

**EFFECTS OF POTENT FUNGAL-BASED BIOPESTICIDES ON PROMISING
INDIGENOUS FALL ARMYWORM (*Spodoptera frugiperda*) (J. E. SMITH)
(LEPIDOPTERA: NOCTUIDAE) ASSOCIATED PARASITOIDS IN KENYA**

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**A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL
FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF
PHILOSOPHY (M.PHIL.) DEGREE IN ENTOMOLOGY.**

**AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE
(ARPPIS) UNIVERSITY OF GHANA, LEGON, ACCRA, GHANA**

DECEMBER 2021

*** JOINT INTER-FACULTY INTERNATIONAL PROGRAMME FOR THE
TRAINING OF ENTOMOLOGISTS IN WEST AFRICA**

COLLABORATING DEPARTMENTS:

ANIMAL BIOLOGY AND CONSERVATION SCIENCE

(SCHOOL OF BIOLOGICAL SCIENCES)

AND

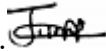
CROP SCIENCE

(SCHOOL OF AGRICULTURE)

COLLEGE OF BASIC AND APPLIED SCIENCES

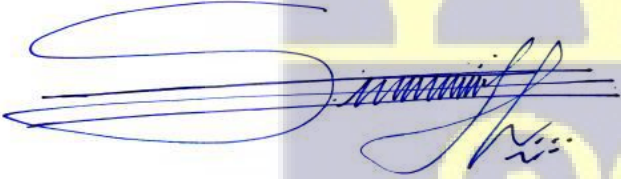
DECLARATION

I hereby declare that this thesis is the result of the original work personally done by me for the award of a Master of Philosophy Degree in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana. All the references to other people's work have been duly acknowledged and this thesis has not been submitted in part or whole for the award of a degree elsewhere.

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ABSTRACT

Maize is a major staple food crop in sub-Saharan Africa, mainly grown by resource-poor farmers. It is the third most important agricultural commodity worldwide after rice and wheat. However, its production is threatened by several biotic and abiotic factors such as cereal pests, parasitic weeds (striga), moisture stress, low fertility and diseases that inflict 15 – 90% yield losses. Arthropod pests are among the main factors leading to low maize yield and are central to many of today's major maize production challenges. These losses have been aggravated by the recent invasion of Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), a highly polyphagous pest that affects many crops with a great preference for cereal crops such as maize, sorghum, and rice. Many control strategies have focused on the use of synthetic chemical pesticides which has a lot of adverse effects to human and environment and has been ineffective. Biological control of FAW using parasitoids; *Telenomus remus*, *Cotesia icipe* and entomopathogenic fungal-based biopesticides have been explored. However, the combination of these biocontrol agents against FAW has not been evaluated in the invaded areas. In this study, four most potent entomopathogenic fungal isolates mainly *Metarhizium anisopliae* (ICIPE 7, ICIPE 41 and ICIPE 78) and *Beauveria bassiana* (ICIPE 621) were screened against FAW in the laboratory through direct and indirect fungal infections to assess their pathogenicity and virulence against *T. remus* and *C. icipe*, and determined their effects on the oviposition, emergence and parasitism rates of these parasitoids. The results showed high susceptibility of the parasitoids through direct infection, where *M. anisopliae* ICIPE 78 caused the highest mortality rate on adult *T. remus* (95.83 ± 4.17%) after direct infection of dry conidia to the adult parasitoids, whereas both *M. anisopliae* ICIPE 7 and ICIPE 41 caused 100% mortality to adult *C. icipe* seven day post-infection. However, the infected adult parasitoids were able to parasitize FAW larvae that were exposed to them prior to their death, but their F1 generation emergence was also significantly affected by direct fungal infection, with highest number of parasitoids recorded in the control (83.25 ± 5.94%) compared to fungal treatments. In the indirect infection where infected second instar FAW larvae were exposed to the parasitoids, *M. anisopliae* ICIPE 7 and ICIPE 41 and *B. bassiana* ICIPE 621 caused moderate mortality rates to *T. remus*, except *M. anisopliae* ICIPE 78 that caused >60% mortality to this egg parasitoid at all the concentrations (1 x 10⁵ – 1 x 10⁹ conidia/ml). However, the mortality rates of the endoparasitoid *C. icipe* increased when

the fungal isolates concentrations also increased. In both direct and indirect infections, additional induced mortality of FAW eggs and larvae was also obtained. Furthermore, high parasitism rates were obtained for both parasitoids in indirect fungal treatments as well as in the controls compared to direct infection experiment where the parasitism rates were lower (~40%) in fungal treatments than the controls (83.25%) for *T. remus*; while high parasitism rates were observed in *M. anisopliae* ICIPE 78 (62%) and *B. bassiana* ICIPE 621 (58%) compared to 35.75 and 36.75% in *M. anisopliae* ICIPE 7 and ICIPE 41, respectively for *C. icipe*. This study demonstrated that effective combination of entomopathogenic fungal-based biopesticides with both parasitoid species through indirect infection provide better suppression of FAW populations and consequently could be integrated into FAW-IPM strategies.



DEDICATION

To my mother, Linner Kamoing, Uncle Wesley Terer and my entire Kamoing family for the love and support. Also, to my colleagues both at *icip*e and UG for their encouragements and all the support during this study. May the Almighty bless you all abundantly.



ACKNOWLEDGEMENT

First, I would like to thank the almighty for his love, blessings and strength throughout this study period. I am also especially grateful in many ways to Dr. Komivi Senyo Akutse for the research opportunity, dynamic supervisory together with professional guidance and for permitting me to conduct my M.Phil. research work at *icipe*. I am also thankful to Dr. Ken Okwae Fening for his supervisory work and encouragement and scientific contributions to this work. Thank you Dr. Felicitas Chaba for your guidance during the data analysis and also manuscript development, proof reading and also your prayers and encouragements.

I also recognize the financial support from German Academic Exchange Service (DAAD) through the scholarship award to pursue this M.Phil. Programme and explore/build a network with other students and scientists across the world. My sincere appreciation also goes to the UK's Foreign, Commonwealth and Development Office (FCDO) by funding this research project through FCDO Fall Armyworm Management (B2329A-FCDO-FAW) and FCDO Biopesticides (B2291A-FCDO-BIOPESTICIDE) projects.

I also thank *icipe*'s graduate students, especially Dr. Mkiga Abdullah, Pascal Ayelo and Vongai Paradza who never got tired of training me on data analysis and positive criticisms which challenged me to research far in cleaning and analyzing my data. I thank *icipe*'s APU team mainly Mr. Levi Ombura, Sospeter Wafula, Francis Obala, Peter Malusi and John Buluma for their much-needed technical support in laboratory bioassays training as well as parasitoid and the host rearing. To my colleagues both at *icipe* and UG: Mercy Kibii, Joseph Munywoki, Bethelihem Mekonnen, Chika Obiekwe, thank you so much for the love and support you demonstrated toward me during our entire journey.

Lastly, I am so grateful to both Kamoing and Kimutai families for the constant love and

support together with their motivation throughout this journey.



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CHAPTER ONE

GENERAL INTRODUCTION

1.1. Background information

Maize is among the most common staple food crops across Africa and is largely grown by resource-poor farmers (FAOSTAT, 2017). In terms of area planted and consumption, it ranks third among the most significant agricultural products globally after wheat and rice. Diverse types are grown for human consumption, industrial processing, and animal feed and production. However, maize production was threatened by several factors such as cereal pests, parasitic weeds (striga), moisture stress, low fertility, and diseases that inflict 15 – 90% losses (IBPO, 2017). Arthropod pests are one of the major factors affecting maize yield, and are key to most, if not all, of today's major maize production challenges. Despite the use of pesticides, loss of crop yields due to arthropod pests are still high, especially in developing nations (Ferdu et al., 2001). More than 40 insect species were recorded in maize field. Of these pests, the main pests are the spotted stalk borer (*Chilo partellus*), maize stalk borer (*Busseola fusca*), and numerous species of termite (*Macrotermes* and *Microtermes* spp.) that cause significant losses.

These losses have been aggravated by the latest invasion of Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) which constitutes a high food security risk in Africa. The pest was reported for the first time in Africa in early 2016, in the rainforest zones of Nigeria (Georgen *et al.*, 2016). In Kenya, the pest was reported first in Trans Nzoia County by growers in March 2017, and the Kenya Plant Health Inspectorate Service immediately confirmed it (FAO, 2017). FAW is native to tropical and sub-tropical America, where it has been commonly termed among the most destructive crop pests. It has been reported to feed on over 80 host plants and devastated most cereals (with a special preference for maize and sorghum causing yield losses of up to 70%)

and has dispersed across Africa and Asia. The appearance and fast scattering of FAW is, therefore, a major risk to maize production and a significant handicap to food security in Kenya and Africa in general. Studies done by the Centre of Agriculture and Biosciences International in 12 states in Africa revealed that the pest leads to a loss of yield ranging from 8.3 -to 20.6 metric tons if not controlled (Day *et al.*, 2017). The pest makes long-distance migration aided by wind but also breeds in areas with suitable climatic conditions. Due to the presence of several host plants, favorable climatic conditions, and probably the limited/absence of its native natural enemies in Sub-Saharan Africa, this pest is capable of persisting over long periods causing serious damage to crops, therefore, affecting the livelihoods of resource poor farmers. Due to the lack of adequate management strategies in the newly invaded zones, fast reactions by governments and growers are highly based on the widespread deployment of synthetic pesticides, which are unsustainable and ineffective in the end, with negative impacts on non-target organisms, humans, and the environment. In the pursuit to develop sustainable and environmentally friendly approaches, some key potent fungal-based biopesticides were identified to be effective against eggs and neonates of FAW (Akutse *et al.*, 2019). The authors reported that *Metarhizium anisopliae* ICIPE 20, ICIPE 40, and ICIPE 78 outpaced all the other tested isolates by decreasing the hatchability of eggs by 79.5, 83 and 87%, respectively. Besides the mortality of eggs, the newly emerged larvae (neonates) get mortality induction from the fungal isolates, where *M. anisopliae* separates, ICIPE 7 and ICIPE 41 outperformed the rest by causing larval mortality of more than 90% in all cases. Moreover, ICIPE 7 and ICIPE 78 have already been commercialized as Achieve[®] and Tickoff[®] to fight ticks and spider mites respectively, and could thus be applied as an ovicidal biopesticide for suppression of FAW population across Africa (Akutse *et al.*, 2019). Furthermore, some parasitoids such as *Cotesia icipe*, *Chelonus*

curvimaculatus, *Charops ater*, *Telenomus remus*, *Trichogramma chilonis*, *Palexorista zonata*, and *Coccygidium luteum* were also locally found to be associated with and efficient against FAW egg and larval stages (Sisay *et al.*, 2018; Mohamed *et al.*, 2021). Therefore, there is an urgent need to evaluate the interactions between these effective biopesticides and the potent parasitoids to boost the control of FAW under the IPM umbrella in Africa. This study's aim, therefore, is to assess the non-target effects of the identified effective biopesticides on two selected FAW-associated parasitoids.

1.2. Justification

FAW is a highly polyphagous pest, that feeds on more than 80 species of crops with the greatest preference for cereals such maize, sorghum, rice, and other crops such as sugarcane, cabbage, pasture grasses, alfalfa, groundnuts, cotton, onions and tomato (Day *et al.*, 2017; FAO, 2018; Prasanna *et al.*, 2018). In Africa, its populations include both rice and corn strains and can cause significant losses of maize yield of between 8.3 and 20.6 metric tons per year based on a study conducted in 12 countries in Africa producing maize. This signifies a range of between 21- and 53% of the yearly maize production averaged over a period of three years in these states. The estimated value of these losses was between US\$2.48 and slightly above billion (Day *et al.*, 2017; CABI, 2017; Prasanna *et al.*, 2018).

FAW invasion into Africa alarmed several countries to use different insecticide types in maize farms as an emergency to come back to the pest attack (Kumela *et al.*, 2018). However, most of the resource-poor farmers in Africa not only cannot afford frequent sprays which are not cost-effective but are also concerned with studies that already reported that over-reliance on chemical pesticides results in the creation of resistance to major classes of insecticide (Yu *et al.*, 2003). Moreover, the abuse of these insecticides may result in residues in food, a higher threat to

human health because of lack of proper safety measures, adverse impacts on valuable and non-target organisms, and risks to the environment and biodiversity (Day *et al.*, 2017).

To curb the harmful consequences of the overuse of chemical pesticides, it is essential to develop new sustainable strategies for an IPM that is appropriate to African resource-poor farmers. Biological methods of control, which is a strong component of IPM strategies, is a more sustainable alternative insect pest control method and is widely recommended, as it has no harmful effects on the environment, or human and animal health. The use of entomopathogenic fungal-based biopesticides and natural enemies (predators and parasitoids) has been one of the best alternatives for pest control in recent years.

Some parasitoids such as *Telenomus remus*, an egg parasitoid, locally identified in Africa were used as a biocontrol agent against Fall armyworm (Tolon-Becerra *et al.*, 2012). Some other species of egg and larval parasitoids have also been reported in East and West Africa as FAW-associated parasitoids. For example, a study conducted by Sisay *et al.*, (2018) recovered five distinct parasitoid species from FAW larvae and eggs, including one Dipteran and four Hymenopteran. *Cotesia icipe* was the main larval parasitoid in Ethiopia, with rates of parasitism found to be between 33.8 and 45.3%. On the other hand, *Palexorista zonata* (or the tachinid fly) was the primary parasitoid in Kenya with a parasitism rate of 12.5%.

Metarhizium anisopliae and *Beauveria bassiana* are some of the Entomopathogenic fungi (EPF) considered being potential suppressors of different kinds of arthropod pests (Leland *et al.*, 2005). Fall armyworm was found to be vulnerable to as many as 16 entomopathogens comprising fungi, viruses, protozoa, bacteria, and nematodes (Wayne *et al.*, 1980; All *et al.*, 1996). Amongst them, *Bacillus thuringiensis*, *B. bassiana*, and *M. anisopliae* caused significant mortality in the FAW population (Molina-Ochoa *et al.*, 2003). Many of these pathogens naturally exist in FAW

populations but are also used artificially through inundative application to increase the natural epizootics. In addition, a recent study by Akutse *et al.* (2019) found two *B. bassiana* isolates are effective against FAW adults and three *M. anisopliae* isolates are effective against FAW eggs and neonates. Since the IPM strategy includes synchronized implementation of different control methods, such management strategies need to be evaluated together to use them for precise pest control. Potential microbial control agents' effects on the parasitoids should be examined for an effective FAW-IPM programme (Hajek and St. Leger, 1994). The findings of the current study could significantly contribute to the sustainable suppression of FAW using both biopesticides and parasitoids in the cereals production systems in Africa and Asia.

1.3. Objectives

1.1.1 General objective

The overall goal of this study was to establish the interactions between potent fungal-based biopesticides and FAW-associated parasitoids for effective management of the invasive pest populations under cereals production systems in Kenya.

1.1.2 Specific objectives

To achieve this main objective, the following specific objectives were proposed:

- 1- To assess the direct effects of the potent *Metarhizium anisopliae* isolates ICIPE 7, ICIPE 78, and ICIPE 41 and *Beauveria bassiana* ICIPE 621 on adult *Telenomus remus* and *Cotesia icipe*
- 2- To evaluate the indirect effects of the potent fungal isolates of *M. anisopliae* and *B. bassiana* on the performance of *Telenomus remus* and *Cotesia icipe*
- 3- To compare the parasitism rates of the potential parasitoids exposed to fungal infected and non-infected FAW larvae/ eggs.

CHAPTER TWO

LITERATURE REVIEW

2.1. Taxonomy of Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

One of the world's largest orders of insects containing butterflies and moths is the Lepidoptera order. Scales on the wings of moths and butterflies, which fall off when handled, distinguish between the two. Economically beneficial pests that feed on seeds, stored food, or cloth are many species in the Lepidoptera order. Insects belonging to this order undergo full metamorphosis throughout the egg, larval, pupal and adult stages (Ferreira, 2015). *Spodoptera* is a Noctuidae family genus where the moths are nocturnal. Noctuidae larvae have five pairs of prolegs in smooth and dull colour; a majority eat plant foliage and few eat fruit (Borror *et al.*, 1989).

2.2. Origin and distribution of Fall armyworm

The Fall armyworm, (FAW; *Spodoptera frugiperda* J E Smith, Lepidoptera: Noctuidae) which is a North and South American native, appeared for the first time in Africa in 2016 (Goergen *et al.*, 2016). Its first detection was in Nigeria and it dispersed rapidly from West Africa across the continent, and in 2017 it was reported in parts of Southern and East Africa (Midega *et al.*, 2018). In Kenya, the Trans Nzoia farmers made the first reports in the County by in March 2017 and the Kenya Plant Health Inspectorate Service confirmed the case (FAO, 2017). Of the 47 counties, the pest has spread rapidly to 43, and the pest primarily infests maize crops (FAO, 2019). As there is no maize production, the Semi-Arid counties such as Garissa, Marsabit, Lamu, Mandera and Wajir have so far not recorded any occurrences.

They feed on more than 80 host plants, devastated most cereals (with special preference for maize and sorghum causing yield losses of up to 70%), and now has dispersed through Africa

and Asia. The entrance and quick spread of FAW is, therefore, a key threat to maize production and significant handicap to food security in Kenya and Africa in general. Centre of Agriculture and Biosciences International conducted studies in 12 countries producing maize in Africa revealed that the pest leads to a loss of yield of between 8.3 and 20.6 metric tons if not controlled (Day *et al.*, 2017). The pest makes long-distance migration aided by wind, but also breeds in areas with suitable climatic conditions. Due to the presence of several host plants, favourable climatic conditions Sub-Saharan Africa, this pest is capable of persisting over long periods causing serious damage to crops therefore affecting the livelihoods of resource poor farmers.

2.3. Biology and ecology of FAW

Fall armyworm life cycle comprises of the egg, six stages of growth of instars (caterpillar development), pupa, and moth. During the warm summer conditions, the life cycle lasts about 30 days (at an average temperature of $\sim 28^{\circ}\text{C}$) and up to 60 days in the seasons of autumn and spring, and between 80 to 90 days in winter period (Capinera, 2014). Similar to other insects, Fall armyworm development rate is affected by temperature. Its development rate is faster at high temperature, though a decline is observed at temperatures above 33.8°C . Just as they cannot survive in freezing temperatures and cannot progress when temperatures lower than 10°C (Silva *et al.*, 2016). Eggs are laid in 100–200 egg masses. The eggs hatch in 2 to 3 days and neonates emerge. The larval instars are six, and the sixth one is the most damaging stage. Typically, larvae feed on the leaves that are featured by moist sawdust-like frass and ragged feeding near the funnel and upper leaves. Early feeding may seem similar to other caterpillars. Deep eating inside the leaves funnel kill the developing tassels and growth points. The older larvae do not leave inside the funnel and mostly eats at night (FAO and CABI, 2019).

The adult wing-size of the fall armyworm can reach 32 to 40 mm (Capinera, 2000), but sexual dimorphism is present. The female forewings without noticeable patterns are gray (Plate 1a). The male usually have brown forewings patterned with white spots (Plate 1b). The last instar larvae has a dark head with an inverted mark of white shape of Y (Plate 1c).

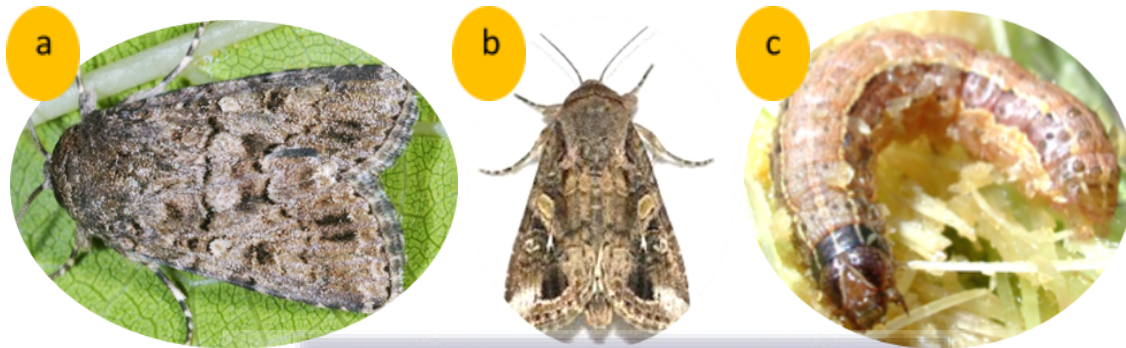


Plate 2. 1; Description of the Fall armyworm; a- female, b- male, c- Sixth instar larva

2.3.1. Eggs

The eggs are pale green/white when freshly laid and are laid in clusters of about 100-200 eggs on the underside of the leaf. The scales from the female's body cover the egg mass. They hatch within 2 to 3 days. The egg of FAW is dome-shaped with a flat base and has a diameter of approximately 0.4 mm and height of 0.3 mm. A single adult female will lay between 1500 and 2000 on average during her lifetime (CABI, 2017).



Plate 2. 2:FAW egg mass with scales

2.3.2. Larva

There are six larval stages. Neonates have a black head and a greenish body (plate 3a), the head in the second instar is orange (plate 3b), while in the third instar the body's dorsal surface becomes brownish and white lateral lines begin to form (plate 3c). The fourth to sixth instars have head colored reddish brown, mottled with white and brownish body bears white sub dorsal and lateral lines. Elevated spots emerge dorsally; usually dark in color, with bear spines (Capinera, 2014; CABI, 2017). The larva has an inverted "Y" dominant on the head capsule. Larvae markings may include a non-incessant white line in middle as well as an abdomen with red and yellow "flecking". Larvae also have a distinct pattern of four "dots" on the eighth abdominal segment which is the best identifying feature of the Fall army worm from other armyworms. The larval length ranges from about 1.68 mm and 34.15 mm, (Luginbill, 1928)

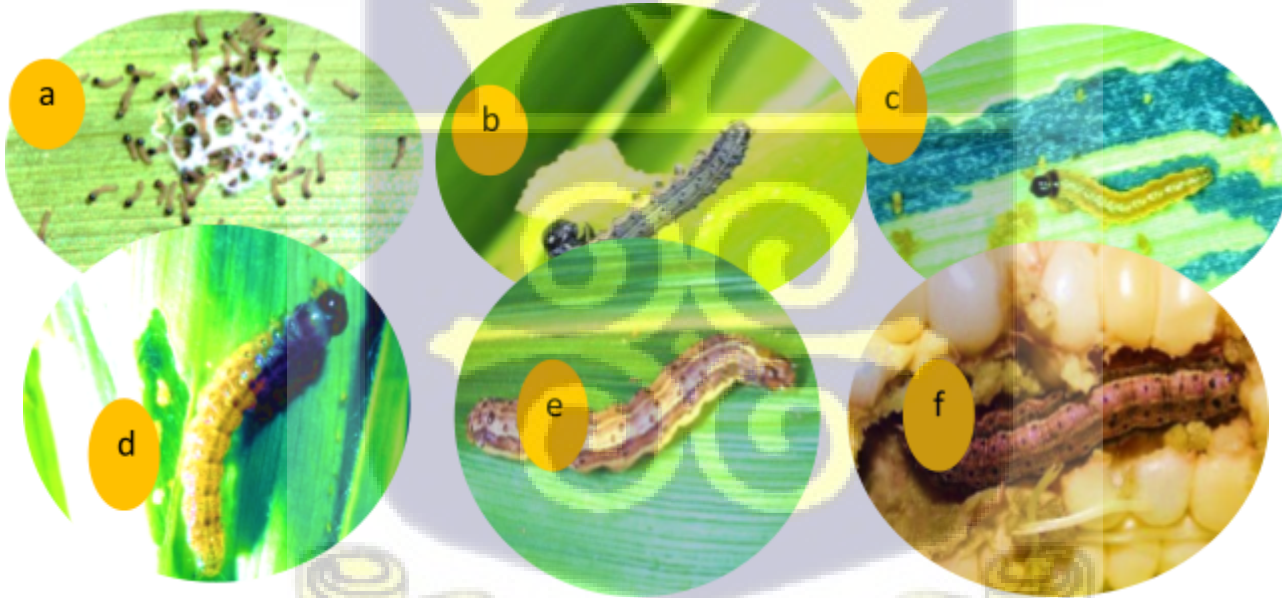


Plate 2. 3: Fall armyworm different larval stages; a- first instar, b-second instar, c- third instar, d- fourth instar, e- fifth instar, f- sixth instar.

2.3.3. Pupa

Pupae are shiny brown and pupation usually occurs in the soil or in reproductive parts including mature maize ears. The larva builds a wobbly cocoon by binding together silk. The cocoon is oval in shape and length is between 20 and 30 mm. If the soil is too hard, larvae may web leaf debris and other material together to form a cocoon on the soil surface. The period of pupal development is about 8 to 9 days during warm temperatures and about 20-30 days during cooler temperatures before an adult emerges (CABI, 2017).

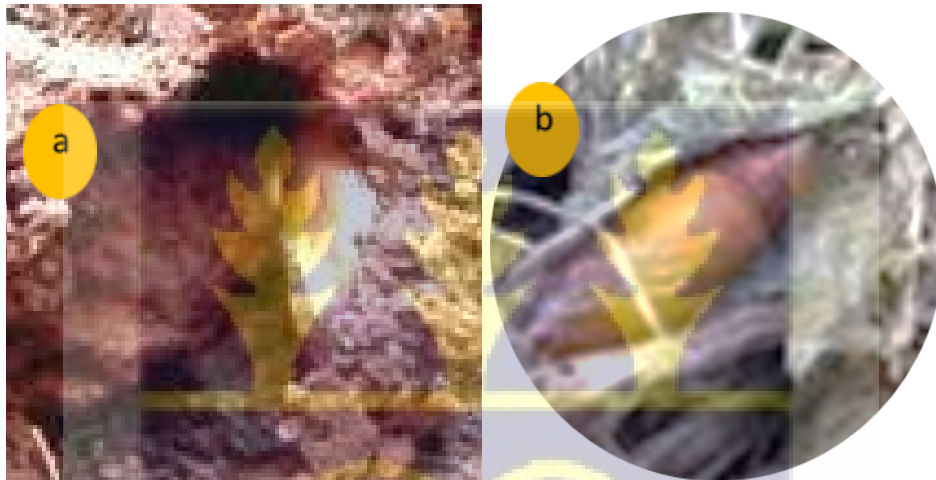


Plate 2. 4: Fall armyworm pupation; a- in the soil, b- in the debris

2.3.4. Adult

The male and female moths are typically different. The forewing is usually shaded in gray and brown in the male moth, with the tip having white triangular spots and in the middle of the wing (plate 5a). Females are less conspicuously colored, with a range of a uniform grayish brown to fine gray and brown mottling (plate 5b). The hind wing is iridescent silver-white, with both sexes having a narrow, dark margin. Adults are nocturnal, and at dry, humid evenings are most active. Following 3 to 4 days of pre-oviposition, the female usually lays most of her eggs within the first 4 to 5 days but some oviposition takes place for up to 3 weeks of life. The adult lifespan is about 10 days on average, with a range of between 7 and 21 days (Luginbill, 1928; Sparks, 1979).

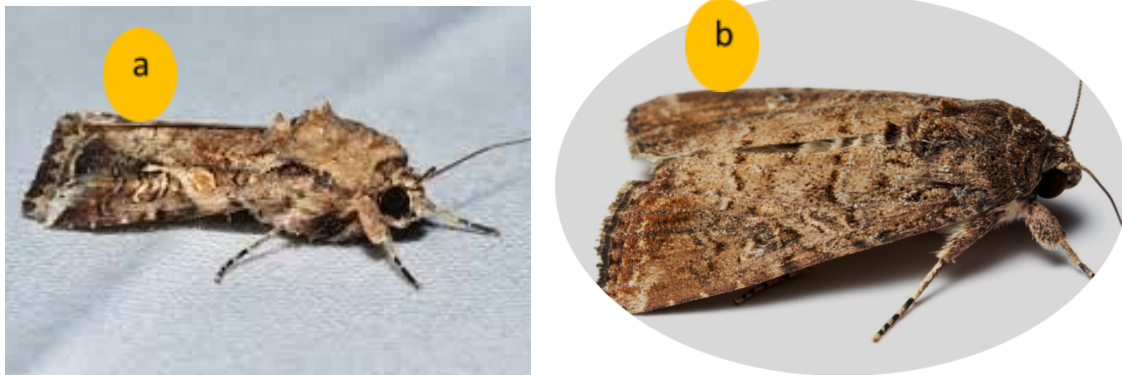


Plate 2. 5: Fall armyworm adults showing; a- male, b- female

2.4. Nature of damage

FAW is a polyphagous pest feeding over 80 different crop species with the highest liking for maize and others including sorghum, rice, sugarcane, cod, pasture grasses, soybeans, groundnuts, alfalfa, cotton, onions, tomatoes and potatoes (B.M. Prasanna *et al.*, 2018). It was not detected previously outside the Americas. However, its two strains are present in Africa and quickly dispersing through tropical and sub-tropical areas. In Africa, the population comprise of both the rice and corn strain.

Larvae consume foliage and thereby cause damage. The young larvae eat leaf tissues from one side and leave intact the opposite epidermal layer. From the second instar, the larvae begin to feed inward from the leaf edge creating holes. Feeding in the corn whorl also yields a typical series of leaf perforations. Older larvae lead to widespread defoliation, in most cases leaving only the stalks and ribs or a ragged torn look of maize plants (Capinera, 2014).

Larvae will also dig tunnel into the growing point, destroying growth potential of the plant, or cutting leaves. In maize, they often dig holes into the ear, eat kernels in the same way as *Helicoverpa zea*, the corn earworm. In comparison to corn earworm, which eat through the silk

an then attacks the ear tip of the kernels. Fall armyworm eat by digging holes in the husk on the ear side (plate 6a,6b and 6c).



Plate 2. 6: Damage caused by the fall armyworm on maize; a- leaves, b- whorl, c-ear

Several factors suggest that *S. frugiperda* is likely to become more damaging to maize than other species of the same genus occurring in Africa which includes adult females of *S. frugiperda* directly oviposit on maize, the mandibles of caterpillars of the fall armyworm have comparatively stronger, serrated cutting edges, which ease the feeding on plants with high silica. Due to its migratory habit and a dispersal habit, FAW moths spreads quickly across large geographic areas. The moths can migrate over 500km before oviposition (Kebede., 2018). The pest can also persist throughout the year in Africa since the host plants are readily available and also the climatic conditions are suitable. Although the patterns of population persistence, dispersal, and migration in Africa are yet to be determined, conditions in Africa, especially where there is a bimodal rainfall pattern, suggest that the pest can persist throughout much of the year.

2.5. Fall armyworm management options

The secret to managing Fall armyworm is detecting it before they inflict economic damage. The moth can be sampled with either black light or pheromone traps. Pheromone traps are more effective than backlight traps. The other plans that have been used for fall armyworm management include Biological control practices, cultural management options, use of synthetic insecticides and botanicals (Viana and Prates, 2003).

2.5.1. Cultural control

Sole maize cropping systems provide an ideal climate for FAW to spread rapidly. The cultural control includes early planting, good soil moisture, and health as healthy plants can endure infestation. The diversity of plants, including multiple varieties and intercropping systems, lessen the oviposition rate by confusing female FAW moth. In addition, rotating and intercropping maize with non-host crops such as beans and sunflower can minimize invasion by FAW (FAO, 2018).

In Africa, Most subsistence farmers do not use pesticides to control; however, they use cultural control methods, such as wood ashes, caterpillar picking and killing, intercropping, and soils to leaf whorls (Abate *et al.*, 2000). A survey in Ethiopia and Kenya found that 39% and 14% of farmers applied cultural methods of FAW management (Kumela *et al.*, 2018).

Recent research has shown that a Push-Pull or climate-adapted version is successful in controlling the Fall armyworm, offering a usable, environmentally friendly, and cost-effective pest management technique. Such results show evidence of a technology that should be implemented for the FAW's efficient management. The study indicated that FAW infestation was about 80 percent lower in plots where Push-Pull was adopted, together with an increase in grain yields relative to monocrop plots (Midega, 2018).

2.5.2. Pheromone lure

Insect traps are effective methods for pest population monitoring in the integrated pest program Management (IPM). Traps detect invasions by new species of pests, the start of Seasonal behavior of the pest, assess the extent and severity of the infestations, and monitor changes in the pest populations, both of which lead to informed pest management decision-making (Wyatt, 1998). Traps are usually used to attract pest insects through olfactory (chemical) and/or visual cues or stimuli. Pheromone lures are a vital tool for identifying and managing several insect pest populations (Spears, 2016).

The use of pheromone traps as a monitoring technique for adult *S. frugiperda* will be useful for indicating the real demand for control of the fall armyworm in maize, particularly when the trap is placed in the field shortly after planting. The decision on the use of seed treatment with appropriate chemical insecticide can also be determined if the trap is placed a few days before planting (Cruz *et al.*, 2010).

2.5.3. Botanical control

The use of botanical pesticides is the alternative to harmful synthetic pesticides that cause environmental pollution, increased consumer prices, the resurgence of pests, and pesticide resistance (Arya and Tiwari, 2013). Because of availability and affordability, the farmers in developing countries use botanical insecticides to combat insect pests (Schmutterer, 2009).

Croton macrostachyus, *Azadirachta indica*, *Phytolacea docendra*, *Milletia ferruginea*, *Jatropha curcas*, *Chrysanthemum cinerariifolium*, and *Nicotina tobaccoum* are some plants that are successfully used for insect pest control (Schmutterer, 2009; Addisu *et al.*, 2014). The plant species have useful properties, like antifertility or growth disrupters, oviposition/ hatching

deterrence, anti-youth hormone activity, repellence, biodegradability, and anti-nutrition (Mochiah *et al.*, 2011).

Recent research has shown that Papaveraceae (*Argemone ochroleuca*) ethanolic extracts led to larval FAW mortality because of decreased feeding and delayed larval development (Martínez *et al.*, 2017). Asmare *et al.* (2006) noted that botanicals like *N. tabaco* and *J. curcas* have been better than and superior crude controls in decreasing insect harm and increasing maize yield. Many plant extracts show insecticidal features that combat FAW, but just a few are commercialized successfully. *Melia azadirachata* is one of the possible bioactive plants that have been studied extensively in the laboratory and also in the field against many insect pests and vectors (Charleston, 2004). Isolated from *Croton macrostachyus* bark, the compound *cisdehydrocrotonin* inhibits the development of lepidopteran pests (Viegas, 2003).

2.5.4. Chemical control

Control of FAW is achieved using synthetic insecticides (Blanco *et al.*, 2014, 2010; Hruska and Gould, 1997), but entails high costs, adverse effects on the environment, and the creation of resistance to chemicals (Colborn, 1995; Crowe and Booty, 1995).

Yu (1991) documented a Fall armyworm strain harvested from maize in North Florida that exhibited resistance to common insecticides. Resistance to pyrethroids varied from 2- to 216-fold; fluvalinate was observed with the highest degree of resistance. Resistance to organophosphorus insecticides fluctuated between 12- and 271-fold; methyl parathion was the greatest resistance degree observed. Carbamate resistance ranged from 14- to > 192-fold, with carbaryl being the highest degree of resistance observed. He further claimed that the broad range of insecticide resistance found in the field strain was due to different resistance mechanisms, including higher detoxication of these insecticides by microsomal oxidase and insensitivity to the

target site. Managing Resistance is a critical part of IPM. Managing pesticide resistance can increase the useful life of essential pesticides that are compliant with IPMs. Combined with daily pest control use of appropriate thresholds for care, it is likely to be effective and allow full use of non-pesticidal approaches such as biological and cultural management, field sanitation, and host plant resistance. The exact timing for applying chemical substances is critical for successful control. Both time of day matter and the life cycle, i.e. applying chemicals when larvae are deep inside the maize ears and whorls is ineffective. Moreover, spraying during the day is useless as larvae emerge from the holes at night, dusk, or dawn to feed (Day *et al.*, 2017).

2.5.5. Biological control

Biological control is one of the most successful alternative control measures offering sustainable and healthy plant safety for the ecosystem. The effectiveness of biological control will rely on knowing how the implemented biological agents are created and adapted. Biocontrol agents and Microbial pathogens from arthropods have been used extensively in agricultural systems due to their safety for the environment and non-target vertebrates, and manufacturing costs have been drastically reduced in recent times (Mahmoud, 2017). Several biological agents have been documented that are effective against the FAW.

2.5.5.1. Parasitoids and predators

Numerous insects experience parasitizing *S. frugiperda* eggs and larvae. Ashley (1979) described 53 parasitoid types; only eighteen are specific to the United States, while twenty-one are in South and Central America, including Mexico. Three hymenopteran and eight dipteran parasitoid kinds parasitize FAW in Argentina. Egg-larval parasitoids, for example, *Chelonus insularis* occurs across North America as FAW parasitoids and are recorded to cause 63% parasitism in Southern Florida (Murúa *et al.*, 2009). In Latin and South America, *C. insularis* is widely distributed and,

due to its overwintering behavior, it is considered to be a good candidate for augmentative release. Thus, when released on specific crops, it can colonize the area early and assist in pest management (Lewis and Nordlund, 1984). The level of parasitism caused by *Ichneumonid* wasps was low in Vipos, and during a four-year analysis, it ranged between 0.8 and 1.3 percent (Murúa *et al.*, 2009). A number of parasitoid species were reported attacking *S. frugiperda* in a survey carried out in different locations in Karnataka, South Asia. For instance, egg parasitoids, *Telenomus* (Hymenoptera: Platygastridae), *Trichogramma* sp (Hymenoptera: Trichogrammatidae) and larval parasitoids, *Glyptapanteles creatonoti* (Hymenoptera: Braconidae), and *Campoletis chloride* (Hymenoptera: Ichneumonidae) (Shylesha *et al.*, 2018).

Parasitoids such as *Telenomus remus* Nixon, a Hymenoptera egg parasitoid, locally identified in Africa was used as a biocontrol agent against Fall armyworm (Tolon-Becerra *et al.*, 2012). Some other egg and larval parasitoid species are in East and West Africa as FAW-associated parasitoids. For example, a study conducted by Sisay *et al.*, (2018) recovered five different types of parasitoids from larvae and eggs of fall armyworm, including one Dipteran and four Hymenoptera. *Cotesia icipe* is dominant in Ethiopia, (Hymenoptera: Braconidae) with parasitism rates of between 33.8% and 45.3%, while the tachinid fly is dominant in Kenya (Diptera: Tachinidae) with parasitism of 12.5%. *Charops ater* and *Coccygidium luteum* are common in Kenya and Tanzania with parasitism of between 6-to12% and 4 to 8.3%, respectively (Birhanu *et al.*, 2018).

Ghana and Benin also recorded *Chelonus bifoveolatus* and *Coccygidium luteum* (Hymenoptera: Braconidae) as the key egg-larval parasitoid species causing up to 75% parasitism while egg parasitoid, *T. remus* caused parasitism ranging between 14.5% and 25.9%, respectively (Agboyi *et al.*, 2020). *Trichogramma* sp was among the egg parasitoids collected. Three predators and

seven parasitoid species were identified in Ghana by (Koffi *et al.*, 2020) *C. luteum* and *C. bifoveolatus* were common with parasitism rates of 1.04% and 0.85% respectively.

Similarly, research on natural enemies of FAW was conducted in maize and sorghum fields in Niger in 2017 and 2018. It showed the existence of three egg parasitoids, Trichogrammatoidea sp. *Trichogramma* sp., and *Telenomus* sp. (Hymenoptera: Platygasteridae); one egg-larval *Chelonus* sp parasitoid (Hymenoptera: Braconidae); four larvae parasitoid, *Cotesia* sp., *Charops* sp., and tachinid fly and unidentified ichneumonid (Amadou, *et al.*, 2018).

FAW's predators attack larvae of other kinds of lepidopterans. The most significant predators are ground beetles, the spined soldier bug, striped earwig, the insidious flower bug, and *Orius insidiosus* (Capinera, 2001). Vertebrate predators are rodents, skunks, and birds, which eat FAW larvae and pupae (Capinera, 2005).

2.5.5.2 Biology of *Telenomus* species

Telenomus remus Nixon is an effective biocontrol agent for several pests belonging to the *Spodoptera* genus (Pomari *et al.*, 2013). Up to 270 eggs are produced by adult female parasitoids before they die, which are typically laid on host eggs. Additionally, *T. remus* is successful in parasitizing *Spodoptera* spp. as it can attack eggs situated in the innermost layers of the egg mass, with its long ovipositor (Pomari *et al.*, 2013). *T. remus* is widely distributed because of its elevated host search capabilities that make it ideal for augmentative release (Cave, 2000). To assess its effectiveness on *S. frugiperda* eggs, several studies have been performed worldwide. (Bueno *et al.*, 2010; Cave, 2000). The level of its parasitism in various populations in Barbados ranged from 50-70% under laboratory conditions, whereas in Venezuela its parasitism rates was between 78-100% in corn fields (Cave, 2000).

Telenomus remus, *T. pretiosum*, and *T. atopovirilia* Oatman & Platner are the most reported egg parasitoids of FAW in Latin America (Beserra *et al.*, 2005; Parra and Zucchi, 2004). Among the three species, *T. remus* is a fit candidate for biological control of crop pests due to its robustness, larger size than *Trichogramma* sp. and its capability to attack inner deposits of the egg masses of FAW (Cave, 2000). Bioassays from Laboratory have shown that *T. remus* is capable of parasitizing *S. frugiperda* egg masses faster than *T. pretiosum* (Carmo *et al.*, 2010). When the two species are exposed to FAW eggs, adult *T. remus* is the majority that emerges (Carmo *et al.*, 2010).

This egg parasitoid was introduced in Brazil in the mid-1980s with the goal of testing its efficacy in a classical control program to reduce FAW. In soybean IPM, it has also been used to control pests such as *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) and *S. eridania* (Cramer) (Lepidoptera: Noctuidae) which attack crops at the reproductive stage feeding on foliage and affecting the pods. In Mexico, *T. remus* is reported to be effective on *S. frugiperda* egg masses with a parasitism rate ranging between 78% -100% when 5000 to 8000 parasitoids are released per hectare of land (Bueno *et al.*, 2010).

2.5.5.3 Biology of *Cotesia* species

Cotesia species belongs to the subfamily Microgastrinae (Hymenoptera: Braconidae) which is the single most important group of parasitoid wasps that are reported to control larvae of lepidopterous species Yu *et al.* (2013). It consists of more than 2,700 described species (Yu *et al.* 2016) with many others yet to be described (Rodriguez *et al.* 2013). Originally, *Cotesia* was considered as a genus by Cameron in the 19th century and definitively split from the genus *Apanteles* by Mason (1981) during his reclassification. 12 species of *Cotesia* have been recorded from the entire Afrotropics (Rousse and Gupta 2013, Yu *et al.* 2016, Kaiser *et al.* 2017).

The females emit sex-pheromones to attract males for mating (Xu 2014). Microgastrine wasps apply a tamed virus (Polydnaviridae: bracovirus) to suppress the immunological reaction of the host larva. They develop Bracoviruses in the ovaries of wasps by integrating genes into their genomes and then injecting the eggs into the host body (Gitau *et al.* 2007). The virus infects the cells of the host creating viral proteins that inactivate the immune cellular response of the host and regulate metabolism, helping wasp larvae. (Herniou *et al.* 2013). To undergo metamorphosis, fully formed larvae exit the host and create cocoons.

2.5.6. Use of entomopathogens

Over 700 fungi species are pathogenic to insects from approximately 90 genera (Roberts and Humber, 1981). *Metarhizium*, *Beauveria*, *Isaria*, and *Lecanicillium* are mainly intensively researched genera for the development of mycoinsecticides, as they are fairly easy to produce in mass production (Vega *et al.*, 2009). Using microbial control is a useful alternative to chemical pesticides (LezamaGutiérrez *et al.*, 2001).

About 16 entomopathogens species, including fungi, viruses, nematodes, bacteria, and protozoa, are vulnerable to fall armyworm (All *et al.*, 1996; Wayne *et al.*, 1980). *Metarhizium anisopliae*, *Beauveria bassiana*, and *Bacillus thuringiensis* cause major mortality rates to FAW among the 12 pathogens and decrease leaf defoliation of plants (Molina-Ochoa *et al.*, 2003).

2.5.6.1 Mode of infection of entomopathogenic fungi

The typical infection route for entomopathogenic fungi is through the cuticle (Goettel *et al.*, 2000). Depending on favorable conditions for temperature and relative humidity within the cuticular insect cuticle, virulent conidia grow via the host cuticle.

2.5.7. Fungal conidia attachment to the host cuticle

Insect cuticles act as the primary propagules of fungal barriers. Both cuticle and conidia of insects are hydrophobic, producing an initial inert interaction. Pathogenicity begins with the host having to adhere to conidia (Brobyn *et al.*, 1977). Initially, Conidia binds by preformed mucilage. The mutual action of actively secreted mucilage and enzymes before germ tube emergence achieves permanent attachment. Adhesion seems to be attributable to conidia-covered rodlets applied by hydrophobic forces for *B. bassiana* and *M. anisopliae* (Charnley *et al.*, 1989). *M. anisopliae* conidial mucoid coating is minimal, indicating that its binding position is poor and that before solid anchorage is formed, the appressorium provides a risk of loss. (Zacharuk, 1970a).

2.5.8. Conidial germination

Entomopathogenic fungi need nutrients, fatty acids ions, free water, integuments, and microflora for germination and are adopted for the use of alkanes and lipids as carbon sources. Water is necessary for mycopathogens to successfully infect their host insects. The cuticle also contains other compounds that act as nutritional sources for fungal germination, mainly amino acids and amino sugars (Butt *et al.*, 1998; Hajek and St. Leger, 1994).

2.5.9. Fungal penetration into the cuticular layer

Insect cuticle penetration is accomplished through enzymatic degradation and insertion of mechanical pressure (Brobyn *et al.*, 1983). Lipases, proteinases, and chitinases are the enzymes responsible (Weiser *et al.*, 1982). Besides penetration, fungal germination is mainly affected by the physical parameters assigned to the cuticle, availability of water and nutrients, and chitin content. Additionally, *Metarhizium anisopliae* produces hydrogen peroxides that bleach insect

cuticle and softens them, aiding in penetration of the hyphae. This is accompanied by the development of extra-cellular that are produced by fungi (St Leger *et al.*, 1986a).

2.5.10 Hosts immune responses

The fungus typically grows in the haemocoel as yeast-like hyphae that multiply by budding during the pathogenic process, sometimes referred to as blastospores (Charnley, 1992). Host defenses involve a system of phenoloxidase that deposits oxidized phenols and cuticle protease inhibitors and can limit the activity of pathogen enzymes (Moore and Prior, 1993). With haemocytes trapping fungal fragments, nodule formation within the haemocoel is the major cellular protection against the fungi (Charnley *et al.*, 1992).

2.6.0 Factors that affect fungal efficacy on insects

Abiotic and biotic variables primarily impact fungal efficacy by inhibiting epizootic growth triggered by entomopathogenic fungi. Abiotic factors consist of water availability, appropriate temperature, length of precipitation periods, canopy nature, and rainfall, whereas biotic factors include strains of the pathogens, host physiology, nourishment, mechanism of defense, and epi-cuticular micro-organisms.

2.6.1 Abiotic factors

2.6.1.1 Temperature

This is a crucial factor that influences the efficiency of entomopathogenic fungi (Watanabe *et al.*, 1987). The majority of entomopathogenic fungi have a wide temperature tolerance range, but usually, the optimal temperature for infection, growth, and sporulation is much more limited (usually 20-30⁰ C) (Goettel *et al.*, 2000). *Beauveria bassiana* and *M. anisopliae* tropical Isolates have shown up high growth and infection at optimal temperatures ranging from 20-25 °C.

Fargues (1997b) demonstrated that four isolates of *M.anisopliae* var. *acridum* were pathogenic to *S. gregaria* and caused mortality of 98-100 percent at temperatures between 25 °C and 30 °C, though when the similar pathogen was applied to *S. gregaria* kept at 40°C, mortality was 0%.

2.6.1.2 Relative humidity

On cadavers, high humidity is required for entomopathogenic fungal spores to germinate, penetrate the cuticle, and sporulate (Benz, 1987; Hajek *et al.*, 1990; Inglis *et al.*, 2001). At least 90 percent free water or humidity is essential for fungal sporulation and spore germination (Goettel *et al.*, 2000). Reports by Daoust (1983) indicated that conidia of *M. anisopliae* thrived best at moderate temperatures of between (19-27^o C) when RH was high (97%). On the other hand, *M. anisopliae* var. *acridum* can infect the desert locust at a relative humidity as low as 13%.

2.6.1.3 Solar radiation and light

Conidia of entomopathogenic fungi are vulnerable to solar radiation, particularly ultraviolet light, which is harmful to all microorganisms (Inglis *et al.*, 2001). For example, after 48 hours of incubation, conidia formed in oil were exposed to radiation below 320 nm for 2 hours, which lowered germination from 99.0 percent to 37.5 percent (Moore *et al.*, 1993). Despite the negative effects of ultraviolet radiation, mycelial development, sporulation intensity, and germination of *B. bassiana* spores have been reported to be stimulated by light. Tang and Hou (2001) found that incubation under full (24 hours) and half-light (12 hours) light increased the pathogenicity of *N. rileyi* against the larval stage of *Helicoverpa armigera* (Lepidoptera: Noctuidae). There are, however, differences in irradiation susceptibility among entomopathogenic fungal species and strains within species (Fargues *et al.*, 1996; Goettel *et al.*, 2000).

2.6.2 Biotic factors affecting the efficacy of entomopathogenic fungi

2.6.2.1 The pathogen properties

The biological characteristics of a pathogen involved in disease transmission include infection mechanisms, virulence, pathogenicity (infectivity), and proliferation. Nonetheless, different types of infections have diverse pathogenic properties (viruses, bacteria, fungi, protozoa, and nematodes). The most significant characteristics are virulence and infection, which are crucial in the selection of a suitable candidate for microbial control (Tanada and Fuxa, 1987).

The specificity of the host controls the capacity of pathogenic microorganisms to invade, establish and replicate potential insect hosts within them. (Hajek *et al.*, 1995). *Metarhizium anisopliae* has 18 described species out of roughly 300 Lepidopteran, Coleopteran, Orthopteran, and Hemipteran species, while *B. bassiana* has over 700 host species (Moore and Prior 1993). Present host ranges, on the other hand, are normally limited to, and sometimes even more narrowly limited to, the original host's family (Moore and Prior, 1993).

2.6.2.2 Host plant

Herbivore existence, development, reproduction, distribution, and disease susceptibility have all been demonstrated to be influenced by inter-and intra-specific diversity in host plants (Price *et al.*, 1980). The host plant of phytophageal insects may have a significant impact on their disease susceptibility, either through nutritional stress or the plant's direct antimicrobial activity (Tanada *et al.*, 1993; Cory *et al.*, 2006). Many plants produce antimicrobial chemicals that impede the activity of entomopathogens (Poprawski *et al.*, 2000). Antimycotic phytochemicals, which can give resistance to fungal infection, are also known to be sequestered by insect herbivores (Poprawski *et al.*, 2000).

2.6.2.3 The host population

Arthropod susceptibility to entomopathogenic fungi can be influenced by a variety of factors including population density, behavior, age, genetics, injury exposure, and nutrition (Tanada and Fuxa, 1987; Inglis *et al.*, 2001). Grooming, cannibalism, and feeding in safe conditions are all behavioral actions that determine how hosts are infected. (Watanabe *et al.*, 1987).

Maniania *et al.* (1998) recorded that when reared on several *Sorghum bicolor* (Linnaeus) cultivars, found that the duration of 50% stem borer larvae mortality (LT_{50}), *Chilo partellus* (Swinhoe), when infested with *M. anisopliae*, is exceedingly variable. At any one time, not all phases of the host's development are equally susceptible to entomopathogenic fungi. Adults and deutonymphs of *Trypanosoma evansi* (Trypanosomatida: Trypanosomatidae) are more vulnerable to *B. bassiana* and *M. anisopliae* than larvae and protonymphs, according to Wekesa *et al.* (2006). When the cassava green mite, *Mononychellus tanajoa* Bondar (Acarida: Tetranychidae), was infested by *Neozygites floridana*, a similar effect was found (Entomophthorales: Neozygitaceae) (Oduor, 1995).

2.6.2.4 Host developmental stage and sex

The effectiveness of entomopathogenic fungi is determined by the stage of development and sex of the insect, and not all stages of the insect life cycle are equally vulnerable (Dimbi *et al.*, 2003). In a study of the effects of tsetse species, age, and sex (*Glossina morsitans morsitans* Westwood and *G. m. centralis* Machado (Diptera: Glossinidae) on infection response by *M. anisopliae*), the age of the host had a significant impact on susceptibility, with females of both species being more susceptible than males (Maniania *et al.*, 1998).

2.6.2.5 Process of infection

Pathogenic fungi infect their host through the cuticle. Some Hyphomycetes infect the host through the respiratory system and digestive tract (Maniania *et al.*, 2002). Three steps are recognized in the mechanism of fungal infection and disease development: adhesion and germination, penetration of the host integument, and intra-haemocoelian proliferation of the fungus. (Ferron, 1978; Inglis and Goettel 2001).

2.7 Safety of entomopathogenic fungi and persistence in environment

Propagule stability during storage, ability to successfully apply pathogen to target insect, and experience with pest-pathogen interactions in the field are all factors to consider when evaluating the propagule. UV-protectors are used throughout the production process to protect propagules from solar radiation and low RH, which improves their performance in the field (Inglis *et al.*, 1996). Optical brighteners, for example, are added to improve their environmental persistence (Inglis *et al.*, 1995a; 1996d).

In addition, studies have shown that fungal biological control agents can be utilized to successfully and safely manage invertebrate pests with little negative effects on non-target organisms. In agricultural, horticultural, and forest systems, hazardous synthetic chemical pesticides have been reduced. They are usually host-specific and do not secrete a significant number of metabolites into the environment (Strasser *et al.*, 2000; Goettel and Hajek, 2001; Vestergaard *et al.*, 2003; Zimmermann, 2007). While they are regarded as environmentally beneficial, they can pose a risk of toxicity, allergies, or direct infection to non-target species (people, domestic animals, and wildlife) and the environment (Vestergaard *et al.*, 2003). A human patient with 28 empyemas induced by *B. bassiana*, for example, has been documented (Gürcan *et al.*, 2006).

2.8 Strategies for insect pest microbial control using fungi

2.8.1 Introduction

The goal of the introduction is to permanently introduce a contagious exotic pathogen in a new area in order to suppress the pest population. The newly introduced exotic infection would be able to survive in the ecosystem and propagate from its release sites. In order to introduce exotic pathogens, a candidate pathogen is usually collected from the region where the introduced pest is native. In contrast to annual crops, this biological management scheme is appropriate for stable ecological systems such as forests, where long-lived entomopathogenic stages can survive. One of the successful projects is the use of the fungus *Entomophaga maimaiga* to suppress the forest defoliator *Lymantria dispar* (Lepidoptera: Erebidae) (Hajek *et al.*, 1995).

2.8.2 Augmentation

There are two approaches to augmentation: inundative and inoculative releases.

2.8.2.1 Inundative release

This entails releasing inoculum in large areas on a regular basis for immediate management rather than waiting for the pathogen to multiply on its own with no intervention.

2.8.2.2 Inoculative release

Inoculation involves releasing relatively small amounts of a pathogen, intended to develop and spread in the targeted population (Goettel *et al.*, 1997). The approach is ideal for the introduction of pathogens targeting hosts that live primarily in cryptic environments. In a wide range of ways, fungal inoculums can be released, e.g. spore-filled cadavers, living-infected hosts, or the release of resilient fungal stages added as sprays or dust directly to substrates on which they feed or live as soil-dwelling pests (Bidochka *et al.*, 1996). On artificial media, these fungal cultures can be

grown and then overturned over target plants infested by pests in the field, so that compulsorily removed conidia can land on vulnerable hosts. Infected insects are destructive to host plants, whereas, on mobile-infected insects, airborne conidia allow the pathogen to spread.

2.8.3 Conservation

Environmental manipulation or conservation (ecosystem) involves enhancing the natural pest control through other methods than the direct accumulation of the already present units of the pathogen (Nordlund, 1984). The pathogen may be able to reproduce at a higher rate than usual as a result of cultural manipulation or it may preserve or improve those that are already present (Fuxa, 1987).



CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Experimental site

The experiments were carried out at the Arthropod Pathology Unit (APU) of the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus in Nairobi, Kenya (S 03.35517°, E037.33861°, and 1616 m.a.s.l.).

3.2 Fall armyworm colony

The FAW colony was reared on both natural and artificial diets.

The FAW colony on a natural diet was initiated from field-collected from maize plants in Siaya and Homa Bay counties (−0.61401° Latitude 34.09095° Longitude 1215 m.a.s.l.) They were reared in small cages (30 × 30 × 30 cm) under laboratory-controlled ambient conditions of 25 ± 2 °C temperature, 70 ± 10% relative humidity, and 12 L:12 D photoperiod. Maize leaves containing FAW egg masses were cut using scissors and kept in plastic jars (1.2 litres, 10 cm diameter, 16 cm height), and the hatching larvae were maintained until the 3rd instar. To minimize the cannibalism by the 4th, 5th, and 6th; the 3rd instar larvae were transferred to larger transparent plastic buckets (0.4 litres, 18 cm diameter, 21 cm height), where they were maintained till pupation. The jars were lined with paper towels for moisture absorption and the lids cut for aeration. The leaves were changed daily until pupation. To prevent the pupae from being cannibalized, they were collected periodically (24–48 hrs). They were then placed in Petri dishes and kept in an oviposition cage (30 × 30 × 30 cm) for moth emergence and oviposition. The moths were held in Perspex cages (30 x 30 x 30 cm) and provided with 20% honey solution with a moistened cotton wool ball placed in a Petri dish (8.6 cm in diameter). They were provided with maize leaves obtained from pesticide-free maize grown at *icipe*, for oviposition.

Preparation of Fall armyworm artificial diet

The FAW artificial diet contained a mixture of nutrients, including carbohydrates, proteins, fats, minerals, and vitamins, each of which has a particular role in the insect’s growth. The artificial diet used in this rearing was prepared at *icipe* in fractions (Fraction A, Fraction B, and Fraction C) as described below.

Table 3. 1: Diet ingredients for Fall armyworm rearing

Ingredients	Quantity (g or ml) per 1L of diet	Purpose
1. Bean powder	62.5g	Source of protein
2. Wheat germ	50g	Mineral source and roughage
3. Maize leaf powder	25g	Natural diet
4. Milk powder	19g	Protein source
5. Torula yeast	32g	Feeding attractant
6. Ascorbic acid	3g	Source of vitamin c
7. Methylparaben	2.5g	Prevents bacterial growth
8. Distilled water	500ml 350ml	Mixing the paste Boiling agar
10. Sorbic acid	1.5g	Prevents mold growing
11. Agar	11.5g	Gelling the diet
12. Vitamin mix Vitamin B complex	1ml	Healthy growth
13. Formalin	40%2ml	Acts as a preservative
14. Suprapen powder (Tetracycline)	2.5g	Acts as an antimicrobial agent

Fraction A: Powdered ingredients (quantities listed in table 3.1) which included bean powder, wheat germ, maize leaf powder, milk powder, torula yeast, ascorbic acid, methylparaben, and sorbic acid were weighed using an electronic balance, transferred into a mixing pot then mixed and put in a clean container under a fume hood except Methylparaben. Distilled water was then boiled, and cooled to 60°C after which it was mixed with the pre-mixed ingredients above and blended for one minute. Lastly, 2.5 g of Methylparaben (melted in 20 ml of absolute ethanol) was added to the mixture in the blender and then blended for another two minutes.

Fraction B: 11.5 g of agar powder was weighed in a separate container, then added to 150ml cold distilled in a separate saucepan. The mixture was then brought to boil while stirring and then allowed to cool to 60°C. The ingredients of fraction A were then added to ingredients of fraction B and blended for 3 minutes.

Fraction C: Lastly, after the mixture of fractions A and B had cooled down, Formaldehyde (40%), suprapen powder (2.5g), and vitamin mix (1 ml) were added and blended and mixed for 3 minutes at room temperature, (25±1°C); 12:12 light: dark photoperiod; and 75±5%, relative humidity (RH). Ten (10) ml of the diet was then dispensed into clean plastic/glass vials and left uncovered in the laminar hood for about 12 hrs to allow the formaldehyde to volatilize before inoculating the larvae.

3.2.1 Diet infestation/inoculation with Fall armyworm larvae

After the diet had cooled down for 12 hrs, the surface of the diet in each vial was perforated using a sterilized laboratory plastic rod to ease larval penetration and feed through the diet. FAW egg masses that had turned black and were ready to hatch were also placed inside plastic jars containing the diet. After the eggs hatched and the larvae reached second instars, two larvae were

transferred into a glass/plastic vial to reduce cannibalism that could arise due to limited food. The vials were then closed using cotton wool plugs that could allow aeration and at the same time prevent larvae from escaping. The vials and jars containing the larvae were then kept on shelves at controlled environmental conditions ($28\pm 2^{\circ}\text{C}$; $65\pm 5\%$ RH; 12:12 light: dark photoperiod). Larval development was monitored daily to record any death or fungal contamination. Harvesting of the pupae was done when 50% of the larvae had pupated. This was achieved by carefully removing the diet from each vial onto a clean tray using a spatula to avoid crushing the pupae. The pupae were first placed in petri dishes lined with a paper towel and later put in a clean oviposition cage ventilated with a fine net on the sides. The cages were incubated at room temperature ($25\pm 1^{\circ}\text{C}$); 12:12 light: dark photoperiod; and $75\pm 5\%$ relative humidity (RH) until the moths emerged.

After emergence, moths were fed with a 10% honey solution soaked in balls of cotton wool. Potted maize plants and waxed paper were placed in the cages for oviposition. Upon oviposition, the plants were removed after 24 hrs and transferred to a separate ventilated cage to facilitate egg hatch. Fall armyworm egg masses which were laid on waxed paper and on the maize plants were cut using a pair of scissors.

3.2.2 Mass rearing of *Telenomus remus* on Fall armyworm eggs

Colony of *T. remus* was initiated from a cohort of fifteen wasps (1:2 - males: females) obtained from parasitized eggs of FAW collected during a field survey in Yatta (01.23044°S ; 37.45789°E). They were maintained in glass vials in the laboratory at *icipe*'s Animal Rearing and Quarantine Unit (ARQU), at room temperature $25 \pm 1^{\circ}\text{C}$, 12 L: 12 D photoperiod, and 60-70% relative humidity (RH). The parasitoid was reared on freshly laid FAW egg masses which were pasted onto rectangular manila cards (1cm x 5cm) using white glue and exposed to wasps to parasitize.

The date of exposure was recorded at the back of the card to calculate the expected adult parasitoid emergence. Plain paper (2cm x 1cm) coated with a thin layer of honey was placed inside the glass vial as a source of food for the emerged parasitoids. Glass vials containing parasitoids were placed on the shelves in the laboratory. Three to four days after exposing the card, the eggs turned black and this showed that it has been parasitized. The parasitized egg cards were removed and placed in a separate clean glass vial and incubated at room temperature to observe for parasitoid emergence. The parasitoids took a period of 6-13 days before emerging. The above process was repeated for colony maintenance. The colony was maintained for 16 generations before starting the bioassays.

3.2.3 Mass rearing of *Cotesia icipe* on Fall armyworm 2nd instar larvae

Cotesia icipe colony was obtained from parasitized larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) collected from Amaranthus plants in East and Central Kenya, particularly from Yatta (01.23044°S; 37.45789°E), Mwea (0.6309°S; 37.35117°E), Kitengela (1.6°S; 36.85°E) and Thika (1.00269°S; 37.07858°E). For multiple generations, the parasitoid was reared on its natural host (*S. littoralis*) at the *icipe* insectary. Following that, a parasitoid colony was established in 2018 using *S. frugiperda* and maintained in the laboratory for several generations prior to conducting the bioassays. The parasitoid wasps were kept in Perspex cages (30 x 30 x 30 cm) in a rearing room at room temperature, 25 1°C, 12 L:12 D photoperiod, and 60-70 percent relative humidity (RH).

Droplets of 20% honey solution placed on the inner top side of the rearing cage and water on a moist cotton wool ball placed in a Petri dish (9 cm in diameter) were fed to the wasps. The wasps in the rearing cage were fed early instar *S. frugiperda* (1st and 2nd instar larvae) on fresh maize leaves for colony maintenance. After 24 hours, the exposed host larvae were removed from the

cage and placed in rectangular plastic containers (20.5 cm in length, 14.5 cm in width, 8 cm in height) with fresh maize leaf pieces for larval feeding. The larvae were kept until parasitoid cocoon formation or FAW pupation occurred (in the case of unparasitized larvae). The cocoons were removed from the leaves on a regular basis with a fine camel hair brush and placed in a clean Perspex cage (40 × 40 × 45 cm) to allow new generations of adult parasitoids to emerge. Before beginning the bioassays, the colony was maintained for five generations on *S. frugiperda* (Mohamed *et al.*, 2021).

3.2.4 Fungal cultures

The four most potent fungal isolates against FAW used in this study were obtained from the Germplasm Centre of the *icipe's* Arthropod Pathology Unit. Their origin and source are summarized in Table 1.0 below. *Metarhizium anisopliae* ICIPE 7, ICIPE78, and ICIPE 41 isolates were cultured on Sabouraud dextrose agar (SDA), while *Beauveria bassiana* ICIPE 621 was cultured on Potato dextrose agar (PDA) in Petri dishes (9 cm diameter) and incubated at 25±2°C in total darkness. After three weeks, conidia were harvested from surface cultures and suspended in 10 ml distilled water containing 0.05 % Triton X100 in universal bottles containing glass beads (beads of 3 mm diameter per bottle). To break up the conidial clumps and ensure a homogeneous suspension, the suspension was vortexed for 5 minutes at 700 rpm. A haemocytometer was used to measure conidial concentrations under a light microscope. Prior to bioassays, the conidial suspensions were adjusted to a concentration of 1×10^9 conidia ml⁻¹ and other concentrations were achieved through serial dilution (Akutse *et.al*, 2013).



Plate 3. 1: Fungal cultures incubated at $25\pm 2^{\circ}\text{C}$.

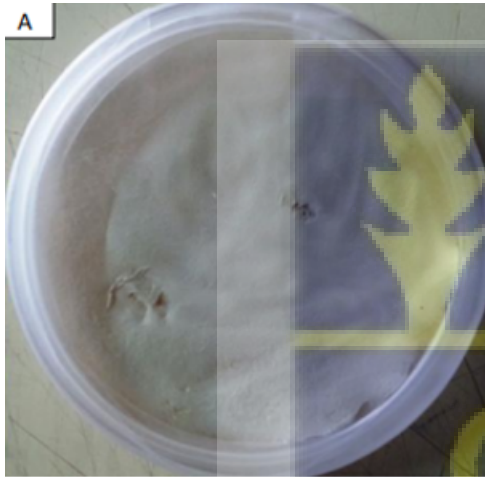


Plate 3. 2: Sporulating cultures of entomopathogenic fungi *Beauveria bassiana* used for the bioassays.

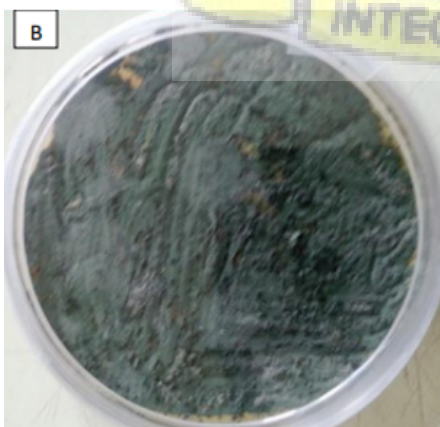


Plate 3. 3: Sporulating cultures of entomopathogenic fungi *Metarhizium anisopliae* used for the bioassays.

3.2.5 Conidial germination viability

The viability of the fungal conidia was determined by spreading 0.1 ml of 3×10^6 conidia ml⁻¹ on 9 cm petri dishes containing medium of SDA or PDA. A sterile microscope cover slip (2 x 2 cm) was placed on top of the agar on each plate. Each plate was sealed with parafilm and incubated in complete darkness at 25 ± 2 °C for 18 - 20 hours before being examined. Lactophenol cotton blue was applied after 18 hours post-inoculation for germination to end and stain the spores to make counting easy. Conidia germination rate was determined from 100 random conidia on the surface area that each coverslip covers under the light microscope (40x) (Goettel and Inglis, 1997). When the germ tube length reached twice the conidium diameter, conidia were said to have germinated (Goettel and Inglis, 1997; Inglis, Enkerli, and Goettel, 2012). Each plate represented a replicate and every isolate was replicated four times.

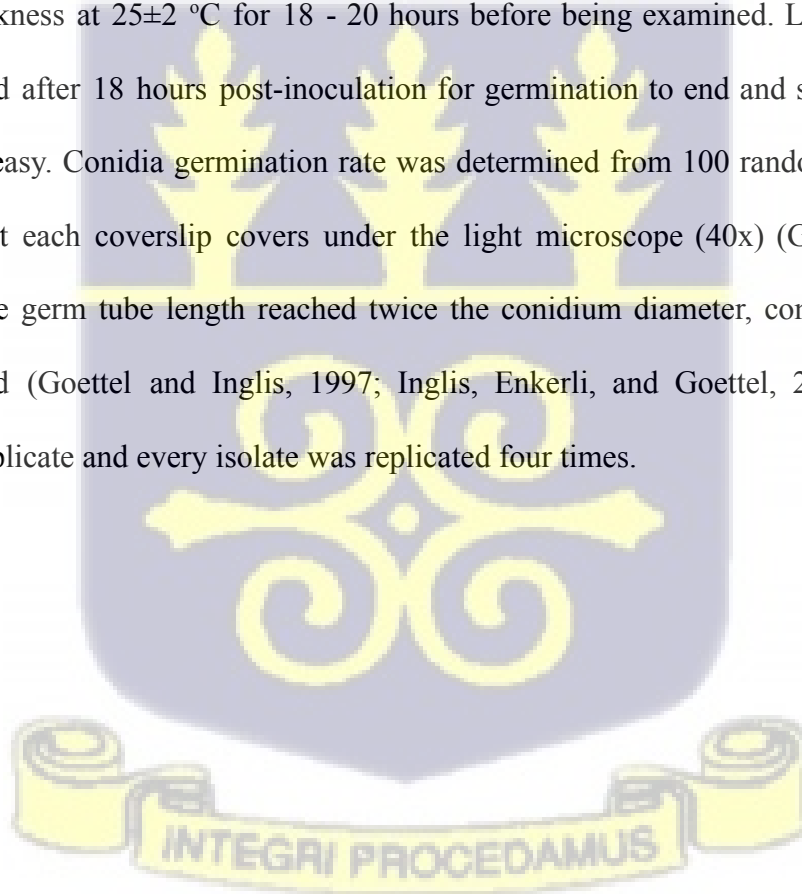


Table 3. 2: Identity of fungal isolates screened against the parasitoid *Telenomus remus* and *Cotesia icipe* for virulence under laboratory conditions.

Fungal species	Isolates	Source	Location/Country	Year of isolation	%Germination \pm SE
<i>Metarhizium anisopliae</i>	ICIPE 7	<i>Rhipicephalus appendiculatus</i>	Rusinga island (Kenya)	1996	93.0 \pm 1.3a
	ICIPE 78	<i>Temnoschoita nigroplagiata</i>	Ungoe (Kenya)	1990	94.2 \pm 1.7a
	ICIPE 41	Soil	Kenya	1990	87.3 \pm 2.3b
<i>Beauveria bassiana</i>	ICIPE 621	Soil	Kericho (Kenya)	2008	91.2 \pm 1.04ab

Means within a column followed by same letter are not significantly different by Student-Newman-Keuls (SKN) test ($p < 0.05$).

3.3 Direct effects of *Metarhizium anisopliae* and *Beauveria bassiana* isolates on *Telenomus remus*

The four entomopathogenic EPF isolates *M. anisopliae* isolates (ICIPE 7, 78, 41) and *B. bassiana* (621) were screened for their virulence against *T. remus* adult parasitoids. For each fungal isolate (treatment), 20, 1-day-old adult *T. remus* parasitoids (at a ratio of 1:2 /male:female) were contaminated with dry conidia of EPF using velvet-coated plastic jars (150 \times 80 mm) following the procedure described by Migiro *et al.*, (2010) and Opisa *et al.*, (2019).). 1 g of dry conidia contaminated the devices. Twenty (20) adult parasitoids (ratio 1:2) were introduced into the device for 3 minutes to pick up spores. Control treatments were exposed to fungus-free velvet plastic jars. After 3 min of exposure, contaminated insects were transferred into clean glass vials and provided with honey as food. Adult mortality was recorded daily for 7 days by counting all dead parasitoids. Mycosis of the dead insects was assessed and presence of conidia and hyphae

on the cadaver was evidence of mortality caused by fungal infection. A complete randomized design was used to arrange all treatments where each treatment was replicated four times like the controls. Twenty adult parasitoids (at a ratio of 1:2) were exposed to 50 freshly laid FAW eggs for 24 hrs after contaminating them with the fungus. The eggs were then removed and kept in separate glass vials until parasitoid adults emerged. FAW egg mortality and parasitoid adult emergence were recorded. In addition to adult emergence of FAW and parasitoids, parasitism rates and sex ratio of the parasitoids were recorded. Adult parasitoids were kept in separate vials and fed with honey. The mortality, fecundity, oviposition rate, and egg hatchability of the infected parasitoids were also assessed. The experiment was replicated four times under a completely randomized design.

3.4 Indirect effects of *Metarhizium anisopliae* and *Beauveria bassiana* isolates on *Telenomus remus*

10 ml fungal suspension was used to spray a hundred freshly laid eggs of *S. frugiperda* at different concentrations (1.0×10^5 , 1.0×10^6 , 1.0×10^7 , 1.0×10^8 and 1.0×10^9 conidia ml⁻¹) of *M. anisopliae* isolates (ICIPE 7, 78, 41) and *Beauveria bassiana* (621) using Burgerjon's (1956) spray tower, before transferring them onto potted maize plants. Control treatments were sprayed with sterile distilled water containing 0.05% Triton X-100. The sprayed eggs were air-dried before being transferred into clean glass vials. Twenty *T. remus* parasitoids at a ratio of 1:2 male:female were introduced into every glass vial with sprayed FAW eggs for 24 hrs for parasitism assessment for a period of 7 days. Replication of the experiment was done four times and also arranged in a complete randomized design. Maintenance of the bioassays was done at 25-27 °C, 50-70% RH, and 12L: 12D photoperiod. After 24 hours of exposure, sprayed eggs were transferred to clean vials, and *T. remus* were maintained with honey. A daily recording of the

mortality rate was kept until all parasitoids die. The dead parasitoids were surface sterilized and placed on Petri dishes lined with damp sterilized filter paper to allow fungal growth on the surface of the cadaver, and the mycosis rate was determined. Mortality of the FAW eggs and adult parasitoids, as well as parasitism rates, were assessed.

3.5 Direct effects of *Metarhizium anisopliae* and *Beauveria bassiana* isolates on *Cotesia icipe*

Four entomopathogenic fungal isolates *M. anisopliae* ICIPE 7, 78, 41, and *B. bassiana* ICIPE 621 were screened for their pathogenicity/virulence against *C. icipe* adult parasitoids. For each fungal isolate (treatment), twenty, 1-day-old adult *C. icipe* parasitoids (at a ratio of 1:2 /male: female) were contaminated with EPF using velvet-coated plastic jars (150 × 80 mm) following the procedure described by Migiro (2010 and Opisa *et al.*, (2019). The devices were contaminated with 1g of dry conidia. The 20 adult parasitoids were introduced into the contamination device for 3 min to pick up spores. Control treatments were exposed to fungus-free velvet plastic jars for also 3 min. After 3 min of exposure, contaminated insects were transferred into perspex cages as described above and provided with honey as food. Adult mortality was recorded daily for seven days by counting all dead parasitoids. Mycosis test was conducted to confirm mortality due to infection by the fungus treated, where the dead insects were surface sterilized with 70% alcohol and then rinsed thrice in distilled water. The surface-sterilized cadavers were kept separately in Petri dishes lined with sterile moistened filter paper to record fungal outgrowth and verify if mortality could be attributed to the respective fungal isolates they were treated with. The presence of hyphae and conidia on the cadaver surface confirmed mortality caused by fungal infection (Opisa *et al.*, 2019; Akutse *et al.*, 2020). A completely randomized design was used to arrange the treatments, where each treatment was replicated four times as the controls.

In addition, after infection, the twenty infected parasitoids (ratio 1:2 male to female) were exposed to fifty 2nd instar larvae for 24 hrs. The larvae were then removed and kept in separate clean lunch boxes (19 x 13 x 8 cm), fed with fresh maize leaves until adults emerged. Adult parasitoids were kept in the cages, where they were fed with honey, and mortality assessed until all parasitoids died. In addition to adult mortality, FAW larval mortality and parasitism rates were also recorded.

3.6 Indirect effects of *Metarhizium anisopliae* and *Beauveria bassiana* isolates on *Cotesia icipe*

10 ml fungal suspension was used to spray Fifty 2nd instar of *S. frugiperda* larvae at different concentrations (1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 and 1×10^9 conidia ml⁻¹) of *M. anisopliae* isolates (ICIPE 7, 78, 41) and *B. bassiana* ICIPE 621, using the Burgerjon's spray tower (Burgerjon, 1956), before transferring them onto potted maize plants. Sterile distilled water sprayed control treatments with 0.05% Triton X-100. The sprayed larvae were first air-dried and then transferred into clean perspex cages containing the potted maize plants. Twenty 1-day-old adult *C. icipe* parasitoids at a ratio of 1:2 /male: female were then introduced into every cage with sprayed 2nd instar FAW larvae for 24 hrs for parasitism assessment. After 24 hrs, sprayed and exposed larvae were transferred into clean cages containing fresh maize leaves, and *C. icipe* were maintained with honey as food source. The bioassays were maintained at 25-27° C, 50-70% RH, and 12L: 12D photoperiod. A daily record of the mortality rates was maintained until all parasitoids died. Dead parasitoids were surface sterilized and placed in Petri dishes lined with damp sterilized filter paper for fungal growth on the cadaver surface, and the mycosis rate was determined. Mortality of the FAW larvae and parasitism rates were also assessed. A

replication of the experiment was done four times and arranged in a complete randomized design.

3.7 Effect of entomopathogenic fungi on *Cotesia icipe* emergence

The cocoons that formed from the parasitized larvae both in the direct and indirect experiments were counted, and kept in petri dishes inside the perspex cages awaiting the emergence of adult parasitoids. The total number of parasitoids that emerged were counted in all treatments and their sex ratios determined.

3.8. Mycosis test

Confirming the fact that fungal infection caused mortality of insects required removal of cadavers from treatments, then surface-sterilized in 1% sodium hypochlorite solution and later in 70% alcohol for 3 seconds in each solution and rinsed three times in sterile distilled water. The cadavers were then placed into Petri dishes lined with filter paper and moistened with sterile distilled water. There were lids covering the petri dishes, and Parafilm sealed the edges, then the dishes were placed in an incubator at 25°C to assess fungal growth. Confirmation of mycosis was under microscopic examination for hyphae and spores at a magnification of 400×. Petri dishes that had cadavers were observed for the growth of fungal for about one week and mycosis rate was recorded.

3.9 Statistical analysis

FAW egg, larval, adult *T. remus* and *C. icipe* mortality data were corrected using Abbott's formula (Abbott, 1925) to correct natural mortality, and their normality was tested using Barlett's before being subjected to one-way analyses of variance (ANOVA). The variances of the percentage mortalities were calculated using a binomial Generalized Linear Model (GLM) with a logit link (McCullagh and Nelder, 1989). Where treatments had significant difference ($p < 0.05$),

the Student–Newman–Keuls (SNK) test was used to separate means. GLM was used to analyze the lethal time for 50% mortality (LT_{50}) was analyzed by GLM, through use of the ‘dose.p’ function from the MASS library, to estimate the lethal time to 50% mortality (LT_{50}). Parasitism rates were calculated as the percentage of the number of parasitoid adults that emerged divided by the sum of eggs or larvae of the host and subjected to ANOVA. One-way ANOVA was also used to analyze Mycosis data. All data analyses were performed using R (version 3.2.5) statistical software packages (R Development Core Team, 2018).



CHAPTER FOUR
RESULTS

4.1. Direct effects of entomopathogenic fungal isolates on *Telenomus remus* and FAW eggs

4.1.1 Effect of different fungal species on mortality of *Telenomus remus* adults

Significant differences were noted in mortality rates among the fungal species at day one ($F = 7.16$; $df = 4, 15$; $P = 0.001$), day 2 ($F = 4.60$; $df = 4, 15$; $P = 0.0128$), three ($F = 6.38$; $df = 4, 15$; $P = 0.003$), four ($F = 8.75$; $df = 4, 15$; $P = 0.0007$), five ($F = 11.47$; $df = 4, 15$; $P = 0.0002$), six ($F = 9.36$; $df = 4, 15$; $P = 0.0005$) and seven ($F = 10.16$; $df = 4, 15$; $P = 0.0005$) after treatment. The lowest mortality was observed in ICIPE 621 ($61.39 \pm 6.50\%$) while ICIPE 7 caused the highest *T. remus* adult mortality ($81.40 \pm 4.08\%$) 7 days after treatment (Fig. 4.1).

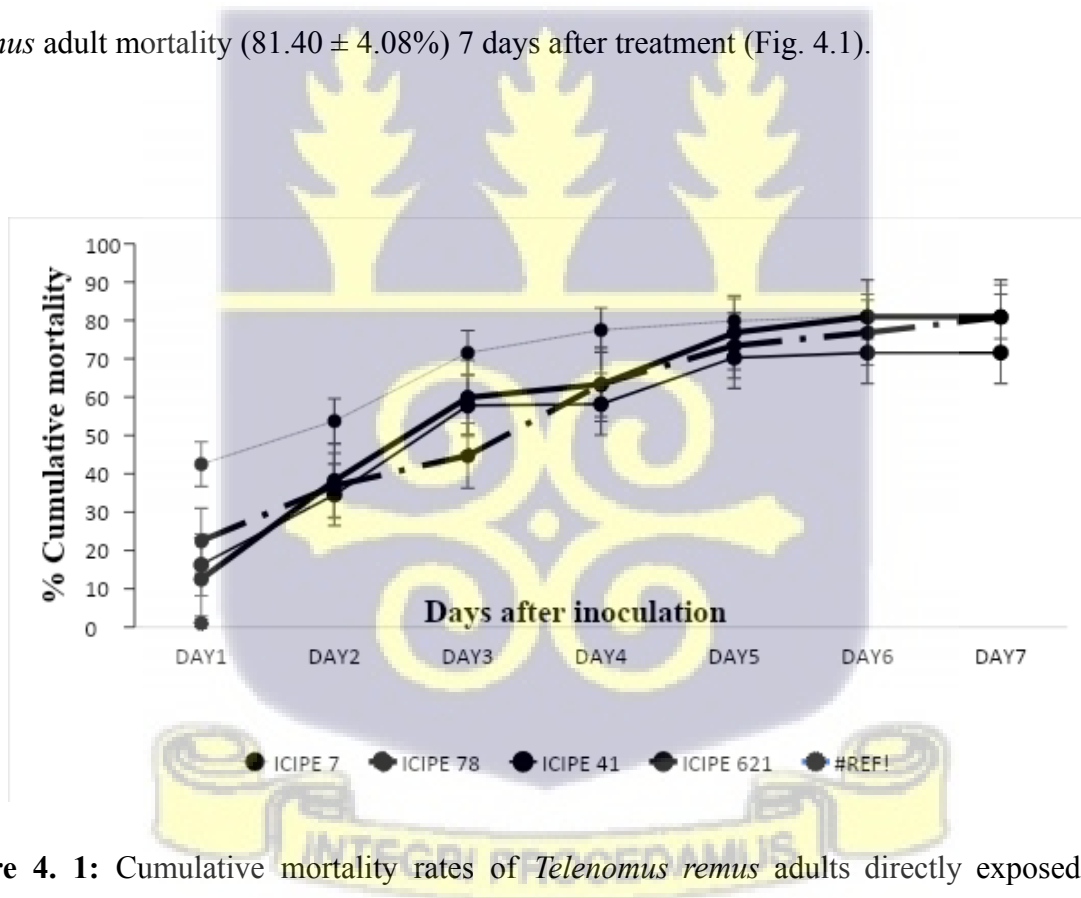


Figure 4. 1: Cumulative mortality rates of *Telenomus remus* adults directly exposed to the different fungal isolates.

When exposed *T. remus* adults to the four different fungal isolates, the most virulent isolates with LT_{50} less than 2 days were *M. anisopliae* isolates ICIPE 7 and ICIPE 78, whereas ICIPE 41 and *B. bassiana* isolate ICIPE 621 had LT_{50} values of 2.52 and 2.89 days respectively (Table 4.1). There were significant differences in mycoses among the isolates ($F = 6.306$; $df = 4, 15$; $P = 0.003$). Calculated LT_{50} values for the isolates show that, in general, the *M. anisopliae* isolates elicited quicker mortality with lower LT_{50} values compared to *B. bassiana* isolate (Table 4.1).

Table 4. 1: Median lethal time (LT_{50}) 7 days post-treatment of egg parasitoid *Telenomus remus* inoculated with different isolates of entomopathogenic fungi (Mean \pm SE)

Fungal species	Isolates	LT_{50} (Days) (95% FL)	%Mycosis of <i>T. remus</i> cadavers
<i>Beauveria bassiana</i>	ICIPE 621	2.89 \pm 0.09	47.50 \pm 9.242a
<i>Metarhizium anisopliae</i>	ICIPE 7	1.00 \pm 0.15	38.75 \pm 10.5a
	ICIPE 78	1.91 \pm 0.07	40.00 \pm 8.4a
	ICIPE 41	2.52 \pm 0.08	16.25 \pm 6.25ab

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

The mean mortality rates also varied significantly among the fungal isolates with *M. anisopliae* ICIPE 7 causing the highest mortality rate 7 days post-treatment (Figure 4.2).



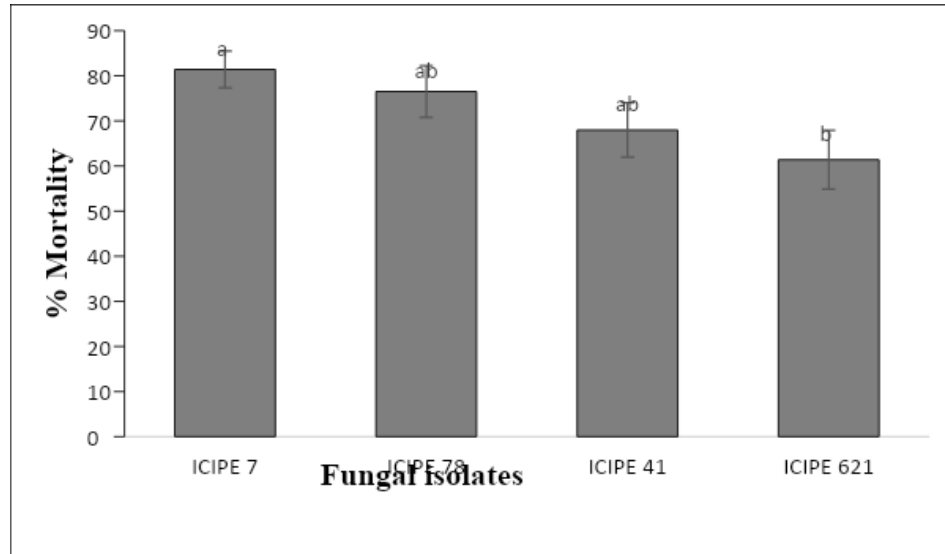


Figure 4. 2: Mean percentage mortality of adult *Telenomus remus* directly exposed to the different fungal isolates.



4.1.2 Effect of different fungal species on mortality of FAW eggs exposed to infected *Telenomus remus* adults

The fungal isolates had no significant effects on FAW egg's hatchability 7 days post-treatment ($F = 5.487$; $df = 4, 15$; $P = 0.632$). The *M. anisopliae* ICIPE 78 caused the highest eggs mortality of 61.25%, whereas ICIPE 41 caused the least at 39.75%. *Metarhizium anisopliae* ICIPE 7 and *B. bassiana* ICIPE 621 caused egg mortality of 44.25 and 55% respectively (Figure 4.3).

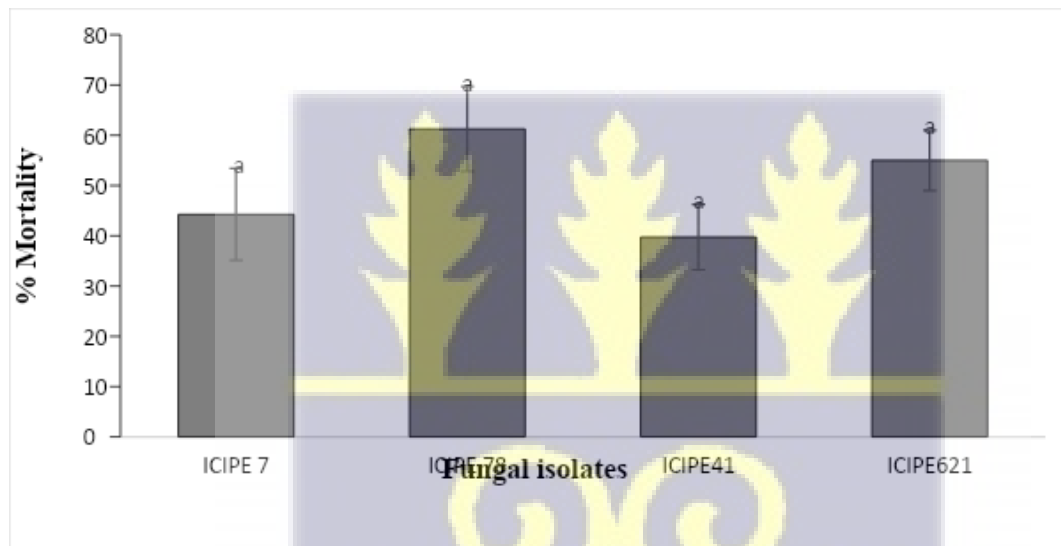


Figure 4.3: Mean percentage mortality of FAW eggs directly exposed to infected *Telenomus remus* with the different fungal isolates.

4.1.3 Effect of fungal species on parasitoid emergence and parasitism rate

The infection of various fungal species affected the mean number of emerged *T. remus* individuals, which varied among the tested isolates. ($F = 9.357$; $df = 5, 18$; $P = 0.0005$). The control treatment had the highest number of parasitoids that emerged ($83.25 \pm 5.94\%$) while

ICIPE 78 treatment had the lowest parasitism rate ($38.75 \pm 8.43\%$) (Fig. 4.4). In addition, there were no significant effects on the sex ratios of the parasitoids that emerged.

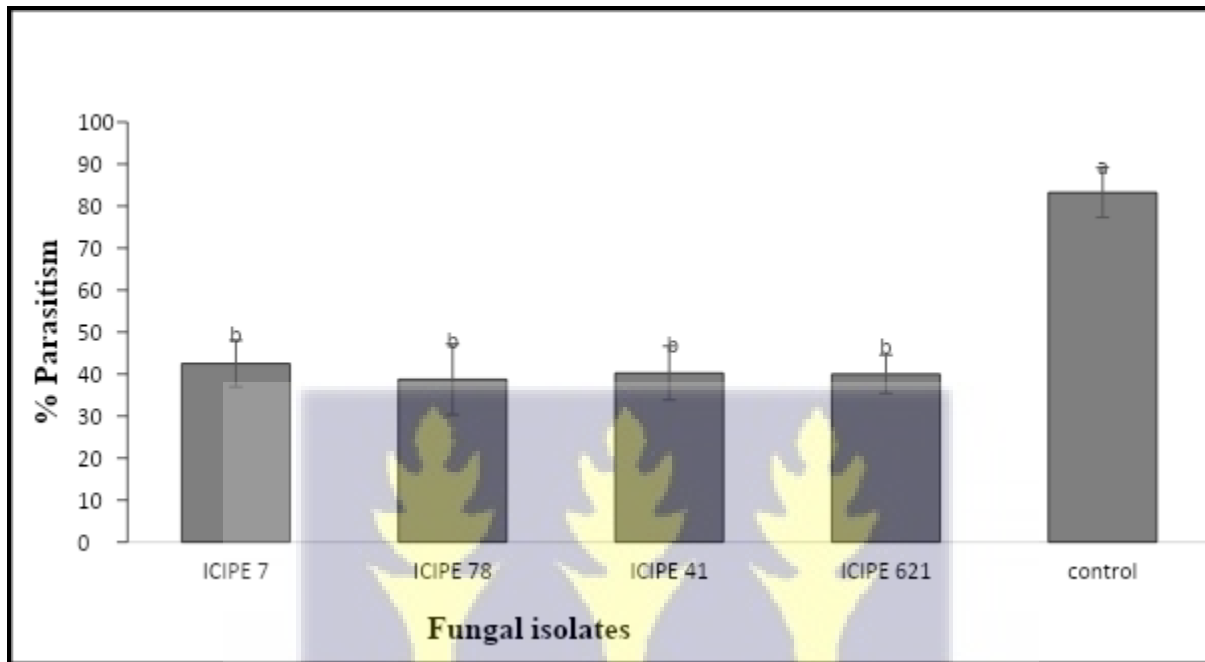


Figure 4.4: Mean percentage parasitism rates of *Telenomus remus* directly exposed to the different fungal isolates.

4.2. Indirect effects of entomopathogenic fungal isolates on *Telenomus remus* and FAW eggs

Generally, only ICIPE 41 isolate had significant differences in mortality among the different concentrations from day one of exposure. The other isolates had however significant differences from day three.

4.2.1 Effect of different fungal species concentrations on mortality of *Telenomus remus* adults

Metarhizium anisopliae ICIPE 78 recorded high mortality rates at all the concentrations as compared to other tested isolates where moderate mortality rates were obtained. At the highest

concentration of 10^9 *M. anisopliae* ICIPE 78 caused the highest mean *T. remus* adult mortality of 78.54%, whereas the lowest mean mortality of 34.46% was recorded for ICIPE 41. However, *B. bassiana* ICIPE 621 caused mortality of 40.17% at its highest concentration (Table 4.2).

Table 4. 2: Mean mortality of *Telenomus remus* adults indirectly exposed to FAW eggs induced by fungal isolates (Mean \pm SE)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Mean adult <i>Telenomus remus</i> mortality				
10^9	37.32 \pm 6.06a	34.46 \pm 4.08a	78.54 \pm 5.85a	40.17 \pm 5.77a
10^8	36.25 \pm 5.60a	30.00 \pm 3.58a	71.96 \pm 5.66a	38.39 \pm 5.13a
10^7	31.60 \pm 4.85a	21.25 \pm 3.08b	66.83 \pm 5.91a	33.92 \pm 4.98ab
10^6	27.67 \pm 4.24ab	17.32 \pm 2.59b	66.24 \pm 5.76a	28.36 \pm 3.97ab
10^5	27.50 \pm 3.62ab	8.57 \pm 1.23c	62.13 \pm 6.59a	20.89 \pm 2.93bc

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls

4.2.1.1 Effect of different concentrations of *Metarhizium anisopliae* ICIPE 7 on *Telenomus remus* adults

There were no significant differences in *T. remus* adult mortality among the various concentrations of *M. anisopliae* ICIPE 7 at day one ($P = 0.42$), two ($P = 0.8$), three ($P = 0.064$), four ($P = 0.21$) and five ($P = 0.051$) after treatment. Significant differences were, however observed as from day six ($F = 6.429$; $df = 5, 18$; $P = 0.001$) and seven ($F = 14.35$; $df = 5, 18$; $P < 0.001$), after treatment (Fig. 4.5). In addition, the mortalities increased with the concentrations. The lowest concentration of 1×10^5 conidia ml^{-1} caused the lowest cumulative mortality rate of $56.25 \pm 2.39\%$, while the higher concentration of 1×10^9 conidia ml^{-1} caused the highest mortality ($87.50 \pm 4.79\%$) of the parasitoid after 7 day post-treatment (Fig. 4.5).

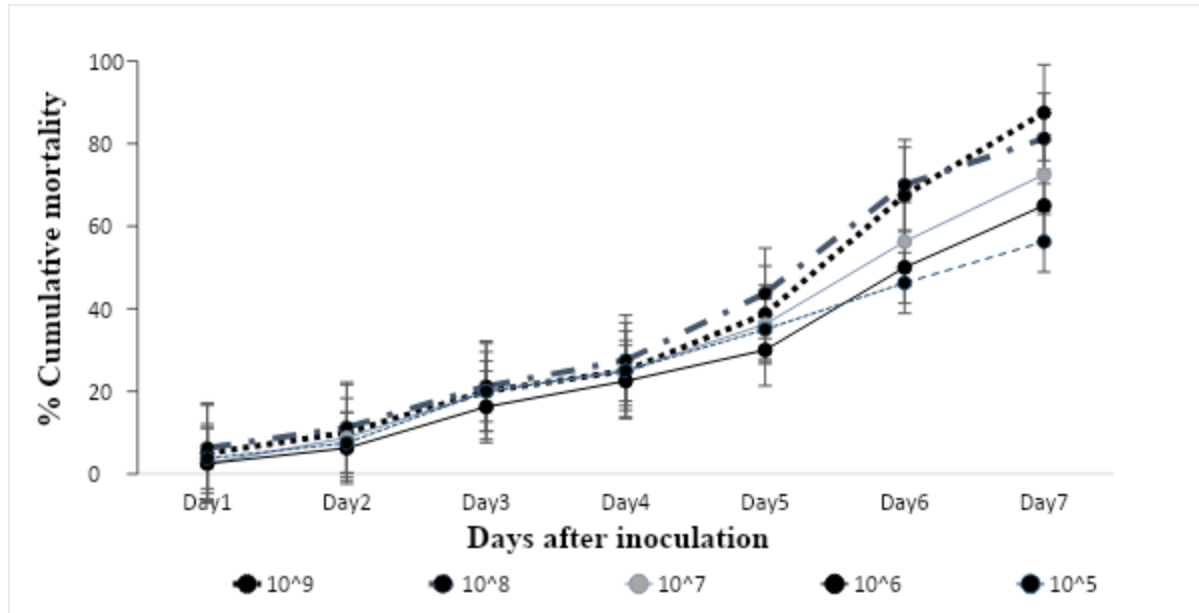


Figure 4. 5: Cumulative mortality rates of *Telenomus remus* adults after indirect exposure to FAW eggs infected with *Metarhizium anisopliae* isolate ICIPÉ 7 at different concentrations (n=20).

4.2.1.2 Effect of different concentrations of *Metarhizium anisopliae* ICIPÉ 41 on *Telenomus remus* adults

There were significant differences in mortality of *T. remus* adults at the various concentrations of *M. anisopliae* ICIPÉ 41 at day one (F =5.023; df = 5, 18; P = 0.005), day two (F = 6.274; df = 5,18; P = 0.002), three (F = 4.527; df = 5,18; P = 0.008), four (F = 6.94; df = 5,18; P < 0.001), five (F = 11.05; df =5,18; P = 0.0009), day six (F = 9.051; df =5,18; P < 0.001), and seven (F = 14.83; df =5,18; P < 0.001) after treatment (Fig. 4.6). The mortalities increased with the concentrations, where the lowest concentration of 1×10^5 conidia ml⁻¹ caused the lowest cumulative mortality of $17.5 \pm 1.44\%$ while the highest concentration of 1×10^9 conidia ml⁻¹ caused $62.50 \pm 5.94\%$ mortality (Fig. 4.6).

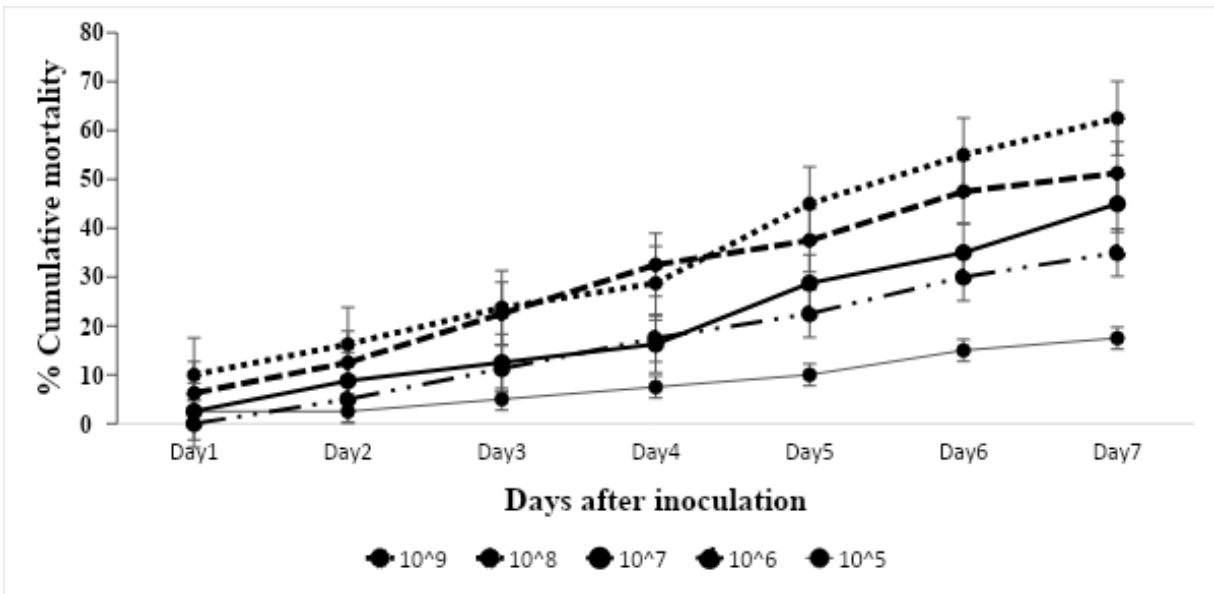


Figure 4. 6: Cumulative mortality rates of *Telenomus remus* adults after indirect exposure to FAW eggs infected with *Metarhizium anisopliae* isolate ICIPE 41 at different concentrations (n=20).

4.2.1.3 Effect of different concentrations of *Metarhizium anisopliae* ICIPE 78 on *Telenomus remus* adults

There were no significant differences in mortality among the various concentrations of *M. anisopliae* ICIPE 78 at day one ($P = 0.073$) after treatment. However, significant differences were observed at day two ($F = 3.71$; $df = 5,18$; $P = 0.017$), three ($F = 13.59$; $df = 5,18$; $P < 0.001$), four ($F = 73.62$; $df = 5,18$; $P < 0.001$), five ($F = 21.27$; $df = 5,18$; $P < 0.001$), six ($F = 34.02$; $df = 5,18$; $P < 0.001$) and seven ($F = 24.16$; $df = 5,18$; $P < 0.001$) after treatment (Fig. 4.7). Similar to other isolates, the mortalities increased with the concentrations, where the lowest concentration of 1×10^5 conidia ml^{-1} caused $85.00 \pm 7.91\%$, while the highest concentration of 1×10^9 conidia ml^{-1} yielded 100 ± 0.00 cumulative mortality (Fig. 4.7).

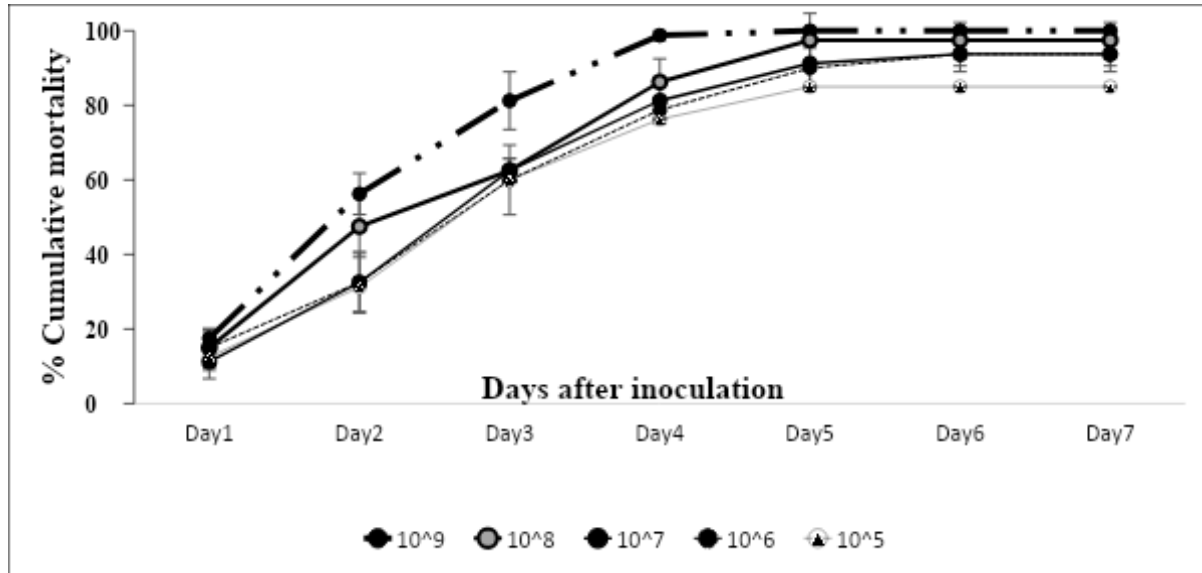


Figure 4. 7: Cumulative mortality rates of *Telenomus remus* adults after indirect exposure to FAW eggs infected with *Metarhizium anisopliae* isolate ICIP 78 at different concentrations (n=20).

4.2.1.4 Effect of different concentrations of *Beauveria bassiana* ICIP 621 on *Telenomus remus* adults

There were no significant differences in adult *T. remus* mortality among the various concentrations of *B. bassiana* ICIP 621 at day one ($P = 0.29$), two ($P = 0.15$), three ($P = 0.092$) and four ($P = 0.06$) after treatment. Significant differences were, however observed from day five ($F = 4.50$; $df = 5, 18$; $P = 0.008$), day six ($F = 9.35$; $df = 5, 18$; $P < 0.001$) and day seven ($F = 14.77$; $df = 5, 18$; $P < 0.001$) after treatment (Fig. 4.8). In addition, the mortalities varied with the concentrations, where 40.00 ± 5.44 and $77.50 \pm 4.79\%$ cumulative mortalities were recorded at 1×10^5 conidia ml^{-1} and 1×10^9 conidia ml^{-1} respectively (Fig. 4.8).

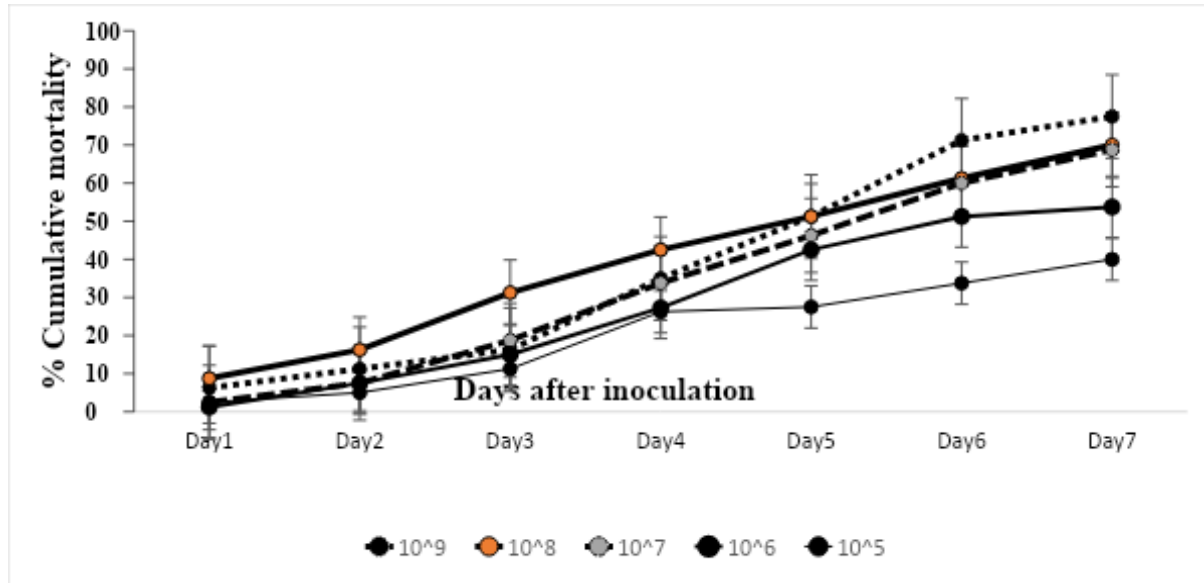


Figure 4. 8: Cumulative mortality rates of *Telenomus remus* adults after indirect exposure to FAW eggs infected with *Beauveria bassiana* isolate ICIPE 621 at different concentrations (n=20). Furthermore, the lethal concentration assessment indicated that, the most toxic isolate to the egg parasitoid *T. remus* was *M. anisopliae* ICIPE 78 with the lowest LC₅₀ of 2.00 x 10⁵ followed by *M. anisopliae* ICIPE 7 with LC₅₀ value of 2.24 x 10⁶ (Table 4.3).

Table 4. 3: Median lethal concentration (LC₅₀) 7 days post-treatment of egg parasitoid *Telenomus remus* adults inoculated with different isolates of entomopathogenic fungi

Fungal species	Isolates	LC ₅₀	%Germination
<i>Beauveria bassiana</i>	ICIPE 621	4.57*10 ⁶	91.20±1.04ab
<i>Metarhizium anisopliae</i>	ICIPE 7	2.24*10 ⁶	93.00±1.3a
	ICIPE 78	2.00*10 ⁵	94.20±1.7a
	ICIPE 41	1.23*10 ⁸	87.30±2.3b

4.2.2 Effect of different fungal concentrations on mortality of FAW eggs exposed to *Telenomus remus* adults

M. anisopliae isolate concentrations had a significant effect on FAW egg hatchability, resulting in the mortality of *S. frugiperda* eggs 7 days after treatment with ICIPE 7 ($F = 7.063$; $df = 5, 18$; $P = 0.0008$), ICIPE 41 ($F = 2.77$; $df = 5, 18$; $P = 0.005$), ICIPE 78 ($F = 2.304$; $df = 5, 18$; $P = 0.004$). *Beauveria bassiana* ICIPE 621, on the other hand, had no significant effects with the concentrations. ($F = 4.79$; $df = 5, 18$; $P = 0.3$). Among all the isolates, the highest concentration caused the highest FAW eggs mortality with *M. anisopliae* ICIPE 7, 41, 78 and *B. bassiana* ICIPE 621 recording 49, 26.25, 51.25 and 30.75% mortality rates respectively (Table 4.4).

Table 4. 4: Mean FAW egg mortality after infection with different isolate concentrations

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Mean FAW egg mortality				
10 ⁹	49.25± 5.42a	26.25± 3.86a	51.25± 7.55a	30.75± 4.03a
10 ⁸	38.50± 5.98ab	21.75± 7.13ab	42.75± 7.85ab	25.25± 3.94a
10 ⁷	35.50± 2.40ab	20.25± 3.84ab	38.25± 6.81ab	27.5± 4.52a
10 ⁶	30.00± 5.96b	14.75± 2.84b	36.00± 3.87ab	22.75± 3.47a
10 ⁵	25.75± 2.50bc	15.25± 1.55b	32.25± 8.08b	21.00± 2.86a

4.2.3 Effect of entomopathogenic fungi concentrations on *Telenomus remus* parasitoid emergence

The mean number of individuals emerged *T. remus* was not affected by *M. anisopliae* and *B. bassiana* isolates, ICIPE 41 ($F = 1.415$; $df = 5, 18$; $P = 0.266$), ICIPE 78 ($F = 0.913$; $df = 5, 18$; $P = 0.495$) and *B. bassiana* ICIPE 621 ($F = 1.197$; $df = 5, 18$; $P = 0.35$), except for ICIPE 7 ($F = 3.174$; $df = 5, 18$; $P = 0.0315$). There were also no significant effects on the sex ratios of the

parasitoids that emerged from the different fungal isolates' treatments compared to the controls (Table 4.5).

Table 4. 5: Effects of fungal isolates on the number of emerged *Telenomus remus* in the laboratory (Mean \pm se)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
10 ⁹	40.25 \pm 8.94a	48.25 \pm 5.71a	48.75 \pm 7.55a	69.25 \pm 4.03a
10 ⁸	48.00 \pm 7.74a	52.00 \pm 9.96a	57.25 \pm 7.85a	74.75 \pm 3.94a
10 ⁷	49.25 \pm 6.49a	60.25 \pm 6.49a	61.75 \pm 6.81a	72.5 \pm 4.52a
10 ⁶	67.50 \pm 6.59a	63.25 \pm 9.59a	64.00 \pm 3.87a	71.5 \pm 5.42a
10 ⁵	69.25 \pm 2.32a	66.00 \pm 2.86a	67.75 \pm 8.08a	75.25 \pm 2.17a
control	72.75 \pm 2.95a	71.50 \pm 6.41a	70.25 \pm 5.44a	82.00 \pm 3.29a

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

4.3. Direct effects of entomopathogenic fungal isolates on *Cotesia icipe* and FAW larvae

There were significant differences in mortality of *C. icipe* adults among all the treatments ($F = 1.72$; $df = 3, 12$; $P = 0.02$) seven days post-treatment (Table 4.5). The lowest adult mortality rate was recorded in the *B. bassiana* ICIPE 621 treatment, whereas the highest mortality was recorded in ICIPE 7 and ICIPE 41 (Table 4.5). The most virulent isolates with LT_{50} less than 3 days were *M. anisopliae* isolates ICIPE 7 and ICIPE 41, whereas ICIPE 78 and *B. bassiana* ICIPE 621 had LT_{50} values of 5.2 and 5.0 respectively days (Table 4.5).

In addition, significant differences in mortality of FAW larvae exposed to the infected parasitoid were observed among all *M. anisopliae* ICIPE 41, 7, 78, and *B. bassiana* ICIPE 621 treatments ($F = 7.27$; $df = 3, 12$; $P < 0.001$), seven days post-treatment (Table 4.5). *M. anisopliae* ICIPE 7 caused the highest FAW larval mortality, while ICIPE 78 recorded the lowest among the isolates (Table 4.5).

Furthermore, significant differences in parasitism rates were also observed among all the tested fungal isolates ($F = 8.61$; $df = 3, 12$; $P < 0.001$) seven days post-treatment (Table 4.5). The highest and lowest parasitism rates were recorded in the ICIPE 78 (62.0%) and ICIPE 7 (35.7%) treatments respectively (Table 4.5). There were also no significant differences in the sex ratios among the different fungal isolates, ($P > 0.05$, χ^2 test) but the F1 parasitoid generations were female bias.

Table 4. 6: Cumulated mortality of *Cotesia icipe* adults, LT_{50} and FAW larvae directly induced mortality by the various fungal isolates

Fungal isolates	Mean mortality %	Larval mortality	Parasitism rates	Sex ratios (F:M)	<i>Cotesia icipe</i> LT_{50}	% Mycosis of <i>C. icipe</i> cadavers
ICIPE 7	73.95 ±7.49a	55.25±6.74a	35.75±4.80b	2:1	2.3±0.0a	65.75±6.69a
ICIPE 78	33.63±6.63b	28.25±4.41b	62.00±5.02a	2:1	5.2±0.0c	43.75±5.91ab
ICIPE 41	66.33±7.29a	53.75±3.91a	36.75±3.82b	2:1	2.8±0.0b	57.25±6.84ab
ICIPE 621	36.59±6.65b	31.00±5.29b	58.00±5.24a	2:1	5.0±0.0d	35.00±4.56b

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls

The results also indicated that *M. anisopliae* ICIPE 7 caused the highest FAW larval mortality whereas ICIPE 78 caused the lowest FAW larval mortality when the fungal infected parasitoids were exposed to the healthy FAW larvae (Fig 4.10).



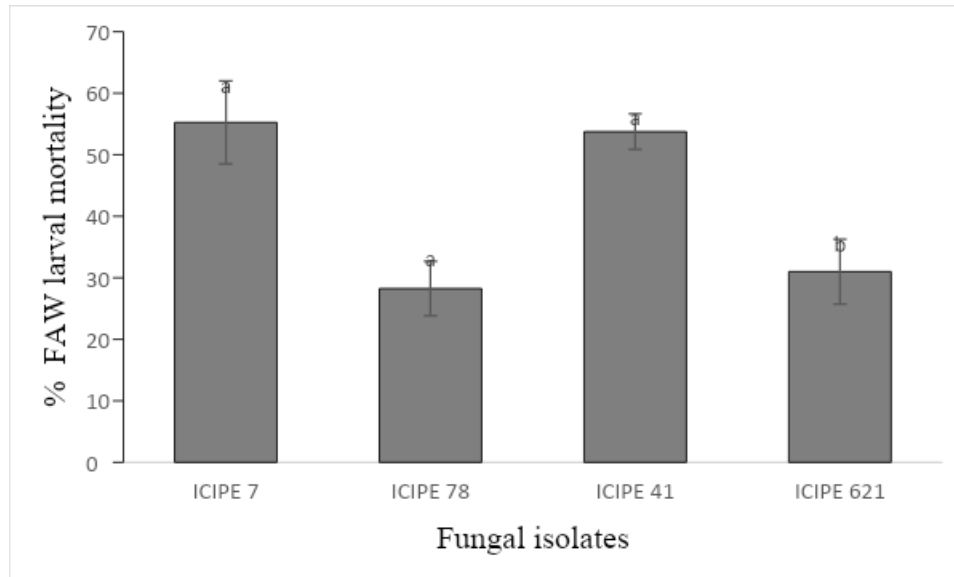
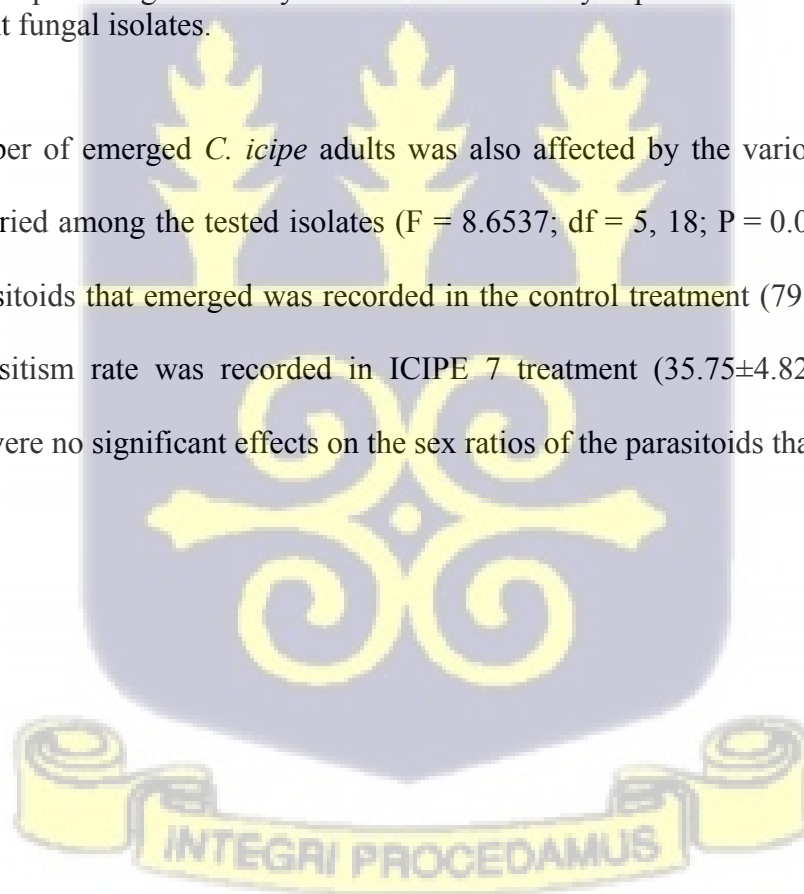


Figure 4. 9: Mean percentage mortality of FAW larvae directly exposed to infected *Cotesia icipe* with the different fungal isolates.

The mean number of emerged *C. icipe* adults was also affected by the various fungal species infection and varied among the tested isolates ($F = 8.6537$; $df = 5, 18$; $P = 0.0005$). The highest number of parasitoids that emerged was recorded in the control treatment ($79.00 \pm 3.96\%$) while the lowest parasitism rate was recorded in ICIPE 7 treatment ($35.75 \pm 4.82\%$) (Fig. 4.4). In addition, there were no significant effects on the sex ratios of the parasitoids that emerged.



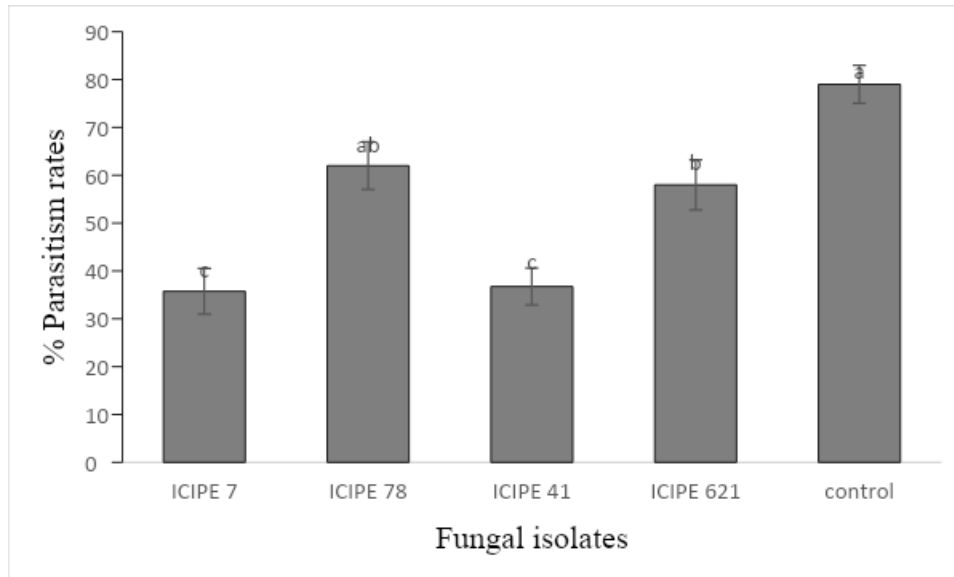


Figure 4. 10: Mean percentage parasitism rates of *Cotesia icipe* directly exposed to the different fungal isolates.

4.4. Indirect effects of entomopathogenic fungal isolates on *Cotesia icipe* adults

4.4.1 Cumulative mortalities of *Cotesia icipe* adults and FAW 2nd instar larvae after indirect exposure to FAW larvae infected with different isolate concentrations

The results showed significant differences in mortality of *C. icipe* adults among all the concentrations of *M. anisopliae* isolates ICIPE 41 ($F = 60.63$; $df = 4, 15$; $P < 0.0001$), ICIPE 7 ($F = 273.8$; $df = 4, 15$; $P < 0.001$), ICIPE 78 ($F = 6.03$; $df = 4, 15$; $P < 0.001$) and *B. bassiana* ICIPE 621 ;; ($F = 152.3$; $df = 4, 15$; $P < 0.001$), seven days post-treatment. For all the tested fungal isolates, the highest concentration always recorded the highest *C. icipe* adult mortality with cumulative mortalities of 94.1, 81.25, 67.00 and 88.75% for *M. anisopliae* ICIPE 41, ICIPE 7, ICIPE 78 and *B. bassiana* 621 respectively (Table 4.6).

Also, significant differences in mortality of FAW larvae were observed among all the concentrations of *M. anisopliae* ICIPE 41 ($F = 17.13$; $df = 4, 15$; $P < 0.001$), ICIPE 7 ($F = 38.88$;

df = 4, 15; $P < 0.001$), ICIPE 78 ($F = 24.85$; df = 4, 15; $P < 0.001$) and *B. bassiana* 621 ($F = 10.86$; df = 4, 15; $P < 0.001$), seven days post-treatment. For all the tested fungal isolates, the highest concentration recorded the highest FAW larval mortality with cumulative larval mortalities of 25.75, 31.58, 29.50 and 19.00% for *M. anisopliae* ICIPE 41, ICIPE 7, ICIPE 78 and *B. bassiana* ICIPE 621 respectively (Table 4.6).

Table 4. 7: Mean mortalities of *Cotesia icipe* adults and FAW larvae after indirect exposure to FAW larvae infected with different isolate concentrations

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Cumulative adult <i>Cotesia icipe</i> mortality				
1 x 10 ⁹	81.25± 8.79a	94.12± 1.20a	67.00± 8.57a	88.75± 3.15d
1 x 10 ⁸	76.25± 4.26ab	86.40± 2.34b	52.38± 4.98ab	77.50± 5.95c
1 x 10 ⁷	71.25± 4.26b	72.20± 3.81c	35.57± 6.98b	63.75± 9.34b
1 x 10 ⁶	55.00± 6.12c	61.50± 2.50d	25.55± 4.22b	61.50± 6.61b
1 x 10 ⁵	55.00± 7.39c	51.30± 1.44e	24.08± 7.52b	52.50± 8.54a
Adult <i>Cotesia icipe</i> percentage mycosis				
1 x 10 ⁹	54.50± 4.86a	73.25± 5.76a	37.25± 4.64a	59.50± 8.15a
1 x 10 ⁸	42.00± 3.03b	49.50± 4.17b	20.25± 4.71b	43.25± 3.45b
1 x 10 ⁷	30.25± 3.94c	30.00± 1.41c	8.75± 1.75c	27.25± 1.11c
1 x 10 ⁶	20.25± 2.29c	21.50± 2.84c	7.55± 1.94c	18.25± 1.25c
1 x 10 ⁵	18.00± 2.06c	20.30± 2.35c	4.00± 0.41c	15.25± 3.42c
Mean FAW larval mortality				
1 x 10 ⁹	31.58± 1.38a	25.75± 3.64a	29.50± 2.72a	19.00± 3.19a
1 x 10 ⁸	14.36± 2.74b	12.25± 1.49b	13.25± 1.65b	9.25± 1.12b
1 x 10 ⁷	6.23± 1.89c	8.75± 1.65b	9.25± 2.39b	6.75± 1.31b
1 x 10 ⁶	4.75± 2.02c	6.25± 0.95b	6.25± 1.65b	5.75± 1.32b
1 x 10 ⁵	3.25± 0.85c	5.75± 0.85b	5.75± 0.85b	5.25± 0.48b

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls

4.4.2 Effect of entomopathogenic fungal isolate concentrations on *Cotesia icipe* emergence

Parasitism rates of *C. icipe* varied among the fungal isolates after exposing the infected FAW larvae to the parasitoid. Significant differences in emergence of *C. icipe* were observed among all the concentrations of *M. anisopliae* ICIPE 41 ($F = 4.70$; $df = 5, 18$; $P = 0.006$), ICIPE 7 ($F = 3.291$; $df = 5, 18$; $P = 0.0027$) and ICIPE 78 ($F = 10.83$; $df = 5, 18$; $P < 0.0001$). However, no significant difference was observed for *B. bassiana* ICIPE 621 ($F = 2.10$; $df = 5, 18$; $P = 0.1$). The highest number of parasitoids that emerged was recorded in the control treatments. For all the tested fungal isolates, the least concentration always recorded the highest parasitoid emergence, while the high concentrations yielded moderate parasitism rates (Table 4.7).

In addition, the sex ratios of the F1 *C. icipe* generations were females bias for all the tested isolates and at all concentrations; except for ICIPE 41 and ICIPE 78 where the sex ratios were balanced at the concentrations of 1×10^9 and 1×10^9 and 1×10^8 respectively (Table 4.7).

Table 4. 8: Effects of fungal isolates on the number of emerged *Cotesia icipe* (parasitism rates) in the laboratory (Mean \pm SE) and their sex ratios (F:M)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
1×10^9	54.75 \pm 3.33b (2:1)	46.50 \pm 7.27c (1:1)	44.75 \pm 2.90d (1:1)	56.25 \pm 3.40a (2:1)
1×10^8	60.25 \pm 5.14b (2:1)	51.25 \pm 5.84bc (2:1)	56.00 \pm 6.56cd (1:1)	59.25 \pm 3.74a (2:1)
1×10^7	64.75 \pm 3.84ab (2:1)	61.50 \pm 5.58abc (2:1)	65.75 \pm 4.53bc (2:1)	61.25 \pm 4.60a (2:1)
1×10^6	66.50 \pm 8.91ab (2:1)	67.25 \pm 4.73abc (2:1)	71.50 \pm 4.33ab (2:1)	66.75 \pm 6.14a (2:1)
1×10^5	70.50 \pm 7.37ab (2:1)	74.25 \pm 10.05ab (2:1)	76.75 \pm 3.66ab (2:1)	69.50 \pm 8.68a (2:1)
Control	85.75 \pm 4.46a (2:1)	83.50 \pm 2.47a (2:1)	82.75 \pm 3.47a (2:1)	77.25 \pm 3.33a (2:1)

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.



CHAPTER FIVE

DISCUSSION

Fall armyworm (FAW) is affected by numerous natural enemies such as pathogens (fungi, viruses, protozoa, and bacteria) with some causing high epizootics (Gardner *et al.*, 1984). Biological agents such as multiple nucleopolyhedroviruses (SfMNPV) belonging to the family Baculoviridae were demonstrated by Gomez *et al.*, (2013) to reduce FAW feeding damage on maize to 2.5-7.5% compared to 70% infestation in the control treatments. Entomopathogenic fungi (EPF) mainly *M. anisopliae* and *B. bassiana* have also proved to be effective biocontrol agents of FAW with laboratory mortalities of 67.8% and 64.3% respectively (Ramanujam *et al.*, 2020). Recently, *Beauveria bassiana* ICIPE 621 and *M. anisopliae* ICIPE 7 were also reported to be highly effective against adult FAW by causing 100% mortality of the moths with the lowest LT_{50} values of 3.6 ± 0.1 and 3.9 ± 0.0 days, respectively. Both isolates were also found compatible with the FALLTRACT lure, as the lure had no effect on the conidial germination in the laboratory (Akutse *et al.*, 2020). Prior to this, Akutse *et al.* (2019) reported that *Metarhizium anisopliae* isolates ICIPE 78, ICIPE 40, and ICIPE 20, outperformed all the other tested isolates, through reduction of its hatchability by 87, 83, and 79.5%, respectively. Also, the fungal isolates induced mortality to the new larvae (neonates) from the fungus-treated eggs 7 days post-emergence, where *M. anisopliae* isolates ICIPE 41 and ICIPE 7 outperformed the rest through larval mortality of 96.49 and 93.66% respectively.

Among major FAW-associated indigenous parasitoids, *C. icipe* has been identified as a key FAW larval parasitoid causing more than 60% parasitism rate, in addition to the efficiency of *T. remus* (Mohamed *et al.*, 2021; Sisay *et al.*, 2018). Compatibility of EPF with other pest control agents

such as these key parasitoids is vital in the development of sustainable integrated pest management strategies to suppress the pest populations.

Despite the availability of data on the efficacy of entomopathogenic fungi on FAW, their effects on other non-target organisms, mainly parasitoids are not well documented. Hence, the need for assessing the effects of combining these two biocontrol agents in controlling FAW through a comprehensive interaction study is very crucial. Our study, therefore, underlined the interactions of four potent entomopathogenic fungal isolates with two major indigenous parasitoids associated with FAW in Africa.

Results of this study showed that the EPF isolates have the potential to infect both *T. remus* and *C. icipe* adults when directly exposed to dry conidia of these entomopathogenic fungal isolates. Upon application, *M. anisopliae* isolates showed more virulence to the two parasitoids as compared to *B. bassiana* isolate when infecting them directly. *Metarhizium anisopliae* ICIPE 7 caused the highest mortality to adult egg parasitoid *T. remus*, whereas both ICIPE 7 and ICIPE 41 caused the highest mortalities to the larval parasitoid *C. icipe*. *Beauveria bassiana* ICIPE 621 caused the least mortality to both parasitoids after direct application of dry conidia. Although the infection process of both entomopathogenic_fungal species is through the insect cuticle, pathogenicity variation could be due to differences in components that mediate variation in virulence, including proteinases, chitinases, and lipases, responsible for cuticle disintegration and insect penetration (Ortiz-Urquiza and Keyhani, 2013).

Pathogenicity variations and virulence of different isolates were reported for fungal pathogens tested in different insects (Migiro *et al.*, 2010; Opisa *et al.*, 2018). Studies by Ansari *et al.* (2011), also indicated that dry conidia considerably outperformed wet-formulated conidia. The

author found that the dry conidia of *M. anisopliae* were shown to be effective in infecting mosquitoes. It is worth noting that applications of dry conidia in the field may lose their viability and virulence due to environmental conditions, which in turn is beneficial to the parasitoids. Furthermore, since dry conidia are always applied in combination with the pest attractants to target the adult pests, there would be less interactions of the fungus with the natural enemies that might not negatively affect the performance of the parasitoid under field conditions.

Generally, results indicated that *M. anisopliae* isolates elicited quicker mortality with lower LT_{50} values lower than the *B. bassiana* isolate for both parasitoids. Transfer of viable conidia from adult eggs *T. remus* parasitoid to *S. frugiperda* eggs was highest from treatments exposed to *M. anisopliae* ICIPE 78 at 61.25%, followed by *B. bassiana* ICIPE 621 at 55.00%, while *M. anisopliae* ICIPE 41 had the least FAW egg mortality effect of 39.75% through the parasitoid contamination. As for the larval parasitoid *C. icipe*, the highest conidial transfer was observed from *M. anisopliae* ICIPE 7 whereas ICIPE 78 recorded the lowest FAW mortality. Such transfer of fungal conidia by a third-party insect that is not the target species is known as entomopathogenic vectoring. Both parasitoids were able to vector at some degree the conidia of the various fungal isolates to FAW eggs and larvae, causing significant induced mortalities to the host. This can be attributed to the fact that parasitoid oviposition renders some hosts to be more susceptible to the fungal infection due to physiological and structural changes in the cuticle caused by parasitism and therefore enabling hyphae penetration into the host's hemocoel as reported by Furlong *et al.*, (2005). Also, reports by Almeida *et al.*, (1984) showed that fungus entry into the host can be through direct penetration, through the parasitoid's ovipositor, or germination on the host surface following germination, then penetrating through the opening left by the ovipositor. Reports by Alves *et al.*, (2002) also confirmed a horizontal transfer of *M.*

anisopliae isolate inoculum from honeybees to the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae).

There was a reduction in parasitism rates of the two parasitoids when treated with dry conidia of the different fungal isolates as compared to the control treatments. Control treatments had more emergence as compared to the different fungal isolates. Comparing the different isolates, *M. anisopliae* ICIPE 7 recorded the highest parasitoid emergence for the egg parasitoid *T. remus*, whereas *M. anisopliae* ICIPE 78 recorded the highest emergence of *C. icipe* adults. Similarly, Broglio-Micheletti *et al.*, (2006) reported that there was a 78.3% reduction in parasitism of *Trichogramma galloi* in *Diatraea saccharalis* eggs, after exposure to *M. anisopliae* (strain IPA 159E). The authors also reported that there was significant variation in the effects and other strains from the same entomopathogenic species.

The level of parasitism of *T. remus* on FAW without influence from other external biopesticides was confirmed by Laminou *et al.*, (2020) after conducting a laboratory study, whose findings were that *T. remus* parasitized an average of 78% of FAW eggs as compared to 25% for *Trichogrammatoidea* sp. (Hymenoptera: Trichogrammatidae). Moreover, *T. remus* was able to parasitize on masses of FAW eggs fully covered with scales unlike *Trichogrammatoidea* sp. which was only able to parasitize only uncovered eggs.

Studies have also shown that there are various degrees of specificity for various fungal isolates. According to Poprawski *et al.*, (1998), spraying predators with *B. bassiana* lowered the survival of *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae), a major whiteflies predator. However, when *L. fumosorosea*, was used, the pathogen's dose rate did not affect the survival.

Results from our indirect infection experiment to determine the most efficient ovicidal/larvicidal inducing entomopathogenic fungal isolate concentrations on *S. frugiperda* eggs/larvae and subsequent effects on the two parasitoids showed that the lethal capacity of all the four isolates induced high mortality as the fungal concentrations increased (from 1×10^5 to 1×10^9). The highest induced egg mortality rate of *S. frugiperda* was observed in *M. anisopliae* ICIPE 78 at 51.25% whereas the highest larval mortality was recorded in *M. anisopliae* ICIPE 7 at 31.58%. Other studies have also reported high mortality of adult parasitoids caused by EPF applications. For example, Abbas *et al.*, (2020) reported 40% mortality from *M. anisopliae* to *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethyridae) adult parasitoid of coffee berry borer (*Hypothenemus hampei*). Sisay *et al.*, (2019) reported that parasitoids *Bracon hebetor* (Hymenoptera: Braconidae) and *Anagyrus lopezi* (Hymenoptera: Encyrtidae) were highly susceptible to infection by 11 strains of *M. anisopliae*. Uma Devi *et al.*, (2008) showed a low risk for the parasitoid wasp, *Trybliographa rapae* recorded after they parasitized larvae of the cabbage root fly, *Delia radicum* previously infected with a strain of *M. brunneum* and recommended the combined use of both agents to control *D. radicum*. Similar results on pathogenicity of ICIPE 41 and ICIPE 7 to FAW larvae when in contact with their cuticle were also reported by Akutse *et al.*, (2019).

According to our findings, the direct fungal infection of the eggs and larvae, and additional mortality rates of the eggs or larvae could be through conidia transfer/vectoring (induced mortality) by the parasitoids. However, as the concentrations of the fungal isolates increased, they also negatively affect the performance of the parasitoids as well as their survival.

A field trial of biocontrol-based IPM on FAW with two sprays of *Bacillus thuringiensis* (NBAIR-BT25) and *M. anisopliae* (NBAIR Ma-35) at 2.0×10^8 cfu/g also resulted in ovicidal

effects with 76 and 71.64% on egg masses respectively. Furthermore, the two products caused larval mortality of 80 and 74.4% respectively within a period of 60 days post-treatment (Varshney *et al.*, 2020). In a different study García *et al.*, (2011) observed that isolates of *B. bassiana* with a concentration of 1×10^9 conidia mL⁻¹ were able to cause 96% mortality to 2nd instar FAW larvae. In a different study, both *B. bassiana* and *M. anisopliae* were demonstrated to cause ovicidal effects on other species of *Spodoptera* Guenée viz. *S. litura* by Anand and Tiwary (2009) and in other lepidopterans such as potato tuber moth *Phthorimaea operculella* Zeller where 63% larval mortality was reported (Khorrami *et al.*, 2018). Shimazu (2004) also reported that adult pine sawyers, *Monochamus alternatus* (Hope) were able to be inoculated with *B. bassiana* at a lethal dose just by walking on fungus-contaminated non-woven fabric strips with 3.5×10^8 conidia cm⁻².

Our results further showed that exposure of the parasitoids to infected FAW eggs and larvae had some effects on the parasitoids with high EPF concentrations causing some significant mortality to the adult parasitoids as compared to lower concentrations. The Highest *T. remus* adult mortality was recorded in *B. bassiana* ICIPE 621 at the concentration of 1×10^9 conidia ml⁻¹ whereas the highest *C. icipe* adult mortality rate was recorded in *M. anisopliae* ICIPE 41 at 1×10^9 conidia ml⁻¹. Similar results were also reported by Förster *et al.* (2004) during the evaluation of the impact caused by the five entomopathogenic fungi, *M. anisopliae*, *Isaria fumosorosea*, *Nomuraea rileyi* and two strains of *B. bassiana* against the predatory lady beetle, *Hippodamia convergens* (Coleoptera: Coccinellidae) in the laboratory. The authors reported that the fungal isolates led to mortality ranging from 56 to 95%, which depended on dose except for *Nomuraea rileyi*. Migiro *et al.* (2010) also reported that *M. anisopliae* has the potential to infect the

ectoparasitoid of *Liriomyza* leafminer *Diglyphus isaea* and thus could not be used indiscriminately.

In addition, the four fungal isolates tested in the current study on the FAW larvae showed low larval mortality rates at all the concentrations, where only *M. anisopliae* ICIPE 7 recorded the highest moderate larval mortality of 31.58%. Reports by Akutse et al. (2019) also revealed that *M. anisopliae* isolates showed 92 and 97% mortality in FAW eggs and neonate larvae, respectively, but the isolates were less pathogenic to the 2nd instar larvae of FAW, where only 30% larval mortality was recorded in *B. bassiana* isolate. Akutse et al., (2020) further reported that *M. anisopliae* and *B. bassiana* isolates caused 19 and 100% mortality in adult FAW. Morales-Reyes et al., (2018) reported that *M. anisopliae* and *B. bassiana* at the concentrations of 1×10^6 and 1×10^7 caused FAW 2nd instar larval mortality ranging between 45 to 65%, respectively. García-Munguía et al. (2011) reported 96.6 and 78.6% mortality on 2nd instar larvae of FAW with *B. bassiana* and *M. anisopliae* strains, respectively at a concentration of 1×10^9 conidia ml⁻¹. Mumo et al., (2018) also reported that entomopathogenic fungal enzymes play an important role in the virulence to insect pests, which varies with different strains. Additionally, Ramanujam et al., (2020), reported that *M. anisopliae* ICAR-NBAIR Ma-35 and *B. bassiana* ICAR-NBAIR Bb-45 isolates were effective against FAW 2nd instar larvae with mortalities of 67.8 and 64.3%, respectively.

Adult *T. remus* resulting from the FAW eggs sprayed with various fungal concentrations was not affected by all isolates except for *M. anisopliae* ICIPE 78 in which the highest concentration showed a significant difference as compared to the control treatments. This shows that the other isolates can be used together with *T. remus* parasitoid for FAW management. It indicates that although the high concentrations may kill the parasitoid, the timing is important such that the

females will have parasitized the hosts. Similarly, Agüero *et al.*, (2014), reported that there were no adverse effects detected in parasitism rates of *T. podisi* (Hymenoptera: Scelionidae) on *Euschistus heros* eggs sprayed with *M. anisopliae*. However, *C. icipe* showed low emergence in all the *M. anisopliae* concentrations except for *B. bassiana* isolate. Similar results on low emergence of *Diglyphus isaea* parasitoids infected with *M. anisopliae* isolate were reported after indirect infection (Migiro, 2010).

The sex ratios of the emerged *T. remus* and *C. icipe* parasitoids were not affected by the fungal isolates in both the direct and indirect applications when compared with the controls. Furthermore, the F1 progenies showed dominant female bias populations at all the concentrations, which is very important for the reproduction of the parasitoids in the system. Further studies are warranted to assess the longevity/survival of the F1 progenies. Similarly, Potrich *et al.*, (2009) did not observe any differences in sex ratios discrimination of *T. pretiosum* emerging from *Ephestia kuehniella* eggs sprayed with *M. anisopliae* (Unioeste 22 strain) as compared to the controls. Moreover, experiments by Polanczyk *et al.*, (2010), indicated that *M. anisopliae* did not affect the sex ratios of *Trichogramma atopovirilia* through diet contamination and egg immersions in conidial suspensions.

Some studies have also shown that the interaction of insect pests, parasitoids, and fungus does not affect the parasitoid (Emami *et al.*, 2013). Such interaction is complementary and not pathogenic. For example, *Encarsia formosa* (Hymenoptera: Aphelinidae), a parasitoid of greenhouse whitefly, was able to detect virus-infected hosts and therefore only oviposits in hosts not infected with *Aschersonia aleyrodis* (Hypocreales: Clavicipitaceae) (Montesinos, 2003). Further reports by Caballero *et al.*, (2013) indicated that the survival of *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae), a whitefly predator was lower when they were sprayed with

B. bassiana as opposed to sprays from *Isaria Fumorosea*; and that the dose of each pathogen did not affect the survival rate. The parasitoid's ability to detect and avoid hosts infected by entomopathogenic fungi seems crucial for combining both biocontrol agents (Abbas, 2020). Thus, one must explore the behavior of the two key FAW-associated parasitoids toward the infected and non-infected host for better timely application decision-making.

There would be a greater selection for antifungal mechanisms directed toward fungi of a broad host range, such as *B. bassiana* and *M. anisopliae* that can infect the parasitoid and the host. *Dimbi et al.*, (2003) found that *Eretmocerus* sp. avoided oviposition on *Bemisia argentifolii* Bellows infected with *B. bassiana*. In addition, antifungal compounds production by host and parasitoid has been reported as a response to the presence of *B. bassiana*.



CHAPTER SIX

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

Our findings showed that mode of autodissemination of the fungal inoculum vectoring by the FAW eggs/larvae to the parasitoids or vice versa, and the fungal concentrations may cause detrimental effects to the parasitoids. The assessment of the direct effects of the different fungal isolates showed that, *M. anisopliae* isolates were more virulent to the two parasitoids as compared to *B. bassiana* isolate. Direct infection of *M. anisopliae* ICIPE 7 was more pathogenic to *T. remus* parasitoid, whereas both *M. anisopliae* ICIPE 7 and ICIPE41 were more pathogenic to *C. icipe*. The parasitoids infected with *M. anisopliae* isolates also showed a higher conidial transfer to their hosts, inducing additional eggs/larval mortality. The indirect exposure of parasitoids on infected FAW eggs and larvae also showed that the highest concentrations had some effects on the parasitoids and thus causing significant mortalities to the parasitoids as compared to the lowest concentrations.

Upon parasitization of FAW eggs and 2nd instar larvae, *T. remus* and *C. icipe* emergence were highest in control as compared to the different fungal isolate treatments. Parasitoid emergence was significantly affected by direct application of dry conidia to the two parasitoid species. However, the indirect application did not affect *T. remus* emergence except for ICIPE 78 which recorded lower emergence rates. As for the larval parasitoid *C. icipe*, *M. anisopliae* isolates recorded low emergence of the parasitoid as compared to *B. bassiana* isolate. This shows that it is important to time the application of the isolates and the release of the parasitoids to avoid negative effects on the parasitoids. Though in some cases, high mortality of *T. remus* parasitoid

was recorded because of direct or indirect exposure to *M. anisopliae* and *B. bassiana*, the use of entomopathogenic fungi increased FAW mortality. Furthermore, since dry conidia are always applied in combination with the pest attractants to target the adult of the pests, there would be fewer interactions of the fungus with the natural enemies that might not negatively affect the performance of the parasitoid under field conditions.

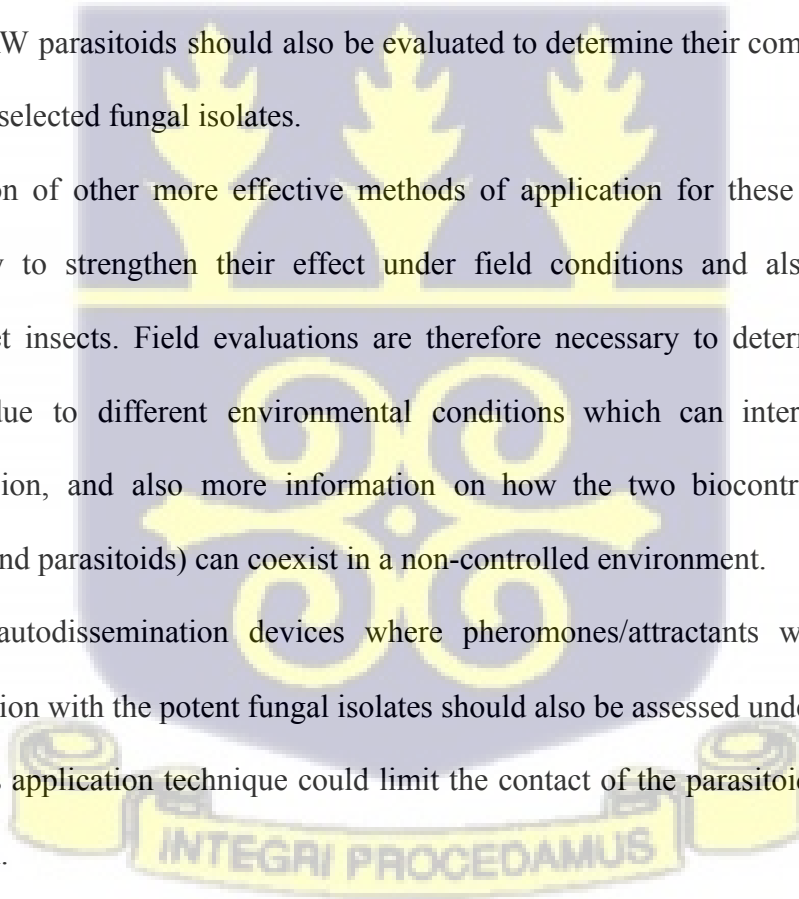
Some field studies however have shown that EPF at low concentrations can be combined with different parasitoids as biocontrol agents of insect pests (Goettel and Hajek, 2001; Sterk et al., 2003). However, a blanket statement on compatibility cannot be assumed given the fact that variations that occur among fungal species and isolates, the specificity of natural enemies, and the environmental factors in which they are coexisting might also interfere during field applications. To accurately assess compatibility, trials must be conducted under field conditions through artificial applications. Results obtained in laboratory bioassays may indicate that a fungus is pathogenic to a natural enemy, though in the agricultural setting systems, mortality of the non-target may not occur (Brown and Khan, 2009; Sterk et al., 2003). However, the timing of the release of the biological control agents (simultaneous or sequential) as well as the specific concentrations that may be compatible with the parasitoid must be determined. In our case, the lower concentrations (1×10^5 to 1×10^7) for all the isolates did not show significant detrimental effects on the two parasitoids, especially with *B. bassiana* ICIPE 621.

6.2. Recommendations

1. This study recommends the use of *B. bassiana* ICIPE 621 dry conidia together with both parasitoids to achieve the combined effect on the FAW, since the isolate was less virulent

to both parasitoid species. However, among *M. anisopliae* isolates, it is recommended to reduce the amount /concentration applied to the parasitoids to reduce their mortalities.

2. Both direct and indirect bioassay results on the effect of the isolates on the two parasitoids' emergence indicated that *M. anisopliae* isolates significantly reduce parasitism rates and should not be used indiscriminately. However, the indirect application recommends that lower concentrations could be applied together with the parasitoids without affecting their performance.
3. Timing the release of the two biological agents is important and therefore the timing should be determined for maximum effectiveness of both agents when used together. Other FAW parasitoids should also be evaluated to determine their compatibility with the different selected fungal isolates.
4. Evaluation of other more effective methods of application for these fungal isolates is necessary to strengthen their effect under field conditions and also friendly to the non-target insects. Field evaluations are therefore necessary to determine the level of impact due to different environmental conditions which can interfere with fungus transmission, and also more information on how the two biocontrol agents (fungal isolates and parasitoids) can coexist in a non-controlled environment.
5. Use of autodissemination devices where pheromones/attractants would be used in combination with the potent fungal isolates should also be assessed under field conditions since this application technique could limit the contact of the parasitoids with the fungal inoculum.



6. It is also critical to investigate the behavior of the two key FAW-associated parasitoids toward infected and non-infected hosts in order to make more timely application decisions.



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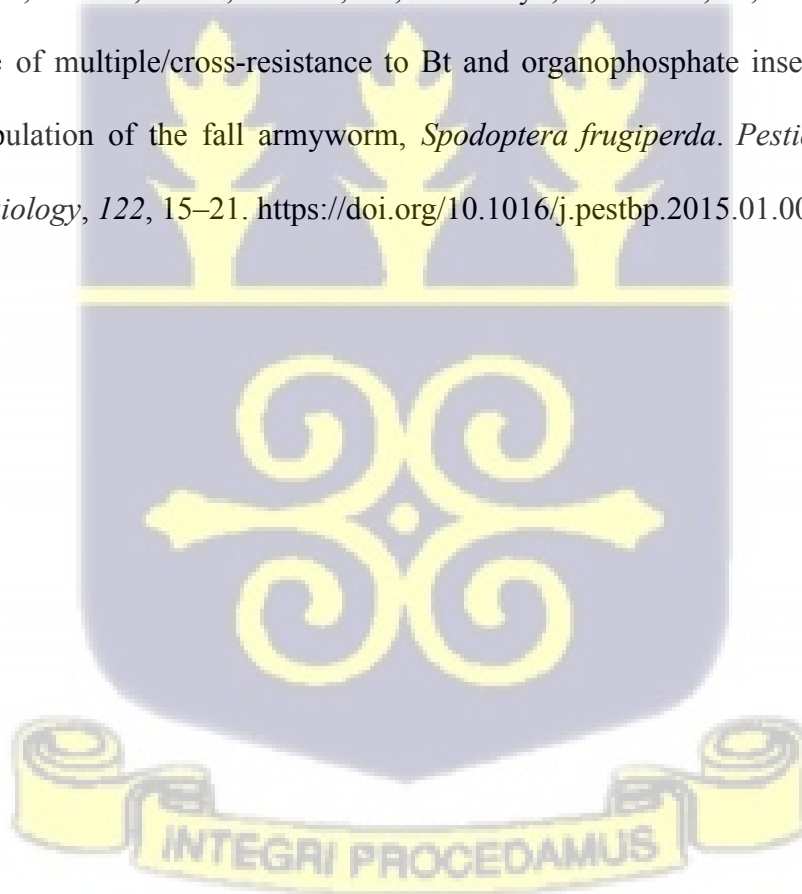
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APPENDICES

Appendix 1. 1: Mean mortality of (*Telenomus remus* adults and FAW eggs) and parasitism induced by fungal isolates (Mean±se)

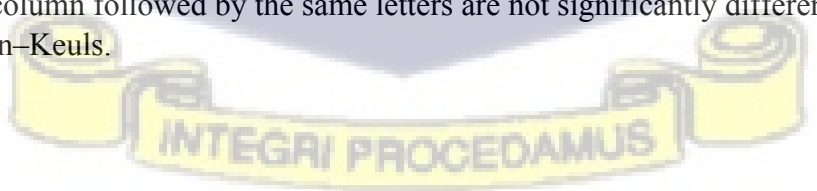
Fungal isolates	<i>Tremus</i> adult mortality %	FAW egg mortality %	Parasitism rates%
ICIPE 7	81.40± 4.08a	44.25± 9.14a	42.50± 5.55b
ICIPE 78	76.51± 5.69ab	61.25± 8.43a	38.75± 8.43b
ICIPE 41	67.97± 6.01ab	39.75± 6.52a	40.25± 6.42b
ICIPE 621	61.39± 6.50b	55.00± 5.99a	40.00± 4.56b
control			83.25± 5.94a

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

Appendix 1. 2: Cumulated mortality of *Telenomus remus* exposed to different entomopathogen fungal species

Fungal isolates	DAY1 Means±Se	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7
ICIPE 7	42.50± 9.24a	73.75± 15.19a	81.53±13.2 5a	87.50± 12.50a	92.86± 7.14a	95.83± 4.17a	95.83± 4.17a
ICIPE 78	22.50± 4.79b	46.90± 10.38ab	84.74± 9.26a	93.30± 3.88a	93.43± 3.57a	95.83± 4.17a	95.83± 4.17a
ICIPE 41	16.25± 4.27b	34.53± 9.76ab	67.80± 10.93a	88.17± 7.12a	90.29± 6.27a	90.59± 5.46a	90.59± 5.46a
ICIPE 621	12.50 ±6.61b	48.22± 17.97ab	59.90± 17.61a	68.30± 18.30a	76.79± 13.48a	80.95± 11.17a	80.95± 11.17a

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.



Appendix 1. 3: Median lethal concentration (LC₅₀) 7 days post treatment of egg parasitoid *Telenomus remus* inoculated with different isolates of entomopathogenic fungi

Fungal species	Isolates	LC ₅₀	%Germination
<i>Beauveria bassiana</i>	ICIPE 621	4.57*10 ⁶	91.20±1.04ab
<i>Metarhizium anisopliae</i>	ICIPE 7	2.24*10 ⁶	93.00±1.3a
	ICIPE 78	2.00*10 ⁴	94.20±1.7a
	ICIPE 41	1.23*10 ⁸	87.30±2.3b

Appendix 1. 4: Mortality of (FAW eggs and *Telenomus remus* adults) induced by fungal isolates (Mean±se)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Mean FAW egg mortality				
10 ⁹	49.25± 5.42a	26.25± 3.86a	51.25± 7.55a	30.75± 4.03a
10 ⁸	38.50± 5.98ab	21.75± 7.13ab	42.75± 7.85ab	25.25± 3.94a
10 ⁷	35.50± 2.40ab	20.25± 3.84ab	38.25± 6.81ab	27.5± 4.52a
10 ⁶	30.00± 5.96b	14.75± 2.84ab	36.00± 3.87ab	22.75± 3.47a
10 ⁵	25.75± 2.50bc	15.25± 1.55ab	32.25± 8.08ab	21.00± 2.86a
Mean adult <i>Tremus</i> mortality				
10 ⁹	37.32± 6.06a	34.46± 4.08a	78.54± 5.85a	40.17± 5.77a
10 ⁸	36.25± 5.60a	30.00± 3.58a	71.96± 5.66a	38.39± 5.13a
10 ⁷	31.60± 4.85a	21.25± 3.08b	66.83± 5.91a	33.92± 4.98ab
10 ⁶	27.67± 4.24ab	17.32± 2.59b	66.24± 5.76a	28.36± 3.97ab
10 ⁵	27.50± 3.62ab	8.57± 1.23c	62.13± 6.59a	20.89± 2.93bc
Cumulative adult <i>Tremus</i> mortality				
10 ⁹	87.50± 4.79a	62.50± 5.95a	100.00± 0.00a	77.50± 7.73a
10 ⁸	81.25± 3.75ab	51.25± 7.74ab	95.83± 7.89a	70.00± 7.36ab
10 ⁷	72.50± 4.79abc	45.00± 4.56ab	93.75± 12.90a	68.75± 6.57ab
10 ⁶	65.00± 6.12bc	35.00± 4.56b	93.75± 7.22a	53.75± 3.15bc
10 ⁵	56.25± 2.39c	17.50± 1.44c	87.50± 2.41a	40.00± 5.44c

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

Appendix1. 5: Effects of fungal isolates on the number of emerged *Telenomus remus* in the laboratory (Mean ± se)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
10 ⁹	40.25± 8.94a	48.25± 5.71a	48.75± 7.55a	69.25± 4.03
10 ⁸	48.00± 7.74a	52.00± 9.96a	57.25± 7.85a	74.75± 3.94
10 ⁷	49.25± 6.49a	60.25± 6.49a	61.75± 6.81a	72.5± 4.52
10 ⁶	67.50± 6.59a	63.25± 9.59a	64.00± 3.87a	71.5± 5.42
10 ⁵	69.25± 2.32a	66.00± 2.86a	67.75± 8.08a	75.25± 2.17
control	72.75± 2.95a	71.50± 6.41a	70.25± 5.44a	82.00± 3.29a

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

Appendix1. 6: Cumulative mortalities of *Cotesia icipe* adults and FAW larvae after indirect exposure to FAW larvae infected with different isolate concentrations

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Cumulative adult <i>C. icipe</i> mortality				
10 ⁹	81.25± 8.79a	94.12± 1.20a	67.00± 8.57a	88.75± 3.15d
10 ⁸	76.25± 4.26ab	86.40± 2.34b	52.38± 4.98ab	77.50± 5.95c
10 ⁷	71.25± 4.26b	72.20± 3.81c	35.57± 6.98b	63.75± 9.34b
10 ⁶	55.00± 6.12c	61.50± 2.50d	25.55± 4.22b	61.50± 6.61b
10 ⁵	55.00± 7.39c	51.30± 1.44e	24.08± 7.52b	52.50± 8.54a
Mean FAW larval mortality				
10 ⁹	31.58± 1.38a	25.75± 3.64a	29.50± 2.72a	19.00± 3.19a
10 ⁸	14.36± 2.74b	12.25± 1.49b	13.25± 1.65b	9.25± 1.12b
10 ⁷	6.23± 1.89c	8.75± 1.65b	9.25± 2.39b	6.75± 1.31b
10 ⁶	4.75± 2.02c	6.25± 0.95b	6.25± 1.65b	5.75± 1.32b
10 ⁵	3.25± 0.85c	5.75± 0.85b	5.75± 0.85b	5.25± 0.48b

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls

Appendix 1. 7 : Effects of fungal isolates on the number of emerged *Cotesia icipe* in the laboratory (Mean \pm se)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
10 ⁹	54.75 \pm 3.33b	46.50 \pm 7.27c	44.75 \pm 2.90d	56.25 \pm 3.40
10 ⁸	60.25 \pm 5.14b	51.25 \pm 5.84bc	56.00 \pm 6.56cd	59.25 \pm 3.74
10 ⁷	64.75 \pm 3.84ab	61.50 \pm 5.58abc	65.75 \pm 4.53bc	61.25 \pm 4.60
10 ⁶	66.50 \pm 8.91ab	67.25 \pm 4.73abc	71.50 \pm 4.33ab	66.75 \pm 6.14
10 ⁵	70.50 \pm 7.37ab	74.25 \pm 10.05ab	76.75 \pm 3.66ab	69.50 \pm 8.68
control	85.75 \pm 4.46a	83.50 \pm 2.47a	82.75 \pm 3.47a	77.25 \pm 3.33

Means within a column followed by the same letters are not significantly different by Student–Newman–Keuls.

Appendix 1. 8: Adult *Telenomus remus* percentage mycosis in the laboratory after indirect infection with the fungal isolates (Mean \pm se)

Concentration	<i>M. anisopliae</i> ICIPE 7	<i>M. anisopliae</i> ICIPE 41	<i>M. anisopliae</i> ICIPE 78	<i>B. bassiana</i> ICIPE 621
Adult <i>T. remus</i> percentage mycosis				
10 ⁹	70.25 \pm 7.25a	51.75 \pm 5.11a	78.75 \pm 3.15a	36.20 \pm 2.39a
10 ⁸	62.20 \pm 4.87a	40.50 \pm 5.40ab	70.00 \pm 5.40a	30.25 \pm 5.76ab
10 ⁷	43.25 \pm 5.65b	31.25 \pm 5.54bc	65.00 \pm 6.12ab	22.50 \pm 3.23bc
10 ⁶	31.28 \pm 3.51bc	18.75 \pm 3.15cd	62.25 \pm 7.12ab	19.25 \pm 2.10bc
10 ⁵	23.20 \pm 3.20c	8.75 \pm 2.39d	45.50 \pm 7.26b	15.00 \pm 2.04c

