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The Effectiveness of Eco Enzymes to Suppress the Development of Sarcoptes scabiei Mites In Vitro

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Abstract. Scabies is a disease caused by the mite Sarcoptes scabiei that attacks goats. It is quite difficult for traditional goat breeders to obtain scabies medicine because of the high price, so alternative treatment is necessary. This study aims to determine the effectiveness of eco-enzymes as an alternative for the treatment of scabies in goats. The study was conducted at the Sei Putih Goat Research Workshop, Galang District, from September to October 2021. This study used a factorial completely randomized design because there were two factors, namely the concentration of ecoenzymes (0%, 25%, 50%, 75%, 100%) and time difference (0 hours, 2 hours, 4 hours, 6 hours, 8 hours) and repeated 4 times. Each replication contained 10 mites. The parameters used were the number of dead mites, mite death rate and mite lethal concentration. The results showed that the ecoenzyme was effective in killing and suppressing the growth of Sarcoptes scabiei mites in vitro. The LC50 value is 40.45% which proves that the eco-enzyme is able to kill mites in goats.

Keywords : eco enzymes, drugs, goats, lethal concentration, sarcoptes scabiei

1. Introduction

The provision of animal protein needs for humans is obtained from the livestock sector. One type of livestock that is able to meet human needs for protein derived from animals is goat. Goats are widely distributed in rural areas which are kept as beef cattle and dairy cattle which can be consumed and the manure can be used for plant fertilizers [1]. In terms of maintenance and business techniques, goats are relatively easier to cultivate when compared to cattle [2]. The challenge that is usually faced by breeders is that goats are often exposed to various types of diseases, one of which is a disease that arises due to parasites, namely scabies, the cause of which is the mite Sarcoptes scabiei. Scabies is a disease that attacks the skin [3]. The scabies mite has a very small size and can only be seen by the eye directly if there is enough light. In some cases, the presence of mites can only be confirmed positive by microscopic examination [4]. [5] The spread of scabies is too fast because cattle often scratch the skin that causes itching or rub their bodies against other livestock or on logs. Scabies is a zoonotic disease so that farmers and their families can be infected after a few hours of contact with infected livestock [6]. Livestock affected

by scabies experience a decrease in livestock body condition, which can have a negative impact on maintenance and the environment because it is zoonotic [7]. In dealing with scabies in general, veterinarians will give certain drugs such as ivermectin, neguvon and asuntol [8]. Medicines to treat scabies are given by injection. It is necessary to find alternative medicine in the treatment of scabies. One alternative medicine to cure scabies in goats is neem leaf extract [9]. Eco-enzymes can be used as bio-disinfectants [10]. [11] Eco enzymes contain acetic acid and lactic acid. So it is necessary to do research on the effect of the concentration of the most effective eco-enzyme in killing Sarcoptes scabiei mites in vitro. [11] Eco enzymes contain acetic acid and lactic acid. So it is necessary to do research on the effect of the concentration of the most effective eco-enzyme in killing Sarcoptes scabiei mites in vitro.

2. Materials and Methods

The research was conducted at the Sei Putih Slaughter Goat Research Workshop, Galang District, Deli Serdang Regency, North Sumatra. The study took place from September 2021 to October 2021.

The tools used include measuring cups to measure eco-enzymes and aquadest, scabies to take samples of scabies scabies, petri dishes for containers for scabies scabs, filter paper to filter eco-enzymes, small bottles to store eco-enzymes, dropper pipettes to drip the eco-enzyme solution onto mites, a microscope to observe the mites, a needle to separate the mites and scabs.

The materials used include the mite Sarcoptes scabiei originating from goats as research subjects, eco-enzymes made from pineapple, papaya and bananas that have been fermented for 3 months as a scabicide liquid, aquadest as a solvent for eco-enzymes and betadine to treat wounds in goats.

2.1. Research design

This research was conducted experimentally using a factorial completely randomized design because there are two factors, namely the concentration of ecoenzymes (0%, 25%, 50%, 75%, 100%) and time differences (0 hours, 2 hours, 4 hours, 6 hours, 8 hours) and repeated 4 times. Tabulation of research data using a factorial completely randomized design as follows:

Table 1. Tabulation of research data using a factorial completely randomized design

Time	Concentration				
	P0 : Control (Aquadest 100%)	P1 : Eko Enzyme 25%	P2 : Eko Enzyme 50%	P3 : Eko Enzyme 75%	P4 : Eko 100% Enzyme
0 hours	P0W0	P1W0	P2W0	P3W0	P4W0

2 hours	P0W1	P1W1	P2W1	P3W1	P4W1
4 hours	P0W2	P1W2	P2W2	P3W2	P4W2
6 hours	P0W3	P1W3	P2W3	P3W3	P4W3
8 hours	P0W4	P1W4	P2W4	P3W4	P4W4

The linear model of factorial completely randomized design is:

$$Yijk = + i + j + (\alpha\beta)ij + ijk$$

i = 1, 2, 3, 4 and 5

j = 1, 2, 3, 4 and 5

k = 1, 2, 3, …, r

Yijk = observational value of factor a at level i, factor b at level j and repetition k

= general mean

i = influence of factor a at level i

j = influence of factor b at the j-th level

(αβ) ij = interaction of factor a at level i and factor b at level j

ijk = Effect of error on factor a at level i, factor b at level j and k-th test

This study used an in vitro method, namely taking samples of mites from goats infected with scabies and then placing samples of mites that had been treated with eco-enzymes and observed with a microscope.

In this study there were 5 treatments, namely control with 0% eco-enzymes, P1 with 25% eco-enzymes, P2 with 50% eco-enzymes, P3 with 75% eco-enzymes and P4 with 100% eco-enzymes. The treatment in this study is as follows:

P0 : Control (0%)

P1 : 25 ml of eco-enzyme + 75 ml of aquadest (25%)

P2 : 50 ml eco enzyme + 50 ml aquadest (50%)

P3 : 75 ml of eco-enzyme + 25 ml of aquadest (75%)

P4 : 100 ml of eco-enzyme (100%)

2.2. Research Parameters

1. Number of Dead Mites
2. Dead Mite Speed
3. Deadly Concentration

2.3. Research Stages

Eco Enzyme Making

In this study, eco-enzymes were made with molasses, fruit and water in a ratio of 1: 3: 10. The fruits used in the manufacture of eco-enzymes for this study were pineapple, papaya and banana. With this comparison, the eco-enzyme was made with 500 grams of molasses, 1.5 kg of fruit (pineapple, papaya and banana each 500 grams) that had been cut into small pieces and 5 liters of water. The resulting mixture of all these ingredients is then put into a 10 liter jerry can then fermented for 3 months in a dark room.

Petri dish preparation

20 petri dishes were prepared which were used as containers for mites to be labeled according to the treatment and replication. Petri dishes are washed with clean water and then dried with a tissue first so that no dust sticks to it, making it easier to observe.

Mite Retrieval

Mites are obtained by scraping the skin of goats infected with scabies. Scraping can be done in the area of the goat's ear and then scraped using a scalpel until the scab part is separated from the goat's ear. The results of the scab from the goat's ear were collected in a petri dish and then taken to the laboratory for selection. Removal of mites from the scab is done using a syringe and carried out under a microscope. The part of the goat's ear that has been scraped is given betadine to prevent infection and accelerate wound healing.

Mite Selection

Selected adult mites with uniform size were then put into a petri dish, 10 mites each in each petri dish. In selecting mites, a needle is used to make it easier to separate the scab mites from the goats. Mites that have been separated from the scab can be directly put into a petri dish.

Making Eco Enzyme Solution

In this research, the eco-enzyme solution will be made with different concentrations, namely 0%, 25%, 50%, 75% and 100%. In making the eco-enzyme solution, aquadest was used as the solvent. For 0% eco-enzyme concentration, 100 ml of eco-enzyme was used completely, 25% eco-enzyme concentration was used 25 ml of eco-enzyme and 75 ml of aquadest, 50% eco-enzyme concentration was used 50 ml of eco-enzyme and 50 ml of aquadest, 75% eco-enzyme concentration was used 75 ml of eco-enzyme and 25 ml of aquadest, and 100% eco-enzyme concentration used 100 ml of eco-enzyme without aquadest mixture. After all the solutions are mixed and then stirred to mix evenly until homogeneous then the solution is placed in different bottles and labeled according to the concentration of the solution.

Eco Enzyme Application

The application of eco-enzymes to mites was carried out by dripping the eco-enzyme solution using a dropper on the mites in a petri dish with varying concentrations of eco-enzymes, namely 0%, 25%, 50%, 75% and 100%. Each petri dish was given one drop of the ecoenzyme solution and the petri dish was left open during the observation.

Observation Method

Observations were made every 2 hours for 8 hours after the treatment was given to count the number of dead mites through a microscope. The data that has been obtained is then calculated and analyzed.

3. Results and Discussion

Number of Dead Mites

Table 2. Number of dead mites

Treatments	Test	Time				
		0 hours	2 hours	4 hours	6 hours	8 hours
P0 : Control (Aquadest 100%)	U1	0	0	0	0	0
	U2	0	0	1	1	1
	U3	0	0	0	1	1
	U4	0	0	0	0	0
P1 : Eko Enzyme 25%	U1	0	0	1	3	4
	U2	0	2	3	5	5
	U3	0	1	3	4	5
	U4	0	3	4	4	4
P2 : Eko Enzyme	U1	0	1	5	7	9
	U2	0	3	5	8	9

50%	U3	0	4	4	6	8
	U4	0	3	6	8	10
P3 : Eko Enzyme 75%	U1	0	6	7	7	7
	U2	0	5	7	8	10
	U3	0	4	6	7	9
	U4	0	5	7	9	10
P4 : Eko Enzyme 100%	U1	0	8	10	10	10
	U2	0	6	8	9	10
	U3	0	7	9	10	10
	U4	0	7	8	10	10

Based on the observations, it can be seen that the increase in the number of dead mites is directly proportional to the increase in the concentration of the applied ecoenzyme. The higher the concentration of the eco-enzyme, the higher the levels of acetic acid and lactic acid contained in the eco-enzyme solution, resulting in an effect on the death of mites. The acetic acid contained in the eco-enzymes is insecticidal and capable of being toxic to insects such as mites which indicates that the acetic acid plays a role in killing mites. This is in accordance with the statement of [12].

Table 3. Analysis of variance in the number of dead mites

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	2980,000a	25	119,200	203.182	.000
Treatment	585,460	4	146,365	249,486	.000
Time	544,060	4	136,015	231,844	.000
Treatment * Time	185,840	16	11,615	19,798	.000
Error	44,000	75	.587		
Total	3024,000	100			

Based on the analysis of variance data, it can be concludedThere was a relationship between the increase in the concentration of the ecoenzyme given to the number of dead mites. This calculation also shows that the higher the dose of eco-enzyme has a higher mortality effect on mites. It can also be seen that the longer the time used, the higher the number of dead mites. There is an interaction between the higher the dose of the eco-enzyme given and the longer the time it affects the mortality rate of mites. The results of the analysis are then continued into the following Duncan test.

Table 4. DMRT test results the number of dead mites

O'clock	Eco Enzyme Concentration					
	P0 : Control (Aquadest 100%)	P1 : Eco Enzyme 25%	P2 : Eco Enzyme 50%	P3 : Eco Enzyme 75%	P4 : Eco Enzyme 100%	Average
0 hours	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a

2 hours	0.00a	1.50b	2.75c	5.00d	7.00e	3.25b
4 hours	0.25a	2.75c	5.00d	6.75e	8.75fg	4.70c
6 hours	0.50ab	4.00d	7.25e	7.75ef	9.75gh	5.85d
8 hours	0.50ab	4.50d	9.00gh	9.00gh	10.0h	6.60e
Average	0.25a	2.55b	4.80c	5.7d	7.10e	20.4

Based on “Table 4”, it can be seen that the concentration of eco-enzymes is directly proportional to the mortality of mites. The highest average of dead mites was found in P4 with a value of 7.1 and the lowest average of dead mites was found in P0 with a value of 0.25. The highest average time is at 8 hours with a value of 6.60 and the lowest average time is at 0 hours with a value of 0.00. It can be concluded that P1 is significantly different from P0. P2, P3 and P4 were significantly different from P0. From the table, it can be shown that P1 at 2 hours showed an interaction between the concentration of the eco-enzyme and the time it was able to kill mites. The best concentration to be applied is P1 because the use of eco-enzymes is more efficient than P2, P3 and P4.

Dead Mite Speed

Table 5. Mite death rate

Treatment	Test	Time				
		0 hours	2 hours	4 hours	6 hours	8 hours
P0 : Control (Aquadest 100%)	U1	0.000	0.000	0.000	0.000	0.000
	U2	0.000	0.000	0.250	0.167	0.125
	U3	0.000	0.000	0.000	0.167	0.125
	U4	0.000	0.000	0.000	0.000	0.000
P1 : Eko Enzyme 25%	U1	0.000	0.000	0.250	0.500	0.500
	U2	0.000	1,000	0.750	0.833	0.625
	U3	0.000	0.500	0.750	0.667	0.625
	U4	0.000	1,500	1,000	0.667	0.500
P2 : Eko Enzyme 50%	U1	0.000	0.500	1,250	1,167	1.125
	U2	0.000	1,500	1,250	1.333	1.125
	U3	0.000	2,000	1,000	1,000	1,000
	U4	0.000	1,500	1,500	1.333	1,250
P3 : Eko Enzyme 75%	U1	0.000	3,000	1,750	1,167	0.875
	U2	0.000	2,500	1,750	1.333	1,250
	U3	0.000	2,000	1,500	1,167	1.125
	U4	0.000	2,500	1,750	1,500	1,250
P4 : Eko Enzyme 100%	U1	0.000	4,000	2,500	1,667	1,250
	U2	0.000	3,000	2,000	1,500	1,250
	U3	0.000	3,500	2,250	1,667	1,250
	U4	0.000	3,500	2,000	1,667	1,250

Based on “Table 5”, it can be seen that the highest rate of dead mites occurred in treatments P1, P2, P3 and P4 occurred at the 2nd hour where each speed was 1.5, 1.5, 3 and 4. This shows that the mites are faster die at the beginning of time.

Table 6. Analysis of the velocity variance of dead mites

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	165,042a	25	6,602	109.109	.000
Treatment	34,297	4	8,574	141,712	.000
Time	28,410	4	7.103	117,388	.000
Treatment * Time	17,691	16	1.106	18,274	.000
Error	4,538	75	.061		
Total	169,580	100			

There was a relationship between the increase in the concentration of the given ecoenzyme and the rate of death of the mites. This calculation also shows that the higher the dose of eco-enzyme has a higher mortality effect on mites. It can also be seen that the longer the time used, the faster the mites die. There is an interaction between the higher the dose of eco-enzyme given and the longer the time it affects the rate of mite mortality. This is necessary because the sooner the mites die, the better because the mites move very quickly. [13] Mites are capable of moving 2.5 cm per minute above the skin surface. The results of the analysis are then continued into the following Duncan test.

Table 7. DMRT follow-up test for dead mites

O'clock	Eco Enzyme Concentration					
	P0 : Control (Aquadest 100%)	P1 : Eco Enzyme 25%	P2 : Eko Enzyme 50%	P3 : Eko Enzyme 75%	P4 : Eko 100% Enzyme	Average
0 hours	0.000a	0.000a	0.000a	0.000a	0.000a	0.000a
2 hours	0.000a	0.750b	1.375cde	2,500f	3,500g	1.625d
4 hours	0.063a	0.688b	1,250cd	1,688e	2.188f	1.175c
6 hours	0.084a	0.667b	1,208c	1,292cd	1,625de	0.975b
8 hours	0.063a	0.563b	1.125c	1.125c	1,250cd	0.825b
Average	0.042a	0.533b	0.992c	1.321d	1,713e	4,601

From “Table 7”, it can be shown that P1 at 2 hours showed an interaction between the concentration of the eco-enzyme and the time it was able to kill mites quickly.

Deadly Concentration

In determining the lethal concentration, it can be done at the time of 50% mortality (LC50) in mites. To determine the value of LC50 using regression analysis. Based on table 2, it can be seen that at the 6th hour the mortality of mites has reached 50%. The LC50 value was obtained by calculating the regression analysis, namely the concentration of the eco-enzyme as the

independent variable along with the number of dead mites as the dependent variable at 6 hours. The number of dead mites that reached 50% can be seen in "Table 8."

Table 8. The average number of mites that died at the 6th hour

n	x(%)	y (tail)
1	0	0.50
2	25	4.00
3	50	7.25
4	75	7.75
5	100	9.75

x = concentration of ecoenzyme

y = average number of mite deaths for 6 hours

This shows that the higher the ecoenzyme concentration is directly proportional to the increase in the number of dead mites. Based on attachment 5, it can be seen that the LC50 value is 40.45%. This shows that the effective concentration of eco-enzymes in killing mites is 40.45%. The LC50 value then becomes the basis for the in vivo application of the ecoenzyme solution dose. The content of the protease enzyme is able to hydrolyze the cell walls of the mites so that the mites die. This is in accordance with [14] protease enzymes [15] protease enzymes capable of hydrolyzing cell walls so as to inhibit microbial growth.

4. Conclusion

The best and most effective concentration of eco-enzyme in killing mites was P1 with 25% eco-enzyme concentration. According to the LC50 calculation, the eco-enzyme can be given and applied to kill and suppress the development of Sarcoptes scabiei mites in goats with an optimal concentration of 40.45%.

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