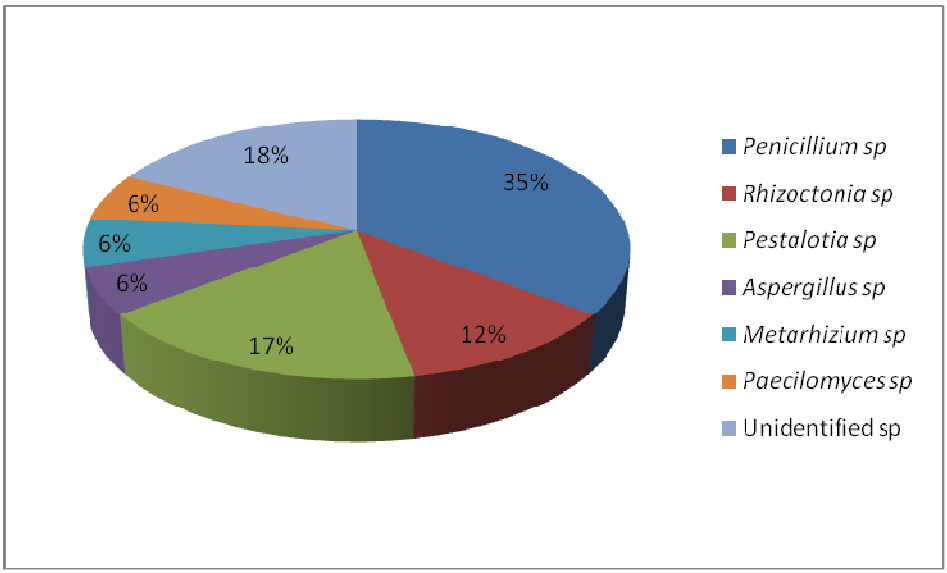


**Figure 1:** Incidence (%) of fungi isolated from cadavers of *C. lameensis* collected from the study

**Table 1:** Identification and characteristics of fungi isolated from cadavers of *C. lameensis*

| Isolate | Growth morphology | Colony colour | Key characteristics | Spore shape | Identity |
|---|---|---|---|---|---|
| Aoprik31 | Cushion-like | White with acervuli dark | Conidia with 3-5 septa, dark at the median but colourless at the terminal end with two or more hyaline apical appendages | Fusoid with hyaline pointed end cells | *Pestalotia* sp. |
| Boprik31 | Fast growing and fluffy | Gray | Threads of mycelia | Lacking | *Rhizoctonia* sp |
| Coprik31 | Concentric growth rings | Olive green | Phialides are penicillate | Globose | *Penicillium* sp |
| Ioprik31 | Cotton like | White | Ropes of hyphae and various types of spore-bearing structures | Elliptical | *Paecilomyces* sp |
| Joprik31 | Dense and upright conidia. | Dark brown | Conidiophores ends in clavate swelling with phialides at the apex | Globose | *Aspergillus* sp. |
| Koprik31 | Powdery and grows in scattered colonies | Violet | Phialides are penicillate | Globose | *Penicillium* sp. |
| Oprik31 | Concentric growth and fluffy | Gray and white | Threads of mycelia | Lacking | *Rhizoctonia* sp |
| Poprik31 | Slow growing | White with yellow background | | | Unidentified |

**Table 1: Contd.** Identification and characteristics of fungi isolated from cadavers of *C. lameensis*

| Isolate | Growth morphology | Colony colour | Key characters | Spore shape | Identity |
|---|---|---|---|---|---|
| Qoprik31 | Concentric growth | White with small black acervuli pustules | Conidia with 3-5 septa, dark at the median but colourless at the terminal end with two or more hyaline apical appendages | Fusoid with hyaline pointed end cells | *Pestalotia* sp |
| Roprik31 | Powdery | Gray with tangerine background | Phialides are penicillate | Globose | *Penicillium* sp |
| AKFF | Powdery | Olive green | Phialides are penicillate | Globose | *Penicillium* sp |
| BKFF | Slow growing and dense | Snowy White | | | Unidentified |
| CKFF | Dense growth and cushion like | Snowy White with acervuli dark | Conidia with 3-5 septa, dark at the median but colourless at the terminal end with two or more hyaline apical appendages | Fusoid, with hyaline pointed end cells | *Pestalotia* sp |
| DKFF | Slow growing | Snowy white | | | Unidentified |
| A/Twifo | Fluffy, cotton-like and dense | White | Conidiophores erect and closely grouped | Ovoid | *Metarhizium* sp |
| B/Twifo | Powdery | Olive green | Phialides are penicillate | Globose | *Penicillium* sp |
| C/Twifo | Concentric growth and powdery | Olive green | Penicillate phialides and pinch off conidia in dry chains | Globose | *Penicillium* sp |

**Pathogenicity test:** The fungal isolates induced varying levels of mortality in adult *C. lameensis* (Table 2). One unidentified isolate BKFF collected from a commercial farmer's field at Kusi induced the highest mortality of 77.5 % at spore concentration of 3.3 x $10^7$ conidia/mL while *Rhizoctonia* sp (Oprik31) induced the lowest mortality (12.5%) within seven days of inoculation. However, *Rhizoctonia* sp lacked spores and therefore the spore concentration could not be determined. Also, *Paecilomyces* sp (Ioprik31) and *Pestalotia* sp (CKFF) induced high mortality (65%) in the adult insects after seven days of treatment.

**Table 2:** Mortalities and conidia emergence post mortem of adult *C. lameensis* seven days after inoculation with the various isolated fungi

| Fungal Isolates | Spore concentration | Mean mortalities seven days after inoculation (± SE) | Growth of mycelia upon death of insect (%) |
|---|---|---|---|
| *Rhizoctonia* sp. (Oprik31) | 0.00 | 12.5 ± 0.75a | 0 |
| *Aspergillius* sp. (Joprik31) | 2.30 x $10^7$ | 17.5 ± 1.12a | 2.5 |
| *Penicillium* sp. (BTwifo) | 2.10 x $10^7$ | 15.0 ± 0.29a | 2.5 |
| *Rhizoctonia* sp. (Boprik31) | 0.00 | 15.0 ± 0.29a | 0 |
| Control | 0.00 | 16.8 ± 0.58a | 0 |
| *Penicillium* sp. (Roprik31) | 1.20 x $10^7$ | 17.5 ± 0.63a | 0 |
| *Penicillium* sp. (Coprik31) | 1.70 x $10^7$ | 20.0 ± 0.71a | 2.5 |
| Poprik31 (unidentified) | 1.10 x $10^7$ | 15.0 ± 0.29ab | 0 |
| *Penicillium* sp. (Koprik31) | 5.47 x $10^7$ | 25.0 ± 0.50ab | 17.5 |
| DKFF (unidentified) | 0.79 x $10^7$ | 27.5 ± 0.75ab | 7.5 |
| *Penicillium* sp. (CTwifo) | 1.60 x $10^7$ | 27.5 ± 0.48ab | 2.5 |
| *Pestalotia* sp. (Aoprik31) | 2.10 x $10^7$ | 32.5 ± 1.18ab | 0 |
| *Penicillium* sp. (AKFF) | 1.41 x $10^7$ | 35.0 ± 1.32abc | 0 |
| *Pestalotia* sp. (Qoprik31) | 3.50 x 106 | 37.5 ± 1.80abc | 0 |
| *Metarhizium* sp. (ATwifo) | 2.80 x 107 | 52.5 ± 0.75bcd | 0 |
| *Paecilomyces* sp. (Ioprik31) | 2.70 x $10^7$ | 65.0 ± 0.65cd | 15 |
| *Pestalotia* sp. (CKFF) | 8.50 x $10^6$ | 65.0 ± 0.65cd | 0 |
| BKFF (unidentified) | 3.30 x$10^7$ | 77.5 ± 0.25d | 47.5 |

Mean mortalities ± SE followed by same letters in same columns are not significantly different at $p < 0.05$ from one another (Duncan's multiple test).

**Re-isolation of fungi from treated insects:** Inoculated insects after death showed different incidence of sporulation and re-isolation confirmed them to be same organisms that were used in inoculation (Figure 2). A 47.5% growth of mycelia was observed for the unidentified (BKFF) fungus and no growth of mycelia was observed for *Pestalotia* sp, *Rhizoctonia* sp, *Metarhizium* sp, Poprik31 (unidentified) and *Penicillium* sp. (Roprik31, AKFF and Doprik31).

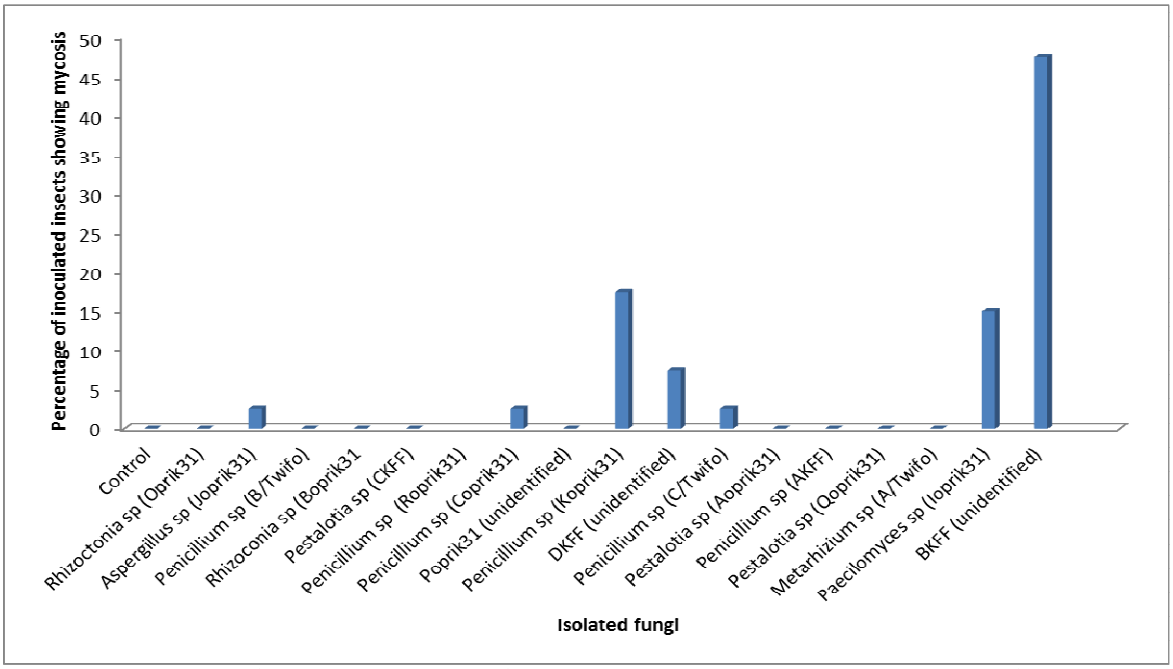


**Figure 2:** Emergence of mycelia post mortem of adult insect which were inoculated with isolated fungi.

## DISCUSSION

The use of Entomopathogenic fungi as alternative to synthetic insecticides is receiving renewed interest (McCoy, 1990). They have been found to be potentially the most versatile entomopathogens because many have wide host ranges and infect different stages and ages of their host, causing natural epizootics (Ferron, 1981). These fungi include *Paecilomyces farinosus, Zoopthora radicans,* (Bredfeld) Batko (Zygomycetes: Entomophorales), *Beauveria bassiana,* (Balsamo) Vuillemin (Deuteromycetes), *B. brogniati, P. fumosoroseus* and *Metarhizium anisopliae* among others (Sairbanu and Rabindra, 2002). In the current study seventeen fungal species were isolated from cadavers of *C. lameensis* suggesting that fungal pathogens are common microbial agents in regulating field populations of *C. lameensis*. This confirms report by Amer *et al.* (2008) that fungal infections are common in the Coleopterans. The fungi isolated in this study belonged to two genera i.e. Ascomycotina (*Penicillium* sp, *Aspergillus* sp, *Pestalotia* sp, *Metarhizium* sp and *Paecilomyces* sp) and Basidiomycotina (*Rhizoctonia* sp.), thus confirming reports by (Talwar, 2005; Dolinski and Lacey, 2007) that most entomopathogenic fungi are in the phylum Ascomycotina and Basidiomycotina. Pathogenicity screening tests showed that all the isolated fungi induced varying degrees of mortality in adult *C. lameensis*. The highest mortality (77.5%) induced by the unidentified fungus coded BKFF (from commercial farmer's farm) seven days after inoculation suggests that the spores are lethal to *C. lameensis*. A 47.5 % emergence of mycelia upon death of beetles further suggests that it has a great potential as a bio-control agent as it can self-perpetuate once the inoculum is introduced into field populations of *C. lameensis*. *Paecilomyces* sp also induced a high mortality of 65% and growth of mycelia (15%) after death of treated insects suggests that the fungus could serves as potential agent against *C. lameensis*. *Paecilomyces* sp has been found to be pathogenic to many insects (Alves *et al*., 2004; Amer *et al*., 2008; Er *et al*., 2008; Sookar *et al*., 2008). Jiji *et al.* (2006) also reported more than 50% cumulative mortality when puparia and adults *Bactocera curcubitae* were inoculated with *Paecilomyces lilacinus*. *Paecilomyces* sp has been isolated from white grubs, *Coccinella septempunctata*, *Galleria mollenella* and *Bactocera cucurbitae* (Lezama-Gutiérrez *et al*., 2000; Er *et al*. 2008; Ceryngier, 2000; Jiji *et al*., 2006). *Paecilomyces ferinosus* has been reported to be very virulent against all immature stages of the diamondback moth (DBM) except the egg and mortality occurs 48-72 hours after exposure of the pest to the fungal inoculum (Gopalakrishnan *et al*., 2000) . *Penicillium* sp, the most prevalent fungus and *Aspergillus* sp isolated have been reported by Er *et al*. (2008) as common saprophytic fungi that invades cadavers of insects. The authors also reported that fungal growths noticed on most of the cadavers of Coccinellids they worked with were generally

those of the saprophytic fungus *Penicillium.* These genera of fungi have been isolated from field populations of diamondback moth in Ghana (Anaisie *et al*., 2011) and *Zonocerus variegatus* in Ibadan (Balogun and Fagade, 2004). However in the present study, these two genera may have contributed to the mortality of adult *C. lameensis* as they were re-isolated from treated insects and identified as such. *Metarhizium* sp have been isolated from many insects and much work has been done on their efficacy and safety. The genus is the very first entomopathogenic fungus that was mass produced and used as pests control agent and commercial formulations of the bio-pesticides are available under many patent names (deFaria and Wraight, 2007). *Metarhizium* sp (ATwifo) and *Pestalotia* sp isolated in the present study each induced mortalities above 50% but doesn't attest to be the causative organism when a re-isolation test was conducted. However, Liu *et al.* (1996) observed that *M. anisopliae* var. *anisopliae* can induce up to 90% mortality in the larvae of the diamondback moth within 3 days. *Penicillium* sp were isolated from all three farms surveyed. The overall cumulative mortalities induced by the various *Penicillium* sp isolated were between 15% and 35 %. However, sporulation tests conducted on dead beetles revealed quite high recovery rate of (17.5%), in the Koprik 31 strain, and low recovery of 2.5% and 0% suggesting that all the species collected may not be important microbial control agents of *C. lameensis*. This confirming report by Humber (2005) that *Penicillium* sp may be a primary pathogen, facultative pathogen or just contaminant saprobes. *Aspergillus* sp also induced a low mortality of 17.5% but the fungus was recovered at 2.5% upon re-isolation. However, Baidoo and Ackuaku (2011) recorded a high mortality of 86.6% when 2nd stage larvae of the maize stem borer (*Eldana saccharina*) were inoculated with *Aspergillus flavus*. *Rhizoctonia* sp and *Pestalotia* sp seems not to have been isolated from any insect. However, Elliot (2005) has reported that *Pestalotia* species is a pest on many palm species. This could probably explain why it was found on cadaver of the beetles. Ivanovic and Ivanovic, (2001) and Vico *et al*. (2005) have also reported *Pestalotia* sp as a plant pathogen of potato, beans, alfalfa, tomato, cabbage and ornamentals. *Rhizoctonia* sp. on the other hand induced the lowest mortality of 12.5% suggesting that it may not be an entomopathogen but perhaps a saprophytic fungus that invaded the cadaver after death. Furthermore, a 0% mycosis on inoculated insects upon death suggests that it may not be important in controlling *C. lameensis*. The isolates BKFF, DKFF (from a commercial farm at Kusi), and P/oprik31 (from CSIR-OPRI plantation) appear from macroscopic and microscopic characteristics to be the same organism. Identification of these fungi were however not possible because the reproductive structures could not be clearly seen under the microscope and requires further studies. This study shows that some fungi are associated with the oil palm leaf miner, *C. lameensis* in nature, some of which are entomopathogens. Efficacy under laboratory conditions demonstrated the ability of these fungal isolates to induce some levels of mortalities in *C. lameensis* and may be useful bio-control agents against *C. lameensis*. The most promising isolates were the unidentified fungi (BKFF) and *Paecilomyces* sp whilst *Rhizoctonia* sp was found to be the least lethal. Further field tests are required on the important species identified in this study for possible incorporation into integrated control of *C. lameensis.*

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