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Original Article

Species composition, population dynamics, and assessment of biopesticide application on thrips (Thysanoptera) in Sacred lotus *Nelumbo nucifera* Gaertn.

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Abstract

Species composition and population dynamics of thrips in sacred lotus, *Nelumbo nucifera* Gaertn, were examined in natural and cultivated areas. The results indicated that three species of thrips (*Scirtothrips dorsalis* Hood, *Selenothrips rubrocinctus* (Giard) and *Frankliniella schultzei* (Trybom)) were present on various parts of the plant. *S. dorsalis* had the widest niche breadth; it was the predominant species and was more abundant in cultivated area than in natural areas. The abundance was related to seasonal variations and farmer's practices. Three biopesticides, namely wood vinegar, azadiractin, and the entomopathogenic fungus *Beauvaria bassiana*, were experimentally found to reduce *S. dorsalis* on lotus. The efficiency against *S. dorsalis* of imidacloprid was not significantly different from that of wood vinegar. The value of Shannon-Wiener diversity index was lower than in the other treatments, but Simpson's index indicated that diversity of the aquatic organism after wood vinegar treatment was highest. Although the efficiency of biopesticides and chemical control did not differ much, from biodiversity perspective the biopesticides are easily biodegradable and do not cause resistance build-up among the pests. Therefore, biopesticides, such as wood vinegar, are alternative methods for thrip control in sacred lotus.

Keywords: *Scirtothrips dorsalis, Selenothrips rubrocinctus, Frankliniella schultzei*, niche, wood vinegar

1. Introduction

Nelumbo nucifera Gaertn (Sacred lotus) is an aquatic plant which is found in wetlands throughout temperate and tropical Asia, including Thailand (La-ongsri, Trisonthi, & Balslev, 2008). *N. nucifera* has a high potential as an economic crop. All parts of *N. nucifera* can be utilized. The seed, stem and rhizome of *N. nucifera* are edible, and can be used in traditional of medicine (Mehta, Ekta, Patani, Patani, & Shah, 2013). Leaves are used for wrapping and cooking. Additionally, the flower has an ornamental value and psychological importance in the Buddhist religion.

Thrip is a sucking insect pest in the order Thysanoptera that has been reported to be an important pest in *N. nucifera* before and after harvest (Bumroongsook & Kilaso, 2018; Seal & Kumar, 2010). Damage caused by direct piercing and sucking by thrip results in severe symptoms such as leaf deformity, deformation of shoots, scarring of the petal, distortions of leaves, discoloration of flowers, and the leaf rolling upward. Such damage directly affects the product quality of *N. nucifera*. However, the species of thrips infesting *N. nucifera* and their population dynamics are poorly understood. Knowledge of their biology and behaviors in feeding, ovipositing, and pupation microhabitat would be very helpful for thrip control.

Insecticides, such as imidacloprid, abamectin, chlorfenapyr, dinotefuran, novaluron, spinosad, spinetoram, thiamethoxam, etc., are the most frequently used method for

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thrip management in many plants (Ludwig & Bográn, 2007; Seal & Kumar, 2010). However, for aquatic ornamental plants, contamination of the water ecosystem with harmful chemicals influencing aquatic animals and water utilization is a restrictive concern. Apart from toxic contamination, overdoses and high frequency of use of pesticides elevates risks of developing pesticide resistance (Loughner, Warnock, & Cloyd, 2005).

Biopesticides are an alternative approach to insect pest management. A biopesticide is defined as a substance from plants and animals that has been used to manage pests in crops (Lengai & Muthomi, 2018). Murray (2006) reported that insecticides extracted from plants have a low risk of insecticide resistance compared with synthetic insecticides. Certain commercial biopesticides, such as wood vinegar, azadiractin, and entomopathogenic fungi, have been reported to control thrips on many crops such as pomelo, peanut, rose, and tea, etc. (Jothityangkoon, Jogloy, & Wongkaew, 2007; Kivett, Cloyd, & Bello, 2016; Roy & Gurusubramanian, 2011; Leibee, Kok-Yokomi, Aristizabal, Arthurs & Morales-Reyes, 2015; Pangnakorn & Chuenchooklin, 2015). Azadirachtin has been used as an insect growth regulator and feeding deterrent with western flower thrip (Kivett *et al.*, 2016; Pearsall & Hogue, 2000), while wood vinegar was used as an insect repellent (Rahmat, Pangesti, Natawijaya, & Sufyadi, 2014). *Beauveria bassiana* is an entomopathogenic fungus that effectively controls thrip species such as *Frankliniella occidentalis* (Pergande) (Gao, Reitz, Wang, Xu, & Lei 2012), *Scirtothrips citri* (Moulton) (Zahn, Haviland, & Stanghellini, 2013), and *S. dorsalis* (Arthurs, Aristizábal, & Avery, 2013) on horticultural crops. However, there is scant information concerning the use of biopesticides against thrips on *N. nucifera* except for the report of Seehavet and Tangkawanit (2019) estimating the median lethal concentrations (LC_{50}) of wood vinegar and azadirachtin on thrip (*Scirtothrips dorsalis*) from lotus in the laboratory. In this study, species composition and population dynamics of thrip in *N. nucifera* were examined, and efficiency of the biopesticides wood vinegar, azadiractin, and *B. bassiana* in sacred lotus fields and their effects on aquatic ecosystems were evaluated. The knowledge from this study will be applied to develop an environmentally friendly method of thrip control in aquatic ecosystems.

2. Materials and Methods

2.1 Species composition and population dynamics of thrip in lotus

This study was conducted in a sacred lotus production area located in Sila district of Khon Kaen province. The thrip population was surveyed twice a month during Febuary 2018 to January 2019 in two cultivated lotus areas (16°29′20″ N/102°51′20″E, 16 °29′14 ″ N/102°52′27″E) and during March 2018 to February 2019 in two natural lotus areas (16 °30 ′11 ″ N/102 ° 52′9″E, 16 °31′18 ″ N/102 ° 51′18″E). Thrips were counted from 20 lotus plants for each area (for each plant one flower of 4–5 cm diameter in size and 1 foliage leaf were counted) by random diagonal sampling. Types of infested leaves (young and old leaves) by thrips species were recorded. The average number of thrips and the identities of thrip species were recorded. Diversity of resources used by thrip species or niche breadth was measured as standardize niche breadth (Hurlbert, 1978) as follows;

$$B_A = \frac{B-1}{n-1} \tag{1}$$

where

B_A = standardized niche breadth

n = number of individuals found in or using resource state

B = Levins' measure of niche breadth

$$B = \sum \frac{1}{pi^2} \tag{2}$$

where

pi = proportion of individuals found in or using resource state (Levins, 1968)

The scale of standardized niche breadth values ranges within 0–1, and 0 means that the species was highly specialized to a resource.

2.2 Efficiency of biopesticides on thrip in lotus plant

2.2.1 Plant preparation

The experiments were conducted in the Entomology Research Area, Faculty of Agriculture, Khon Kaen University. Lotus rhizomes (n=5) were planted in the soil within a cement container 1.2 m in diameter and 50 cm in height. Clay soil was added to approximately 15 cm depth in the pot. Water was added to a level of about 30 cm over the soil. When small leaves emerged from the mud, 15-15-15 NPK fertilizer was applied to the lotus container. The containers were kept outside under natural sunlight conditions. Experiments used lotus plants that were 45–50 days old, or when the 7–8 emerging leaves were held above the water.

2.2.2 *S. dorsalis* rearing

Adults of *S. dorsalis* were collected from a lotus field. Then they were maintained in the Entomology research area, Faculty of Agriculture, Khon Kaen University for artificial outbreak. *S. dorsalis* were inoculated onto the lotus plants (50 days) which were growing in clay pots (50 cm in height and 80 cm in diameter). Each lotus clay pot was covered with a mesh cage (70×70×120 cm). A new lotus plant was transferred to the cage when the symptom of dry and curled leaves occurred. Then, the old plant was removed from the cage when the new one had become infested. After 3 generations the number of thrips was large enough for release in the next experiment to induce an artificial outbreak. Thrips were counted and then transferred to the experimental pot by using a small paintbrush.

2.2.3 Bioassay

The effects of the three biopesticides were separately examined for the predominant species, *Scirtothrips dorsalis*. Biopesticides were evaluated for controlling insects in the lotus plant using a completely randomized design

(CRD) with 4 trials for each treatment. The experimental treatments comprised (1) imidacloprid (Provado®) 0.021 g/l; (2) *Beauvaria bassiana* (Isolates 6241) 5.71×10^{10} conidia/l; (3) wood vinegar (TPIPL®) 4.734 ml/l; and (4) azadiractin (Thai neem 111, 0.1% W/V) 85.822 ml/l; and water was used as control (concentration rates followed LC_{90} as reported by Seehavet and Tangkawanit, 2019). The lotus pot was covered with a mesh cage (90×100×120 cm) and *S. dorsalis* from a mass rearing was released onto the lotus leaves at 50 insects per experimental pot to create an artificial outbreak. After 1 week, the biopesticides were applied once in the evening to prevent the effects of heat from sunlight on entomopathogenic fungi. After application, each lotus pot was covered with a mesh cage to protect against other lotus pests. Insect abundance was randomly recorded daily in the foliage leaf (4 leaves per pot) at 24 hr after each treatment. The means of insect numbers were analysed by Least Significant Difference (LSD) ($P < 0.05$) using Statistix 10 software. The percent reduction of thrips was statistically calculated according to the equation of Henderson and Tilton (1955)

$$\%\, reduction = 100\left(1 - \frac{Ta\times Cb}{Tb\times Ca}\right) \quad (3)$$

where

Ta = population of insect counts after treatment (treatment group),

Cb = population of untreated insect counted before treatment (control group),

Tb = population of insect counted before treatment (treatment group),

Ca = population of untreated insect counted after treatment (control group).

2.3 Effect of pesticides on species richness and diversity of aquatic organisms inhabiting in lotus ponds

The experiment was performed in the field research area of the Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University. Field conditions were simulated in the lotus pond (1.5×4.0×0.5 m). Lotus rhizomes (n=20) were planted in the clay soil under the pond essentially as described in Section 2 with 30 cm of water above the soil and application of lotus fertilizer (15-15-15) to the pond. Experimental units were used after the lotus was 45–50 days old.

The most effective biopesticide from the preceding experiment was evaluated for its effects on organisms (pest, natural enemy, and other) in the aquatic environment and compared with imidacloprid. The treatments were randomly applied to each lotus pond when symptoms of damage by sucking insects were present (feeding scars, wilting and distortion of leaves). Water was used as control. The experiments were conducted with 4 replications for each treatment.

The field survey was conducted 9 days after application of biopesticide. Sampling of insects and other organisms was conducted between 16.00 to 17.00 pm using a random directed count method from lotus plants (4 plants) and with an aquatic sweep net (4 sampling sites in the pond). A total of 4 lotus plants and 4 area points were sampled for each lotus pond. Aquatic insects and other animals were collected by dragging the aquatic sweep net through the substrate of aquatic habitats and then placing all accumulated material into a tray for sorting. All collections were preserved in 70% ethanol and stored in the laboratory, Department of Entomology, Khon Kaen University, before identification of their contents, and classified according to their ecological roles such as plant pest, natural enemy of plant pest, and other species.

Shannon-Wiener (H′), and Simpson's Index (λ) (Simpson, 1949) were used to assess aquatic diversity after the different treatments:

$$H' = -\sum_{i=1}^{s} pi\ln(pi) \quad (4)$$

$$\lambda = \sum_{i=1}^{s} pi^2 \quad (5)$$

where pi is the proportion of individuals belonging to the i^{th} species

3. Results and Discussion

3.1 Population dynamics of thrip in lotus

The study revealed that 3 species of thrip were found in sacred lotus, namely *S. dorsalis*, *Se. rubrocinctus,* and *F. schultzei*. The results showed that these 3 species inhabit different habitat types. *S. dorsalis* was the predominant species in young leaves and flowers. *Se. rubrocinctus* was only present in foliage leaves, whereas *F. schultzei* was present in the flowers (Figure 1). Determination of standardized niche breadth revealed that the widest niche breadth was exhibited by *S. dorsalis* (BA = 0.754), followed by *Se. rubrocinctus* (BA = 0.006) while *F. schultzei* had the narrowest (BA = 0). *S. dorsalis* was the predominant species, and it was found to be more abundant in cultivated areas (34.31 insects/leaf) than in natural lotus areas (12.10 insects/leaf). In contrast to *S. dorsalis*, *Se. rubrocinctus,* was always found in older foliage leaves rather than in younger growth. It was found at higher density in the natural area than in the cultivated area. *F. schultzei* was found to be more abundant in cultivated than in natural lotus area (1.62 and 0.42 insects/flower, respectively). The differentiation of thrips density may relate to the cultural practices of farmers. In the cultivated area, the farmer usually removed older foliage leaves, and therefore the number of *Se. rubrocinctus* was lower than in a natural area as they preferred the old leaves. Resource partitioning of thrip species in lotus plant represents the avoidance of competition. Ananthakrishnan (1993) suggested that resource partitioning of thrip involved interspecific and intraspecific competition for mating and egg laying as has been reported for other insects, such as *Thrips hawaiiensis* and *Elaeidobius kamerunieus* on oil palm (Anggraeni *et al.*, 2013). Griffin and Silliman (2011) suggested that one species may prefer a different part of host plant than another species, allowing them to coexist and help to maintain species diversity.

The abundance of thrips is presented in Figures 2A and B. In the cultivated lotus area, the density of *S. dorsalis* gradually increased during February until late March 2018. During this period, insecticide was applied 2 times for thrip control (Figure 2). After a week, the thrip population

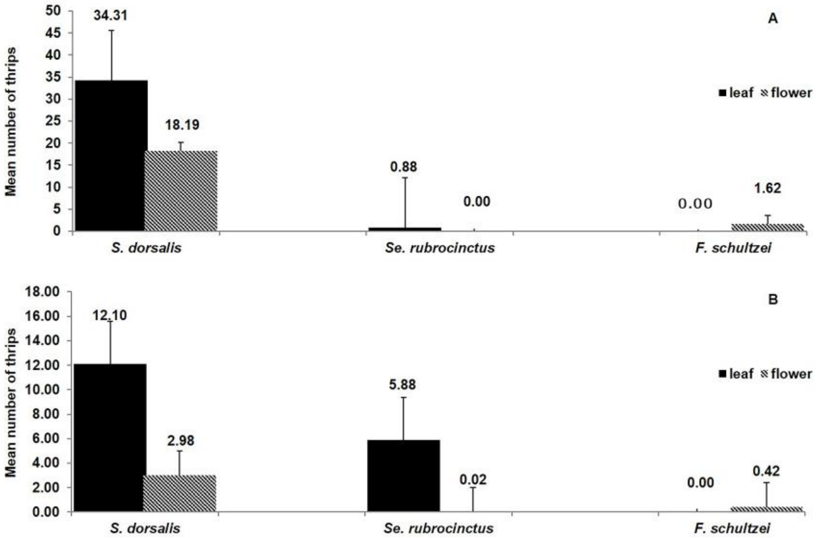

Figure 1. Mean numbers of the thrip species *Scirtothrips dorsalis*, *Selenothrips rubrocinctus*, and *Frankliniella schultzei* found on leaves and flowers of *Nelumbo nucifera* in cultivated areas (A), and in natural areas (B).

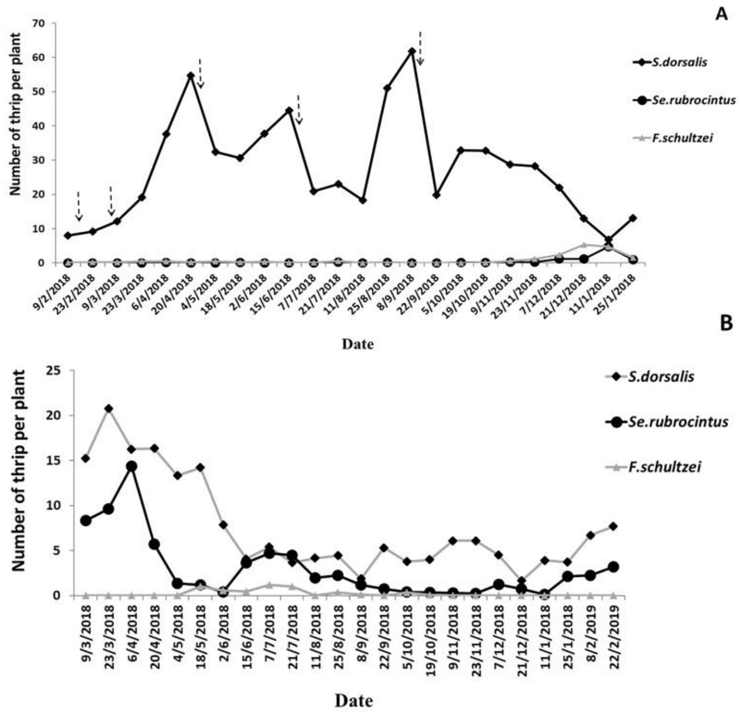

Figure 2. population dynamics of *Scirtothrips dorsalis*, *Selenothrips rubrocinctus*, and *Frankliniella schultzei* on *Nelumbo nucifera* in cultivated areas (A), and in natural areas (B). Arrows indicate insecticide application in the cultivated area.

increased rapidly, and then it fluctuated and tended to decrease because of the application of insecticide during May to August 2018. The highest peak of thrip population was found in September with 61.8 insects/plant. Then, the population began to decrease. The reason for this may involve dormancy of lotus during the short photoperiod occurring during the winter time (Masuda, Urakawa, Ozaki, & Okubo, 2006). The populations of *Se. rubrocinctus* and *F. schultzei* were comparatively low in the cultivated lotus area throughout the whole year of study.

Population dynamics of thrip in the natural lotus area were different than in the cultivated area. *S. dorsalis* slightly increased during March to April 2018, which is a dry season (20.79 insect/plant). Then it slightly decreased until the beginning of June 2018 (rainy season). Then, the population trend was stable (2–5 insects/plant). The different population dynamics of *S. dorsalis* between the 2 types of lotus areas relates to farming management. In cultivated areas, old foliage leaves were cut to promote production of young leaves. Therefore, the population of *S. dorsalis* increased rapidly.

Population dynamics of *Se. rubrocinctus* in the natural area were correspondingly similar to *S. dorsalis*. However, population density of *Se. rubrocinctus* was lower. In contrast to cultivated areas, the population density of *Se. rubrocinctus* in the natural area was high, possibly because of the prevalence of much older leaves in the natural area. The population of *F. schultzei* was low (less than 1 insect/plant). Species and population dynamics of thrips have been reported to vary in composition and size depending on host plant and environmental factors (Aliakbarpour, Che Salmah & Dieng, 2010: Pobozniak, Palacz & Rataj, 2007). Antonio *et al.* (2015) revealed that rainfall was the main abiotic factor exerting a suppressive effect on thrip population. These results were consistent with the report of Bumroongsook (2018), in which *S. dorsalis* was much more abundant in summer than in the rainy season, whereas *F. schultzei* was found most often during April to September.

3.2 Efficiency of biopesticide to *S. dorsalis* in lotus plant

The results revealed that all 4 biopesticide treatments could reduce *S. dorsalis* on sacred lotus (Table 1). However, there were significant differences in the effects of the applied substances. Percent reduction of *S. dorsalis* caused by all 4 substances tended to increase up to 9 days after application. Imidacloprid was the most effective for *S. dorsalis* control in this experiment, followed by wood vinegar, azadiractin and *B. bassiana*, in rank order. Application of imidacloprid reduced *S. dorsalis* by 82.82 % after the first day of application. The results correspond to the results reported by Seehavet and Tangkawanit (2019) in laboratory conditions. Application of *B. bassiana* resulted in the least reduction of *S. dorsalis* among the treatments. This may have resulted from environmental factors such as strain of *B. bassiana*, temperature, sunlight and humidity, etc. (Joronski, 2010). Lengai and Muthomi (2018) revealed that although the experimental tests produce excellent results, there are always inconsistencies in the field due to short shelf life of *B. bassiana* and some environmental factors. Although activity of wood vinegar against *S. dorsalis* was not significantly different from that of imidacloprid, there were some effects on the lotus leaves. It was observed that splashing of wood vinegar solution caused brown spots on upper surfaces (but did not affect the lower leaf surface that experienced direct application of wood vinegar to thrip). The application of high doses of wood vinegar under field conditions needs care in order to avoid plant damage.

3.3 Effects of pesticides on species richness and diversity of aquatic organism inhabiting in lotus pond

The insecticidal activity of wood vinegar against *S. dorsalis* was not significantly different from activity of imidacloprid. Therefore, wood vinegar was selected for diversity impact assessment in field conditions. After nine days of insecticide and wood vinegar application, the results indicated that the number of natural enemies of plant pest species (predators and parasitoids) in wood vinegar application was highest, followed by the control and imidacloprid treatments in rank order (Figure 3). The numbers of pest species and other species were similar among the treatments. This suggested that wood vinegar was less toxic to the natural enemies than imidacloprid. Diversity indices of aquatic animals found in the treatments were compared, see Table 2. The highest Shannon diversity index was found with wood vinegar treatment (H′ = 1.778) followed by control and imidacloprid in this order. These results agree with Arfan, Anshary, Basri & Toana, (2018) who found that the Shannon-Wiener (H') diversity index in red onion ecosystem was higher with no insecticide application compared to a chemical treatment.

The high Simpson’s Index indicates a high probability that two individuals randomly selected from the sample will be of the same species. Therefore, aquatic organisms in wood vinegar treatment were more species rich and had greater evenness than in the other treatments. However, the indexes for different treatments did not much differ from each other. The results agree with Amali, Peña, Duncan Leavengood and Koptur (2009) who reported there

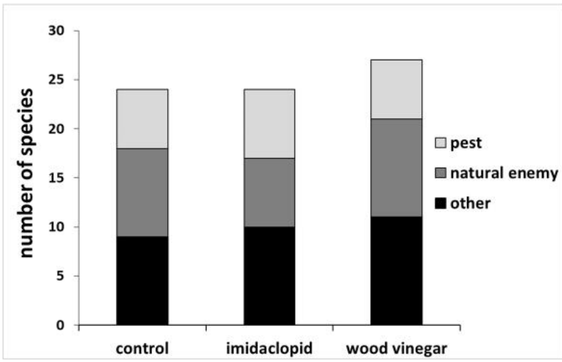

Figure 3. Numbers of aquatic species from sacred lotus ponds by treatment (control, imidacloprid, and wood vinegar)

Table 1. Bioefficacy of insecticides and biopesticides against thrips (*Scirtothrips dosalis* hood) in sacred lotus (*Nelumbo nucifera* Gaerth)) on days 1, 2, 3, 5, 7 and 9 after treatment

Treatment	Conc. (%) dosage	Mean percent reduction of thrip population[1/]					
		1 day	2 day	3 day	5 day	7 day	9 day
1. imidacloprid	0.021 g/l	82.82a	93.91a	86.36a	93.73a	97.39a	95.28a
2. Wood vinegar	4.734 ml/l	66.48a	77.12a	84.08a	85.32ab	84.53ab	87.23ab
3. Azadiractin	85.822 ml/l	65.95a	76.95a	75.05a	77.55b	74.44bc	83.71ab
4. *Beauvaria bassiana*	5.71 x 10^{10} (conidia/l)	23.56b	46.93b	50.60b	59.62c	66.67c	74.38b
F-Test	-	**	**	**	**	*	*
CV (%)	-	20.64	17.35	19.07	11.39	13.78	11.78

1/ values followed by the same small letter within a column are not significantly different (P≥ 0.05, according to LSD)

Table 2. Diversity indices of aquatic organisms 9 days after pesticide application.

Index	Treatment		
	Control (water)	Imidacloprid	Wood vinegar
Shannon - Wiener	0.521	0.508	0.427
Simpson's Index	1.243	1.304	1.154

were more arthropod taxa in a biologically treated area than in a chemically treated area. Apart from biodiversity loss, contaminating substances or residues of pesticide may be taken up by non-target aquatic organisms and accumulate in their tissues (bioaccumulation). Chemical substances are passed through the food chain to the next consumer in the ecosystem, which will have a higher concentration of substance than the source (enrichment known as biomagnification) (Zenker *et al.*, 2014). Although the efficiencies in thrip control of biopesticides and chemical control were not much different, from an environmental perspective the biopesticides are easily biodegradable and do not cause resistance build-up among pests. Therefore, wood vinegar can be applied in crop systems by rotating it with a chemical pesticide product for reducing pesticide residues and for delaying the development of resistance in the pests.

4. Conclusions

Thrips species were found to inhabit different parts of sacred lotus. *S. dorsalis* inhabited young leaves and flowers. *Se. rubrocinctus* utilized foliage leaves, and *F. schultzei* was found in flowers of sacred lotus. *S. dorsalis* had the widest niche breadth and it is the predominant species in sacred lotus. Thrip was abundant throughout the year, especially during the hot and dry season from March to May. The efficiency against *S. dorsalis* of wood vinegar was not significantly different from that of imidacloprid, followed by azadiractin and *B. bassiana* in this order. The diversity index indicated that diversity of aquatic organisms after wood vinegar treatment was the highest among the alternative treatments. Biopesticides such as wood vinegar are possible methods for thrip control in sacred lotus.

Acknowledgements

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