



Efficacy of Neem Oil (*Azadirachta indica*) and Clove Oil (*Syzygium aromaticum*) against Ear Mites in Rabbits (*Oryctolagus cuniculus*)

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ABSTRACT

Ear mites are a common ectoparasite that causes a skin disease known as mange in rabbits. Its infestation will contribute to poor growth, decrease production performances and in severe cases, cause mortality. To date, chemical pesticides are still widely used to control rabbit mites, where long-term use will contribute to the evolution of mite resistance. Essential oils have become an alternative method due to their efficacy and safety. This study was aimed to determine the efficacy of clove oil and neem oil against rabbit ear mites. The rabbits were divided into four treatment groups: T1, T2 and T3 and T4, each having three rabbits. These groups were topically treated with ivermectin (control group), clove oil, neem oil, 50:50 of clove and neem oil respectively on days 0, 7, 14, 21 and 28. The number of mites was counted and recorded as post-treatment evaluations by microscopic examination of skin scrapings from each group. The number of mites were progressively decreased in T2, but fluctuated in T1, T3 and T4. The number of live mites for each treatment was not statistically different ($p>0.05$) between treatments for days 0, 7 and 21, but the number of mites was statistically different ($p<0.05$) between all treatments for days 14 and 28. T4 that was treated with both clove and neem oil showed the highest efficacy with 94% for day 14 post-treatment, while treatment with neem oil (T3) showed the highest efficacy (89.7%) for day 28 after treatment. The results revealed that the mixture of both oils was the most effective against rabbit ear mites, however comparing the two oils, neem was more effective than clove oil in controlling the mites.

Keywords: Rabbit, essential oil, ectoparasite, ear mites, mange

INTRODUCTION

Rabbits (*Oryctolagus cuniculus*) are small mammals in the family Leporidae that originated from Europe. They are widely distributed across the continents of the world, and people have treated rabbits as companion animals,

for laboratory use and for meat consumption (Gunalan et al., 2019). Malaysia's rabbit industry has excellent potential as demand for rabbit meat has been rising and could be an alternative to beef and mutton (Bernama, 2020). According to current industry figures, there are 600 farmers registered with the Department of Veterinary Services (DVS) Malaysia, and the number is increasing due to the high demand for rabbit meat (Gunalan et al., 2019). However, despite increasing rabbit farming, parasite infection management is not well established and practiced (Mohamad-Radzi, 2021).

Ectoparasitic infestation of rabbits, particularly by mites, is causing economic losses to rabbit farmers. The mites are responsible for blood loss and irritation, which results in poor growth, decreased production and reproduction performances and in severe cases, caused mortality in rabbits (Bowman, 2009). A study conducted by Mohamad-Radzi et al. (2021) showed that the prevalence of mites in rabbit farming in Selangor was over 50 percent. The mites' infestation has an important health impact on rabbits, therefore it needs to be fully considered to develop guidelines to overcome the infestations in rabbits.

To date, chemical pesticides are still widely used to control rabbit mites, where long-term use will contribute to the evolution of mite resistance (Seddiek et al. 2013). Botanical pesticides have become an alternative to this issue due to their efficacy and safety. Studies have shown that essential oils from several plants have acaricidal effects on animal mites (Altinok Yipel et al., 2016), such as neem (*Azadirachta indica*) and clove (*Syzygium aromaticum*). These herbs are common and can be easily found in Malaysia. Tabassam et al. (2007) said that neem extracts had demonstrated acaricidal activity against sheep mites while Fang et al. (2016) reported that clove oil was the most effective oil as compared to the other nine essential oils to kill poultry mites. However, little study has been conducted to observe the efficacy of neem and clove oils on rabbit ear mites. Therefore, this study was aimed to determine the effectiveness of neem and clove oil against rabbit ear mites.

MATERIALS AND METHODS

Study area

The study was conducted in the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, Terengganu, Malaysia. The experimental animals' procedures were approved by the Universiti Sultan Zainal Abidin Animal Ethics Committee (UAPREC/007/040).

Experimental animals

Twelve domestic rabbits (*Oryctolagus cuniculus*) with an average weight of 3-4 kg were selected for this study. The selected rabbits did not receive any treatment three months before the study. They were also examined for the presence of ear mites at least in one ear before the study was conducted. The rabbits were then separated into four categories of three rabbits (n=3): Treatment 1 (T1) was treated with ivermectin as the control group, Treatment 2 (T2) was treated with clove oil, Treatment 3 (T3) was treated with neem oil, Treatment 4 (T4) was treated in a 50:50 ratios with both oils (clove and neem) (Fichi et al., 2007). The rabbits were fed the commercial rabbit feed and given water ad libitum.

Essential oil treatments

This study used two essential oils: neem oil (*Azadirachta indica*) and clove oil (*Syzygium aromaticum*). Both oils were 100% pure oil without additives and store-bought (Now Solutions brand). Each oil was diluted with jojoba oil in a ratio of 2:5 ml to reduce the irritations of pure essential oil to the rabbits. For each oil treatment, 2 ml of oil was applied topically to the same spot that had been marked on the ear of the infected rabbits (Treatment 2, Treatment 3, and Treatment 4) on days 0, 7, 14, 21 and 28th (Altinok Yipel et al., 2016). In addition, two drops of ivermectin (i-Dectin 0.5% pour-on) were given on the back of the neck of the rabbits of Treatment 1 on day 1 for the four weeks' study as the control group.

Data collection

Samples of mites (Fig. 1) were obtained the next day after each treatment day (0, 7, 14, 21 and 28th day) from the infected rabbits by a skin scraping from the ear lesions. The skin scraping method was adopted from Tagesu (2018) with some modification. The skin scraping was collected by placing the mineral oil on the scalpel blade and was vigorously scraped six or seven times to remove the top of the papule. The sample of scraping and oil was transferred to a glass slide, and a few drops of mineral oil were added and mixed. The scrape and mineral oil mixture was covered with a cover slip, and then taped to prevent them from coming apart. A big sterile cup was used to hold the sample. Finally, the sample was sent to the Laboratory of Microscopic, Faculty of Bioresources and Food Industry, UniSZA for further analysis.

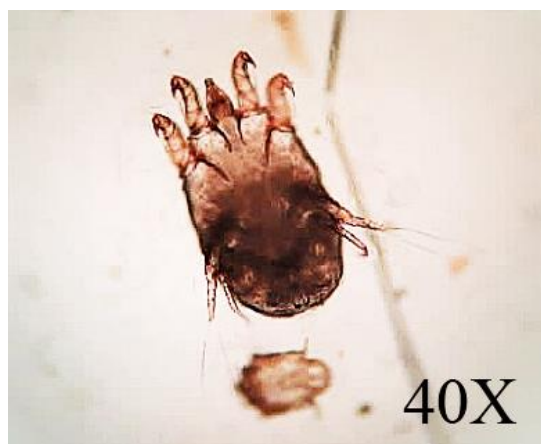


Fig. 1. Rabbit ear mite under microscope (40× magnification).

Determining the efficacy percentage

The efficacy percentage of the essential oils applied to different groups was determined using equation 1 after treatment (days 7 and 28) adopted from Altinok Yipel et al. (2016).

$$\text{Efficacy (\%)} = \frac{100 \times (\text{Tbt} - \text{Tat})}{\text{Tbt}} \quad \text{Eqn. 1}$$

Where,

Tbt = Total number of mites before treatment

Tat = Total number of mites after treatment (days 7 and 28)

Data analysis

Data were analysed with One-way ANOVA to measure the mortality rate of the mites for each essential oil. The significance of the mean was made using the Tukey test. The analysis was done at a significance level of $p < 0.05$. Results were expressed as mean and standard error. Analysis was done using Minitab Software version 2018 and Microsoft Excel 2019.

RESULTS AND DISCUSSION

Number of live mites after treatments

The number of live mites progressively decreased from day 0 to day 28 after treatment for Treatment 2 (T2), which was the treatment with clove oil as shown in Fig. 2. Treatment 3 (T3) and Treatment 4 (T4) also showed a progressive decrease in the number of live mites, however, the mite's count was increased on day 21 post-treatment for T3 and day 28 post-treatment for T4. The control group (T1) displayed a sharp decrease in the number of mites on day 7 after application, and the number fluctuated until the end of the treatment.

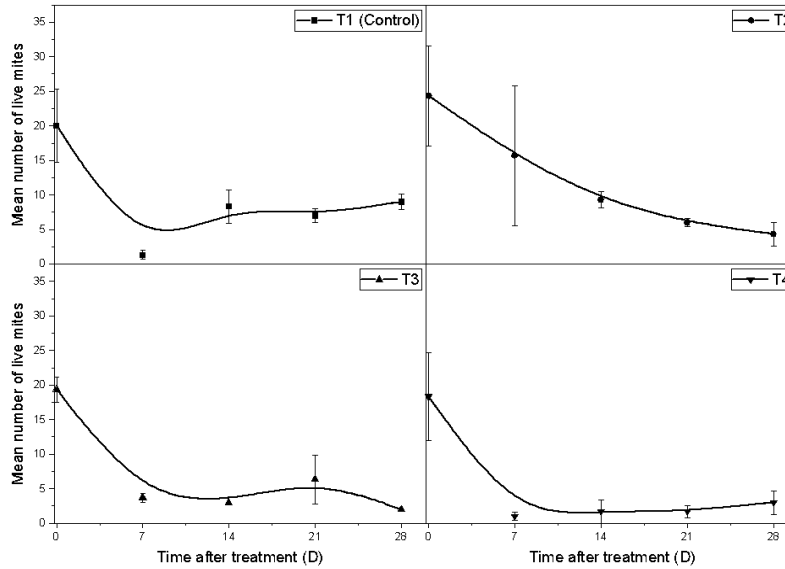


Fig. 2. Mean number of live mites ($\pm$ Standard Error) for all treatments in 28 days.

Generally, mites have four life stages: egg, larva, protonymph and adult that take about 21 days to complete the life cycle (Swarnakar et al., 2014). The eggs will hatch after four days, while larval and protonymph stages will remain for 18 days before they moult into an adult. The fluctuation in the number of live mites in this study may be influenced by the mite's life cycle because the study was conducted *in vivo*. Therefore, the mite count showed an increase after the 7th day of treatment because the mite eggs on the ears of the treated rabbits would hatch into larvae, and then develop into an adult. This in turn caused the number of mites to increase when samples were taken and counted after 14th day post treatment.

Although the mite count showed a reduction after treatment in some of the treated groups, the number of live mites for each treatment was not statistically different ($p > 0.05$) between treatments for days 0, 7 and 21 as shown in Table 1. However, the number of mites was statistically different ($p < 0.05$) between all treatments for days 14 and 28.

Table 1. Comparison of mean number of live mites ($\pm$ Standard Error) between different treatment.

Day after treatment	Mean number of live mites ($\pm$ SE)				P value
	T1 (Control)	T2	T3	T4	
Day 0	20.00 $\pm$ 5.29 ^a	24.33 $\pm$ 7.26 ^a	19.33 $\pm$ 1.86 ^a	18.33 $\pm$ 6.36 ^a	0.516
Day 7	1.33 $\pm$ 0.67 ^a	15.7 $\pm$ 10.1 ^a	3.67 $\pm$ 0.67 ^a	1.00 $\pm$ 0.58 ^a	0.213
Day 14	8.33 $\pm$ 2.40 ^{ab}	9.33 $\pm$ 1.20 ^a	3.00 $\pm$ 0 ^{ab}	1.67 $\pm$ 1.67 ^b	0.021
Day 21	7.00 $\pm$ 1.00 ^a	6.00 $\pm$ 0.58 ^a	6.33 $\pm$ 3.53 ^a	1.67 $\pm$ 0.88 ^a	0.261
Day 28	9.00 $\pm$ 1.15 ^a	4.33 $\pm$ 1.67 ^{ab}	2.00 $\pm$ 0 ^b	3.00 $\pm$ 1.73 ^{ab}	0.025

Mean with the same letters was not significantly different at $p < 0.05$

For day 14 post-treatment, T4 which was treated with clove and neem oil showed the highest efficacy (94.0%) as shown in Fig. 3. The control group (T1) treated with ivermectin showed the second highest efficacy (93.0%), followed by T3 with neem oil with 81.0%. On the other hand, treatment with clove oil (T2) showed the least efficacy percentage in killing the mites with only 35.6%.

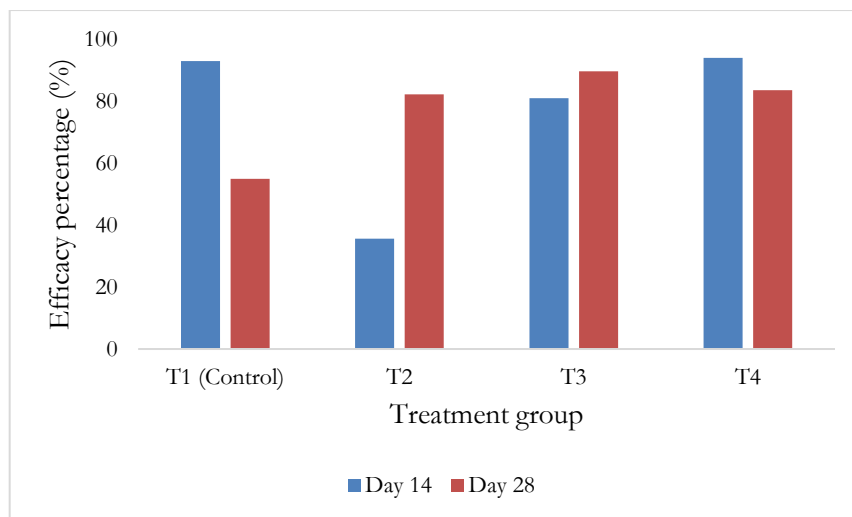


Fig. 3. Efficacy percentage for each treatment group for day 14 and day 28.

After 28 days of treatment, the treatment with neem oil (T3) showed the highest efficacy (89.7%) to diminish the ear mites (Table 2), followed by T4 with 83.6% and then T2 with 82.2%. Treatment with ivermectin showed the lowest efficacy percentage with only 55.0%.

Table 2. Comparison of efficacy percentage of each treatment for days 14 and 28 after treatment.

Day after treatment	Efficacy percentage (%)			
	T1 (Control)	T2	T3	T4
Day 14	93.0	35.6	81.0	94.0
Day 28	55.0	82.2	89.7	83.6

Studies reported that neem extracts from various parts of the plant possess pesticidal properties (Mahran et al., 2020). The potent active compound in neem, azadirachtin, was reported to have pesticide activity as insecticides (Chaudhary et al., 2017). A study by Tabassam et al. (2008) reported mange infection in sheep caused by *Sarcoptes scabiei* var. *ovis* wholly cured with a treatment from neem extract. Cloves contain an active compound called eugenol that accounts for at least 50% of the compounds in cloves. Clove was reported to possess several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties (Haro-González et al., 2021). A study conducted by Fang et al. (2016) on ten essential oils to treat *S. scabiei* showed that clove oil was effectively kills the mites.

The results of efficacy percentage showed that both clove (*Syzygium aromaticum*) and neem (*Azadirachta indica*) oil have acaricidal effects in treating ear mites in rabbits. The treatment with the mixture of clove and neem oil presented the highest percentage after 14 days of application, while the treatment with neem oil was most effective in controlling the mites 28-day post-treatment. Therefore, it can be concluded that the mixture of both oils was the most effective in killing ear mites in rabbits due to the presence of both potent compounds found in neem and clove oils. However, comparing the two oils, neem oil was found to be more effective than clove oil in controlling rabbit ear mites. The efficacy results also suggested that the effect of the essential oils can be observed after 14 days post-treatment.

CONCLUSION

The present study demonstrated that both neem and clove oils have acaricidal effects on rabbit ear mites. The results showed that the mixture of both oils was more effective in killing the ear mites. In comparison, neem oil was more effective than clove oil against the mites. The study also suggests that the oil's effectiveness can be observed after 14 days of the oil application. The findings of this study suggest that rabbit breeders use a mixture of neem oil and cloves to achieve optimal effectiveness of ear mite treatment with essential oils in rabbits.

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