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POTENCY OF JATROPHA CURCAS SEED CRUDE EXTRACT AGAINTS NEZARA VIRIDULA (HEMIPTERA: PENTATOMIDAE)

A. Asmanizar (a)*, A. Aldywaridha (b), Edy Sumantri (c), Ratna Mauli Lubis (d)
*Corresponding author

(a) Universitas Islam Sumatera Utara, Jl. Sisingamangaraja Teladan, Medan, Indonesia asnmanizar_az@fp.uisu.ac.id
(b) Universitas Islam Sumatera Utara, Jl. Sisingamangaraja Teladan, Medan, Indonesia
(c) Universitas Islam Sumatera Utara, Jl. Sisingamangaraja Teladan, Medan, Indonesia
(d) Universitas Islam Sumatera Utara, Jl. Sisingamangaraja Teladan, Medan, Indonesia

Abstract

Green Stink Bug *Nezara viridula* (Hemiptera: Pentatomidae) causes soybean yield losses and moreover decreasing of seed quality. Controlling this insect by using chemical insecticides might cause a variety of negative impacts. Hence, using of botanical insecticides is an alternative. *Jatropha curcas* seed crude extract contains bioactive components that can affect insects. The purpose of this study was to know the effect of seed crude extract concentration of *J. curcas* against Green Stink Bug *N. viridula*. Two serial laboratory studies were done to determine the residual and oral toxicities of the extract. The study was arranged as Completely Randomized Design (CRD) and seed crude extract of *J. curcas* concentrations as the treatment viz. Control (water); 0.015 %; 0.0312 %; 0.0625 %; 0.125 %; 0.25 % and 0.5 %. Results showed that the concentration of *J. curcas* seed crude extract affected significantly on *N. viridula* mortality, number of eggs and nymphs. The crude extract showed high effect as residual toxicity because the highest mortality (95-100 %) of *N. viridula* occurred at 0.5 %, 0.25 % and 0.125 % concentrations. Eggs and progenies were not produced when applied at 0.5 %, 0.25 % and 0.125 % concentrations. In the oral toxicity test, 100 % of insect mortality was observed at 0.50 %, 0.25 % and 0.125 % concentrations; whereas at 0.125 % eggs and nymphs were still produced. The application of *J. curcas* seed crude extract as residual toxicity (contact poison) showed rapid effect of insect mortality compared to oral toxicity (stomach poison). Thus, *Jatropha curcas* seed crude extract is potent to control *N. viridula* and needs further studies to examine its effects in soybean field.

Keywords: Jatropha curcas, crude extract, Nezara viridula, residual toxicity, oral toxicity.

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1. Introduction

In Indonesia, soybean (*Glycine max* L.) is an important commodity. It is used to prepare food items such as tempe, tahu and tauco which are largely consumed by almost all local communities. The productivity of soybean in Indonesia however is still low (1.2 ton/hectare), whilst the yield potency is 2.0-2.5 ton/hectare. As the consequence, Indonesia needs to import soybean in a large scale. There are various obstacles to achieve maximum productivity of soybean in Indonesia. Loss yield due to pests and disease damages is the main cause of the low production of soybean. Farmers lack of interest to cultivate soybean as the costs for controlling the pests and diseases become high (Sudaryanto & Swastika, 2007). One of the soybean pests is Green Stink Bug *Nezara viridula*. The insect is polyphagous and is recognisable by its uniform green colour. *N. viridula* attacks on soybean cause reduction in seed quality and quantity. The mouthparts of the sucking insect are developed for piercing and sucking, whereby it penetrates the young pods, injects its saliva and ingests the resulting liquid. Damages are caused by the saliva which contains toxic substances which destruct the tissues directly. The infested young pods are empty of seeds and kernels whilst mature pods show necrotic tissues and reduced seed quality (Kalshoven, 1981; Panizzi, McPherson, James, Javahery, & McPherson, 2000, Marwoto, Sri Hardaningsih, & Taufiq, 2017). According to Marwoto (2007), soybean caused by insect pest attack could decrease up to 80 % of total yield. Hence farmers commonly rely on synthetic insecticides to control the pests.

2. Problem Statement

Reliance on synthetic insecticides to control pests have given rise to a number of problems such as the destruction of beneficial non-target organisms (parasitoids and predators) and environmental negative impacts such as contamination with toxic materials. The use of botanical insecticides is an alternative for synthetic insecticides. Many plants contain compounds which include alkaloids, terpenoids and phenolics as secondary metabolites. These compounds are able to control insecticidal properties such as mortality rate, growth disruption and as antifeedant or antifertility (Dodia, Patel, & Patel, 2008). Botanical insecticides are less expensive and easily available because of their natural occurrence, especially in developing countries. There is a need to examine botanical insecticides to substitute or complement synthetic insecticides in controlling insect pests of soybean such as *Nezara viridula*.

3. Research Questions

Jatropha curcas (Euphorbiaceae) is a perennial plant, drought resistance and easily be grown on almost any soil and marginal land. In Indonesia, the plant is also used in traditional medicine and being planted as living fences by village communities. The seeds contain 30-50 % oil, therefore in the East of Indonesia the plants are planted to obtain the oil for the source of biodiesel. Adebowale and Adedire (2006) reported that *J. curcas* seed crude extract contains several sterols and terpen alcohols known to exhibit insecticidal properties. It gave significant protection to seeds of cowpea *Callosobruchus chinensis*. Ahmed and Salimon (2009) added that the *Jatropha* oil from Indonesia has the highest phorbol ester content compared to from Malaysia and India. Makkar and Becker (1997) reported among *J. curcas* extracts, the main biocidal action has been ascribed to the phorbol ester fraction from the seed oil. Devappa, Angulo-

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Escalante, Makkar, and Becker (2012) also reported that phorbol ester from *J. curcas* oil exhibited contact toxicity and declined the armyworm (Spodoptera frugiperda) pests. Therefore, *J. curcas* has the potent as a botanical insecticide to control *N. viridula*. Moreover, *J. curcas* plants are locally available around Kabupaten Deli Serdang and easily obtained by farmers. It is important to know the effective concentration of *J. curcas* crude extract in controlling of *N. viridula*.

4. Purpose of the Study

The present study was conducted to evaluate the potencial of *J. curcas* seed crude extract in controlling *N. viridula*, a pest of soybean**.**

5. Research Methods

5.1. Crude extracts of *J. curcas*

Seeds of *J*. curcas seed were obtained from the ripe fruits (yellow to brownish) collected in Kabupaten Deli Serdang Indonesia during April 2018. The *J. curcas* seeds were hulled to get the kernels. After a week of air dried under shade, the kernels were grinded. An amount of 50 g of material was wrapped in a filter paper for extraction using soxhlet extractor (with 200 ml acetone solvent). Total crude extract was obtained after the solvent was dried using a rotary vacuum evaporator.

5.2. Rearing of insects

Nezara viridula was obtained by mass rearing with the initial population from a soybean field. Male and female adults were collected in a wired cage (50x50x60 cm) and fed with fresh long beans *Vigna unguiculata subsp. sesquipedalis*. After about 4 weeks, new adults emerged which were used in the study (5-10 days old).

5.3. Application of crude extracts

Study was done at the plant protection laboratory Faculty of Agriculture, Islamic University of North Sumatra, Indonesia from May to August 2018. The assay was performed in a laboratory at temperature 29 ± 2 ºC, relative humidity 70 ± 10 % and photoperiod of 12 hours. Tests for residual toxicity (contact poison) and oral toxicity (stomach poison) was performed according to Tilman (2006) with 0.50 %, 0.25 %, 0.125 %, 0.062 %, 0.031 % and 0.015 % concentrations.

Residual toxicity (contact poison) test was done by smearing 2 ml of crude extract on top and bottom of petri dishes (150 by 15 mm). Water was used as the control. After an hour when the solution has dried, 10 *N. viridula* adults were placed in each petri dish. After 24 hours, the adults were transferred into a transparent plastic jar (9.5 cm diameter and 7 cm height) with a piece of filter paper at the bottom. The jars were covered with muslin cloth. These insects were provided with 5 young soybean pods per jar, as their food. The pods and filter paper were replaced every 2 days.

Oral toxicity (stomach poison) test was done by soaking soybean pods in the crude extract for 30 seconds and then air dried on a screen wire for an hour. The pods were then wrapped with a muslin cloth (to prevent contact of insects) and placed inside a transparent plastic jar (9.5 cm diameter and 7 cm height)

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with a piece of filter paper at the bottom. Ten *N. viridula* adults were placed in each jar. After 3 days, the pods were replaced with untreated pods.

For both tests, the mortality rate was recorded daily until ten days after treatment. Number of egg production and nymphs were counted. Statistical analysis was done using one way ANOVA ($P<0.05$). Data of insect mortality were transformed using Arc Sine Transformation, whilst the number of eggs and nymphs were transformed by log (x+10) (Gomez & Gomez, 1984) for normalization before analysis. Duncan's Multiple Range Test (DMRT) at 5 % confidence interval was used to measure specific differences between pairs of means. All statistical analyses were done using SPSS Statistic 24 program.

6. Findings

6.1. Residual toxicity (Contact poison)

There was a significant difference in *N. viridula* mortality ($F = 46.43$, df = 6 & 27, $P < 0.05$), number of eggs ($F = 5.19$, df = 6 & 27, $P < 0.05$) and number of nymphs ($F = 6.08$, df = 6 & 27, $P < 0.05$) caused by *J. curcas* seed crude extract concentrations. The mean percentage of *N. viridula* mortality, and number of eggs and nymphs is shown in Table 01.

The results showed that all concentrations of *J. curcas* seed crude extract caused *N. viridula* mortality and were significant different compared to control. The insect mortality was 75-100 %. The concentrations of 0.25 % and 0.50 % of *J. curcas* seed crude extract gave total percentage of mortality and therefore act as the best contact poison. There was a trend of increased *N. viridula* mortality with the increase of extract concentration. The effect was probably due to the bioactive componenst of *J. curcas* seeds. According to Habou, Haougui, Adam, Haubruge, and Verheggen (2014) *J. curcas* seed crude extract caused *Callosobruchus maculatus* mortality up to 72 % at 7 days after treatment. Devappa et al. (2012) reported that phorbol ester from *J. curcas* oil exhibited contact toxicity. Bourga (2018) also reported a high contact toxicity of the phorbol ester in *J. curcas* seed oil, and that the oil was observed on the grain aphid *Sitobion avenae*. The topical and spray applications of a concentration of 2 % of phorbol ester and the seed oil caused a sudden death of the aphids. With both compounds, the aphids died soon after the application of the compounds on the insect cuticle.

Table 01. Percentage of mortality, and number of eggs and nymphs of *N. viridula* after treatment with *J. curcas* seed crude extract as residual toxicity. Means in a column followed by different letters are significantly different (P=0.05) by Duncan's Multiple Range (DMRT) Test

Crude Extract Concentration (%)	% Mortality (± SEM)	Number of Egg (± SEM)	Number of Nymph (± SEM)
0.000	10.00 ± 4.08 e	31.25 ± 4.27 a	28.75 ± 3.57 a
0.015	75.00 ± 2.89 d	32.50 ± 11.09 a	25.00 ± 8.66 ab
0.031	80.00 ± 4.08 cd	17.00 ± 10.48 ab	16.25 ± 9.87 ab
0.062	90.00 ± 4.08 bc	7.50 ± 7.50 b	7.00 ± 7.00 c
0.125	95.00 ± 2.89 ab	0.00 ± 0.00 b	0.00 ± 0.00 c
0.250	100.0 ± 0.00 a	0.00 ± 0.00 b	0.00 ± 0.00 c
0.500	100.0 ± 0.00 a	0.00 ± 0.00 b	0.00 ± 0.00 c

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Treatment of the crude extract at 0.062 % concentrations caused the lowest number of eggs and nymphs. There were no eggs and nymphs produced when the *J. curcas* seed crude extract was applied at concentrations of 0.125 %, 0.250 % and 0.5 %. Thus, these concentrations were considered as the contact poison. Although *J. curcas* seed crude extracts at concentrations of 0.015 %, 0.031 % and 0.062 % caused 75-90 % *N. viridula* mortality, there were still survival insects producing eggs and progenies.

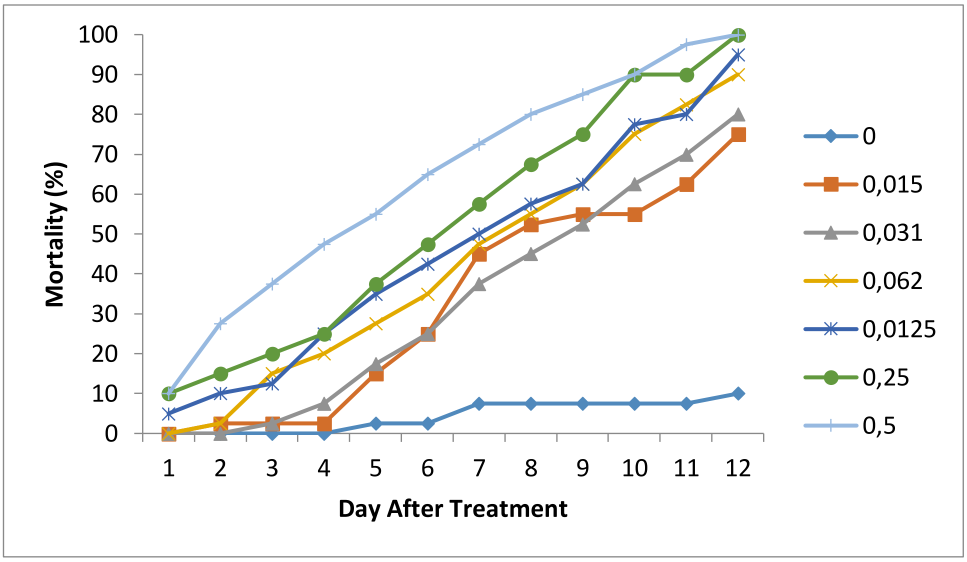

Figure 01. Mortality percentage of *N. viridula* when treated with *J. curcas* crude extract by residual toxicity

The mortality pattern of the insect at 1 to 12 days after treatment is presented in Figure 01. The graph shows that insect mortality at 0.5 % concentration of *J. curcas* seed crude extract occurred faster than other concentrations. The concentration of 0.5 % caused 50 % mortality at 5 days after treatment, whilst 0.25 % and 0.125 % concentrations caused lower than 40 % mortality. Although the percentage of mortality reached 75-90 % at lower concentrations, these were not recommended because there were still presence of eggs and progenies at these concentrations.

6.2. Oral toxicity (stomach poison)

There was a significant difference in *N. viridula* mortality ($F = 72.25$, $df = 6$ & 27, $P < 0.05$), number of eggs ($F = 2.90$, $df = 6$ & 27, $P < 0.05$) and number of nymps ($F = 3.28$, $df = 6$ & 27, $P < 0.05$) caused by *J. curcas* seed crude extract concentrations. The mean percentage of *N. viridula* mortality, number of eggs and nymphs is shown in Table 02.

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Table 02. Percentage of mortality, and number of eggs and nymphs of *N. viridula* after treatment with *J. curcas* seed crude extract as oral toxicity. Means in a column followed by different letters are significantly different (P=0.05) by Duncan's Multiple Range (DMRT) Test

Crude Extract Concentration (%)	% Mortality (± SEM)	Number of Egg (± SEM)	Number of Nymph (± SEM)
0.000	12.50 ± 4.79 [d]	53.75 ± 12.81 [a]	53.50 ± 12.70 [a]
0.015	75.00 ± 2.89 [c]	47.50 ± 8.54 [a]	47.50 ± 8.54 [a]
0.031	82.50 ± 2.50 [c]	45.25 ± 26.22 [a]	42.50 ± 24.62 [a]
0.062	92.50 ± 2.50 [b]	29.75 ± 13,90 [ab]	29.75 ± 13.90 [ab]
0.125	100.00 ± 0.00 [a]	18.75 ± 10.87 [b]	17.50 ± 10.31 [b]
0.250	100.00 ± 0.00 [a]	00.00 ± 0.00 [c]	00.00 ± 0.00 [c]
0.500	100.00 ± 0.00 [a]	00.00 ± 0.00 [c]	00.00 ± 0.00 [c]

The results showed that all concentrations of *J. curcas* seed crude extract caused *N. viridula* mortality and were significant different compared to control. The 0.25 % and 0.5% of *J. curcas* seed crude extract concentration had the best effect with 100 % mortality, and without production of eggs and progenies. This revealed that *J. curcas* seed crude extracts could act as stomach poison (oral toxicity). The results are agreement with those obtained by Ingle et al. (2017), who observed 80 % *Plutella xylostella* mortality when fed with cabbage leaves dipped in *J. curcas* seed extracts at 0.5 % concentration.

Tukimin, Soetopo, and Karmawati (2010) stated that the mortality of *Achaea janata* larva was 75 - 85 % at 3 days after feeding with *Ricinus communis* leaves reated with *J. curcas* seed oil solution (5 ml seed oil + 1 g detergent/l water). The source of the *J. curcas* seeds which contain the phorbol ester was from South of Sulawesi and East of Java, Indonesia. Bourga (2018) reported the seed oil of *J. curcas* caused the mortality of aphids after feeding on wheat plants sprayed with 2% of the extract. The oil is rich with dipterpenoids which are considered to be the most important compound synthesized by *J. curcas* (Devappa, Makkar, & Becker, 2011). Ratnadass and Wink (2012) reported that the seed oil and the phorbol ester of *J. curcas* had high ingestion toxicity on Hemipteran species (*Myzus persicae*, *Aphis gossypii* and *Lipaphis erysimi*).

The concentration of 0.125% has shown good effect (insect mortality has reached 100%), but some of the insects had managed to lay eggs and produced progenies (nymph) before dying. The number of eggs and progenies were 18.75 and 17.5 respectively. The concentrations of 0.15 % to 0.062 % showed the insect mortality of 75 to 93 %, and the insects still also produced eggs and progenies. These low concentrations were not recommended because the progenies produced could be a source of infestation in further period.

The oral toxicity of the crude extract showed a slower mode of action than the contact toxicity. It needed a longer period to cause mortality effect because the insects need to suck the soybean pods before the toxic materials reached the mid gut. Figure 02 shows the 0.5 % extract concentration caused only 40% at 5 days after treatment and 70 % mortality at 8 days after treatment. Adolf, Opferkuch, and Hecker (1984) reported that *J. curcas* seeds contain curcin and phorbol ester which could affect the insects. Sauerwein, Sporer, and Wink (1993) found that phorbol esters from *J. curcas* seed have insecticidal properties. In this study, *N. viridula* which sucked the soybean pods caused the inset mortality. The insects ingested the toxic material and response occurred when the crude extract reached the mid gut.

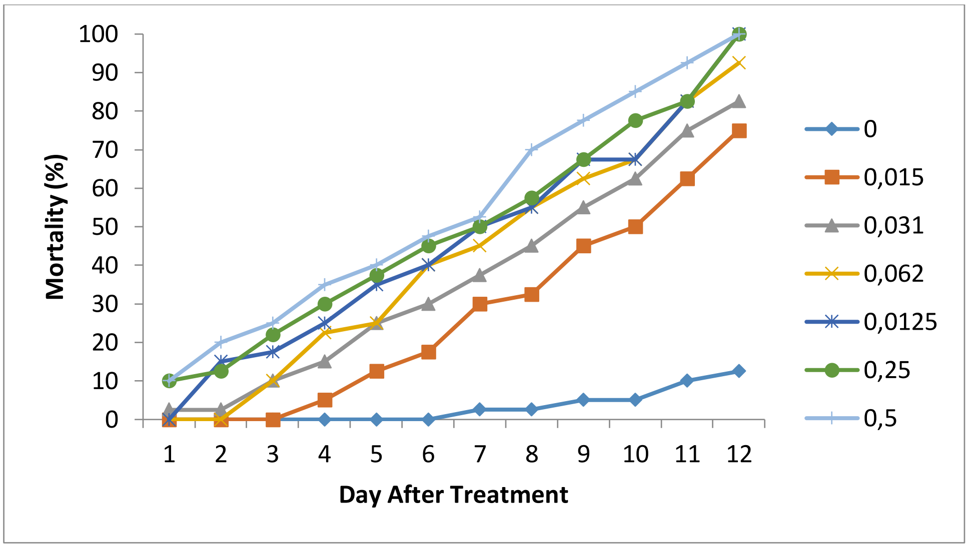

Figure 02. Mortality percentage of *N. viridula* when treated *J. curcas* crude extract by oral toxicity

7. Conclusion

7.1. The *Jatropha curcas* seed crude extract showed a residual toxicity (contact poison) and oral or ingestion toxicity (stomach poison) against Green Stink Bug *N. viridula*.

7.2. The crude extract exhibited a high mortality at concentrations of 0.5 % and 0.25% (100% of mortality, no egg and progeny production).

7.3. The crude extract could be an alternative for the control of *N. viridula*.

7.4. *Jatropha curcas* seed crude extract showed rapid action as contact poison (residual toxicity) if compared to as stomach poison (oral toxicity) against *N. viridula.*

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