EVALUATION OF POTENTIAL PLANT CRUDE EXTRACTS AGAINST GREEN STINK BUG *Nezara viridula* Linn. (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

The Green Stink Bug *Nezara viridula* (Hemiptera: Pentatomidae) is an important pest of soybean crop in Indonesia, causing losses of seed production. A study was conducted to evaluate the insecticidal potential crude extract on the mortality of Green Stink Bug, *N. viridula*. Fourteen of crude extracts used in this study were extracted by Soxhlet extractor. The experiment was conducted using residual toxicity (contact poison) by applying crude extract solution of 0.5 and 0.25% (volume of extract/volume of water, v/v) on adult *N. viridula*. Results revealed that *N. viridula* mortality was affected significantly by crude extracts. The crude extracts of *Annona muricata* seed, *Alpinia galanga* rhizome and *Jatropha curcas* seed, at 0.5 and 0.25% concentration showed insects mortality up to 80-100%. Whilst, crude extracts of *Citrus aurantifolia* peel, *Carica papaya* leaf, *Lantana camara* leaf, *Tagetes erecta* leaf, *Ageratum conaizoydes* plant, *Cymbopogon nardus* leaf and *Murraya koenigii* leaf caused insect mortality between 45.00 to 72.5%. Probit analysis showed that *J. curcas* seed crude extract was highly toxic (LC_{50}=0.026%) to *N. viridula* adult followed by *A. galanga* rhizome crude extract (LC_{50}=0.031%) and *A. muricata* seed crude extract (LC_{50}=0.038%). These crude extracts proved to be highly potent to control *N. viridula*. Further studies are needed to actually verify these crude extracts and its potential application as botanical insecticides.

Keywords: Insect mortality, *Nezara viridula*, plant crude extract, residual toxicity

ABSTRAK

Piangang Hijau *Nezara viridula* (Hemiptera: Pentatomidae) ialah perosak utama tanaman kacang kedelai di Indonesia, ianya menyebabkan kerosakan biji yang dihasilkan. Kajian ini dijalankan untuk menilai potensi insektisidal dari pada ekstrak krud terhadap kematian Pianggang Hijau *N. viridula*. Empat belas ekstrak krud digunakan dalam kajian ini yang diperoleh melalui ekstraksi dengan Soxhlet. Kajian dijalankan dengan residual toksisiti (racun kontak) dengan aplikasi larutan ekstrak krud pada 0.5 dan 0.25% (volume ekstrak/volume air, v/v) terhadap *N. viridula* dewasa. Keputusan mendapati bahawa kematian *N. viridula* dipengaruhi secara nyata oleh ekstrak krud. Ekstrak krud biji *Annona muricata* dan rizom *Alpinia galanga* dan biji *Jatropha curcas* pada kepekatan 0.5 dan 0.25%

menunjukkan serangga mati hingga 80-100%. Manakala, ekstrak krud kulit *Citrus aurantifolia*, daun *Carica papaya*, daun *Lantana camara*, daun *Tagetes erecta*, tanaman *Ageratum conyzoides*, daun *Cymbopogon nardus* dan daun *Murraya koningii* menyebabkan kematian serangga antara 45.00 hingga 72.5%. Keputusan analisis Probit menunjukkan bahawa ekstrak krud biji *J. curcas* sangat toksik (LC_{50}=0.026%) terhadap *N. viridula* diikuti oleh ekstrak krud rizom *A. galanga* (LC_{50}=0.031%) dan biji *A. muricata* (LC_{50}=0.038%). Ekstrak krud ini membuktikan potensi yang tinggi untuk kawalan *N. viridula*. Kajian lanjutan perlu dilaksanakan bagi memastikan potensi ekstak krud sebagai insektida botanis.

Kata kunci: Kematian serangga, *Nezara viridula*, ekstrak krud tanaman, residual toksisiti.

INTRODUCTION

Green stink bugs *Nezara viridula* Linn. were distributed worldwide. This species is polyphagus that feeds on various parts of the herbaceous plants. This bug is recognisable by its uniform green colour. In Indonesia the species has been reported on rice, maize, tobacco, potatoes, chilli, cotton and pod of various legumes where the pods are also sucked. The major damage is not only caused by sucking but by a toxin in the salivary secretion which causes wilting, withering and death of leaves and shoots (Kalshoven 1981; Tengkano et al. 1991). There were various control methods have been reported for *N. viridula*. In spite of many cultural practices were available, the farmers still prioritize the use of chemical (insecticide). According to Marwoto (2007), monitoring in soybean production centre in Indonesia showed the control of insect pest still rely on synthetic insecticide. Some planting area used insecticide intensively in controlling insect pest on soybean with high dose and frequency of application.

There were many negative effects of using synthetic insecticides in crop protection programmes. Agrochemical residues not only resulted in environmental degradation and direct toxicity to the humans but also increase pest resistance, resurgences, and toxic effect to non-target organism (Ahmed et al. 1983; Sharma 1983). Moreover, pest controls using pesticides is costly and reduces farmer profit margin. Compared to financing for other food crops, the cost of pesticides in soybean plants is 2.2%, while in rice and maize are 1.9 and 1.2%, respectively (Badan Pusat Statistik 2015). Hence, an alternative method is needed to overcome various problems in soybean crop cultivation. In order to overcome the negative effects of using synthetic pesticides that harm human health and the environment is by utilizing natural products (Koul et al. 2008). Plants contain compounds which include alkaloids, terpenoids, phenolics as secondary metabolites. These compounds show some insecticidal effects such as antifeedant, antifertility, or growth disturbance (Dodia et al. 2008) and decline the insect resistance probability (Hanif et al. 2015). Belmain et al. (2001) reported that plant material contains bioactive components with insecticidal properties have been used traditionally for generation throughout the world. Abdullah et al. (2015b) also reported that crude extract of *Gliricidia sepium* (Fabaceae) cause high mortality of *Globitermes sulphurous* (Termitidae).

There is a need to screen plants or/and part of plant which is locally viable in order to control Green stink bug, *N. viridula*. A number of plants or part of plants which grow around

Kabupaten Deli Serdang, North Sumatra which are known to have insecticidal properties were studied the mortality effect against *N. viridula.*

MATERIALS AND METHODS

Plant Materials Collection and Extraction

Plants that have been reported to possess insecticidal property (Prakash & Rao 1997; Prijono 2003; Dodia et al. 2008) were collected from Kabupaten Deli Serdang Indonesia in April 2018. The plants or part of plant were washed thoroughly with tap water then air dried under shade for 1 week. The seed of *A. indica*, *A. muricata* and *J. curcas* were hulled to get the kernel. The rhizome of *A. galanga* was cut into 5 mm to make it was dry finely. The leaf and clump of *C. nardus* were used for this study. The dried plant material was ground by using electric grinding and passed through sieve (10 mm mesh size). The material (50 g) was transferred into a filter paper then placed in the soxhlet extractor and acetone was used as solvent (200 ml) in the receiving flask. The extraction process took about 10 hours and a 50 ml stock solution was obtained after evaporation by using Rotary Vacuum Evaporator.

Insect Rearing

The adults of *N. viridula* used in this study were obtained by mass rearing of individual insects collected from soybean field. The collected adult individuals were reared in a wired caged (50x50x60 cm) and fed with fresh long bean *Vigna unguiculata* subsp. *sesquipedalis*. The newly emerged adults were obtained after about 4 weeks. Adults of *N. viridula* between 5-10 days old were used for toxicity test.

Contact Toxicity Test

The study was carried out at the Plant Protection Laboratory, Faculty of Agriculture, Islamic University of North Sumatra, Indonesia during May to August 2018. The assay was performed under laboratory conditions at 29±2^{o}C temperature, 70±10% relative humidity and photoperiod of 12 hours. Methods were adopted from Tilman (2006). Each crude extract with the 0.5 and 0.25% concentration was smeared with 2 ml crude extract on the top and bottom of petri dish (150 by 15 mm). Water was used as the control. After about 1 hour (the solution was dry), 10 *N. viridula* adult were placed in the petri dish. After 24 hours, the adult was moved to a transparent plastic jar (9.5 cm diameter and 7 cm height) with filter paper at the bottom. The insects were provided with food (5 young soybean pods each cup). The jar was covered with muslin cloth. The pods and filter paper were replaced every 2 days. The mortality rate was recorded daily from day-1 to day-10 after treatment. Statistical analysis was done using one-way ANOVA ($P<0.05$). Data of insect mortality were transformed using Arc Sine Transformation (Gomez & Gomez 1984) for normalization before analysis. To determine the different of mortality of *N. viridula* each crude extract, Duncan's Multiple Range Test (DMRT) at 5% confidence interval was used. All statistical analyses were done using SPSS Statistic 24 Programme. The crude extract which showed high effect (80-90% of mortality) was continued for toxicity study. It was determined by Probit Analysis (Finney 1971) using POLO-PC (LeOra software 1987).

RESULTS AND DISCUSSIONS

There was a significant difference in 0.25% mortality (F = 26.44, df = 14 & 45, P < 0.05) and 0.5% mortality (F = 19.26, df = 14 & 45, P < 0.05) of *N. viridula* among plant extracts. The mean *N. viridula* mortality was showed at Table 1

Table 1. Mortality (%) of *N. viridula* after contacted with crude extract as residual toxicity

No	Extract	Percent Mortality (Mean±SEM)	
		0.25%	**0.5%**
1	*Annona muricata* (seed)	$80.00±4.08^{b}$	$100.00±0.00^{a}$
2	*Alpinia galanga* (rhizome)	$95.00±5.00^{a}$	$100.00±0.00^{a}$
3	*Jatropha curcas* (seed)	$90.00±7.07^{a}$	$97.50±2.50^{a}$
4	*Citrus aurantifolia* (peel)	$50.00±4.08^{cde}$	$65.00±6.45^{b}$
5	*Carica papaya* (leaf)	$50.00±12.91^{cde}$	$60.00±5.77^{bc}$
6	*Isotoma longifora* (leaf)	$40.00 ±8.66^{def}$	$40.00±4.72^{bc}$
7	*Lantana camara* (leaf)	$67.50±4.79^{c}$	$72.50 ± 2.50^{b}$
8	*Tagetes erecta* (leaf)	$57.50±8.54^{cde}$	$65.00±6.45^{b}$
9	*Ageratum conyzoides (*plant)	$45.00±15.00^{def}$	$50.00±12.25^{bc}$
10	*Cymbopogon nardus* (leaf)	$22.50±4.79^{egh}$	$45.00±6.45^{bc}$
11	*Cymbopogon nardus* (clump)	$27.50±7.50^{efg}$	$32.50±7.50^{c}$
12	*Ocimum citriodorum* (leaf)	$5.00±5.00^{ij}$	$15.00±8.66^{d}$
13	*Piper betle* (leaf)	$17.50±2.50^{gh}$	$32.50±2.50^{c}$
14	*Murraya koenigii* (leaf)	$7.50±4.79^{i}$	$45.00±6.45^{bc}$
15	Control	$0.00±0.00^{k}$	$0.00±0.00^{e}$

Means in a column followed by different letters are significantly different (P=0.05) by Duncan's Multiple Range (DMRT) Test.

All of plant extract tested (residual toxicity) showed certain degree of ability to control *N. virudula*. As expected, the mortality effect of plant extract to control *N. viridula* varied among plant species. Crude extract of *A. muricata*, *A. galanga*, and *J. curcas* caused mortality effect of 80-100% at 0.5 and 0.25% concentration. Whilst, *C. aurantifolia, C. papaya, L. camara* and *T. erecta* showed 50-70% mortality effect. The others crude extract showed less effect. In this study, the adult was actively moved hence contacting with crude extract was high. According to Bayu (2015) adult of *N. viridula* attack the pod of soybean actively. The insect punctured and sucked the pod. *Cymbopogon nardus* leaf and clump extract and *M. koenigii* leaf extract caused 30-45% *N. viridula* mortality. Both plants were used for food flavouring ingredients. Lemon grass *C. flexuosus* which has also citral as main components caused *Sitophilus zeamais* mortality up to 46.67% at 5% concentration (Islam et al. 2017). *Murraya koenigii* was known to contain the chemical constituents mahanimbin, koenimbin, koenigicine, curryanine and curryangine. The *M. koenigii* leaf extract at 0.5 and 1% concentration caused reduction of linseed bud fly *Dasyneura lini* (47.46 and 56.52%, respectively) and its leaf powder cause 62.2% mortality of *Sitophilus oryzae* 14 days after treatment in store wheat (Dodia et al. 2008). It was also promising sign of seed protection and insecticidal properties against *Tribolium castaneum* (Gandhi et al. 2010). On the other hand, *C. nardus* rich with essential oil cymbopogone, cymbopogonol, α and β-citrals, myrcene, linalool, lynalyl acetate, citronellal and nerol. The essential oil of *C. nardus* citonella oil exhibited insecticidal properties against *Sitophilus zeamais* adult (Doumbia et al. 2014).

All crude extract tested contain bioactive component against insect and contacted the *N. viridula* adult with crude extract as residual effect causing mortality. Isman (2006) reported *A. muricata* contains acetogenins with a wide variety of biological activities that act as botanical insecticides. Whilst, *J. curcas* seed contain curcin and phorbol esters (Adolf et al. 1984). Sauerwein et al. (1993) found that phorbol esters from *J. curcas* seed have insecticidal properties. The mortality effects of *C. aurantifolia* peel may cause by insecticidal substances in the extracts. Simon Oke and Akeju (2017) reported that peel crude extracts contains citral, limonene, β-pinene and other terpenoids. The *L. camara* extracts have been reported to have insecticidal properties against various insect pests (Abdel-Hady et al. 2005). The study against worker termites of *Reticulitermes flavipes* showed leaf crude extract of *L. camara* exhibited strong repellency, moderate reduction in feeding and toxicity (Yuan & Hu 2012).

Tagetes erecta (Marigold) have been used for centuries by people in India and East Asia to control pests. Marigold water extracts exhibits insecticidal effects against ant, aphids and grasshoppers (Dodia et al. 2008). Salinas-Sanchez et al. (2012) reported that the leaf extracts of *T. erecta* are toxic to *Spodoptera frugiperda* larvae with significant toxic effect extending into the pupal stage. Certain terpenes have very great antifeedant, phagodeterent, and toxic effects on herbivorous insects. A variety of chemical constituents have been isolated from *Tagetes* species and their structures elucidated. They belong to the classes as essential oils, thiophenes, flavonoids, carotenoids and phenolic compounds (Gupta & Vasudeva 2012). The 0.5% concentration of *C. aurantifolia* peel crude extracts, *L. camara* and *T. erecta* leaf crude extracts in this study caused up to 65% of *N. viridula* mortality as contact effect. It seems that these crude extracts were potent to use for controlling this insect. But, there is a need for further studies to evaluate their effectiveness as stomach poison.

The crude extract which caused high effect in this study was continued with probit analysis to evaluate the toxicity of the extract. The crude extract for probit analysis were *A. muricata* (seed), *A. galanga* (rhizome) and *J. curcas* (seed). The LC_{50} probit statistics estimates and their 95% fiducial limits (FL) is presented in Table 2.

Table 2. Toxicity of crude extracts to *N. viridula* adults

Crude Extract	**Insects* (N)**	**LC_{50} (%)**	**95% Fiducial Limit**	**Slope±SE**
A. muricata	40	0.038	0.022-0.058	0.97±0.16
A. galanga	40	0.031	0.019-0.045	1.23±0.18
J. curcas	40	0.026	0.003-0.059	1.09±0.18

*Number of insects tested for each concentration.

The crude extract of *J. curcas* showed the lowest LC_{50} (0.026%), means it was more toxic than *A. muricata* seed and *A. galanga* rhizome crude extract (0.038 and 0.031%, respectively) against *N. viridula* adult. The probit regression for *A. muricata* is Y= 6.38+0.97 logX, *A. galanga* is Y= 5.85+1.23 logX, and *J. curcas* is Y= 6.72+1.09 logX. The graph of concentration-probit mortality of crude extract against *N. viridula* is presented in Figure 1. The cumulative of percent mortality of *N. viridula* over 10 days caused by *J. curcas* and *A. muricata* seed and *A. galanga* rhizome crude extracts as shows at Figure 2.

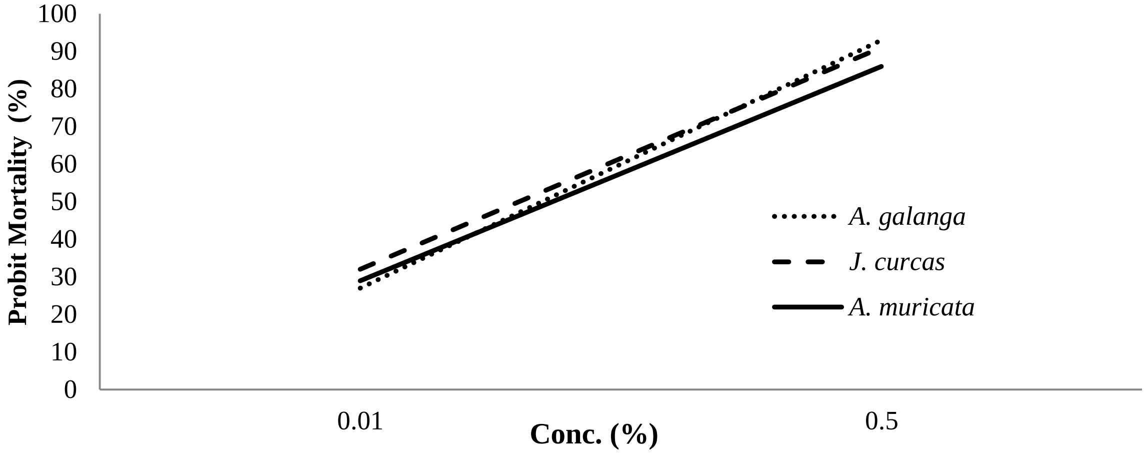

Figure 1. Concentration–probit mortality graph for crude extracts against *N. viridula* adult

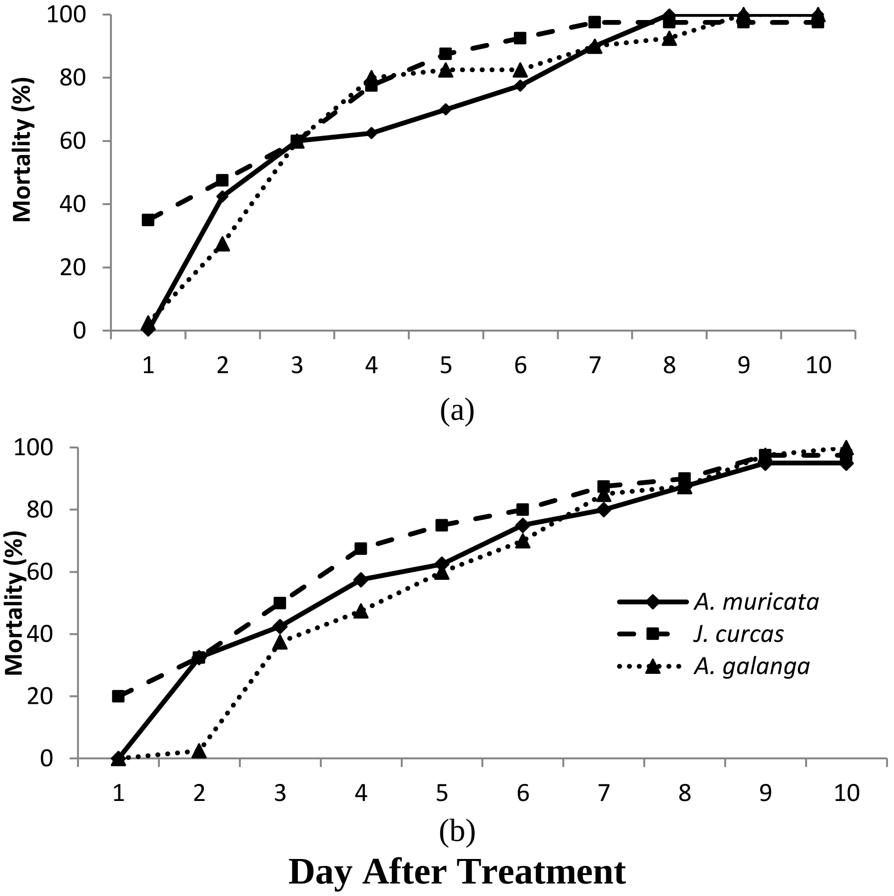

Figure 2. Cumulative percent mortality of *N. viridula* treated by *A. muricata* (seed), *J. curcas* (seed) and *A. galanga* (rhizome) crude extract at (a) 0.5 and (b) 0.25% of concentrations

The graph showed that *J. curcas* seed crude extract at 0.5% concentration showed the rapid effect because 1 DAT cause 35% of *N. viridula* mortality, whilst, no insect mortality when treated with *A. muricata* (seed) and only 2.5% with *A. galanga* (rhizome). Some studies showed that *J. curcas* seed extract caused insects mortality effect. Seed extract of *J. curcas* at 35% (weight of seed powder/volume of water) concentration incorporated into the worker termites *Macrotermes* spp. diet caused 93.33, 98.33 and 100% mortality at 24, 48 and 72 h after exposure, respectively (Addidu et al. 2014). Habou et al. (2011) reported 0.5 and 1% of *J. curcas* seed oil-based formulation (50% *J. curcas* seed oil, 30% pure ethanol as a stabiliser and 20% gum arabic as an adjuvant) caused the mortality of *Aphis fabae* 13,1 and 30% at 24 h. Despite *A. muricata* seed and *A. galanga* rhizome crude extract at 0.5% concentration showed less effect on insect mortality at 1 DAT, but at 3 DAT both crude extracts showed similar effect with *J. curcas* seed crude extract (60% of *N. viridula* mortality). Another study of *A. muricata* seed crude extract incorporated to *Sitophilus zeamais* diet at 0.5% concentration caused 100% weevil mortality at 4 DAT, whilst when it treated with *J. curcas* seed crude extract, the 100% weevil mortality occurred at 3 DAT (Asmanizar et al. 2008). Khattak (2005) reported, *Alpinia galanga* rhizomes have medicinal properties, and many compounds of this plant have various biological activities. Abdullah et al. (2015a) added that the most abundant compound (61.9%) in the rhizome of *A. galanga* identified as 1,8-cineole and caused mortality of *Coptotermes gestroi* and *C. curvignathus* adults, and also caused 92.50% of *Callosobruchus chinensis* mortality when treated on Mung bean *Phaseolus radiatus* and fed to the insects (Asmanizar et al. 2016). Generally, *J. curcas* and *A. muricata* seed and *A. galanga* rhizome crude extract tested at 0.5 and 0.25% of concentration caused 90-100% of *N. viridula* mortality at 10 DAT, although 0.25% of concentration showed longer effect than 0.5%. From the results, more studies are essential to determine the potency of crude extract on eggs and immature insects.

CONCLUSION

The present study revealed that *J. curcas*, *A. muricata* seed and *A. galanga* rhizome crude extract exhibited effective contact toxicity against adult *N. viridula*. Applications for pest management using plant extract such as *J. curcas*, *A. muricata* seed and *A. galanga* rhizome crude extract may provide a better solution to suppress insect pests' populations while reducing agrochemical impacts on the environments and better human safety for the long term compared to synthetic insecticides. There is a need in the up coming study to explore the potency of *J. curcas*, *A. muricata* seed and *A. galanga* rhizome crude extract against *N. viridula* on eggs and immature insects.

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