DIURNAL ATTRACTION OF FRUIT FLIES (DIPTERA: TEPHRITIDAE) TO METHYL EUGENOL IN A VILLAGE ECOSYSTEM IN TANJUNG BUNGAH, PENANG, MALAYSIA

Suk Ling Wee[1]* & Alvin Kah-Wei Hee[2]
[1] Centre for Insect Systematics, Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, Bangi Selangor, Malaysia
[2]Department of Biology, Faculty of Science, Universiti Putra Malaysia,
Serdang Selangor, Malaysia
Corresponding author: slwee@ukm.edu.my*

ABSTRACT

Diurnal rhythm of male *Bactrocera* fruit fly attraction to methyl eugenol (ME) was investigated using clear traps design in a village ecosystem in Tanjung Bungah, Penang, Malaysia. The diurnal rhythm pattern studied by half-hourly sampling from 07:00 to 18:00 hr showed significant male fruit fly attraction to ME in the morning from 07:30 to 09:30 hr, with a distinct peak at 08:30-09:00 hr. The male attraction to ME was significantly lower in the afternoon and remained low approaching late afternoon. The ME-responding fruit fly species captured were *B. dorsalis* (86%), which dominated the local *Bactrocera* community, followed by *B. umbrosa* (5.8%) and *B. carambolae* (0.1%). This showed that *B. dorsalis* is a more dominant species than its sibling species, *B. carambolae* in the area studied. In addition, *ca.* 8.1% of *Bactrocera* male flies that bore intermediate morphological characteristics between *B. dorsalis* and *B. carambolae* were also captured in those traps. The present study shows that for ME-responding *Bactrocera* spp., male attraction to ME occurs throughout the day with peak period of attraction to ME occurring *ca.* 30 mins following sunrise for 2 hrs before gradually tapering off.

Keywords: *Bactrocera* spp., methyl eugenol, diurnal rhythm

ABSTRAK

Ritma harian penarikan lalat buah jantan *Bactrocera* spp. terhadap metil eugenol (ME) telah dikaji dengan menggunakan perangkap lutsinar di sebuah ekosistem kampung di Tanjung Bungah, Pulau Pinang. Kajian ritma harian melalui pensampelan lalat buah setiap setengah jam dari jam 07:00 hingga 18:00 menunjukkan waktu penarikan lalat buah jantan terhadap ME paling tinggi dari jam 07:30 hingga 09:30, dengan puncak penarikan berlaku pada jam 08:30-09:00. Penarikan lalat buah jantan terhadap ME adalah rendah sepanjang waktu petang sehingga lewat petang. Spesies lalat buah yang terperangkap melalui gerak balas terhadap ME adalah *B. dorsalis* (86%), yang merupakan spesies dominan di komuniti tempatan, diikuti oleh *B. umbrosa* (5.8%) dan *B. carambolae* (0.1%). Ini menunjukkan *B. dorsalis* adalah lebih dominan daripada spesies beradiknya, *B. carambolae* di kawasan yang dikaji. Tambahan pula, terdapat kira-kira 8.1% lalat buah jantan *Bactrocera* spp. yang mempunyai morfologi

perantaraan antara *B. dorsalis* dan *B. carambolae* telah diperangkap. Kajian ini menunjukkan bahawa lalat buah jantan *Bactrocera* spp. adalah tertarik kepada ME sepanjang hari dengan satu puncak penarikan yang berlaku kira-kira 30 min selepas waktu subuh dan berlanjutan selama dua jam sebelum trend penarikan mengurang ke satu tahap yang lebih rendah.

Kata kunci: *Bactrocera* spp., metil eugenol, ritma harian

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are fruit pests of economic importance in the tropic and subtropic regions (Bateman 1972). The pest causes direct losses at pre- and post-harvest of fruits and vegetables as well as indirect losses through stringent quarantine rules and regulations imposed on export and market access (White & Elson-Harris 1992). Tephritid fruit flies in tropical regions are prolific species with a relatively long-life span, high reproductive capability and dispersal ability, as well as wide host range which made fruit fly control a huge challenge (Allwood et al. 1999; White and Elson-Harris, 1992).

Methyl eugenol (ME) is a potent male attractant for many fruit fly species and attracts over 80 species out of almost 800 identified *Bactrocera* species (IAEA 2003; Drew & Romig 2013). ME is effective in minute quantities and with a long-lasting effect (*ca.* 3 months in the field) making it suitable for use in fruit fly control-related programs such as population detection, monitoring, suppression and eradication (see review by Tan and Nishida 2012). For a population monitoring programme, a reliable trapping system and an efficient bait are of utmost importance. ME has been shown to be a very useful male attractant for this purpose (Tan & Lee 1982; Tan & Nishida 2012). Apart from the abovementioned use of ME, ME could also be utilized to study the diurnal rhythm of male fruit flies. The most active period of an insect species in a day for any activities, for instance food foraging, feeding, host searching, oviposition, and mating or even unique/specific attraction to natural resources (like in the case of male fruit flies' response to male attractants) that form a predictable pattern, could be a useful input for timing or a more effective strategy in insect control (Bayoumy & El-Metwally 2017). Therefore, the objectives of this study are: (i) to determine the diurnal rhythm of *Bactrocera* spp. male attraction to ME, and (ii) to determine the presence and abundance of ME-responding species in a village ecosystem.

MATERIALS AND METHODS

The study was conducted in Kampung Melayu Tanjung Bungah (GPS coordinates 5.462428, 100.280715), Pulau Pinang, Malaysia from 07:00 to 18:00 hr on four separate occasions on a weekly basis from January to February, 1996. Average daily temperature and relative humidity of the study site were 28 ± 3ºC and 73–74 %, respectively. A total of four ME-baited trap were used in each sampling occasion. A clear-trap design (Tan 1984) was used. The trap was made from cylindrical transparent acetate sheet (15 cm length x 9 cm diameter) and with two entrances (2 cm diameter) on each side. Cotton wool impregnated with 1-ml of pure ME in liquid form was hung at the centre of the trap. Traps were set up at least 1.5 m from the ground on tree branches and placed at least 50 m apart in a transect line. Two sets of traps were used inter-changeably when traps were serviced at every 30 min interval. During trap service, the entrances of a trap were closed by using cotton wools to prevent trapped flies from escaping and the trap was replaced by a new trap. Flies trapped in the trap were then anesthetized using carbon dioxide and transferred to a clean specimen vial filled with 95%

ethanol. All captured flies were brought back to the laboratory and identified according to species level based on the identification keys by Drew & Hancock (1994).

For comparison of species abundance, data were subjected to Kruskal-Wallis One Way Analysis of Variance (ANOVA) on ranks since the data obtained was not normally distributed despite data transformation. For the analysis of male attraction to ME versus time, one-way ANOVA was used and means were separated by Holm-Sidak method (P=0.05). All analysis was performed by Sigma Plot 12.0 software.

RESULTS

A total of 6,769 male fruit flies were captured by ME-baited traps with a significant difference in the abundance of different *Bactrocera* spp. (H=13.413, df=3; *P*=0.004, Kruskal-Wallis on ranks). Out of the total capture, about 86% were *B. dorsalis* which was significantly higher than other species, i.e. *B. umbrosa* (5.8%) and *B. carambolae* (0.1%) (*P*<0.05; Tukey's test) (Figure 1). There was about 8.1% of *Bactrocera* male flies of intermediate morphological characteristics between *B. dorsalis* and *B. carambolae* (Wee & Tan 2005; hereafter referred to as intermediates).

Since initial analysis showed that the fruit fly capture versus time was not significantly different between species, hence the data were pooled to produce mean *Bactrocera* fruit flies captured versus time for further analysis. The results showed that there was a temporal effect in the diurnal attraction of male *Bactrocera* spp. to ME from 07:00 to 18:00 hr (F=13.375, df=21,66; *P*<0.001). A low number of *Bactrocera* spp. males (28.3 ±10.3 flies) started to respond and captured by ME-baited trap at 07:00-07:30 hr (Figure 2). After just 30 min, a significant increase in male attraction was observed from 07:30 onwards. The attraction peaked at 08:30-9:00 hr (235.3 ± 46.0 flies) (*P*<0.001; Holm-Sidak method) before the attraction slightly decreased to 135.0 ± 29.0 flies (*P*>0.05). From 10:00-10:30 hr and thereafter, male fruit fly capture was significantly decreased (*P*<0.05) and the trend of attraction remained low until 18:00 hr.

DISCUSSION

There diurnal rhythm of *Bactrocera* males' attraction to ME clearly displayed a temporal pattern. A low number of males began to respond to ME as early as 07:00 hr and male attraction increased with time in a day. The period of optimum response to ME was from 07:30 to 09:30 hr with a prominent peak between 08:30 to 09:00 hr. The temporal attraction to ME for males of *B. dorsalis* and *B. umbrosa* were almost similar in this study while those of *B. carambolae* cannot be verified as the *B. carambolae* capture was too low to make any meaningful conclusion. The diurnal rhythm of fruit fly is closely associated with the changes in the daylight intensity where most fruit flies engage in food foraging, feeding and oviposition activities in the morning (Arakaki et al. 1984). This result showed that the male attraction to ME is also corresponded with food foraging and feeding activities in fruit flies, as in the case of other ME-attracted male species such as *B. cacuminata* (Brieze-Stegeman et al. 1978) as well as those from another group of species such as *Zeugodacus cucurbitae* (Manoukis & Jang 2013) and *B. tryoni* (Brieze-Stegeman et al. 1978) that are attracted to cuelure. Once attracted, the males displayed voracious feeding behaviour on the chemical source.

The attraction of the male flies to lures such as ME has been a subject of discussion for many years (Brieze-Stegeman et al. 1978; Cunningham 1989; Metcalf 1990) until it was shown in the case of *B. dorsalis* that the acquisition of ME by males of the aforementioned species was a part of the co-evolution between those flies as pollinators and their flowers as in the case of certain *Bulbophyllum* orchids (Tan & Nishida 2012 & references therein) and plants such as the golden shower blossom, *Cassia fistula* (Shelly 2000). Male flies acquired sexual advantage from consumption of ME and this led to attainment of earlier and higher copulation rates (Hee & Tan 1998; Tan & Nishida 2012; Wee et al. 2018). Thus, the determination of peak period of male response to ME is important especially for fruit fly behavioural study in relation to phytochemical lure consumption. Hence, the preparation of phytochemical lure feeding should be done within the optimum time of lure response to ensure optimum feeding by the tested flies (Wee et al. 2002, 2018).

Present study also suggests that within the peak period of male attraction to ME, population also especially using mark-release-and recapture technique would be significantly improved as males' ME attraction is highest at those periods. This allows optimization to achieve higher levels of precision leading to a more accurate interpretation of the population estimates.

Bactrocera dorsalis was found to be the most abundance species with a very low number of sibling species, *B. carambolae* in the sampling site in Tanjung Bungah, Penang. The result is corroborated with previous report that *B. dorsalis* is widely distributed in the northern region while *B. carambolae* is found more readily in the southern region of Peninsular Malaysia (Wee & Tan 2005; Clarke et al. 2001). The findings of *Bactrocera* spp. with intermediate moprphological characteristics were first reported by Wee and Tan (2005). Both *B. dorsalis* and *B. carambolae* are closely related sibling species within the *B. dorsalis* complex with almost similar morphological characteristics except for a recurve pattern at the apex of wing at costal band and the presence of bar-shaped abdominal bands at terga III-V in the latter. The presence of a dark spot on the fore femore in *B. carambolae*, formerly used as a morphological trait to differentiate between *B. dorsalis* and *B. carambolae*, was found to be an unreliable character for species differentiation (Schutze et al. 2014). However, both species have a pronounce difference in the pheromone make up (Wee & Tan 2005a, b). The presence of these intermediates have sparked speculation that they were resulted from natural interbreeding between the two sibling species in the wild as both species interbred readily in the laboratory and produced viable offspring up to F_3 (Wee 2002). While no direction observation in the field can confirm the occurrence of natural hybrids, indirect inference was obtained from the pheromone analysis higher that shown the intermediates had intermediate pheromonal contents in the rectal gland (Wee & Tan 2005).

CONCLUSIONS

The present study suggests that both *B. dorsalis* and *B. umbrosa* males' attraction to ME occurs throughout the day; with peak period of attraction to ME occurring *ca.* 30 mins following sunrise for 2 hrs from 07:30 to 09:30 hr and a distinct peak at 08:30-09:00 h, before gradually tapering off. Such information is valuable for ecological and behavioural studies involving phytochemical lures and their interactions with lure-responding *Bactrocera* males as well as operational level of fruit fly control programmes.

ACKNOWLEDGEMENTS

We wish to thank KH Tan for his earlier guidance in the course of this work and CH Ong for his assistance in conducting this survey.

REFERENCES

Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hameck, E.L., Hancock, D.L., Hengsawad, J.C., Jipanin, M., Kon Krong, C., Kritsaneepaiboon, S., Leong, C.T.S. & Vijaysegaran, S. 1999. Host plant records for fruit flies (Diptera: Tephritidae) in South East Asia. *The Raffles Bulletin of Zoology*. In supplement No. 7–92.

Arakaki, N., Kuba, H. & Soemori, H. 1984. Mating behavior of the Oriental fruit fly, *Dacus dorsalis* Hendel (Diptera: Tephritidae). *Applied Entomology and Zoology 19*: 42-51.

Bayoumy, M. H. & El-Metwally M. M. 2017. Daily flight activity rhythms of the peach and mediterranean fruit flies using sexual and olfactory attractants. *Acta Phytopathologica et Entomologica Hungarica* 52. doi.org/10.1556/038.52.2017.022.

Bateman, M.A. 1972. The ecology of fruit flies. *Annual Review of Entomology* 17: 493-518.

Brieze-Stegeman, R., Rice, M.J. & Hooper, G.H.S. 1978. Daily periodicity in attraction of male tephritid fruit flies to synthetic chemical lures. *Journal of the Australian Entomological Society* 17: 341-346.

Clarke, A.R., Allwood, A.J., Chinajariyawong, A., Drew, R.A.I., Hengsawad, C., Jirasurat, M., Kong Krong, C., Kritsaneepaiboon, S. & Vijaysegaran, S. 2001. Seasonal abundance and host use patterns of seven *Bactrocera* MacQuart species (Diptera: Tephritidae) in Thailand and Peninsular Malaysia. *Raffles Bulletin of Zoology* 49: 207–220.

Cunningham, R.T. 1989. Male annihilation. In Robinson, A.S. & Hooper, G. (Eds.). *World Crop Pests: Fruit Flies. Their Biology, Natural Enemies and Control*, Vol. 3B, pp. 345-351. Amsterdam: Elsevier.

Drew, R.A.I. & Hancock, D.L. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. *Bulletin of Entomological Research*, Supplement 2.

Drew, R.A.I. & Romig, M. C. 2013. *Tropical Fruit Flies of South-East Asia: Indomalaya to North-West Australia*. UK: CAB International.

Hee, A.K.W. & Tan, K.H. 1998. Attraction of female and male *Bactrocera papayae* to conspecific males fed with methyl eugenol and attraction of females to male sex pheromone components. *Journal of Chemical Ecology* 24: 753-764.

IAEA. 2003. Trapping Guidelines for Area-wide Fruit Fly Programmes. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Austria.

Manoukis, N.C. & Jang, E.B. 2013. The diurnal rhythmicity of *Bactrocera cucurbitae* (Diptera: Tephritidae) attraction to cuelure: Insights from an interruptable lure and computer vision. *Annals of the Entomological Society of America* 106: 136-142.

Metcalf, R.L. 1990. Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America* 83: 1017-1030.

Shelly, T.E. 2000. Flower-feeding affects mating performance in male Oriental fruit flies, *Bactrocera dorsalis*. *Ecological Entomology* 25: 109-114.

Schutze, M.K., Aketarawong, N., Amornsak, W., Armstrong, K.F., Augustinos, A., Barr, N., Bo, W., Bourtzis, K., Boykin, L.M., Cáceres, C., Cameron, S.L., Chapman, T.A., Chinvinijkul, S., Chomič, A., De Meyer, M., Drosopoulou, E.D., Englezou, A., Ekesi, S., Gariou-Papalexiou, A., Hailstones, D., Haymer, D., Hee, A.K.W., Hendrichs, J., Hasanuzzaman, M., Jessup, A., Khamis, F.M., Krosch, M.N., Leblanc, L., Mahmood, K., Malacrida, A.R., Mavragani-Tsipidou, P., McInnis, D.O., Mwatawala, M., Nishida, R., Ono, H., Reyes, J., Rubinoff, D.R., San Jose, M., Shelly, T.E., Srikachar, S., Tan, K.H., Thanaphum, S., Ul Haq, I., Vijaysegaran, S., Wee, S.L., Yesmin, F., Zacharopoulou, A. & Clarke, A.R. 2015. Synonymization of key pest species within the *Bactrocera dorsalis* complex (Diptera: Tephritidae): Taxonomic changes based on 20 years of integrative morphological, genetic, behavioural, and chemoecological data. *Systematic Entomology* 40: 456–471.

Tan, K.H. 1984. Description of a new attractant trap and the effect of placement height on catches of two *Dacus* species (Diptera: Tephritidae). *Journal of Plant Protection in the Tropics* 1: 117-120.

Tan, K.H. & Lee, S.L. 1982. Species diversity and abundance of *Dacus* (Diptera: Tephritidae) in five ecosystems of Penang, West Malaysia. *Bulletin of Entomological Research* 72: 709-716.

Tan, K.H., Nishida, R. 2012. Methyl eugenol – its occurrence, distribution, and role in nature, especially in relation to insect behavior and pollination. *Journal of Insect Science* 20:56.

Wee, S.L. 2002. Behaviour and Reproductive Ecology of *Bactrocera carambolae* and *B. papayae.* Ph.D. thesis. University Science Malaysia, Penang, Malaysia.

Wee, S.L. & Tan, K.H. 2005a. Evidence of natural hybridization between two sympatric sibling species of *Bactrocera dorsalis* complex based on pheromone analysis. *Journal of Chemical Ecology* 31: 845–858.

Wee, S.L. & Tan, K.H. 2005b. Female sexual response to male rectal volatile constituents in the fruit fly, *Bactrocera carambolae* (Diptera: Tephritidae). *Applied Entomology and Zoology* 40: 365-372.

Wee, S.L., Abdul Munir, M.Z. & Hee, A.K.W. 2018. Attraction and consumption of methyl eugenol by male *Bactrocera umbrosa* Fabricius (Diptera: Tephritidae) promotes conspecific sexual communication and mating performance. *Bulletin of Entomological Research* 108: 116-124.

Wee, S.L., Hee, A.K.W. & Tan, K.H. 2002. Comparative sensitivity to and consumption of methyl eugenol in three *Bactrocera dorsalis* (Diptera: Tephritidae) complex sibling species. *Chemoecology* 12: 193–197.

White, I.M. & Elson-Harris, M.M. 1992. *Fruit Flies of Economic Significance: Their Identification and Bionomics*. Oxford: CAB International.

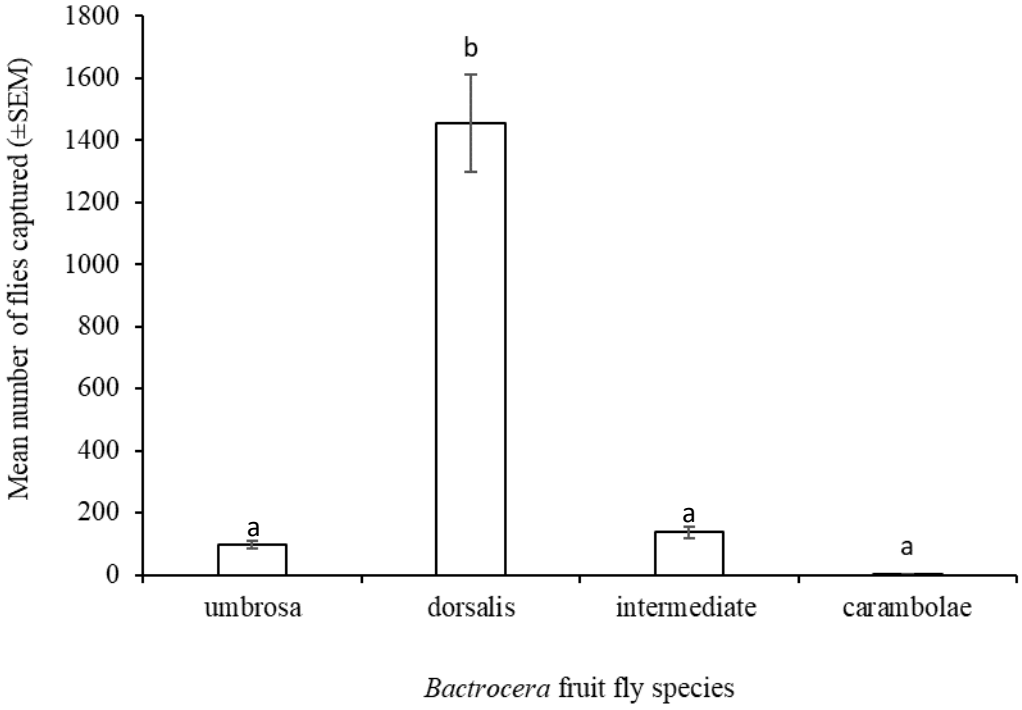

Figure 1 Fruit fly males of *Bactrocera* spp. captured in methyl eugenol-baited traps from 07:00 to 18:00 hour in Tanjung Bungah, Penang, Malaysia (n=4). Bars (mean number ±SEM) designated by different alphabets are significantly different (Tukey's test, $P<0.05$).

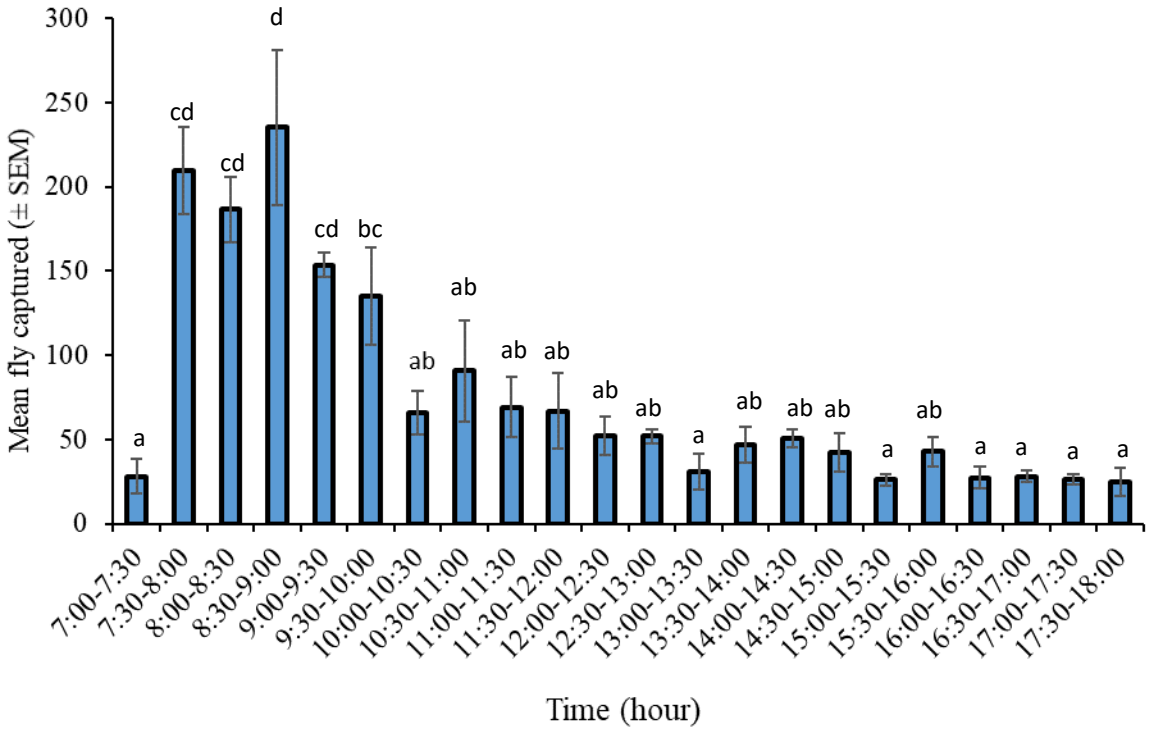

Figure 2 Diurnal attraction of males *Bactrocera* spp. (mean number ±SEM) to methyl eugenol-baited traps from 07:00 to 18:00 hour in Tanjung Bungah, Penang, Malaysia (n=4). Bars (mean number ±SEM) designated by different alphabets are significantly different (Holm-Sidak method, $P<0.05$).