SEARCHING AND PARASITISM BEHAVIOUR OF *Trichospilus pupivorus* (HYMENOPTERA: EULOPHIDAE), A POTENTIAL PARASITOID FOR COCONUT BLACK-HEADED CATERPILLAR, *Opisina arenosella* (LEPIDOPTERA: OECOPHORIDAE)

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ABSTRACT

Black-headed caterpillar, *Opisina arenosella* (Lepidoptera: Oecophoridae) is one of the major pests of coconut in Asian countries including India, Sri Lanka, Bangladesh, Pakistan, Myanmar, China and Thailand. Recently, the pest has been detected in two (2) acres of aromatic (Pandan) coconut palms (*Cocos nucifera*) in Cherating, Pahang. Nearly 60% of coconut palms have been infested by the caterpillar and caused extensive defoliation of coconut leaves. For interim control measure, chemical pesticides have been applied for several times, however the infestations still exist. Therefore, an alternative effort to manage the pest by using biological control method has been initiated. As an initial experiment, a study on searching and parasitism behaviour of naturally occurred parasitoid namely as *Trichospilus pupivorus* (Hymenoptera: Eulophidae) has been conducted. In a no choice and choice experiment, three (3) types of host were prepared as follows: (i) unparasitized, (ii) parasitized and (iii) empty (control) host in ventilated glass vials which served as parasitism areas with ten hosts per category. Female parasitoids were then released into the areas and allowed to search for their host for two (2) hours. The experiments were done in complete randomised design (CRD) with five (5) replications each. Data on parasitoids behaviour, time duration for each behavioural stages and host preferences were recorded throughout the activity. Results showed that *T. pupivorus* significantly preferred the unparasitized host compared to the other treatments ($P<0.05$). Overall, there were six (6) stages of behaviour as they completed the parasitism process. The females spent most of their time parasitizing a host and successfully completed the entire parasitism phase in 98.12 minutes. Further observation should be carried out to ensure whether its parasitism ability would outweigh the long time-duration of parasitism process.

Keyword(s): Pupal parasitoid, gregarious parasitoid, *Opisina arenosella*, *Trichospilus pupivorus*, searching behaviour, parasitism behaviour

ABSTRAK

Ulat '*black-head*', *Opisina arenosella* merupakan salah satu daripada perosak utama tanaman kelapa di negara-negara Asia termasuk India, Sri Lanka, Bangladesh, Pakistan, Myanmar, China dan Thailand. Kebelakangan ini, perosak tersebut telah dikesan menyerang kawasan penanaman kelapa (*Cocos nucifera*) beraroma (Pandan) berkeluasan dua (2) ekar di Cherating, Pahang. Dianggarkan hampir 60% tanaman kelapa di kawasan tersebut telah diserang dan menyebabkan kerosakan yang teruk pada daun kelapa. Aplikasi kawalan menggunakan racun perosak kimia telah dilaksanakan sebagai kawalan sementara. Namun, serangan masih dikesan. Oleh itu, kaedah kawalan alternatif menggunakan agen kawalan biologi telah dimulakan dengan mengkaji kelakuan mencari dan memparasit oleh parasitoid *Trichospilus pupivorus*. Sebanyak tiga (3) jenis perumah disediakan seperti berikut: (i) perumah baru, (ii) perumah yang telah diparasit, dan (iii) perumah kosong. Sebanyak sepuluh (10) perumah bagi setiap jenis diletakkan di dalam bekas kaca telus udara yang berfungsi sebagai ruang memparasit. Kajian berbentuk pilihan dan tanpa pilihan dengan lima (5) replikasi dilaksanakan dengan melepaskan parasitoid betina untuk mencari dan memparasit perumah dalam tempoh dua jam. Data berkaitan kelakuan parasitoid, tempoh masa bagi setiap peringkat kelakuan dan pemilihan perumah telah dicatat bagi sepanjang tempoh aktiviti dijalankan. Keputusan mendapati bahawa *T. pupivorus* memilih perumah baru secara signifikan berbanding dengan perumah yang lain (P<0.05). Secara keseluruhannya, terdapat enam (6) peringkat kelakuan yang diperhatikan sepanjang aktiviti tersebut. Parasitoid betina menghabiskan lebih banyak masa ketika memparasit perumah dan melengkapkan fasa memparasit dalam tempoh masa 98.2 minit. Kajian lanjutan perlu dilaksanakan bagi memastikan sekiranya keupayaan memparasitnya adalah lebih besar berbanding dengan tempoh masa yang lama dalam fasa memparasit.

Kata kunci: Parasitoid pupa, parasitoid gregarius, *Opisina arenosella*, *Trichospilus pupivorus*, kelakuan mencari, kelakuan memparasit

INTRODUCTION

Opisina arenosella (previously known as *Nephantis serinopa*) or known as black-headed caterpillar is a polyphagous pest infesting coconut palms (*Cocos nucifera*) in many Asian countries including India (Jayaprakash 1992; Kumara 2015), Sri Lanka (Perera 1989), Bangladesh (APPPC 1987), Pakistan, Myanmar (EPPO 2017), China (Baoqian, et al. 2016; Jin, et al. 2018) and Thailand (DOA Thailand 2017). The pest has never been documented in Malaysia previously until late 2017, it was found infesting coconut area in Cherating, Pahang. The pest's morphological characteristics and symptoms of infestation has been recorded and discussed by Nor Ahya and Tajul Ariffin (2018) to confirm its identity. Aside from coconut, this caterpillar also attacks other crops including oil palm, jackfruit and cashew (Shameer et al. 2017). *Opisina arenosella* larvae consists of 8 instars and completed its life cycle within 2-3 months under laboratory condition (Santhosh 1989).

Trichospilus pupivorus Ferriere is a gregarious pupal parasitoid of many agricultural pests (Tavares et al. 2012a, 2013; Silva et al. 2016) including *O. arenosella.* It was first observed as pupal parasitoid by Hutson (1920) in Ceylon. Even though it is a pupal parasitoid, it has been used to control the pest in South India along with other mechanical control methods (Anantanarayanan 1934). Its efficiency to be used as a potential biocontrol agent of *O. arenosella* in certain localities during favorable season also agreed by Remadevi et al. (1980). The characteristics of the parasitoid has been described in detail by Ferriere (1930) and Narendran (2011). Extensive studies on various aspects of *T. pupivorus* has been done by previous workers such as the biological aspects (Jayaratnam 1941; Rao et al. 1948; Dharmaraju 1952; Nirula 1956), factors affecting the realization of the biotic potential of the parasitoid (Dharmaraju & Pradan 1976) and its hosts range (Tavares et al. 2012a; Tavares et al. 2013; Silva et al. 2016). However, very limited information recorded on the behaviour of this parasitoid which is vital in determining its parasitism efficiency.

Parasitism process involves several behavioural steps that require to be followed. The steps are host habitat preference, host location, host acceptance, host adaptation and host regulation (Vinson 1985; Xu 2000). Many workers have shortened the process by eliminating the first step through strategic delivery system of parasitoid to ensure successful parasitism is achieved. Meanwhile, host adaptation and host regulation are considered as unimportant for idiobiont parasitoids (Xiaoyi & Zhongqi 2008) such as *T. pupivorus*. Thus, leaving the two steps of host location and host preference as the keys step which are important in determining the parasitoid's efficiency.

Since *O. arenosella* is a new invasive pest in Malaysia, there is no available information on the suitable control method of the pest under local condition. Even though interim control using chemical pesticides has been done to each infested palm, infestations still exist. Thus, it is vital to look for alternative control method such as biological control using parasitoid to manage this pest. Therefore, this study was initiated to understand the searching and parasitism behaviour of *T. pupivorus* in order to evaluate its efficiency as biocontrol agent of the coconut black-headed caterpillar, *O. arenosella*.

MATERIALS AND METHODS

Sampling of Parasitoid

Coconut fronds showing symptoms of black-headed caterpillar infestation as described by Nor Ahya and Tajul Ariffin (2018) were randomly cut from the two (2) acres coconut planting area in Cherating, Pahang and brought back to the laboratory. Alive samples of the pest were later sorted out according to its developmental stages and placed into labelled container (40cm x 25cm x 15cm) covered with muslin cloth for ventilation. Samples were observed daily for emergence of *T. pupivorus*. Upon emergence, the parasitoids were isolated into labelled ventilated glass vial (2cm diameter x 5cm height) using aspirator and offered with honey as source of food.

Host Preparation

Late instar larvae of *O. arenosella* were reared in transparent plastic containers (10cm diameter x 15cm height) supplied with 10 fresh coconut leaflet cuttings as food source until pupation. The leaflet cuttings were replenished every two days to ensure its freshness. The pupae with same size, age and sex (Nor Ahya & Tajul Ariffin 2018) were later collected and placed into three labelled glass vial (unparasitised, parasitised and empty). Unparasitised/healthy pupae characterised by brownish orange colour (Nor Ahya & Tajul Ariffin 2018) were selected directly from rearing container. Meanwhile, parasitized/unviable pupae denoted by black colour (Tavares et al. 2012b) were prepared by offering another group of unparasitised pupae to *T. pupivorus* to be parasitised. After 24 hours, the presumably parasitised pupae were observed regularly until they turned black. Empty pupae denoted by presence of holes on the pupae served as control (Fig. 1).

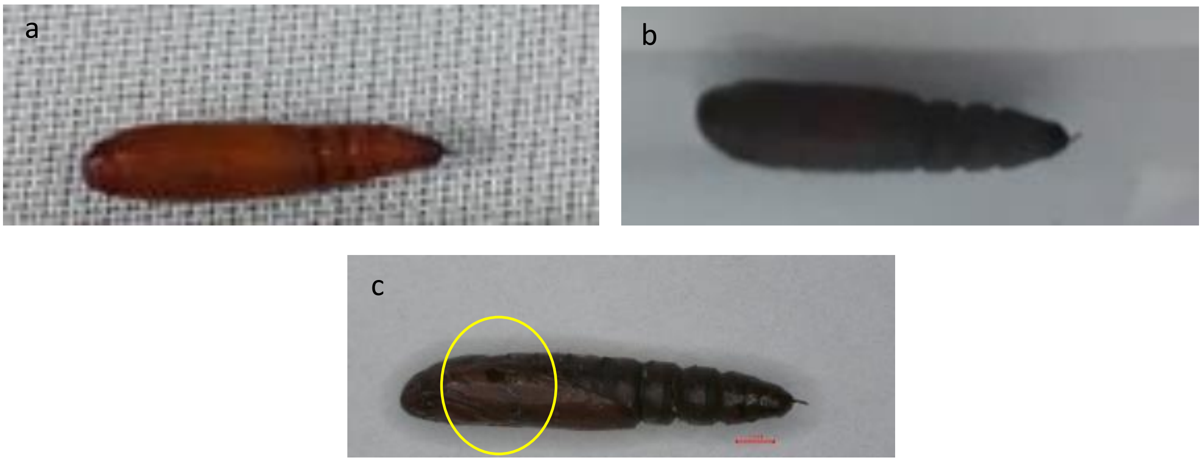

Figure 1. Pupae of *O. arenosella* (a) Unparasitised/healthy pupae (b) Parasitized pupae (c) Empty pupae

Experimental Design

Three ventilated glass vials (4cm diameter x 12cm height) were prepared as parasitism areas where 10 pupae of each type were placed separately. On the other hand, another parasitism area was prepared by placing all three types of pupae together in one area. The cocoons covering the pupae also placed inside the vial as they are part of the host. Diluted honey (50%) were prepared and swabbed onto the wall of glass vials as source of food. Mated female parasitoids (Kumar et al. 1995) were released into the area and allowed to parasitize the hosts. Observations were done for two hours while data on behaviour and host preference of *T. pupivorus* were recorded.

The treatments were arranged following the Complete Randomized Design (CRD) with five replications. All treatments were kept in laboratory at 30±3 ºC, 80±5% RH and illumination of 12h:12h (Light: Dark). All data were analysed using Analysis of Variance (ANOVA) and multiple mean comparisons were done using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Behaviour of *T. pupivorus*

Table 1 shows that *T. pupivorus* undergo two main behavioural phases in microhabitat i.e searching (four events) and parasitism (two events) phases before successfully parasitised its host. This behavioural pattern is different with the ones expressed by other type of beneficial insects such as predator in which the predatory phase of a predator consists of more events (Noor Farehan et al. 2018) than parasitism phase of a parasitoid. During the searching phase, *T. pupivorus* explored the parasitism area while flying and walking in short distance for 18.68±1.9 minutes. Antenna movements were observed but not frequent. Later, they walked onto the host (cocoon) when they found one (15.25±0.5 minutes). Cocoons or protective silk cases were likely attracted by the parasitoid as they spent longer time walking there. It is probably due to the volatile substances emitted from the cocoon that eventually triggered the parasitoid's response in locating its host such as reported by Glasser and Farzan (2016) in which *Melittobia acasta* showed strong attraction to the *Osmia lignaria*'s cocoon in a Y-tube bioassay. Many studies by previous workers has proved that host-derived stimuli such as faeces (Faraone et al. 2017), host habitat (Buonochore Biancheri et al. 2019), saliva (Tang 2016) and others are the most reliable indicators of host presence. Time durations for these first two events did not differ from one to another probably due to the fact that they used both olfactory and visual responses while searching within short distances during these events. Graziosi and Rieske (2013) proved that both olfactory and visual cues able to boost response percentage of *Torymus sinensis,* a Hymenopteran parasitoid, during host searching.

Next, the parasitoids started to move their antennae frequently (drumming) while walking before falsely probing their ovipositors into the host repeatedly. According to Silva et al. (2016), *T. pupivorus* has high concentration of trichodea sensilla on their antenna with mechanosensory function to detect air currents and vibration signals which are important for searching in females. During these events, they used the mechanosensory functions to check the suitability of the host and it lasted for a short time. Ruschioni et al. (2015) claimed that a parasitoid able to discriminate between hosts with the aid of neurons present in the sensillum of its ovipositor. This could indirectly have explained the behaviour of repeated probing by *T. pupivorus* in which the females outweighing the decisions either to oviposit or not.

During parasitism phase, *T. pupivorus* spent significantly longer time bending its abdomen and inserting its ovipositor into the host (56.4±0.7 minutes). This could be attributed by the fact that it lays more than one egg per time. Previous study showed that the number of progenies emerged from a host parasitized by a single female of *T. pupivorus* ranging from 50 to 75 individuals (Tavares et al. 2013).

Ovipositor released and body cleaning by *T. pupivorus* lasted for 1.94±0.2 minutes, the shortest time taken among all other events. The sequence of cleaning process was indistinguishable between individuals but mainly involved cleaning of antenna and both hind and fore legs. Even though the activity may be considered as auxiliary phase and does not seem to contribute directly to the host acceptance process (Hcidari & Jahan 2000), it could however become an indicator of parasitoid preparation to search for the next host.

Table 1 Mean duration (min) spent by *T. pupivorus* for searching and parasitism behaviour

Phase	**Events**	***Mean±S.E (minutes)**
Searching	Flying and walking	18.68±1.9b
	Walking onto the host	15.25±0.5b
	Walking while moving its antenna	3.32±0.5c
	Poking ovipositor while walking	2.53±0.4c
Parasitism	Abdomen bending, inserting ovipositor into the host	56.4±0.7a
	Releasing ovipositor, body cleaning	1.94±0.2c

*Mean in column with different letters indicate significant at $\alpha = 0.05$

Choice and No Choice Experiment

Table 2 shows that the results of parasitism activity between the two experiments and host types are differed significantly with P value 0.0102 and <0.0001 respectively. Host preference of *T. pupivorus* is significantly higher on unparasitized host for both choice and no choice experiments as compared to parasitized and empty hosts (Fig. 2 and 3). It indicates that the parasitoid able to distinguish between types of host by parasitizing the most suitable ones that would give advantage to the progenies development. Li and Sun (2011) reported that a gregarious parasitoid, *Sclerodermus harmandi* able to perfectly discriminate among host in a manner that maximizes its offspring fitness. In other word, it selected the most profitable host for its progeny development.

Selecting a readily parasitized host by a parasitoid would theoretically affected the availability of host resources for more eggs laying and optimum progenies development, thus could explain the less preferred parasitized host by *T. pupivorus* in this experiment. This is supported by Magdaraog et al. (2013) in which both *Cotesia kariyai*, a gregarious parasitoid and *Meteorus pulchricornis*, a solitary parasitoid, able to discriminate between parasitized and unparasitised host. However, the degree of superparasitism, even though less preferred, should be explored further to ensure that it would not cost its parasitism efficiency in the field. Meanwhile, empty hosts of *O. arenosella* pupae were not attractive to *T. pupivorus* and not preferred due to the low quality of the host and not suitable for ovipositions in both choice and no-choice experiments. Hackermann et al. (2007) mentioned that host quality is dependent on the availability of host's resources but not necessarily on host size by referring to the ability of *Hyssopus pallidus* to indicate nutritional quality of its host before making decision for egg laying.

Table 2. Results of ANOVA for parasitism activity on different host types and experiments

Source	**Df**	**F- value**	**P-value**
Experiments	1	7.78	0.0102*
Host types	2	73.03	<.0001*
Experiments x Host types	2	3.41	0.049

Asterisk indicates significant at α = 0.05

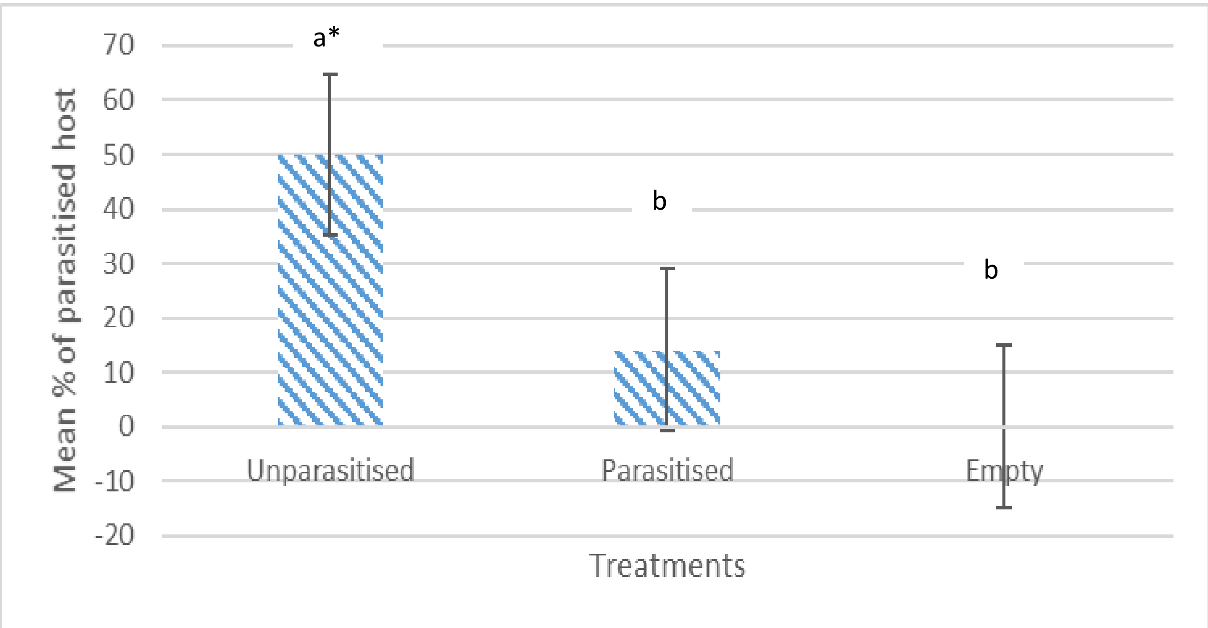

Figure 2. Host preference of *T. pupivorus* in no choice experiment

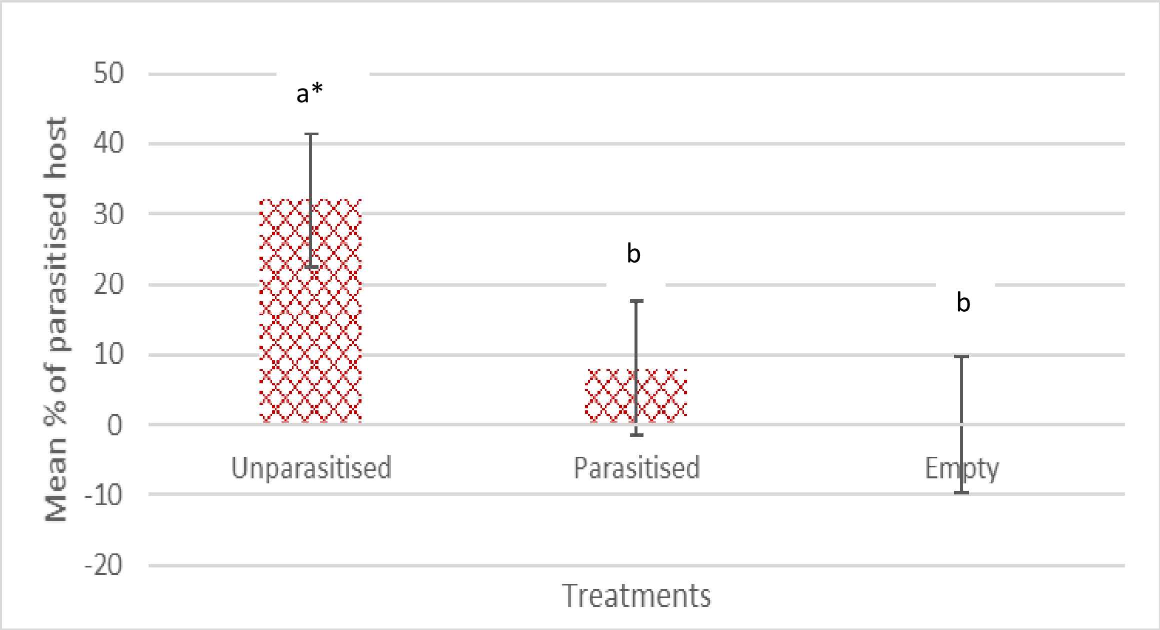

Figure 3 Host preference of *T. pupivorus* in choice experiment

CONCLUSION

Based on this study, we concluded that *T. pupivorus* undergo six (6) behavioural events (flying and walking; walking onto the host; walking while moving antenna; poking ovipositor while walking; abdomen bending and inserting ovipositor; and releasing ovipositor and body cleaning) before successfully parasitized a host and able to complete the entire searching and parasitism process approximately in 98.12 minutes. The longest time duration in parasitism phase which could be attributed by the number of eggs laid is advantageous for the mass rearing. This information could be helpful in developing mass rearing procedure of the parasitoid. However, in-depth study should be conducted to understand whether its parasitism ability would outweigh the long time-duration of parasitism phase as well as the relationship with super parasitism to ensure the parasitoid efficiency.

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