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Research Article

Diversity of Natural Enemies Associated with Refuge Flowering Plants of *Zinnia elegans*, *Cosmos sulphureus*, and *Tagetes erecta* in Rice Ecosystem

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ABSTRACT

The presence of flowering plants is essential to the conservation of natural enemies in some particular ecosystems, such as the agroecosystem. The objectives of this research were to determine the natural enemies associated with refugial flowering plants *Zinnia elegans*, *Cosmos sulphureus*, and *Tagetes erecta* planted in the rice bunts. The research took place in the rice farms in the villages of Jatisarono, Wijimulyo, and Tanjungharjo, Nanggulan Regency, Kulon Progo District. The natural enemies were observed using three different methods: insect nets, pitfall, and direct observation. The observations were conducted in the morning, between 07.00–10.00 a.m., with an interval twice a week for 8 weeks. The natural enemies were identified to the family level. Diversity index (Shannon-Wienner), evenness index, and dominance were calculated for each plant. The diversity index for *Z. elegans, C. sulphureus,* and *T. erecta* fell in the category of medium ranging from 1.328–1.581 with medium evenness (0.365–0.574) and high dominance of 0.314–0.453. Natural enemies associated with *C. sulphureus* and *Z. elegans* were more abundant compared to those associated with *T. erecta*.

Keywords: diversity, dominance, evenness, natural enemy, plant refuge

INTRODUCTION

Rice is a seasonal crop that has a dynamic ecosystem and very vulnerable to changes. This is showed by the pest outbreaks occurred frequently. According to Price (1984), pest outbreaks were partly due to the change in the natural ecosystem with high species diversity into low-diversity monoculture cultivation. Increasing the intensity of pest infestation in rice cultivation can not be separated from the improper use of conventional pesticides. According to Mahrub (1998), the improper use of pesticides reduced the diversity and abundance of arthropod species in the ecosystem of rice and soybean crops. In addition to impacting target insects, pesticides greatly affect natural enemies. The direct impact of the application of sublethal concentration of insecticides caused the mortality of *Anagrus nilaparvatae*, a parasitic wasp of *Nilaparvata lugens*, by up to 100%, decreased the parasitic rate by 76.03% and the adult emergence by 99.67% (Sasmito *et al*., 2017). The application of insecticide reduced the parasitic level of *A. nilaparvatae* through decreasing fecundity and adult longevity (Meilin *et al.*, 2012; Haryati *et al*., 2016).

Because of the negative impact of the improper use of pesticides, the government then instructed a more environmentally friendly pest control strategy called Integrated Pest Management (IPM) (Untung, 2004). One approach in IPM is to maximize the role of natural enemies. One of the efforts is by intensive ecosystem-based control, namely ecological engineering with the planting of flowering plants around the rice cultivation (refuge) (Altieri, 1999; Trisyono, 2015). Refuge provides food sources, shelter or other resources for natural enemies, i.e. predators and parasitoids (Nentwig, 1998; Wratten *et al*., 2004). The presence of refuge plants provides shelter and source of food for various types of natural enemies in an effort to improve ecosystem services (Long *et al*., 1998; Wilkin *et al*., 2016). Furthermore, the presence of refuge can increase the number of natural enemies by 72% and reduce the number of pest populations by 74% (Letourneau *et al*., 2011). According to Sugiharti *et al.* (2018), the flowers of *Turnera subulata* and *Cosmos sulphureus* provided for *A. nilaparvatae* adults increased the fecundity of their offsprings by 45–91%.

The use of refuge is an effective strategy to increase biodiversity in the rice cultivations and species richness in ecosystems (Vandermeer, 1995; Price *et al*., 2011). The type of plant species used in an ecological engineering effort to attract carnivorous arthropods is important (Kaplan, 2012). This is because the volatile compounds of various plants used as decoys have a very wide range of functions. Flowering plants attract the insects using the morphological and physiological characteristics of the flower, i.e. size, shape, color, fragrance, flowering period, and the content of nectar and pollen (Haydak, 1970; Menzel *et al*., 1988). Refuge plants are very important in the efforts to conserve natural enemies in rice cultivation. Therefore, the objective of this study was to determine the diversity level of the natural enemy associated with refuge plants in the rice bunts as an effort to improve ecosystem services.

MATERIALS AND METHODS

This study was carried out on rice fields in the Villages of Jatisarono, Wijimulyo and Tanjungharjo, Nanggulan Regency, Kulon Progo District, Yogyakarta from February to May 2017. The refuge plants used in this study were *Zinnia elegans*, *Cosmos sulphureus* and *Tagetes erecta* planted in the rice bunts with the same rice variety. The sampling technique used was purposive sampling. The refuge plants observed were previously identified to the species level in the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. Observations were conducted on the one row of each refuge plants with an observation plot of 1×1 m at 15 points. Four plants of each species were observed in each observation plot. Observations were conducted between 07.00–10.00 Western Indonesian Time with the observation interval twice a week for eight weeks.

Abundance and Diversity of Natural Enemies in Refuge Plants

Three different samplings were employeed. Observation of natural enemies with pitfall traps was conducted by immersing a plastic container (7 cm in diameter and 10 cm in height) at the same height as the ground. The trap was filled with water mixed with liquid soap (one-third the height of the trap). At each sample point, 1 pitfall trap was installed for 24 hours. The observations of natural enemies using the sweep net were carried out by swinging a net (1 m in length and 50 cm in diameter) for 10 double swings in each sample plot. Direct observation (*in situ*) was carried out by visually observing natural enemies approached the refuge plants and then recorded and photographed them. The aspirator was used to take the tiny insects from refuge plants and then put them into a plastic vial, and were then identified in the laboratory.

Natural Enemies Identification

The identification of natural enemies was performed in the laboratory to the family level using (a) *An Introduction to the Study of Insect* (Borror *et al*., 1992) and (b) *Hymenoptera of the World: An Identification Guide to Family* (Goulet & Hubner, 1993).

Data Analysis

The type and number of natural enemies were analyzed descriptively and quantitatively. The dominance index (D) (Simpson, 1949), Shannon-Wienner diversity index (H') and evenness index (E) were calculated to analyze community differences (Price, 1984; Krebs, 2014).

RESULTS AND DISCUSSION

Structure of Natural Enemies Associated with Refuge

The diversity index (H') for *Z. elegans, C. sulphureus,* and *T. erecta* of the three locations fell in the category of medium ranging from 1.328–1.581 (Table 1). According to Price (1984), if the value of H 'is 1.0–3.322 then the diversity was categorized as medium, where the ecosystem condition is quite balanced with medium ecological pressure. Ecological pressure is also influenced by evenness (E) and dominance (D). The medium diversity in the three refuge plants also influenced the value of evenness index (E) which was categorized as medium as well. However, this was not balanced by the low dominance (D) of the three refuge flowers. The dominance of the three flowers was < 0.6 revealed that there was real dominance hence the population development becomes unbalanced (Krebs, 2014).

Diversity and dominance are negatively correlated. Evenness values tend to be high if the population in one family-level does not dominate the population of other families (Oka, 1995). Furthermore, the diversity and evenness of natural enemies are also influenced by food availability and microhabitat conditions.

Table 1. Structure of natural enemies associated with three refuge plants in Villages of Jatisarono, Wijimulyo, and Tanjungharjo, Nanggulan Regency, Kulon Progo District

Taxon	*Zinnia elegans*	*Cosmos sulphureus*	*Tagetes erecta*
Order	10	10	7
Family	29	38	15
Individual	1115	1582	414
H'	1.581	1.328	1.515
E	0.469	0.365	0.574
D	0.360	0.453	0.314

Arthropod changes, diversity and abundance are in line with the development of the growth and development of the plants as their habitat. The older the plants, the lower the population and composition of the arthropod, because the habitat conditions become less suitable thus lead the insects to migrate to the new habitats or die if they fail to adapt. Therefore, the number of species therein is relatively constant (Mahrub, 1999; Widiarta *et al*., 2006).

The natural enemy that dominates the three refuge flowers was Formicidae (1.014 insects) that may occur due to the competition for food sources. Marc *et al.* (1999) reported that dominant Formicidae could interfere with other natural enemies. Formicidae was found most likely because the soil at the research location was relatively dry and not flooded like in the rice fields, thus the ants prefer to make their nests on that ground. Besides Formicidae, the predator that dominates was Lycosidae (316 insects). Week & Holitzer (2000) also reported that Lycosidae was effective to suppress the pest population, but also had a negative impact on other natural enemies if they were too dominant. This may be because of polyphagous predators have a high ability to adapt, to spread quickly, and be able to switch prey when the amount of the main prey is less.

Natural Enemies Associated with Refuge and their Role

Five families of parasitoids and 25 families of predators were found as the natural enemy associated with *Z. elegans* (Table 2). Parasitoids which were only associated with *Z. elegans* compared to the other refuge flowers were Ichneumonidae and Chalcididae. Ichneumonidae are ectoparasitoid of larvae and pupae of Lepidoptera. After the insect emerged from its host, Ichneumonidae adult will feed flower nectar (Driesche *et al*., 2008). The parasitoid that also associated with *Z. elegans* was Chalcididae. Chalcididae is an endoparasitoid gregarious of pupae of Lepidoptera (Borror *et al*., 1992). The presence of this parasitoid may relate to the presence of Lepidoptera as a host which is one of the important pests in rice cultivation. According to Pathak & Khan (1994), *Scirpophaga incertulas*, *Chilo suppressalis, S. innotata*, and *Sesamia inferens* are important pests of rice spread widely in Indonesia.

Eight families of parasitoids were found associated with *C. sulphureus* (Table 3). A family of the parasitoids associated with *C. sulphureus* from Hymenoptera Order was Mymaridae. For example, *Anagrus* spp., which is an important parasitoid egg of brown planthopper (*Nilaparvata lugens*) in Asia

Table 2. Natural enemies associated with *Zinnia elegans* and their roles in Villages of Jatisarono, Wijimulyo, and Tanjungharjo, Nanggulan Regency, Kulon Progo District

Role	Order	Family
Parasitoid	Hymenoptera	Ichneumonidae, Mymaridae, Braconidae, Eulopidae, Chalcididae
Predator	Coleoptera	Coccinellidae, Carabidae
	Diptera	Conopidae, Sciomyzidae, Syrphidae, Dolichopodidae, Asilidae
	Hemiptera	Reduviidae
	Hymenoptera	Formicidae, Sphecidae, Eumenidae, Vespidae
	Odonata	Libellulidae, Coenagrionidae, Gomphidae
	Orthoptera	Gryllidae, Tettigoniidae, Gryllotalpidae
	Mantodea	Mantidae
	Araneae	Tetragnathidae, Lycosidae, Linyphiidae, Araneidae
	Dermaptera	Forficulidae
	Neuroptera	Chrysopidae

Table 3. Natural enemies associated with *Cosmos sulphureus* and their roles in Villages of Jatisarono, Wijimulyo, and Tanjungharjo, Nanggulan Regency, Kulon Progo District

Role	Order	Family
Parasitoid	Diptera	Bombyliidae, Acroceridae, Tachinidae
	Hymenoptera	Mymaridae, Braconidae, Eulopidae, Proctotrupidae, Scoliidae
Predator	Coleoptera	Coccinellidae, Carabidae, Staphylinidae
	Diptera	Conopidae, Sciomyzidae, Syrphidae,Dolichopodidae, Asilidae, Scenopinidae, Empididae, Tachinidae
	Hemiptera	Reduviidae, Gerridae, Nabidae, Miridae
	Hymenoptera	Formicidae, Sphecidae, Eumenidae, Vespidae
	Odonata	Libellulidae, Coenagrionidae, Gomphidae
	Orthoptera	Gryllidae, Tettigoniidae
	Mantodea	Mantidae
	Araneae	Tetragnathidae, Lycosidae, Linyphiidae, Araneidae
	Dermaptera	Forficulidae

Table 4. Natural enemies associated with *Tagetes erecta* and their roles in the Villages of Jatisarono, Wijimulyo, and Tanjungharjo, Nanggulan Regency, Kulon Progo District

Role	Order	Family
Parasitoid	Hymenoptera	Braconidae, Encyrtidae, Eurytomidae
Predator	Coleoptera	Carabidae
	Hymenoptera	Formicidae, Vespidae
	Odonata	Libellulidae, Coenagrionidae
	Orthoptera	Gryllidae
	Mantodea	Mantidae
	Araneae	Tetragnathidae, Lycosidae, Linyphiidae
	Dermaptera	Forficulidae

(Gurr *et al.*, 2011). Based on research conducted by Zhu *et al.* (2013), *A. nilaparvatae* is attracted by chemical compounds released by *C. sulphureus*. This then indicates that the presence of *C. sulphureus* in rice cultivations has an important role in the presence of brown planthopper egg parasitoids. Thirty families of predators were found associated with *C. sulphureus* (Table 3). There were two families of Araneae Order mostly found, i.e. Lycosidae and Linyphiidae. Linyphiidae is a small spider that is often found at the base of rice stalks. These spiders prey the nymphs of leafhoppers and brown planthopper, and mostly found in the humid place (Shepard *et al.*, 1987). Linyphiidae are often found in the canopy of *C. sulphureus* and its abundance is quite high (39 insects). Because *C. sulphureus* has a dense and lush canopy than the other two refuge.

Predators from Syrphidae Family are only associated with *Z. elegans* and *C. sulphureus*. According to Zborowski & Storey (2003), when Syrphidae lay eggs, they will look for leaves contain a lot of honeydew. Factors influence the behavior of that insect are the availability of pollen and nectar in the flowers. Pollen is an important food for insects including *Syrphidae* (Stanley & Linskens, 1974). Pollen was also able to improve fitness and abundance of insects (Wong & Frank, 2013). Besides Syrphidae, predators from Coccinellidae Family also only associated with *Z. elegans* and *C. sulphureus*. *N. lugens* and *Nephotettix virescens* are prey for Coccinellidae. Adult insects sometimes also eat pollen and nectar from flowers as nutrients for growth and development of eggs in the ovary (Reissig *et al*., 1986). The number of natural enemies associated with *T. erecta* was less compared to *Z. elegans* and *T. erecta*. The only parasitoids visited *T. erecta* were Encyrtidae and Eurytomidae (Table 4). Encyrtidae is an egg and larval parasitoid of Coleoptera, Diptera, Lepidoptera, Hymenoptera, Neuroptera, Orthoptera, and spiders (Driesche *et al*., 2008).

The differences in natural enemies associated with *Z. elegans, C. sulphureus*, and *T. erecta* may be caused by flower morphology, nectar and pollen composition, and volatile compounds. The important morphological factors in obtaining the nectar or pollen are the width and depth of the petals, in relation to the size of the head and mouth structure of the parasitoids (Farell, 2013). *T. erecta* has the largest pollen size (39 µm) compared to the two other flowers (Mazari *et al*., 2012). *Z. elegans* has a smaller pollen size (14.05 µm) than *C. sulphureus* (32.63 µm) (Wróblewska, 2016). Erdtman (1943) stated that the pollen fell in the small category if it has a size ranging from 10-25 µm and medium-size if ranging from 25–50 µm. Therefore, the different flowering plant species will most likely associate with different natural enemies (predators and parasitoids). Because each flower has varying attractiveness and suitability for certain natural enemies (Sivinski, 2011; Farell, 2013). Arthropods found in refuge plants were mostly beneficial arthropods, for example as natural enemies. In addition, many insects may just stay in the plantation to rest and will migrate to another plant or may also stay temporarily to go through one phase of their life cycle. This condition may illustrate that flowering plants are beneficial in efforts to improve ecosystem services of natural enemies in rice cultivation.

CONCLUSION

The diversity index for *Z. elegans, C. sulphureus,* and *T. erecta* fell in the category of medium ranging from 1.328–1.581 with medium evenness (0.365–0.574) and high dominance of 0.314–0.453. Natural enemies were more associated to *C. sulphureus* and *Z. elegans* than that to *T. erecta*.

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