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Mass infestation of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) on colonies of the Indo-Malayan stingless bees *Geniotrigona thoracica* and *Heterotrigona itama*

Nur Aida Hashim*[1], A. R. Shamsul Bahri[2], Norasmah Basari[3], Nur Hafizah Sharudin[4]

[1]*School of Food Science and Technology, Universiti Malaysia Terengganu,Terengganu, Malaysia*

[2]*UMT COE for Apis and Meliponine, Universiti Malaysia Terengganu, Terengganu, Malaysia*

[3]*School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, Terengganu, Malaysia*

[4]*Rice, Industrial Crops and Floriculture Division, Department of Agriculture, Perak, Malaysia*

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Abstract

Meliponiculture, a stingless bees beekeeping has become an important industry in Malaysia. Reports on mass infestation of pests on colonies of domesticated stingless bees (*Geniotrigona thoracica* and *Heterotrigona itama*) were received in mid-June 2016 from the beekeepers in northern peninsular Malaysia. The infestation destroyed thousands of stingless bee colonies within one week of attack, resulting in huge losses to the bee industry. This research was conducted to determine the species of pest infested the bees colonies in the northern peninsular Malaysia. Samples of larvae from the infested colonies were collected manually using entomological forceps from a stingless bees farm in the state of Kedah and the larvae were reared in the laboratory until adult stage. Identification of the larvae and adult pest confirmed that the invading species were the black soldier fly (BSF) (*Hermetia illucens*) that had not hitherto been regarded as a pest, but as a beneficial insect. The four major signs of attack by this pest on the stingless bee colonies are: 1) the nest entrance became smaller, 2) Honey in the honey pots developed bubbles 3) The presence of white larvae in the nest topping, and 4) honey spilled from the honey pots and flowed out from the nest topping. This is the first report on mass infestation of domesticated stingless bee colonies by the BSF. Therefore it is important to monitorthe spread and the potential threat poses by BSF on the stingless bee for sustainability of stingless bees itself and meliponiculture in Malaysia.

***Corresponding Author:** Nur Aida Hashim ✉ aida.hashim@umt.edu.my

Introduction

Stingless bees are a group of eusocial insects which play an important role in the pollination process of plants, particularly wild flowers, in most tropical countries (Heard, 1999).The stingless bees constitute the Meliponini tribe of the family Apidae. Their nests are made from wax mixed with resin and gum; some species add mud collected by worker bees. The honey of stingless bees is a bit sour to the taste, and has a tangy smell. The acidity of stingless bee honey is related to the maturation state of honey, and it intensifies with fermentation (Vit *et al.,* 2004).In Malaysia, several species are commonly cultured for their honey. Until August 2016, a total of about 3000 stingless bee colonies were maintained by 55 stingless-beekeepers in northern peninsular Malaysia, with aid received from the Department of Agriculture (Department of Agriculture Malaysia, unpublished). The most common species of stingless bee domesticated for honey in Malaysia are *Geniotrigona thoracica, Heterotrigona itama*, *Tetragonula laeviceps* and *Lepidotrigona terminata* (Department of Agriculture Malaysia, unpublished).

The insect pests associated with beehives of honey bees are well known. The most relevant pests associated with honey bees in North America and Australia are the small hive beetle (SHB) *Aethina tumida* (Coleoptera:Nitidulidae) and dusky sap beetle (DSB) *Carpophilus lugubris* (Coleoptera: Nitidulidae) (Cuthbertson *et al.,* 2013).

Meliponiculture or stingless-beekeeping is still in its infancy in Malaysia. Hence, reports on pests associated with stingless bees are very limited, with only one paper describing the nitidulid beetle, *Haptoncus luteolus* as a pest of three local species of stingless bees, viz. *G. thoracica, H. itama and T. laeviceps* (Krishnan *et al.,*2015).

However, on the 25th June 2016, a report on mass infestation of pests on stingless bees colonies was received from a local stingless-beekeeper in the northern peninsular Malaysia.

Hence, a thorough investigation was conducted at the infested area. This paper reports the association of the pest with two Indo-Malayan stingless bees in Malaysia that caused huge losses to beekeepers in northern peninsular Malaysia.

Materials and methods

Description of study site

Samples in this study was collected from a stingless bees farm namely Kelulut Jelutong Sdn. Bhd. located in Kuala Nerang, Kedah (6.2528° N, 100.6072° E) (Fig. 1). The bees farm is surrounded by a rubber plantation and fruit orchards. Approximately 300 m from the farm there is a smallpoultry processing house. Kelulut Jelutong Sdn. Bhd. Accommodated 350 colonies (log/nest)of stingless bees. Of these, 25 were *G. thoracica*,305 were of *H. itama*, ten were *Lepidotrigona terminata*,

Five were *Tetragonula laeviceps* and *Lophotrigona canifrons* respectively (Table 1).

Collecting larvae samples

Various larval stages of the invading pest were collected from each infested colony in the vicinity of the honey pots by using entomological forceps. The specimens were transferred into 250 ml plastic containers labelled for the different colonies. A small amount of honey was also pipetted into the container to keep the larvae alive.

The containers were covered with fine netting for ventilation, to prevent ant predation and also to prevent the larvae from escaping. Fifty representative larvae were collected from each infected colony.

The rest of the infected colonies were destroyed by burning. All the samples were brought back to the Animal Science Laboratory, School of Food Science and Technology, Universiti Malaysia Terengganu for further processing. In the laboratory, the larvae were observed under the stereomicroscope (Olympus SZX16) and identified based on keys by Smith (1989) and Thyssen (2010).

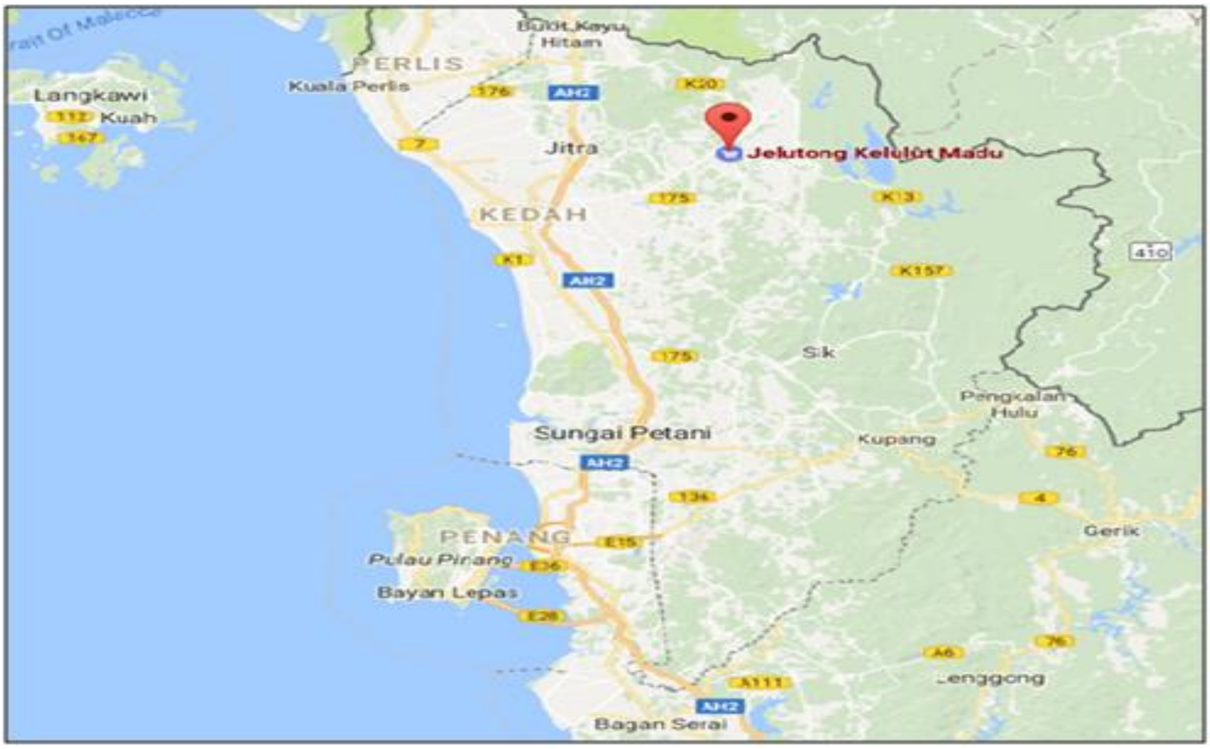

Fig. 1. Location of sampling area; Kelulut JelutongSdn. Bhd., Kuala Nerang, Kedah. The red button indicate study location (6.2528° N, 100.6072° E).

Observation of infested logs

In addition to larvae samples, five logs of stingless bee nests were packed in biohazard plastic bags and brought back to the green house of Universiti Malaysia Terengganu for further examination.

The logs were cut open vertically using a chainsaw and the extent of the damage by the pest was observed. Pre-pupae from each log were collected using forceps and placed into plastic containers which were then moved into separated insect cages (30cm x 30cm x 30cm). The pre-pupae developed into pupae before emerging as adults. The identification of adults was based on keys by Carvalho and Mello-Patiu (2008).

Results and discussion

All the pest samples collected were identified as the black soldier fly (BSF) larvae, *H. illucens* (Fig. 2A). The larva of *H. illucens* was approximately 18 to 20 mm long and whitish in colour (Fig. 2B).

It had a small head that was sclerotized and clearly differentiated from the rest of the body.

Table 1. Details on the number of infested and non-infested stingless bee colonies at Kelulut Jelutong Sdn Bhd farm.

Species	Infested	Non-infested	Total
Geniotrigona thoracica	25	-	25
Heterotrigona itama	225	80	305
Lophotrigona canifrons	-	5	5
Lepidotrigona terminata	-	10	10
Tetragonula laeviceps	-	5	5
Total	250	95	350

The head consisted of a mouth hook moving vertically, parallel to each other like a pair of hooks, matching the description by Thyssen (2010). The body was flattened and covered with dense and pressed yellow hairs. The posterior spiracular opening was almost apical, consistent with the observation by Smith (1989).

Of the 350 stingless bees colonies that were investigated, 250 colonies were collapseddue to damage done by *H. illucens*. Of these, 250 stingless bee colonies, 25 colonies were of *G. thoracica* and 225 were of *H. itama* (Table 1).

The five stingless bee colonies in the infested logs which were brought back to the Universiti Malaysia Terengganu were in total collapsed since no queen, brood or workers were found in the nests (Fig.2C). Initial observations revealed that each log contained BSF larvae at various stages of development. While the larvae were distributed all over the nest, those in the earlier stages were found more in the upper section of the log which contained the honey pots (Fig.2D). Late instar larvae and pre-pupae were found abundantly at the bottom of the log. There were no pupae inside the log/nests.

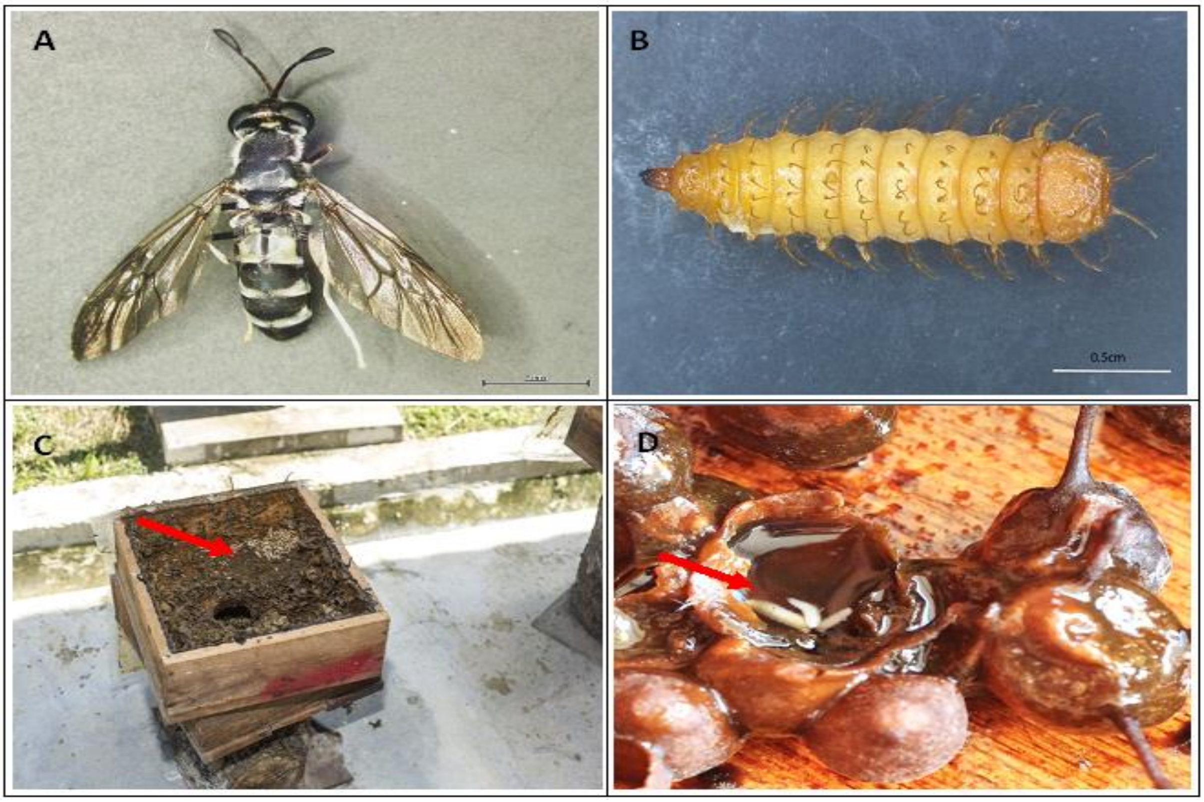

Fig. 2. Sign of pest infestation and life stages of *H. illucens* A) adult of *H. illucens* B) larva of *H. illucens* C) *Hermetia illucens* larvae in the rectangular nest topping (red arrow) D) early stage larvae of *H. illucens* in honey pot (red arrow).

The eggs of BSFtake 3.4 days to hatch (Kim *et al.*,2008) and the larvae pass through six instars after that (Hall and Gerhardt, 2002; Kim *et al.*, 2010). At a temperature of 27 °C and with a standard manure diet, the larvae need another 15 to 20 days to complete the larval duration (Kim *et al.*, 2008). Based on the aforementioned information, the presence of pre-pupae, and the absence of pupae, it was estimated that the stingless bee colonies in the bee farms had been infested for 24 days. Eggs were laid approximately in mid May 2016 which coincided with the start of the heavy rainy season in Kedah. Furthermore, the pre-pupae were found at the bottom of the log, indicating they were ready to pupate.

According to Hall and Gerhardt (2002), the late instar larvae would search for shielded and dry sites to pupate.

The BSF has never been reported as a pest of stingless bees.This fly is generally considered as beneficial insect in agriculture. The fly larvae feed on organic materials including decomposing materials such as rotting fruit, animal corpses, manure, and food waste (Kim *et al.,* 2008; Diener *et al.,* 2009; Nguyen *et al.,* 2015). This insect is well distributed in the tropics and in warm temperate regions (Sheppard *et al.,* 2002). The adult of the black soldier fly has a short lifespan ranging from 5 to 18 days (Kim *et al.,* 2008). It feeds only at the larval stage. The adult does not need to feed and relies on the fats stored from the larval stage (Newton *et al.,* 2005).

Another pest was also found in three out of five log colonies that were brought back for examination.

The infected colonies harboured adults and pupae of the sap beetle *Haptoncus luteolus* but they were fewer in number than the *H. illucens* larvae. The association of *H. luteolus* with stingless bee has been reported by Krishnan *et al.* (2015).

Based on beekeepers' accounts, early signs of the pest infestation were spotted at the start of the rainy season. The nest entrance became smaller, and in some instances, the entrance was blocked by propolis. Honey in the honey pots developed bubbles, indicating active fermentation. After a day, the beekeepers observed that the honey spilled from the pots, and flowed out from the nest topping. During this time the presence of several hornets hovering over the spilled honey could be observed. Small white coloured larvae started to emerge in the vicinity of the honey pots. Upon close inspection, it was observed that the larvae bore holes into the honey pot walls in the course of their feeding activities, causing the honey to flow out. The nest entrance was darkened in colour and dried up, indicating that the stingless bees were trying to protect their colony.

Two local domesticated stingless bee species, *H. itama* and *G. thoracica* colonies were severely affected. However, colonies of another three domesticated local species, *T. laeviceps*, *L. canifrons*and *L. terminata*,in the same bees farm escaped infestation.

The drastic change in climate from the dry season to wet season could have caused insects to proliferate. The sour smell of stingless bees honey might have attracted female black soldier flies to lay their eggs on the nest/log. Normally, this insect would prefer to oviposit in dry cracks and crevices above and around moist decomposing organic matter (Gonzalez *et al.,* 1963). In this instance, the abundance of food sources during the honey flow season (honey, propolis and bee bread) provided a conducive environment as an alternative breeding ground.

Pre-pupae of BSF collected from the logs successfully emerged as adults. The BSF adults are black in colour and have a wasp-like appearance (Fig. 2A). The antenna has three segments with a spatulated apical flagellum. Basal segments of the adult abdomen are transparent (Carvalho and Mello-Patiu, 2008). The legs have a white coloration near their ends. The adults are from 15 to 20 mm in length (Sheppard *et al.,* 2002). The first adults from the collected logs emerged on 20th July 2016. This showed that the black soldier fly larva could successfully complete its life cycle on stingless bee honey.

Hitherto, no insecticide has been used to control BSF for fear it would harm the bees or contaminate the honey. The infestation of stingless bee colonies by BSF larvae cannot be overcome once the attack is well established in the late stages. Nevertheless, there is a fair chance to save the colonies at the very early stages of infestation by replacing the top box on the log with a clean one. Another option is to transfer the healthy stingless bee queen and the brood cells into another starter box. At late stage infestation, however, it is advisable to burn the infested logs and keep the area clean to prevent further spread of the pest.

At the same time, beekeepers might consider according greater attention to the sanitation of areas nearby which can be breeding grounds for unintended pest such as the BSF. In addition, *T. laeviceps*, *L. canifrons* and *L. terminata*, three species of stingless bees that appear to resist invasion by BSFalso need to be examined.

Conclusion

Hermetia illucens (BSF) attack have severely affected the colonies of stingless bees at the abovementioned bee farm. Since stingless bees not only essential for honey production but also have great importance as pollinators of the forest and agriculture ecosystems, thus it is important to highlight the danger posed by BSFon sustainability of stingless bees and meliponiculture in Malaysia. If not controlled, BSF infestation can cripple the stingless bee industry in Malaysia and in neighboring countries such as Thailand and Indonesia. More research needs to be carried out on BSF to understand their development, life history, mode of entry into the nests of stingless bees, as well as the factors that attract this pest to stingless bee nests. Understanding the pest will assist in developing effective early detection methods or repellent approaches to combat the infestation.

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