CO-OCCURRENCE OF DIFFERENT INSECT SPECIES IN OVIPOSITION MEDIA OF BLACK SOLDIER FLY, *Hermetia illucens* (DIPTERA: STRATIOMYIDAE)

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ABSTRACT

The black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae) is well known as a non-pest tropical insect which is distributed in warm temperature and tropic regions. The larvae of this species consume mainly on decomposing organic materials. In this study, fermented coconut waste (CW) was used as oviposition media for black soldier fly. For each sampling, the oviposition media were placed inside five (5) black bins (43 cm diameter x 50 cm height) for ten days in Taman Flora, School of Biological Sciences, Universiti Sains Malaysia (USM). There was co-occurrence of other insect species found in oviposition media of black soldier fly. A total of 3554 insects belonging to four (4) orders were encountered during the study period of eight months, namely, Blattidae, Coleoptera, Diptera and Hymenoptera. Highest number of individuals was recorded from the order Diptera of the family Drosophilidae (N=1890, 53.18%) and followed by the family of Phoridae (N=1350, 37.99%). The percentage of insect species occurred in the following order, *Epunaea luteolus* (3.71%) > Scarabaeidae (3.38%) > *Oecophylla smaragdina* (0.90%) > *Symploce pallens* (0.59%) > *Periplaneta fuliginosa* (0.23%) > and *Paederus littoralis* (0.03%). The finding of this study is of great important to identify the multiple species of insects that contribute either as visitor, decomposer, competitor, or predator to the black soldier fly colony.

Keywords: Species, co-occurrence, black soldier fly, oviposition media, Diptera

ABSTRAK

Lalat askar hitam (BSF), *Hermetia illucens* (Diptera: Stratiomyidae) terkenal sebagai serangga bukan perosak yang tersebar luas di kawasan suhu hangat dan tropika. Larva spesies ini makan terutamanya bahan yang telah diurai atau bahan organik. Dalam kajian ini, sisa kelapa (CW) yang telah difermentasi digunakan sebagai media oviposisi bagi lalat askar hitam. Bagi setiap persampelan, media oviposisi diletakkan di dalam tong hitam (diameter 43 cm x 50 cm tinggi) selama sepuluh hari di Taman Flora, Pusat Pengajian Sains Kajihayat, Universiti Sains Malaysia (USM). Walau bagaimanapun, terdapat kejadian bersama serangga lain yang dijumpai di dalam media oviposisi lalat askar hitam. Sejumlah 3554 spesies serangga yang terdiri daripada empat (4) order telah ditemui dalam tempoh lapan bulan kajian iaitu Blattidae, Coleoptera, Diptera dan Hymenoptera. Jumlah tertinggi individu telah dicatatkan daripada order Diptera daripada keluarga Drosophilidae (N=1890, 53.18%) dan

diikuti oleh keluarga Phoridae (N=1350, 37.99%). Peratusan spesies serangga berlaku dalam susunan berikut, *Epunaea luteolus* (3.71%) > Scarabaeidae (3.38%) > *Oecophylla smaragdina* (0.90%) > *Symploce pallens* (0.59%) > *Periplaneta fuliginosa* (0.23%) > dan *Paederus littoralis* (0.03%). Penemuan kajian ini sangat penting untuk mengenal pasti pelbagai spesies serangga yang menyumbang sama ada sebagai pelawat, pengurai, pesaing, atau pemangsa kepada koloni lalat askar hitam.

Kata kunci: Spesies, kejadian bersama, lalat askar hitam, media oviposisi, Diptera

INTRODUCTION

The black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae) is one of the non-pest tropical insect, considered a native of southern America but currently widespread throughout the world including tropical and sub-tropical regions (Ustuner et al. 2003; Nyakeri et al. 2017). BSF is beneficial in several ways. The larvae of this species consume mainly on decomposed organic materials and effectively reduce nutrient and moisture contents as well as produces non-polluted residues. This species also converts food waste, organic material and manure into nutritionally valuable feedstuff for other animals (Newton et al., 2005; Diener et al. 2009). Furthermore, the adults of BSF are not attracted to human habitation and do not come into contact with any degrading or fresh organic material such as foodstuffs, thus this species was not considered as a vector of diseases (Newton et al. 2005).

Adult BSF usually found near the places with abundant of rotting food, dead animals and manures. Female of BSF attracted to decaying materials and ready to lay eggs near these resources, whereas males prefer to rest on vegetation during daylight (Caruso et al. 2014). The life of adult BSF is short between seven to eight days as its life expectancy being dependent on body size which associated with energy reserves, while mating occurs two to three days after emergence. Mating is exclusively diurnal where illumination and space availability are particularly important during mating (Tomberlin & Sheppard 2002). The cycle of BSF life varies between populations because it is highly influence by the temperature, humidity, light intensity and the availability of the food sources (Tomberlin et al. 2009). The larvae use surrounding organic materials as food sources and gradually develop into pre-pupae. Pre-pupal BSF migrate from the development media to a drier and protected site for pupation. Based on the temperature and ambient humidity, the pupation last five to seven days before emerged into adult (Win et al. 2018). Adult of this species do not feed but relies solely on its body fat reserve. Myers et al. (2014) also indicated that they live longer when a continuous source of water was provided during adult stage.

In nature, decomposing organic matter is the natural habitat for BSF. Other microorganisms such as yeast, fungi, bacteria and small invertebrates are mostly found in decomposing organic matter. Galante & Marcos-Garcia (2008) documented that numerous insect species found actively involved in the recycling process, feeding on plant and animal origin wastes. The BSF is one of the beneficial insect species that completed their life cycle within the organic matter which consequently contributing and speeding up the composting process. The duration of the composting process depends on the type of organic matter and also other factors such as aeration and humidity (Butler et al. 2001). Many insect species naturally feed and develop in organic waste, incorporating the nutrients into their bodies and reducing the amount of waste material in the process. Insects feed almost any substance that has nutritional value, adapted to a broad range of habitats and successfully finding their own role in many ecosystems.

The succession of the insect community in decomposition process has been investigated by several experimental studies using animal models such as demonstrated by Azwandi et al. (2013), Benbow et al. (2013), Mabika et al. (2014) and Zuha et al. (2016) but very few contributions on insect diversity documented during composting process on organic matters such as food waste, agricultural waste, industrial waste and manures. Therefore, this is the study of co-occurrence of different insect species found in oviposition media of BSF which related to the decomposition material. Organic matter attracts an important diversity of arthropods and therefore, the objective of the study is to focus on the identification of insect species and further knowledge of insect's roles occurred in BSF oviposition media. The existence of different species found were further discussed either as visitor, decomposer, competitor, or predator to the BSF population.

MATERIALS & METHODS

Trapping Method

The study was conducted from the month of August 2017 to the month of March 2018. A mixture of 10 kilogram of coconut waste (CW) and 10 litre of effective microorganism (EM) was allowed to be fermented for four days inside five trapping bins each measuring (43 cm diameter x 50 cm height) as oviposition media for BSF. Each of the trapping bin contained two kilograms (2kg) of oviposition media. The pH of oviposition media was recorded using pH meter (Trans Instruments, Model BP3001) to identify the association of pH value with habitat preference by BSF larvae and other insect species during the study. Several holes (2 cm diameter) were drilled around the bins to increase oviposition surface area for female BSF to lay eggs. For each sampling, five media were prepared and placed inside the trapping bins with distance of 10m from one another. The preparations left to stand for the period of ten days inside the trapping bins under the annual average temperature of 29±2 ℃ and 70% relative humidity in Taman Flora, School of Biological Sciences, Universiti Sains Malaysia.

Insect Collection and Identification

The sampling method was adapted from Morales & Wolff (2010). The set up were inspected twice daily at 10am and 4pm for both immature and adult specimens for eight months of the study. Insect species were thoroughly separated and collected continually from the trapping bins until the BSF larvae ready to be transferred into food waste media after ten days. Larvae, pupae or adult of the insects were collected by using forceps and immediately placed into glass vials containing 20 mL of 70% ethanol. Pupae were also collected and brought to the laboratory for adult emergence. Adult insects were killed in chloroform, pinned and kept in insect box. The external morphological characteristics of collected insect species were observed under stereoscopic microscope (Olympus SZ61), connected to Program Cell'senes 1 (Dell Inc. computer) and identified using pictorial keys published by Centers for Disease Control and Prevention (2006), Scudder & Cannings (2006) and Oosterbroek (1998).

Statistical Analysis

For analysis, the identified insects counted for each sampling were pooled together to represent the community of insects throughout the study. Number of insects for each species recorded were subjected to one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) multiple range test was applied to further detect any statistically significant differences at $p<0.05$, using IBM SPSS Statistics Campus Edition V24.0 for Win/Mac.

RESULTS

A total of 3554 insects belonging to four (4) orders were encountered during the study period. These include Blattidae, Coleoptera, Diptera and Hymenoptera respectively. The highest number of individuals was collected from the order Diptera from the family Drosophilidae (N=1890), corresponding to 53.18% whereas family Phoridae (N=1350) corresponding to 37.99%. Coleoptera represented three families namely Scarabaeidae, Nitidulidae and Staphylinidae followed by Blattodae with two families namely Blattidae and Blattellidae while the least was Hymenoptera with one family namely Formicidae (Table 1). Apart from Diptera, the percentage of insect species occurred in the following order of abundance were *Epunaea luteolus* (3.71%) > Scarabaeidae (3.38%) > *Oecophylla smaragdina* (0.90%) > *Symploce pallens* (0.59%) > *Periplaneta fuliginosa* (0.23%) > and *Paederus littoralis* (0.03%). The existing orders recorded in the media during the study period showed that Diptera had 91.17% of the total insects followed by Coleoptera (7.12%), Blattodae (0.82%) and Hymenoptera (0.90%). *D. melanogaster* was the most dominant species followed by *M. scalaris*. Furthermore, the result also showed that the number of *D. melanogaster* and *M. scalaris* found in oviposition media of BSF were significantly higher (df =7, F=59.17, p<0.05) compared to the other insect species and there were also significant different in number of *D. melanogaster* compared to *M. scalaris* (Table 1).

Table 1 List of the recorded family, genera, species, percentage of present and role of insects found in oviposition media of black soldier fly, *H. illucens.*

No	Order	Family	Genus	Species	Number of insects	Percentage (%) of insects	Role of insects
1	Blattodae	Blattidae	*Periplaneta*	*Periplaneta fuliginosa*	8	0.23 [a]	Decomposer
2	Blattodae	Blattellidae	*Symploce*	*Symploce pallens*	21	0.59[a]	Decomposer
3	Coleoptera	Scarabaeidae			120	3.38[a]	Decomposer
4	Coleoptera	Nitidulidae	*Epunaea*	*Epunaea luteolus*	132	3.71[a]	Decomposer
5	Coleoptera	Staphylinidae	*Paederus*	*Paederus littoralis*	1	0.03[a]	Visitor
6	Diptera	Drosophilidae	*Drosophila*	*Drosophila melanogaster*	1890	53.18[b]	Competitor
7	Diptera	Phoridae	*Megaselia*	*Megaselia scalaris*	1350	37.99[c]	Competitor
8	Hymenoptera	Formicidae	*Oecophylla*	*Oecophylla smaragdina*	32	0.90[a]	Predator
					3554	100	

Percentage followed by the same letters in a column within a species were not significantly different (Tukey HSD tests, $p>0.05$)

DISCUSSION

Initially, the number of BSF larvae were significantly lower while after two months, the BSF larvae dominated the oviposition media and gradually reduce the numbers of other insect species. Previous study showed that, once the population of BSF established inside the oviposition media, this species released pheromones for abolishing other insects and flies (Bradley & Sheppard 1984). According to Furman et al. (1959), BSF does not only deter, and control others flies but may feed on dead larvae of others flies as they find them while searching through and feeding on organic sources. Previous study shown that the existence of BSF larvae may inhibit the oviposition and development of housefly due to the rapid growth of the BSF larvae which therefore, interfered and physically render the habitat less suitable for the house fly and other Muscidae (Sheppard 1983). Bradley & Sheppard (1984) also suggested the existence of allomone once inhabited by the BSF larvae which prevent oviposition of other species of flies. They also found that wild populations of house flies, *Musca domestica* strongly avoided ovipositing into media containing BSF larvae.

Among the species recorded, *M. domestica* from the family Muscidae was excluded from the result as this species only observed before the study was conducted. We believed that the decline of *M. domestica* population was due to the fermentation process of coconut waste and effective microbes (EM) in trapping bins. In support of this concept, previous work by Lam et al. (2010) found that female house flies, *M. domestica* detect the presence of harmful cause in the odour profile of animal faeces, and that females accordingly avoid ovipositing in these resources. The selection is likely to favour insects that can distinguish unsuitable substrate by their volatile profiles and respond accordingly by avoiding the sources of certain organic materials.

The common species mostly found growing together with BSF larvae were *D. melanogaster* and *M. scalaris* (Figure 1). This could attribute to the range of pH that are required by the two species which correlated with the pH value required for BSF oviposition media, recorded as 4.83. Study conducted by Deshpande (2015), food source with pH 5 for *D. melanogaster* resulting in significantly longer median lifespan. For *M. scalaris,* pH of growth media was 4.5-5.0 (Gullan & Cranston 2014) which were similar to pH oviposition media of BSF. The trapping bins were also infested by the pupae of *D. melanogaster*. Additionally, *D. melanogaster* has a complete metamorphosis and development of this species mainly depends on temperature which takes about 10 to 11 days at 25°C but extended up to three weeks at lower temperature, 18°C (Resh & Cardé 2009), thus, its ability to survive in the media of BSF may have associated with the temperature range that are suitable for the development of both BSF and *D. melanogaster.* Similarly, *M. scalaris* pupae covered many places around the trapping bins. The existence of this species usually does not reduce the oviposition by the BSF however the presence of this species in a large number indicated that the colony was unhealthy.

Several species such as *D. melanogaster*, *M. scalaris*, *E. luteolus*, *S. pallens* nymphs and the family of Scarabaeidae were found colonizing the oviposition media of BSF (Figure 1). However, after three days, the number of those species were replaced by the population of *D. melanogaster* and *M. scalaris*. Furthermore, only few *P. fuliginosa* nymphs and *O. smaragdina* were found in the trapping bins while *P. littoralis* was once observed during the first month of the study (Figure 1). The BSF larvae dominated the oviposition media mostly at day seven until day ten after the placement of trapping bins each month. This corresponds to results reported by Morales & Wolff (2010), according to which a decrease in number of

insect species is attributable to depredation by other Diptera species at the larval stage, further influencing their abundance (Labud et al. 2003).

According to Galante & Marcos-Garcia (2008), the group of arthropods that involved in the process of decomposition of plant remains includes Diptera larvae (Muscidae, Sarcophagidae, Scatophagidae or Calliphoridae), termites (Isoptera), ant (Hymenoptera), beetles (Coleoptera) and other microorganisms (bacteria, fungi and protozoa). This support the finding that most of the species found this study were the decomposer of the plant remains. Plant part such as fruits were extensively exploited by arthropods such as Diptera (Drosophilidae) and Hymenoptera (Vespidae) to feed into the product resulting from the fermentation (Galante & Marcos-Garcia 2008). The coconut waste used as oviposition media also caused by the fermentation process, therefore attract more *D. melanogaster* in this study.

Stratiomyidae - The larvae of BSF are voracious feeder which feed most of the organic matters in nature. The larval feed on any decomposing matter and therefore, this species is greatly known as converters of organic waste (Diener et al. 2009).

Blattidae - The smokybrown cockroach or *P. fuliginosa* from the family Blattidae usually found outdoors with specific preference on moist, warm, protected area and abundant of organic matter (Gold et al. 2005). This species is omnivorous, feeding on a wide variety of potential sources such as plant materials, scraps of human, the bark and catkins of pecan trees outdoors, dead conspecifics and excrement (Appel & Smith 2002).

Blattellidae - *S. pallens* is a small cockroach which is considered as urban pest, found to infest residences area. This species is scavenger, feeds on plant material and dead animal available in its habitat (Peck 2001).

Scarabaeidae - The family Scarabaeidae is the biggest family of insects which included more than 30,000 species worldwide. This family is known as dung beetles that usually found in every biological niche, which consume on a variety of substrate such as fungi, rotting fruits, carrion and organic matters (Falqueto et al. 2005; Chandra & Gupta 2011).

Nitidulidae - *E. luteolus* is commonly found in various habitats and mostly are classified as saprophagus which is associated with flowers, carrion and stored product pests. This species mainly feed on fruits, flowers, stored products, fungi, decaying and fermenting plant tissues from crops to diverse trees (Rondon et al. 2011). The adult female laid eggs near decomposing plants matter while the larvae consume on whatever is available, then emerge and become pupae in the soil (Myers 2004).

Staphylinidae - Rove beetle is morphologically diverse group of beetles and commonly encountered in terrestrial ecosystem. This species is commonly found in wet agriculture areas such as banks of rivers, lakes and creeks (Anlas et al. 2017). Female of *P. littoralis* lay eggs singly in moist habitats while the larval have two instars before developing into pupa. Adult and larvae known as predators which consume on other insects and invertebrate, living on moist soil and decaying plant matter (Abdullah & Sina 2009; Nikhita et al. 2014).

Drosophilidae - *D. melanogaster* feed on various rotting fruits in their natural environment (Resh & Cardé, 2009). The existence of yeast that enhance the fruit decomposition, allowing this species to feed on the vegetative remains (Galante & Marcos-Garcia 2008).

Phoridae - The larvae of *M. scalaris* are able to develop in varied organic materials such as a living or decaying organism, alive animals and plants, faeces and also paint. This species is able to consume on a wider range of organic materials than any other insect. Adult has unusual locomotory behaviour which capable to burrow deep into the ground. Larval of this species are usually found in water-filled containers such as tree holes and decaying coconuts because they have specific unique behaviour of swallowing air when exhibited to a liquid environment (Varney & Noor 2010; Alcaine-Colet et al. 2015).

Formicidae – *O. smaragdina* is a social insect colony which is considered as a pest. This species is arboreal ants, they make a nest using living leaves by stitched together with their silks. *O. smaragdina* are both herbivores and carnivores, mainly require animal protein, honeydew and other small insects for their diet (Bagchi 2015; Henriksen et al. 2015).

Muscidae – *M. domestica* is one of the importance insect pests of public health and veterinary. *Musca domestica* breed and feed on decaying organic material such as poultry manure, faecal matter, decomposing plant material, carrion and food waste (Van Zanten et al. 2015; Zito et al. 2015). Heo et al. (2008) reported that the presence of *M. domestica* eggs on fresh animal remains concluded that this species could be an initial visitor.

Most of the species insects found during this study were consume and develop on organic materials such as *P. fuliginosa*, *S. pallens*, the family of Scarabaeidae, *D. melanogaster* and *M. scalaris.* Although exploiting the same habitat as BSF larvae, these species have different life cycle which able them to develop together inside the same habitat. The appearance of *D. melanogaster* has no significant effect on BSF larvae, but the great number of this species could lead to potential nuisance and exhibit as an unhealthy colony of BSF. *Megaselia scalaris* was also found inhabit the same habitat as BSF larvae. The oviposition media that have been dominated by BSF larvae were further decrease the population of *M. scalaris* at the later period of time. However, the oviposition media that greatly dominated by *M. scalaris*, were finally become mouldy and unattracted to the female BSF to lay their eggs.

In contrast, the presence of weaver ant, *O. smaragdina* has contributed to the loss of eggs and detrimental effects on BSF adult. Although they do not consume on the fermented coconut waste in oviposition media, but this species feed on other small insects or plants part for their diets. This species has been known to prey on many insect species as well as nectary exudates from plants and sugary secretions produced by other insects (Tsuji et al. 2004). Although the nests of *O. smaragdina* are always found in tree canopies (Dejean et al. 2007), this species also can be found foraging on the ground. During the study, *O. smaragdina* was found to feed on BSF eggs whereas this species was also observed to attack certain parts of the newly emerged BSF body such as wings and abdomen using their powerful mandibles. The adult of BSF were often found surrendered and not be able to fight back once attack by *O. smaragdina.* The weaver ants use to have a painful bite into which can produce irritant chemical secretion from the abdomen called formic acid as a venom and defensive substance (Peerzada et al. 1990). The physical attack by *O. smaragdina* has caused mortality of adult BSF and therefore various possible harmful could be derived from this species.

CONCLUSION

This study evaluated co-occurrence of different insect species in oviposition media of BSF, *H. illucens*. The results found that different insect species, with the same needs and habits competed among themselves for food nutrients and habitat. *Drosophila melanogaster* and *M. scalaris* believed to appear as competitors to BSF larvae because of their significant numbers in the oviposition media. Although the number of *O. smaragdina* was much lower than *D. melanogaster* and *M. scalaris*, the presence of this species had harmful effect on BSF population. *Oecophylla smaragdina* was considered as predator because they preferentially feed on eggs and physically attack on newly emerged BSF adults. *Paederus littoralis* was once found during the study, therefore only regarded as accidently found or visitor. Infestation of other species such as Scarabaeidae family, *P. fuliginosa* nymphs, *S. pallens* nymphs and *E. luteolus* in oviposition media of BSF showed that these populations greatly living in the same media as BSF larvae, considered mainly as decomposer but declined once become unsuitable, exhausted and decreased in nutrient supplies.

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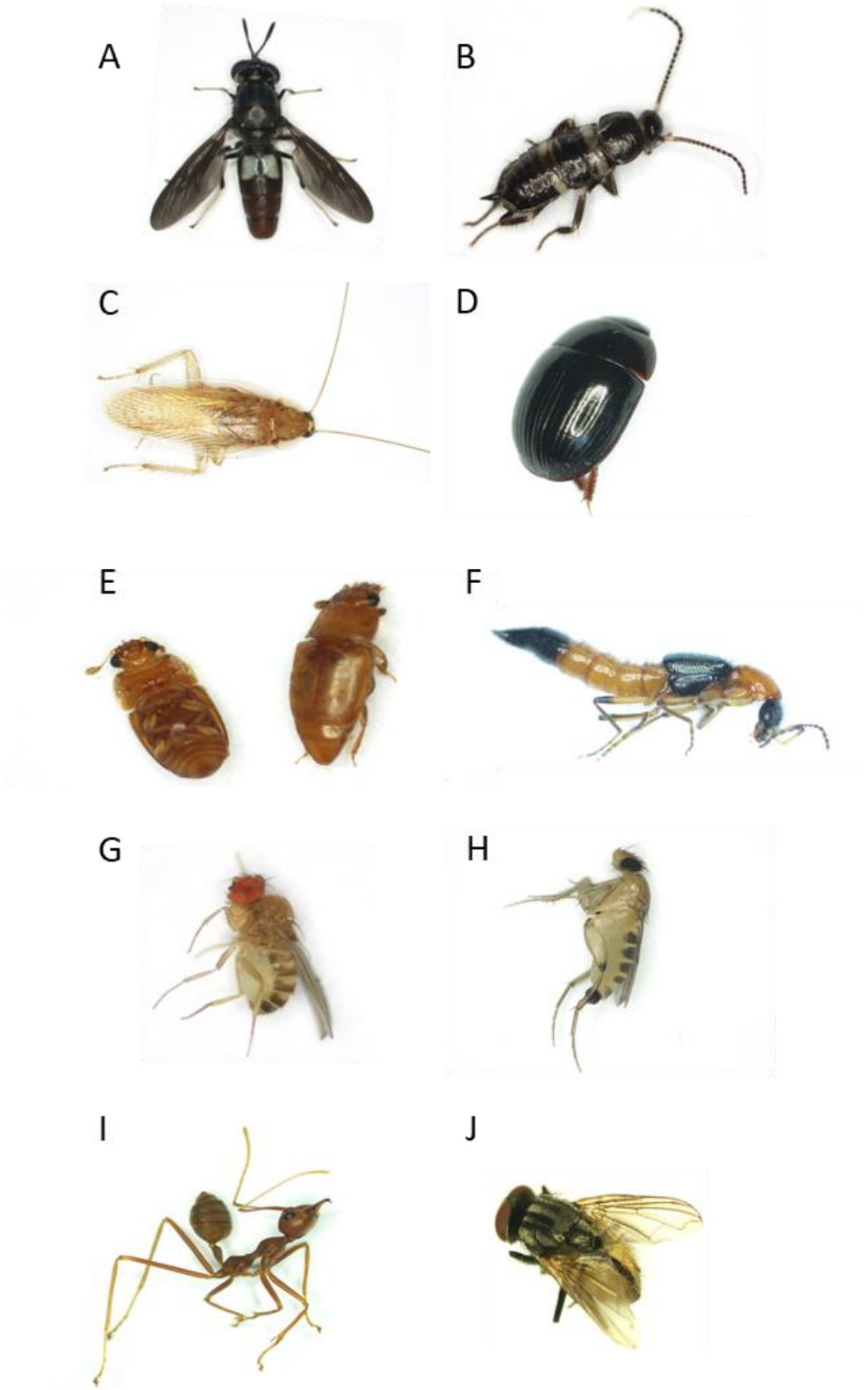

Figure 1. Species found in oviposition media of black soldier fly, **A.** *H. illucens* (2 mm); **B.** *P. fuliginosa* nymph (500 µm); **C.** *S. pallens* nymph (500 µm); **D.** Adult of Scarabaeidae (500 µm); **E.** *E. luteolus* (500 µm); **F.** *P. littoralis* (500 µm); **G.** *D. melanogaster* (500 µm); **H.** *M. scalaris* (500 µm); **I.** *O. smaragdina* (2 mm) and **J.** *M. domestica* (1 mm). Scale for each of the picture (500 µm - 2 mm).