THE BIOACTIVITIES OF SELECTED PIPERACEAE AND ASTERACEAE PLANT EXTRACTS AGAINST BROWN PLANT HOPPER (*Nilaparvata lugens* Stål.)

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ABSTRACT

Brown planthopper (*Nilaparvata lugens*; BPH) is one of major insect pests of rice and farmers often resort to synthetic insecticides to control this pest. Intensive and excessive use of synthetic insecticides cause several deleterious effects to human, beneficial organisms as well as the environment. Therefore, an alternative control strategy of BPH which is environmentally friendly and safer than the synthetic insecticides should be developed, such as botanical insecticides.This research sought to study mortality, feeding inhibition, and oviposition deterrent activities of *Piper retrofractum, P. crocatum, Chromolaema odorata, Tagetes erecta, Tithonia diversifolia* and *Ageratum conyzoides* extracts against BPH. Probit analysis was used to estimate LC_{50} and LC_{95} values of the extracts. *P. retrofractrum* extract exhibited highest mortality compared to the other extracts with LC_{95} values of 0.71%, followed by *T. erecta* 3.28%. The extract of *P. retrofractum* also indicated the highest feeding inhibition of 86.40% at LC_{75} value, which gradually decreased on *T. erecta, T. diversifolia*, *P. crocatum, A. conyzoides*, and *C. odorata* extracts were 72.68, 71.92, 68.89, 68.25, and 65.34%, respectively. The treatment of *P. retrofractum* extract at LC_{75} level caused the highest oviposition deterrence against BPH at 80.40%. All of the above results indicate that *P. retrofractum* extract has great potential to be developed as a botanical insecticide for controlling BPH.

Key words: botanical insecticide, feeding inhibition, mortality, oviposition deterrence

INTRODUCTON

Brown planthopper (BPH) (*Nilaparvata lugens* Stål.;Hemiptera: Delphacidae) is one of the major rice insect pests in Asia (Zheng et al. 2014). This insect frequently causes heavy damage on rice cultivation called hopperburn. This insect is also known as a vector of grassy stunt and ragged stunt viruses (Cabauatan et al. 2009). In Indonesia, rice plant damage caused by BPH occurs throughout the year, along with the continuous rice plant cultivation in every season (Baehaki 2014). Farmers commonly used synthetic insecticides to control the pest, however, most rice farmers (90%) improperly sprayed the insecticides and majority (71%) used unrecommended insecticides (Baehaki

2014). Intensive and excessive use of synthetic insecticides cause several deleterious effects to human, beneficial organisms, the environment as well as cause the occurrence of resurgence and resistant insect pest populations (Basanth et al. 2013). In Asia, the BPH population became resistant to imidacloprid, fipronil, and ethiprole insecticides (Nakao 2017). Therefore, an alternative control strategy of BPH which is environmentally friendly and safer than the synthetic insecticides should be developed. One of the alternatives are botanical insecticides which are more biodegradable and selective (Scott et al. 2003; Sparks and Nauen 2015).

Plant families of Meliaceae, Annonaceae, Asteraceae, Piperaceae, and Rutaceae are considered to be potential sources of botanical insecticides (Isman 1995). Among the Piperaceae members, a Javanese chili (*Piper retrofractum*) has been studied to be one of the most promising botanical insecticides (Isman 2014). The fruit extract of *P. retrofractum* suppressed the population of green stink bugs *Nezara viridula (*Hasnah and Rusdy 2015). Among Asteraceae plants, Siam weed (*Chromolaena odorata*), tree marigold (*Tithonia diversifolia*), and big marigold (*Tagetes erecta*) have been recommended as botanical insecticides in many tropical countries. Other studies showed that *C. odorata* leaf extract has insecticidal activity against *Periplaneta americana* (Udebuani et al. 2015), *T. diversifolia* leaf extract is toxic and has oviposition deterrent effects on the cowpea beetle (*Callosobruchus maculatus*) (Green et al. 2017) and has phagodeterrent effect on *Bemisia tabaci* adults (Bagnarello et al. 2009). *T. erecta* flower extract was found toxic to *Spodoptera frugiperda* (Sánchez et al. 2012). Bioactivities of these plants against BPH have not been studied intensively.

This research sought to evaluate the toxicity, feeding inhibition, and oviposition deterrence activities of selected Piperaceae and Asteraceae plant extracts against BPH.

MATERIALS AND METHODS

Insect and plant sources. The BPHs were obtained from Center for Rice Research of Indonesia, Muara, Bogor and mass-reared on rice cv. Ciherang in the Laboratory of Insect Physiology and Toxicology of the Department of Plant Protection, Bogor Agricultural University. Six different plant species; *Ageratum conyzoides* (leaves), *Chromolaema odorata* (leaves), *Tithonia diversifolia* (flowers), and *Tagetes erecta* (flowers) (Asteraceae), *Piper crocatum* (leaves), and *P. retrofractum* (fruits) (Piperaceae) were collected from Central and East Lampung Regencies, Lampung, Indonesia.

Rice plant seedling preparation. Seeds of rice plant cv. Ciherang were sown in plastic trays (35 cm in diameter and 9.0 cm in height). After 21 days, three rice seedlings were each transferred to plastic pots (30 cm in diameter and 20 cm in height) containing soil and green manure compost (1:1, w/w). Seven weeks old rice seedlings were used for mass rearing of BPH while other rice seedlings were used for bioassays.

Mass rearing of BPH. Five pairs of adult BPH (3 days old) were infested into rice plants covered with a cylindrical insect cages made of mica plastic (30 cm in diameter, 75 cm high). The second and fourth instars of nymphs and adults BPH were used for bioassays.

Plant extract preparation. Each plant material was cut into small pieces, air-dried for 7-14 days and ground using a blender to get plant powders. Crude plant extracts were obtained using maceration method by soaking for 24 hrs each of 200 g of *P. crocatum* and *P. retrofractum* fruit powders within 2 L ethyl acetate (Indriati et al. 2015), *T. erecta* flower powder in 2 L ethanol (Sánchez et al. 2012), and *C. odorata*, *A. conyzoides* leaves, and *T. diversifolia* flower powders in 2 L methanol (Bernard et al. 2012). The soaked plant material was stirred with a magnetic stirrer, filtered and the filtrate evaporated using a rotary evaporator at 50 $^{\circ}$C at a pressure of 400-450 mm Hg, until a crude extract was obtained. Each crude plant extract was then refrigerated at 4 $^{\circ}$C before use.

Preliminary contact toxicity assays. The toxicity of the plant extract was tested against the second instar BPH nymphs. The preliminary assay was conducted to determine a range of concentrations of extracts that caused 5-99% mortality. Five concentrations of the crude extract diluted in solvent (same solvent when extraction of plant materials) were 0.125, 0.25, 0.5, 1.0, 2.0 %, and control (solvent without extract) were prepared for bioassay (Dadang and Prijono 2011). Stock solutions were prepared by weighing the crude extract and diluting with a solvent system containing 0.2% emulsifier (Tween 80) and 1% of organic solvent and making up to volume with distilled water. This was subsequently was stirred using a magnetic stirrer at 750 rpm for 30 minutes.The organic solvent used for the test solution was the solvent that was used in the extraction process.

Ten BPH nymphs were introduced in a cylinder plastic cage (6 cm in diameter, 20 cm in height) and sprayed with 0.4 ml of each test solution using a small hand sprayer. The treated BPHs, were transferred to rice seedlings which were planted in a plastic pot. Mortality was assessed at 24, 48, 72, and 96 hours after treatment. The LC values of the plant extracts were determined using probit analysis (Finney 1997).

Advanced contact toxicity assays. Based on the preliminary test results, further bioassays of each extract were conducted by using the solutions representing the LC_{15}, LC_{35}, LC_{55}, LC_{75}, and LC_{95} values.

Feeding inhibition test. Fourth instar BPH nymphs were assayed using the honeydew collection method on filter paper (Paguiaet al. 1980). The concentration of each extract solution used for the treatment was equivalent to LC_{25}, LC_{50}, and LC_{75} values. Preparation of extract solution was made similar to the preparation on the mortality test described above.

Three rice seedlings (21 days old) were dipped in each test solution for 10 seconds, and then planted in a plastic pot as described before. One piece of plastic petri dish (9 cm in diameter), that had a hole (1 cm in diameter) in its center, was placed on the top surface of the plastic pot. A sheet of Whatman filter paper no.41 was sprayed with 0.3 ml of 0.1% ninhydrin (2,2-dihydroxyindane-1,3-dione) in acetone, air-dried and was layered above a petri dish. The planted rice seedlings were then covered with a transparent plastic cup (400 ml) with few holes on top for aeration. Five fourth instar BPH nymphs were released into the rice seedlings and allowed to feed for two days (Fig. 1). Each treatment was replicated five times.

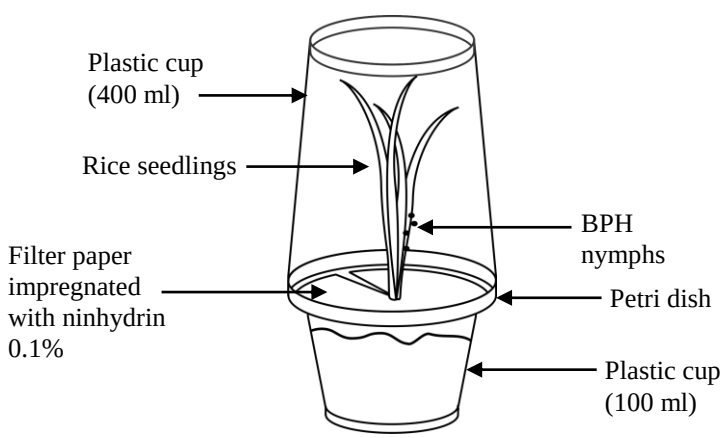

Fig. 1. Feeding inhibition set-up for BPH

The splash of honey dew excreted by BPH is absorbed on the filter paper and reacts with ninhydrin to produce a purple spot (Paguiaet al.1980; Ito et al.1994). The area of the purple spots on the filter paper were qualitatively measured. The percent feeding inhibition was calculated using the following formula:

$$PD = \frac{HC - HT}{HC} \times 100\%$$

PD = percent feeding inhibition
HC = total area of spots on control treatment
HT = total area of spots on crude extract treatment

The intensity of feeding inhibition was categorized based on Mokodompit et al. (2003), and is shown in Table 1.

Table 1. The categories of feeding inhibition of BPH

Categories	Percent feeding inhibition range
Very high	$X \geq 80\%$
High	$60\% \leq X < 80\%$
Medium	$40\% \leq X < 60\%$
Low	$0\% \leq X < 40\%$
No inhibition	$X = 0$

Oviposition deterrence test. The oviposition deterrence of the plant extracts was tested against the adult BPH on the rice seedlings. The concentrations of crude plant extract used were equivalent to the LC_{25}, LC_{50}, and LC_{75} values. The preparation of extract solution was the same as the method described in the preparation of the solution in the mortality test. Three rice seedlings (35 days old) were dipped for 3 minutes in the extract solution.The treated seedlings are planted in a soil medium placed in a plastic cup (250 ml), then covered with a cylinder plastic cage (7 cm in diameter and 20 cm in height). Two pairs of adult BPH were released inside the plastic cage and allowed to oviposit on the rice seedling for 48 hours. The presence of eggs was observed by dissecting the epidermal tissue of rice straw and then the number of eggs was counted under a binocular microscope. The percentage of the oviposition deterrence was calculated using the following formula (Pavela, 2009):

$$PA\ (\%) = \frac{NC - NT}{NC} \times 100\%$$

Where, PA = Percentage of oviposition deterrence (%)
NC = Number of eggs laid on control treatment
NT = Number of eggs laid on crude extract treatment

All the experiments were planned using a complete randomized design. Data were statistically analyzed using the One-way ANOVA and the means were compared using the Tukey's Test.

RESULTS AND DISCUSSION

Contact toxicity. All plant extracts caused different mortality levels by contact on the BPH nymphs. *P. retrofractum* extract was the most toxic. Treatment of *P. retrofractum* extract resulted in the lowest LC_{50} and LC_{95} values against BPH nymphs by 0.07% and 0.71%, respectively followed by *T. erecta* extract treatment by 0.20% and 3.09%, respectively (Table 2).

Table 2. Toxicity of Piperaceae and Asteracae plant extracts against BPH nymphs

Plant extract	$a^a \pm$ SE	$b^b \pm$ SE^c	LC^d_{50} (CI^e 95%) (%)	LC_{95} (CI 95%) (%)
T. diversifolia	0.63±0.99	1.83±0.25	0.45 (0.33-0.57)	3.57 (2.41-6.76)
T. erecta	0.93±0.10	1.37±0.25	0.20 (0.06-0.35)	3.09(1.77-15.19)
C. odorata	0.43±0.94	1.84±0.24	0.62(0.43-0.74)	5.56 (3.11-8.49)
A.conyzoides	0.61±0.96	1.85±0.25	0.50 (0.34-0.59)	4.24 (2.49-6.63)
P. crocatum	0.29±0.89	1.54±0.23	0.65 (0.45-0.85)	7.52 (4.57-17.67)
P.retrofractum	1.89±0.22	1.68±0.25	0.07 (0.03-0.11)	0.71(0.41-2.52)

[a]a= intercept of probit regression line, [b]b=probit regression slope, [c]SE: Standard Error. [d]LC: Lethal Concentration, [e]CI: confidence interval

P. retrofractum extract showed toxicity against *H. antonii* adult with the LC_{95}value of 0.49% (w/v) (Indriati 2015). *P. retrofractum* fruit extract was toxic against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (Subsuebwong et al.2015) and is known to contain piperin, piperiside, and retrofractamide A which have neurotoxin activity with a quick knockdown effect (Scott et al. 2008; Musthapa and Gumilar 2016). This present study demonstrated that *P. retrofractum* extract has a high potential to be developed as a botanical insecticide to control BPH nymphs.The second lowest LC_{50} and LC_{95} values were shown by the treatment of *T. erecta* extract, i.e. 0.20% and 3.09%, respectively. According to (Sánchez *et al*. 2012) ethanolic extracts of *T. erecta* and *T. diversifolia* were toxic against *S. frugiperda* and *T. diversifolia* larvae. *T. diversifolia* flower extract with topical application method has insecticidal activity against *Sitotroga cereallea (*Lepidoptera: Gelechiidae) (Fouad et al. 2014). *T. diversifolia* flower extract contains several secondary metabolite compounds, including glycosides, tannins, flavonoids, saponins, and alkaloids (Essiett and Unung 2013). From this result, the contact mortality activity of *T. diversifolia* to BPH may be the first report.

Feeding inhibition activity. Piperaceae and Asteraceae test plant extracts inhibited feeding activity of BPH nymphs. *P. retrofractum* extract at LC_{75} resulted in the highest feeding inhibition by 86.40%, followed by *C. odorata* (70.63%), *P. crocatum* (70.32%), *T. diversifolia* (68.25%),*T. erecta* (60.61%), and *A. conyzoides* (53.88%) (Table 3). The percentage of feeding inhibition increased with increasing the plant extract concentration. *P. retrofractum* extract at LC_{75} gave the highest feeding inhibition by 86.40% and is categorized as strong feeding inhibition. This might be associated with the presence of alkaloid, flavonoid, and tannin compounds. *P. retrofractum* fruit extract contains piperidine alkaloid, piperoctadecalidine, together with three known alkaloids, i.e. piperine, pipernonaline, and guineensine (Ahn et al. 1992). Many alkaloid compounds have strong feeding inhibition activity against various insects. The high feeding inhibition activity may cause decrease in fitness of insects and consequently may decrease insect fertility. Feeding inhibition was also demonstrated by other plant extracts with varying percentages. *C. odorata*, *T. erecta* and *T. diversifolia* extracts showed lower feeding inhibition activity than *P. retrofractum* extract. Although, Asteraceae plants have been known to contain alkaloid, flavonoid, and tannin compounds, these plant extracts caused medium feeding inhibition only at LC_{75}. The concentration and the type of compounds influence the strength of feeding inhibition activity.

Several alkaloids and flavonoids with stomach poison action may affect the digestive system of insects. Some sesquiterpene compounds from Asteraceae plants, such astagnitin A, B, C, and F, tirotudin, tithonine, and sulphurein, showed feeding inhibition activity (Mwanauta et al. 2014). These compounds are also capable of inhibiting the taste receptors in the insect mouth, making the insect unable to recognize the food and eventually starve to death. Tannins are components that serve as

plant defense against insects by blocking food digestion. by binding with digestive proteins, which is necessary for growth, thus causing impaired protein absorption (Yunita et al. 2009).

Table 3. Feeding inhibition activity of Piperaceae andAsteraceae plant extracts on BPH nymphs

Treatment (%)	Feeding inhibition(%) ± SE	Category
P. retrofractum		
LC_{25} (0.03)	30.67±7.38	Very weak
LC_{50} (0.07)	50.98±11.72	Weak
LC_{75} (0.18)	86.40±5.87	Strong
P. crocatum		
LC_{25}(0.19)	18.26±4.39	Very weak
LC_{50}(0.71)	40.68±8.31	Weak
LC_{75} (2.58)	70.32±10.74	Medium
T. diversifolia		
LC_{25} (0.19)	25.39±10.64	Very weak
LC_{50} (0.45)	46.58±15.24	Weak
LC_{75} (1.05)	68.25±9.61	Medium
T. erecta		
LC_{25} (0.06)	30.34±5.09	Very weak
LC_{50} (0.21)	46.62±5.92	Weak
LC_{75} (0.63)	60.61±6.82	Medium
A. conyzoides		
LC_{25} (0.21)	30.93±9.98	Very weak
LC_{50} (0.50)	40.28±8.84	Weak
LC_{75} (1.21)	53.88±4.77	Weak
C. odorata		
LC_{25} (0.71)	5.72±5.82	Very weak
LC_{50} (1.81)	33.86±5.32	Very weak
LC_{75}(4.61)	70.63±10.73	Medium

Oviposition deterrence activity. Piperaceae and Asteraceae plant extracts could cause oviposition inhibition activity against BPH. *P. retrofractum* extract at LC_{75} showed the highest oviposition deterrence activity by 80.40% against BPH (Fig. 2).This might inhibit the enzyme activities which decompose toxic compounds into cells. Therefore, the toxic compounds accumulate in the insect digestive system and distrupt the physiological processes, such as the insect reproduction (Scott et al. 2008).

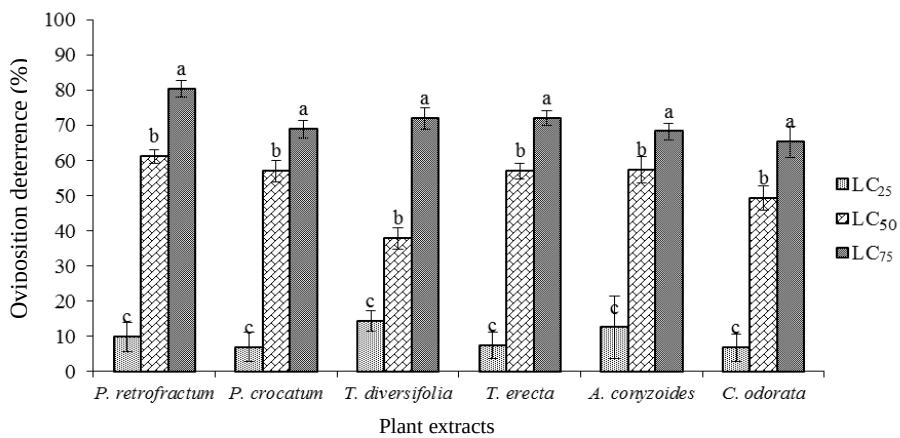

Fig. 2. Oviposition deterrence of BPH treated with six plant extracts at three concentrations

These results show that percent deterrence increased with the increase of extract concentrations. *Murraya koenigii* leaf extract suppressed oviposition of *Spodoptera litura* (Senrung et al. 2014), *Clerodendrum infortunatum* leaf extract caused repellent and deterrence activities against the pulse beetle (*Callosobruchus chinensis*) (Valsala and Gokuldas 2015). *T. minuta* had significant effect on reducing the fecundity of aphids (*Brevicoryne brassicae*) along with the increase of crude extract concentration (Phoofolo et al. 2013). Female adults have sensory receptors that are sensitive to host plant biochemical components for determining oviposition suitability of host plants (Chen et al. 1999).

P. retrofractum extract is the most bioactive plant extract and can be considered for development as a botanical insecticide for controlling BPH. Further research is necessary to improve the efficacy and stability of the extract by improving the formulation.

CONCLUSION

Piper retrofractum (Piperaceae) and *Tagetes erecta* (Asteraceae) plant extracts showed a high mortality activity against BPH nymphs. *P.retrofractum* extract also showed highest feeding inhibition and oviposition deterrent activities by 86.40% and 80.40% inhibition, respectively at LC_{75}. The study implies that *P. retrofractum* extract can be considered with the most potential to be developed as a botanical insecticide for controlling BPH.

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