EVALUATION ON PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* ON THE ROVE BEETLE, *Paederus fuscipes* CURTIS

by

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Thesis submitted in fulfilment of the requirements for the degree of Master of Science

December 2018

ACKNOWLEDGEMENT

I would like to thank my supervisor, Associate Professor Dr. Wan Fatma Zuharah, for the patient guidance, encouragement and advice she has provided throughout my time as her student. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly. I continually felt thankful by her willingness to proof read countless pages of my work and help me to do so much improvement all the way round. I would also wish to express my gratitude to my co-supervisor Associate Professor Dr. Hideyuki Nagao for his guidance and advice on my research work. Especially at the part deal with the fungi. It was a great honor for me to work under their guidance.

I would also like to thank the staff of the School of Biological Sciences for their helping in my study. My sincere thanks also to all my fellow friends, which are Maryam, Ahbirami, Rohaiyu, Dinesh, Katherine, Syuhada, Meha, Fatin, and Sumayyah. I appreciate the friendship and memories we had together.

Besides that, I would definitely like to express my gratitude and thanks to Mr Mohd Fadzli, Ms Noor Shafawati, Mr Hamzah and Ms Manorenjitha which guide me a lot during my first and second semester when I worked as a lab assistant for the genetic course. It was the most memorable moments, which I able to have the chances stand in front of many students to teach and conduct the practical sessions. At the same time, I would like to say thanks to my fellow students in the genetic course by accompany me and listen to my lecture in the laboratory. Until now, I still nostalgic those moments with you all together. It was my pleasure to be your teacher during your all first semester.

Finally, and the most important, I would like to say thanks to these persons, my mother, my other beloved half and their family members. I am very lucky to have my mother who supports me a lot during my master's degree journey; I always understand that you want respect and me to be the person who can stand tall by others. I hope that I as your eldest son able to make you feel proud and when I will live happily and work for my career at the not long further time, you can support me in spirit and rest assured I would always be a better man. Then, to my beloved person, Lock Mei Chen. I am very lucky to have you accompany me along the whole journey of my master's degree. Knowing and be together with you could be the most lucky the destiny arranged for me. Although we have unhappy moments, but mostly you make me happier precious moments at all time. Travel from Johor to Penang here to study was not easy to me; however I does not have worries on accommodation and meals at the very beginning as well as the feeling of warm family I felt at here. These must specially thanks to Madam Cheng Swee Heok who not selfish and treat me as one of her family members always, your delicious food that accompanies so long would never be forgotten. In addition, the moments and time together with my beloved Lock Mei Chen's brother and sisters are part of the great moments to me.

For all the persons dedicated here, I truly wish for your support towards me. You all will always be in my memories during my Master's degree journey until the eternal.

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ABSTRAK

Kumbang rove, *Paederus fuscipes* Curtis adalah agen kawalan biologi kepada perosak tanaman dalam agro-ekosistem, tetapi kadar pencerobohan yang tinggi ke kawasan perumahan manusia menyebabkan mereka menjadi kebimbangan kepada kesihatan awam disebabkan oleh infeksi Paedarus dermatitis pada manusia. Kajian ini dilaksanakan bertujuan untuk menilai keberkesanan kulat entomopatogenik, *Metarhizium anisopliae* sebagai agen kawalan biologi terhadap *P. fuscipes*. Kepatogenan kulat entomopatogenik, *M. anisopliae* terhadap kumbang *P. fuscipes* telah diuji dengan menggunakan tiga teknik; jangkitan melalui tanah, infeksi umpan dan transmisi melintang melalui kelakuan endo-kanibalisme. Pertama, kepekatan suspensi konidia dari 10^6 hingga 10^{10} konidia/ml telah diinokulasi ke permukaan tanah yang telah sterilkan dan *P. fuscipes* diletakkan di dalam tanah tersebut secara berasingan. Kepekatan paling tinggi iaitu 1.3×10^{10} menyumbang kepada kepatogenan yang hebat dengan mencapai 100% kematian paling cepat dan purata masa kematian yang signifikan rendah (10.2 ± 1.28 hari) berbanding dengan kawalan pada $P < 0.001$. Bangkai *P. fuscipes* yang diinkubasi dijumpai mempunyai mikosis kulat *M. anisopliae*. Kajian kedua yang telah dilakukan adalah dengan inokulasi konidia *M. anisopliae* berdasarkan umur 7, 10, 13, 16, 19 dan 22 hari kepada lipas (*Nauphoeta cinerea*) yang bertindak sebagai umpan makanan dijangkiti kepada kumpulan *P. fuscipes* secara

berasingan. Konidia berumur hari-16 menunjukkan kadar kematian 100% yang terpantas dengan purata masa kematian signifikan rendah (11.7 ± 1.22 hari; $P < 0.05$) berbanding dengan kawalan, walaupun paling kurang jumlah berat umpan dimakan (13.3%). Secara keseluruhan, *P. fuscipes* tidak menunjukkan tanda pengelakan terhadap umpan makanan yang telah terjangkit dengan kulat. Kajian ketiga adalah memerhatikan transmisi kulat melalui tindakan kelakuan endo-kanibalisme. Bangkai *P. fuscipes* yang dijangkiti melalui inokulasi kulat konidia berbeza umur (Hari 7, 10, 13, 16, 19 dan 22) dan diperkenalkan kepada kumpulan *P. fuscipes* lain yang sihat secara berasingan. Kumpulan rawatan hari 16 memberi hasil yang terbaik kerana menyumbang purata masa kematian yang tercepat secara signifikan pada 10.2 ± 1.80 hari berbanding dengan kawalan ($P < 0.05$). Secara keseluruhan, *P. fuscipes* yang sihat tidak gemar melakukan kelakuan endo-kanibalisme terhadap bangkai *P. fuscipes* yang dijangkiti dengan kulat konidia *M. anisopliae*. Kumpulan rawatan hari 7 menunjukkan tindakan endo-kalibalisme yang tertinggi sebanyak 60%, manakala hari 13 menunjukkan tiada endo-kalibalisme yang terjadi. Melalui perbandingan ketiga-tiga teknik, kami mendapati teknik jangkitan melalui tanah adalah cara paling efektif kerana mampu memberikan kepatogenan yang tinggi serta berjaya dalam mentransmisi kulat kepada *P. fuscipes*. Oleh itu, kulat *M. anisopliae* telah menunjukkan keupayaan sebagai agen kawalan biologi dan boleh digunakan dalam strategi Intergrasi Perosak Bersepadu pada masa hadapan.

EVALUATION ON PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* ON THE ROVE BEETLE, *Paederus fuscipes* CURTIS

ABSTRACT

Rove beetle, *Paederus fuscipes* Curtis is a natural predator of several crop pests in agricultural ecosystem, however their high intrusion into human settlements causes them to become a public health concern due to Paedarus dermatitis infection among human. This study was undertaken to investigate the effectiveness of *Metarhizium anisopliae* fungi as a biocontrol agent for *P. fuscipes*. The pathogenicity of entomopathogenic fungi, *Metarhizium anisopliae* against the beetle *P. fuscipes* were tested using three techniques; soil infection, bait infection, and horizontal transmission via endo-cannibalistic behaviour. Firstly, the conidia suspension concentrations from 10^6 to 10^{10} conidia/ml were inoculated to the sterilized soil surface and *P. fuscipes* were placed inside that soil separately. The highest concentration of 1.3×10^{10} contributed excellent pathogenicity effect, which was fastest to reach full 100% mortality, and significantly shortest mean mortality time (10.2 ± 1.28 days) compared with the control at $P < 0.001$. The *P. fuscipes* cadavers were incubated and found the presence of fungi mycosis. The second study was performed by inoculating *M. anisopliae* conidia age of 7, 10, 13, 16, 19 and 22 day, on the cockroaches (*Nauphoeta cinerea*) which was served as treated food baits to *P. fuscipes* separately. The Day 16 conidia age showed the fastest to cause the 100% mortality with significantly shortest mean mortality (11.7 ± 1.22 days; $P < 0.05$) as compared to control, even at the least total bait consumption weight recorded (13.3%). Overall, the *P. fuscipes* showed no

sign of repellency towards the fungi inoculated on food baits. The third study was to observe the transmission of the fungi through their endo-cannibalistic behaviour. The infected *P. fuscipes* cadavers was inoculated with different age of conidia (Day 7, 10, 13, 16, 19 and 22) and introduced to another group of healthy *P. fuscipes* separately. The Day 16 treatment group gave the best outcome as it contributed the significantly fastest mean mortality time of 10.2 ± 1.80 days as compared to control ($P < 0.05$). Overall, the healthy *P. fuscipes* does not prefer to perform an endo-cannibalism behaviour on the *P. fuscipes* cadaver that was infected with *M. anisopliae* conidia. The Day 7 treatment group has performed the highest endo-cannibalism at 60%, while Day 13 showed none endo-cannibalism occurred. By comparison, of the three techniques, we observed that soil infection technique was more effective since it is able to provide high pathogenicity and successful transmission of fungus towards the *P. fuscipes*. Therefore, this *M. anisopliae* has shown its potential as a biocontrol agent and can be used in Integrated Pest Management strategies.

CHAPTER ONE

INTRODUCTION

Coleoptera is the largest order in the class Insecta and has occupy more than quarter of all the identified organisms in the universe (Powell, 2009). Some of the families of Coleoptera have been studied in distribution, biological and ecological aspects because of their toxic products that causes human dermatitis in many different countries. The various kind of ill effects they produce were ranged from the instant wound of bite to the effects of blister beetle's products that induces the different forms of skin dermatitis (Ghoneim, 2013). There are only three families of Coleoptera known capable of releasing the vesicant chemicals including Oedemeridae, Meloidae, and Staphylinidae, the first two releases the toxin named Cantharidin and the latter releases Paederin (Gnanaraj et al., 2007).

Rove beetles from the family Staphylinidae are the second largest family of beetles after the Curculionidae (True weevil). Their elongated body form and the short elytra identify these beetles, which makes part of their abdomen visible (Lott and Anderson, 2011). *Paederus* beetles are considered as a useful insect in different agriculture fields because of their natural behaviour of consuming numerous smaller insect pests. It is recognized as one vital biocontrol agent on the important rice pests, they prefer to prey on and scavenge on the soft-bodied pests such as whitefly, planthoppers and green leafhoppers of many different crops (Nasir et al., 2012). The adult *Paederus* spp. is highly active during the daytime to look for their prey, while at night they are attracted to the sources of building lights that indirectly brings them to be contact to humans (Frank and Kanamitsu, 1987). Although the beetles do not bite and sting humans, but inside the haemolymph of these beetles contains a blistering chemical, the 'paederin' ($C_{24}H_{43}O_9N$), produced by the endosymbiont bacteria closely similar with *Pseudomonas aeruginosa* and largely found in adult beetles (Kellner, 2002; Piel, 2002). Paederus dermatitis (PD) is a severe skin disease when *Paederus* beetles are crushed on the skin that causes irritation and blistering effect to the skin. This dermatitis is world-widely recognise as causing serious health threat to the public (Nasir et al., 2015). Since the 1990's, *Paedarus fuscipes* beetle has become a public health threat due to many outbreaks that were reported worldwide. The PD was most often reported in the regions with hot tropical climate except for Antarctica as no *Paederus* species was discovered in the extremely cold weather condition (Frank and Kanamitsu, 1987).

Paederus beetle are endemic in Southeast Asia (Khan et al., 2009) and the first cases of PD in Malaysia were noted during year 1919 (Raju, 2002). Ever since then the number of *P. fuscipes* beetles has become abundant and received highly public attention within the country. The species reported by the Seberang Perai Municipal Council (MPSP), Malaysia that causes the PD is *Paederus fuscipes* Curtis also locally known as 'Semut Semai', 'Semut Kayap', or 'Charlie'. The *P. fuscipes* are abundant in paddy fields, that causes it to be present in large number in Penang (Ahmad et al., 2010; Bong et al., 2013), Kelantan (Mokhtar et al., 1993), Terengganu (Rahmah and Norjaiza, 2008), and Selangor (Heo et al., 2013) state. The tropical climate of Malaysia has become an important rice growing region, which makes it an ideal habitat for the Rove beetle (Khan et al., 2009). According to Bong et al. (2013a), massive dispersal of *P. fuscipes* was due to human activities during the stage of the crop harvesting which disturbs their habitats. In addition, environmental factors contributed to their peak dispersal periods, under the ideal climatic condition the onset dispersal flights of most insects are stimulated (Neoh and Lee, 2009). *Paedarus fuscipes* infestation is not only limited to land houses but the high-rise apartment buildings as well (Raju 2002; Huang et al., 2009).

Chemical insecticides have been used widely by several application methods in different agricultural fields for the control of economic important pest insects for many years. Until now, the control methods towards the swarming of rove beetles near the rural and urban areas in many countries including Malaysia are through applying insecticides via thermal fogging, target spraying using commercial pest killer products or glue traps for household use (Raju, 2002). Constant usage of insecticides for controlling insect pests by applying to ground or aerial is known to cause problems such as groundwater contamination and destruction of soil fauna (Ghany, 2015). Most importantly, over use of insecticides could cause adverse effects towards non-target organisms and human beings. In addition, the unmonitored usage of many herbicides, fungicides and insecticides may create chemical-resistant beetles (Shahid et al., 2012), resulting in the phenomenon of a massive number of *P. fuscipes* beetle populations in Malaysia. These problems have driven those related industries and scientists to pay attention on the development of alternative measures to control the *P. fuscipes* infestation into affected household areas.

In recent years, the biological control method as alternative way that includes entomopathogenic fungi (EPF) were implemented. Although these were strongly emphasized and encouraged in other countries, it is not yet implemented in Malaysia. The use of EPF as bio-control agent is known for its benefits for controlling insects because it creates little environmental impact and averting the evolution of resistance among the vector. The utilization of EPF can replace the repetitive application usage of some insecticides for controlling insect pest because fungi show persistence and able to provide prolong pest control (Singh et al., 2017). Besides, the application of

EPF are non-toxic and less hazardous. Humans and non-target organisms are safe from infection; decreased the pesticide residues in food and maintains the biodiversity in managed ecosystems (Shahid et al., 2012). Amongst these fungi, one of the most frequently used in commercial products and the work of research is *Metarhizium anisopliae*, the 'green muscardine' fungi. One of the famous soil inhabiting EPF known worldwide. It is a Deutromycetes belonging to Hyphomycetes from the taxonomy profile. The EPF *M. anisopliae* known as the natural enemies to vast insects and arachnids (Roberts and Leger, 2004). The fungus *M. anisopliae* have been use in many researches to study their mode of infection and transmission through several strategies to exhibit pathogenicity and virulence towards important insect pests. The EPF *M. anisopliae* being studied and shows the potential in pest management strategies. However, the effectiveness of *M. anisopliae* in controlling *Paedarus* beetle has not been yet discovered anywhere as to our knowledge. Therefore, the main aim of this study is to evaluate the pathogenicity effectiveness for EPF *M. anisopliae* towards the *P. fuscipes* beetles through control methods under laboratory conditions.

Objective

1) To evaluate the pathogenicity of entomopathogenic fungi, *M. anisopliae* inoculated into treated soil as a source of infection towards *P. fuscipes*.
2) To examine the toxicity of *M. anisopliae* inoculated to cockroach (*Nauphoeta cinerea*) as food bait against *P. fuscipes*.
3) To study the transmission potential of *M. anisopliae* from infected *P. fuscipes* cadavers towards uninfected *P. fuscipes* based on their endo-cannibalism behaviour.

CHAPTER TWO

LITERATURE REVIEW

2.1 General introduction of rove beetle genus *Paederus*

Beetles are the highest number of species that have identified and described which makes the beetles the largest order of insects in the world (Mullen and Durden, 2002). The presence of elytra (wing covers) are the most distinct characteristic for beetles, but some family group of the Coleoptera such as Staphylinidae, have soft elytra and body parts that makes them flexible. There are three major families of soft-bodied beetles named: Oedemeridae (False blister beetle), Meloidae (Blister beetle) and Staphylinidae (Rove beetle).

Staphylinidae is the largest family among the beetles, with over 63,000 species identified (Howard and Thomas, 1999). They are agile insects, which move swiftly and acts as active predator and scavengers of aphids. The rove beetle of the genus *Paederus* has more than 600 species described (Nasir, 2011). The genus *Paederus* is widely distributed in the world, especially in all temperate and tropical regions (Frank, 1988) with the exception in Antarctica (Frank and Kanamitsu, 1987). Most of the Paederus beetles have the morphology of short elytra makes part of their abdomen revealed compared with the common beetles which the elytra cover their whole abdomen (Figure 2.1). However, not all the beetles with the short elytra are belonged to the family Staphylinidae (Howard and Thomas, 1999).

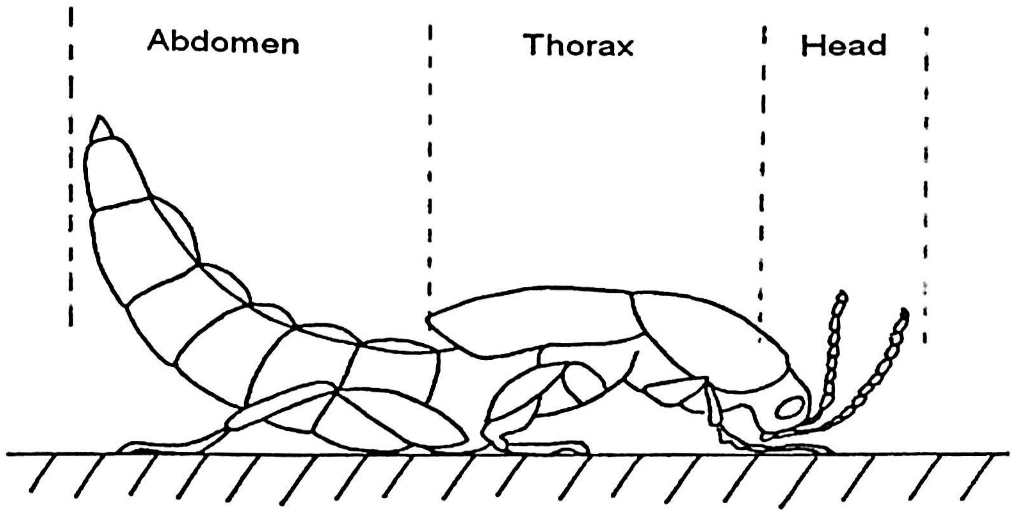


Figure 2.1: General morphology of rove beetle

Adult *Paederus* beetles are small-elongated insects often mistaken for ants (Cressey et al., 2013). They neither bite nor stings their victims, which in the past some authors incorrectly have written about this (Cressey et al., 2013). The *Paederus* beetle species are very abundant, especially in tropical countries. The genus *Paederus* was getting high attention in the research field due to certain *Paederus* species beetles (Paederinae) capable releasing a compound called 'Paederin', which cause them to become a serious health threat to humans in many countries.

They have wings present and could perform flight for some distance, sensitive to artificial lights, which attracted them to fly and gather around the light sources near their habitat. In Malaysia, rove beetles are abundant in paddy fields and marshes land (Khan et al., 2008). These rove beetles are commonly known as the 'Beetle Tomcat', 'Skirt and Blouse Beetle' and the term 'Ch'ing yao ch'ung' was used to describe Paederus beetle in China (Cressey et al., 2013). Their bright orange color body pattern somehow exhibited the aposematic coloration warning (Mullen and Durden, 2002).

2.2 Life Cycle

Paederus beetles in temperate continents have a single annual breeding season in the warmer periods, but the breeding season of beetles in tropical regions appears to depend upon rainfall (Ghoneim, 2013). Beetle *Paederus fuscipes* undergo complete metamorphosis (holometabolous) which includes egg, larvae (two instars), pupae and adult stages (Manley, 1977). The number of generations varies with climate (Cressey et al., 2013). Overall, the immature stages develop rapidly range from two to three weeks, and the adult stage was longevity for several months and produce two or more generations per year (Manley, 1977; Howard and Thomas, 1999).

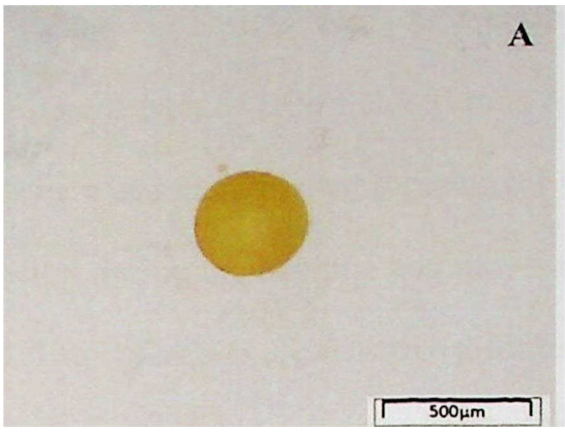
Typical rove beetle life cycle begins with female adults laying eggs in small clutches near potential food sources and moderately moist habitats on the soil litter, laying eggs singly (Nikhita et al., 2014). Adult rove beetles preferred moist areas for laying their eggs to prevent eggs and larvae from desiccation (Bong et al., 2012). According to Nasir and Akram (2012), these beetles avoid the dry soils to lay their eggs throughout the season, even if the soils contain potential organic matter. This highlight that rove beetle life cycle is highly associated with moist habitat conditions.

The *P. fuscipes* eggs is a spherical shape with yellowish white or light orange color (Figure 2.2A). Egg incubation period ranged depends on the surrounding environment in nature, but in the laboratory rearing conditions, the egg incubation period until hatching was ranged from four to six days (Bong et al., 2012).

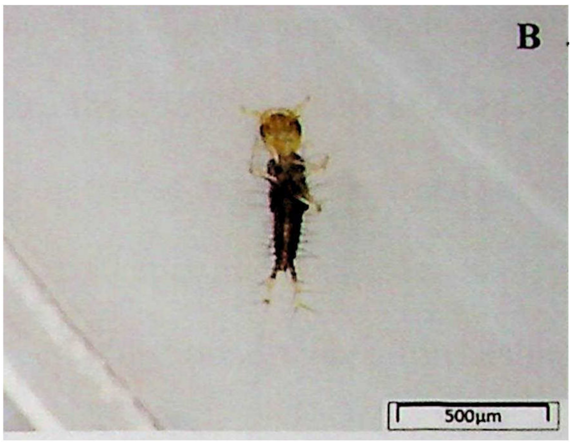
Larvae of *P. fuscipes* are mostly campodeiform; meaning have well developed legs, head, antennae, thorax and abdomen with segments. Their shape is elongate and cylindrical but slightly flattened, white brownish head capsule, three pairs of notable legs and ten segments with two slim projections at the abdomen tip. The newly emerged larvae are soft-bodied and fragile; the larvae need more humid conditions compare to their adults stage. Based on Bong et al. (2012) in laboratory rearing condition, first larval instar (L1) development range from three to five days to reach second larval instar (L2) (Figure 2.2B). Then, the L2 larvae develop until pupae range from five to seven days (Figure 2.2C). The larval instars are predatory same as the adult *P. fuscipes*. Both L1 and L2 larvae are highly active and seek for food in sheltered areas after emerging. The very small size larvae feeds on dead soft-bodied insects or organic matters.

Pupae of *P. fuscipes* are obtect and sclerotized. The body structure consists of three pairs of obviously elongated legs, chewing jaws parts, well-developed head with red orange compound eyes and pairs of antennae (Figure 2.2D). The pupae body are yellowish white color. During this stage, it does not require any feeding. Yet, the moist condition is essential for the pupae to emerge as the adult beetles (Ghoneim, 2013). The pupae stage lasts for three to four days under laboratory conditions and developed as an adult beetle (Bong et al., 2012).

Adults of *P. fuscipes* are typical rove beetles, elongate body shape, usually 7 to 10 mm long and 0.5 to 1 mm wide (Gnanaraj et al., 2007), commonly consisting of the dark color head, orange and black thorax, metallic blue elytra, orange abdomen and black tips with two projections on the tip of the abdomen (Figure 2.2E). The adult *P. fuscipes* are predators of soft bodied insects (Nikhita et al., 2014). Bong et al. (2012) recorded the adult *P. fuscipes* could live for one to two months long, and the female lays substantial numbers of eggs in her lifetime under laboratory conditions. While Laba and Kilin (1994) mentioned that the adult longevity of both male and females was more than 100 days under laboratory conditions. It was calculated that *Paederus* required only about 40 days to reach maturity and produce a new generation of offspring (Bong et al., 2012).

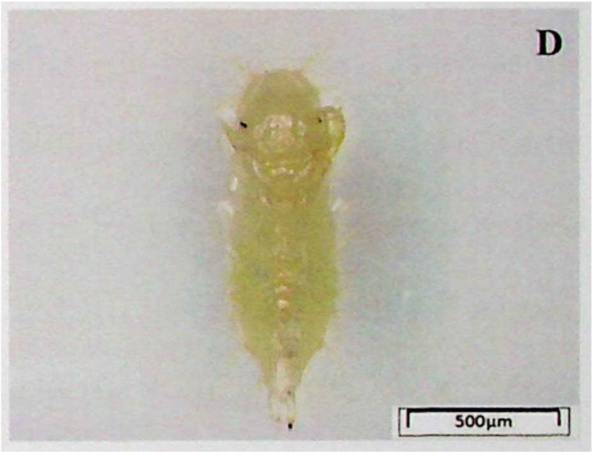


Egg stage

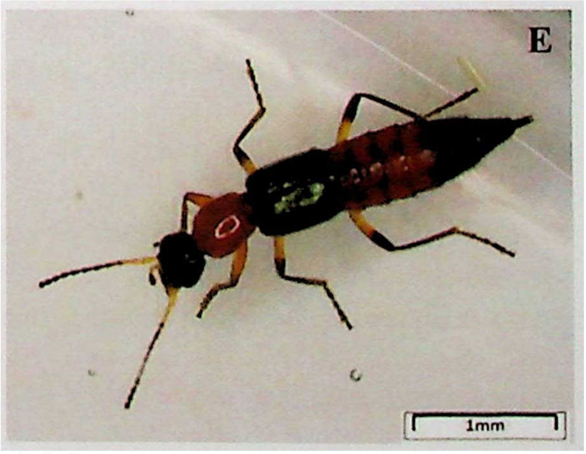


First larval instar stage

Second larval instar stage



Pupal stage



Adult stage

Figure 2.2: The morphology of immature stages of the *Paedarus fuscipes*; (A) Egg, (B) first instar larva, (C) second instar larva, (D) Pupal stage and (E) Adult stage.

2.3 Role and Behaviour in the Environment

Paederus beetles were found inhabited mostly at slightly moist environments at a different type of crop fields (Frank and Kanamitsu, 1987). Adults of *Paederus fuscipes* are commonly found foraging for food at the leaf of the rice plants and search for shelter during the daytime and move actively at night at the rice fields (Manley, 1977). The *P. fuscipes* are sensitive towards the light; at nighttime, those artificial light sources from the buildings nearby their shelter have triggered the beetles to disperse towards those light sources through flights (Davidson et al., 2009). Indirectly bringing them into contact with human beings.

Paedarus beetles are mostly predaceous on soft-bodied insects, such as the aphids that are found abundantly in the agricultural crops (Figure 2.3). Large populations of *Paederus* beetles have been observed in agricultural fields due to their natural feeding choices on the pests of different crops (Figure 2.4) (Nasir and Akram, 2012). More than 20 different pests were predated by the *P. fuscipes* (Devi et al., 2003). They particularly fed on insect pests such as the rice moth, cotton aphid, rice leaf folder, soybean aphid, fruit fly, green leafhoppers, zigzag leafhoppers and brown plant hopper in different crops (Manley, 1977; Frank and Kanamitsu, 1987; Berglind et al., 1997; Krakerb et al., 2000; Devi et al., 2003; Nasir et al., 2012; Ghoneim, 2013).

In a laboratory study, the lobster cockroach (*Nauphoeta cinerea*) was provided as protein-rich food sources to the *P. fuscipes* beetle (Bong et al., 2012). The great variation in *P. fuscipes* feeding behaviour has reflects these insects were live in great diverse habitats. Overall, their food intake includes many varieties of insect pests apart from the living tissues of the higher plants.

Although rove beetle *Paederus fuscipes* Curtis resembles the ants but they do not live as social insects in the natural environments. Cannibalism occur among their populations. Another unique behaviour of these *Paederus* beetles is they often raise their end of the abdomen to resemble the scorpion when they are disturbed or threatened. These beetles were highly sensitive to the change of weather conditions. During the rainfall or windy conditions they would hide in their shelter areas (Ghoneim, 2013).

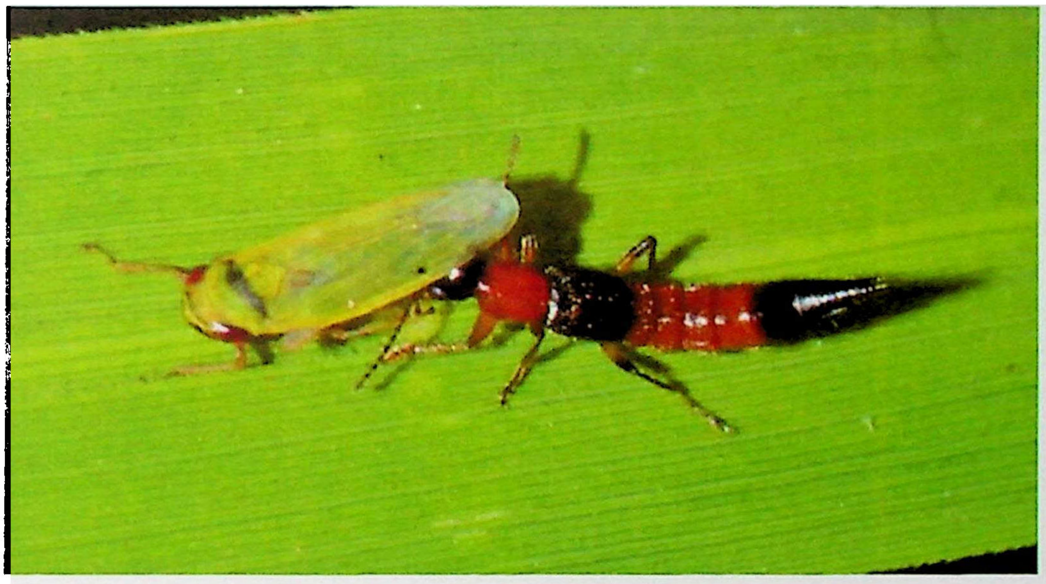


Figure 2.3: The *P. fuscipes* beetles prey on the green leafhopper. Retrieved from: http://www.nbair.res.in/Featured_insects/Paederus-fuscipes.php

Figure 2.4: *P. fuscipes* beetle found at the edge of a rice field. Retrieved from: http://natural-japan.net/?p=92

2.4 Medical Importance (Paederus Dermatitis)

There are only three different genera from Staphylinidae able to cause Paederus Dermatitis (PD) including *Megalopaederus*, *Paederidus* and *Paederus* (Capinera, 2008). While the primarily focus genus *Paederus* has hundreds of species described, but less than half of them only contributed to the PD problems (Mullen et al., 2002). These species include *P. fuscipes* in Asia and Europe, *P. sabaeaus* in Africa, *P. columbinus* and *P. brasiliensis* in South America. However, this does not limit to only one species present at the continents (Cressey et al., 2013).

The hemolymph in the beetle's entire body (except the wings) contains the very poisonous contact toxin called 'paederin' (C_{24} H_{43} O_9 N) named in 1953. It was described as 15 times poisonous than venom from cobra and capable of maintaining its toxicity even after the beetle has been dried for few years (Verma and Agarwal, 2006). It has been proven that the *Paederus* beetles do not produce the paederin by themselves but rely on the activities of the endosymbiont bacteria species that has closest relationship with *P. aeruginosa* present within the beetles (Kellner, 2002). Not all species of the genus *Paederus* contain the endosymbiotic bacteria (Kellner, 2003).

Female *Paederus* beetles infected with the bacteria will produce eggs containing with paederin in the outer shell of eggs; it was transmitted vertically to the offspring because paederin circulates in the haemolymph of beetles throughout their development process (Ghoneim, 2013). The non-producing female is capable of producing offspring with paederin and male beetles store the paederin after acquire these endosymbiont bacteria through ingesting the infected egg shells or cannibalizing larvae containing the bacteria (Piel, 2002; Nikbakhtzadeh and Tirgari, 2008).

PD symptoms begin between 1 to 2 days after contact with the paederin. Paederin was released when these beetles land on the human body part and the victims are accidentally brushed or crushed them while on the skins. Paederin penetrates the skin without any abrasion, meaning the toxin could penetrate into the skin without any need of skin scraping or infected to injured parts. Most common symptoms were the redness, burning sensation and the pain at the affected site (Khan et al., 2008). The affected part was developed to erythematous lesions, followed by some vesicles appeared at the center of the brass and became a pustule which hurt if broke it (Figure 2.5).

Figure 2.5: Paederus dermatitis infection on the victim's hand (A), neck (B), eye (C) and kissing lesion on elbow flexure (D). Redness and swelling symptoms shown.

2.5 Occurrence of Paederus Dermatitis

The skin effects triggered by the paederin have few common names used worldwide, but the frequently used name is dermatitis linearis and Paederus Dermatitis (PD) (Nikbakhtzadeh and Tirgari, 2008; Ghoneim, 2013). During the past, researchers studied regarding the outbreak of this dermatitis in most countries blamed the PD on other insects such as spiders, caterpillars or moths (Raju, 2002).

PD has been spread in different parts of the world and outbreaks were reported in more than 50 countries with mostly comprising of the country with tropical climate (Ghoneim, 2013). According to many case reports of PD, the outbreaks commonly happened in residential areas, which close to the *Paederus* beetle habitat or shelter. The outbreaks usually took place once in seasonal countries every year. However, more than once outbreaks has been reported in the tropics countries. The dispersal of adult *Paederus* beetles was related to the change of temperature and mostly happened during the temperate weather months (Frank and Kanamistu, 1987) in seasonal countries. As the weather conditions in the tropical country are hot or warm and humid throughout the year, therefore dispersal elicited when their shelter was seriously disrupted and forced to escape from their living areas (Bong et al., 2015).

The earliest case reported in Malaysia was as early as the year of 1919 (Raju, 2002). There are many cases of PD outbreaks were reported at the north region in mainland Penang primarily at Permatang Pauh, Kepala Batas and Seberang Perai areas every year since 2004 (Bong et al., 2015). While for Penang Island the common outbreaks were reported at Ayer Itam and Gelugor areas. In 2002, two thousand people living in high-rise flats and dormitories in Penang were affected by PD (Heo et al., 2013). Besides, PD also occurred among 12 medical students at Universiti Sains Malaysia, Kubang Kerian, Kelantan (Mokhtar et al., 1993). More than 36 cases in one primary school at Kuala Nerus, Terengganu (Rahmah and Norjaiza, 2008). A total of 360 cases were recorded in the residential college in Puncak Alam, Selangor near an oil palm plantation (Heo et al., 2013). It should be highlight that the incidence of outbreaks is underestimated, as there are still many cases are unreported.

2.6 Control methods of *Paedarus* Beetle

The contradictory roles of *P. fuscipes* as insect pests regulators in the ecosystem and agriculture fields but poses serious health problems towards human when getting into contact causes limited efforts being implemented to control *Paederus* beetles in rice fields. The control towards swarming of *P. fuscipes* beetles was by applying insecticide or commercial pest killer products such as Pesguard FG-161 (d-tetramethrin 4.4% / cyphenothrin 13.2%) (Raju, 2002), aerosol insecticide (Rahmah and Norjaiza, 2008), Baygon (2-isopropoxyphenyl N-methylcarbamate) and Malathion (Gnanaraj et al., 2007) via thermal fogging or direct spraying. However, these methods need a continuous application as this just provide only temporary elimination (Bong et al., 2015).

Those infested housing areas have the insect-proof mesh mounted to windows and doors for preventing *Paederus* beetle enter into the house, however the elongated and slender body of these beetles could burrow to narrow crevices. The sticky traps are placed beside the fluorescent lamps to manage *Paederus* beetles even this method could not capture all the beetles but may partially decrease the incidence of contact with them (Bong et al., 2015). In addition, overall public awareness was promoted and help to decrease the incidence of paederus dermatitis by informing the public do not brush but blow away the *Paederus* beetles if landed on the body parts.

2.7 Biological Control as Alternative Measure

The high concerns on the environment and health problem created by great application of chemical insecticides to control insect pest become more serious. The development of resistance somehow was developed in those important urban pests including the *Paederus* beetles. Therefore, these problems had led to an interest in the development of alternative methods for the control of insecticides resistant insects (Rahimzadeh et al., 2012). During these years, the chemical pesticides have become less preferred because of the increased cost; more amount has to be used in crop fields; the problem of human health hazards and side effects towards the non-target species. There has been great interest in biological control measures to replace the use of the chemical pesticide. The interest is especially being focused on the development of microbial biocontrol agents or better known as Entomopathogens, which includes certain species of fungi, was highly suggested as controlling agents of insect pests.

2.8 Entomopathogenic Fungi

A group of fungi that kill an insect by attacking and infecting its insect host called as entomopathogenic fungi (EPF) (Singkaravanit et al., 2010). EPF had occurred in diverse habitats and had a broad range of insect as susceptible hosts (Ramachandran et al., 1997). History of control insects by using the EPF was done by Augostino Bassi's in early of 1835, showed the successful transmission of EPF and induced disease in silkworm (Steinhaus, 1956). Successful control using EPF to fight against the insect pests whether presents at terrestrial and aquatic environments have been developed since then by employing fungi from the list of genera such as *Metarhizium*, *Beauveria*, *Isaria* and *Enthomophthora* (Alves and Lopes, 2008).

The majority of EPF identified was belong to these classes including: Zygomycetes, Pyrenomycetes, Laboulbeniales and Hyphomycetes (Ghany, 2015). The EPF occur naturally was found at insect hosts through infections and was collected from the field, incubated under laboratory conditions for identification. Overall, they able to infect a wide range of insect pests, but there are strains of fungus that are host specific.

2.9 General description of *Metarhizium anisopliae*

Metarhizium anisopliae, one of the well-known soil EPF has great pathogenicity potential on many insect pests (Tajick et al., 2009). The use of *M. anisopliae* for the biological control of insect pest was firstly reported in the early of 19th century, the Russian scientist Metschnikoff started with his first practical by used *M. anisopliae* to control the insect pest, the wheat cockchafer in 1879 (Samson et al., 1998). The research works done since the past has inspired many studies and implemented products based on entomopathogens and commercially used. There have been numerous studies on the infection mode of the *Metarhizium* spp. on many important arthropod pests (Aw and Hue, 2017). In these few years, *M. anisopliae* is used to control many insect pests including termites, cockroaches, soil-dwelling pest insects, whiteflies, mosquitoes and even ticks (Zimmermann, 2007).

It is also known as the 'green muscardine' fungus because of the dark green color sporulating colonies found on the insect host body or different medium cultures (Figure 2.6). Fungal spore is an elongated shape from the view of the scanning electron microscope (SEM) (Figure 2.7). The *M. anisopliae* do not grow saprophytic within or under the soil surface but existed as the dormant conidia which then infect susceptible hosts when they get into contact with the spores by attaching to the cuticle surfaces (Tiago et al., 2014).

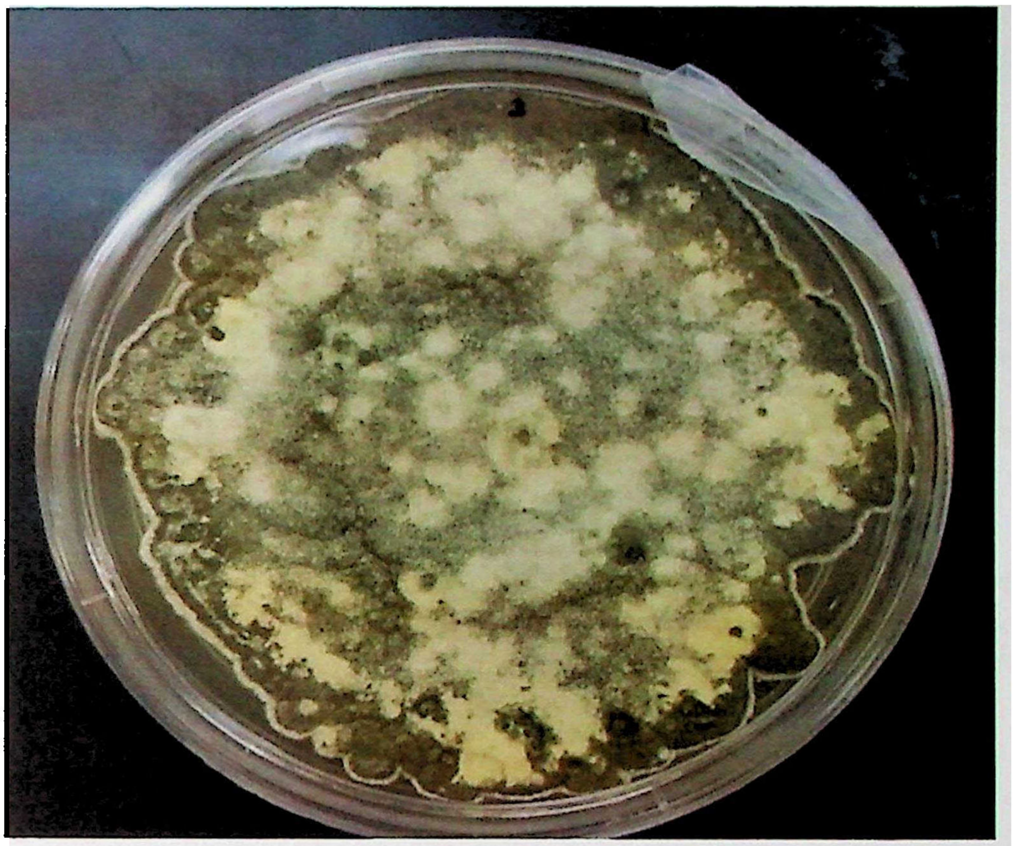


Figure 2.6: Morphology of the colonies, *M. anisopliae* on the Potato Dextrose Agar (PDA) plate

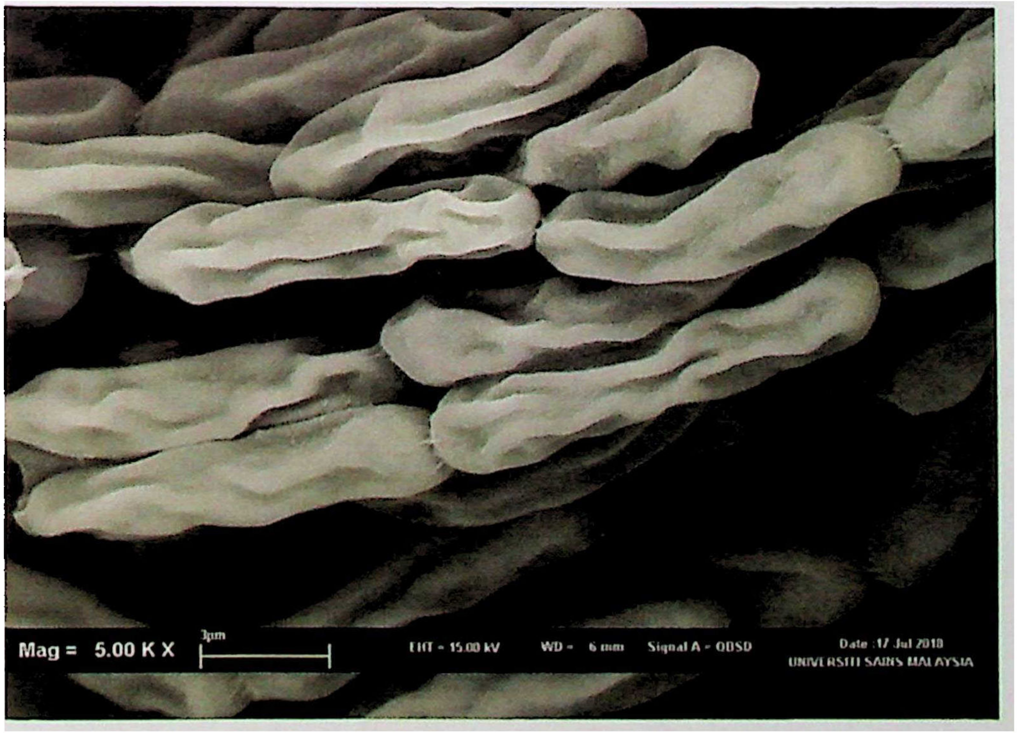


Figure 2.7: Morphology view on the dry conidia of *M. anisopliae* under Scanning Electron Microscope (SEM).

2.9.1 Infection of *Metarhizium anisopliae*

Metarhizium anisopliae and the active compounds it generates do not appear to be harmful to human, other mammals, fish, useful insects such as honeybees, or plants (Ritter, 2006). *M. anisopliae* infects susceptible hosts via direct penetration through the cuticle, and the process as shown in Figure 2.8.

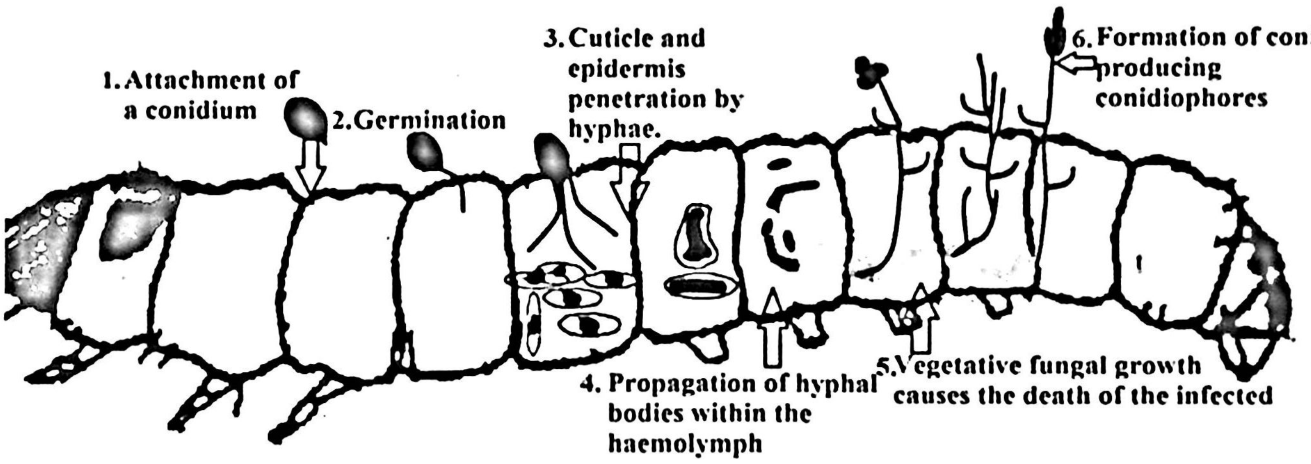


Figure 2.8: General action Mechanisms of Entomopathogenic Fungi.

Generally, the fungus penetrates the insect's host from the outer parts like joints between segments, even though infections through the buccal cavity in beetles and the siphon tip in mosquito larvae or the gut have also been reported. The hydrophobic dry conidia of *M. anisopliae* and interactions between cuticle surfaces are responsible for adherence of the spore (Boucias et al., 1988). Germination and successful infection relied on factors included the susceptibility of insect, the life stages of that hosts and abiotic factors. In addition, successful germination is influenced by the level of nutrients present in the insects and suitable humidity as well (Dillion and Charnleya, 1991). Ibrahim et al. (1999) suggested that *M. anisopliae* conidia need a high level of humidity to germinate. The barrier of cuticle lipids on the insects mostly consisted the antimicrobial agents are another difficulty need to overcome for successful germination of conidia. However, the cuticle contained the crucial substances that highly associated with fungal recognition such as free amino acids that necessary to trigger the attachment and germination. Before the penetration, germinated conidia of *M. anisopliae* are produced germ tube term as 'appressorium', which then develop the peg for the penetration. The penetration process is supported with the making of some enzymes include the proteases, chitinases, and lipases. After successful penetration of the host insect, the fungus produces blastopores or hyphal bodies, which distributed passively in the haemolymph, enabling the fungus to invade other tissues of the host insect by extensive vegetative growth. During the invasion of the insect body, nutrients in the haemolymph and the fat body are depleted. This is followed by the death of the insect and the end of the pathogenic process. During the invasion of the host insect by *M. anisopliae*, a wide range of secondary metabolites or toxins are produced. The incubation period depends on the host, the host stage, the temperature and the virulence of the fungus strain. After the host death and under humid conditions, the fungus starts its saprophytic growth out of the body. Conidia are produced outside of the cadaver. Under very dry conditions, the fungus may also persist in the hyphal stage inside the cadaver.

The rapid kill by *M. anisopliae* on its host could be caused not only through direct physical invasion but through different entry routes as well (Jiang et al., 2003). They can also be ingest and enter the organism through the digestive tract, trachea, or wounds and cause infection (Holder et al., 2005). Fungus *M. anisopliae* can infect all the life stages of insects (Gul et al., 2014).

Based on previous studies of *M. anisopliae* effects on targeted insects' pest and behavioural changes had been observed in a few aspects. Insect species infected with the *M. anisopliae* have demonstrated a significant reduction in feeding phenomenon as early as 1 to 4 days after introduced the source of the pathogen to the targeted insects

(Tefera and Pringle, 2003). Feeding then decreases through time until the death (France et al., 2002). It has been hypothesized that the reduction in feeding may be due to toxic substances by the EPF. The *M. anisopliae* was used the auto-dissemination strategy by mixed with attractant traps or contamination baits to infect the attracted pest insects which then transmit the fungus to them (Kaakeh et al., 1996; Klein and Lacey, 1999; Quesada-Moraga et al., 2004). Thus, with all the statements mentioned the potential of *M. anisopliae*, it could have suggested for biological control towards many insect pests.

CHAPTER THREE

EVALUATION ON PATHOGENICITY OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* TOWARDS *Paederus fuscipes* CURTIS THROUGH SOIL INFECTION

3.1 Introduction

Rice fields is an ideal habitat for the *P. fuscipes* (Khan et al., 2009). Their maximum activity was observe near the ground (Nasir et al., 2012). The *Paederus* beetles play an important role as a biological control of 'rice pests' by feeding on them at different crops. However, this beetle is also an important public health insect worldwide as it causes Paederus dermatitis (PD) towards people once in contact with them. PD is cause by the released paederin when *P. fuscipes* beetles is crush on the skin accidentally and created a burning effect to the affected part. It is widely reported around the world and has caught many public interests whenever these beetles infests human settlements that lives near their natural habitats. The dermatitis are most frequently reported in regions with a hot and tropical climate such as India, Egypt, Australia, Sri Lanka, Brazil, and in Malaysia (Gnanaraj et al., 2007). Previous outbreak cases were reported in states of Malaysia including Penang, Kelantan, Terengganu, and Selangor. Mostly the control methods applied towards the swarming of *Paederus* beetles in affected residential areas are through applying insecticide via fogging, target spraying using pest killer products or glue traps for household (Raju, 2002).

Usage of insecticide for controlling pests either by ground or air application are known to cause environmental problems such as ground water contamination. Therefore, these warrant the search for biological control as alternative methods which including the entomopathogens such as bacteria, nematodes, and fungi. In recent years, the use of entomopathogenic fungi (EPF) has been studied as biocontrol agents of several important arthropod pests. Amongst these fungi, *Metarhizium anisopliae* was the most frequently used in commercial products and the work of research. The fungus *M. anisopliae* is a worldwide famous soil-inhabiting entomopathogen could potentially exhibit virulence towards insect pests (Ghanbary et al., 2009). This fungus has been reported able to infect some insects that lived and active on the soil (Bruck, 2005). St. Leger (2008) indicated that *M. anisopliae* could survive for various years in the soil and multiply within the soil. Ghanbary et al. (2009) mentioned that *M. anisopliae* was capable of shifting into the saprophytic phase and remain viable within the soil. Therefore, these fungi could truly thrive in soil or survive in a dormant state while wait for the susceptible host (Inglis et al., 2001).

The EPF, *M. anisopliae* has been studied and showed its potential in the pest management strategies. However, the effectiveness of *M. anisopliae* in controlling *Paedarus* beetle has not yet discovered anywhere as to our knowledge. The information of biological management methods on the *P. fuscipes* is therefore necessary. Therefore, the main aim of this study was to evaluate the pathogenicity effect of the selected EPF *M. anisopliae* inoculated into the treated soil as a source of infection towards the *P. fuscipes* beetles.

3.2 Materials and Methods

3.2.1 Insect Sampling

Adult *Paedarus fuscipes* were captured using blacklight trap (CDC Fay-Prince Blacklight (UV) Trap) from the residential area that is close to the rice field situated at Jalan Sejahtera Indah, Teluk Air Tawar, Butterworth, Penang (N 5° 29' 7.2171" E 100° 23' 20.9012") (Figure 3.1). This site has a high occurrence of *P. fuscipes* as reported by the Seberang Perai Municipal Council (MPSP). The UV blacklight traps are located at 110 cm elevation from the ground to attract *P. fuscipes* beetles, then captured alive by using a battery-operated aspirator (Figure 3.2). The alive beetle sample was kept in a transparent container (11 x 17 cm) and brought back to the laboratory for the rearing. Sampling was conducted from 1900 until 2300.

Figure 3.1: The sampling site. Google maps (2018). *Jalan Sejahtera Indah, Teluk Air Tawar, Butterworth, Penang*. Retrieved from: https://www.google.com/maps/dir//5.4853062,100.3891363/@5.4853979,100.3884453,233m/data=!3m1!1e3!4m2!4m1!3e0