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EVALUATIONS OF ENTOMOPATHOGENIC FUNGI, *Metarhizium anisopliae* INOCULATE ON THE TREATED SOILS TOWARDS *Paederus fuscipes*

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ABSTRACT

Rove beetle, *Paederus fuscipes* Curtis is a natural predator of several crop pests in the agriculture ecosystem, however, their high intrusion into human settlements caused them to become public health concern due to Paedarus dermatitis infection among humans. The entomopathogenic effectiveness of *Metarhizium anisopliae* Mechnikov was tested as biological control towards adults *Paederus fuscipes* by inoculating on soils. The mortality of *P. fuscipes* was observed and data were subjected to analysis using ANOVA and Kaplan-Meier method. Results show that *P. fuscipes* tested with the highest concentration at 1.3×10^{10} conidia/mL exhibited the shortest mean mortality time at 11.0 ± 2.5 days and survival time of 7.6 ± 0.7 days, yet the second-highest concentration exhibited at 2.2×10^{9} showed mean mortality of 18.4 ± 4.2 days and survival time of 11.9 ± 0.8 days. Log Rank (Mantel-Cox) pairwise comparison indicated the significant differences between the highest concentration of 1.3×10^{10} with the control ($\chi^2 = 62.3$, df=1 $p<0.0005$). Both mean mortality time and survival time of *P. fuscipes* showed inconsistent trends from the highest concentration of *M. anisopliae* towards the lowest. Pathogenicity was observed at the concentrations of 10^6, 10^9, and 10^{10} after performing Koch's postulates. The results were unexpected but could indicate that *M. anisopliae* has the potential to be a biocontrol agent at a higher concentration.

Key words: Entomopathogenic, *Metarhizium anisopliae*, *Paederus fuscipes*, pathogenicity

INTRODUCTION

Rove beetles (Coleoptera: Staphylinidae) are endemic in Southeast Asia (Khan *et al*., 2009). *Paederus* sp. beetles are beneficial insects because they are carnivorous and consume smaller pests. It plays an important role as biological control of 'rice pests' and feeds on soft-bodied insect pests like aphids, whitefly, mites, and maggots of fruit fly also leafhoppers of different crops (Frank & Kanamitsu 1987). However, this beetle is also a public health important insect worldwide (Yasri & Wiwanitkit, 2014). Since the 1990s, the *Paedarus fuscipes* beetle has become a public health concern after many outbreaks were reported worldwide because it creates a kind of skin dermatitis to people once contact them. The common name called Paederus dermatitis is widely reported in the world and has caught public interest whenever these rove beetles infested human settlements near their natural habitats. *Paederus* spp., especially the adults, is active during the day in search of prey and attracted by luminescent and fluorescent lights at night which bring them into contact with human beings (Maryam *et al*., 2016). Although the beetles do not bite nor sting humans, inside the hemolymph of Rove beetle contains a blistering chemical, the 'paederin' ($C_{24}H_{43}O_9N$) which was named in 1953 and described as 15 times more poisonous than cobra venom (Verma & Agarwal, 2006). Paederus dermatitis caused by paederin is a peculiar irritant contact dermatitis when beetles of the genus *Paederus* were crushed on the skin, causing irritation and blistering effect to the skin part. Dermatitis is most frequently seen in regions with a hot and tropical climate. Paederus dermatitis has been reported in many countries such as India, Egypt, Australia, Sri Lanka, Brazil, and also in Malaysia (Gnanaraj *et al*., 2007).

In Malaysia, rove beetles are abundant in paddy fields, which make them appear in large numbers in Penang, Kelantan, Terengganu, and Selangor. This is due to the availability of large areas of tropical rain forest and rice-growing areas in these states, which made an ideal habitat for the Rove beetle (Khan *et al*., 2009). The species being reported by the Seberang Perai Municipal Council (MPSP), Malaysia which caused the paederus dermatitis is *Paederus fuscipes Curtis* (Semut Semai, Semut Kayap, or Charlie). Until now, the control methods towards the swarming of rove beetles in residential areas in Malaysia are by applying insecticides such as permethrin, deltamethrin, and malathion via

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thermal fogging or target spraying using commercial pest killer products or glue traps for households (Raju, 2002).

Insecticide use for controlling pests is applied either by ground or air applications. The use of persistent insecticides to control soil insects, like termites, is known to cause problems such as groundwater contamination and the destruction of soil fauna (Ghany, 2015). Most importantly, over-use of insecticides could cause adverse effects on human health which might affect the nervous system. The intensive and unmonitored use of herbicides, fungicides, and insecticides by the farmers, may create chemical-resistant beetles, thereby contributing to the current massive explosion of *P. fuscipes* populations in Malaysia. These problems, therefore, warrant the search for 'Biological Control' as alternative ways which include entomopathogenic bacteria, nematodes, and fungi. However, these were being highly emphasized and encouraged in other countries, but not yet implemented in Malaysia.

In recent years, the use of Entomopathogenic fungi has been examined for the biological control of several arthropod pests. Amongst these fungi, most frequently used in commercial products and the work of research is the *Metarhizium anisopliae*, which belong to Order Hypocreales: Family Clavicipitaceae, of its taxonomy profile. The anamorphic entomopathogenic fungi *M. anisopliae* is the natural enemies of a wide range of insects and arachnids (Roberts & Leger, 2004). *Metarhizium anisopliae* has been extensively studied and it is the best-characterized entomopathogen used for biological control of several pests including a wide range of insect species such as termites, fruit fly, and acari ticks' parasites (Zimmermann, 2007).

The entomopathogenic fungi, *M. anisopliae* were being studied and showed their potential in pest management strategies. However, the effectiveness of *M. anisopliae* in controlling the *Paedarus* beetle has not yet been discovered anywhere as to our knowledge. Therefore, the main aim of this study is to investigate the effectiveness and infection method of entomopathogenic *M. anisopliae* on the treated soil towards the *P. fuscipes* beetles.

MATERIALS AND METHODS

Insect sampling

Paedarus fuscipes were captured from the residential area close to the rice field situated at Jalan Sejahtera, Teluk Air Tawar, Butterworth, Penang (N 5º 29' 9.3171" E 100º 23' 1.3012"). The UV backlight traps located at 110 cm elevation from the ground were used to attract the beetles then captured alive by using an aspirator. Sampling was conducted from 1900 until 2300.

Insects maintenance

The beetles were reared in the insectarium of the Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia. The beetles were reared in the transparent plastic container (11.0 cm diameter, 17.0 cm height) with open-top tied and covered with muslin cloth for air ventilation. The samples were kept at 28.0 ºC, 65% RH, and photoperiod of 12 h: 12 h (L:D). The beetles were provided with lobster cockroaches (*Nauphoeta cinerea*) as the food source, and moist cotton was provided as a water source and a site for oviposition. New food and water were provided every 48 hr.

Metarhizium anisopliae Strains and Culture conditions

The fungal strain of *M. anisopliae* was cultured from the insecticide product Ory-X manufactured by FELDA Agricultural Services Sdn Bhd. The fungal were cultured in Petri dishes on Potato dextrose agar containing chloramphenicol (0.05 g/L) and incubated for 14 days at 28 ºC before the conidia were harvested for the experiment. The conidia were scraped off using a sterile metal loop and suspended in sterile distilled water containing 0.01% Tween 80. The suspension was filtered with eight layers of cheese clothes to remove mycelium before being poured into the sterile glass tube. The required conidia concentration was then determined by using a Neubauer hemocytometer.

Preparation of soil and inoculation of *M. anisopliae*

A total of 50 g black soil and 20% potato powder (TESCO brand) were mixed in the Erlenmeyer flasks. Then, 15 mL of sterile distilled water was then added to the mixture. The soil mixture was sterilized by autoclaving at 121 ºC for 40 min before the introduction of *M. anisopliae*. A total of 1 mL *M. anisopliae* spore suspensions were inoculated on the surface of cool sterilized soil mixtures at different concentrations (10^6, 10^7, 10^8, 10^9, 10^{10} conidia/mL). While the untreated controls were added with 1 mL of sterile distilled water. The fungi suspension was allowed to grow at 28 ± 3 ºC under laboratory conditions before the experiments were conducted. The flasks were sealed up with parafilm to avoid contamination.

Experimental design

The soil surface was inoculated with *M. anisopliae* grown in the Erlenmeyer flasks (10^6, 10^7, 10^8, 10^9, and 10^{10} conidia/mL) on day 2. A single adult *P. fuscipes* was introduced into the treated flask. This is to avoid data loss due to cannibalistic behavior if the number of testing subjects is more than one. A piece of moist cotton wool and cat food was added as food supply. Cat food contains carbohydrates and protein that are useful to maintain the energy of the beetles. Food and moist cotton wool were newly replaced every

48 h. The flasks were kept at room temperature (28 ± 3 ºC) with a photoperiod of 12:12 (L:D). Each of the testing concentrations was replicated five times with control were incorporated in this experiment. Testing subjects were observed daily, mortality was reached once the beetle had an absence of movement. Koch's postulates steps were performed on the dead samples of *P. fuscipes* to check for the existence of *M. anisopliae* fungal infection by placing their cadavers on the Potato Dextrose Agar (PDA) plates separately and incubated at the temperature of 28 ± 3 ºC.

Data analysis

Data were log-transformed before analysis to fulfil the assumption of ANOVA after the normality test was performed. The differences in mortality time between the fungal concentrations treatments were compared by using analysis of variance (ANOVA) in the SPSS (version 20.0) statistical software. When a significant difference was detected by ANOVA, Tukey's Honest Significant Difference (HSD) test was applied to separate means ± SE. In all analyses, $p<0.05$ was taken to indicate statistical significance. Survival analysis was applied to estimate the mean survival time on the mortality time at different fungal concentrations at 95% confidence interval (CI 95%), nonparametric estimates of the survivor function by the Kaplan-Meier method and the Log-Rank (Montel-Cox) test with α=5% probability for pairwise comparisons among different exposure fungal concentrations were also computed using SPSS (version 20.0).

RESULTS

The highest concentration at 1.3 × 10^{10} conidia/mL exhibited the shortest mean mortality time of 11.0 ± 2.5 days, which indicated the fastest time to cause mortality to *P. fuscipes* after the exposure to *M. anisopliae* (Figure 1). Positive infection case of *M. anisopliae* was only observed at concentrations of 1.3 × 10^{10} and 2.2 × 10^{9} conidia mL^{-1} (both have only one *P. fuscipes* infected out of five replicates). At a concentration of 2.3 × 10^{6} conidia mL^{-1}, two out of five replicates showed infection of *M. anisopliae* after performing Koch's postulate. No symptoms of *M. anisopliae* were observed for concentrations of 1.5 × 10^{8} and 3.1 × 10^{7} conidia mL^{-1}. However, the longest mean mortality time at 18.4 ± 4.2 days was observed from the second-highest concentration of 2.2 × 10^{9} conidia mL^{-1}. Mean mortality time of *P. fuscipes* showed inconsistent trends from the highest concentration of *M. anisopliae* towards the lowest. There was no statistically significant difference detected between the *M. anisopliae* concentrations tested on the *P. fuscipes* as determined by one-way ANOVA (F (5, 24) = 2.002, p=0.115).

The duration of the whole experiment was 39 days, and all the tested *P. fuscipes* reached full mortality. Based on the survival curve, the longest survival time exhibited by the control *P. fuscipes* at 40 days. Whereas, the shortest survival time exhibited at the concentration of 1.3 × 10^{10} conidia/mL at 20 days same as at the concentration 2.3 × 10^{6} conidia/mL (Figure 2). As estimated by the Kaplan-Meier analysis, the survival time of the *P. fuscipes* statistically showed a significant difference between the tested concentration (χ^2= 19.136, DF=4, $p<$ 0.005; Table 1). The mean survival time showed by *P. fuscipes* at the untreated control was 18.2 ± 0.8 days. Among the treated group, the lowest value of the mean survival time by *P. fuscipes* was observed in the soil treated with the highest concentration of 1.3 × 10^{10} conidia mL^{-1} (7.6 ± 0.7 days) showed a significant difference with the control ($p<0.05$). The second-highest concentration (2.2 × 10^{9} conidia/mL) surprisingly exhibited the highest value of mean survival time of *P. fuscipes* among all treatments at 11.9 ± 0.8 days (Table 1).

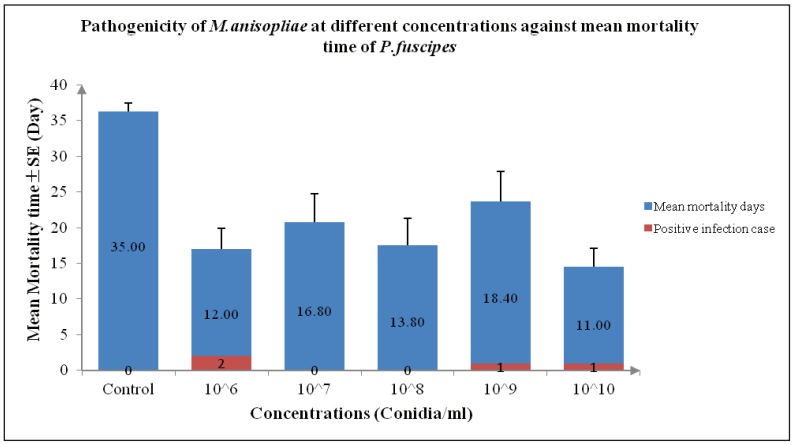

Fig. 1. Mean mortality time of *Paedarus fuscipes* exposed to soil inoculated with different concentrations of *Metarhizium anisopliae* [28 ± 3 ºC and photoperiod of 12:12 (L:D)]. Bars depict the mean ± SE (standard error)

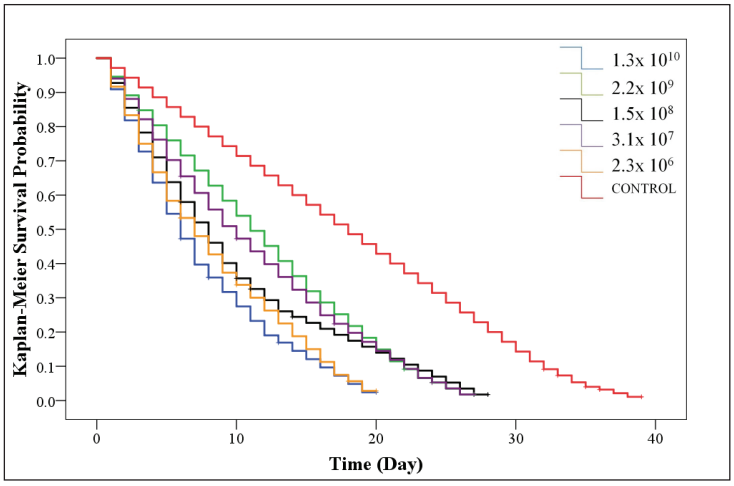

Fig. 2. Kaplan-Meier survival curve of *Paedarus fuscipes* exposed to soil inoculated at different tested concentrations of *Metarhizium anisopliae* with a control group [28 ± 3 ºC and photoperiod of 12:12 (L:D)]

Table 1. Mean survival time of *Paedarus fuscipes* exposed to soil surface treated with *Metarhizium anisopliae* [28 ± 3 ºC, photoperiod of 12:12 (L:D)]

M. anisopliae concentration (conidia/mL)	Survival Time (Days)	
	Mean[a] ± SE	CI (95%)
Control	18.2 ± 0.8 [a]	12.1 - 13.7[a]
2.3×10^6	8.3 ± 0.7 [b]	6.9 - 9.7 [b]
3.1×10^7	11.1 ± 0.8 [c]	9.5 - 12.7 [c]
1.5×10^8	9.9 ± 0.9 [b,c]	8.1 - 11.8 [b,c]
2.2×10^9	11.9 ± 0.8 [c]	10.4 - 13.4 [c]
1.3×10^{10}	7.6 ± 0.7 [b]	6.2 - 9.0 [b]

Mean survival times (± standard error) were determined by the Kaplan-Meier method.; CI – Confidence Interval; Estimation is limited to the largest survival time if it is censored; same small letter indicates no significant differences using Log Rank test pairwise comparison between concentrations.

DISCUSSION

Our study revealed using the treated soil method, the infections of *M. anisopliae* are successfully delivered in causing the mortality towards *P. fuscipes*. At the concentration of 1.3×10^{10} conidia/mL *M. anisopliae*, the survival time of *P. fuscipes* is reduced more than two times than in control. In our study, we observed that *M. anisopliae* was able to grow on the treated nutrient soils under direct microscopic observations. The soil may act as a storage place (Gul *et al*., 2013) and form a normal habitat for spores in nature. *Metarhizium*, *Beauveria*, *Tolypocladium,* and *Isaria* are naturally occurred fungi and normally found in soil (Keller *et al*., 2003) The optimum temperature for *M. anisopliae* to grow is 28 ºC and at this temperature, many insects have a high activity level and will be more likely to pick up the conidia than at a lower temperature (Grund & Hirsch, 2010). Even though *P. fuscipes* do not burrow into the soil, the conidia of *M. anisopliae* can be found on their body. Thus suggested, if it does not grow saprophytically in soil, the dormant conidia still exist on the soil which may infect the susceptible hosts by contact.

It has been suggested that the targeted host mortality should be increased alongside the increasing concentration of conidia. A short period of lethal infection indicates a high level of pathogenicity or virulence of a pathogen, and in contrast, a long period of lethal infection indicates a low level of pathogenicity (Tanada & Kaya 1993; Fuxa & Richter 2004). Yet, from our data, inconsistent trends of mortality and survival time against *P. fuscipes* were shown from the highest concentration of *M. anisopliae* treated towards the lowest concentration, which is against the statement mentioned above. Thus, indicated the spore viability might not be the only factor that affects the virulence or pathogenicity. There might be a relationship between the rate of germination and the rate of mortality (Herlinda, 2010). Mnyone *et al*. (2009) stated that different experimental designs and procedures involving fungal strains, formulations, targeted insects, or arthropods would have affected the end product causing different results. Also, the virulence depends on the ability of *M. anisopliae* to penetrate directly through the cuticle

using physical and enzymatic mechanisms (Kirkland *et al*., 2004).

From our results, lengthy mean mortality and survival time showed that *P. fuscipes* might be less susceptible to the *M. anisopliae* infection. Steenberg *et al*. (1995) has mentioned that the infection levels of the microbial pathogen were low towards the adult rove beetle from research conducted on the prevalence of entomopathogenic fungi that occurred naturally in the field of white cabbage. Also, it's been commonly known the insects' cuticle has a certain degree of defensive mechanism against penetration of microbial pathogens such as fungi, varied by different species and abiotic factors (Pedrini *et al*., 2013). Aside from the cuticle defensive system, some insects contained symbiotic microbial inside their bodies in defending against microbial (fungal) pathogens (Ortiz-Urquiza & Keyhani, 2013). It has been confirmed that within the body of *P. fuscipes* has the endo-symbiont bacteria (*Pseudomonas* ssp.) and the tail of this insect is known to contain the highest pederin concentration compared to other body parts for the defensive mechanism (Zuharah & Sufian, 2014). The endosymbiont bacteria inside *P. fuscipes* likely enhance their defensive system against microbial such as *M. anisopliae*, yet these remained unclear.

Pathogenicity test showed the existence of *M. anisopliae* was able to cause mortality in the *P. fuscipes*. Although Koch's postulates do not show 100% fungi infection for all concentrations tested, all treated *P. fuscipes* showed a short survival time compared with the control group. It might be due to the soil surface treated with the *M. anisopliae* causing food contamination with the fungus and leading to reduce food intake by the tested *P. fuscipes* (Magalhaes *et al*., 2001). In addition, there are several examples of reduction in food consumption in insects infected by pathogenic fungi (Thomas *et al*., 1997; Ekesi, 2001; Tefera & Pringle, 2003).

Also, from our observation, the tested *P. fuscipes* which infected or under the altered environment displayed symptoms of lack of behavioral activity, similar to the results from Herlinda (2010) who observed that infected *A. gossypii* nymphs by *M. anisopliae* displayed a lack of appetite and decreased mobility. Lower food intake was also displayed by infected red palm weevil and/or from the energetic cost of confrontation with *M. anisopliae* infection (Gindin *et al*., 2006). *Metarhizium* has been evaluated as a Biological Control Agent (BCA) on target hosts showed that their feeding activities are reduced before it dies after the infection with the fungi (Makaka, 2008). The Destruxin A toxins may increase mortality (Yin *et al*., 2021) by weakening the host immune defences, damaging the muscular system and the Malpighian tubules, affecting excretion, and leading to feeding and mobility difficulties.

CONCLUSIONS

In conclusion, the *M. anisopliae* was able to grow saprophytically on the treated soil surface and caused mortality to the adult stages of *P. fuscipes* under laboratory conditions. More extended research is required to increase the infection rate of *M. anisopliae* towards the adult *P. fuscipes* on the treated soil. Conidia formulated in oil suspensions are suggested for further experiment since few studies stated that oil suspension is generally more effective than water formulations conidia. Therefore, a high rate of attachment on the body of *P. fuscipes* can be achieved and increase the penetration rate of this fungi.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Ekesi, S. 2001. Pathogenicity and antifeedant activity of entomopathogenic hyphomycetes to the cowpea leaf beetle, *Ootheca mutabilis* Shalberg. *Insect Science and its Application*, **21**: 55-60. https://doi.org/10.1017/S174275840002004X

Frank, J.H. & Kanamitsu, K. 1987. *Paederus*, Sensu Lato (Coleoptera: Staphylinidae): natural history and medical importance. *Journal of Medical Entomology*, **24**: 155-191. https://doi.org/10.1093/jmedent/24.2.155

Fuxa, J.R. & Richter, A.R. 2004. Effects of soil moisture and composition and fungal isolate on prevalence of *Beauveria bassiana* in laboratory colonies of the red imported fire ant (Hymenoptera: Formicidae). *Environmental Entomology*, **33**: 975-981. https://doi.org/10.1603/0046-225X-33.4.975

Ghany, T.M.A. 2015. Entomopathogenic fungi and their role in biological control. OMIC International. 42pp.

Gindin, G., Levski, S., Glazer, I. & Soroker. V. 2006. Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the Red Palm Weevil *Rhynchophorus ferrugineus*. *Phytoparasitica*, **34(4)**: 370-379 https://doi.org/10.1007/BF02981024

Gnanaraj, P., Venugopal, M.K. & Pandurangan, C.N. 2007. An outbreak of *Paederus dermatitis* in a suburban hospital in South India: A report of 123 cases and review of literature. *American Academy*

of Dermatology, **57(2)**: 297-300. https://doi.org/10.1016/j.jaad.2006.10.982

Grund, J. & Hirsch, L. 2010. The potential of entomopathogenic fungal isolates as an environmentally friendly management option against *Acanthoscelides obtectus*. Faculty of Landscape Planning, Horticulture and Agricultural Science - LTJ-Fakulteten Department, Plant Protection Biology Alnarp.

Gul, H.T., Saeed, S. & Khan, F.Z.A. 2014. Entomopathogenic fungi as effective insect pest management tactic: A review. *Applied Sciences and Business Economics*, **1(1)**: 10-18.

Herlinda, S. 2010. Spore density and viability of entomopathogenic fungal isolates from Indonesia, and their virulence against *Aphis gossypii* glover (Homoptera: Aphididae). *Tropical Life Sciences Research*, **21(1)**: 11-19.

Keller, S., Kessler, P. & Schweizer, C. 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae*. *BioControl*, **48(3)**: 307-319. https://doi.org/10.1023/A:1023646207455

Khan, T., Hassali, M., Gillani, S. & Hameed, M. 2008. Clinical presentation of "Rove Beetle Dermatitis". *Australasian Medical Journal*, **1(7)**: 19-24. https://doi.org/10.4066/AMJ.2009.77

Kirkland, B.H., Cho, E.M. & Keyhani, O.N. 2004. Differential susceptibility of *Amblyomma maculatum* and *Amblyomma americanum* (Acari, Ixodidea) to the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Biological Control*, **31**: 41-42. https://doi.org/10.1016/j.biocontrol.2004.07.007

Laba, W. & Kilin, D. 1994. *Paederus fuscipes* Curtis. biology and feeding ability of brown planthoper (*Nilaparvata lugens* Stall). *Risalah Hasil Penelitian Tanaman Pangan*, **4**: 240-245.

Magalhaes, B.P., De Faria, M.R., Lecoq, M., Schmidt, F.G.V., Silva, J.B.T. & Frazao, H.S., Balanca, G. & Foucart, A. 2001. The use of *Metarhizium anisopliae* var. *acridum* against the grasshopper *Rhammatocerus schistocercoides* in Brazil. *Journal of Orthoptera Research*, **10**: 199-202. https://doi.org/10.1665/1082-6467(2001)010[0199:TUOMAV]2.0.CO;2

Makaka, C. 2008. The efficacy of two isolates of *Metarhizium anisopliae* (Metschin) Sorokin (Deuteromycotina: Hyphomycetes) against the adults of the black maize beetle *Heteronychus licas* Klug (Coleoptera: Scarabidae) under laboratory conditions. *African Journal of Agricultural Research*, **3(4)**: 259-265.

Maryam, S., Fadzly, N. & Zuharah, W.F. 2017. Abundance, distribution and dispersal time of *Paedarus fuscipes* (Coleoptera: Staphylinidae) and its association to human settings. *Tropical Biomedicine*, **34(1)**: 224-236.

Mnyone, L.L., Kirby, M.J., Lwetoijera, D.W., Mpingwa, M.W., Knols, B.G. & Takken, W. 2009. Infection of the malaria mosquito, *Anopheles gambiae,* with two species of entomopathogenic fungi: Effects of concentration, co-formulation, exposure time and persistence. *Malaria Journal*, **8**: 309. https://doi.org/10.1186/1475-2875-8-309

Ortiz-Urquiza, A. & Keyhani, N.O. 2013. Action on the surface: Entomopathogenic fungi versus the insect cuticle. *Insects*, **4**: 357-374. https://doi.org/10.3390/insects4030357

Pedrini, N., Ortiz-Urquiza, A., Huarte-Bonnet, C., Zhang, S. & Keyhani, N.O. 2013. Targeting of insect epicuticular lipids by the entomopathogenic fungus *Beauveria bassiana*: hydrocarbon oxidation within the context of a host pathogen interaction. *Frontiers in Microbiology*, **4**: 24. https://doi.org/10.3389/fmicb.2013.00024

Raju, M. 2002. Information on rove beetle. Seberang Perai Municipal Council Bull. URL http://www.mpsp.gov.my/image/roveeng.pdf (accessed 12.07.21).

Roberts, D.W. & Leger, R.J. 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. *Advances in Applied Microbiology*, **54**: 1-70. https://doi.org/10.1016/S0065-2164(04)54001-7

Samson, R.A., Evans, H.C. & Latgé, J.P. 1988. Atlas of Entomopathogenic Fungi. Springer, Verlag. 187 pp. https://doi.org/10.1007/978-3-662-05890-9

Steenberg, T., Langer, V. & Esbjerg, P. 1995. Entomopathogenic fungi in predatory beetles (Col.: Carabidae and Staphylinidae) from agricultural fields. *Entomophaga*, **40**: 77-85. https://doi.org/10.1007/BF02372683

Tanada, Y. & Kaya, H.K. 1993. Insect Pathology. Academic Press, New York. 490 pp.

Tefera, T. & Pringle, K.L. 2003. Food consumption by *Chilo partellus* (Lepidoptera: Pyralidae) larvae infected with *Beauveria bassiana* and *Metarhizium anisopliae* and effects of feeding natural versus artificial diets on mortality and mycosis. *Journal of Invertebrate and Pathology*, **84**: 220-225. https://doi.org/10.1016/j.jip.2003.11.001

Thomas, M.B., Blanford, S. & Lomer, C.J. 1997. Reduction of feeding by the variegated grasshopper, *Zonocerus variegatus*, following infection by the fungal pathogen *Metarhizium avoviride*. *Biocontrol Science and Technology*, **3**: 327-334. https://doi.org/10.1080/09583159730730

Verma, L.C.R. & Agarwal, M.S. 2006. Blistering beetle dermatitis: An outbreak. *Medical Journal Armed Forces India*, **62**: 42-44. https://doi.org/10.1016/S0377-1237(06)80154-1

Yasri, S. & Wiwanitkit, V. 2014. *Paedarus dermatitis*.

Journal of Coastal Life Medicine, **2(2)**: 124.

Yin, F., Xiao, M., Berestetskiy, A. & Hu, Q. 2021. The *Metarhizium anisopliae* toxin, Destruxin A, interacts with the SEC23A and TEME214 proteins of *Bombyx mori*. *Journal of Fungi*, **7(6)**: 460. https://doi.org/10.3390/jof7060460

Zimmermann, G. 2007. Review on safety of the entomophatogenic fungus *Metarhizium anisopliae*. *Biocontrol Science and Technology*, **17**: 879-920. https://doi.org/10.1080/09583150701593963

Zuharah, W.F. & Sufian, M. 2014. Human health hazards caused by Rove Beetles. Bio-Bulletin June/July 2014, School of Biological Sciences, Universiti Sains Malaysia, 6-7 pp.