USE OF ENTOMOPATHOGENIC FUNGUS AND NEMATODES INDIGENOUS TO THAILAND FOR CONTROLLING OF FALL ARMYWORM (*SPODOPTERA FRUGIPERDA* (J.E. SMITH))

ONGPO LEPCHA

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in (Agricultural Science)
2020

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By ONGPO LEPCHA

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science in Agricultural Science of Naresuan University

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Title	USE OF ENTOMOPATHOGENIC FUNGUS AND NEMATODES INDIGENOUS TO THAILAND FOR CONTROLLING OF FALL ARMYWORM (*SPODOPTERA FRUGIPERDA* (J.E. SMITH))
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Academic Paper	M.S. Thesis in Agricultural Science, Naresuan University, 2020
Keywords	Entomopathogenic fungi, Entomopathogenic nematodes, Fall armyworm

ABSTRACT

The Fall armyworm, *Spodoptera frugiperda* is an insect pest indigenous to tropical and subtropical regions of the Americas. It is a polyphagous pest reported to affect more than 353 plant species belonging to 76 plant families. This pest was never reported outside America until 2016 when it was reported for the first time in Africa, since its invasion, the pest is causing huge economic losses in Africa. In December 2018, this pest was reported for the first time in Thailand. The invasions of this alien species have threatened maize growers in Thailand. Currently, pesticides are used to control and minimize the distribution of Fall armyworm in the maize field. The use of chemical pesticides is not sustainable in long run. Moreover, synthetic chemical pesticides are not good for human health and the environment. Therefore, research is being conducted to identify and develop alternatives to synthetic chemical pesticides that are more sustainable and safer for the environment. Entomopathogens like viruses, bacteria, nematodes, fungi, etc., are reported as the best alternatives. This study was conducted to evaluate the efficacy of entomopathogenic fungus and nematodes indigenous to Thailand for controlling of Fall armyworm under laboratory and greenhouse conditions. Two isolates of entomopathogenic fungus *Beauveria bassiana* (TBRC 2781 and TBRC4755) and each isolate of indigenous

entomopathogenic nematode *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3, were selected for this study. Two different larval stages of Fall armyworm were selected for the test, the second instar representing the younger stage and the fifth instar representing an older stage of the pest. The susceptibility of these larval stages was compared, and the most susceptible stage was selected for the greenhouse experiments.

The result from our study showed that all isolates of entomopathogens used were effective in both tested conditions. In the laboratory, the isolates of entomopathogenic fungus *Beauveria bassiana* TBRC 2781 and TBRC 4755 caused mortality of 73% and 64% respectively and similarly, the highest mortality of fifth instar larvae 35% and 25% were obtained with TBRC 2781 and TBRC 4755. In the greenhouse, these isolates caused mortality of 35% and 33%. The entomopathogenic nematodes *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3 caused mortality of 83% and 68% of second instar larvae and 45% and 33% of fifth instar larvae. In the greenhouse, the highest tested dosage gave mortality of 58% and 45% for *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3, respectively. From the result, it is confirmed that mortality was positively correlated to the dosage and younger larvae of Fall armyworm were more susceptible to these isolates.

The results from this study confirm that all these isolates have the potential to be used as a biocontrol agent to control Fall armyworm. However, the host stage, dosage, and environmental conditions need to be considered for effective results. Our results are based on the experiments conducted in the laboratory and the greenhouse conditions and it is not known how these isolates would perform in the field conditions. Therefore, future work would focus on evaluating the efficacy of these isolates in the field and develop a commercial form of these isolates, and test their efficacy. Furthermore, the effectiveness of these isolates can be increased by integrating them with other biocontrol agents and this can be explored in future works.

ACKNOWLEDGEMENTS

Firstly, I would like to extend my profound gratitude to his Majesty the King of Bhutan and Naresuan University, Thailand for giving me the scholarship to pursue my postgraduate study in Master of Science in Agriculture. I would also like to extend my gratitude to the Royal University of Bhutan and College of Natural resources for granting two years of study leave to undergo the master's program.

Secondly, I would like to extend my profound gratitude to my advisor Assoc. Professor Dr. Det Wattanachaiyingcharoen and co-advisors Assoc. Professor Dr. Wandee Wattanachaiyingcharoen and Assoc. Professor Dr. Apichat Vitta. This thesis would not have come into this shape without the continuous support, inspiring guidance, encouragement, and valuable suggestions from them.

Thirdly, I would like to thank the Center of Excellence on Biodiversity (BDV-PG4-161011) for financially supporting this study. I acknowledge all the members in the entomology laboratory at the Biology Department, Faculty of Science, and also staff at Parasitology and Microbiology Department, Faculty of Medical Science, Naresuan University, for providing all resources and support for conducting this research.

Lastly, I would like to thank my parents and my friends for always encouraging me throughout my entire study period.

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CHAPTER I
INTRODUCTION

1.1. Background of the study

Maize (*Zea mays* L.) is the third most important crop after wheat and rice in the world and it is mostly used as food for man and feed for an animal (Ali & Anjum, 2017; Huma, Hussain, Ning, & Yuesuo, 2019). It is grown throughout the world and has remained a source of staple food and food security in many parts of the world in Africa and America (Mulungu & Ng'ombe, 2019; Ranum, Peña-Rosas, & Garcia-Casal, 2014). Maize is rich in starch, protein, fat, dietary fiber, and Vitamins (Ali & Anjum, 2017). The kernels are used for making many different products like starch, flour, oil, animal feed, biofuel, etc., which may differ from place to place in the world (Ranum et al., 2014).

Maize is grown throughout the world and most of the maize growing area falls in developing countries. In these areas, maize production is challenged by many factors like poor soil fertility, frequent occurrence of droughts, high incidence of insect pests, diseases, and weeds, farmers' limited access to fertilizer, and lack of access to improved maize seed (Shiferaw, Prasanna, Hellin, & Bänziger, 2011). One important factor according to Ali et al, 2007 as cited in (Sori & Ayana, 2012) is insect pests. It is estimated that on average annual yield loss of 18, 80, and 44-55.9% were reported due to stem borers, grain weevils, and ear rots in many maize-producing regions (Shiferaw et al., 2011). Some common insect pest that attacks maize is maize weevil, *Sitophyllus zeamais* and maize grain moth, *Sitotroga cerealla* which are common storage pests. Corn earworm *Helicoverpa zea* Cutworms (Black cutworm, Variegated cutworm) *Agrotis ipsilon Peridroma saucia* Fall armyworm *Spodoptera frugiperda* Corn leaf aphid *Rhopalosiphum maidis* (C. James, 2003) are common in standing crop.

The Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is indigenous to tropical and subtropical regions of the Americas (Day et al., 2017). This insect is a polyphagous pest reported to affect more than 353 plant

species belonging to 76 plant families (Montezano et al., 2018). They are reported to be an economically important pest in maize and other crops belonging to the Gramineae family (Jeger et al., 2017; Silva et al., 2017). Damages to the crops occur when the pest is in its larval stage. There are six larval instars, younger larvae feed on the leaves tissue while older larvae cause extensive defoliation, it also burrows into the growing point (bud, whorl, etc.), resulting in "dead heart", wilting, and death of the unfurled leaves (Day et al., 2017; Marenco, Foster, & Sanchez, 1992).

The Fall armyworm was never reported outside the Americas until 2016 when it was reported for the first time in Africa (Goergen, Kumar, Sankung, Togola, & Tamò, 2016), and since its invasion, the pest is causing huge economic losses in Africa (De Groote et al., 2020). In December 2018, this pest was reported for the first time in Thailand from a few sub-districts of Kanchanaburi and Tak Provinces, along the border of Myanmar (IPPC, 2018). Maize is an important crop in Thailand as it forms an important part of the food and feed system and contributes significantly to income generation for rural households (Ekasingh, Gypmantasiri, Thong Ngam, & Krudloyma, 2004), and the invasions of this pest was a threat to many maize growers in Thailand. Currently, synthetic chemical pesticides are generally used to control and minimize the distribution of the Fall armyworm in the maize fields. The use of synthetic chemical pesticides may initially decrease the attack of insect pests, but it may not be sustainable in the long run. There are many reports on the development of insecticides resistant populations in Fall armyworm (Gutiérrez-Moreno et al., 2019; Yu, Nguyen, & Abo-Elghar, 2003). Moreover, synthetic chemical pesticides are not good for human health and the environment (Carvalho, 2017). This pest has also shown resistance against some maize strain that is genetically modified with Cry protein from bacteria *Bacillus thuringiensis* (Huang et al., 2014; Storer et al., 2010; Storer, Kubiszak, King, Thompson, & Santos, 2012). Research is still being conducted to develop alternatives that are more sustainable, environmentally friendly, and good for human health.

The entomopathogens like viruses, bacteria, nematodes, fungi, etc., are important alternatives for managing various arthropod species, they are sustainable and good for the environment (Charnley & Collins, 2007). Entomopathogenic fungi are a group of fungus that cause epizootic disease in the insect population (Ghulam,

2020). The most common entomopathogenic fungus belongs to the class Hyphomycetes and includes genus like *Verticillium, Metarhizium, Aschersonia, Beauveria, Paecilomyces, Nomuraea,* and *Hirsutella. Beauveria bassiana* (Balsamo) Vuillemin, is the most common entomopathogenic fungus that has a broad host range of approximately 700 insect species (Amutha, Banu, Surulivelu, & Gopalakrishnan, 2010). They are a potential biocontrol agent and are being produced commercially worldwide for the control of many insect pests (Pell, Eilenberg, Hajek, & Steinkraus, 2001). Similarly, entomopathogenic nematodes (EPNs) are nematodes that can infect and kill insects (Kaya & Gaugler, 1993). There are about 23 nematode families studied for biocontrol, but the most common are Steinernematidae and Heterorhabditidae (Koppenhöfer, 2007, as cited in (Lacey & Georgis, 2012) *Steinernema* and *Heterorhabditis* are the two most important genus each belonging to the family. All species of *Steinernema* are associated with symbiotic bacteria of the genus *Xenorhabdus* and all *Heterorhabditis* nematode species are associated with *Photorhabdus* bacteria (Boemare, Akhurst, & Mourant, 1993) and this unique combination helps in killing the host. Entomopathogenic nematodes are widely distributed and are recovered from soils throughout the world (Hominick, Reid, Bohan, & Briscoe, 1996). They are a promising alternative for pest control because of the wide host range (Arthurs, Heinz, & Prasifka, 2004). Many reports are stating the occurrence of entomopathogenic fungi (Mar, Suwannarach, & Lumyong, 2012; Vilarinho, Fernandes, Hunt, & Caixeta, 2011) and nematodes (Stock, 1998; Tangchitsomkid & Sontirat, 1998; Vitta et al., 2015) in Thailand. The effectiveness of entomopathogens differs from genus to genus and species to species, strain to strains (Molina Ochoa et al., 1996; Tavassoli, Ownag, Pourseyed, & Mardani, 2008). However, limited studies are focusing on the evaluation of these indigenous entomopathogens against the Fall armyworm. Therefore, there is need to investigate how these entomopathogens indigenous to Thailand would respond to new insect pest the Fall armyworm.

1.2. Problem Statement

Fall armyworm have been reported susceptible to species of entomopathogenic fungi and nematodes in the Americas and newly invaded areas like Africa. There are many species of entomopathogenic fungi and nematodes indigenous to Thailand. However, there are limited study focusing on evaluation of these indigenous entomopathogens against Fall armyworm in Thailand. Therefore, the purpose of this study was to evaluate the efficacy of some isolates of entomopathogenic fungus and nematodes that are indigenous to Thailand against Fall armyworm larvae.

1.3. Scope of the study

In this study, the efficacy of two isolates of the entomopathogenic fungus *Beauveria bassiana* from Thailand Bioresource Research Centre (TBRC 2781 and TBRC 4755) and two isolates of the indigenous entomopathogenic nematode *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3 initially collected from Agricultural Land in Phitsanulok and Uttaradit Provinces of Thailand were tested against second and fifth instar of FAW under laboratory and greenhouse conditions.

1.4. Objectives

- To evaluate the efficacy of *Beauveria bassiana* isolates (TBRC 2781 and TBRC 4755) and indigenous entomopathogenic nematodes *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3 against second and fifth instar larvae of Fall armyworm.
- To compare the degree of leaf damage of maize when applied with both entomopathogenic fungus and nematodes.

CHAPTER II
LITERATURE REVIEW

2.1. Taxonomy and classification of fall armyworm

Fall armyworm (FAW) *Spodoptera frugiperda* belonging to the order Lepidoptera which consists of moth and butterfly. Many species in this order are economically important pests feeding on plants, stored grains, or fabrics (Simmons & Wiseman, 1993). Lepidopterans undergo complete metamorphosis passing through the egg, larva, pupa, and adult stages. The genus *Spodoptera* belongs to the family Noctuidae where the moths are nocturnal. Noctuidae larvae are smooth and dull-colored having 5 pairs of prolegs, most of them feed on the foliage of the plant and few on fruits (Simmons & Wiseman, 1993). The genus *Spodoptera* consists of many species that are important crop pests including *S. littoralis* (Boisduval) (Egyptian cotton leafworm), *S. exempta* (Walker) (African armyworm), *S. litura* (Fabricius) (tobacco caterpillar), *S. exigua* (Hübner) (beet armyworm), *S. ornithogalli* (Guenée) (yellow striped armyworm), and *S. frugiperda* (J.E. Smith) (fall armyworm) (Capinera, 2002).

2.2. The Life cycle of fall armyworm

The life cycle of the Fall armyworm depends on the season. It completes its life cycle in about 30 days during the summer, 60 days in the spring and autumn, and 80 to 90 days during the winter (Capinera, 2002). Adult moths vary in color, male have a shaded grey and brown forewing with triangular white spots at the tip and near the center of the wing. While forewings of females are less distinctly marked, ranging from a uniform greyish brown to a fine mottling of grey and brown. The hind wing of both sexes is shining silver-white with a narrow dark border. They are also varied in size with their wingspan can reach 32 to 40 mm Adults of Fall armyworm are nocturnal and are most active during the nights (CABI, 2017). The duration of adult life is estimated to average about 10 days, with a range of about 7 to 21 days (Capinera, 2002).

Figure 1 Adult Male of Fall armyworm

Source: Biology of invasive Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize.

Figure 2 Adult female of Fall armyworm

Source: Biology of invasive Fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) on maize.

The female normally deposits most of her eggs during the first four to five days of life, but some oviposition occurs for up to three weeks. The egg of the Fall armyworm is dome-shaped with a flattened base that measures about 0.4 mm in diameter and 0.3 mm in height. Eggs are laid in mass and the number of eggs per mass can vary from 100 to 200. A single adult female can lay on average 1500 to 2000 during its lifetime (Visser, 2017).

Figure 3 Egg of Fall armyworm(0.4mm diameter and 0.3mm height)

There are six instars in Fall armyworm larvae, and they are normally distinguished from one another by the size of head capsule, and the length. Larvae attain lengths of about 1.5 to 40mm, during these instars. Young larvae are greenish with black head, the head turns orangish in the second instar. In the third instar, the dorsal surface of the body becomes brownish, and lateral white lines begin to form. In the fourth to the sixth instars, the head is reddish-brown, mottled with white, and the brownish body bears white subdorsal and lateral lines. Elevated spots occur dorsally on the body; they are usually dark in color, and bear spines (CABI, 2021).

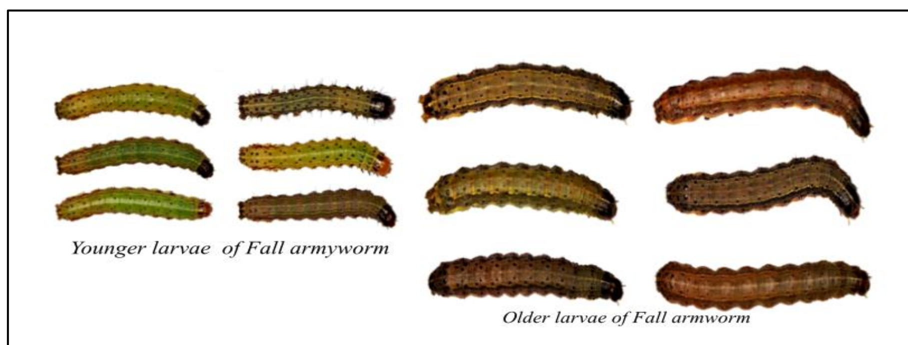

Figure 4 Larvae of Fall armyworm(1.5 to 40mm)

Source: D Visser ARC-VOP Roodeplaat

The face of the mature larva is also marked with a white inverted "Y" and the epidermis of the larva is rough or granular in texture when examined closely (Capinera, 2002). The four black dots at the last abdominal segment are also distinctive to Fall armyworm larvae (CABI, 2021). The duration of the larval stage tends to be about 14 days during the summer and 30 days during cool weather (Capinera, 2002). Pupation normally takes place in the soil, at a depth of 2 to 8 cm. The larva constructs a loose oval cocoon, and 20 to 30 mm in length, and binding together particles of soil with silk. If the soil is too hard, larvae may web together leaf debris and other material to form a cocoon on the soil surface. The duration of the pupal stage is about eight to nine days during the summer but reaches 20 to 30 days during the winter in Florida (Capinera, 2002).

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Figure 5 A: Matured larvae of Fall armyworm, B: The four dots at the last abdominal segment, B: White inverted 'Y' on the forehead

2.3. Origin and distribution of the Fall armyworm

Fall armyworm is native to the tropical and subtropical regions of the Americas. It is the most common lepidopteran pest in the United States and especially is an important pest of maize. The outbreak of this pest occurs sporadically in North and South America and causes severe damage. This pest was never reported in the region outside the Americas until 2016 when this pest was reported for the first time from the African continent. It has been confirmed in Nigeria, Ghana, Zimbabwe, and some cases have been recorded in Malawi, Mozambique, Namibia, and South Africa. In Ethiopia, the Fall armyworm was reported for the first time in Bench Maji zones in January 2017 and by August, it was reported from 28 countries in Africa (De Groote et al., 2020; Goergen et al., 2016). In the Asian continent, this pest was first reported in India in 2018 (Chormule, Shejawal, Sharanabasappa, Asokan, & Swamy, 2019), and by January 2018 it was reported from countries like Thailand, Sri Lanka, Myanmar, Yamen, and China.

2.4. Management of the Fall armyworm

Maize is grown extensively in American continents. Fall armyworm is a major pest of maize and control of this pest relied on the intensive use of chemical insecticides. Fall armyworm larvae have shown a high level of resistance to many taxological groups of insecticides (Yu et al., 2003) reported Fall armyworm as the top 15th most resistant insect pest species in the world. Chemical insecticides are also associated with problems like unavailability and high costs (Kumela et al., 2019) as well as other adverse effects to humans and the environment (Lewis, Silburn, Kookana, & Shaw, 2016). They are also harmful to beneficial biocontrol agents (Bateman et al., 2018). In addition, genetically engineered maize hybrids containing *Bacillus thuringiensis* (*Bt*) are used to battle Fall armyworm problem but Fall armyworm has been reported to show a high level of resistance to the insecticidal proteins expressed by genetically engineered crops. Storer et al. (2012) reported that Fall armyworm showed resistance to *Cry1F* and *Cry1Ac* protein in Puerto Rico, *Cry1F* in the Southern USA (Huang et al., 2014), and *Cry1F*, *Cry1Ab* and reduced susceptibility to *Cry1Ac* in Brazil (Omoto et al., 2016).

2.5. Biological Control

The development of resistance to synthetic insecticides was one of the driving forces that changed the foresight of insect pest management (Mahmoud, 2016). One of the new approaches was using biological control i.e., biological agents (predators, parasites, and pathogens) for managing insect pests. Eilenberg, Hajek, and Lomer (2001) defined biological control or biocontrol as "The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be.". Biological control is considered as one of the most important alternative control measures and a powerful tool for environmentally safe and sustainable plant protection (Lacey et al., 2015) The success of biological control depends on how wisely biocontrol agents are applied and how these agents could adapt in the agricultural ecosystem. Microbial pathogens and arthropod biocontrol agents are safe for non-target vertebrates, environment, and inexpensive than synthetic pesticides (Mahmoud, 2016).

2.5.1. Entomopathogenic Fungi

Fungi are a group of microorganisms that is phylogenetically diverse, heterotrophic, eukaryotic, unicellular, or multicellular (filaments) in nature (Strasser, 2001). They have chitinized cells and reproduce via sexual or asexual spores or both. Entomopathogenic fungi are fungal species that are pathogenic to insects. Many of the genera of entomopathogenic fungi belong to the class Entomophthorales in the Zygomycota or the class Hyphomycetes in the Deuteromycota. *Beauveria* is one of the most common and most important entomopathogenic fungal genus belonging to the class Hyphomycetes in Deuteromycota (Samson, Evans, & Latge, 2013). There are 6 important species of this genus: *B. bassiana, B. clade, B. brongniartii, B. caledonica, B. vermiconia,* and *B. amorpha* (Rehner et al., 2011). *B. bassiana* is a well-known entomopathogenic fungus in the biological control of various insect pest species.

2.5.1.1. Beauveria bassiana

Beauveria bassiana was discovered after white muscardine disease that affected silkworm industry in Italy. The disease was called after the French word for bonbons, as the disease resulted in fluffy white corpses resembling pastries. The disease was discovered by an Italian scientist Agostino Bassi (1836) and later the microbe responsible for the disease was named *Beauveria bassiana* in honor of Bassi's discovery (Lord, 2005). *Beauveria bassiana* is a cosmopolitan, haploid, soil-borne entomopathogenic fungus (Rehner & Buckley, 2005). It is a filamentous fungus with typically white mycelium. The primary means of reproduction in the Deuteromycetes is the conidium, a non-motile, usually deciduous spore. *Beauveria bassiana* can produce three spore types: aerial conidia, submerged conidia, and blastospores (Hegedus, Bidochka, Miranpuri, & Khachatourians, 1992). The conidia are grown on free hyphae or aggregates of hyphae (synnemata), submersed conidia are formed in liquid culture and blastospores arise from the hypha once penetration of the insect cuticle has occurred. They are capable of reproductive budding. These structures are the obligatory parasitic phase of the fungus (Boucias, Pendland, & Latge, 1988).

2.5.1.2. Mode of Infection of Beauveria bassiana

Most entomopathogenic fungi have a similar mode of infection and many (Rehner & Buckley, 2005) infect their hosts through the external cuticle, although a few taxa (e.g., Culicinomyces) can invade through the alimentary canal (Inglis, Goettel, Butt, & Strasser, 2001). The infection pathway consists of the following steps (Fig 6): (1) dispersion of inoculum form sporulated regions of the insect, (2) attachment of the spore to the insect cuticle, (3) spore germination on cuticle, (4) penetration through the cuticle, (5) overcoming the host immune response and proliferation within the host, (6) saprophytic outgrowth from the dead host and production of new conidia (de Faria & Wraight, 2007).

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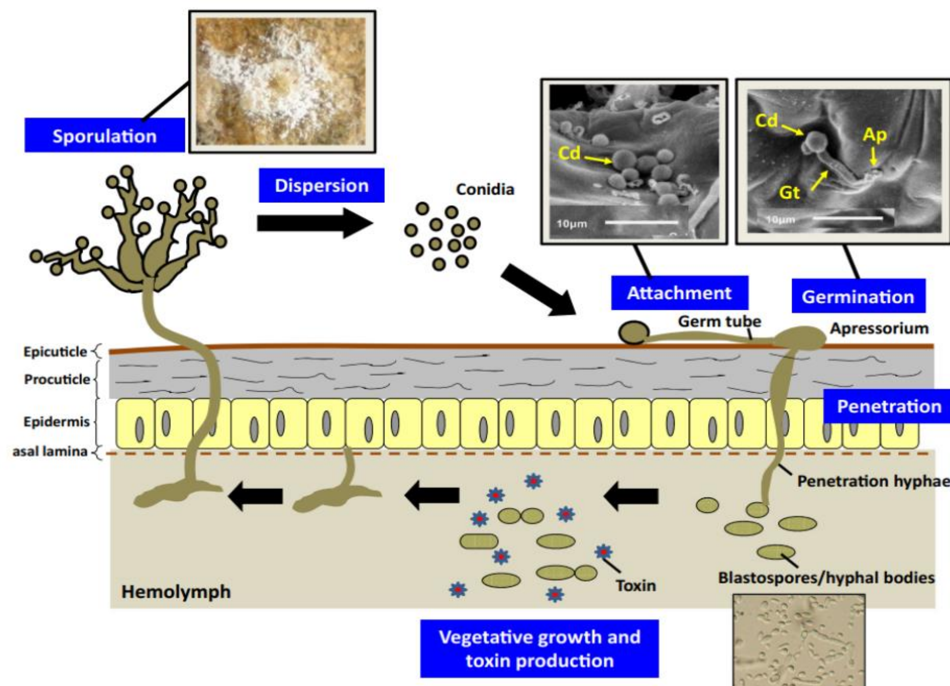

Figure 6 Summary of the events involved in the infection cycle of an entomopathogenic fungus attacking an insect.

Source: *By (Mascarin & Jaronski, 2016).* The production and uses of *Beauveria bassiana* as a microbial insecticide

Primary infection occurs through spores (conidia) that may be present in the infected cadaver, soil, or plants. When conidia encounter the host, they get attached to the cuticle. The attachment of conidia to the host cuticle is brought about by hydrophobic mechanisms. Under favorable conditions, conidia will germinate and penetrate through the host cuticle. During this process, the fungus produces enzymes that enable the growing hyphae to penetrate the host integument (Ortiz-Urquiza & Keyhani, 2013). Once the conidia have germinated on the cuticle of the host insect, the penetration of the cuticle, is brought about by a combination of enzymatic and mechanical mechanisms. Non-sclerotized areas of the insect cuticle like spiracles, mouthparts, and between segments are weaker parts for the germinated spore to penetrate (Ortiz-Urquiza & Keyhani, 2013). During the penetration process enzymes such as chitinases, proteases, endoproteases, esterases, lipases, and chitobiases are

produced which aid in the penetration process (Boucias et al., 1988; Holder & Keyhani, 2005). After the successful penetration, the fungus invades other tissues such as muscle tissues, fatty bodies, Malpighian tubes, and hemocytes of the host insect by extensive vegetative growth and the production of toxic secondary metabolites such as (Beauvericin, Beauverolides, bassianolide, bassianin, tenellin, and cyclosporin A) leading to the death of the insect 3 to 14 days after infection (Hajek & Leger, 1994).

2.5.1.3. Use of Beauveria bassiana for controlling of insect pests

Beauveria bassiana is widely distributed species of the genus. They are reported from infected insect from places throughout the world (Zimmermann, 2007). The occurrence and distribution of this species makes them potential biocontrol agents. They are widely used for the management of numbers of insect pest. It is reported that they have more than 700 species of hosts belonging to 15 orders, including various species of Acari (Zimmermann, 2007). In addition, they are an environmentally friendly alternative to chemical insecticides (Faria & Wraight, 2001) and widely preferred biocontrol agents because their insecticidal activity is faster than other entomopathogenic agents, their conidia can survive for longer duration in the environment, it addition it also has been reported to show endophytic association with plants (Mantzoukas et al., 2021). *B. bassiana* is available commercially as mycoinsecticide to control several insect pests, providing a biological alternative to synthetic chemical insecticides (Pell et al., 2001).

2.5.2. Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) are those nematodes that have facultative or obligate parasitic associations with insects (Vashisth, Chandel, & Sharma, 2013; Zimmermann, 2007). There are about 23 nematode families studied for biocontrol, but the most common are Steinernematidae and Heterorhabditidae (Hazir, Kaya, Stock, & Keskin, 2004). *Steinernema* and *Heterorhabditis* are the two most important genera, belonging to the families Steinernematidae and Heterorhabditidae, respectively. All species of *Steinernema* are associated with symbiotic bacteria of the genus *Xenorhabdus* and all *Heterorhabditis* nematode species are associated with *Photorhabdus* bacteria and this unique combination helps in killing the host (Boemare et al., 1993). These symbiotic bacteria live in the intestine of the nematodes and once the nematodes reach the hemocoel of the host, the symbiotic bacteria are released into the host body. These bacteria serve mainly two functions as a food source for nematode and indirectly supply nutrients through degradation of the insect carcass (Mohan, 2015; Stock & Blair, 2008).

2.5.2.1. Life cycle of entomopathogenic nematodes

The life cycle of EPNs includes the egg stage, four juvenile stages, and adult stage. Generally, these different stages of EPNs are divided into two phases (1) free-living within the soil and (2) saprophytic worms within the host. The free-living stage of any species of EPNs is the third stage (J3), also called an infective juvenile stage (IJ) (Kaya & Gaugler, 1993). At this stage, they are morphologically and physiologically adapted to remain in the environment for a prolonged period. They are non-feeding and soil-dwelling larva, encased in a double cuticle with a closed mouth and anus, and able to survive for long-terms in the soil (Smart Jr, 1995). This stage is responsible for finding and penetrating a suitable insect host. Once the IJ stage locates and finds a suitable host, they penetrate to the hemocoel of the insect host. The symbiotic bacteria are released, and they quickly multiply and help in killing the insect and degradation of the insect tissues. Then, IJs feed on those degraded host tissues. When food is abundant nematodes, and their symbiotic bacteria continue to multiply, and when food gets limited the symbiotic bacteria reunite with

nematodes and become non-feeding IJ (Stock & Blair, 2008).

Infective juveniles developing into adults are different in the two families of EPNs. Steinernematids are amphimictic (fertile offspring are produced after interbreeding of male and female) so infective juveniles may develop into either males or females. The reproduction is only possible if the host is invaded by both sexes (Koppenhöfer & Gaugler, 2009) Heterorhabditids, on the other hand, are hermaphrodite and the infective juveniles mature into self-fertilizing hermaphrodites with ovotestes, permitting host colonization when even a single individual finds and infects an insect. Subsequent heterorhabditid generations are a mix of amphimictic and hermaphroditic stages. Mating behaviors differ sharply in the two genera, with *Steinernema* males coiling around the female at the vulva, whereas *Heterorhabditis* males align parallel to the female or hermaphrodite (Koppenhöfer & Gaugler, 2009; Poinar, 1990).

Both genera are oviparous, initially with eggs laid within the hemocoel hatching in 2 days. If food is abundant, the developing juvenile nematodes proceed through 4 molts to become adults. Several generations may be produced in a single host until the cadaver is completely colonized. When nematode density is high and nutrients become limited within the insect cadaver, nematodes convert into non-feeding IJs. Here second-stage juveniles do not develop into normal third-stage juveniles but take an alternative developmental pathway to become third-infective juveniles encased within the cuticle of the second-stage juvenile. IJs are formed during 'endotokia matricida' in which development results in the death and consumption of the parent hermaphrodite or female by the developing infectives (Johnigk & Ehlers, 1999; Wang & Bedding, 1996). Thus, the parent provides a nutrient supply critical for juvenile development when insect nutrients are depleted. When insect resources have been exhausted, infective juveniles armed with a fresh supply of bacteria emerge from the empty shell of the insect. Steinernematids infective juveniles emerge mainly from natural openings of the maternal cadavers, while heterorhabditids emerge mostly trans-cuticular from the parent (Desta, 2016).

2.5.2.2. Mode of infection

EPNs locate their host in response to carbon dioxide, vibration, and other chemical cues (Kaya & Gaugler, 1993). Once they locate their host, they may enter through natural body openings like the anus, mouth, and spiracles and much less commonly through wounds that permit direct access to the host's hemocoel. Heterorhabditids may use their terminal tooth to cut and penetrate the softer intersegmental area of the cuticle (Bedding & Molyneux, 1982). The nematodes infect the host hemocoel and the bacterial symbiont is released. The bacteria quickly replicate in the nutrient-rich insect blood and the connective tissues surrounding the insect midgut (Silva et al., 2017). The symbiotic bacteria overcome the immune system of the host and release endotoxins and exotoxins. Septicemia develops, with the death of the host insect usually occurring within one to two days (Boemare et al., 1993).

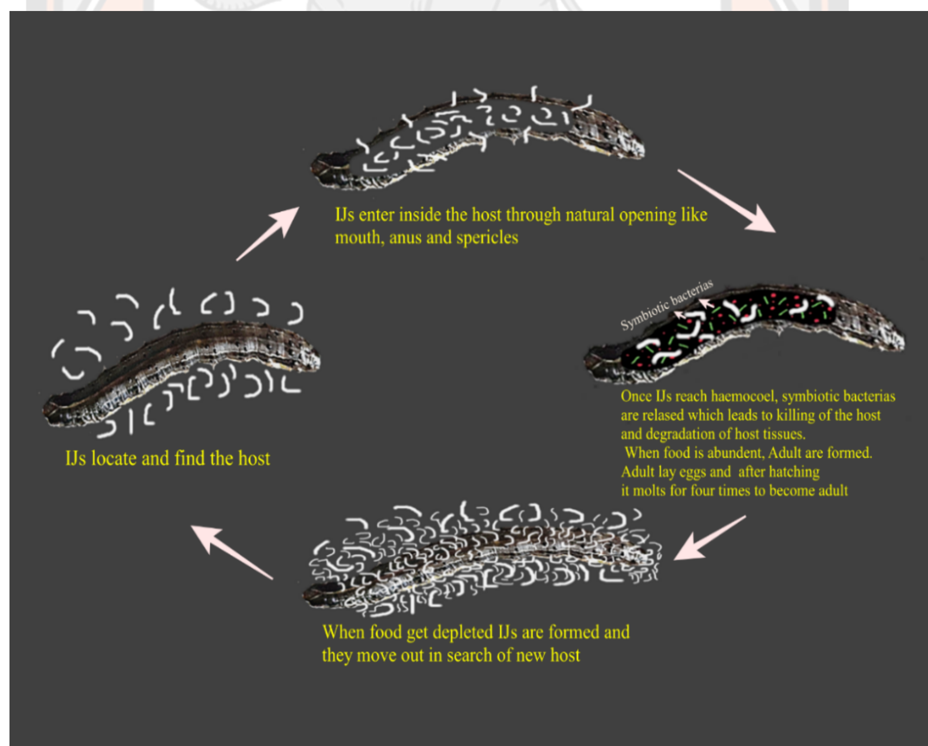

Figure 7 Generalized life cycle of entomopathogenic nematodes

2.5.2.3. Symptom of infection by entomopathogenic nematodes

Symptoms of the infection by entomopathogenic nematodes are highly characteristics that are attributed to the pigments produced by symbiotic bacteria. The insect infected by most Steinernematid-Xenorhabdus become beige, brown, tan, or greyish whereas insects killed by Heterorhabditids-Photorhabdus become brown, or greenish and the host tissue become ropey and highly viscous. Moreover, Photorhabdus produces an enzyme that causes the insect cadaver to glow with a faint but visible green luminescence (Koppenhöfer & Gaugler, 2009).

Figure 8 Characteristics symptom shown by greater wax moth larvae when infected by indigenous entomopathogenic nematode used in the study

2.5.2.4. Entomopathogenic nematodes as a biocontrol agent

Entomopathogenic nematodes in two families (Heterorhabditidae and Steinernematidae) have been effectively used as biological insecticides in pest management programs (Lacey & Georgis, 2012). The entomopathogenic nematodes are very unique because (1) they are the only nematodes that have evolved the ability to carry and introduce symbiotic bacteria into the body cavity of the insect, (2) they are only insect pathogens with a host range that includes the majority of the insect order and families, and (3) they can be culture on large scale on, or in artificial solids or liquid media (Poinar, 1990). Other attributes that make these EPNs suitable for biological control agents like broad host range, safe to vertebrates, plants, and other non-target organisms, have no known negative effect on the environment. They are capable of mass production *in vivo* and *in vitro*, can be applied easily using standard spray equipment, potentially multiply in the environment, are amenable to genetic selection for desirable traits (Hazir, Keskin, Stock, Kaya, & Özcan, 2003). Entomopathogenic nematodes fit nicely into integrated pest management or IPM programs because they are considered non-toxic to humans, relatively specific to their target pest, and can be applied with standard pesticide equipment (Shapiro-Ilan, Han, & Dolinksi, 2012).

CHAPTER III
RESEARCH METHODOLOGY

3.1. Collection and rearing of the Fall armyworm

Fall armyworms were collected from maize filed in Phitsanulok, Sukhothai, and Uttaradit provinces of Thailand. Identification and confirmation of the larvae were carried out as per identification guidelines provided by (Visser, 2017). They were mass multiplied at the Entomology Laboratory belonging to the Department of Biology, Faculty of Science, Naresuan University. Larvae were put in 20ml plastic containers and fed with the leaves of sweet corn variety ‘Super sweet corn” which was grown without any chemical pesticides. The pupae formed were collected and put in a plastic container and placed inside a rearing cage (30cm length, 30cm height, and 30cm width). Adults formed were fed with a 10% sugar solution from a ball of cotton wool soaked in the solution placed at the bottom corner of the rearing cage. The young plant dipped in a glass of water was adjusted and placed inside the chamber for oviposition. The larvae formed were used for the experiments.

Figure 9 A: Plastic container containing maize leaves and FAW larvae. B: Insect cage used for rearing adults.

3.2. **Culture of entomopathogenic fungus (*Beauveria bassiana*)**

The entomopathogenic fungus *Beauveria bassiana* used in the study was obtained from the Thailand Bioresource Research Center (TBRC) in Thailand. The pure fungus culture of two isolates of *Beauveria bassiana* TBRC 2781 and TBRC 4755 was employed in this study. They were further multiplied in the Department of Biology, Faculty of Science, Naresuan University. The isolates were cultured and maintained on Potato dextrose agar (PDA) in a petri dish (9cm diameter) and incubated at the temperature of 25°C under a14:10 hours (light: dark).

3.3. Preparation of the spore suspension

Spores were harvested from three weeks old culture with a sterile spatula in a Laminar flow chamber. Spores were scraped carefully from Petri dishes and were suspended in sterilised distilled water and suspension was vortexed to get a homogenous state. The suspension was strained through a double-layered muslin cloth to separate spores from other fungal parts. The final volume of spore suspension was made to 500ml (stock). 1ml of fungal suspension was taken from the stock and diluted with sterilised distilled water in 1:10 dilution, afterward it was used for the determination of the spore concentration. Spore concentration was determined by using Haemocytometer (Neubauer chamber) under a 40x magnification in a compound microscope. From stock, five different fungal suspensions with spore density $1x10^5$, $1x10^6$, $1x10^7$, and $1x10^8$ and $1x10^9$ spores per milliliter of sterilised distilled water were prepared. The spore suspension was sealed with Parafilm and kept at 4°C in a refrigerator until use.

3.4. Entomopathogenic nematodes

Two indigenous isolates of entomopathogenic nematodes (EPNs) *Heterorhabditis indica* isolate AUT 13.2, and *Steinernema siamkayai* isolate APL 12.3 were used in this research. Both isolates were collected from the Uttaradit and Phitsanulok provinces of Thailand. The EPNs were multiplied in the laboratory using the Greater wax moth *Galleria mellonella* larvae as the host.

3.5. Rearing of Greater wax moth (*Galleria mellonella*)

The adults of Greater wax moth were put in a plastic box with folded papers identical to the laying cage. Adults were fed with a 10% sugar solution from a ball of cotton wool soaked in the solution placed at the bottom corner of the rearing box. Eggs that were laid in folded papers were collected with the help of a paintbrush and were placed in the plastic box with an artificial diet. The artificial diet was prepared by mixing 200g cornflour + 100ml honey + 100ml glycerol + 50g yeast. Larvae emerging from eggs fed on the artificial diet and for the multiplication of EPNs, the last instar larvae were used.

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Figure 10 Last instar larvae of the Greater wax moth

3.6. Collection of infective juveniles of the EPNs from the White trap

Healthy last instars larvae of Greater wax moth were used for the multiplication of the nematodes. Five larvae were put in a Petri dish containing filter paper inoculated with EPNs. The Petri dishes were incubated at 25±2°C. Larvae killed by EPNs developed characteristic symptoms as shown in figure 2 after 48 to 72 hours of infection. When such larvae were observed in Petri dishes they were collected and kept for a White trap technique. The White trap was observed for the emergence of infective juveniles (IJs) of the EPNs. The IJs emerging from the cadavers were separated from other debris by the decantation process and were kept in a sterilised plastic centrifuge tube and stored in the refrigerator at a temperature of 18±2°C.

Figure 11 Greater wax moth larvae infected with nematode placed for White trap technique

3.7. Preparation of the EPNs suspension

The infective juveniles preserved in a sterilised plastic centrifuge tube were counted under a stereomicroscope with help of a micropipette. Six different nematode suspension i.e., 50, 100, 150, 200, 250 and 300 IJs per milliliter (ml) were prepared for both isolates. These suspensions were used against Fall armyworm larvae to determine the efficacy.

3.8. Efficacy test of entomopathogenic fungi and entomopathogenic nematodes against Fall armyworms in the laboratory conditions.

3.8.1. The efficacy of entomopathogenic fungus under laboratory conditions

3.8.1.1. Experimental design

The efficacy of *Beauveria bassiana* isolates TBRC 2781 and TBRC 4755 against Fall armyworm larvae was conducted through larval bioassay of second and fifth instar larvae. A completely randomized design (CRD) was used for the laboratory experiment. For each isolate, six treatments were replicated four times. The treatments included five different fungal suspensions each varying in spore densities per milliliter (ml) of sterilised distilled water i.e., $1x10^5$, $1x10^6$, $1x10^7$, and $1x10^8$, $1x10^9$ and the same volume of sterilised distilled water without fungal spore as control.

3.8.1.2. Application of fungal spores on Fall armyworm larvae

When Fall armyworm larvae reached the second and fifth instar stages, they were removed from the rearing container and each larva was placed into a Petri dish (5.5cm diameter) with detached maize leaf as food. With the help of a micropipette, 1ml fungal suspension was applied topically covering the larvae and the leaf. Treated larvae were incubated at 25°C under a 14:10 (light: dark) photoperiod with 60±5% relative humidity and the food was changed every 24 hours. 40 larvae (10 larvae/replication) each from the second instar and fifth instar were treated with each concentration mentioned above. Therefore, for each fungal isolate, 400 larvae were treated (200-second instar + 200 fifth instar larvae).

Figure 12 inoculating Fall armyworm larvae with the fungal suspension of *Beauveria bassiana*

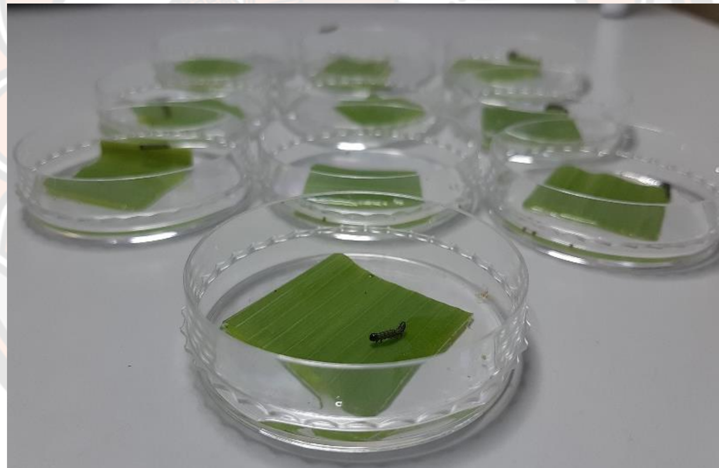

Figure 13 second instar larvae after inoculation with the fungal suspension of *Beauveria bassiana*

3.8.1.3. Assessment of mortality and data collection

The assessment for mortality was carried out 48 hours after inoculation. Larvae were recorded dead when they failed to respond to the touch by the forceps. Dead larvae were kept in a clean Petri dish containing moist filter paper to observe for mycosis. Only those larvae that showed mycosis was recorded. For each treatment, the number of dead larvae was recorded every 24 hours for 10 days. The number of dead larvae showing sporulation was recorded separately. The mortality of the test sample was calculated by summing the number of dead larvae across all exposure replicates and then expressing this as a percentage of the total number of exposed larvae:

$$Observed\ mortality = \frac{(Total\ number\ of\ dead\ larvae)}{(Total\ number\ of\ exposed\ larvae\ )}\ x\ 100$$

A similar calculation was made to obtain a value for the control mortality. If the control mortality was ≥20%, the tests were discarded. When control mortality is < 20%, then observed mortality was corrected using Abbott’s formula, as follows

$$Corrected\ Mortality = \frac{(\%\ test\ mortality - \%\ control\ mortality)}{(100 - \%\ control\ mortality)}$$

3.8.1.4. Data Analysis

The data collected were subjected to the normality of variance. The number of dead larvae in different treatment (dosage) by the isolate TBRC 4755 and TBRC 4755 was subjected to statistical analysis of variance (ANOVA) and the mean of each treatment was compared using Duncan multiple range tests (DMRT) to find a significant difference between treatments ($p \leq 0.05$) in SPSS software.

3.8.2. The efficacy of entomopathogenic nematodes under laboratory conditions

3.8.2.1. Experimental design

To determine the efficacy of EPNs on Fall armyworm larvae under laboratory conditions, two isolates of EPNs *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3 were used. Each of these isolates was used against the second and fifth instar larvae of the Fall armyworm. A completely randomized design (CRD) was used for each isolate. There were six treatments for each isolate which were replicated four times. Treatments included five different nematode suspensions each varying in the number of EPNs per milliliter of sterilised distilled water i.e., 50, 100, 150, 200, 250, and 300 IJs per milliliter of sterilised water, and the same volume of sterilised distilled water without EPNs was used as control.

3.8.2.2. Application of entomopathogenic nematode on Fall armyworm larvae

When Fall armyworm larvae reached the second instar stage or fifth instar stage, they were removed from the rearing container and each larva was placed into a Petri dish (5.5cm diameter) with detached maize leaf as food. With the help of a micropipette, a 1ml nematode suspension containing IJs was applied topically covering the larvae and the leaf. The same volume of sterilised distilled water without IJs was used as control. Treated larvae were incubated at 25±2℃ under a 14:10 (light: dark) photoperiod with 60±5% relative humidity and the food was changed every 24 hours. Ten second instar and fifth instar larvae were treated with each concentration of nematode suspension mentioned above. Each treatment was repeated four times. Therefore, for each nematode isolate, 480 larvae (240-second instar + 240 fifth instar larvae) were treated.

3.8.2.3. Assessment of mortality and data collection

The assessment for mortality was carried out 48hours after inoculation. Larvae were recorded dead when they failed to respond to the touch made by the forceps. Dead larvae were kept for the White trap technique to observe any emergence of the nematodes from the cadaver. Only those larvae that gave the emergence of nematodes were recorded as the ones killed by the nematodes. For each treatment, the number of dead larvae was recorded every 24 hours for 10 days. The number of dead larvae showing the emergence of nematodes was recorded separately. The mortality of the test sample is calculated by summing the number of dead larvae across all exposure replicates and then expressing this as a percentage of the total number of exposed larvae:

$$Observed\ mortality = \frac{Total\ number\ of\ dead\ larvae}{(Total\ sample\ size)}\ x\ 100$$

A similar calculation was made to obtain a value for the control mortality. If the control mortality is ≥20%, the tests were discarded. When control mortality is < 20%, then observed mortality was corrected using Abbott’s formula, as follows

$$Corrected\ Mortality = \frac{\%\ test\ mortality - \%\ control\ mortality}{100 - \%\ control\ mortality} * 100$$

3.8.2.4. Data Analysis

The data collected were subjected to the normality of variance. The number of dead larvae in different treatment(dosage) by the *H. indica* isolate AUT 13.2 and *S. siamkayai* APL 12.3 was subjected to statistical analysis of variance (ANOVA) and the mean of each treatment was compared using Duncan multiple range tests (DMRT) to find a significant difference between treatments(p≤0.05) in SPSS software.

3.9. Efficacy test of entomopathogenic fungi and entomopathogenic nematodes (EPNs) against Fall armyworms in the greenhouse conditions

3.9.1. The efficacy of entomopathogenic fungus under greenhouse conditions

3.9.1.1. Experimental design

A Randomized Complete Block Design (RCBD) was used for conducting greenhouse experiments. Three treatments were replicated eight times. The treatment included the best concentration that caused the highest mortality percentage in a laboratory experiment for each fungal isolate. i.e., best dosage of *B. bassiana* isolate TBRC 2781, best dosage of *B. bassiana* isolate TBRC 4755, and an equal amount of sterilised distilled water as control. The volume and spore density of the fungal suspension used in the greenhouse experiments were calculated based on the volume and spore density used in the laboratory experiments. Therefore, spore density i.e., $1x10^8$ spores ml^{-1}, was selected corresponding to the highest mortality in a laboratory experiment. When mortality obtained at $1x10^8$ spores ml^{-1} obtained was not promising the experiment was repeated by increasing dosage to $1x10^9$ and $1x10^{10}$ spores ml^{-1}.

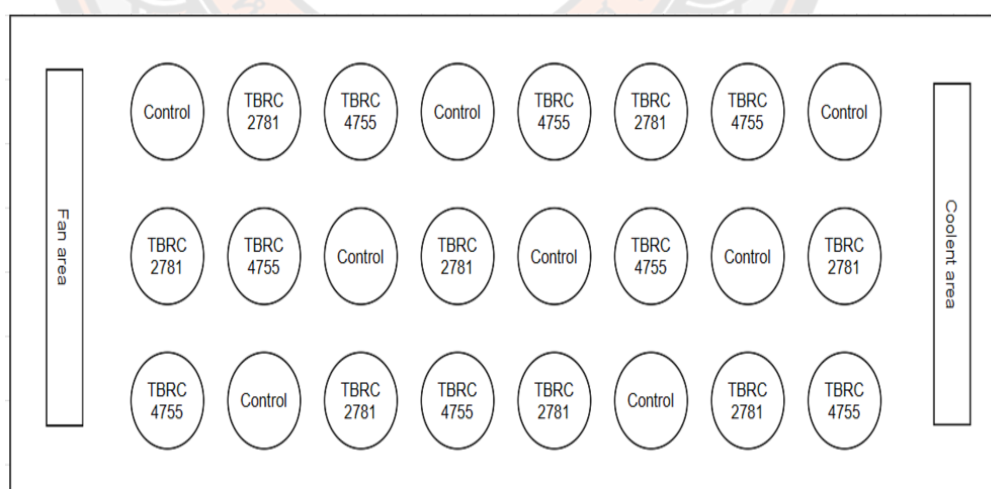

Figure 14 Experimental layout of pots in the greenhouse

3.9.1.2. Planting maize in greenhouse

Sweet corn was selected for this study because this variety of maize was reported as the most susceptible to Fall armyworm. Thai variety of sweet maize "Super sweet" were grown in a 50 cm diameter earthen pot. Initially, ten maize seeds were grown in each pot and watered daily. After germination, the seedling number was reduced to five per pot. When the seedling reached four leaves fully emerged stage (Hanway, 1966) approximately two weeks after emergence, each pot was covered vertically and from the top with insect mesh (figure 15).

Figure 15 A: Maize seedlings in an earthen pot, B: Earthen pot containing maize seedlings covered with insect mesh

3.9.1.3. Release of Fall armyworm in the pot

For the greenhouse experiment, second instar larvae of Fall armyworm were selected because of the higher susceptibility of this larval stage to the entomopathogenic fungus in the laboratory experiments. When the plant reached two-week-old (4 leaves fully emerged stage), Fall armyworm infestation was done manually by introducing larvae that were about to complete the first instar stage with some detached maize leaves in each pot. The rate of infestation was 10 larvae per pot. After infestation, the pots were caged and covered from the top with insect mesh as shown in (figure 15).

3.9.1.4. Application of fungal suspension

The fungal suspension was sprayed 24 hours after the release of the Fall armyworm larvae. 100 millilitres of fungal suspension containing 1 $x10^8$ spore per milliliter of sterilised water was applied with the help hand sprayer. The plants were sprayed three times. The first spray was done 24 hours after the release of the larvae, the second spray and third spray were done 48 and 96 hours after the first spray. 100ml sterilised distilled water without fungal spores was used as control. The mortality of the larvae, the number of leaves damaged, and the number of stems destroyed was assessed daily for a period of 10 days.

3.9.1.5. Assessment of Mortality and data collection

The pots were assessed daily for dead larvae. When dead larvae were observed, they were picked and kept for mycosis to confirm the death of larvae by the fungus. The mortality of the test sample was calculated by using the formula.

$$Observed\ mortality = \frac{Total\ number\ of\ dead\ larvae}{Total\ exposed\ larvae}\ x\ 100$$

When control mortality is < 20%, then observed mortality was corrected using Abbott, s formula, as follows

$$Corrected\ Mortality = \frac{\%\ test\ mortality - \%\ control\ mortality}{100 - \%\ control\ mortality}\ x\ 100$$

The number of dead larvae was collected every 24 hours for 10 days. The mortality percentage was calculated and corrected using Abbott's formula. The corrected mortality was compared among treatment and reported.

3.9.1.6. Assessment of damage caused on leaves and data collection

Fall armyworm feeds on both vegetative and reproductive stages of maize and the yield depends on the damages caused on plants. Maize can tolerate a certain degree of damages done on their leaves tissue. Therefore, it was very important to access the leaf-feeding rating score among the treatments because the rating score indicates damages caused on leaves. The infestation of maize with Fall armyworm larvae was carried out at the most susceptible stage (V5 stage). Damages on leaves were assessed by following the rating system developed by Davis and William (1992). The degree of damage caused was visually scored from 0 to 9 with 0 indicating no damage and 9 indicating heavy damage (Table 1). All the plants in each pot were assessed for damage to the leaves.

3.9.1.7. Assessment of damage caused on stalk and data collection.

The number of maize plants with stalk destroyed by Fall armyworm larvae was counted and recorded. The percentage of plants destroyed was calculated for each treatment using the following formula.

$$Percentage\ of plants\ damaged = \frac{Number\ of\ plants\ damaged}{Total\ number\ of\ plants\ in\ treatment} x\ 100$$

3.9.1.8. Data analysis

The number of dead larvae, leaf-feeding score, number of plants with stalk destroyed in different treatment (dosage) were subjected to statistical analysis of variance (ANOVA) and the mean was compared using the Tukey test to find a significant difference between treatments ($p \leq 0.05$) in SPSS software.

Table 1 Explanation of the leaf-feeding rating score by Davis and William (1992).

Explanation/definition of damage	Rating
No visible damage	0
Only pin-hole damage	1
Pinhole and small circular hole damage to leaves	2
Pinholes, small circular lesions, and a few small elongated (rectangular shaped) lesions of up to 1.3cm in length present on whorl and furl leaves	3
Several small to mid-sized 1.3 to 2.5 cm in length elongated lesions present on a few whorls and furl leaves	4
Several large, elongated lesions greater than 2.5 cm in length present on a few whorls and furl leaves and/or a few small- to mid-sized uni-form to irregularly shaped holes (basement membrane consumed) eaten from the whorl and/or furl leaves	5
Several large, elongated lesions present on sever-al whorl and furl leaves and/or several large uniforms to irregularly shaped holes eaten from furling and whorl leaves	6
Many elongated lesions of all sizes present on several whorl and furl leaves plus several large uniform to irregular shaped holes eaten from the whorl and furl leaves	7
Many elongated lesions of all sizes present on most whorl and furl leaves plus many mid- to large-sized uniform to irregular shaped holes eaten from the whorl and furl leaves.	8
Whorl and furl leaves almost destroyed	9

3.9.2. The efficacy of entomopathogenic nematodes under greenhouse conditions

3.9.2.1. Experimental design

A Randomized Complete Block Design (RCBD) was used for conducting greenhouse experiments. Like our experiment with entomopathogenic fungus, the best concentration that caused the highest mortality percentage in the laboratory experiment was selected for our greenhouse experiment. There were three treatments i.e., best dosage of *Heterorhabditis indica* isolate AUT 13.2 *and Steinernema siamkayai* isolate APL 12,3 and an equal amount of sterilised distilled water as a control. The volume and EPNs density used in the greenhouse experiments were calculated and adjusted based on the volume and density of EPNs used in the laboratory experiments. Therefore, two dosages were selected 20,000IJs and 50,000 per milliliter of sterilised distilled water, and each dosage was tested separately.

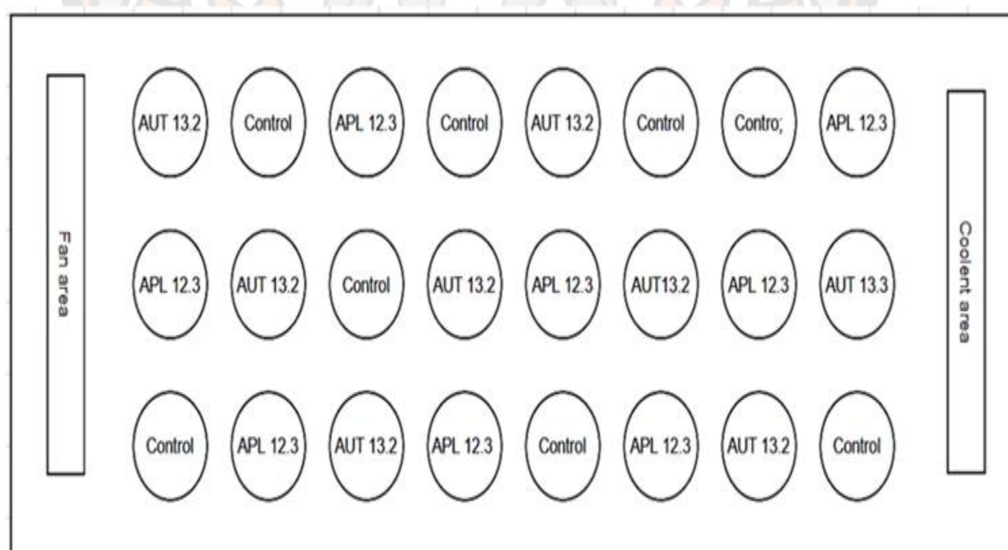

Figure 16 Layout of the experimental design in greenhouse experiments

3.9.2.2. Planting maize in greenhouse

Planting of maize was done similar to our experiment with entomopathogenic fungus. Sweetcorn variety "Super sweet" was grown in an earthen pot and the seedling number was reduced to five during the time of the infestation.

3.9.2.3. Release of the Fall armyworm

Second instar larvae of Fall armyworm were used for the greenhouse experiment. The larvae were released like our experiment with entomopathogenic fungus.

3.9.2.4. Application of nematode suspension

The first spray of nematode suspension was done 24 hours after the release of Fall armyworm larvae in the pot and the second and third after 48 and 96 hours of the first spray. Hundred milliliters fungal suspension containing 20,000 infective juveniles per milliliters of sterilised distilled water was applied with the help hand sprayer. Hundred milliliters of autoclaved distilled water without nematode suspension was used as a control.

3.9.2.5. Assessment of Mortality and data collection

The pots were assessed daily for dead larvae. When dead larvae were observed they were picked and kept for the White trap technique to confirm the death by the nematodes. The emergence of IJs from larvae was used to confirm the death by nematodes.

The mortality percentage was calculated with the following formula

$$Observed\ mortality = \frac{Total\ number\ of\ dead\ larvae}{Total\ exposed\ larvae}\ x\ 100$$

When control mortality is < 20%, then observed mortality was corrected using Abbott's formula, as follows

$$Corrected\ Mortality = \frac{\%\ test\ mortality - \%\ control\ mortality}{100 - \%\ control\ mortality}\ x\ 100$$

For each treatment, the number of dead larvae was recorded every 24 hours for 10 days. The dead larvae showing the emergence of IJs were recorded and the percentage mortality was calculated. Mortality is compared among treatments and reported.

3.9.2.6. Assessment of damage caused on leaves and data collection

For assessing leaf feeding damage by Fall armyworm, the rating system developed by Davis and William (1992) was used. The degree of damage caused was visually scored from 0 to 9 with 0 indicating no damage and 9 indicating heavy damage. The plants were numbered 1 to 5 and damage scores for the fifth and tenth day were recorded. The degree of damage caused was visually scored from 0 to 9 with 0 indicating no damage and 9 indicating heavy damage for the fifth and tenth days after the release of Fall armyworm larvae. The average damage score was calculated, compared between treatments, and reported.

3.9.2.7. Assessment of damage caused on stalk and data collection.

The number of maize plants with stalk destroyed by Fall armyworm larvae was counted and recorded. The percentage of plant damaged in treatment was calculated and compared. The percentage of plants destroyed was calculated for each treatment using the following formula and reported.

$$Percentage\ of plants\ damaged = \frac{Number\ of\ plants\ damaged}{Total\ number\ of\ plants\ in\ treatment}\ x\ 100$$

3.9.2.8. Data analysis

The number of dead larvae, leaf-feeding rating scores, were subjected to the normality of variance and subjected to statistical analysis of variance (ANOVA), and the mean of each treatment was compared using the Tukey test to find a significant difference between treatments (p≤0.05) in SPSS software.

3.9.3. The combined efficacy of entomopathogenic fungus and nematodes under greenhouse conditions

3.9.3.1. Experimental design

The entomopathogenic fungus and nematodes were combined by sequentially spraying maize plant infested with second instar Fall armyworms larvae. The entomopathogenic fungus *Beauveria bassiana* isolates TBRC 2781 at $1x10^{10}$ spores density and nematode *Heterorhabditis indica* isolates AUT 13.2 at 50,000IJs per milliliter of sterilised water was used. Two combinations were tested, in the first combination, nematodes spray was sequentially followed by fungus and nematode (*H. indica* AUT 13.2 + *B. bassiana* TBRC 2781+ *H. indica* AUT 13.2) and in the second combination fungus spray was followed by nematode and fungus (*B. bassiana* TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781). The same amount of sterilised distilled water was used as a control. A Randomized Complete Block Design (RCBD) was used for conducting this experiment.

3.9.3.2. Planting maize in greenhouse

Planting of maize was done similar to our previous experiment with fungus and nematodes.

3.9.3.3. Release of the Fall armyworm

Second instar larvae were released when maize seedlings were two weeks old. Each pot was manually infested with ten larvae.

3.9.3.4. Application of the spray

The first spray was done 24 hours after the release of the larvae. The second and third spray was done 48 and 96 hours after the first spray. In the first treatment, the spray was done according to this sequence; *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781+ *H. indica* AUT 13.2) and in the second treatment spray was done in the following this order; *B. bassiana* TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana*

TBRC 2781. One hundred millilitre of each suspension were sprayed at a time and the same volume of sterilised distilled water was used as control.

3.9.3.5. Assessment of larval mortality and data collection

The pots were assessed daily for dead larvae. When dead larvae were observed, half of the dead larvae were placed on moist filter paper for mycosis to determine the sporulation and the White trap technique to confirm the death from nematodes. The sporulation or emergence of IJs from larvae was used to confirm the death fungus and nematodes.

3.9.3.6. Assessment of leaf and plant damage and data collection

For assessing leaf damage, leaf-feeding rating scores were recorded as it was done in our previous experiment with fungus and nematodes. The plant damaged was counted at the end of the 10th day and the percentage of plant damaged was calculated.

3.9.3.7. Data analysis

The number of dead larvae, leaf-feeding rating scores, was subjected to statistical analysis of variance (ANOVA), and the mean of each treatment was compared using the Tukey test to find a significant difference between treatments ($p \leq 0.05$) in SPSS software.

CHAPTER IV
RESULT

4.1. The efficacy test of entomopathogenic fungi and entomopathogenic nematodes against Fall armyworms in the laboratory conditions

4.1.1. The efficacy of entomopathogenic fungus under laboratory conditions

The effect of the two isolates of *Beauveria bassiana* TBRC 2781 and TBRC 4755 on mortality of second and fifth instar larvae of Fall armyworm was determined by spraying larvae with the fungal suspension of different spore densities $1x10^5$, $1x10^6$, $1x10^7$**,** $1x10^8$, and $1x10^9$ spores per milliliter of sterilised distilled water. Dead larvae initially show no sign of infection and it was kept for mycosis in a clean Petri dish with moist filter paper. At 4-7 days after the death of the larvae, mycelia and sporulation structures were observed on most of the larvae treated with a fungal suspension of TBRC 2781 and TBRC 4755 (figure 17 and 18). Larvae that died in the control did not develop sporulation, suggesting that the two isolates of *B. bassiana* used in the study were able to infect and kill fall armyworm larvae of different stages.

The mortality of second instar larvae varied significantly according to the density of the spore suspension. The mortality of second instar larvae treated with a fungal suspension of *B. bassiana* isolates TBRC 2781 and TBRC 4755 was statistically significant between treatments as determined by one-way ANOVA (F(5,18)=64.34, p=0.00) and ($F_{(5,18)}$=36.34, p=0.00), respectively. *B. bassiana* isolate TBRC 2781 had higher mortality at both lower and higher spore densities. The highest mortality of 72.23% and 63.89% was obtained at spore density of $1x10^8$ for TBRC 2781 and TBRC 4755, respectively. The lower density of spore gave a lower percentage of mortality and at $1x10^5$ spore density TBRC 2781 and TBRC 4755 caused 30.55% and 22.22% mortality of second instar larvae. Control had mortality but it was always lesser than 20%.

Similarly, in the case of the fifth instar larvae of the Fall armyworm, both isolates were able to infect and cause mortality. The mortality was statistically significant between treatments as determined by one-way ANOVA ($F_{(5,18)}$=17.86,

p=0.00) and ($F_{(5,18)}$=6.14, p=0.002), respectively for isolates TBRC 2781 and TBRC 4755. The mortality of fifth instar larvae also varied according to spore densities. The isolate TBRC 2781 caused higher mortality than TBRC 4755. The highest mortality 34.82% and 24.62% was obtained with TBRC 2781 and TBRC 4755 at $1x10^9$ spore density.

Table 2 Effect of different dosages of *B. bassiana* isolates TBRC 2781 and TBRC 4755 on mortality of second instar larvae of Fall armyworm after 10days.

Larval stage	Treatments	*B. bassiana* TBRC2781 Mean ±SD	*B. bassiana* TBRC 4755 Mean ±SD
Second instar	Control	10.00±8.16a	15.00±5.77a
	$1x10^5$	30.55±5.56b	22.22±9.07b
	$1x10^6$	47.22±5.56c	30.55±5.56b
	$1x10^7$	58.34±5.55d	55.56±9.06c
	$1x10^8$	72.23±6.41e	63.89±5.56c
	$1x10^9$	72.23±5.05e	61.12±6.41c

The average of four replications. In each column, the mean followed by a different letter differs significantly. Mean separated by Duncan Multiple Range Test at p<0.05.

Table 3 Effect of different dosage of *B. bassiana* isolate TBRC 2781 and TBRC 4755 on mortality of fifth instar larvae of Fall armyworm after 10days

Larval stage	Treatments	*B. bassiana* TBRC2781 Mean ±SD	*B. bassiana* TBRC 4755 Mean ±SD
Fifth instar	Control	2.50±5.00a	5.00±5.77a
	$1x10^5$	17.30±4.88b	10.56±8.20a
	$1x10^6$	22.32±4.65b	15.56±5.13a
	$1x10^7$	29.85±7.91c	13.33±5.95a
	$1x10^8$	32.32±5.13c	21.11±1.28b
	$1x10^9$	34.82±5.98c	24.62±5.82b

The average of four replications. In each column, the mean followed by a different letter differs significantly. Mean separated by Duncan Multiple Range Test at p<0.05.

Figure 17 Sporulation of *Beauveria bassiana* on Fall armyworm larvae

Figure 18 Sporulation of *Beauveria bassiana* isolates on pupae of the Fall armyworm

4.1.2. The efficacy of entomopathogenic nematodes under laboratory conditions

The effect of entomopathogenic nematodes *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolates APL12.3 on the mortality of second and fifth instar larvae of Fall armyworm was determined by exposing larvae to five different dosages of EPNs 50, 100, 150, 200, 250, and 300IJs per milliliter of sterilised distilled water. The mortality percentage among treatments was compared at the end of 10 days. It was found that both EPNs isolates caused mortality of the Fall armyworm larvae, but the percentage of mortality varied significantly (Table 4). The mortality percentage was significant as determined by one-way ANOVA ($F_{(6,21)}$=118, p=0.000) and ($F_{(6,21)}$=102.7, p=0.000) respectively for isolates AUT 13.2, and APL 12.3. There was a proportional increase in mortality to the dosage of EPNs, however, when the dosage of EPNs was increased from 250 to 300IJs the difference in mortality was not very significant. The highest mortality percentage (82.5%) of the second instar Fall armyworm larvae was obtained when applied with *H. indica* isolate AUT 13.2 at a dosage of 250IJs. But *S. siamkayai* isolate APL12.3 caused only 67.5% mortality at the dosage of 300IJs.

Table 4 Effect of *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3 over mortality (Mean ± SD) of FAW larvae inoculated at second instar after 10 days.

Larval stage	Density (IJs/ml)	EPNs	
		H. indica AUT 12.3	*S. siamkayai* APL 12.3
		Mean±SD	Mean±SD
Second instar	Control	0.00±0.00a	0.00±0.00a
	50IJs	27.50±5.00b	17.50±5.00b
	100IJs	42.50±5.00c	27.50±5.00c
	150IJs	55.00±5.80c	35.00±5.00d
	200IJs	65.00±5.80d	47.50±5.00e
	250IJs	82.50±5.00e	65.00±5.80f
	300IJs	80.00±8.10e	67.50±5.80f

The average of four replications, treatments means followed by a different letter in column differ significantly at p<0.05 (ANOVA and Duncan Multiple Range Test).

When the fifth instar larvae were inoculated at a different dosage of EPNs, both isolates were able to infect and kill Fall armyworm larvae. The mortality percentage was significant as determined by one-way ANOVA ($F_{(6,21)}=39.24$, $p=0.000$) and ($F_{(6,21)}=19.62$, $p=0.00$) respectively for isolate AUT 13.2 and APL 12.3. Mortality was positively correlated to the dosage of the EPNs however, increasing dosage above 250IJs did not show many differences. The highest mortality 45% and 32.5% were obtained with isolate AUT 13.2 and APL 12.3 at a dosage of 250IJsml^{-1} at the end of 10 days of the exposure time.

Table 5 Effect of *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3 over mortality (Mean ± SD) of FAW larvae inoculated at fifth instar stage after 10 days

Larval stage	Density (IJs/ml)	EPNs	
		H. indica AUT 12.3	*S. siamkayai* APL 12.3
		Mean±SD	Mean±SD
Fifth instar	Control	0.00±0.00a	0.00±0.00a
	50IJs	17.50±5.00b	15.00±5.80b
	100IJs	25.00±5.80c	20.00±8.16b
	150IJ	32.50±5.00d	22.50±5.00b
	200IJ	35.00±5.80d	27.50±5.00c
	250IJs	45.00±5.80e	32.50±9.60d
	300IJs	42.50±5.00e	30.00±8.20d

The average of four replications, treatments means followed by different letters in columns differ significantly (ANOVA and Duncan Multiple Range Test, $p<0.05$).

4.2. The efficacy test of entomopathogenic fungus and nematodes against Fall armyworms under Greenhouse conditions

4.2.1. The efficacy of entomopathogenic fungus under greenhouse conditions

The effect of the isolates of *B. bassiana* TBRC 2781 and TBRC 4755 on mortality of Fall armyworm larvae under the greenhouse condition was determined by spraying second instar larvae of Fall armyworm with three different dosages of fungi i.e., 1 x 10^8, 1x$10^{9,}$ and 1 x 10^{10} spores. The isolates were sprayed three times, the first spray was made 24 hours after releasing the larvae, the second and third spray was done 48 and 96 hours after the first spray. The mortality was assessed daily and compared at the end of 10 days. The two isolates were able to infect and cause mortality of Fall armyworm larvae at all three dosages of fungal suspension tested. The mortality caused by the two isolates was statistically significant. At spore density of 1x10^8 the isolate TBRC 2781 caused higher mortality of 21.25% than the isolate TBRC 4755 which caused 18.76% mortality. Similarly, the isolate TBRC 2781 caused higher mortality 25% than isolate TBRC 4755 at a spore density of 1x10^9. At a spore density of 1 x 10^{10}, the isolate TBRC 2781 caused 35% mortality and TBRC 4755 caused 32.50%. At all three tested dosages, the isolate TBRC 2781 showed better result (Table 6).

Figure 19 Dead larvae observed on maize plant after the spray

Table 6 Effect of *B. bassiana* isolates TBRC 2781 and TBRC 4755 on mortality of Fall armyworm under greenhouse conditions at spore density of 1 x10^{8}, 1x10^{9}, and 1x10^{10} at the end of 10 days

Treatments	Spore density	% mean mortality± SD
B. bassiana TBRC 2781	1 x 10^{8}	21.25±8.35a
B. bassiana TBRC 4755	1 x 10^{8}	18.76±6.41a
Control	Distilled water	0±0.00b
B. bassiana TBRC 2781	1 x 10^{9}	25.00±5.35a
B. bassiana TBRC 4755	1 x 10^{9}	20.00±7.56a
Control	Distilled water	0±0.00c
B. bassiana TBRC 2781	1 x 10^{10}	35.00±5.35a
B. bassiana TBRC 4755	1 x 10^{10}	32.50±7.07a
Control	Distilled water	0±00b

The average of eight replications, treatments means followed by the same letter do not differ significantly (ANOVA and Tukey test, $p>0.05$)

4.2.2. The effect fungal spray on leaf-feeding rating score and plant damaged by Fall armyworm larvae

The average leaf-feeding rating score taken on the fifth and tenth day after the release of larvae in the pot is given in Table 7. Our result showed that even after spraying with fungus, the leaf damages were significant. According to the descriptive leaf-feeding rating score decreased when spore concentration was increased. The highest leaf-feeding rating score was 4 and 8.68 for the fifth and tenth days from the control treatment. The lowest leaf-feeding rating score 2.23 and 6.69 for the fifth day and tenth day was obtained from a treatment sprayed with *B. bassiana* isolate TBRC 2781 at 1 x 10^{10} spore density.

Regarding the number of plants damaged by the larvae, the control treatment had a higher number of plant damaged than the treatments sprayed with fungal isolates. The highest percentage of plant destroyed was 88% which was observed in control and the lowest 65% was observed from treatment sprayed with isolate TBRC 2781 at 1x10^{10} spore density (Table 7).

Table 7 Leaves feeding rating scores (Mean ± SD) taken at the fifth and tenth days and percentage of plant that was completely damaged at the end of 10 days

Treatments	Spore density (Spores/ml)	Leaf feeding rating score Mean±SD		Plant Damaged (%)
		Day 5	Day 10	
Control	0	3.975±0.29a	8.58±0.31a	85
B. bassiana TBRC 2781	1 x 10^8	2.90±0.75a	7.73±0.32a	75
B. bassiana TBRC 4755	1 x 10^8	2.98±0.55a	7.88±0.34a	78
Control	0	3.85±0.41a	8.68±0.28a	88
B. bassiana TBRC 2781	1 x 10^9	2.65±0.50a	7.40±0.39a	73
B. bassiana TBRC 4755	1 x 10^9	2.85±0.77a	7.80±0.34a	70
Control	0	4.00±0.30a	8.48±0.35a	85
B. bassiana TBRC 2781	1 x 10^{10}	2.23±0.37b	6.69±0.63b	65
B. bassiana TBRC 4755	1 x 10^{10}	2.70±0.81b	7.10±0.55b	70

The mean of eight replications. The mean rating scores followed by different letters differ significantly (ANOVA and Tukey's test; P≤0.05).

Figure 20 A and B Damages by larvae, C: Larvae feeding on a stem, and D: Plant completely defoliated by Fall armyworm larvae.

4.4.1. The efficacy of entomopathogenic nematodes under greenhouse conditions

The efficacy of entomopathogenic nematode *Heterorhabditis indica* isolates AUT 13.2 and *Steinernema siamkayai* isolates APL 12.3 on mortality of Fall armyworm larvae under the greenhouse condition was determined at two different dosages i.e., 20,000 and 50,000IJs per milliliter of sterilised distilled water. The density of EPNs and volume of suspension applied were calculated based on the dosage of IJs that caused the highest mortality and volume applied in the laboratory condition. The second instar larvae of Fall armyworm were used for our greenhouse experiment because this stage was found susceptible to EPNs isolates of more than fifth instar larvae. The mortality of Fall armyworm larvae in each treatment was recorded daily and compared at the end of 10 days. At EPNs density of 20,000IJs, the mortality of Fall armyworm larvae was statistically significant as determined by one-way ANOVA ($F_{(2,21)}$=101.43, p=0.00). *H. indica* isolates AUT 13.2 caused higher mortality (37.50%) than *S. siamkayai* isolates APL12.3 (28.75%). Similarly, at EPNs density of 50,000IJs, the mortality was statistically significant ($F_{(2,21)}$=113.82, p=0.00). It can be concluded from our study that *H. indica* isolates AUT 13.2 caused higher mortality compared to *S. siamkayai* isolate APL 12.3 (Table 8).

Table 8 Mortality percentage (Mean±SD) of Fall armyworm larvae under greenhouse condition at EPNs dosage of 20,000 and 50,000IJs

Treatments	IJs ml^{-1}	Mean ± SD
H. indica AUT 13.2	20,000	37.50±7.07a
S. siamkayai APL 12.3	20,000	28.75±6.41a
Control		0.00±0.00b
H. indica AUT 13.2	50,000	57.50±11.64b
S. siamkayai APL 12.3	50,000	45.00±7.56a
Control		0.00±0.00c

The mean of eight replications, treatments mean followed by the different letter differ significantly (ANOVA and Tukey test p<0.05).

4.4.2. The effect of nematodes spray on leaf-feeding rating score and plant damaged by Fall armyworm larvae

The effect of different treatments on the degree of leaf-feeding damage by the Fall armyworm larvae was determined by comparing means of leaf-feeding rating score as described by Davis and William (1992) among the treatments for the fifth day and the tenth day after the release of the larvae. The leaf-feeding rating score was not significant at 20,000IJsml^{-1}. However, at 50,000IJsml^{-1}, the average damage score differs significantly between treatments and control (Table 9).

Table 9 Average leaf damage rating scores (Mean ± SD) for 5th and 10th days and percentage of plant damaged after 10 days at 20,000 and 50,000IJs

Treatments	IJs ml^{-1}	Mean ± SD		Plant damaged (%)
		Day 5	Day 10	
H. indica AUT 13.2	20,000	3.35±0.64a	7.95±0.79a	70.00
S. siamkayai APL 12.3	20,000	3.82±0.35a	7.90±0.26a	75.00
Control	0	4.08±0.37a	8.55±0.26a	85.00
H. indica AUT 13.2	50,000	3.30±0.49a	7.70±0.34a	63.00
S. siamkayai APL 12.3	50,000	3.20±0.30a	7.67±0.50a	65.00
Control	0	3.98±0.29b	8.48±0.32b	83.00

The average of eight replications. Mean followed by the different lower-case letters in the column differ significantly at ($P \leq 0.05$) according to the Tukey test.

The percentage of plant destroyed was calculated from each treatment and compared among the treatments. We found that the control treatment had a higher percentage of plant damaged than the treatment treated with EPNs in both experiments. At EPNs dosage of 20,000IJs per milliliter of sterilised distilled water, the lowest percentage of plant damaged was 70% which was observed in treatment treated with *H. indica* isolate AUT 13.2 and highest 85% in control. Similarly, at 50,000IJs per milliliter of sterilised distilled water, the lowest percentage of plant destroyed was 63% which was observed in treatment treated with *H. indica* isolate AUT 13.2 and the highest 83% was seen in control.

4.4.3. Combined efficacy of entomopathogenic fungus and nematode under greenhouse conditions

Two combinations were tested, in the first combination, nematodes spray was sequentially followed by fungus and nematode (*H. indica* AUT 13.2 + *B. bassiana* TBRC 2781+ *H. indica* AUT 13.2) and in the second combination fungus spray was followed by nematode and fungus (*B. bassiana* TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781). The same amount of sterilised distilled water was used as a control. Dead larvae were observed from the treatments where fungus and nematode were sprayed. The mortality obtained in the two treatments was statistically significant as determined by one-way ANOVA ($F_{(2,21)}$=135.80, p=0.00). The first combination treatment (*H. indica* AUT 13.2 + TBRC 2781 + *H. indica* AUT 13.2) had the highest mortality of the Fall armyworm larvae (55%) than (40%), in the second treatment (TBRC 2781 + *H. indica* AUT 13.2 + TBRC 2781). The mortality percentage of both treatment were significantly different (Table 10).

Table 10 Mortality percentage (Mean±SD) of Fall armyworm larvae using combined treatment under greenhouse condition.

Treatments	**Mean±SD**
H. indica AUT 13.2 + TBRC 2781 +*H. indica* AUT 13.2	55.00±9.26a
TBRC 2781 + *H. indica* AUT 13.2 + TBRC 2781	40.00±7.56b
Control(Distilled water)	0.00±0.00c

The mean of eight replications. The mean followed by the different lower-case letter in the column differs significantly at (p≤0.05) according to the Tukey test.

Our result showed that spraying entomopathogenic nematode followed by fungus and nematode would give better results than spraying with entomopathogenic fungus followed by nematode and fungus. Most larvae died earlier in the first treatment than the second treatment (Figure 21). In the first combination spray the Fall armyworm larvae begin to die from the third day and majority of larvae were killed between 3rd to 7th day. Whereas, in second combination spray most larvae died 5th to 8th day.

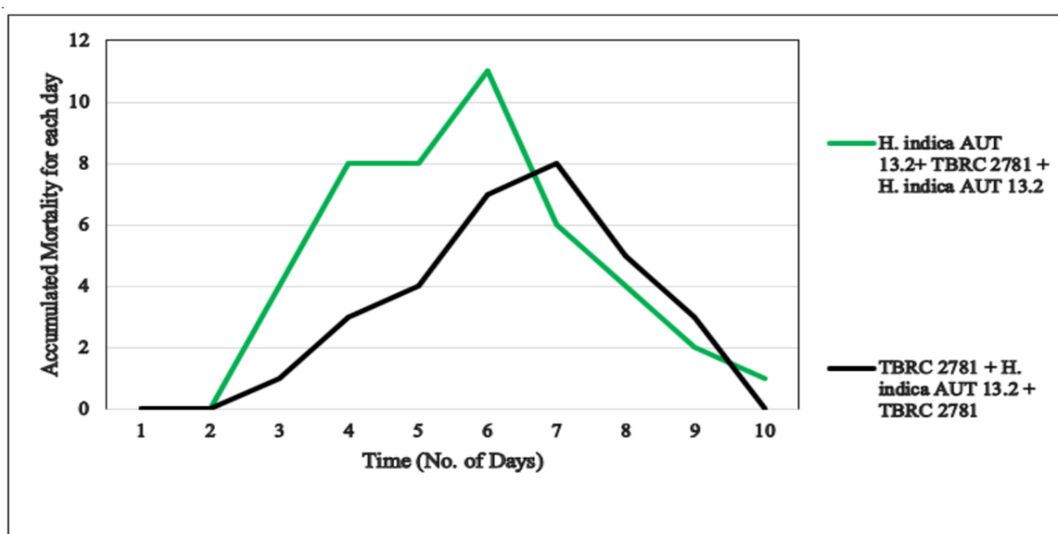

Figure 21 Accumulated number of Fall armyworm larvae died using combined treatment under greenhouse condition.

4.4.4. The effect of combined spray on leaf-feeding rating score and plant damaged by the Fall armyworm

The damages by Fall armyworm larvae on the leaf were determined by following the description given by Davis and William (1992). The leaf-feeding rating score was taken on the fifth and tenth days and compared among the treatments. There were statistically significant differences in the leaf-feeding rating score taken on the fifth day between treatments as determined by one-way ANOVA ($F_{(2,21)}$=20.78, p=0.00). The lowest leaf-feeding rating score was 1.78 for first treatment (*H. indica* AUT 13.2 + *B. bassiana* TBRC 2781 + *H. indica* AUT 13.2). The second treatment (*B. bassiana* TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781) had a leaf

damage rating score of 2.65, while the highest score of 3.35 was observed in control.

Similarly, the tenth-day leaf-feeding rating score was statistically significant when determined by one-way ANOVA ($F_{(2,21)}$=16.10, p=0.00). The lowest leaf-feeding rating score of 6.00 was observed in the first treatment (H. indica AUT 13.2 + B. bassiana TBRC 2781 + H. indica AUT 13.2). The second treatment (B. bassiana TBRC 2781 + H. indica AUT 13.2 + B. bassiana TBRC 2781) had the score as 6.58 and 7.53 was observed in control. Therefore, the result indicated that combination treatment one and two reduced the leaf-feeding damages caused by Fall armyworm larvae. In addition, the result also showed that spraying entomopathogenic nematodes followed by fungus was more effective than spraying entomopathogenic fungus followed by nematodes.

Table 11 Average leaf damage rating scores (Mean ± SD) for 5th and 10th days and percentage of plant damaged after 10 days

Treatments	Leaf-feeding rating score		Plant damaged (%)
	Day 5	Day 10	
H. indica AUT 13.2 + *B. bassiana* TBRC 2781 + *H. indica* AUT 13.2	1.78±0.64a	6.00±0.57a	72
B. bassiana TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781	2.65±0.40b	6.58±0.61a	76
Control (Distilled water)	3.35±0.40c	7.53±0.44b	88

The mean of eight replications. The Mean followed by the different lower-case letters in the column differs significantly at (P≤0.05) according to the Tukey test.

The result also showed reduced number of plant damaged by the Fall armyworm larvae. The lowest percentage of plant damaged was 72% which was observed in first treatment (*H. indica* AUT 13.2 + *B. bassiana* TBRC 2781 + *H. indica* AUT 13.2) and second treatment (*B. bassiana* TBRC 2781 + *H. indica* AUT 13.2 + *B. bassiana* TBRC 2781) had 76% while control had 88% of total plant damaged.

CHAPTER V
DISCUSSION

5.1 The efficacy of entomopathogenic fungus under laboratory conditions

Fall armyworm is reported susceptible to entomopathogenic fungus *B. bassiana* (Ramirez-Rodriguez & Sánchez-Peña, 2016). However, entomopathogenic fungi largely differ among genus, species, and isolates for infecting and killing the host (Sengonca, Thungrabeab, & Blaeser, 2006). This is the first study evaluating the efficacy of *B. bassiana* isolates TBRC 2781 and TBRC 4755 indigenous to Thailand against larval stage of newly introduced pest Fall armyworm. Our result demonstrated that *B. bassiana* isolates TBRC 2781 and TBRC 4755 were able to infect and kill larvae of Fall armyworm inoculated at the second and fifth instar stage.

There was a difference observed in the percentage of mortality caused by *B. bassiana* isolates TBRC 2781 and TBRC 4755. The difference in mortality is related is determined by many factors which are classified as biotic and abiotic factors. Biotic factors include host response to the pathogens, the susceptibility of the host, virulence of pathogens (Sengonca et al., 2006). Abiotic factors include environmental conditions specifically temperature and humidity (Maina, Galadima, Gambo, & Zakaria, 2018). Our laboratory experiment was carried under controlled temperature and relative humidity and similar sized larvae were used. Therefore, the difference in the mortality of Fall armyworm in our study is assumed to be due virulence of *B. bassiana* isolates TBRC 2781 and TBRC 4755. The infection of the host by any entomopathogens is determined by successful interaction between entomopathogens and the immune response of the target host (Dunn, 1986). All entomopathogens differ from genus to genus and species to species in terms of their ability to produce toxins and enzymes and this is true for entomopathogenic fungi also (Feng, Poprawski, & Khachatourians, 1994). Many earlier studies have also reported differences in the virulence of entomopathogenic fungi. García, González, and Bautista (2011) evaluated 8 strains of *B. bassiana* but reported only two isolates Bb18 and Bb42 as most effective against Fall armyworm larvae. In another study carried out by (Ramanujam, Poornesha, & Shylesha, 2020) they also claimed a varying degree of

mortality among 10 different fungal strains tested in their study with *M. anisopliae* ICAR-NBAIR Ma-35 which caused 67.8% mortality followed by *B. bassiana* ICAR-NBAIR Bb-45 with 64.3% and Bb-11 with 57.1% mortality. The rest of the strains showed 10.7–28.6% mortality. Akutse et al. (2019) evaluated 20 isolates of fungus (14 isolates of *M. anisopliae and* 6 isolates of *B. bassiana*) against the neonate larvae of Fall armyworm and among six isolates of *B. bassiana,* only ICIPE 281 and ICIPE 676 and caused mortality of 83.9% and 53.9%, respectively. It is evident from these studies that isolates differ in virulence even when tested against the same stage of the insect pest. The difference in the virulence among different strains of entomopathogenic fungi is determined by their ability to produce enzymes which play important role in the infection process (Fang et al., 2005). Therefore, the difference in the mortality percentage of the *B. bassiana* isolates TBRC 2781 and TBRC 4755 used in our study may be due to the difference in the virulence between these isolates.

In our result, we also observed that the mortality of second instar larvae was higher than the mortality of fifth instar larvae. This can be related to the differences in susceptibility of the second and fifth instar stage of the Fall armyworm larvae. Opisa, Du Plessis, Akutse, Fiaboe, and Ekesi (2018) stated that entomopathogens can cause infection in all host stages but the susceptibility of each stage of the host may differ significantly. Our results were similar to the finding of (Ramanujam et al., 2020) who reported susceptibility of second instar larvae to *M. anisopliae* and *B. bassiana,* respectively. Garcia, Raetano, and Leite (2008) reported 96.6 and 78.6% mortality of the second instar larvae of Fall armyworm with *B. bassiana* and *M. anisopliae,* respectively. Morales-Reyes et al. (2013) also reported 45 to 65% mortality of second instar larvae of Fall armyworm in laboratory bioassay by *M. anisopliae* and *B. bassiana*. However, the result obtained was in contrast with the study carried out by (Wraight, Ramos, Avery, Jaronski, & Vandenberg, 2010) who reported the second instar larvae was least effective against fungal isolates used in their study. (Akutse et al., 2019) also reported some isolates of *B. bassiana* tested were less effective against second instar larvae of Fall armyworm larvae. The immune response of the host determines the susceptibility of the host to particular entomopathogens (Dunn, 1986). The underlying mechanism for what made second instar larvae of Fall armyworm more susceptible to *B. bassiana* isolates TBRC 2781 and TBRC 4755 needs to be

investigated in future works. We assume that the difference in the immune response of younger and older larvae might have resulted in the differences in overall mortality. Our result shows that *B. bassiana* isolates TBRC 2781 and TBRC 4755 have great potential to be used as biocontrol agents to control Fall armyworm larvae.

5.2. The efficacy of entomopathogenic fungus under greenhouse conditions

The result obtained from our greenhouse experiments showed that the *B. bassiana* isolates TBRC 2781 and TBRC 4755 were effective in greenhouse conditions. The efficacy of entomopathogenic fungi is determined by environmental conditions including moisture, temperature, relative humidity (RH) and ultraviolet (UV) radiation determine (Fernandes, Rangel, Braga, & Roberts, 2015; Maina et al., 2018). In the greenhouse, where environmental conditions were subjected to change, a lower percentage of mortality was expected. Variation in temperature and relative humidity can affect the growth and development of *B. bassiana* (Ekesi, Maniania, & Ampong-Nyarko, 1999; Luz & Fargues, 1998; Seid, Fredensborg, Steinwender, & Meyling, 2019; Sosa-Gómez & Alves, 2000; Walstad, Anderson, & Stambaugh, 1970). Therefore, the efficacy of the *B. bassiana* isolates TBRC 2781 and TBRC 4755 were affected due to variation in temperature and relative humidity. The optimum temperature for growth and development of the two isolates was 20-25°C as mentioned in the description provided by Thailand Bioresource Research Centre but in the greenhouse, the average temperature was 28.9°C. The lower efficacy could also be due to exposure of fungal spore to sunlight and UV radiation. Inglis (2001), stated that exposure to fungal spore to UV radiation leads to inactivation of the spores.

In addition, to extreme environmental conditions, the larval movement to settle and establish a feeding site on a suitable host (Vilarinho et al., 2011), can be responsible for lower efficacy. Zalucki, Clarke, and Malcolm (2002) reported that many arthropods move away from infested plants. In the greenhouse larvae freely moves from plants to plants making it difficult for fungus to thus making it difficult for proper host contact. It was also difficult to direct the spray against larvae because some larvae remain on the lower surface of the leaves and are not affected. This was also reported by (El-Husseini, Agamy, Mesbah, El-Fandary, & Abdallah, 2008) who

stated that insect present on the lower surface of leaves of sugar beet was poorly affected with two formulations of *B. bassiana*. Larvae of many Lepidoptera are concealed feeders (Porter, 1982) and we observed similar behaviours with Fall armyworm larvae. We observed that second instar larvae quickly establish by moving to the region where softer plants part are available i.e., whorl region maize plant. Once they reach the whorl, they burrow and conceal themselves in a maize funnel (Day et al., 2017). This behavior not only makes larvae avoid fungal spray but also makes them grow faster by feeding on the host. These factors are very important to consider while using entomopathogenic fungus for pest control in the greenhouse or field. In greenhouse conditions, the *B. bassiana* isolates TBRC 2781 and TBRC 4755 showed great potential to be used as biocontrol agents.

5.3. The efficacy of entomopathogenic nematodes under laboratory conditions

This is the first study evaluating the efficacy of indigenous entomopathogenic nematodes, *Heterorhabditis indica* isolates AUT 13.2, and *Steinernema siamkayai* isolates APL 12.3 against the Fall armyworm. The result demonstrates that the EPNs *Heterorhabditis indica* isolates AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3 are capable of infecting and killing different stages of Fall armyworm larvae in laboratory conditions. Both isolates showed high efficacy against the different larval stages at different dosages. There was a proportional increase in the mortality of Fall armyworm larvae with an increase in density of infective juveniles per milliliter of sterilised distilled water however increasing IJs dosages above 250 did not show much difference.

The differences observed in mortality of Fall armyworm larvae by the isolates may be due to the differences in the virulence of the nematode isolates which is determined by the presence of symbiotic bacteria and the ability of nematode to find and proliferate inside the host (Shapiro-Ilan, Gouge, Piggott, & Fife, 2006). Other factors like the environment, the immune response of the host also play an important role in the process of killing the host (Batalla-Carrera, Morton, & García-del-Pino, 2010). However, in the laboratory condition where most environmental conditions were uniform, the differences in mortality were expected to be due to the presence of

different symbiotic bacteria in the two isolates which is responsible for killing the host, and also the ability of nematode isolates to find and infect the host. *Photorabdus* bacteria are associated with nematode belonging to the *Heterorhabditidae* family *and Xenorhabdus* bacteria with the family Steinernematidae (Boemare et al., 1993). These bacteria are reported to release many toxins (Hinchliffe, Hares, & Dowling, 2010) that suppress the immune response of the target host or interrupt the normal development by disrupting host tissues (Dowling & Waterfield, 2007). However, some studies have reported that *Photorabdus* bacteria performing better than *Xenorhabdus* bacteria because *Photorabdus* bacteria can survive better immune response and also release more toxin in the host (Abdel-Razek, 2003). Therefore, higher mortality obtained with *H. indica* isolates AUT 13.2 over *S. siamkayai* isolates APL 12.3 can be related to the presence of symbiotic bacteria *Photorabdus* and *Xenorhabdus*, respectively.

In addition, the ability to find the host and cause infection by nematodes also differ among the families and species of the nematodes (F. James & Randy, 1997; Lortkipanidze, Gorgadze, Kajaia, Gratiashvili, & Kuchava, 2016). Generally, most *Heterorhabditis* sp., are cruiser in their natural habitat, they are motile, and they move around their environment to find their host, whereas most *Steinernema* sp are ambusher, they wait for the host to come in contact to cause infection (Campbell, Lewis, Stock, Nadler, & Kaya, 2003; Lewis, Gaugler, & Harrison, 2009). In our study we obtained higher mortality with *H. indica* isolates AUT 13.2 over *S. siamkayai* isolate APL 12.3. *H. indica* isolates AUT 13.2 being more motile caused more mortality of the Fall armyworm larvae. Our results were similar to (Molina Ochoa et al., 1996) who evaluated virulence of six entomopathogenic nematodes and reported *Heterorhabditis bacteriophora* most efficient when considering the larval phase, causing 65% mortality in second instar larvae. The result of lower mortality of fifth instar larvae in our study was similar to (Fuxa, Richter, & Acudelo-Silva, 1988) who reported decreased mortality of older larvae. In contrast to our result (Acharya, Hwang, Mostafiz, Yu, & Lee, 2020) reported 100% mortality of second instar larvae by *H. indica*, *S. carpocapsae*, and *S. longicaudum*, whereas *S. arenarium* and *H. bacteriophora* caused 77% and 53% mortality, but *Heterorhabditis sp*. and *S. kushidai* used in their study caused little or no mortality of older Fall armyworm larvae than the second instar. This result indicates that there are other factors responsible for

causing differences in mortality which needs to be investigated in the future study for our isolates. Both isolates of EPNs used in our study were effective against second instar and fifth instar Fall armyworm larvae under laboratory conditions. Therefore, both isolates were selected for the greenhouse experiment to evaluate these isolates for their capacity to find and infect the host.

The result from our study showed that fungal spray and nematodes did not make significant differences, leaf-feeding scores and plant damaged by Fall armyworm were very high even after the spray at all tested dosage. There was a slight decrease in the leaf-feeding score and plant damaged when dosages were increased for both fungus and nematodes spray. This reaches us to speculate that it may be an effect of the spray. The slightest differences observed in leaf-feeding rating score and percentage of plant damaged is due to the higher number of active spores or IJs which might have suppressed the larval activity of the Fall armyworm. . In addition, some larvae died in pots treated with fungus and the differences in the number of larvae also played an important role. In the laboratory where host contact was assured these isolates took on average 5-6. days to infect and kill Fall armyworm larvae. It took more days to observe dead larvae in the greenhouse experiment. Therefore, larvae had enough time to cause damages before it was killed by the fungus. In our experiment with entomopathogenic nematodes, a slight difference was observed in leaf damage and plant with stalk destroyed at EPNs density of 20,000IJs and 50,000IJs per milliliters of sterilized water. This result speculates us of some effect by EPNs spray. However, the leaf damage and plant destruction by Fall armyworm larvae were significant. In the experiment where we combined entomopathogenic fungus and nematodes, the leaf-feeding rating score and plant destroyed by Fall armyworm were very high. All these results from our greenhouse experiment speculate of some effect by the spray.

5.4. The efficacy of entomopathogenic nematodes under greenhouse condition

Entomopathogenic nematodes live in the soil and they are reported very effective against soil-dwelling insects but ineffective or less effective against insect pests that live outside the soil (Grewal, Lewis, Gaugler, & Campbell, 1994; Kaya &

Gaugler, 1993). The main objective of our greenhouse experiment with EPNs was to evaluate their efficacy under different conditions so that they are recommended for using them as biocontrol agents in an Integrated pest management system. The result from our experiment showed that the entomopathogenic nematodes *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate 12.3., were effective under greenhouse conditions although the percentage mortality was very low compared to what we achieved in the laboratory condition. The lower efficacy of EPNs in the greenhouse is due to inactivation of an infective juvenile by desiccation, UV radiation, higher temperature, and characteristic of exposed foliage (Poinar, 1990) and Glazer (2002, cited in (Vashisth et al., 2013). In addition, proper host contact by EPNs also plays an important role in causing infection and killing the larvae (Kaya & Gaugler, 1993). EPNs must be tested under different conditions (temperature and relative humidity) for assessing their ability to find the host and cause infection (Garcia et al., 2008). In the greenhouse where conditions were different from what was provided in the laboratory, the sunlight, UV radiation may have desiccated infective juveniles that were directly exposed. Moreover, Fall armyworm larvae freely move from plants to plants, and this might have created a lesser chance for IJs to make proper host contact and cause infection. In our second experiment where we increased IJs concentration to 50,000IJs per milliliter of sterilised water, mortality was slightly higher when compared to what we obtained at dosage 20,000 IJs. This may be due to application of EPNs directed on the feeding site of the Fall armyworm larvae. Therefore, in the field were EPNs is exposed to harsh condition, the rate and application methods need to be considered for better result. In the greenhouse due to the availability of food, higher temperature, and relative humidity, it was observed that Fall armyworm larvae were able to complete their life cycle faster. This also may be a reason why EPNs were unable to penetrate and enter the integument of the larvae.

5.5. Combined efficacy of entomopathogenic fungus and nematodes under greenhouse conditions

Pest control can be significantly enhanced by using combinations of biocontrol agents (Pal & Gardener, 2006). When entomopathogenic fungus and nematodes are combined and applied for pest and disease control, the effect on pest and disease control can be additive, synergistic, or sometimes antagonistic (Ansari, Shah, & Butt, 2008). In our study we combined entomopathogenic fungus *B. bassiana* isolate TBRC 2781 and nematode *H. indica* isolates AUT 13.2. The combined effect was assessed for number of Fall armyworm killed in each treatment. After 10 days of the assessment, we observed high percentage mortality in combination treatment (*H. indica* isolate AUT 13.2 + *B. bassiana* isolate TBRC 2781+*H indica* isolate AUT 13.2) and treatment (*B. bassiana isolate* TBRC 2781 + *H. indica* isolate AUT 13.2 + *B. bassiana isolate* TBRC 2781) than control. However, the percentage mortality obtained was lesser than what we obtained when these isolates were used separately. We observed that the mortality in the first treatment was slightly higher than the second treatment. The underlying mechanism for lower mortality can be further investigated under laboratory conditions and production of inhibitory compounds as done by (Guetsky, Shtienberg, Elad, Fischer, & Dinoor, 2002).

Generally, in the nature where one or more pathogens are present, the presence of one pathogen can antagonize by the presence and activities of another pathogen they encounter (Pal & Gardener, 2006). The differences in mortality can be attributed to the interaction between fungus and the nematode isolates used in our study. The symbiotic bacteria in entomopathogenic nematodes produces antibiotic that inhibits growth of *B.bassiana.* On the other hand, entomopathogenic fungi produces mycotoxin which are detrimental to nematodes (Barberchek & Kaya, 1990; Isaacson & Webster, 2002). In the present study there was evidence that nematodes and fungus isolates used are antagonistic because the mortality was lesser than what we obtained when these isolates were used separately and in addition when dead larvae were kept for sporulation and White trap, some larvae developed sporulation and some showed emergence of IJs, but same larvae did not show both sporulation and IJs emergence. Ansari et al. (2008) evaluated the mutual effect of symbiotic bacteria in

entomopathogenic nematodes and entomopathogenic fungi. They found an antagonistic effect of *Photorhabdus luminescens* (symbiotic bacteria in *Heterorhabditis* sp.) to *Metarhizium anisopliae, Beauveria bassiana, Beauveria brongniartii,* and *Paecilomyces fumosoroseus* whereas *Xenorhabdus poinarii* (symbiotic bacteria in *Steinernema* sp.) did not show antagonistic effect. *Heterorhabditis* sp. of entomopathogenic nematode are associated with *Photorhabdus* bacteria and in the present study, the presence of this *Photorhabdus* bacteria in *H. indica* isolate AUT 13.2 might have inhibited the growth of fungus *B. bassiana* isolate TBRC 2718. Similarly, mycotoxin produced by *B. bassiana* isolate TBRC 2781 might have inhibited *H. indica* isolate AUT 13.2. However, in future research, the mutual interaction can be further studied.

We conclude that when we apply entomopathogenic fungi *B. bassiana* isolate TBRC 2781 and nematode *H. indica* isolate AUT 13.2 sequentially, the mutual interaction was not observed as the number of Fall armyworm died was comparatively lower than what we achieved when they were used separately. There is growing evidence that pest control can be greatly improved by using combinations of biocontrol agents However, all combinations are not synergistic or additive in nature and this mutual interaction must be studied to achieve effective pest control.

5.6. Effect of different treatments on the degree of leaf-feeding damage and plant damaged by FAW larvae in the greenhouse

The result from our study showed that fungal spray and nematodes did not make significant differences in leaf-feeding scores and plant damaged by the Fall armyworm were very high even after the spray at all tested dosage. There was a slight decrease in the leaf-feeding score and plant damaged when dosages were increased for both fungus and nematodes spray. This reaches us to speculate that it may be an effect of the spray. The slightest differences observed in leaf-feeding rating score and percentage of plant damaged is due to the higher number of active spores or IJs which might have suppressed the larval activity of the Fall armyworm. In addition, some larvae died after the spray with fungus and nematodes and the differences in the number of larvae also played an important role.

The higher rating score of leaf damage and a higher percentage of the plant completely destroyed by Fall armyworm larvae can be attributed to the longer time taken by biocontrol agents. Normally, in the laboratory experiment *B. bassiana* isolates (TBRC 2781 and TBRC 4755) took on average 5-6 days to kill Fall armyworm larvae. EPNs isolates took on average 3-4 days to kill Fall armyworm larvae. It might have taken more days to kill Fall armyworm larvae in the greenhouse due to conditions like larval mobility and environmental conditions. In the greenhouse, experiment Fall armyworm larvae had wider space for mobility and this made it difficult for fungal spores and IJs of the EPNs to settle and make good contact. Only a few spores or IJs which were able to settle and make good contact might have caused mortality of the larvae. In addition, many fungal spores and EPNs under extreme environmental conditions in the greenhouse led to inactivation due to sunlight, UV radiation, and higher temperature. The larvae continued to feed and cause damages even after spraying with EPNs suspension.

There is a limited study done on assessing leaf damages and stalk damaged by Fall armyworm by spraying with biocontrol agents. However related studies done with insecticides showed that even when pesticides are used the leaf damage was significant (Kumela et al., 2019), which were previously reported very susceptible to Fall armyworm damages. The higher leaf damage and stalk damage also might have resulted because we selected the young stage of the maize. Maize which is two weeks old is reported very susceptible to Fall armyworm in a previous study. However, there are need to confirm these finding and future work may be focused on exposing maize germplasm to Fall armyworm and compare the leaf-feeding damages in detail.

CHAPTER VI
CONCLUSION

6.1.Conclusion

This research was carried out to evaluate two isolates of entomopathogenic fungus *Beauveria bassiana* (TBRC 2781 and TBRC 4755), and two isolates of indigenous entomopathogenic nematodes *Heterorhabditis indica* isolate AUT 13.2 and *Steinernema siamkayai* isolate APL 12.3., for controlling Fall armyworm under laboratory and greenhouse conditions. The result from our laboratory and greenhouse reveals that all isolates were able to infect and kill Fall armyworm larvae when inoculated at the second and fifth instar. In the laboratory where conditions (temperature and humidity) are controlled and host contact by these isolates were certain. Higher mortality was obtained with both fungus and nematodes.

In the laboratory, the isolates of *B. bassiana* TBRC 2781 caused higher mortality than TBRC 4755 at all tested dosages. The mortality increased with the increase of densities of the spores. At all tested densities, the Fall armyworm larvae inoculated at the second instar stage showed higher susceptibility than the one inoculated at the fifth instar stage. The highest mortality of second instar larvae was 72% and 64% for TBRC 2781 and TBRC 4755 respectively at $1x10^9$ ml per milliliter density. Similarly, for the fifth instar, the highest mortality was 35% and 25% for TBRC 2781 and TBRC 4755 at $1x10^9$ per milliliter spore density. In the greenhouse experiment, the *Beauveria bassiana* isolates were found least effective at $1x10^8$ and $1x10^9$ dosage of fungal spores but it was effective when the dosage of fungal spores was increased to $1x10^{10}$. The isolate *B. bassiana* TBRC 2781 caused higher mortality than *B. bassiana* TBRC 4755 at all tested densities.

The entomopathogenic nematodes were also more effective against second instar larvae. The mortality increased with the increased dosage of the infective juveniles. The isolate *Heterorhabditis indica* AUT 13.2 caused the highest mortality (83%) of second instar larvae at 250 IJ per milliliter. At the same dosage, the isolate *Steinernema siamkayai* AUT 12.3 was able to kill 66% of the second instar larvae. Fifth instar Fall armyworm larvae were less susceptible to the EPNs isolates compare

to second instar larvae. The highest mortality 45% and 33% were obtained with *Heterorhabditis indica* AUT 13.2 and *Steinernema siamkayai* AUT 12.3 respectively, at 250IJs per milliliter. The entomopathogenic nematodes were also found very effective in the greenhouse although mortality of Fall armyworm larvae was comparatively lesser than in the laboratory. The isolates *Heterorhabditis indica* AUT 13.2 were more effective at 20,000IJs per milliliter and 50,000IJs per milliliter than *Steinernema siamkayai.* The entomopathogenic fungus isolates *B. bassiana* TBRC 2781 and entomopathogenic nematode *H. indica* isolate AUT 13.2 were also applied in combination. The mortality was not very promising, and it was lower compared to what we achieved in our previous experiments where we used nematodes and fungus separately.

Therefore, we conclude that all isolates of entomopathogenic fungus and indigenous entomopathogenic nematodes tested in our study were able to infect and kill Fall armyworm larvae in laboratory and greenhouse conditions. We achieved higher mortality of Fall armyworm larvae in the laboratory with all the isolates than in the greenhouse. In the greenhouse due to extreme environmental conditions, the effectiveness of isolates was reduced which resulted in lower efficacy. When compared, the entomopathogenic nematodes performed better-controlling agents than the fungus in both tested conditions. The entomopathogenic nematodes were also able to infect and kill the larvae in a shorter period than the fungus. Between two isolates of entomopathogenic nematode used *Heterorhabditis indica* isolate AUT13.2 was more effective than *Steinernema siamkayai* isolate APL12.3.

6.2. Limitation of the study

The current investigation was carried out with *B. bassiana* isolates (TBRC 2781 and TBRC 4755) and indigenous entomopathogenic nematodes *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3. These isolates were never tested against Fall armyworm and there was limited knowledge on how these isolates would perform against this new pest.

6.3. Future work

The result of our investigation with entomopathogenic fungus *B. bassiana* isolates (TBRC 2781 and TBRC 4755) and indigenous entomopathogenic nematodes *H. indica* isolate AUT 13.2 and *S. siamkayai* isolate APL 12.3. is based on the mortality of second and fifth instar larvae of Fall armyworm under laboratory and greenhouse conditions. Therefore, in the future, we would like to suggest other researchers conduct research and compare the susceptibility of other stages of the Fall armyworm like eggs, prepupae, pupae, and adults against the isolates used in our study. The effectiveness of these isolates also needs to be tested under field conditions. When *B. bassiana* TBRC 2781 and *H. indica* isolate AUT 13.2 were combined they gave lower percentage mortality, and the underlying mechanism of the interaction needs to be thoroughly investigated and production of inhibitory compounds since this will help determine which isolate would be combined for pest management. There are many isolates of entomopathogenic fungus and nematodes that are indigenous to Thailand and in the future, similar studies can be done to find more virulent isolates so that they can be effectively used as a biological control agent.

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