

Screening insectary refugia plants that increase the performance of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) against diamondback moth larvae

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Manuscript received: 6 September 2021. Revision accepted: 20 September 2021.

Abstract. Asmoro PP, Dadang, Pudjianto, Winasa IW. 2021. Screening insectary refugia plants that increase the performance of *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) against diamondback moth larvae. *Biodiversitas* 22: 4254-4260. Habitat manipulation through the planting of insectary refugia plants is one way of controlling pests. These refugia plants serve as the source of nutrients that improve the fitness of parasitoid against insect pests. In the cabbage agroecosystem, the presence of refugia plants is expected to benefit the parasitoid *D. semiclausum*. This study aimed to select insectary refugia plants beneficial for the *D. semiclausum* in term of attracting and increasing their longevity, and parasitization ability against *P. xylostella* larvae. Ten species of refugia plants were used in this study, i.e., *Ageratum conyzoides*, *Galinsoga parviflora*, *Sphagneticola trilobata*, *Synedrella nodiflora*, *Portulaca oleracea*, *Rorippa indica*, *Brassica rapa*, *Apium graveolens*, *Ocimum basilicum*, and *Arachis pintoi*. The methods used in this study included testing the response of the imago olfactory to refugia plants and its effect on the longevity and parasitization level. The results showed that of the ten species of investigated, *R. indica* and *A. graveolens* were the most attractive for the presence of parasitoids. The two refugia plants could also increase the longevity and parasitization rate compared to the water treatment. *R. indica* and *A. graveolens* are recommended as refuge plants in the cabbage agroecosystems to control *P. xylostella*.

Keywords: Biological control, habitat manipulation, insect olfactory, insectary plant, *Plutella xylostella*.

INTRODUCTION

Ecological engineering is one of the strategies in pest control implemented using an ecological-based approach. Compared to other approaches (e.g., chemical and physical methods), ecological engineering could reduce farmers' dependence on synthetic chemical insecticides. Ecological engineering is a complex approach and requires careful decision-making (Cloyd 2020). However, the more effective use of flowering plants to maximize biological control of insect pests in agroecosystems depends on selecting plant species that are more beneficial to natural enemies than to pests (Lu et al. 2014). One method of ecological engineering in pest control is by using the natural enemies of pests through the manipulation of an agroecosystem (Gonzalez-Chang et al. 2019; Herz et al. 2019). The increased performance of natural enemies of the pest is expected to reduce pest populations that might harm crops or reduce crop's yields (Heimpel and Mills 2017; Zada and Saljoqi 2019).

One example of habitat manipulation in pest control is the addition of flowering non-host plants, which serve as the habitat of the natural enemies of pest insects (Saldanha et al. 2019; Palsson 2019). It is hoped that the flowering plants to be used will attract natural enemies and increase traits related to their fitness so that they have the capacities

to control pest insects, for example, through parasitization or predation.

Insectary flowering plants attract the presence of pest's natural enemies by providing nectar or pollen (Parolin et al. 2012a). Plants generally use flowers throughout the season to maintain the availability of nutrients which might increase the chance of being inhabited by pest's natural enemies. These plants must also have a specific flower structure accessible to natural enemies (Colley and Luna 2000; Parolin et al. 2012b). The flowering plants in agroecosystems can positively affect natural enemies, such as an increase in longevity or fitness, which will impact pest control's sustainability (Meiadi et al. 2015; Wuriyanto and Tjahyaningrum 2016).

Besides being a source of nutrition, the scent of flowers and flower nectar can also attract insects by emitting volatile compounds, which will serve as clues to find hosts (Dannon et al. 2010; Liu et al. 2019). Volatile compounds emitted by plants are essential clues for insects (both herbivores and parasitoids) to find hosts, prey, or sources of nutrition or to carry out various stages/processes of insect life. Volatile compounds emitted by plants are also possible to mediate or influence the result of trophic interactions between plants, herbivores, and their natural enemies (Desurmont et al. 2020). On the other hand, guard plants' volatile compounds can be detrimental, such as repellents (Chai et al. 2017).

In addition, insectary flowering plants might increase the parasitic ability of insects. Several studies have shown that the presence of a flowering plant can increase parasitoid parasitization. Increased levels of parasitization of *Cotesia rubecula*, *Diaretiella rapae*, and *Copidosoma koehleri* are closely related to the presence of nectar-producing plants (Baggen and Gurr 1998). The presence of flowering plants, *Fagopyrum esculentum*, and *Lobularia maritima* were able to increase the parasitization of *Cotesia orobena* against *Evergetis rimosaris* (Shrestha et al. 2019). There are at least two mechanisms that can explain how the availability of refugia plants can increase the parasitoid ability of parasitoids. The first mechanism is that flowering plants can prolong the life of the parasitoid. The implication of the longer life of a parasitoid is that more hosts will be parasitized, and more offspring will be produced (Sugiharti et al. 2018). The second mechanism is that the presence of refugia plants that are easily accessible will make the parasitoid energy to search for sources of nutrients more efficiently (Shrestha et al. 2019). Most of the energy possessed by parasitoids will be used more to find and parasitize the host (Rivero and Casas 1999; Lu et al. 2014).

Diadegma semiclausum Hellen (Hymenoptera: Ichneumonidae) is one of the important natural enemies of the diamondback moth *Plutella xylostella* L. (Lepidoptera: Yponomeutidae). In order to support the sustainability of biological control by *D. semiclausum*, the presence of flowering plants is expected to occur among the main crops, for example, in the cabbage agroecosystem. This study aimed to investigate refugia plants that are beneficial for the parasitoid *D. semiclausum* in regard to the ability to attract *D. semiclausum* presence and parasitic ability against *P. xylostella* larvae. The result of this study is expected to provide a reference of plants that can be used to control diamondback moth pests in the cabbage agroecosystem.

MATERIALS AND METHODS

Diadegma semiclausum rearing

The *D. semiclausum* cocoon was taken from a cabbage plantation in Pacet Sub-district, Cianjur District, West Java, Indonesia. The cocoon was then placed in a gauze cage (60 cm x 30 cm x 30 cm) and left for several days until the adult emerged. A 10% honey solution was provided as feed for insect adults. The adults of *D. semiclausum* were then exposed to 20-25 third instar larvae of *P. xylostella* in another cage for 24 hours. The parasitized *P. xylostella* larvae were transferred to plastic boxes with screen windows and fed with pesticide-free cabbage leaves. The *P. xylostella* larvae were placed until they came out to become parasitoids. The new adult that came out was used for testing. Rearing was carried out indoors with a temperature of ± 25 °C and RH 76%.

Propagation and maintenance of insectary refugia plants

The insectary refugia plants to be selected have several criteria: i) it can grow well in a cabbage growing environment, ii) plant height is around 10-40 cm, iii) it

does not compete with the main crop, and iv) it is known or has been reported to be repellent or attractant for pest insects (adapted from Gonzales et al. 2016). There were ten refugia plants used in this study, namely the Asteraceae family: *Ageratum conyzoides* (L.) L., *Galinsoga parviflora* Cav., *Sphagneticola trilobata* (L.) Pruski, *Synedrella nodiflora* (L.) Gaertn.; Portulacaceae family: *Portulaca oleracea* L.; Brassicaceae family: *Rorippa indica* (L.) Hiern, *Brassica rapa* L.; Apiaceae family: *Apium graveolens* L.; Lamiaceae family: *Ocimum basilicum* L.; and Fabaceae family: *Arachis pintoii* Krapov. & W.C.Gregory.

Olfactory response test of *Diadegma semiclausum* against insectary refugia plants

Testing the response of *D. semiclausum* against refugia plants was carried out using an olfactometer following the method developed by Belz et al. (2013). As a source of aroma, flowers or leaves were cut on the stalk, wrapped in wet cotton, and then placed in a small tube. The flowers or leaves used for testing should not be more than 30 minutes after being cut to avoid wilting. The test flower was placed on a glass tube connected to the end of the olfactometer tube sleeve. The olfactometer shaft end was covered with gauze, and then the olfactometer shaft was connected by a hose to the olfactometer suction pump (Dyna-Pump Model 3). Between the olfactometer and the suction pump, a flowmeter (Gilmont GF-6541-1215) was installed to monitor the airflow rate. After the olfactometer was installed, the insects were placed on the olfactometer tip. The flowmeter was run at an air rate of 10 ml/minute. The test was carried out at room temperature ± 26 °C and an RH of 76% (Figure 1). The test consisted of three experiments, i.e., (i) refugia flowers vs. clean air, (ii) refugia flowers vs. refugia leaves (same plant), and (iii) refugia flowers vs. other refugia flowers. The *D. semiclausum* adults used were female with the age of fewer than 24 hours old. Each series of experiments used 15 female adults.

Test of the effect of refugia plants on the longevity of *Diadegma semiclausum*

The objective of the experiment was to examine the effect of refugia flowers as a food source on the longevity of *D. semiclausum* adults. The refugia plants used in this experiment were plants whose flowers showed high attractiveness in the previous olfactory test (Experiment 3). Each flowering refugia polybag was placed in a mica-gauze tube cage (diameter: 15 cm, height: 30 cm). A pair of *D. semiclausum* adults that had just emerged from the cocoon was put in a cage. Every two days, refugia plants was replaced with a new one. A positive control treatment used 10% honey solution, which had been applied to a cotton ball, while water was used as a negative control. The variables observed were the longevity of male and female *D. semiclausum*. Observations were carried out until all the adults died.

Test of the effect of refugia plants on the parasitization rates of *Diadegma semiclausum*

This experiment examined the effect of refugia flowers as a food source on the parasitization of *D. semiclausum* to *P.*

xylostella larvae. The type of refugia plant used in this experiment was the plant that showed the best effect on the parasitoids' longevity. Each refugia plant polybag was put into the wood-framed screen cage (60 cm x 60 cm x 60 cm). Furthermore, 30 larvae of *P. xylostella* instar III were placed on cabbage seeds. Two pairs of *D. semiclausum* adults aged 24 hours were inserted and allowed to populate in a cage. After 24 hours, the male *D. semiclausum* was removed from the confinement. Refugia plants and *P. xylostella* larvae were replaced every day until the female's adult died. *P. xylostella* larvae that had been removed from the cage were then kept in another plastic box. The positive control action used 10% honey solution, which had been applied to a cotton ball, while the negative control used water. The variables observed included the proportion of parasitization, the number of emerging adults, and the sex ratio.

Data analysis

All experiments used Completely Randomized Design (CRD). Data analysis used Minitab 17 software to analyze each treatment's effect and using Tukey's real difference test at the 5% level. The analysis of the significant difference in the selection of aroma sources in the olfactory test used the x^2 test.

RESULT AND DISCUSSION

Olfactory response of *D. semiclausum* against refugia plant

The results showed that five out of ten species had high attractiveness of flowers for *D. semiclausum* imago ($p < 0.01$) (Figure 1.A). The five species are *B. rapa*, *R. indica*, *A. conyzoides*, *G. parviflora*, and *A. graveolens*. The highest attractiveness of *D. semiclausum* was shown to *R. indica* flowers, while *P. oleracea* had the lowest. In experiment 2 (Figure 1.B), *D. semiclausum* females were generally more attracted to flowers than to plant leaves. The four flowers from experiment 2 ($p < 0.05$) (except *B. rapa*) were used again for experiment 3 to compare parasitoid attractiveness between one flower to another. The *R. indica* and *A. graveolens* flowers showed higher attractiveness than *S. trilobata* and *A. conyzoides* (Figure 1C). This attractiveness (as well as unattractiveness) in *D. semiclausum* adults can be considered as an inherited trait of the field (agricultural land) because the insect samples used in this experiment were the first offsprings that had not been exposed to various types of feed before (only 10% honey solution).

The parasitoid's attractiveness to some flower species can also be caused by the quantity and quality of the compounds emitted by the flower or other plant parts. Kugimiya *et al.* (2010) reported that *Cotesia vestalis* females could orient the flower to the nearest nutrient source using olfactory signals. Belz *et al.* (2013) stated that the low attractiveness of *Fagopyrum esculentum* was due to the low quality of volatile compounds compared to *Centaurea cyanus* and *Iberis amara*, so that these compounds were not detected by *Microplitis mediators* (Hymenoptera: Braconidae). Another possibility is that the quality of the compounds produced by the three

flowers was the same. Still, the quantity produced by *F. esculentum* flowers was lower than the other two flowers. The insect's unattractiveness to a flower can also be possible because of its repellent compound content. The volatile compounds emitted by a flower are of specific interest to a parasitoid. The floral aroma of the buckwheat plant *Fagopyrum esculentum* is attractive to egg parasitoid *Nezara viridula* (Hemiptera: Pentatomidae), *Trissolcus basalis*, but on the other hand, it is also repellent for another parasitoid, *Ooencyrtus telenomicida* (Foti *et al.* 2019). The attractiveness of plant's flowers can be caused by the scent of flowers or nectar captured and responded by parasitoids (Raguso 2004; Foti *et al.* 2017). The olfactory response is a crucial ability in insects in the early stages for searching and finding the host. The attractiveness of flowers is an essential factor in selecting flower plants for use in habitat manipulation. The easily detected flower odor (or flower nectar) will create the parasitoids' time and energy to find a more efficient food source (Fataar *et al.* 2019).

The allocation of time and energy for the parasitoids will then be more widely used, affecting their performance in agriculture. Failure or difficulty in finding nectar sources will cause more significant energy loss, but it also causes high mortality risk (Wackers and van Rijn 2012). Therefore, flowering plants with high attractiveness to parasitoids can affect parasitoids' performance in agriculture (Bianchi and Wackers 2008). However, Wackers (2004) states that flower attractiveness does not correlate with nectar accessibility. It means that when a parasitoid is attracted to a flower, it may not necessarily access or utilize the nectar in that flower. Therefore, after an attraction to a flower or plant, the accessibility of the parasitoid flower nectar is an important indicator for assessing the plant's suitability to manipulate the habitat.

The effect of refugia plants on the longevity of

Diadegma semiclausum

Four species of refugia plants showed high olfactory attractiveness to *D. semiclausum* adults, namely *S. trilobata*, *A. conyzoides*, *A. graveolens*, and *R. Indica*, and were used in this test. The results showed that all refugia flower treatments had a better effect on the longevity of the *D. semiclausum* adult compared to the water treatment. Still, three of them showed a better impact than the honey solution treatment (Table 1). The *A. graveolens* treatment showed the best effect on the increase in female adult longevity, while the male longevity was shown in the *R. indica* treatment.

The *A. graveolens* treatment increased the adult longevity to 4.75 times (female) and 4.44 times (male) than the water treatment, while the *R. indica* treatment increased the longevity up to 4.44 times (female) and 4.65 times (male). The female longevity was higher than males, as is the typical report of other Hymenoptera parasitoids (Onagbola *et al.* 2007). It is different from Kumarawati *et al.* (2018) that the longevity of male *D. semiclausum* is longer than that of females, namely 12.10 ± 0.74 days and 9.80 ± 1.87 days, respectively. It is hoped that the longer life of the parasitoids will increase egg production and the number of parasitized hosts.

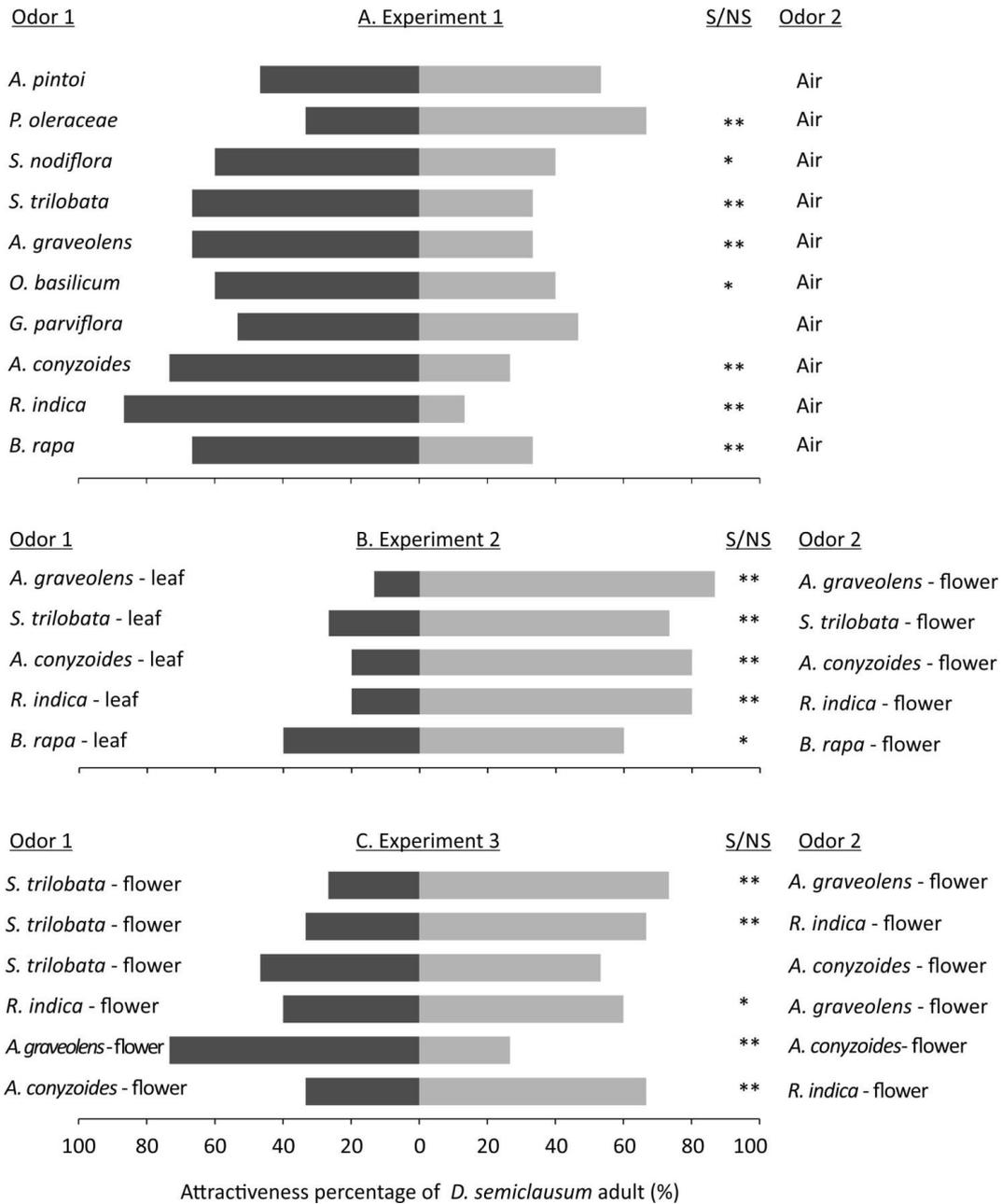


Figure 1. The olfactory response of *D. semiclausum* adult in insectary refugia plants. (A) the percentage of attractiveness between refugia flowers and clean air, (B) the attractiveness between the flowers and leaves of the same refugia plant, (C) the percentage of attractiveness between the species of refugia flowers. **= $p < 0.01$, *= $p < 0.05$

Table 1. The longevity of *D. semiclausum* adult in the refugia plant treatment

Treatments	Adult longevity ($\bar{x} \pm SD$) (days)	
	Female	Male
Honey solution (10%)	10.20 \pm 1.69 bc	9.10 \pm 1.66 c
Water	3.60 \pm 1.26 d	3.40 \pm 0.97 d
<i>R. indica</i>	16.90 \pm 1.37 a	15.80 \pm 2.39 a
<i>S. trilobata</i>	9.80 \pm 1.69 c	9.20 \pm 1.99 c
<i>A. conyzoides</i>	12.20 \pm 1.40 b	12.00 \pm 1.70 b
<i>A. graveolens</i>	17.10 \pm 1.66 a	15.10 \pm 1.29 a

The numbers in the same column follow the same letters show that they are not significantly different based on Tukey's test at the level of $\alpha = 5\%$.

Flower nectar and other carbohydrate sources can increase the length of life and parasitoid meridians in laboratory and field tests (Foster and Ruensink 1984; Winkler et al. 2006). Nectar contains water, carbohydrates, and amino acids which are needed by insects (Heil 2011). Carbohydrates generally affect the life span of parasitoids, while fat and protein are required for the production or maturation of eggs (Benelli et al. 2017).

The existence of flower nectar is essential as a source of nutrition that determines the parasitoid's survival. Parasitoids generally emerge first from their host with a limited mass of energy so that they can last only 1-2 days without food (Olson and Nechols 1995; Steppuhn and

Wakcers 2004). Winkler et al. (2005) also stated that the *D. semiclausum* adult could survive less than two days without eating. Therefore, after leaving their host, the parasitoids will first start searching for food sources rather than searching for hosts (Takasu and Lewis 1995). The *D. semiclausum* are synovigenic parasitoids, which must continue to ripen eggs during their reproductive period. The presence of nectar as a nutrition source will increase fecundity and longevity (Gurr et al. 2005; Jervis et al. 2008).

Nectar of kale flowers (*Brassica oleracea* var. *Sabellica*) as a source of carbohydrates can increase the survival of *Diadegma insulare* adult (4-28 days) and is not significantly different from 15% honey treatment (27-28 days), while water treatment is only 0-1 days (Gourdine et al. 2003). Johanowicz and Mitchell (2000) stated that the survival of *D. insulare* was supported by *Lobularia maritima* (L.) Desc (1-53 days) compared to water treatment (1-4 days). *Barabarea vulgaris* flower can increase the longevity to more than 20 days, not significantly different from honey treatment, while water treatment is only about two days (Idris and Grafius 1995). The average *D. semiclausum* adult longevity was feeding on the flowers of *F. esculentum* Moench cv. Katowase is higher than the water treatment and not significantly different from the honey treatment (Wratten et al. 2003).

The effect of refugia plants on the parasitization rates of *Diadegma semiclausum*

The treatment of *A. graveolens* and *R. indica* showed a better effect on all fitness indicators than the water control treatment (Table 2). The *A. graveolens* treatment gave the best effect on the fitness indicators of *D. semiclausum*, both the level of parasitization and the percentage of females that emerge. In contrast, the imago percentage that emerges was not significantly different from the honey solution treatment and *R. indica*.

The honey solution in the control treatment and flower nectar provides a nutrition source to enhance their fitness, indicated by a higher level of parasitization than in the water treatment as feed. Chau et al. (2019) stated that the average number of the emergence of *Cotesia vestalis*, a larval parasitoid of *P. xylostella*, was significantly influenced by *Cosmos sulphureus*, *Lantana camara*, and *Coriandrum sativum* compared to water as feed. The companion plant *Centaurea cyanus* in cabbage has a significant positive effect on the parasitism rate of *Microplitis mediator* against the larvae of *Mamestra*

brassicae (Balmer et al. 2013). Parasitism of *Nezara viridula* (L.) adult by *Trichopoda pennipes* (F.) was higher in cotton planted with milkweed (*Asclepias curassavica*) or buckwheat (*F. esculentum*) rows compared to only cotton (Tillman 2017). Buckwheat, cilantro, and fennel provided the best nectar sources for *Trissolcus japonicus*, the egg parasitoid of *Halyomorpha halys*, with an average survival rate of 15, 3.5, and 17.5 days compared with water treatment (McIntosh et al. 2020).

The sex ratio of *D. semiclausum* adults showed male bias in all treatments (Table 2). The highest percentage of female adults emerged in the honey treatment (0.86%), followed by *A. graveolens* and *R. indica* treatments. The sex ratio of *D. semiclausum* to male bias was also stated in several previous studies, both in the laboratory and in the field (Yang et al. 1993; Kwon et al. 2003; Khatri et al. 2008). Khatri et al. (2011) stated that the offsprings of *D. semiclausum* had a high male bias, reaching more than 70%.

Several studies related to the sex ratio of other Hymenoptera parasitoids also tended to be male bias. The sex ratio of offsprings in the parasitoid *Dolichogenidea tasmanica* (Hymenoptera: Braconidae) is very biased for males in the absence of *Lobularia maritima* flowers. Still, when female outsiders have access to flowers, the sex ratio of offspring between males and females tends to be the same (Berndt and Wratten 2005). In laboratory conditions, the sex ratio of offsprings was male bias in both host species, namely *Galleria mellonella* (L.) and *Ephesia kuehniell* (Zell) on the parasitoid *Bracon hebetor* (Hymenoptera: Braconidae) (Gunduz and Gulel 2005). The male-biased sex ratio can be caused by a variety of factors such as sperm depletion, sperm death, physiological ageing, active sperm digestion by female imago. The disintegration of sperm when stored in their sperm, copulation number, and differences in sex mortality during development. Larvae (Uchan and Gulel 2002; Damiens et al. 2003).

Insect access to flower nectar will be closely related to the shape or morphology of flowers. Other flower morphological structures such as petals and sepals also affect the access of parasitoids to nectar. As reported on *Brassica kaber* flowers by Idris and Grafius (1995), *B. rapa* and *R. indica* flowers have separate petals and sepals, causing the nectar found in the basal part of the flower to be exposed. It causes the nectar of *B. rapa* to remain accessible to *D. semiclausum* imago even though it has a long (deep) corolla.

Table 2. Parasitization rate of *D. semiclausum* against *P. xylostella* larvae in the refugia plant treatments

Treatments	Parasitization rate (%) ($\bar{x} \pm SD$)	Emerge, adult, (%) ($\bar{x} \pm SD$)	Emerge female (%) ($\bar{x} \pm SD$)	Sex ratio ($\sigma:\rho$)
Honey solution	69.10 \pm 1.47 a	56.12 \pm 4.50 a	46.33 \pm 1.95 a	1 : 0,86
Water	0.00 \pm 0.00 c	0.00 \pm 0.00 b	0.00 \pm 0.00 c	-
<i>R. indica</i>	46.94 \pm 2.24 b	50.70 \pm 6.20 a	40.78 \pm 1.39 b	1 : 0,69
<i>A. graveolens</i>	69.65 \pm 2.98 a	50.68 \pm 2.79 a	43.89 \pm 1.91 ab	1 : 0,78

The numbers in the same column follow the same letters show that they are not significantly different based on Tukey's test at the level of $\alpha = 5\%$.

Likewise, the treatment of *A. graveolens* has a short flower corolla and a wide opening so that the nectar is easily accessible. Nave et al. (2016) stated that several parasitoids such as *Chelonus elaeophilus* Silvestri (Hymenoptera: Braconidae), *Apanteles xanthostigma* (Haliday) (Hymenoptera: Braconidae), *Ageniaspis fuscicollis* (Dalman) (Hymenoptera: Encyrtidae), and *Elasmus flabellatus* (Fulmencolombe), and the predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), can easily reach the nectar of the Apiaceae plants, namely *Conopodium majus* (Gouan) Loret, *Daucus carota* L. and *F. vulgare* Mill.

According to Gurr et al. (2004), hierarchical steps are required for successful ecological engineering effort through the planting of insectary flowering plants as biological control. The two earliest stages are the screening of flowering plants or refugia, which can attract parasitoid aggregations on or near the flowers, then increase their fitness and increase the level of parasitization. From this study, two insectary refugia plants, *R. indica* and *A. graveolens*, were able to meet these criteria, namely attracting attendance and showing a positive effect on the fitness of *D. semiclausum*. Further research is needed to evaluate these two plants' effect on the insect pest, *P. xylostella*. The two plants that are known to provide benefits to natural enemies are also expected to have a negative effect on pests.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Education and Culture, Directorate General of Higher Education, the Republic of Indonesia, with *Penelitian Pendidikan Magister menuju Doktor untuk Sarjana Unggul* (PMDSU).

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