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Research Article

The Role of *Turnera subulata* and *Cosmos sulphureus* Flowers in the Life of *Anagrus nilaparvatae* (Hymenoptera: Mymaridae)

Manfaat Bunga Turnera subulata *dan* Cosmos sulphureus *bagi Kehidupan* Anagrus nilaparvatae *(Hymenoptera: Mymaridae)*

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ABSTRACT

Anagrus nilaparvatae is a potential egg parasitoid to control the rice brown planthopper (*Nilaparvata lugens* Stal.) The parasitoid needs to consume suitable food to maximize its biotic potential and parasitizing ability. This study was aimed to determine the benefits provided by the presence of *Turnera subulata* and *Cosmos sulphureus* flowers on the life of *A. nilaparvatae*. This study consisted of two experiments. The first experiment was designed to determine the effects of the tested flowers on the parasitism and hatching rate of *A. nilaparvatae* on *N. lugens* eggs. The flowers were set inside the rearing cage of parasitoid in the presence of *N. lugens* eggs in Ciherang rice seedlings. In addition, honey and the control (no feed added) were included into the treatments, totalling of four treatments. The parasitism rate of *A. nilaparvatae* fed with the flowers or honey was similar to those unfed. However, the hatching rate of *A. nilaparvatae* was much higher on those fed with flower or honey than those unfed. The number of *A. nilaparvatae* unable to eclose from eggs of *N. lugens* for the unfed parasitoid was 37.4% in comparison with 8.19 to 15.67% for those fed with flower or honey. The second experiment was a follow-up to address the question on the fitness of progeny of *A. nilaparvatae* fed with the tested flowers. The flowers and honey did not increase the longevity of *A. nilaparvatae* progeny. However, *A. nilaparvatae* fed with flowers or honey produced progeny that resulted in higher number of offspring compared to those unfed. *C. sulphureus* flower significantly increased the number of offspring produced by *A. nilaparvatae*. This suggest that the diet of the parental parasitoid determines the fitness of the progeny. Improving the hatching rate and the fecundity of progeny produced by the adults of *A. nilaparvatae* fed with the flower of *T. subulata* and *C. sulphureus* would contribute to the increasing population of this parasitoid which could lead to a better control of *N. lugens* in the rice field.

Keywords: *Anagrus nilaparvatae*, *Cosmos sulphureus*, *Nilaparvata lugens*, parasitism, *Turnera subulata*

INTISARI

Anagrus nilaparvatae *merupakan parasitoid telur yang potensial untuk mengendalikan serangan wereng batang cokelat (*Nilaparvata lugens *Stal.). Parasitoid perlu mendapatkan sumber pakan yang sesuai untuk memaksimalkan potensi biologis dan kemampuan memarasit inang. Penelitian ini bertujuan untuk mengkaji manfaat pemberian bunga* Turnera subulata *dan* Cosmos sulphureus *pada kehidupan* A. nilaparvatae*. Penelitian ini terdiri dari dua percobaan. Percobaan pertama dirancang untuk mengetahui pengaruh bunga yang diuji terhadap tingkat parasitasi dan penetasan telur* A. nilaparvatae *pada telur* N. lugens*. Bunga diletakkan di dalam tabung rearing parasitoid yang di dalamnya terdapat bibit padi varietas Ciherang yang mengandung telur* N. lugens*. Selain itu, terdapat pula perlakuan madu dan kontrol (tanpa bunga dan tanpa madu), dengan total empat perlakuan.* A. nilaparvate *yang diberi pakan bunga atau madu memiliki tingkat parasitasi yang sama dengan yang tidak diberi pakan. Namun, tingkat penetasan telur* A. nilaparvatae *yang diberi pakan bunga atau madu jauh lebih tinggi daripada yang tidak diberi pakan. Jumlah* A. nilaparvatae *yang tidak menetas dari telur N. lugens pada parasitoid yang tidak diberi pakan adalah 37,4% dibandingkan dengan parasitoid yang diberi pakan bunga atau madu yang berkisar 8,19−15,67%. Percobaan kedua adalah tindak lanjut untuk menjawab pertanyaan tentang kebugaran keturunan* A. nilaparvatae *yang diberi pakan dengan bunga yang diuji. Bunga dan madu tidak meningkatkan lama hidup keturunan* A. nilaparvatae*. Namun,* A. nilaparvatae *yang diberi pakan dengan bunga atau madu menghasilkan keturunan yang memiliki fekunditas lebih tinggi dibandingkan dengan keturunan dari* A. nilaparvatae *yang tidak diberi pakan. Bunga* C. sulphureus *secara signifikan mampu meningkatkan jumlah keturunan yang dihasilkan oleh* A. nilaparvatae*. Ini menunjukkan bahwa jenis pakan induk parasitoid menentukan kebugaran keturunannya. Peningkatan penetasan dan fekunditas dari keturunan* *yang dihasilkan oleh induk* A. nilaparvatae *yang diberi pakan bunga* T. subulata *dan* C. sulphureus *akan berkontribusi pada peningkatan populasi parasitoid sehingga dapat mengendalikan serangan* N. lugens *di pertanaman padi dengan lebih baik.*

Kata kunci: Anagrus nilaparvatae*,* Cosmos sulphureus*,* Nilaparvata lugens*, parasitasi,* Turnera subulata

INTRODUCTION

The rice brown planthopper (*Nilaparvata lugens* Stal.) (Hemiptera: Delphacidae) is a major pest of rice plants in Asia with the damage could reach 90% (Liu *et al*., 2012). This pest is a serious threat in rice production because it may cause hopperburn (Catindig *et a*l., 2009), and vector for *Rice grassy stunt virus* (RGSV) and *Rice ragged stunt virus* (RRSV) (Reissig *et al*., 1986).

Anagrus nilaparvatae (Hymenoptera: Mymaridae) is a potential egg parasitoid for controlling *N. lugens* (Gurr *et al*., 2011). Parasitism rate of *A. nilaparvatae* on *N. lugens* eggs ranged from 15.7 to 35.7% with average of 24.9% (Yaherwandi & Syam, 2007). *A. nilaparvatae* parasitizing ability may reach 38.21% when *N. lugens* lived in rice plants, 64.09% in grasses (Atmadja & Kartohardjono, 1990), and decreased at the end of rice plants (66 Days After Planting, DAP) to 1.12−8.51% (Haryati *et al*., 2017). In Malaysia, parasitism rate of *N. lugens* eggs by *A. nilaparvatae* could reach 68% (Watanabe *et al*., 1992), while in Sri Lanka could be as high as 54% (Fowler *et al*., 1991). In Japan, parasistism rate of *N. lugens* eggs by *Anagrus flaveolus* Waterhouse was 67% (Otake, 1970), and 40−60% by *A. nilaparvatae* (Liu *et al*., 2012).

Parasitoids have been negatively impacted by the use of chemical pesticides, starting from reduction in their fitness and parasitizing ability to their death (Hardin *et al*., 1995; Meilin *et al*., 2012a). Improvement in the role of parasitoids as Biological Control Agents (BCA) can be done through ecological engineering so that optimized ecosystem services may result in a significant control of *N. lugens*. The existence of parasitoids can be preserved by increasing the biodiversity of rice ecosystems. Planting flowering plants (non-crop plants) around the crops will be able to provide a source of food (pollens and nectars), a shelter, as well as alternative hosts for parasitoids (Wratten *et al*., 2003).

Several previous studies have shown that addition of flowering plants could increase insect fecundity and longevity (Pianka *et al*., 1977; Wratten *et al*., 2003; Berndt and Wratten, 2005; Abd El-Kareim *et al*., 2011). Zhang *et al*. (2004) reported that the longevity and fecundity of *Trichogramma brassicae* increased after feeding the parasitoid with honey and corn pollen. *A. nilaparvatae* and *A. optabilis* fed with sesame flowers had longer life and better parasitizing ability (Zhu *et al*., 2013). The longevity of *A. nilaparvatae* also became longer after being treated with the flowers of *Phacelia tanacetifolia* (Farrell, 2013). Egg parasitoids, including *A. nilaparvatae*, could control the population of *N. lugens* in the early stage and it made these parasitoids into potential BCA (Godfray, 1994). Adding flowering plants surrounding rice field could reduce insecticide applications by 70%, increased grain yield by 5%, and delivered an economic advantage of 7.5% (Gurr *et al*., 2016).

This study aimed to assess the effects of *Turnera subulata* and *Cosmos sulphureus* flowers on the parasitizing capacity of *A. nilaparvatae*, as indicated by the parasitism rate, parasitoid emergence, and the longevity and fecundity of parasitoid progeny after their parents were fed with these two flowers. *T. subulata* and *C. sulphureus* are widely adapted and planted crops and recently used by farmers as refuge in the rice fields in the Special Province of Yogyakarta and the Province of Central Java. In addition, *T. subulata* was selected to represent perennial flowering plants, while *C. sulphureus* represented seasonal flowering plants.

MATERIALS AND METHODS

Rearing of Nilaparvata lugens

The starting population of *N. lugens* was collected from the rice field in Ngestiharjo Village, County of Kasihan, the District of Bantul, Yogyakarta and reared in the greenhouse of Crop Protection Department, Faculty of Agriculture, Universitas Gadjah Mada (UGM). *N. lugens* was reared on Ciherang rice variety employing an established procedure at the Laboratory of Pesticide Toxicology, UGM.

Rearing of Anagrus nilaparvatae

Anagrus nilaparvatae initial population was obtained by trapping in the rice field located in

Tanjungharjo Village, Nanggulan County, the District of Kulon Progo, Yogyakarta in November 2016. The rice variety used for trapping was Ciherang (one-month old) and this variety was similar to the rice field where the trapped crops were placed. Rice plants were grown in plastic pots with a diameter of 14 cm (top) and 11 cm (bottom), and 12 cm tall. Potted rice plants were individually covered with a plasic tube (50 cm tall, 10 cm diameter) constructed from mica plastic sheet. The tops of the tubes were covered with cotton gauze. Plants were infested with 50 gravid females of *N. lugens* and incubated for three days for oviposition. Subsequently, *N. lugens* and the plastic tube were removed. Rice plants contained with eggs of *N. lugens* were transferred to one month-old rice plants and placed randomly. Plants were left in the rice field for three days. Subsequently, leaves and roots of the plants were removed and left only the stems. The cut end of each stem was wrapped in tissue paper moistened with water. The stems were inserted in a plastic cup with 8 cm in diameter (top), 5.5 cm in the bottom, and 12 cm tall. Each plastic cup with rice stems were then placed inside a plastic tube (8 cm diameter, 25 cm tall). The plastic tubes were covered with cotton gauze on the top. The newly emerged *A. nilaparvatae* was transferred and reared in the Laboratory using established laboratory procedure (Meilin *et al*., 2012b) on Ciherang rice variety.

Flowers of Turnera subulata *and* Cosmos sulphureus

Turnera subulata and *C. sulphureus* were obtained from Banyumas, Central Java. *T. subulata* was propagated by cuttings and *C. sulphureus* was propagated by sowing the dried seeds in polybag of 30×30 cm in size. Flowers at the full bloom were used for the testing.

Effect of Turnera subulata *and* Cosmos sulphureus *on* Parasistism *of* Anagrus nilaparvatae

The experiment employed a Complete Randomized Design (CRD) with four treatments: *T. subulata* and *C. sulphureus* flowers, honey, and the control (no flower and no honey). The rice plant used was two-weeks old of Ciherang variety.

Ten rice seedlings were placed in plastic cups (6.5 cm top diameter, 4.5 cm bottom diameter, 4.5 cm tall). The cup was then placed inside a plastic tube (8 cm diameter, 23 cm tall) and the plants were maintained their freshness by adding water into the cup. Twenty *N. lugens* gravid females were released in the plastic tube for two days for oviposition when the adults were then removed. One flower of *T. subulata* or *C. sulphureus* was placed into each cup inside the plastic tube. For the treatment of honey, 10% honey solution (Madu Murni Nusantara) was smeared on plastic tube wall. The honey and control treatments were applied in the absence of flowers. Five *A. nilaparvatae* females were transferred into each plastic tubes set for the four different treatments. The flowers were replaced every other day until all the parasitoids died. Subsequently, the leaves of rice seedlings were removed and the roots were wrapped with tissue of 5×5 cm, moistened with water and wrapped in aluminum foil on the outside and placed into a test tube, and covered with cotton gauze. Each treatment was replicated 6 times.

Observation was conducted everyday by counting the number of the newly emerged *A. nilaparvatae* and *N. lugens* nymphs. After three days in a row without any emergence, the rice seedlings were dissected to record the number of *N. lugens* eggs being parasitized but not hatching (unhatched parasitized eggs) and the number of healthy (not parasitized) *N. lugens* eggs but not hatching. Parasitism rate was calculated by dividing the sum of emerged and died parasitoids inside the host by the sum of unhatched eggs, *N. lugens* nymphs and the newly emerged A. nilaparvatae. Analysis of Variance (ANOVA) was done using SAS 9.3, and was continued with LSD ($P < 0.05$) when significant differences existed (Gomez & Gomez, 1995).

Effect of Turnera subulata *and* Cosmos sulphureus *on the Progeny of* Anagrus nilaparvatae

The previous study was designed to determine the direct effects of *T. subulata* and *C. sulphureus* flowers on *A. nilaparvatae* (F_0). This study was the continuation of the previous one to address the questions whether the adults of *A. nilaparvatae* fed with the flowers produced better progeny (F_1) than those of unfed parasitoid. The design of this study was similar to the previous one, consisting of four treatments. *A. nilaparvatae* emerged from the all treatments were used according to their respective treatments. However, no matter from what treatments the parasitoids came from they were not fed with any materials (flower or honey). Therefore, it was expected that if the flowers had something good for the parent, the progeny would be beneficially affected by the life of their parents.

The rice plant used was Ciherang aged of two weeks. Five rice seedlings were placed in a glass vial (3.5 cm in diameter, 4.5 cm tall). The vial was then placed inside a plastic tube (5 cm diameter, 17 cm tall) and the plants were maintained their freshness by adding water into the vial. Five *N. lugens* gravid females were released in the plastic tube. One day after *N. lugens* release, one *A. nilaparvatae* adult was released into the plastic tube without food until the parasitoid insidedied . Subsequently, the leaves of rice seedlings were removed. The roots were wrapped in tissue of 5×5 cm, moistened with water followed by the second wrapping using aluminum foil on the outer side. The rice stems were placed inside the test tubes (one treatment in one tube) and covered with cotton gauze. Each treatment was replicated 20 times.

Observation was conducted everyday to determine the longevity and fecundity of *A. nilaparvatae* progeny. The longevity of *A. nilaparvatae* was calculated by summing up the number of days since adult emergence until died. Fecundity of the progeny was counted based on the number parasitoid emerged from the parents being treated with no food in this experiment added with the number of *N. lugens* eggs being parasitized but not hatching (unemerged parasitoid). Rice stems were dissected three days after the last parasitoid had emerged to count the number of unemerged *A. nilaparvatae*.

$Sin^{-1}\sqrt{X}$ transformation was performed to normalize the data distribution. ANOVA test was performed using SAS 9.3, and was continued with LSD test ($P < 0.05$) when significant differences existed (Gomez and Gomez, 1995).

RESULTS AND DISCUSSION

Effect of Turnera subulata *and* Cosmos sulphureus *on Parasistism of* Anagrus nilaparvatae

Addition of *T. subulata* and *C. sulphureus* flowers or honey had no effect on the parasitism rate of *A. nilaparvatae* on *N. lugens* eggs (Figure 1). This result might be due to the fact that *A. nilaparvatae* is pro-ovogenic nature which does not require food source for eggs production but energy is needed for the host-searching process and survival (English-Loeb *et al*. 2003; Farrell, 2013). Gravid female of *A. nilaparvatae* could immediately oviposit when she found a host (Lou *et al*., 2014). Furthermore, *A. nilaparvatae* might also collect carbohydrates from the honeydew excreted by *N. lugens* (Lee *et al*., 2006; Wackers *et al*., 2008), although in smaller amounts.

In contrast to the data on parasitism rate, parent parasitoids not receiving any food (control) resulted to high number of unsuccesful parasitoid progeny to emerge from *N. lugens* eggs. Close to 40% of parasitoid were not able to hatch when the parents were not fed with flowers or honey, while those fed with flowers or honey caused significantly lower number of unsuccesfull hatching (Figure 2). Furthermore, differences between the tested flowers and honey was not significant. Nectar or honey could provide additional capacity of parasitoids in storing glycogen (Olson *et al*., 2000), slowing the declining rate of lipids (Lee *et al*., 2004), and increasing the egg maturation (Tylianakis *et al*., 2004). Pro-ovogenic parasitoids can resorb eggs and divert their energy for host searching process (Rivero & Casas, 1999). These results indicate that providing flowers and honey could improve the

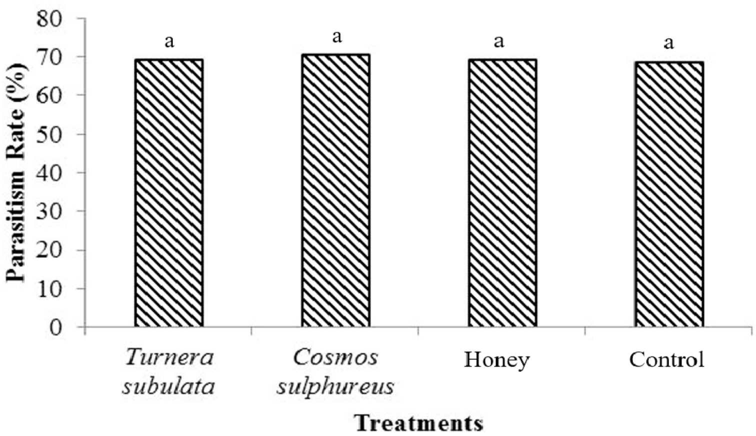

Figure 1. Effect of two species of flowers on the parasitism of *Nilapavarta lugens* eggs by*Anagrus nilaparvatae*; *A. nilaparvatae* (F_0) were released in the cage containing rice plants infected by *N. lugens* eggs and the flower

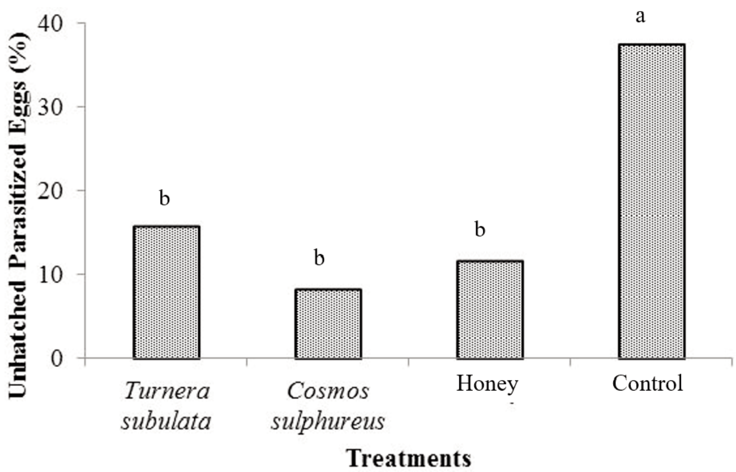

Figure 2. Effect of two species of flowers on hatchability of parasitized *Nilaparvatae lugens* eggs by *Anagrus nilaparvatae*

quality *A. nilaparvatae* eggs. High parasitism rate and high success of parasitoid emergence from the host would contribute to high population of parasitoid for the following generation which in turn could lead to provide a better control for *N. lugens*.

The emergence of *A. nilaparvatae* exposed to *T. subulata* and *C. sulphureus* flowers started at the 11th day and ended at the 15th day after infestation. However, the emergence of parasitoids from the control and honey treatments occured one day after those fed with flowers and ended at the same time. The peak of emergence of *A. nilaparvatae* occurred at the 12th and 13th day (Figure 3). Haryati (2016) reported that *A. nilaparvatae* fed with honey started to emerge at the 12th to 14th day. The early emergence of *A. nilaparvatae* in the treatments of *T. subulata* and *C. sulphureus* flowers indicates that nutrients from the flowers may be able to optimize the level of vitellogenin (a precursor protein of yolk) (Zhang *et al*., 2016) which then increasing the maturation of eggs (Tylianakis *et al*., 2004). Thus, eggs hatch quickly and accelerate the process of parasitization.

Effect of **Turnera subulata** *and* **Cosmos sulphureus** *on the progeny of* **Anagrus nilaparvatae**

The addition of flowers or honey for the diet of parents parasitoids had no effect on the longevity of *A. nilarpavatae* progeny. However, it significantly increased the number of parasitoids produced by the progeny (Table 1). In other words, when the parents of *A. nilaparvatae* were fed with flower of *C. sulphureaus*, their progeny would resulted more offsprings than those not receiving any food supplement. The absence of any positive effects on the longevity of F_1 progeny might be related to the fact that females would die immediately after laying their last eggs and they did not spend time or energy on activities that did not contribute to their fitness (Segoli, 2013). On the other hand, the flower of *C. sulphureus* consumed by F_0 parent had its effect on the fecundity of F_1 progeny. These results confirm that food sources from flowers (pollen and nectar), which contains sugar, protein, amino acids, and lipids, could increase the fecundity of parasitoids, such as *A. nilaparvatae* (Baggen & Gurr, 1998; English-Loeb *et al*., 2003; Vattala *et al*., 2006; Sivinski *et al*., 2011; Zhu *et al*., 2013).

Turnera subulata and *C. sulphureus* flowers had different effects which likely due to differences in morphology, composition of nectar and pollen, as well as volatiles (Farrell, 2013). The most important morphological factor in the suitability of flowers is corolla width and depth, in relation to parasitoid head width and mouthpart structure. Previous research reported that *Phacelia tanacetifolia* flowers had no effect on the longevity of *A. nilaparvatae* (Farrell, 2013), even decreasing the longevity of Hymenopteran parasitoid than that of water (Vattala *et al*., 2006). Biological clock of *T. subulata* (the flower blooms at 7−8 a.m. and closes at 11−12 a.m.) is one of the biological factors that may limit the access of the parasitoid to its food sources. This result confirms that different species of flowering plant were associated with different families of parasitoids (Sivinski, 2011). Each flowering plant has variability in the attractiveness and suitability for certain parasitoid species (Farrell, 2013). This information is important for selecting the suitable flowering plants to enhance the role of parasitoids as BCA in agricultural ecosystems.

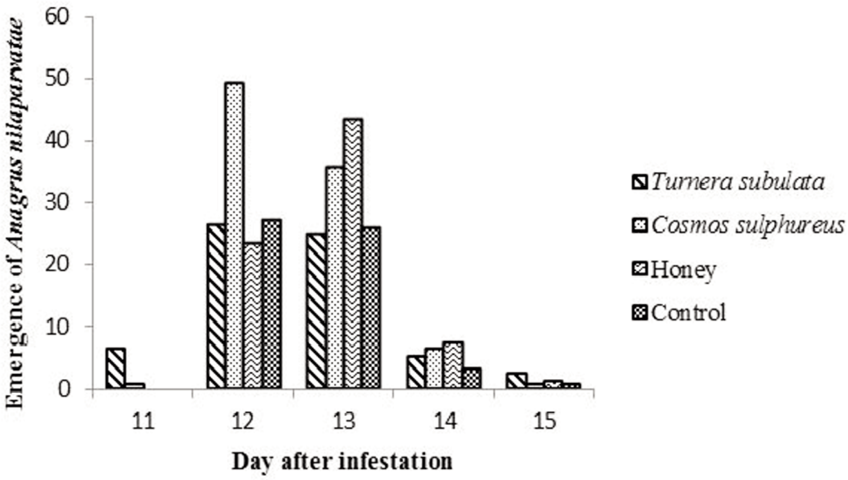

Figure 3. Daily emergence of *Anagrus nilaparvatae* from eggs of *Nilaparvatae lugens* supplied with two species of flowers

Table 1. Effects of two species of flowers on the longevity and fecundity of *Anagrus nilaparvatae* progeny

Treatment for Parents (F_0)	Progeny (F_1)	
	Longevity (day)	No. Parasitoid Produced by F_1*
Turnera subulata	1.85 ± 1.14 a	23.45 ± 10.98 ab
Cosmos sulphureus	1.70 ± 0.86 a	26.85 ± 17.05 a
Honey	1.65 ± 0.75 a	23.35 ± 14.42 ab
No supplements	1.85 ± 1.18 a	18.45 ± 13.30 b

Remark: The parents were fed with flowers and honey but the progeny were not. Mean ± standard deviation followed by the same letter were not significantly different according to LSD ($P < 0.05$) * Data was transformed to $\text{Sin}^{-1}\sqrt{X}$.

Parasitizing ability of parasitoids in an agro-ecosystem will increase following the increase in fecundity, egg quality, longevity, and the acceleration of the emergence. The stability of sustainable parasitoid population must always be managed to optimize the ecosystem services (Altieri, 1999). The presence of flowering plants (non-crop plants) on crop cultivation is one of the natural enemies' conservation and contribution of biodiversity to optimize the ecosystem services (Gurr *et al*., 2016).

CONCLUSION

The presence of *T. subulata* and *C. sulphureus* flower improved the hatchability of *A. nilaparvatae* from *N. lugens* eggs but they did not affect the parasitism rate. Under laboratory condition, the progeny of *A. nilaparvatae* produced from the parents reared with the flowers emerged one day earlier than those not receiving any food supplement. Although their progeny had the same longevity than those unfed parents, the flowers provided for the parents of *A. nilaparvate* were able to increase the number of parasitoids produced by their progeny. Improving hatchibility, shortening the embryonic developmental time, and increasing the progeny produced by *A. nilaparvatae* fed with the flowers of *T. subulata* and *C. sulphureus* would contribute the overall role of this parasitoid in managing the population of *N. lugens* in the field.

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