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Research Article

Benefits of Flowering Plants as Refuge to Improve the Ecosystem Services by Egg Parasitoids of the Rice Brown Planthopper*)

Nike Grace Hanjelina Sinulingga[1)]*, Y. Andi Trisyono[1)], Edhi Martono[1)], & Buyung Hadi[2)]

[1)]*Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada*
Jln. Flora No. 1, Bulaksumur, Sleman, Yogyakarta 55281 Indonesia

[2)]*Crop and Environmental Sciences Division, International Rice Research Institute (IRRI)*
DAPO Box 7777, Metro Manila 1301 Philippines

**Corresponding author. E-mail: nike.grace.h@mail.ugm.ac.id*

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ABSTRACT

Outbreaks of the rice brown planthopper, *Nilaparvata lugens* Stal., tend to increase in 2017. There has been significant interest to reduce reliance on pesticides by manipulating habitat plant species and communities to benefit natural enemies of insect. Flowering plants as refuge can contribute in enhancing the ecosystem services. This study aimed to assess the benefit of flowering plants as refuge to improve the role of egg parasitoids of brown planthopper. We sampled three rice fields: rice field adjacent to refuge, far from refuge, and rice field with no refuge using trapping procedure. We found two genera of parasitoid in Banyumas: *Oligosita* and *Anagrus*. The parasitism in the three rice fields was 46.14, 43.05 and 42.32%, respectively, showing no differences. However, the number of parasitoids emerged from the traps placed in the rice field with refuge was higher (31.08 adults/trap) than the other two rice fields (25.67 and 20.71 adults/trap). In addition, the number of unhatched parasitoids was lower in the rice with refuge (5.9%) compared to no refuge (14.54%). These findings show that the refuge provides better environments for the parasitoid by improving the number of progeny produced which eventually could increase their role in managing *N. lugens* population.

Keywords: egg parasitoid, *Nilaparvata lugens*, plant refuge

INTRODUCTION

Brown planthopper, *Nilaparvata lugens* Stal. (Hemiptera: Delphacidae), is one of the main pests of rice in Indonesia, first reported in 1854 and it remains until now. Brown planthopper is a minor pest in Asia until 1960 (Pathak & Dhaliwal, 1981) and became the main pest after the Green Revolution (Bottrell & Schoeny, 2012). The control of brown planthopper generally relied on insecticides, and the previous researches showed these practices induced to resurgence and resistance (Untung, 2006; Bottrell & Schoeny, 2012; Londingkene *et al*., 2016). Moreover, insecticides decreased *Anagrus nilaparvatae* activity and caused its mortality up to 50−100% (Wang *et al*., 2008; Meilin *et al*., 2012a).

Parasitoid has an important role in controlling brown planthopper populations (Manjunath, 1978; Gurr *et al*., 2010). Egg parasitoid is more effective and more efficient in controlling brown planthopper than those for the nymphs or adults. The egg parasitoid of *N. lugens* found in Klaten and Bantul were *A. nilaparvatae*, *Oligosita*, and *Gonatocerus* (Meilin, 2012b) and in Sukamandi and Puwokerto were *O. yasumatsui*, *O. nephotetticum*, and *Anagrus* sp. (Claridge *et al*., 1999). In the wet season, the parasitism by *Anagrus* and *Oligosita* was 18−61% (Claridge *et al*., 1999). The highest activity of *A. nilaparvatae* was at 11.00-13.00 with the parasitism of 36.13% (Haryati *et al*., 2016).

There has been significant interest to reduce the reliance on pesticides by manipulating the ecosystems (Marino & Landis, 1996; Gurr *et al*., 2004, Gurr *et al*., 2016). Polyculture planting system enhanced the parasitoid diversity and the high parasitation level compared to that in the monoculture (Marino & Landis, 1996; Gardiner *et al*., 2010). Manipulating habitat could be done through planting the trapping plants to divert the pest, applying polyculture techniques to reduce migration, and providing feed and alternative hosts for natural enemies (Gurr *et al*., 2010; Gurr *et al*., 2016). Manipulating habitat is a conservation effort in increasing the diversity of

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plants to enhance the role of natural enemies by providing better microclimates, alternative hosts and nutritional sources e.g. pollen, nectar or honeydew (Landis *et al*., 2000; Pfiffner & Wyss, 2004), and to reduce the pest population of Delphacidae family (Hemiptera) up to 50% (Gurr *et al*., 2000).

Integrated Pest Management (IPM) has been implemented in Indonesia since 1989. However, the number of registered pesticides increases every year and the outbreaks of brown planthopper are still reported. The implementation of Landscape IPM to improve the ecosystem services was conducted by planting flowering plants in the bunds as refuge (Trisyono, 2015). FAO and the Ministry of Agriculture (2014−2016) collaborated to implement Landscape IPM in Kembaran District, Banyumas Regency, Central Java Province. The implementation of Landscape IPM was able to reduce yield loss caused by pests, thus increasing farmer's yield 1-2 ton/ha than to the previous planting seasons and the other farmers not applying Landscape IPM (Sucipto, 2016; personal communication). Therefore, this study aimed to assess the benefit of flowering plants as a refuge in providing ecosystem services to improve the role of egg parasitoids of brown planthopper.

MATERIALS AND METHODS

Mass Rearing of Brown Planthopper

The founding population of brown planthopper (150 insects) was collected from Sariharjo Village, Ngaglik District, Sleman Regency, the Special Region of Yogyakarta. Mass rearing of brown planthopper was conducted using an established method in the Pesticide Toxicology Laboratory since 1985. 100 g of Cisadane rice seeds were washed, soaked for 24 hours at the room temperature. Those rice seeds were germinated in a plastic jar (20 cm in diameter and 19 cm in high) and water was added. Seven days after germination, the rice seedlings were used as a natural diet of brown planthopper. The new rice seedlings (7 days old) were supplied every 5−7 days before the plants turn into yellow and dry. Mass rearing was carried out continuously until the the research was completed.

Trapping Plants

Trapping plants (Ciherang variety) were germinated in the plastic pots (20 cm in top diameter, 16 cm in bottom diameter, and 16 cm in height). Rice seeds were soaked in water for 24 hours and sown. Fourteen days after planting (dap), rice seedlings were moved to the pots and watered accordingly until the rice plants were ready (2, 4, 6, 8, 10, and 12 weeks after planting [wap]). Trapping plants were prepared 4 days before the trapping time. The trapping plants were acclimatized and covered with a mica tube (18 cm in diameter and 70 cm in height) for 2 days, then the next day they were infested with gravid brown planthopper (30 females/pot for 2 days). The infested plants were moved to the field to trap parasitoids. The parasitoid trapping was conducted for 36 hours starting at 07.00 until 19.00 the following day.

Trapping Procedure

The trappings were conducted in Kembaran and Sokaraja Districts, Banyumas Regency, Central Java. The parasitoid trapping employed a Randomized Complete Block Design consisting of three types of rice field: adjacent to refuge, far from the refuge and no refuge (Figure 1). Rice field adjacent to refuge was indicated by having flowering plants (*Cosmos caudatus* and *Turnera subulata*) surrounding the rice fields. The area had a simultaneous cropping practice, and the biological and conventional pesticides was only applied when it was necessary. The rice field far from refuge was also surrounded by flowering plants with the nearest refuge was 12 meters. In addition, this area was not simultaneous in the planting time and cropping practices, and the improper use of buprofezin insecticide (Applaud® 10WP), i.e. 3 times in one planting season (Munaryo, 2016; personal communication). The rice field far from refuge was located in Sokaraja District with a distance of 5 km from refuge. Similarly, the area was not simultaneous in the planting time and cropping practice. Moreover, the applications of pesticides was done every two weeks. The insecticides used were carbofuran (Furadan® 3GR) before transplanting, imidacloprid (Confidor® 5WP) and buprofezin (Applaud® 10WP). The farmers experienced the attack of the golden snail at the beginning of the planting season, the brown planthopper, and the rice ear bug (Tohir, 2016; personal communication).

The trapping was conducted every two weeks, starting from the plant aged 2 weeks until 12 weeks. Eight trapping plants were employed using the same variety. The distance between trapping plants was at least 3 meters. After 36 hours in the field, trapping plants were brought to the laboratory to be observed.

The leaf and the root tip of the rice plant were cut then the bottom part was covered with a moist tissue and placed in a mica container (7.8 cm in diameter and 23 cm in high) at room temperature. Daily observations were conducted to calculate the number of brown planthopper nymphs emerged and the number of parasitoid emerged during 14 days. The last observation was carried out to calculate the number of parasitized brown planthopper eggs by dissecting the stem tissue of rice plants using a Nikon 7314 20x Mini Field Microscope. Five parasitoids having the same morphological characteristics were sampled for identification to the genus level. The parasitism was calculated by the following formula (Haryati *et al*., 2016):

$$\text{Parasitism} = \left(\frac{A + T}{W + A + T}\right) \times 100\%$$

A is the number of parasitoids emerged, W is the number of brown planthopper nymphs emerged, and T is the number of unhatched parasitoids.

Data Analysis

The number of parasitized brown planthopper eggs and the number of parasitoids emerged were analyzed using Variant Analysis (ANOVA) with a Randomized Complete Block Design by R-analysis statistical software packages 1.5. The LSD test was carried out when the ANOVA showed a significant difference at the significance level of 5%.

RESULTS AND DISCUSSION

Effect of Flowering Plants as a Refuge on the Parasitism of the Brown Planthopper Eggs

The parasitism at plants aged of 4−12 wap showed a similar trend between the three treatments, with the rice field adjacent to refuge had higher parasitism than the other treatments (Figure 2). The plant aged 2 wap with no refuge had the lowest parasitism which might be due to the applications of herbicide and carbofuran to control golden snail before planting. The parasitism in the three rice fields increased at 6 wap and decreased at 12 wap. Similarly, the previous report showed that the number of the parasitoid in the rice fields without the applications of pesticides and the pesticides applied based on IPM principles was high at 6 wap (Meilin, 2012b). The use of pesticides at the beginning of planting caused a decrease in the parasitism level. However, there was no difference in the parasitism level during the whole rice planting season (Table 1).

The density of brown planthopper in the field influenced the ability of parasitoid to detect the volatile compounds produced by plants to find their hosts. Decrease in the fecundity, sex ratio, the index of population increase, and the developmental time of parasitoid was associated with the exessive number of brown planthopper eggs in the field surpassing the the ability of *A. nilaparvatae* to parasitize

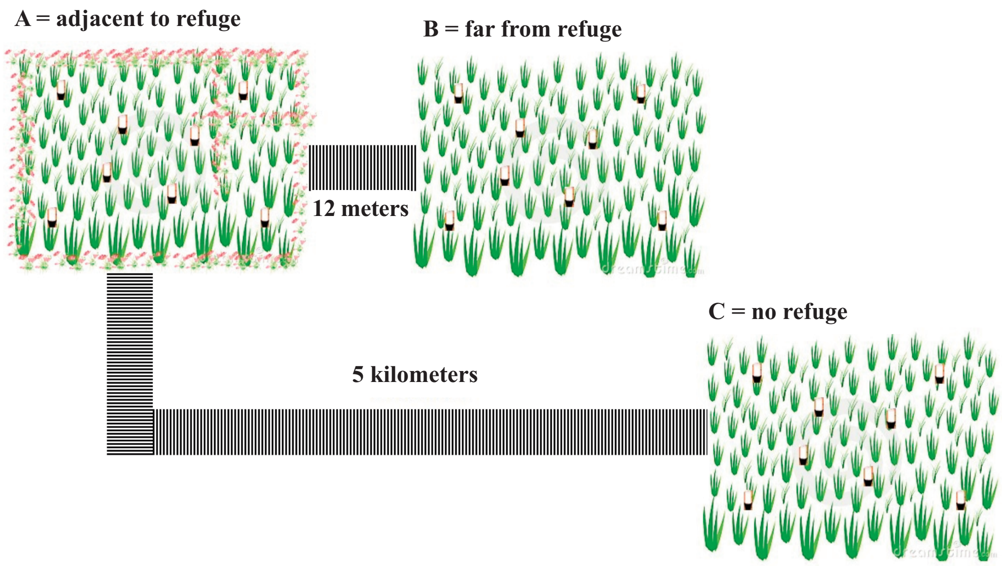

Figure 1. Type of rice fields in Kembaran and Sokaraja Districts, Banyumas Regency, Central Java Province (A = adjacent to refuge means the flowering plants surrounded the rice fields; B = far from refuge means the rice field was not surrounded by refuge and the nearest refuge were 12 meters in distance; C = no refuge means the nearest refuge was found in 5 km)

(Xiang *et al*., 2008). *A. nilaparvatae* was proovigenic insect in which 75% of the mature eggs was produced before reaching the adult stage. The absence of host at the early stage of adulthood caused a decrease in the parasitism (Abdilah *et al*., 2016).

The parasitism level was not significantly affected by the presence of refuge. However, the refuge improved the fitness of parasitoids as indicated by more parasitoids emerged from brown planthopper eggs placed closed to refuge. The number of parasitoids emerged from the rice field adjacent to refuge was higher (31.08 adults/trap) than that far from refuge (25.67 adults/trap) and with no refugia (20.71 adults/trap) (Table 1). Furthermore, the number of unhatched parasitoids in the rice field adjacent to refuge was lower (5.9%) than that far from refuge (12.27%), and with no refugia (14.54%) (Table 1). This results showed that refuge plants benefited the development of parasitoids as indicated by high number of parasitoids emerged. Nectar, pollen, and honeydew produced by refuge plants were carbohydrate sources for adult parasitoids to enhance their fitness (ability to parasitize and to lay eggs), fecundity, number of offspring, and adult longevity (Ouyang *et al*., 1992; Baggen *et al*., 1999; Lee *et al*., 2004; Rahat *et al*., 2005; Wratten *et al*., 2004; Begum *et al*., 2004; Heimpel & Jervis, 2005; Zhu *et al*., 2013).

The Genera of Parasitoid and their Potency

The two genera of parasitoid were found: *Oligosita* and *Anagrus* (Figure 3). *Oligosita* belongs to Trichogrammatidae (Hymenoptera) characterized with a body size of 0.15−1 mm; 3 segments of tarsi (t); and the antenna consists of scape, pedicel, 2 funnel segments, and the club-shaped in the last segment. The characteristic of the *Oligosita* is the marginal fringe (Mf) in the middle of the front wing and distal cilia (Dc) (Achterberg *et al*., 1991; Barrion & Litsinger, 1994; Begum & Anis, 2013). *Anagrus* belongs to Mymaridae (Hymenoptera) characterized by tarsus of 4−5 segments, a narrow front wing with distal cilia (Dc) rarely and a rear wing is narrower and shorter than the front wing. The female antenna consists of 9 segments with the club-shaped in the last segment and a long gaster ovipositor (Barrion & Litsinger, 1994).

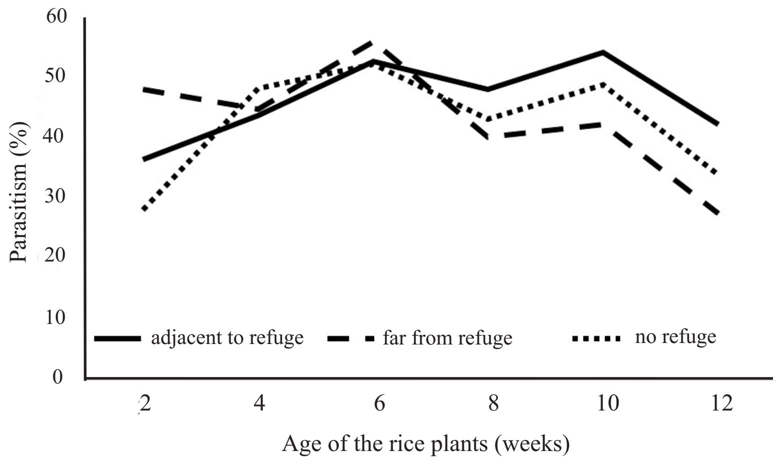

Figure 2. The parasitism level of brown planthopper eggs during the whole rice season (November 2016−February 2017) in Kembaran and Sokaraja Districts, Banyumas Regency, Central Java Province

Table 1. Effect of refuge plants on the parasitism of the brown planthopper eggs

Rice Field	Parasitism (%)	Parasitoid Emerged (adults/trap)	Unhatched Parasitoids (%)
Adjacent to refuge	46.14 ± 9.42a	31.08 ± 8.61a	5.90 ± 1.12c
Far from refuge	43.05 ± 6.72a	25.67 ± 20.83ab	11.27 ± 2.56b
No refuge	42.32 ± 9.40a	20.71 ± 9.17b	14.54 ± 4.10a

Remarks: Adjacent refuge = flowering plants surrounded the rice fields. Far from refuge = the rice field was not surrounded by the flowering plants and the nearest refuge was found 12 meter from the research area. No refuge = the nearest refuge was found in 5 km from the reaserch area. Values (mean ± SD) followed by the same letter for each column were not significantly different at 5%.

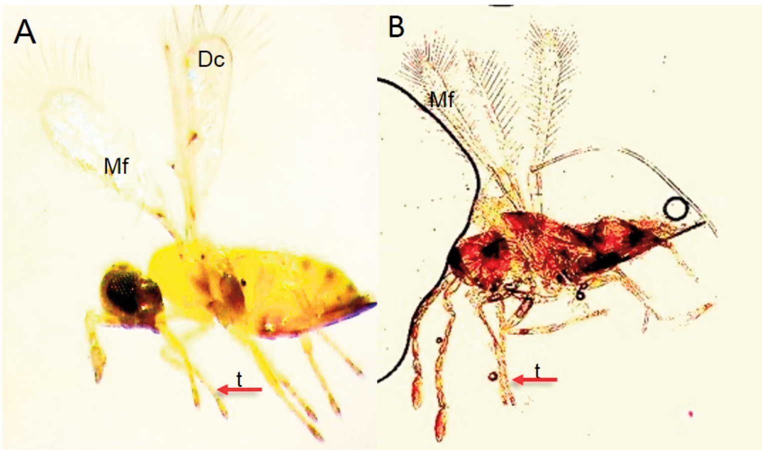

Figure 3. The two genera of the brown planthopper eggs parasitoid found in Banyumas: (A) *Oligosita* and (B) *Anagrus*; (Mf) marginal fringe in the center of the front wing and (Dc) distal cilia

Table 2. Parasitoids of *Oligosita* and *Anagrus* emerged from the brown planthopper eggs

Rice Field	*Oligosita* (adults/trap)	*Anagrus* (adults/trap)
Adjacent to refuge	16.25 ± 4.21a	14.83 ± 6.89a
Far from refuge	17.25 ± 16.25a	8.42 ± 6.44b
No refuge	12.75 ± 6.52a	7.96 ± 4.85b
Mean	15.41c	10.25d

Remarks: Adjacent refuge = flowering plants surrounded the rice fields. Far from refuge = the rice field was not surrounded by the flowering plants and the nearest refuge was found 12 meters from the research area. No refuge = the nearest refuge was found in 5 km from the reaserch area. Values (mean ± SD) followed by the same letter for each column were not significantly different at 5%.

The number of parasitoid, fecundity, and parasitoid fitness in agricultural ecosystems are very diverse. The number of collected *Oligosita* was higher (15.48 adults/trap) than *Anagrus* (10.25 adults/trap). The refuge did not affect the number of *Oligosita* emerged from the brown planthopper eggs but it did affect *Anagrus*. The number of *Anagrus* emerged from eggs placed adjacent to the refuge was higher (14.8 parasitoids/trap) than that far from refuge (8.42 parasitoids/trap), and rice field with no refuge (7.96 parasitoids/trap) (Table 2). The flowering plants, e.g. *Sesanum indicum*, *Emilia sonchifolis* and *Impatiens balsema* attracted *A. optabilis* and *A. nilaparvatae* (Zhu *et al*., 2013). Moreover, *Anagrus* parasitation in Erythroneuna eggs was higher when black sorghum flowered in the vineyards plantation (Loeb *et al*., 2003). The existing of flowering plants in the vineyards increased the fecundity, longevity, and parasitism of *Trichogramma carverae* (Begum *et al*., 2004).

The nutrients produced by refuge provided benefits for the development of parasitoid in the brown planthopper eggs and the number of the parasitoid emerged. Refuge enhanced the ecosystem services provided by th egg parasitoids. The continuity of refuge in the fileds would determine the quality of ecosystem services. Therefore, the implementation of Landscape IPM, e.g. through habitat management, should be consistently practiced in addition to regular monitoring of the pest and natural enemies population. To gain the benefits from the ecosystem services, pesticides must only be used in accordance with the IPM principles.

CONCLUSION

Flowering plants as refuge provide a better environment for parasitoids by enhancing the number of progeny emerged and reducing the number of unhatched parasitoid from the brown planthopper eggs. These findings showed the practical and sustainable way to contribute maintaning the brown planthopper population under its economic threshold.

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