Toxicity Test on Wedusan (*Ageratum* spp.) Extract Against *Sitophilus* spp. (Coleoptera: Curculionidae)

Erinus Mosip, Bambang Tri Raharjo, Ludji Pantja Astuti

Faculty of Agriculture, Brawijaya University, Malang, Indonesia

ABSTRACT

Tropical condition in Southeast Asia has contributed around 30% of post harvest damage as it provide optimum condition for the growth of *Sitophilus* spp. pest. Therefore, this study was aimed to investigate the effect of wedusan extract on controlling *Sitophilus* spp. Growth. Wedusan extract was given in different level of concentrations. The research used the Randomized Complete Design. Wedusan (*Ageratum* spp.) extract affected the imago mortality of *Sitophilus* spp. There were two kinds of Wedusan extract used in this study, dried extract (DE) and fresh extract (FE). *Sitophilus* imago were put into bottles filled with organic rice and were treated with Wedusan extract. The mortality level was observed at 24 Hours After Application (HAA), 48 HAA, and 72 HAA. The highest toxicity was found by the treatment of dried extract of wedusan 5 tablets, by LC50 55% on 24 HAA and 82.25% on 72 HAA. Extract of dry matter has higher toxicity effect than fresh extract. The application of wedusan extract is environmental friendly and specific-range against pest. It is promising for future development as natural product in agriculture area.

Keywords: *Sitophilus* spp., *Ageratum* spp., toxicity, storehouse pest

INTRODUCTION

The quality and quantity of food and seed storages have become frequently reduced. One of the causes is the infestation of storehouse pest (*Sitophilus* spp.). Tropical climate and moist condition at Southeast Asia have damaged post harvest, which is estimated to reach 30% [1]. Storehouse pest controls have been done chemically, physically, and biologically. However, the chemical control is the most frequently used since the other more effective control methods have not been found yet [2]. Despite of the drawbacks that chemical pesticide bring, it still become the superior in controlling the storehouse pests [3].

The increasing serious problems, such as resistance, pesticide residue, and biosphere contamination as the effects of synthetic pesticide application in large-scale has encouraged various research on diverse materials including floral materials as the alternative insecticide to control pest [4]. Floral insecticide has some superiorities,

such as environmentally safe, short spectrum, easily degradable, safe residue for the stored products, and easily obtained [5].

The vegetation biodiversity in Indonesia has various potential of active materials that can also be used as the source of floral insecticides [6]. *Wedusan* (*Ageratum* spp.) is one of vegetations that is easily found at almost the whole areas in Indonesia, which is belongs to *asteraceae* family [7]. *Wedusan* contains some active compounds, such as saponin, flavanoid, polyphenol, and ageratochmone that have been known as toxic substances to insects [8]. It has been reported that these compounds may cause death on *Sitophilus* spp., inhibit insects growth, and may cause some defects. The application of *Wedusan* powder on *Sitophilus* spp. may increase its mortality and affect on its population. *Wedusan* chemical properties have been also reported in eliminating and controlling *Sitophilus* spp. pest as it is environmentally safe and harmless for human health [9]. Furthermore, it

*Corresponding author:

Erinus Mosip
Faculty of Agriculture, Brawijaya University
Jalan Veteran, Malang, Indonesia 65145
E-mail: leksimosip@yahoo.co.id

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is required to conduct study about the toxicity test of *Wedusan* extract against *Sitophilus* spp. in order to investigate the effectiveness of *Wedusan* as floral insecticide [10].

MATERIALS AND METHODS

Research design and sample preparation

The research design was Randomized Complete Design (RCD) by two factors and six treatments (four replications). *Sitophilus* spp. was collected from rice for the poors to be put into new contained filled with 0.5 kg and placed in a room at $\pm 29^{\circ}\text{C}$ on average and average humidity $\pm 75\%$. Rearing procedures of *Sitophilus* spp. was performed is modified from established method by International Rice Research Institute (IRRI) [11]. Three hundred grams of *Wedusan* extract was homogenized with 50 ml Tween 80 and 300 ml aquadest. The homogenized extract was then mixed with 300 g rice flour and stirred well until form pasta. Furthermore, the pasta was mold into tablet.

A total 5 kg of organic rice was previously kept at cold temperature for 24 hours in order to avoid any infestation of pests and other pathogens. Rice were ten divided into 250 bottles using analytic scale or 20 gram rice for each bottle (height: 9 cm, diameter: 5 cm). Each bottle was labeled according to the treatment. The imago were then infested approximately at the same age, which each bottle containing 20 *Sitophilus* spp. imago.

Toxicity test on *Wedusan* extract

The mortality of insects was observed following the treatment at 24 hours, 48 hours, and 72 hours after application (HAA). The percentage of insects' mortality could be a good indicator for insecticidal activity [12]. Mortality percentage of *Sitophilus* spp. was counted using the equation below:

$$\text{Mortality} = \frac{\text{Died } \textit{Sitophilus} \text{ spp.} \times 100\%}{\text{Tested } \textit{Sitophilus} \text{ spp.}}$$

If death was found on the control (not more than 20%), then death percentage should be corrected by equation [13] as follow:

$$p = \frac{(x - y)}{x} \times 100\%$$

Note:

p is the percentage of the corrected death

x is the percentage of the life of the control

y is a percentage of the living pest at the treatment

LC₅₀ was calculated using probit analysis program (probit value 50%) or manually using the obtained regression equation. The relationship between extract's concentration and mortality level of insects was analyzed through regression analysis. The obtained probit regression determined the value of LC₅₀ (the optimum dose of extract to eliminate 50% of the insect population). It can be calculated using the equation [14] below:

$$y = bx + a$$

Note:

y is concentration log

x is probit number

Data analysis

Data was analyzed using analysis of variance (ANOVA) with significant level of 0.05. If any difference between treatments was found on the results, it may be followed by further test using Least Significant Difference (LSD) of 5%.

RESULTS AND DISCUSSION

The mortality level of insects (Table 1) shows that *Wedusan* extract treatment against *Sitophilus* spp. at 24 hours after application are significantly difference at different concentrations. Treatment by DEy5 (dry extract of the concentration of 5 tablets) contributed the highest mortality level of *Sitophilus* spp. at all observation times. However, the lowest mortality level was obtained from the control groups of DEy0 and FEy0. Furthermore, DE (dry extract of *Wedusan*) performs better in inducing the mortality of *Sitophilus* spp. than FE (fresh extract of *Wedusan*).

In a closer look, the effect of DE y4 (4 tablets) in inducing mortality of *Sitophilus* spp. is better than of DE y3 (3 tablets). Moreover, DE y3 performance is better than DE y2 (2 tablets). In the other words, the higher concentration of *wedusan* extract was given the higher mortality level of *Sitophilus* spp. Likewise, the fresh material of *Wedusan* at concentration of FE y4 (4 tablets) performs better in inducing the mortality of *Sitophilus* spp. than FE y3 (3 tablets).

In average, the mortality level of insects induced by dry extract was higher than induced by fresh extract. It is expected caused by the active materials contained in dry extract are more concentrated than in fresh extract due to low water content. On the other hand, fresh extract has more water content and less rendement in the form of fine extract. Therefore, in order to obtain an

Table 1. Average mortality of *Sitophilus* spp.

Concentration Level	Types of Extract											
	DE (dry extract)						FE (fresh extract)					
	Hours after application (HAA)						Hours after application (HAA)					
	24	48	72	24	48	72	24	48	72	24	48	72
y0	0.71	d	0.71	d	0.71	e	0.71	e	0.71	e	0.71	e
y1	1.76	c	2.21	c	2.39	d	1.48	d	2.06	d	2.73	d
y2	2.05	c	2.49	b	2.87	c	1.86	c	2.45	c	2.91	cd
y3	2.39	b	2.64	b	3.81	b	2.12	bc	2.45	c	3.81	c
y4	3.12	a	3.9	a	4.15	a	2.34	b	2.78	b	3.31	b
y5	3.39	a	4.12	a	4.21	a	3.08	a	3.39	a	3.37	a
LSD 5%	2.028											

Note : - Average numbers followed by the same letter in the same column show insignificant differences on level test of 5%, HAA = Hours After Application, DE = dry extract, FE = fresh extract.
 - Different alphabets indicate significant differences between treatment.

insecticidal extract, it should be aerated dry first to reduce water content [15].

Overall, there were some differences found between the lowest and the highest concentrations (1 tablet and 5 tablets of *Wedusan* extracts) respectively, which leads to different effect of mortality. The higher concentration of *Wedusan* extracts induce the higher mortality of *Sitophilus* spp. imago. As it was presumed that such response was caused by more and stronger chemical compounds of *Wedusan*, which become toxic to *Sitophilus* spp.

Insects with low survival ability died more quickly within 24 hours after application (HAA) or less than 24 HAA. Meanwhile, the resistant insects might die within 48 and 72 HAA. The insect's death and its adaptation towards extract treatment are the natural mechanisms as self-defence from the environmental stress.

Mortality on *Sitophilus* spp. was presumed to be the effect of activity of floral insecticide compounds in of *Wedusan* extract. Those active compounds including coumarin, flavonoid, alkaloid, saponin, triterpenoid, phenol, and ageratochmone. It was presumed that *Wedusan* extract entered into insects' body through respiratory organs. As the result, imago died immediately following the treatment [16].

Insecticide that enter into the insect's body through respiratory tract as a result of fumigation will be distributed and accumulated, which furtherly form lethal toxin for the insects. Besides, it was presumed that the pest's death was caused by toxic compound in *Wedusan* extra-

ct that entered into insect's body through digestive tract [17].

Wedusan contains coumarine compounds, which can interrupt the electron transport in respiration process. Coumarin plays role as inhibitor in electron transport process on mitochondria and reduce ATP production. Therefore, it may result in muscular disturbance due to the lack of energy, which is required for cellular activities. This would make the insects die. Surangin B is one of Coumarin molecule compounds. Another research has reported activities of these compounds group to affect other insect species as well. It has been reported that these compounds are considered as antifeedant and growth inhibitor for insects, and they may disturb the hormonal mechanism. Surangin B is a protein inhibitor of electron in mitochondria transport trough in vitro tha can be toxic for insects [18].

The flavonoid contain in *Wedusan* has catecholic ring-B, which can be toxic against the insects [19]. In this context, there is a correlation between Rotenone antimycin A, and hydramethylnone as floral insecticides in which all of those chemical elements have blocked the electron transport in insects' transpiration process [20]. In addition, coumarin and furanocoumarins may change the detoxification ability of an organism, which reversibly and irreversibly inhibits cytochrome detoxification enzyme.

After all, *Wedusan* is potentially can be utilized as the substitute of synthetic insecticide. The extract is packed in tablets that works slowly by releasing aromatic

Table 2. Result of analysis LT_{50}

Concentration (Tablet)	Dry Extract		Fresh Extract	
	Equation	LT_{50}	Equation	LT_{50}
y1 (4.4)	$y = 2.590 + 0.967 x$	310.82	$y = 0.775 + 2.041 x$	117.56
y2 (8.8)	$y = 2.377 + 1.247 x$	126.72	$y = 1.690 + 1.635 x$	105.80
y3 (13.1)	$y = 2.927 + 1.017 x$	108.97	$y = 2.096 + 1.452 x$	99.92
y4 (17.5)	$y = 1.773 + 2.278 x$	26.12	$y = 2.206 + 1.509 x$	70.98
y5 (21.9)	$y = 2.209 + 2.140 x$	20.15	$y = 3.504 + 0.984 x$	33.16

Note: LT_{50} = Lethal timeTable 3. Result of analysis LC_{50}

Time (Hour)	Dry Extract		Fresh Extract	
	Equation	LC_{50}	Equation	LC_{50}
24	$y = 2.525 + 1.850 x$	21.757	$y = 2.434 + 1.661 x$	35.062
48	$y = 2.365 + 2.429 x$	12.157	$y = 3.205 + 1.260 x$	26.56
72	$y = 2.528 + 2.496 x$	9.7744	$y = 3.901 + 0.972 x$	13.491

Note: LC_{50} = Lethal concentration

gas in a closed room. It can be used as fumigant tablet. Fumigation using *wedusan* extract could induce mortality of insects [21]. Toxicity test using LC_{50} method is an acute toxicity test method, in which the toxic effect of compounds could be determined in a short time following the application of tested concentration [21].

LC_{50} values are the required concentrations to eliminate 50% of the tested insects. LC_{50} values in Table 2 reduced along with the longer duration of observation. The longer duration of the observation, the higher mortality percentage of the insects.

In order to accelerate the toxicity reaction on the insects to higher concentration of *Wedusan* extract is required to eliminate 50% of the insects for 24 hours. If the concentration was reduced, it might take longer time to eliminate 50% of the insects as the insecticide may react systemically.

Data of the research showed that the lower concentration of the floral insecticide extract needs the longer time to eliminate 50% of the insects. It is presumed due to less toxin contained in the low concentration of insecticide that reduces the effectivity of extract to induce insect mortality. If the chemical reaction is slow, it may take longer to eliminate 50% insects, while under the higher concentration of insecticide it may take the relatively shorter time. Furthermore, LC_{50} values reduced along with the observation duration, in which the longer

the observation time, the higher level mortality of *Sitophilus* spp. will be observed [22].

CONCLUSION

Wedusan (*Ageratum* spp.) extract affects against mortality of *Sitophilus* spp. imago. The highest toxicity level was found by the application of 5 tablets of dry *wedusan* extract treatment, by the value of LC_{50} 55% at 24 HAA and 82.25% at 72 HAA. Dry extract showed higher toxicity effect than fresh extract.

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