The toxicity and effectiveness of nanoemulsion formulas from *Piper retrofractum* essential oil against brown plant hopper (*Nilaparvata lugens* Stål)

N S P Nuryanti [1], Jurayrah[1], L Budiarti [1]

[1] Department of Food Crops Production, Politeknik Negeri Lampung, Jalan Soekarno Hatta 10, Lampung, Indonesia

Email: siluhputu@gmail.com

Abstract. Secondary metabolite compounds of plants have a role as a self-defence against herbivores and pathogens attacks. The objective of research was to study the toxicity, persistence, and effectiveness of extracts and essential oils of *Piper retrofractum* against brown plant hopper (BPH). Plant extracts were obtained through maceration method using ethyl acetate solvent for *P. retrofractum* fruit and essential oils obtained by distillation. Extract toxicity testing uses the contact application method. Polo PC program was used to estimate the LC50 and LC95 value. The results showed that essential oil nanoemulsion from *P. retrofractum* was the most toxic against BPH nymphs compared to other treatments. The toxicity values from nanoemulsion of essential oils, maceration extracts, and non-nano formula from *P. retrofractum* extracts 0.19, 0.26, and 0.81 percent respectively. The persistence of the three formulas to BPH nymphs seen starting from one day after the application the average mortality decreased to less than 45 percent. The effectiveness showed that the nanoemulsion from extract and essential oil of *P. retrofractum* were the most effective use to control BPH nymphs in the green house. The results indicated that extracts and essential oils nanoemulsion of *P. retrofractum* is the most promising as a botanical insecticide to BPH.

1. Introduction
Plants use a variety of defences to deal with herbivorous attacks, one of which is by producing secondary metabolites [1]. Secondary metabolites found in plants vary, both in type and concentration. The effectiveness of secondary metabolites as botanical insecticides is influenced by the type and parts of plants used, the place of extracting plant material, the way plants are handled, the type of solvent used, and the method of extraction [2]. Secondary metabolites can be obtained using various extraction methods including simple maceration using organic solvents [3, 4]. Secondary metabolites can also be obtained by distillation, the results are called essential oils. Mostly, essential oils from plants are widely used in pharmaceutical, sanitary, cosmetics, agriculture and food industries for their bactericidal, virucidal, fungicidal, antiparasitical and botanical insecticidal [5].

Plant secondary metabolites can be developed and formulated as botanical insecticides. The botanical insecticides is an alternative control strategy of BPH which is environmentally friendly and safer than the synthetic insecticides [2, 6]. The botanical insecticides can have various effects on the behaviour of

insects (feeding and oviposition deterrent activities), insect physiology (growth and development of insects), and several other secondary metabolites influence indirectly through organisms from other tropics [7-8].

Some plant families such as *Meliaceae, Piperaceae, Annonaceae, Asteraceae*, and *Rutaceae* can be used as sources of botanical insecticides [9]. One of the Piperaceae members known to have activity as a botanical insecticide is the Java chili *Piper retrofractum*. This fruit extracts are reported to contain piperamide compounds that are insecticides [10] such as guininsin, pelitorin, pipericides, piperin, and retrofractamide A. The extract is known to have activity to control *Helopeltis antonii* [11], *Culex quinquefasciatus* larvae, and *Aedes aegypti* [12], brown planthopper nymphs. However, bioactivity of botanical insecticides from *P. retrofractum* nanoemulsion formulas on brown plant hopper (BPH) has never been reported, so testing is needed for the purpose of controlling BPH in the field.

The research was aimed to study the toxicity, persistence and effectiveness of *P. retrofractum* nanoemulsion formulas against BPH. The selection of brown planthopper as a target pest because the pest is the most damaging in rice plantation in South-East Asia.

2. Materials and methods

2.1. Mass rearing of BPH
Five pairs of BPH adult (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 instars of BPH nymphs were used for bioassays.

2.2. Maceration extract method
The *P. retrofractum* fruits was cut into small pieces, air-dried for 14 days and ground using a blender to get plant powders. Crude plant extracts were obtained using maceration method by soaking for 24 hour, 200 g of *P. retrofractum* fruit powders within 2 L ethyl acetate [11]. The soaked plant material was stirred with a magnetic stirrer, the filtrate evaporated using a rotary evaporator at 50 ºC at a pressure of 400-450 mm Hg, until a crude extract was obtained. The crude plant extract was then refrigerated at 4 ºC before use. The distillation follow the Patil's *et al*. methods [13].

2.3. Emulsification method
The nanoemulsion formulation was made using low-energy emulsification method by phase inversion. The emulsification method was performed by [14] with a slight modification, i.e. adding the water phase into the organic phase little by little. The formulation consisted of *P. retrofractum* extract and emulsifier (1:1; v:v) were added to formulation. The *P. retrofractum extracts* or essential oil was added with an emulsifier (Tween-80) and then homogenized by using a magnetic stirrer at 750 rpm for 30 minutes. The emulsion formation was carried out by dropping water at a rate of 4 mL min^{-1} while stirred by a magnetic stirrer at 1250 rpm for 60 minutes. The emulsification was performed at a room temperature (< 29º C).

2.4. Formulas toxicity assay to BPH nymphs
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 emulsifier and aquades were 0.125, 0.25, 0.5, 1.0, 2.0 %, and control (aquades without extract) were prepared for bioassay [15]. 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 [16].

2.5. Formulation persistence test
The testing method used was residue on rice seedlings. The 21 DAS rice seedlings were planted in a plastic cup (v = 250 ml) which already contained planting media, then sprayed with each treatment as much as 0.5 ml per pot evenly using a hand sprayer (20 ml volume). Then the rice plants were placed in an open room for 7 hours from 8.00 a.m to 03.00 p.m. Then the rice seedlings were given a mica plastic lid (d = 7 cm, h = 20 cm), at the top covered with a leotard cloth. Every day from H0 (the day after spraying) to H +3 (three days after spraying). Each treatment was infested with 50 second instar of BPH nymphs. Observations were made at 24, 48, and 72 hours after treatment. The persistence value is seen by calculating the mortality of BPH nymphs after 24, 48, and 72 hours after infestation.

2.6. The effectiveness assay
The formulation tested for the effectiveness assay was same with the persistence assay. The parameters observed were the number of BPH nymphs still alive in each treatment. The effectiveness of the insecticide tested was calculated by the Abbott formula [17]:

$$EI = \frac{Ca - Ta}{Ca} \, x \, 100\%$$ (1)

EI = the effectiveness of insecticides formulas (%)
Ca = the number of BPH in the control plot
Ta = amount of BPH in the treatment plot after insecticide application.

3. Results and discussion

3.1.   Toxicity of formulas
All formulas from secondary metabolites of *P. retrofractum* caused different mortality levels by contact on the BPH nymphs. Treatment nanoemulsion from essential oils of *P. retrofractum* resulted the lowest LC_{50} and LC_{95} values against BPH nymphs by 0.02% and 0.19% respectively. The highest by non-nanoformula treatment by 0.09% and 0.81%, respectively. This shows that the nanoemulsion formulation of *P. retrofractum* from essential oil is the most toxic formulas among the others formulas. According to [18], the advantage provided by nanotechnology includes the unique functional properties of its particles, that is nanoparticle size with the much larger surface area and higher mass than those of non-nanomaterial, therefore it allows bigger chance to contact and penetrate the target. Hence, it can improve the efficacy and effectivity of the active compounds. The treatment of the masteration extract and essential oil extract from *P. retrofractum* as a treatment was formulated and characterized by [19] in the nanoemulsion formulation by producing particle size <200 nm.   *P. retrofractum* secondary metabolites were reported to contain piperamide compounds which are insecticides such as guininsin, pelitorin, pipericides, piperin, and retrofractamide A. Some researchers reported three types of isobutyl amide isolated from Piper nigrum namely pipericide, pelitorin, and piperin which are toxic to *Callosobrucus chinensis* each with LD_{50} values 0.15, 2.0, and 20 µg /male [10, 20, 21]. Guineensin which was isolated from *P. guineense* also had a similar activity with pypericides when tested topically on *Callosobruchus maculatus* with an LD50 value of 0.25 µg / male. According to [22], extracts containing isobutyl amide mixtures have very effective insecticidal activity. Piperamide from Piperaceae has the potential as an insecticide that works as a nerve poison with a rapid knockdown effect [23].

Table 1. Probit regression parameter prediction on the correlation of concentration of nanoemulsion formulas of *Piper retrofractum* to the mortality of brown panthopper nymphs observed at 48 hours after the treatment.

Formulas Treatment	$a^a \pm GB$	$b^b \pm SE^c$	LC_{50}^d (CI^e 95%) (%)	LC_{95} (CI 95%) (%)
Non-nano Formula	1.79 ± 0.28	1.71 ± 0.32	0.09 (0.05-0.12)	0.81 (0.49-2.25)
Nanoemulsion from ME of *Piper retrofractum*	1.89 ± 0.10	1.37 ± 0.25	0.07(0.04-0.09)	0.26 (0.17-1.22)
Nanoemulsion from EO of *Piper retrofractum*	2.22 ± 0.30	1.30 ± 0.29	0.02 (0.01-0.04)	0.19(0.16-0.36)

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

3.2. Formulation persistence test

Nanoemulsion formulas from secondary metabolite of *P. retrofractum* experienced a decrease in persistence marked by decreased mortality from day 1 to day 3 after treatment. Abamectin treatment showed that BPH mortality remained relatively high until the 3rd after treatment (Figure 1). This shows that the persistence of the insecticide with the active ingredient abamectin is longer than that of the three botanical insecticide formulas.
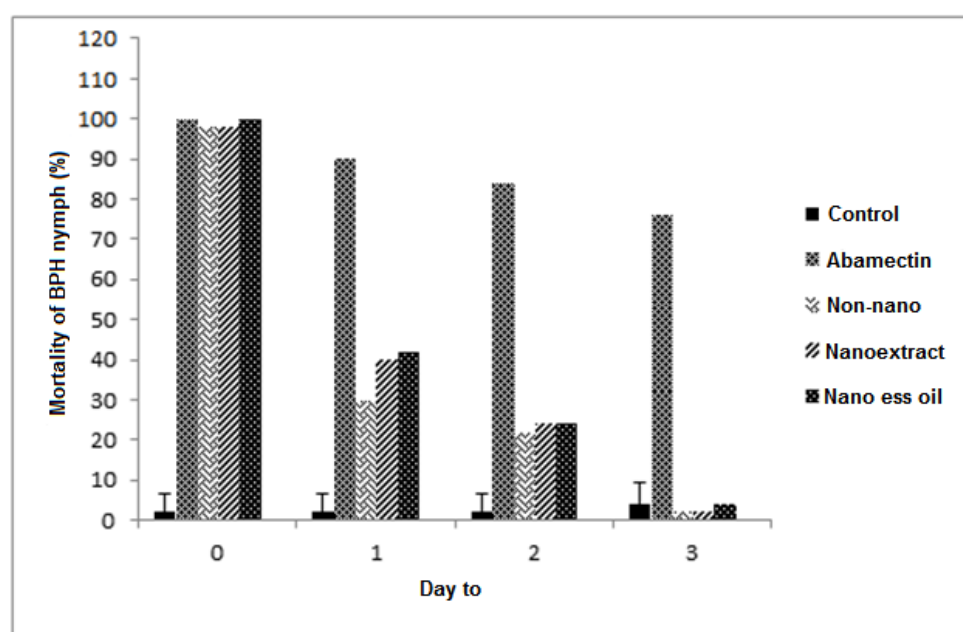

Figure 1. Mortality of brown planthopper nymphs in persistence test.

The toxicity of three botanical insecticide formulations *of P. retrofractum* on the first day decreased significantly, the average mortality of BPH nymphs was less than 42%, on the second day the BPH mortality continued to decrease by less than 26%. Botanical insecticides contain several secondary metabolite compounds as active components, generally have lower persistence in the environment compared to synthetic insecticides, but are relatively safer against untargeted vertebrates and beneficial arthropods, such as natural enemies [7, 3]. Reference [6] reported that the risk of toxicity to non-target organisms will increase in line with the persistence of an insecticide. Therefore, the use of botanical insecticides derived from secondary metabolites of *P. retrofractum* has a better effect because it does

not pose a risk of poisoning for other organisms. Piperamide is a compound that is quickly degraded by sun exposure, so it is more recommended for pest control in post-harvest products or garden plants [10]. Pure piperine compounds can be degraded quickly in an average half-life of 40 minutes [24]. Reference [25] reported that reactive botanical insecticides are safer than synthetic chemical insecticides, because the active ingredients are derived from plant secondary metabolite compounds whose residues are readily biodegradable in nature [26]. Insecticides made from Rosemary (*Rosmarinus officinalis L.*) essential oils are known to be non-persistent because they show lethal and sublethal doses in 1 to 2 days making them safe for the environment and planting of tomatoes [27]. Research [28] active ingredients of several plant extracts such as sesquiterpene lactones from *Tithonia diversifolia*, pentacyclic triterpenoids from *Lantana camara*, vernodalin, vernodalol, and epivernodalol from *Vernonia amygdalina* have a short endurance period. Reference [29] reported the persistence of botanical insecticides from neem and pyrethrum still affected the mortality of BPH until the fourth day after spraying, although their effectiveness decreased with increasing time. The average mortality on the first day after spraying ranges from 53-73% and on the fourth day after spraying between 18-25%.

3.3. Effectiveness test formulation to BPH nymphs
Effectiveness of the formulations at 24, 48, and 72 hours after treatment showed a nanoemulsion formulation of botanical insecticides derived from maceration extract and essential oil of *P. retrofractum* the most effective to BPH nymph control in the green house, and it was not significantly different from Abamectin synthetic insecticide (Table 2).

Table 2. The effectiveness of formulas to BPH nymphs.

Perlakuan	Effectiveness of formulas (%) at (HAT)*		
	24	48	72
Non-nano Formulas	66.10±5.54b	79.31±5.63b	86.21±5.63b
Nanoemulsion from ME of *Piper retrofractum*	64.41±6.49b	77.59±3.55b	90.38±3.45ab
Nanoemulsion from EO of *Piper retrofractum*	84.75±10.17a	93.10±7.96a	97.28±3.45a
Abamektin	83.05±3.91a	91.38±6.60a	96.55±3.98a

* The percentage of effectiveness of the formulation followed by different letters in the same column shows a marked difference based on Duncan's multiple range test at 5% (Steel &Torrie 1993). HAT = hours after treatment.

This shows that nanoemulsion formulations derived from essential oils and maceration extracts from *P. retrofractum* are able to suppress the BPH population in the green house and are not significantly different from commercial insecticide abamectin. Nanoemulsion formulas of *P. retrofractum* essential oils at 24, 48, and 72 HAT were able to suppress BPH populations with values of 84.75%, 93.10%, and 97.28% respectively, compared to controls. Zarkani *et al*. reported the results of *T. vogelii* fraction which has an equivalent ability of profenofos with *B. thuringiensis* in reducing *C. pavonana* larvae populations in broccoli plants [30].

4. Conclusions
Nanoemulsion formulas of *P. retrofractum* essential oil caused the highest toxicity to BPH nymphs. The persistence of nanoemulsion formulas of *P. retrofractum* extracts from the second day decreased less than 45% and was more rapid compared to the synthetic insecticide abamectin. The effectiveness of the nanoemulsion formulation of essential oils from *P. retrofractum* is able to suppress BPH pests in the greenhouse.The study implies that nanoemulsion of *P. retrofractum* essential oil can be considered with the most potential to be developed as a botanical insecticide for controlling BPH.

5. References

[1] Fürstenberg-Hägg J, Zagrobelny M and Bak S 2013 *International Journal of Molecular Sciences (IJMS)* **14** 10242–10297

[2] S D Sharma S N, Jha Z, Tiwari M S and Baghel D 2010 *IOSR Journal of Pharmacy and Biological Sciences* **11** 89–100

[3] Duke A R S O, Dayan F and Romagni J G 2000 *Weed Research* **40** 99–111

[4] D S Cantrell C L and Dayan F E 2012 *Journal of Natural Products* **75** 1231–1242

[5] J Nirmala M and N R 2017 *Journal of Nanomedicine and Nanotechnology* **8**

[6] Koul D G and Walia S 2008 *Biopesticides International* **4** 63–84

[7] Isman M B 2006 *Annual Review of Entomology* **51** 45–66.

[8] Mithöfer A and Boland W 2012 *Annual Review of Plant Biology* **63** 431–450

[9] Isman M B 2014 *ACS Symposium Series* **1172** 21–30.

[10] Scott I M, Jensen H, Philogène B J R and Arnason J T 2008 *Phytochemistry Reviews* **7** 65–75

[11] Indriati G, Dadang D and Prijono D 2016 *Jurnal Penelitian Tanaman Industri* **21** 33

[12] Chansang U *et al*. 2005 *Journal of Vector Ecology* **30** 195–200

[13] Patil K, Choudhary P and Bhatia T 2009 *National Conference on Advances in Heat & Mass Transfer*

[14] Ostertag F, Weiss J and McClements D J 2012 *Journal of Colloid and Interface Science* **388** 95–102

[15] Dadang and D Prijono 2011 *Jurnal Ilmu Pertanahan Indonesia* **16** 100–111

[16] Finney D J 1952 *Annals of the Entomological Society of America* **45** 686

[17] Abbott W S 1925 *Journal of Economic Entomology* **18** 265–267

[18] Peters *et al*. 2014 *EFSA Supporting Publications* **11**

[19] Nuryanti N, Martono E, Ratna E and Dadang 2018 *Jurnal Hama dan Penyakit Tumbuhan Tropis* **18** 1

[20] Musthapa I and Gumilar G G 2017 *Indonesian Journal of Fundamental and Applied Chemistry* **2** 6–9

[21] Dev S and Koul O 1997 I*nsecticides of Natural Origin* (Reading: Harwood Academic Publishers)

[22] Scott I M *et al*. 2002 *Agricultural and Forest Entomology* **4** 137–144

[23] Gbewonyo W S K and Candy D J and Anderson M 1993 *Journal of Pesticide Science* **37** 57–66

[24] Scott I M *et al*. 2004 *Journal of Economic Entomology* **97** 1390–1403

[25] Wiratno, Siswanto and Trisawa I 2004 *Jurnal Penelitian dan Pengembangan Pertanian* **32** 150–155

[26] Regnault-Roger C, Vincent C and Arnason J 2012 *The Annual Review of Entomology* **57** 405–424

[27] Isman M and Machial C 2016 *ACS Symposium Series* **3** 28–44

[28] Mpumi N, Mtei K, Machunda R and Ndakidemi P A 2016 *American Journal of Plant Sciences* **7** 138–151

[29] Kardinan A, Wahyono T E and Tarigan N 2018 *Buletin Penelitian Tanaman Rempah dan Obat* **28** 191

[30] Zarkani A, Prijono J and Pudjianto 2009 *Akta Agrosia* **12** 35–44