



## RESEARCH ARTICLE

# Antibiosis interaction of black soldier flies (*Hermetia illucens*) (Diptera: Stratiomyidae) with house fly (*Musca domestica*) (Diptera: Muscidae)

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## ABSTRACT

Black soldier fly (*Hermetia illucens* (Linnaeus, 1758)) larvae inhibit oviposition of house fly (*Musca domestica* Linnaeus, 1758) by releasing a semiochemical, although in some situations, inhibition is only partial. We hypothesized that there is a certain period in the life cycle of black soldier fly when it can cause antibiosis of the house fly. Choice and non-choice tests were used to separately examine the effectiveness of each developmental stage of black soldier fly (egg, larval (phase I, II, and III), prepupal and pupal stages) and crude extract of larvae on house fly oviposition. Tests using each developmental stage were compared with controls lacking black soldier fly. The effects of black soldier fly on the number of newly hatched house fly larvae were evaluated and there was a significant difference between the test and control in the number of eggs laid by house fly for all phases of the black soldier fly larval stage. Strong inhibition was found in some black soldier fly larval phases. Significant differences in the numbers of house fly eggs oviposited in food containers treated with crude extract were found when compared with a control, confirming that chemicals from black soldier fly larvae resulted in inhibition of oviposition of house fly. The results from experiment also indicated that chemicals from black soldier larvae influenced the number of house fly larvae newly hatched from eggs.

**Keywords:** Semiochemical; interspecific competition; niche; oviposition.

## INTRODUCTION

House flies (*Musca domestica* Linnaeus, 1758) are a major pest in urban and veterinary areas. High population densities of house fly can cause irritation and annoyance to people (Gerry *et al.*, 2007), domestic animals and livestock, as well as a reduction in egg and milk production in poultry and dairy farms (Mishra *et al.*, 2011). Even though these flies do not carry vector-borne diseases, they can transmit animal and human pathogens including helminth eggs, protozoa cysts and trophozoites, bacteria, fungi, and viruses (Issa, 2019). Furthermore, they are intermediate hosts of cestodes in poultry and nematodes in horses. (Kettle, 1995). Insecticides such as cyromazine, permethrin and dichlorvos have been used for house fly control but the flies have rapidly developed resistance to the insecticides used against them (Khan, 2019; Scott *et al.*, 2013; Wang *et al.*, 2019).

Black soldier flies (*Hermetia illucens* (Linnaeus, 1758)) are known as decomposers of organic waste. Newton *et al.* (2005) reported using black soldier fly to digest swine manure solids. Their report showed that 45,000 larvae will consume 24 kg of swine manure in 14 days. Black soldier flies are found in a variety of decaying plant and animal matter as well as dung, garbage, and other organic materials in which the females lay their eggs (Hall & Gerhardt, 2002). These habitats are similar to and overlap the habitats of house fly immature stages. Adults do not need to feed and rely on the fats stored from the larval stage (Newton *et al.*, 2005). Therefore, the adult black soldier fly is not a pest, and also, it does

not carry vector-borne diseases. Apart from being decomposers, the black soldier fly has been reported to decrease house fly populations in poultry (Furman *et al.*, 1959). However, the mechanism involved in the interaction between house flies and black soldier flies is unclear, although competition for food space, and inhibition of house fly development and survival have been suggested (Furman *et al.*, 1959; John & Schoof, 1956). Furman *et al.* (1959) reported that black soldier fly larvae did not feed on house fly larvae when they were in combined culture in the laboratory. Additionally, Miranda *et al.* (2019) reported that the house fly and black soldier fly can both attain the pupal stage when newly hatched larvae of both species were grown in combined culture on fresh manure. Bradley and Sheppard (1984) reported that black soldier fly could inhibit house fly oviposition. Interspecific chemical communication or semiochemical (allomone) transmission has been suggested as a mechanism for this. An allomone is a chemical which is released by a species to its own advantage (Sbarbati & Osculati, 2006). It can have defensive or repellent functions which may provide a mechanism for the coexistence of competitive species exploiting a common food source. Adjavon *et al.* (2021) suggested that chemicals from symbiotic bacteria associated with black soldier fly are probably involved in reducing substrate attractiveness to adult house flies. The report of Bradley and Sheppard (1984) showed that the inhibition of the oviposition of wild house fly by black soldier fly larvae was not complete. In addition, incidents have been noted in which house fly colonized the same resource as the black soldier fly (such as in swine manure and waste) (by observation and

personal communication). Until now there have been no reports concerning which developmental stage of black soldier fly is involved in production of allomone. Additionally, there have not been any reports determining whether or not house fly eggs deposited onto the oviposition site of a black soldier fly will survive. Also, apart from the semiochemical (allomone), behavioral or physical features (i.e. larval size and their movement) of black soldier fly larvae might affect house fly oviposition.

The objectives of this study were, 1) to determine which life stage of black soldier flies is important for inhibition of house fly oviposition, 2) to prove that inhibition of house fly oviposition is mediated by semiochemicals rather than effects of behavioral or physical characteristics, 3) to determine if black soldier fly larvae can affect the number of newly hatched larvae of house fly. Information from this study will be important for controlling dipteran pest species in urban and veterinary areas, and for finding chemical insect repellants in the future.

## MATERIALS AND METHODS

### House fly rearing

House flies (*Musca domestica* L.) were collected from an animal farm at Khon Kaen University. They were then identified using identification guides in Tumrasvin and Shinonaga (1977). House flies were maintained in the laboratory at room temperature,  $28\pm 5^\circ\text{C}$ , relative humidity of  $60\pm 10\%$ . They were reared in colonies in which 10 male/female pairs were separately maintained in a nylon mesh cage measuring  $30\times 30\times 30$  cm. Adults were fed a food containing a mixture of powdered milk and sugar (3:1 (w/w)) (Tangkawanit *et al.*, 2018). A Petri dish with moist tissue paper and fish meal was provided in the cage to provide a site for oviposition. Egg batches were transferred to a container with a mixture of water, powdered milk, yeast, and rice bran (Tangkawanit *et al.*, 2018). After a few days, food and larvae were transferred to a sieve container (sieve size 4.75 mm). Mature larvae then passed through the sieve and became pupae in the dry container underneath. Pupae were moved to a new cage in readiness for the emergence of new adults. The house flies studied were from the 3<sup>rd</sup> generation of the colony.

### Black soldier fly rearing

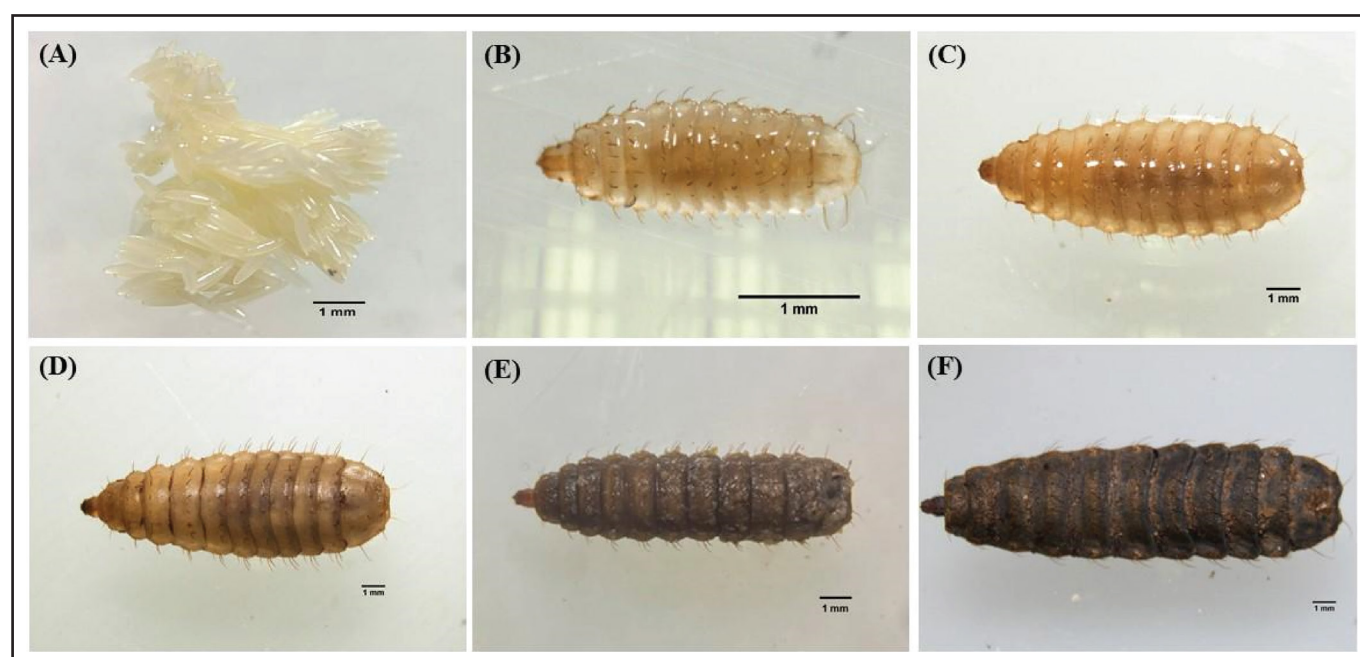
Black soldier fly (*Hermetia illucens* L.) eggs from Artit's farm (a commercial black soldier fly farm in Khon Kaen Province, Thailand) were maintained in a plastic container with a mixture of water (65 ml), powdered milk (7.5 g), yeast (2.5 g) and rice bran (25 g) (house fly larvae diet) as a larval food for 1<sup>st</sup> instar larvae of black soldier flies. When two days old, larvae were transferred to a new plastic container (20 cm in height and 50 cm diameter). Ripe fruits (pineapple and jack fruit) were provided in a container as larval food, and also for attracting adults to oviposition (Burana, 2010). A new container with ripe fruit was placed next to the old container to attract emerging adults to oviposition sites. A piece of paper pulp egg tray was used as egg substrate.

### Effect of life stage of black soldier flies on the oviposition efficiency of house flies

A bioassay was performed using a choice test (modified from Machtinger *et al.*, 2014) to evaluate the inhibition of oviposition induced by different developmental stages (egg, larval, prepupal and pupal stages) of black soldier fly (Figure 1). The choice tests were designed as 4 trials for each developmental stage. The total developmental time of black soldier fly for the larval stage before they reached the prepupa stage was 14-15 days. The larval stage of black soldier fly was classified and divided into 3 phases in this experiment because the exact larval stage is difficult to define and compare with other reports. Also, the life cycle is very variable depending on the environmental conditions (Tomberlin *et al.*, 2002; Liu *et al.*, 2017). Three phases of larva were classified into groups the same age as larvae used in the experiment and were designated as follows:-

- phase I (1<sup>st</sup>-2<sup>nd</sup> instar, 2 days old larvae)
- phase II (3<sup>rd</sup>-4<sup>th</sup> instar, 8 days old larvae)
- phase III (5<sup>th</sup>-6<sup>th</sup> instar, 13 days old larvae) (Figure 1).

Ten pairs of newly hatched house flies were released in a small cloth netting mesh cage ( $30\times 30\times 30$  cm) for mating. Seven days after a preoviposition period, ten female house flies were released into



**Figure 1.** Developmental stages of black soldier fly used in the experiment: a, eggs, b, phase I larva, c, phase II larva, d, phase III larva, e, prepupa, and f, pupa.

the experimental cage, measuring 50x70x50 cm covered with a cloth netting mesh. One side of the cage was provided with a plastic food container (20x30x9 cm) containing 65 g of a mixture of water, powdered milk, yeast and rice bran (larval food). The opposite side-cage was provided with a similar larval food container, but with each individual stage of black soldier fly (n=20) added to investigate the female egg-laying choices of female house flies. A sponge soaked with water was provided in the cage as a water source for adults. Four different cages were used for each experiment. For each experiment, the number of house fly eggs from the food container in each side-cage was recorded for every 24 h until 72 h after the first cluster of eggs was found. Means of total egg number from each experiment were compared by paired t-test ( $P < 0.05$ ) using Statistix10 (Analytical software, 2013). The deterrence Index (DI) (Kramer & Mulla, 1979) was calculated using the formula.

$$DI = \frac{(NT - NC)}{(NT + NC)}$$

where NT = total number of eggs in the treated sample and NC = total number of eggs in the control sample. When DI values  $\leq -0.3$ , the treatment was considered as repellent.

Larvae which were most efficient at inhibiting house fly oviposition were selected for a non-choice test. In the non-choice test, one food container with an individual stage of black soldier fly (n=20) was placed in the center of the cage (50x70x50 cm) to investigate the egg-laying of female house flies. Another cage contained a food container without black soldier fly larvae. The experiment was designed as 4 replications. Two different cages were used for each replication. Therefore a total of 8 different cages were simultaneously being used for the test. Ten pairs of newly hatched house flies were released into each cage. Means of the numbers of house fly eggs from different treatments were compared by paired t-test ( $P < 0.05$ ) using Statistix10 (Analytical software, 2013), and the deterrence Index (DI) was estimated.

#### Effect of chemical crude extract from black soldier fly larvae on the oviposition of house flies

To prove that physical features and behavior of black soldier fly did not affect oviposition of house fly, an experiment was conducted in the laboratory using a choice test. Extracts of the black soldier fly larva were used as a source of putative bioactive chemicals. Twenty examples of phase I larvae were ground with pestle and mortar. The pestle was washed with 1 ml of distilled water and then 1ml of the crude extract was dropped onto a filter paper (3.5 cm of diameter) and put into the food container before treatment. To provide for another choice, 1 ml of distilled water was dropped onto the paper. The experiment was conducted with 4 replications in a 50x70x50 cm netting mesh cage. The number of house fly eggs in each side-cage container was counted. Data were compared by paired t-test ( $P < 0.05$ ) using Statistix10. The deterrence Index (DI) (Kramer & Mulla, 1979) was evaluated.

#### Effect of black soldier fly larvae on the number of newly hatched house fly larvae

The experiment was conducted in the laboratory using Completely Randomized Design (CRD). Four treatments (Phase I, II and III larvae and crude extract of larvae) were investigated. The experiment was performed in a plastic container (20x30x9 cm) containing 65 g of larval food. Fifty newly laid house fly eggs (day 0) were transferred into each of the containers used in each individual treatment. Larval food without treatment was used as control. Hatched or unhatched eggs were difficult to examine in the experiment because they became mixed in with the larval food by the movement of larvae after hatching. Therefore, for each treatment, the number of hatched

larvae was determined under a stereo microscope after 24 h. The experiments were conducted with four replications. Means of hatched larvae from house fly eggs were statistically analyzed by least significant difference (LSD), using Statistix 10 software ( $P < 0.05$ ). The percent reduction of hatched house fly larvae was statistically determined according to Abbott's formula (Abbott, 1925).

$$\% \text{ reduction} = \left( 1 - \frac{\text{insect in treatment after treatment}}{\text{insect in control after treatment}} \right) \times 100$$

## RESULTS

#### Effect of life stage of black soldier flies on the oviposition efficiency of house fly

This experiment tested how the presence or absence of each life stage of black soldier fly larvae at a site influenced the choice of oviposition by house flies at that site. The results indicated that when the data were compared by paired t-tests, there was a significant difference ( $P < 0.05$ ) in numbers of house fly eggs found in food containers with and without black soldier fly for all of the three larval phases. The mean number of house fly eggs on larval food containers from egg, prepupal and pupal stages of black soldier fly was not significantly different from their control ( $P > 0.05$ ) (Table 1). Values of deterrence index (DI) clearly confirmed that oviposition was inhibited by phase I, II, and III of larval stages (DI  $\leq -0.3$ ), but not by the other stages. The results showed that treatment with phase II larvae could completely inhibit house fly oviposition, with no eggs appearing in the food container. Phases I and III could inhibit house fly oviposition (99.78% and 82.85%, respectively), but not completely.

The non-choice test indicated that there was a significant difference in the egg numbers of house fly found in food containers (with larval phase II in a food container) and a control cage (without black soldier fly in a food container). Eggs laid by house fly in a food container in which black soldier larvae were present were not deposited in a cluster but were scattered in the food. Most house fly eggs were found to be deposited outside the container with some of them found in a sponge containing water provided to house fly adults (this did not occur in the control cage).

#### Effect of chemical crude extract from black soldier fly larvae on the oviposition of house fly

The numbers of housefly eggs laid are presented in Table 1. The results revealed that the number of eggs in the treatment with crude extract of phase I larvae black soldier fly was significantly lower than in the control ( $P < 0.001$ ). Deterrence Index strongly corroborated this inhibition of egg-laying (DI = -0.902, Table 1). The results indicated that crude extract treatment did not completely inhibit house fly oviposition. Approximately 29 eggs were deposited in each food tray used in the crude extract treatment. The efficiency of crude extract in inhibiting house fly oviposition was less than with live phase I larvae (0.5 eggs were deposited).

#### Effect of black soldier fly larvae on the number of newly hatched house fly larvae

The efficiency of the effects of phase I, II and III black soldier fly larvae, and crude extracts of them, on newly hatched house fly larvae were determined. The means of the number of hatched larvae from house fly eggs were significantly different among those treatments (Table 2). Means of the number of hatched larvae were compared with the control group to find the percent change in the number of eggs hatched. The results revealed that percent reduction of house fly larvae that emerged reached 96.04% in phase II treatment, following by phase I (80.62%), crude extract (85.02%), phase III (9.69%) of black soldier fly larvae, and control (0%), respectively.

**Table 1.** Mean number of eggs laid by house fly and percent egg hatch in the food tray with different treatment conditions of black soldier fly

Treatment (Black soldier flies)	Egg laid (Mean ± SD)		T-test	P-value <sup>1/</sup>	DI (Mean±SD)	Classification <sup>2/</sup>
	Treatment	Control				
Choice test						
Egg	575.5±132.53	591.5±197.23	-0.23	0.830 ns	-0.004±0.096	non- inhibitor
Phase I	0.5±1	229±37.81	-12.01	0.0012**	-0.995±0.009	inhibitor
Phase II	0±0	308.75±79.39	-8.41	0.0035**	-1±0	inhibitor
Phase III	90.50±70.98	417±71.61	-11.66	0.0014**	-0.686±0.238	inhibitor
pupa	492.5±42.74	470.25±115.33	0.52	0.64 ns	0.031±0.084	non- inhibitor
prepupa	329.75±53	387±34.76	-3.06	0.055 ns	-0.083±0.060	non- inhibitor
crude extract larvae	29±10.29	568.25±37.16	-26.53	0.0001**	-0.902±0.033	inhibitor
Non-Choice test						
Phase II	90.75±14.08 (110.5±25.37) <sup>3/</sup>	283.5±45.67	-8.13	0.0039**	-0.51±0.088	inhibitor

<sup>1/</sup>\*\* p-value < 0.05 is statistically significant, ns is not statistically significant.

<sup>2/</sup> Deterrence Index classification where non-inhibitor: DI > -0.3, inhibitor DI ≤ -0.3.

<sup>3/</sup> Number of eggs deposited in a cage outside a container.

**Table 2.** Mean number hatched larvae of house fly with different treatment conditions of black soldier fly

Treatment (Black soldier flies)	Number of hatched house fly larvae <sup>1/</sup> (Mean±SD)	% Reduction
Phase I	8.80±1.48c <sup>2/</sup>	80.62
Phase II	1.80±1.30d	96.04
phase III	41.00±2.91b	9.69
crude extract larvae	6.80±1.92c	85.02
control	45.40±1.14a	0

<sup>1/</sup> n = 50.

<sup>2/</sup> Within each column, values followed by the same small letter indicate no significantly different (P>0.05).

## DISCUSSION

### Effect of life stage of black soldier flies on the oviposition efficiency of house fly

The selection of a site for oviposition by female insects is important for the survival of their offspring. Chemical, visual, olfactory and tactile cues are factors involved in locating oviposition sites (Navarro-Silva *et al.*, 2009). The results of the experiment support the results of Bradley and Sheppard (1984) who reported that black soldier fly larvae can cause a decrease in egg oviposition of house flies. One possible reason suggested by Bradley and Sheppard (1984) was semiochemical (allomone) effects, and this suggestion is consistent with findings for other dipteran species. For example, in the mosquito family Culicidae, chemical cues have a role in the final consideration in the selection of oviposition sites (Navarro-Silva *et al.*, 2009). The results from the present experiment revealed that the efficiency of inhibition of house fly oviposition was different for different life stages of black soldier fly. The early larval stage was the most effective. Inhibition effects may involve competition for resources between the two species of fly. The early stages of an insect are critical periods for survival and development. Imaginal structures grow little during larval life but achieve most of their

growth during the prepupal and pupal period (Nijhout & Wheeler, 1996). Thus, nutrition during this period is important.

Black soldier flies usually lay their eggs in dry and sheltered places near to crevices close to the food source (Boaru *et al.*, 2019; Booth & Sheppard, 1984). Newly hatched larvae then move to the wet food source. House flies are different to black soldier flies, in that they prefer to oviposit their eggs into the wet food substrate (Machtlinger *et al.*, 2014). According to their biology, house flies may arrive first. However, this also depends on what fly generally arrives to a food source first. Therefore, food sources such as fresh manure or fresh garbage may be occupied by black soldier fly larvae before the first larval instar of house flies emerge or *vice versa*. Niche overlap based on food resources may occur when different species coexist in the same place. Interspecific competition results in a decrease in the number and a niche shift of the co-existing species. The release of repellent allomone might help the black soldier fly larvae to gain a greater chance of occupying ecological niches and decrease competition for larval food source. Our results and those of Bradley and Sheppard (1984) indicated that black soldier fly larvae did not completely inhibit house fly oviposition. With the detailed focus of this study, it was seen that phase II larvae of black soldier flies entirely inhibited house fly oviposition. Phase I almost entirely inhibited, whereas the 3<sup>rd</sup> phase did not. The results may relate to food acquisition by the early stages of larva. Black soldier fly is of large size and has a long developmental time in the larval stage. The larvae have to uptake sufficient nutrients for their entire development and to serve as a source of energy in the adult stage. For this reason, the first stage larvae need to develop a competitive strategy (such as release of a potent allomone) for occupying the food source to provide for their further development. These results suggest a trade-off between allomone production and the nutrients for their entire development. The possible reason why phase II of black soldier fly larvae was more effective than phase I might relate to their greater larval weight and size, and the greater amount of released substance (20 larvae of each stage were used for the experiment). Allomone may decrease in the last stage larvae because they stop consuming food for several days, and it may also decrease in prepupae because they commence migration to a dry place for pupation. The results correspond to a report from Adjavon *et al.* (2021) discussing observations that black soldier fly puparia could not inhibit house fly oviposition. However, their results indicated

that the larval stage also could not inhibit. The possible reason for this apparent discrepancy may relate to the stage of larvae used in the trials.

Even though the egg laying decisions of a female may benefit her offspring, house fly females may have to deposit their eggs in unsuitable places without conscious selection (in this experiment, eggs were deposited outside the food container). However, there will be a lower chance of survival for their offspring. This is opposite to some other insects; in *Pieris* butterflies (Lepidoptera: Pieridae) for example, which refuse to lay eggs in no-choice trials (Schäpers *et al.*, 2017).

#### Effect of crude extract of chemicals from black soldier fly larvae on the oviposition of house fly

The low average number of house fly eggs laid during the crude extract treatment indicated that a chemical inside the larvae is likely to be an important factor impeding house fly oviposition. This proved the hypothesis that the inhibition of house fly oviposition was not because of behavior or physical characteristics of black soldier fly larvae. The efficiency of crude extracts of soldier fly larvae on inhibition of house fly oviposition was less than for live phase I larvae. This may relate to the degradation of the substance during the experiment. The inhibiting substance may be continuously released by live larvae which could result in a high concentration of the substance in the larval container. However, if this is the case, we do not know which organ produces and releases the allomone and it might be released from different organs, depending on the insect involved. In the moth families Arctiidae, Hypsiidae, and Zygaenidae, the chemicals are secreted from an area near or on the prothorax and are fortified with haemolymph (Blum, 1996). Some grasshopper species produce chemicals from tracheal air and glandular secretion (Blum, 1996). Adult Hemiptera store the chemical in the metathoracic scent glands that open at metapleural grooves (Zhang & Aldrich, 2004). Hydrocarbons serve as semiochemicals in insects, and they are synthesized by oenocytes which are located in the integument or hemocoel. Schal *et al.* (1998) hypothesized that hemolymph lipoprotein is a carrier of hydrocarbons in many insects. Hydrocarbons are transported through the hemolymph and deposited in tissues such as the epicuticle and emitting glands. Davis *et al.* (2013) suggested that volatile secretions by insect symbiotic bacteria can have a role in semiochemical production. Recently, Adjavon *et al.* (2021) revealed that the symbiotic bacterium, *Paenaltigenes* sp. associated with black soldier fly, may be involved to the inhibition of house fly oviposition. A chemical inside the larvae or crude extract is likely to be an important factor impeding house fly oviposition. However, its source and the role of this allomone are still unknown. It may be synthesized by some organs in the black soldier larvae or by symbiotic bacteria inside the larval gut (Adjavon *et al.*, 2021), originate from antimicrobial peptides in the crude extract.

#### Effect of black soldier fly larvae on the number of newly hatched house fly larvae

The reduction in the number of house fly larvae may be due to unhatched eggs or the mortality of the larvae. However, Miranda *et al.* (2019) reported that the house fly and black soldier fly can attain the pupal stage when newly hatched larvae of both species were grown in combined culture on fresh manure. Therefore, the larvae of black soldier fly do not affect housefly mortality. Hatching of eggs of house fly was most strongly inhibited by phase II larvae of black soldier fly followed by phase I, crude extract larvae, and phase III. In phase III treatment, the efficiency of reducing the number of hatched larvae was lowest. Miranda *et al.* (2019) revealed that black soldier fly development and survivorship were negatively impacted when house flies were introduced 1-4 days after black soldier fly were established in a food source. Newly hatched larvae from house fly eggs have no effect on the development of phase III larvae (13 days old). These results may explain the situation where the house

fly and the black soldier fly colonized the same source. Resource sharing may occur if they oviposit their eggs and colonization occurs during the last phase of black soldier fly larval stage.

It is worth noting that house fly larvae require living bacteria in their media for successful development and metamorphosis (Zurek & Nayduch, 2016). Antimicrobial peptides derived from black soldier fly (Xia *et al.*, 2021) may affect the survival of house fly larvae and the decision of gravid house flies to oviposit their eggs.

Finally, the concentration of the semiochemical is an important factor as a low abundance of larvae may not affect the house fly. Additionally, environmental factors can influence the concentration of chemicals. For example, temperature influences enzymatic activity and kinetics of chemical reactions, and influences the vapor pressure of compounds (Becker *et al.*, 2015) and also affects the active space (the distance in which a chemical cue can be detected from the source).

## CONCLUSIONS

House fly oviposition was strongly inhibited by phase I, II, and III of black soldier fly larval stages but not by the other stages (egg, prepupal and pupal stages). Inhibition of oviposition of house fly by crude extract from phase I of black soldier fly larvae confirmed that chemicals from black soldier fly larvae result in oviposition inhibition of house fly. The results indicated that chemicals from black soldier fly larvae influenced the number of newly hatched house fly larvae. However, function and mechanism of action of the chemical from black soldier fly on house fly oviposition requires further investigation.

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## Conflict of Interests

The author declares that they have no conflict of interests.

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