Developmental effect of cashew nut shell extract against nymphal instar of Silver leaf Whitefly (Bemisia tabaci Genn.)

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Submission date: 05-Aug-2022 06:15AM (UTC+0700)
Submission ID: 1878909577
File name: IOP_Conf._Ser.__Earth_Environ._Sci._293_012039.pdf (635.23K)
Word count: 3967
Character count: 19208

IOP Conference Series: Earth and Environmental Science

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Abstract. Cashew nut shell (CNS), which are one of the most still is tropical biomass waste. This study was aimed to test mortality rates of CNS extract against the first to third instar nymphs of Silverleaf Whitefly (*Bemisia tabaci* Genn.1889). The concentration of CNS extract were: 0.22 %, 0.67 %, 2.00 %, 6.00 %. The LC_{50} and LC_{90} values indicated that CNS extract at concentration of 2.00 % were the most toxic compound to the first instar stage followed by CNS extract at concentration of 6.00 %, 0.67 %, imidacloprit, and CNS at concentration of 0.22 %, respectively. The concentration of 6.00 % showed the most toxic effect to the second stage followed by CNS extract at concentration of 2.00 %, 0.67 %, imidacloprit, and CNS at concentration of 0.22 %, respectively. The lethal concentration (LC_{50-90}) value of the CNS extract at concentration of 6.00 %, at concentration of 2.00 %, 0.67 %, imidacloprit, CNS extract at concentration of showed the toxicity against the third instar nymph. This CNS extract at concentration of 2.00 % could be used to suppress of the *B. tabaci* nymphs of different stages (first to third instar) and negative effect of phytotoxicity on the soybean leaves.
Keywords: Botanical pesticide, concentration, nymphs, mortality rate, phytotoxicity.

1. Introduction

Silverleaf Whitefly (*Bemisia tabaci* Genn. 1889, Hemyptera: Aleurodidae) is the most problematic pest during the dry seasons on soybean plants. Therefore low rainfall and temperatures high could cause significant outbreaks. On the other hand the whitefly is vector for mosaic disease. The mosaic disease incidence increased with the increases of the whitefly populations, if there was inoculum source. Utilization of traditional breeding for whitefly resistance have mostly not been successful [1].

The use of synthetic insecticide is generally not recommended against whitefly because insecticide induced resurgency of insect on farm condition. Botanical pesticides have been used as attractive alternatives to synthetic chemical. Cashew plant was found in many regions in Indonesia. It is known as *jambu mete* (Java), *jambu mede* (Sunda). Cashew nut shell liquid (CNSL) had the potential to be used as ecofriendly insecticide against *Helicoverpa armigera* (Hübner,1809) and *Spilarctia oblique* (Walker, 1855). The shell liquid of cashew nut contain a complex secondary metabolite, which affected growth and developmental of two lepidopteran insects [2]. Anacardic acid is the main constituents of natural CNSL that obtained by extraction of cashew shell [3, 4]. The high mortality of insect was caused a complex mixture of the compounds anacardic acid in the CNSL. The bioactive compound acts as antifeedant with blocking sensoryc cell to starve. Oils have increased risk of causing

phytotoxicity. Although capsicin was not phytotoxicity because the plants posses a wax which have protected them [5].

These authors also observed the antifeedant active compound of CNS extract against *B. tabaci*. However, the the nymphal stage of *B. tabaci* most susceptible to CNS extract and no phytotoxic on the leaves were not performed. Therefore, concentration of 0.22 %, 0.67 %, 2.00 %, 6.00 % are used to test the efficacy of CNS extract against the soybean young nymphs (first and second instar) and old nymphs (third instar) of *B. tabaci*. Thus, before recommending of CNS extract based on efficacy in controlling the *B. tabaci*.

The objective of this results was to evaluate the activity of CNS extract for reducing phytotoxicity on soybean plant and determine the nymphal stage of *B. tabaci* most susceptible to CNS extract.

2. Materials and methods

Greenhouse experiments were carried out in Kedunggalar District in Ngawi City, East Java, with annual averages of 30 °C, and 90 % RH.

2.1. Rearing B. tabaci

Seedling of soybean were planted in the care cage net at 30 °C ± 1°C , RH 85 % ± 5 %. A large colony of adults *B. tabaci* was introduced on seedling of soybean in pot for 14 d. Thereafter, the insect were removed by the opening the net and shaking the plant. The plants covered with the cage net and the nymphal stage last for 9 d to 10 d before the adults stage. The abaxial part of the soybean leaves was checked the presence of eggs and introduce to the soybean plant until adult *B. tabaci*.

2.2. Cashew nut shell extraction

Shell of cashew nut were sliced and air dried to ground using blender. The powdered cashew nut shell was carried out by maceration in n-hexane and stirred for 1 h. Thereafter, the extract was incubated for 24 h and filtered until three times. The filtrate was distilled at 50 °C to separated oil and the solvent using rotary evaporator vacuum in water bath until viscous extract.

2.3. Acute toxicity (LC_{50} and LC_{90})

The lethal dose (LC_{50} and LC_{90}) of CNS extract was evaluated by seedling of soybean in the rearing cage net to get specific nymphal stages of *B. tabaci*. The seedlings containing nymphs of each instar in blocks were sprayed on the abaxial parts of the soybean leaves with CNS extract at concentration of 0.22 %, 0.67 %, 2.00 %, 6.00 %, which based in preliminary test.

2.4. Data analysis

2.4.1. Mortality effect of cashew nut shell extract on B. tabaci. Cashew nut extract concentration was compared with Imidacloprid active compound of insecticide (Movento Energy 240 SC). Its recommended commercial dose (0.50 mL L^{-1}). Control treatment used distilled water. After 6 d application, the number of living and dead nymphs were counted using a stereo-microscope. Nymphs were considered dead when all appendages were dark brown to black colour. Two leaves per replicate per treatment were removed to record total number of dead and living nymphs at each observation.

2.4.2. Comparative toxicity of cashew nu shell extract and imidacloprit against B.tabaci. In the experiment four concentration of Cashew nut shell extract Cashew nut extract (0.22 %, 0.67 %, 2.00 %, 6.00 %) and imidacloprit active compound of insecticide (Movento Energy 240 SC) with commercial dose (0.50 mL L^{-1}) was used. LC_{50} and LC_{90} was determined on the first, second and third instar nymphs of B. tabaci. Fifty of first, second, and third instar nymph were used per concentration and placed individually in glass vials with soybean leaves in the dark, respectively. The number of dead insect was assessed after 6 d application.

2.5. Analysis statistic.

The analysis of factorial experiment (nymphal instar and CNS extract concentration) was carried out according to a completely randomized design with four replications. Regression analysis were performed to assess of the relationship between nymphal mortality and CNS extract concentration. The normality and homogeneity of variance (HOV) tests were performed using GLM procedure with Welch option in SAS [6]. Non-homogeneity, percent mortality values were transformed using arc sin $(x/100)^{0.5}$ before analysis. Data were submitted to the analysis of variance to determine the interaction effect for instar, CNS extract concentration, and day after spraying. If there is a *significant* interaction effect was observed between factors. One for the factor level was compared to each of the other levels by by Tukey's test ($P < 0.05$). CNS extract concentrations were transformed using $\log_{10}(X)$. The probit analysis was used to determine LC_{50} and LC_{90} values with their associated 95 % confidence intervals of CNS extract at different concentration against *B. tabaci* nymphs. The probit regression (slope) was used to determine the correlation of log concentration with probit mortality at each CNS extract concentration against *B. tabaci* nymphs. The efficiency of extract were determined by the following formula Abbot.

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100\ \% \qquad (1)$$

Pt : Corrected mortality (%)
Po : Observed mortality
Pc : Control mortality

3. Results

The CNS extract at concentration of 2.00 % there was the potential mortality of the first, second and third instar nymphs of *B. tabaci* was compared insecticide with imidacloprit active compound. The mortality of increased gradually with the rise concentration of CNS extract. The mortality of first nymph reached the highest at concentration of 6.00 %. However, the mortality of second nymph decreased with the rise concentration (figure 1).

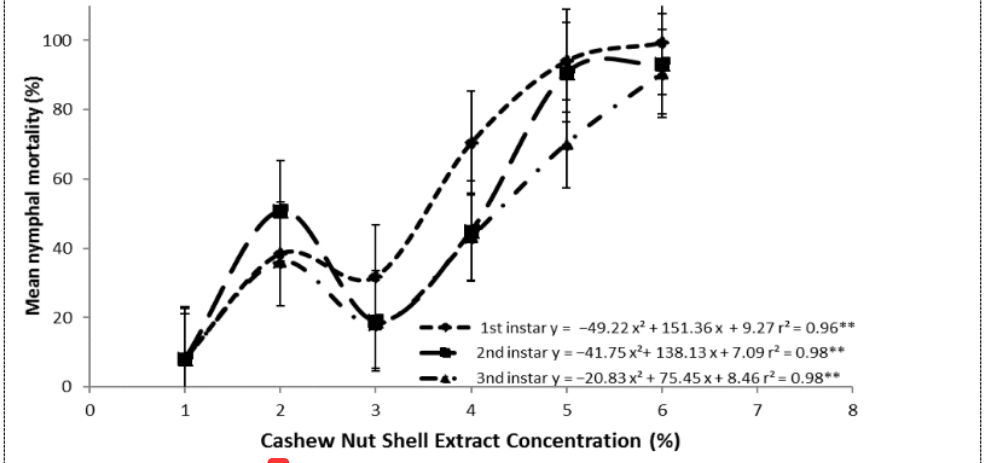

Figure 1. The mortality of the first to third instar nymphs of *B. tabaci* on leaves of soybean seedling after treatment with CNS extract at different concentration. **significant at 1% probability.

Each of the CNS extract at concentration of 6.00 % caused the greatest mortality rate of the *B. tabaci* nymphs of different stages (first to third instar) with 95.6 %, 90.9 % and 87.9 % efficiency, respectively. The CNS extract at 2.00 % and 6.00 % concentrations killed not significantly different for all nymphal stages of *B. tabaci*. Likewise, the ability of imidacloprit killed the second instar nymph not significantly different with CNS extract at concentration of 0.67 %. However, the mortality of second nymph showed significantly with the CNS extract at concentration of 0.22 %. (table 1).

Table 1. Mortality of the *B. tabaci* nymphs (first to third instars) on soybean leaves after application of CNS extract.

Compounds (%)	1st instar mortality (%)[1,*]	Efficiency (%)[2]	2nd instar mortality (%)[1,*]	Efficiency (%)[2]	3rd instar mortality (%)[1,*]	Efficiency (%)[2]
0.00	8.6 ± 3.7 d	0.0	8.1 ± 3.9 d	0.0	8.4 ± 0.9 d	0.0
Imidacloprit	38.2 ± 2.9 c	44.3	50.2 ± 6.6 b	63.1	36.2 ± 1.7 c	42.8
CNS (0.22 %)	31.7 ± 3.1 c	41.6	23.6 ± 1.5 c	32.6	20.3 ± 1.1 cd	30.6
CNS (0.67 %)	70.2 ± 4.8 b	80.4	48.1 ± 6.2 b	61.0	43.5 ± 4.8 b	57.4
CNS (2.00 %)	94.6 ± 3.6 a	91.2	91.2 ± 2.2 a	88.4	72.0 ± 2.7 a	82.6
CNS (6.00 %)	99.1 ± 3.0 a	95.6	92.7 ± 2.2 a	90.9	88.5 ± 2.7 a	87.9

[1] or analysis, the data were transformed using arc sin $(x/100)^{0.5}$
[1] Means ±SE followed by different letters in the columns differ by Tukey's test ($P < 0.05$)
[2] Determined after correcting the control mortality by Abbott's formula [8].

The highest mortalities of first instar nymphs were obtained with 2.00 % and 6.00 % of CNS extract. The two different lethal concentration levels (LC50 and LC 90) of each treatment was estimated by Probit analysis (X^2; $P < 0.0001$) and fiducial limits values of CNS extract agains *B. tabaci* nymphs of different stages are summarized in table 2.

Table 2. Lethal concentration of CNS extract (antifeedant active compound) compared with imidacloprit (neurotoxic insecticide) against *B.tabaci* nymphs (first to third instars).

Compounds (%)	Stage instar	[a]LC	[b]EV	[c]CI	[d]χ^2
Imidacloprit	1st	50	9.82	8.01 to12.41	14.10
		90	15.23	13.46 to 19.01	
	2nd	50	28.16	11.24 to 17.68	18.56
		90	36.82	28.19 to 39.36	
	3rd	50	9.73	7.62 to 12.33	13.65
		90	14.25	11.88 to 18.28	
CNS (0.22 %)	1st	50	11.43 (0.26*)	9.28 (0.21*) to 15.41 (0.33*)	10.22
		90	25.21 (0.53*)	19.08 (0.47*) to 22.86 (0.49*)	
	2nd	50	6.75 (0.14*)	5.85 (0.11*) to 8.41 (0.16*)	12.25
		90	11.01 (0.26*)	9.57 (0.22*) to 17.20 (0.43*)	
	3rd	50	6.45 (0.14*)	5.28 (0.10*) to 7.00 (0.13*)	9.85
		90	10.98 (0.25*)	8.95 (0.17*) to 12.80 (0.27*)	
CNS (0.67 %)	1st	50	19.18 (0.47*)	9.55 (0.23*) to 16.19 (0.31*)	21.18
		90	30.42 (0.44*)	27.61 (0.41*) to 32.75 (0.46*)	
	2nd	50	19.82 (0.48*)	18.02 (0.46*) to 23.11 (0.50*)	26.40
		90	21.25 (0.49*)	14.78 (0.28*) to 19.46 (0.47*)	
	3rd	50	18.25 (0.46*)	15.75 (0.34*) to 19.42 (0.47*)	20.01
		90	19.05 (0.47*)	16.86 (0.32*) to 21.07 (0.48*)	
CNS (2.00 %)	1st	50	68.95 (0.85*)	57.42 (0.74*) to 74.45 (0.89*)	33.57
		90	81.17 (0.92*)	77.65 (0.90*) to 82.17 (0.93*)	
	2nd	50	66.82 (0.84*)	55.42 (0.72*) to 68.45 (0.85*)	33.02
		90	79.17 (0.91*)	75.65 (0.89*) to 78.17 (0.90*)	
	3rd	50	20.15 (0.48*)	18.70 (0.46*) to 22.12 (0.49*)	29.98
		90	22.45 (0.49*)	19.96 (0.47*) to 21.27 (0.48*)	

Continue on next page

Table 2. Continued.

Compounds (%)	Stage instar	[a]LC	[b]EV	[c]CI	[d]χ^2
CNS (6.00 %)	1st	50	85.95 (0.95*)	93.80 (0.98*) to 95.15 (1.75*)	33.51
		90	91.15 (0.97*)	93.65 (0.98*) to 97.28 (1.28*)	
	2nd	50	83.77 (0.93*)	89.65 (0.96*) to 91.42 (0.97*)	31.42
		90	89.45 (0.96*)	91.22 (0.97*) to 95.55 (1.03*)	
	3rd	50	82.99 (0.92*)	86.94 (0.95*) to 89.42 (0.96*)	31.16
		90	86.20 (0.95*)	89.46 (0.96*) to 93.47 (0.98*)	

[a]LC_{50} and LC_{90} concentration of CNS extract causing 50 % and 90 % mortality, respectively; Fiducial limits based on a log scale with significance level at 95 % confidence limits; C, CNS extract concentration; χ^2, Chi square value for lethal concentration. *Estimated value (mg L^{-1}) in mg L^{-1}.

The LC_{50} and LC_{90} values indicated that CNS extract at concentration of 2.00 % (X^2 = 33.57 %; df = 5) were the most toxic compound to the first instar stage followed by CNS extract at concentration of 6.00 %, 0.67 %, imidacloprid, and CNS at concentration of 0.22 %, respectively. The concentration of 6.00 % showed the most toxic effect to the second stage followed by CNS extract at concentration of 2.00 %, 0.67 %, imidacloprid, and CNS at concentration of 0.22 %, respectively. The lethal concentration (LC_{50-90}) value of the CNS extract at concentration of 6.00 % (X^2 = 31.16; df = 5), at concentration of 2.00 % (X^2 = 29.98; df = 5), at concentration of 0.67 % (X^2 = 20.01; df = 5), imidacloprit (X^2 = 13.65; df = 5), CNS extract at concentration of (X^2 = 9.85; df = 5) showed the toxicity against the third instar nymph.

4. Discussion

The toxicity contained in the CNS extract will increase, if the concentrations used are the highest, so the physiological and developmental processes of the nymph are disrupted, but its reduced the number of third instar nymphs. No efficiency of mortality was recorded in the control treatment (0.00). A mortality rate of over 90 % was observed for the first and second instars at concentration of 2.00 and 6.00 %. Both first and second instars had higher mortality than the third instar. The third mortality percentage decreased to less than 90 %. Cashew nut shell compounds can penetrate into the leaves to cause antifeedant effect of early instar after the hatching of viable eggs and toxic effect. These compounds can be prevented ingestion of instar nymphs. Based on the qualitative observation, at concentration of 6 % caused phytotoxic, viz. brown spot on leaves, whitering followed by the death of plant tissues.

The previous studies have been carried out using neem oil at concentration of 1.00 % higher mortality of first instar stage than the other instars. Neem oil at concentration of 1.00 % could kill over 80 % of the first to third instar stages without phytotoxic effect of *Phaseolus vulgaris* L. and *Glycine max* L. plants in a screenhouse [7]. Increasing cashew nut shell liquid extract had negative effect of lettuce and tomato germination that was caused bioactive compound, viz. anacardic acid and cardol. Cashew nut shell liquid (CNSL) could be delayed larva and pupal periods of *Helicoverpa armigera* Hübner. It has been either shown that toxic effect of CNS extract has activity as antifeedant larva and reduced the number of eggs deposited by *Crocidolomia pavonana* F. Likewise, the mortality of nymphs was substantially inhibit the landing of the *B. tabaci* adults on the leaves. Ideally, phytochemical insecticides should be toxic pests with low or no impact on predators [8−10].

The LC_{50} and LC_{90} values with their limits at 95 % confidence interval indicated that lethality of imidacloprid compound were lower on *B. tabaci* than CNS compound. The lethal concentration (LC_{50} and LC_{90}) values was indicator to the level resistance of instar stage population response to percentage compound of CNS extract and imidacloprit. Therefore, the lethality of CNS extract is confined on stage instar of *B. tabaci* depending on the concentration evaluated. Mortality of *B. tabaci* was not restricted to the first instar nymphs. Lethal effect on the second instar nymps stage is also apparent after application with CNS extract compound at concentration of 2.00 %. Here, the toxicological

selectivity of CNS extract compound target are suitable as botanical insecticide for organic agriculture. It showed that CNS extract at concentration of 2.00 % could be developed as source of botanical insecticide to reduce the number of first to second instar nymphs of *B. tabaci* on soybean plant. On the other hand, CNS extract at concentration of 2.00 % as a botanical pesticide has many excellent attributes including its rapid degradation in the environment, and no phytotoxicity effect on the leaves of soybean seedlings. However, these botanical insecticides should not be considered to be the only solution. They may have toxic effects on parasitoids and predators [11].

5. Conclusion

First and second instar nymphs are more susceptible to CNS extract at concentration of 2.00 % than third instar nymphs. Cashew nut shell extract at concentration of 2.00 can reduce the population of first to second nymphal instars of *B. tabaci* by over 90 %, and no phytotoxicity effect on the leaves of soybean seedlings. The lethality of CNS extract is confirmed on stage instar of *B. tabaci* depending on the concentration. CNS extract at concentration of 2.00 % could be developed as source of botanical insecticide to reduce the number of first to second instar nymphs of *B. tabaci* on soybean plant.

Acknowledgments

The authors are very grateful to The *Penelitian Unggulan Dasar Perguruan Tinggi* Batch 2018 Contract No.004/SP3DUPT/Unmer.Mdn/LPPM/II/2018 February 27th 2018 fom Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding this research.

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