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The Effectiveness of Bandotan (*Ageratum conyzoides* L.) Leaf Extract on Mortality of *Boophilus microplus In Vitro*

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Abstract

The purpose of this research is to find the effectiveness of bandotan's (*Ageratum conyzoides* L.) leaf extract on mortality of *Boophilus microplus in vitro* and also to get of *lethal concentration* and *lethal time*. This research used 125 *Boophilus microplus*. This research used five treatments, namely neguvon suspensionon (K+), ethanol 1% (K-), 1% concentration (P1), 2% concentration (P2) and 3% concentration (P3). The observation of mortality was done every six hours. *Boophilus microplus* dies when there is no movement. The data were analyzed using *one-way* ANOVA and followed by Duncan's Multiple Distance Test. The result is said to be significant if $p<0.05$. Probit analysis of LC_{50} is 1.63% at the first hour, 0.85% at the second hour, 0.54% at the third hour, 0.34% at the fourth hour, 0.04% at the fifth hour and 0.04% at the sixth hour, LC_{90} reached by using 5.6%, 2.9%, 1.8%, 1.2%, 0.16 and 0.16% concentration in 1, 2, 3, 4, 5 and 6 hours, respectively. The LT_{50} of ethanol 1% (K-) was at 10.142 hours, neguvon suspension on (K+) was at 0.747 hours, 1% concentration (P1) was at 2.184 hours, 2% concentration (P2) was at 1.579 hours, 3% concentration (P3) was at 0.923 hours and LT_{90} was at 23.734, 1.749, 5.110, 3.695 and 2.159 hours. The conclusion of this study was bandotan's (*Ageratum conyzoides* L.) leaf extract was effective as an acaricide based on the mortality of *Boophilus microplus*.

Keywords: *Ageratum conyzoides*, *Boophilus microplus*, leaf extract, neguvon, probit analysis, leaf extract

Introduction

Tick is one of the important ectoparasites of the subphylum Chelicerata and often infests cattle by sucking blood on the body surface of livestock. *Boophilus microplus* is a tick that has thick skin and is important in cattle farming because it causes great losses. Ticks can act as hosts between diseases caused by protozoa, bacteria, viruses, spirochaetes, rickettsiae, chlamydia and other disease agents (Hendrix, 2012). Wounds from the bite of a large number of ticks can attract Chrysomia flies (green flies) to lay eggs on the wound and then cause myiasis. These conditions can cause the livestock body to become weak, decreased appetite, fever accompanied by decreased milk production and body weight. Another disadvantage of tick infestation is that the selling price of cowhide is cheap or it is rejected, which is detrimental to farmers (Sukarsih *et al*., 1999; Gunandini, 2006).

The losses caused by *B. microplus* infestation are quite large in livestock. This loss can be minimized in various ways. The way farmers deal with *B. microplus* infestations is by taking and killing ticks one by one, scraping using a knife, bathing the livestock using soap and using synthetic acaricides from the officers (Manurug, 2002). Indiscriminate and repeated use of synthetic acaricides can cause various problems. These problems are related to environmental pollution, development of resistance in target species and the subsequent increase in costs (Jonsson and Piper, 2007). Many problems arise due to the use of synthetic acaricides. It is necessary to look for medicinal materials that are able to kill ticks without any resistance, prices are relatively cheap and easy to obtain, so that they are affordable by farmers

in rural areas, safe for the environment and human health.

Bandotan plant (*Ageratum conyzoides* L.) is a nuisance weed that lives in the wild and is found in many areas, although people often think of it as a nuisance plant. Kardinan (in Shinta, 2010) explains the potential of bandotan as a vegetable pesticide/insecticide because it contains compounds including saponins, flavonoids, polyphenols, alkaloids, edultin, friedelin, and prekosenkumarine, 5% eugenol, HCN, and essential oils. Bandotan plants as insecticides and nematicides (Octavia *et al*., 2008; Samsudin, 2008). Rosida (2005) proved that bandotan leaf extract functions as a larvicide that can eradicate *Aedes aegypti* mosquito larvae. The content in bandotan leaves is the basis of reference that bandotan leaves can be used as a vegetable acaricide against *B. microplus*. Research on bandotan leaves as an acaricide is still very rare or not even done. Based on the above, the researchers want to test the effectiveness of acaricide in bandotan leaf extract on mortality of *B. microplus* in vitro by using the spraying method.

Materials and Methods

This study used a post test control only group design. Each treatment used five repetitions and each replication used 5 *B. microplus*. The treatments consisted of 1% ethanol solvent (K-), neguvon suspension (K+), bandotan leaf extract at a concentration of 1% (Pl), 2% (P2), and 3% (P3).

Observation of tick death time was carried out every 1 hour for 6 hours. Observations were made by calculating the percentage of dead ticks. Dead ticks are characterized by a rigid body condition with irregular foot positions, not moving, and not responding to stimuli when touched (Wahyu, 2017).

The research sample was obtained from adult ticks which have the same relative size from Ongole Peranakan cattle in Tahunan Village, Sale Sub-District. The ticks were taken carefully so that no parts were cut off, then put into a sample bottle and immediately brought to the Laboratory of the Fisheries and Livestock Service Office of Blora District for observation and testing.

Observations and records were made of the number of dead ticks. The data obtained were analyzed using a one-way analysis of variance parametric test (One Way ANOVA), which was followed by Duncan's follow-up test. Probit analysis was used to determine the LC50, LC90, LT50 and LT90 of bandotan leaf extract at each hour of observation.

Results and Discussion

The identification of *B. microplus* ticks in this study was carried out macroscopically. The results of macroscopic identification showed that the *B. microplus* tick has a body covered with thick skin (integument), so it is included in the hard tick category. The dorsal part of this tick has a scutum or shield that covers the entire dorsal body of the male tick, while in the female the scutum only covers the anterior third of the body, therefore the female tick's body can grow larger than the male after sucking blood (Fig. 1).

Figure 1. *Boophilus microplus* after suck blood of host.

The results showed that *B. microplus* that had been given bandotan leaf extract by spraying had differences with the controls who were not given any treatment. The treated ticks changed color to green due to exposure to bandotan leaf extract. Another visible change is the tick's body becomes stiff with an irregular foot position, not moving. Based on the results of statistical analysis using one- way ANOVA, it was found that there was a significant difference between treatment groups in the average number of deaths of *B. microplus* at each hour of observation ($p < 0.05$). In Duncan's multiple-distance test, the mean and standard deviation of the mortality of *B. microplus* were obtained. The mean and standard deviation of mortality of *B. microplus* due to the administration of bandotan leaf extract at every hour of observation can be seen in Table 1.

Table 1. Mean and Standard Deviation of Mortality of *B. microplus* given Bandotan Leaf Extract.

Treatment	Observation Time					
	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
K(+)	64.00^{d} ± 16.733	92.00^{c} ± 10.954	100.00^{c} ± 0.000	100.00^{c} ± 0.000	100.00^{b} ± 0.000	100.00^{b} ± 0.000
K(-)	0.00^{a} ± 0.000	4.00^{a} ± 8.944	16.00^{a} ± 8.944	24.00^{a} ± 16.733	36.00^{a} ± 16.733	44.00^{a} ± 21.909
P1	16.00^{b} ± 8.944	44.00^{b} ± 8.944	64.00^{b} ± 16.733	72.00^{b} ± 17.889	92.00^{b} ±10.954	100.00^{b} ± 0.000
P2	36.00^{c} ± 8.944	56.00^{b} ± 8.944	76.00^{b} ± 16.733	88.00^{c} ± 10.954	100.00^{b} ± 0.000	100.00^{b} ± 0.000
P3	56.00^{d} ± 16.733	84.00^{c} ± 8.944	96.00^{c} ± 8.944	100.00^{c} ± 0.000	100.00^{b} ± 0.000	100.00^{b} ± 0.000

Different superscripts a.b,c in the same column showed significant differences ($p<0.05$).

Based on probit analysis, it showed that the Lethal Concentration of 50 bandotan leaf extract against the *B. microplus* at 1, 2, 3, 4, 5 and 6 hours respectively was 1.63%, 0.85%, 0.54%, 0.34%, 0.04%, 0.04% and LC_90 bandotan leaf extract at 1,2,3,4,5,6 hours were 5.6%, 2.9%, 1.8%, respectively. 1.2%, 0.16 and 0.16%. LT_{50} values in negative control, positive control and bandotan leaf extract concentrations of 1%, 2%, 3% respectively were 10.142 hours, 0.747 hours, 2.184 hours, 1.579 hours and 0.923 hours while the LT90 values were 23.734 hours, 1.749 hours, 5.110 hours, 3.695 hours and 2.159 hours. Based on LC50, LC90, LT50 and LT90, it was explained that bandotan leaf extract with a concentration of 3% showed a faster time to kill ticks by 50% and 90% compared to other concentrations and the ability to kill the test ticks was almost the same as the positive control. This shows that bandotan leaf extract with a concentration of 3% is more effective than the concentration of 1% and 2%. Aquades is used as a solvent for bandotan leaf extract because it is a neutral and harmless compound, so it is safe to use and does not interfere with the health of the test ticks. The addition of 1% ethanol to bandotan leaf extract is intended as a suspensator so that bandotan leaf extract can be optimally dissolved in distilled water.

Acaricide is a substance that contains chemical compounds that are used to kill or eradicate mites (Melinda, 2020). According to Melinda (2020), acaricides usually have a dual function, namely as a mite killer and also as an insect killer, because mites are sometimes classified as insects. One of the plants thought to have potential as an acaricide is bandotan leaf. The content of bioactive compounds in bandotan leaves include saponins, flavonoids, polyphenols, and essential oils that can prevent pests from approaching plants (repellents) and inhibit larvae from becoming pupae (Samsudin, 2008). Phytochemical analysis of *A. conyzoides* L. which was carried out by Amadi *et al.* (2012) showed that the main compounds in bandotan plants, namely alkaloids and flavonoids, accumulate in the leaves. According to Mahendra (2010), bandotan leaves can be developed as a botanical insecticide because they contain active ingredients, namely saponins, tannins, flavonoids, polyphenols and contain essential oils.

Astriani (2010) stated that the chemical content of bandotan is saponins, flavonoids, polyphenols, eugenol and bandotan roots contain essential oils, so bandotan plants can be used as environmentally friendly pesticides. According to Kardinan (2001), bandotan plants have advantages as vegetable pesticides because bandotan plants contain the active compound precocene, besides bandotan leaves also contain alkaloids, flavonoids, coumarins, saponins, polyphenols, and essential oils. Gani (2010) stated that precocene compounds work by interfering with the work of the nervous system in the insect body. Alkaloids are bitter compounds that can cause pests/insects to have no appetite and then die. Alkaloids are also able to inhibit insect growth so that it can cause metamorphosis failure (Dewi, 2016).

Conclusion

Bandotan leaf extract was effective as an acaricide for *B. microplus* in vitro. The lethal concentration of 50 bandotan leaf extract against the *B. microplus* at 1, 2, 3, 4, 5 and 6 hours respectively was 1.63%, 0.85%, 0.54%,

0.34%, 0, 04% and 0.04%, while the LC_90 bandotan leaf extract was 5.6%, 2.9%, 1.8%, 1.2%, 0.16 and 0.16%, respectively. Lethal Time 50 bandotan leaf extract concentrations of 1%, 2%, 3% respectively were 2.184 hours, 1.579 hours and 0.923 hours while the LT90 values were 5.110 hours, 3.695 hours and 2.159 hours.

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