UNIVERSITI PUTRA MALAYSIA

TICKICIDAL PROPERTY OF TUBA ROOTS WATER EXTRACT AND CITRONELLA OIL

NAJIHAH BINTI SHOBAT SETTIC

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Tuba roots and Citronella as acaricides", by Najihah Shobat Settic and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project

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DEDICATIONS

This project paper is dedicated to the One Almighty Allah S.W.T, who had created me and made all things possible,

To my family,

Father

Mother

Brother, Sister

and my friends

And to all my teachers who have committed themselves towards the noble cause of education.

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CONTENTS

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

CIRI TIKISIDAL EKSTRAK AIR AKAR TUBA DAN MINYAK SITRONELA

Oleh

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Ayam hutan (*Gallus gallus*) juga rentan terhadap serangan sengkenit. Akar pokok tuba (*Derris elliptica*) dan pokok sitronela (*Cymbopogon nardus*) telah pernah diguna secara berkesan sebagai insektisid. Kajian ini dijalankan untuk menentukan kesan ekstrak air akar tuba dan minyak sitronela terhadap serangan sengkenit pada ayam hutan. Akar tuba diperolehi daripada negeri Pahang, Malaysia, dan sitronela daripada Institut Biosains, Universiti Putra Malaysia. Sengkenit yang dikenal pasti sebagai *Haemaphysalis sp*. dikumpul daripada telinga dan kepala ayam hutan yang dipelihara dalam sebuah ladang ternakan lepas-bebas, di Jenderam Ulu, Selangor, Malaysia. Ampaian akar pokok tuba direbus dalam air hingga mendidih untuk memperolehi ektrak air akar tuba. Pokok sitronela yang dicincang halus dikenalkan penyulingan stim untuk mendapat minyak pati. Dalam kajian ini extrak akar tuba, minyak sitronela, pada kepekatan 100, 50, dan 25 % dan gabungan nisbah 1:1 estrak

kepada minyak pada kepekatan 100, 50, dan 25 % telah diguna untuk menentukan kesan dalam menyebabkan kematian 100 % sengkenit. Masa untuk 100 % kematian sengkenit direkodkan. Hasil kajian menunjukkan yang kedua-duanya ekstrak air akar tuba dan minyak sitronela menyebabkan 100 % kematian sengkenit. Bagaimanapun, tiada perbezaan tererti ($p>0.05$) dalam masa untuk mencapai 100 % kematian sengkenit di antara perlakuan ekstrak air akar tuba dan minyak sitronela. Juga, tiada perbezaan tererti ($p>0.05$) dalam kemujaraban di antara kepekatan 100 dan 50 % ekstrak air akar tuba atau minyak sitronela. Kesimpulannya, ekstrak air akar tuba dan minyak sitronela secara bersendirian dan sebagai gabungan, berpotensi diguna sebagai tikisid.

Kata kunci: ekstrak air akar tuba, minyak sitronela, sengkenit, tikisid.

ABSTRACT

Abstract of the project paper presented to Faculty of Veterinary Medicine in partial requirement for the course VPD4999-Project

TICKICIDAL PROPERTY OF TUBA ROOT WATER EXTRACT AND CITRONELLA OIL

By

Najihah Binti Shobat Settic

2016

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The Red Jungle Fowl (*Gallus gallus*) is susceptible to tick infestation. Tuba roots (*Derris elliptica)* and citronella plant (*Cymbopogon nardus)* have been used effectively as insecticides. This study was conducted to determine the effect of tuba root water extract and citronella oil on tick infestation in the Red Jungle Fowl. Tuba root was collected inaPahang, Malaysia, while citronella was obtained from the Institute of Bioscience, Universiti Putra Malaysia. The ticks, identified as of the *Haemaphysalis sp*., were collected from the ears and head of the Red Jungle Fowl a small reared in a free-ranging farm in Jenderam Ulu, Selangor, Malaysia. The tuba root suspension was boiled in water to obtain tuba root water extract. Fine chopped citronella plant was subjected to steam distillation to obtain the essential oil. In this

study 100, 50 and 25 % concentrations of the tuba root extract and citronella oil and combination of 1:1 ratio of extract to oil of 100, 50 and 25 % concentrations were used to determine their effect in causing 100 % tick mortality. One millilitre of the extract or oil was added to the petri dish containing 10 live ticks. The time taken for 100 % tick mortality was recorded. The results show that both the tuba root water extract and citronella oil caused 100% tick mortality. However, there was no significant ($p>0.05$) difference in time taken for 100% tick mortality to occur between treatments with tuba root water extract and citronella oil. There was also no significant ($p>0.05$) difference in efficacy between 100 and 50% of either the tuba root water extract or citronella oil. In conclusion, tuba root water extraction and citronella oil alone and their combination have potential to be used as tickicides.

Keyword: tuba root water extract, citronella oil, tick, tickicide

1.0 INTRODUCTION

1.1 Ticks

Ticks are parasitic arthropods of the class Arachnida, order of Acarina. They are divided into two families, which are Ixodidae and Argasidae. The species of the Ixodidae family includes *Ixodes*, *Amblyomma*, *Haemaphysalis*, *Rhipicephalus* and *Dermacentor,* whereas there is only one species in family of Argasidae that is *Ornithodoros.*

Haemaphysalis sp. is commonly found in chicken. Tick species such as *Haemaphysalis cinabarina*, *H. leporispalustris, H. chordeilis, Amblyomma haebraeum* feed on poultry and wild ground birds. In Malaysia, the species of tick commonly infesting birds are the Malaysian Parasites XLIX (Audy *et al.*, 1960) and they consist of the genera *Amblyomma*, *Haemaphysalis and Dermacentor*. *Haemaphysalis sp.* are inornate ticks with festoons but without eyes. The sensory palps for this genus are short and broad with the second segment extending beyond the basis capituli.

1.2 Tuba roots

Tuba roots is poisonous to fishes and insect. These toxic properties of this plant are mainly due to rotenone (Evan, 2002). Since rotenone is effective against the ticks, the plant extract can potentially be used as an acaricides to replace the current chemical-based acaricides.

1.3 Citronella

Citronella oil is used in traditional medicine as an antiseptic, bactericidal, deodorant, tonic, weedicide and acaricide. Citronella has been shown to have insect repellant effect (Ansari and Radzan,1995). It was shown that 1% citronella oil had anthelmintic effect on live *Fasciola gigantica* (Jeyathilakan *et al.*, 2010). Citronella had been showed to be effective against ticks and insects. Thus, we believed that citronella can be used to control tick infestation in chicken and other animal.

1.4 Objective

The objective of this study was to determine the effect of tuba root water extract and citronella oil and their combination on the ticks infesting the Red Jungle Fowl.

1.5 Hypothesis

It is hypothesised treatments with tuba root water extract, citronella oil and their combinations cause fowl ticks mortality.

2.0 LITERATURE REVIEW

2.1 Ticks infestation

Ticks are blood feeders and transmit disease-producing organisms to man and domestic animals. Ticks must have a meal of blood if they were to produce eggs. Hard ticks feed for extended periods on their hosts that vary from several days to weeks,

depending on life-stage, host-type and species of tick. The surface or cuticle of hard ticks can actually grow to accommodate the large volume of blood ingested, which in adult ticks can be between 200 to 600 times the unfed body weights (Sonenshine, 1991). Argasidae, also known as soft ticks can endure starvation and can live for years without blood meal (Furman and Loomis, 1984). The cuticle of soft ticks expands, however, unlike the hard ticks, the soft tick accommodates only slightly to the ingestion of blood by expanding between 5 to 10 times of their unfed body weights (Sonenshine, 1991).

The medical and economic importance of ticks has long been recognised due to their ability to transmit diseases to humans and animals. Ticks cause great economic losses to livestock and adversely affect livestock hosts in several ways. Ticks, besides of acting as potential vector for haemo-protozoa and helminth parasites, can cause loss of blood is a direct effect. Blood loss from heavy tick infestation causes anaemia and weight loss in animals. Infected animal may die resulting in economic loss to the farmer. Ticks can transmit protozoal, rickettsial, and viral diseases of livestock. These organisms are also carriers of other disease, which may affect farm animal production. Thus, control the tick infestation in animal is one of the most important management procedures in farms to ensure animal health.

Ticks are very important and harmful blood-sucking external parasites of mammals, birds, and reptiles (Furman and Loomis, 1984). The saliva of the blood sucking arthropod has been identified as the sources of toxins and allergens (Ribeiro, 1987). Thus, tick-bite lesions are characterised by dermal changes with infiltration by variable numbers of inflammatory cells, oedema, haemorrhage and vascular dilatation. Tissues affected by tick bites also exhibit erythrocyte-engorged capillaries. The tick

saliva contains toxins that cause dermal changes at the site of bite bring discomfort to the animal and affecting their welfare. Tick-infested animals show discomfort that may cause them to lose appetite and eventually resulting in decrease in body weight.

2.2 Tuba roots

The *Derris elliptica* is a rambling climber with branches covered with brown hairs. The leaves are pinnate and 30 to 50 cm long. It is originated East Asia and the plant can be found in abundance in the Tropics, notably in India. It is locally known as "Tuba" and in Thailand as *Lotin* or *Hang lai daeng*. The racemes of the plant are lax, 15 to 30 cm in length, with reddish flowers in stalked clusters. The pods are 5 to 8 cm long and contain 1 to 3 seeds, flat and reniform, olive, brown or black. The pod requires least 75 % moisture and temperature of 25 to 30°C to survive.

Figure 1. Root of *Derris elliptica* (arrow).

The root of *Derris elliptica* has been reported to contain rotenone, derrid, anhydroderrid, derrin, tubotoxin ,and tubain. Rotenone is a selective, non-specific botanical insecticide with some acaricidal properties and is used in home gardens for insect and on pet for lice and tick control. It is also used to fish eradicate fish during water body management. Rotenone is slightly soluble in water at 15 mg rotenone in in 1 L of water at 100 °C. Rotenone is also soluble in acetone, carbon disulfide and chloroform and only slightly in alcohol and carbon tetrachloride (Kid and James, 1991). There are various methods that can be used to extract rotenone from the derris root. However, extract of rotenone is easiest with water. This can be done by boiling tuba root suspension in water. The half-life of rotenone in the environment is between 1 and 3 days but it is not readily leached from soil (Augustijn-Beckers *et al.*1994) and does not become groundwater pollutant. Rotenone breaks down readily upon exposure to sunlight (Kidd and James 1991) thus the compound loses almost all its toxicity after 5 to 6 days exposure of spring sunlight or 2 to 3 days to summer sunlight.

Since early times, the root has been used as fish poison in the Philippines, the Dutch East Indies and the Malay Peninsula. The water extract of tuba root is traditionally use to catch fish in streams and rivers. Some farmer used the plant as the pesticides to control vegetable pest. According to Gupta (2007), Tuba roots has been shown to be an important source for compounds with broad spectrum insecticidal properties.

2.3 Citronella

Citronella grass, *Cymbopogon nardus*, also known as nardus, nard or grass is well-known for its mosquito repellent property. The citronella grass is coarse and clumpy and can grow to 1.5 to 1.8 m tall. The stems are cane-like and the leaves greyish green and flat. The grass originated in Southeast Asia and can now be found in tropical Asia. In Florida and Southern California, United States of America, the plant is grown as an ornamental.

Figure 2: Citronella grass

Although, essential oil of citronella grass has been typecast as an insect repellent especially for malaria-carrying mosquitoes, it has also been shown to be beneficial in the clearing of the mind, refreshing rooms, skin softening, combating oily skin and sweaty feet. Citronella, for its insect repellent properties is a popular ingredient in wax candles. It is also used widely in perfumes, soaps, skin lotions and deodorants.

The citronella oil are antiseptic, bactericidal, deodorant, diaphoretic, insecticididal, parasitic, tonic and stimulant. Citronella oil act as antibacterial in infections associated with the bladder, urethra, kidney, prostate and urinary tract. Citronella oil at its full strength is very potent. Therefore, use of the oil must be with caution to avoid absorption through open wounds or by ingestion.

3.0 MATERIALS AND METHOD

3.1 Sample collection

Tuba roots were collected from a district in Pahang whereas the citronella were obtained from Institut Biosains, Universiti Putra Malaysia. The ticks were collected from Red Jungle Fowls reared in a small free-ranging farm in Jenderam Ulu, Selangor, Malaysia. The ticks were collected from the comb, wattle and inside the ear using a fine forceps. The ticks were carefully and gently removed to ensure their viability. Only ticks that were active with complete body structure were selected for the study.

3.1 Sample preparation

3.1.1 Tuba root preparation

Tuba roots (480 g) with some stem were used in this study. The roots and stem of the plant were properly cleaned and cut into the smaller pieces and mixed with 1 L distilled water. The suspension was heated to boiling for approximately 7 hours to obtain the water extract (Figure 3).

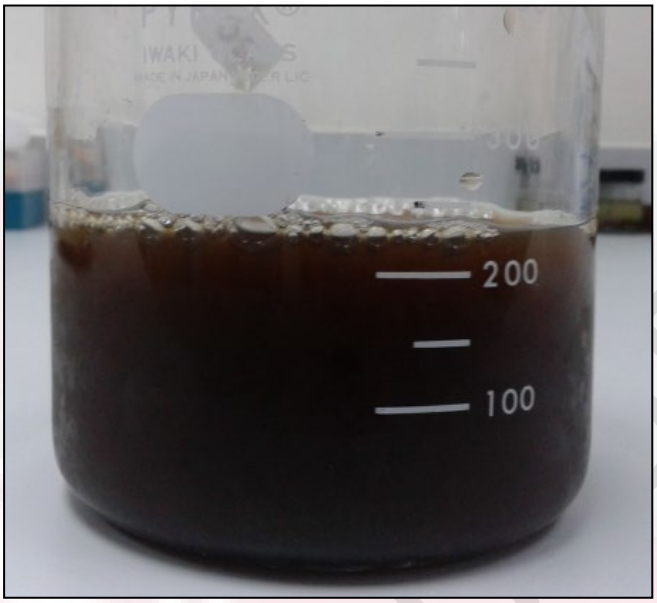

Figure 3: Tuba root water extract.

3.1.3 Citronella preparation

The citronella were subjected to steam distillation to obtain essential oil. The citronella (2.3 kg) were properly cleaned and cut into the smaller pieces. The chopped material consisting of leaves and the stem were placed inside the flask with 6 L distilled water added. The suspension was heated to boiling temperature for approximately 7 hours. About 15 mL of citronella oil was obtained by the steam distillation extraction method (Figure 3).

Figure 3: Citronella essential oil

3.1.3 Extract and oil preparation

Stock tuba roots extract (100%) was the undiluted extract obtained after boiling the roots in water. The stock citronella oil (100%) was the product of undiluted steam distillated citronella grass. The concentration of tuba root extract and citronella oil used in this study were 100, 50, and 25 %. To obtain the different concentrations, tuba root extract was diluted appropriately in distilled water and citronella oil in 30% ethanol. The tuba root water extract and citronella oil combination was obtained by mixing at 1:1 of either 100, 50 or 25 % concentration of tuba root extract and citronella oil.

3.2 Experimental design.

Ten live ticks were placed in each of 9 labelled petri dishes; 3 for tuba root extract, 3 for citronella oil and 3 for extract-oil combination treatments. One millilitre of either 100, 50, or 25 % extract or oil or 100, 50, or 25 % 1:1 extract-oil combination

was added to the respective plates. The time taken for the ticks in each plate to reach 100% mortality was recorded.

4.0 Results

The ticks used in the experiment were active and showed good motility before treatment with either tuba root extract or citronella oil. The motility of the ticks gradually declined after treatment with the extract and oil and eventually all ticks died (Table 1).

Table 1. Time for 100% tick after treatment with tuba root extract and citronella oil.

Treatment	Concentration (%)			Mean± std. error
	100	50	25	
	Time for 100 % mortality (hours)			
Tuba Root extract	4.7	5	11	$7.02^{a} \pm 0.70$
Citronella oil	1.2	0.89	1.4	$1.17^{b} \pm 0.05$
Tuba root extract-citronella oil Combination	1.2	1.1	1.3	$1.25^{b} \pm 0.07$

[ab] Means in the same column with different superscripts and significantly different at $\alpha = 0.05$

The longest time taken for the ticks to reach 100% mortality was with 25% tuba root extract that took 11 hours; while the shortest time was with 50 % citronella oil that took < 1 hour.

There was no significant ($p>0.05$) difference between citronella oil and combination of tuba roots water extraction and citronella oil treatment in time taken

to cause 100 % tick mortality. It is took approximately 1 hour for 100 % tick mortality to occur after the two treatments. However, ticks were comparatively more resistant to treatment with tuba root water extract alone. In this case, it took 7 times longer for attainment of 100% tick mortality. Thus, this study shows that the most effective treatments in causing tick mortality are citronella oil alone and combination citronella oil and tuba root water extract.

A comparison between concentrations of the treatment versus 100 % tick mortality was made (Table 2). The study showed that there was no significant ($p>0.05$) difference in time for 100 % tick mortality between 100 and 50 % concentrations of the treatments. The time for attainment of 100 % tick mortality after treatment with 100 and 50 % treatment concentrations was approximately 2 hours. Whereas is took approximately 4 hours for 100 % tick mortality to occur when they were treated with 25% concentration of extract, oil or their combination. Thus, the minimum effective tuba root water extract, citronella oil or their combination concentrations to cause 100 % tick mortality was 50%.

Table 2. Relationship between times taken for ticks to reach 100% mortality among different concentration.

Concentration	Mean ± Std. error
100%	$2.41^{a} \pm 0.28$
50%	$2.34^{a} \pm 0.32$
25%	$4.70^{b} \pm 0.86$

[ab]Means in the same column with different superscripts and significantly different at $\alpha = 0.05$.

5.0 Discussion

This study showed that tuba root water extract and citronella oil contain components that are effective tickicides. In this study, however, characterisation of the extract and oil was not done. Thus, it cannot be concluded as to which component of these compounds are responsible for causing tick mortality. Previous studies showed that the major component of *Derris elliptica* is rotenone (Sae-Yun *et al*., 2006). Rotenone is a respiratory enzyme inhibitor that acts on NAD+ and coenzyme Q reactions. This compound inhibit NAD+ and coenzyme Q, resulting in failure of the respiratory functions (Hien et al., 2003). Thus, it is possible the mortality of ticks caused by tuba root extract treatment is due to failure in this enzymatic reaction.

Citronella oil is one of the essential oil obtained from the *Cymbopogan nardus* plant (Wei and Wee, 2013). It was shown previously that the main compounds of citronella oil are the monoterpene, geraniol, citronellal and citronellol, (Labinas and Crocomo, 2002; Quintas-Júnior *et al.,* 2008; Pinheiro *et. al.,* 2013). The essential oil of another species of the grass, *C. winterianus,* was also shown to contain high concentrations of citronellal, geraniol and citronellol (Leite *et al.,* 2013). Citronellal, geraniol and citronellol being monoterpenes are effective insecticides and it is suggested that they are also effective tickicides. This tickicidal effect of monoterpenes was in fact demonstrated in an earlier study on the southern cattle ticks, *Rhipicephalus (Boophilus) microplus* (Prates *et al.*, 1998). That study showed that the efficacy of monoterpenes in controlling cattle tick infestion is in the following order; citronellol > α-pinene = β-pinene > linalool.

In our study, three concentrations of tuba root water extract, citronella oil and their combinations was used in the treatment of ticks. The results show the most effective treatment to cause 100 % tick mortality are with citronella oil and tuba root extract-citronella oil combination.

The components of tuba root extract and citronella oil, particularly rotenone, are quite toxic. For example the LD_{50} of rotenone on mallards and pheasants were approximately 2000 and 1680 mg/kg body weight, respectively. Rotenone is believed to be moderately toxic to humans. Human fatalities from rotenone exposure is rare, but the compound can cause mild gastric irritation and vomiting. Tuba roots can cause acute and chronic toxicity. Acute local exposure to Tuba roots extract, among others, results in conjunctivitis, dermatitis, sore throat, and congestion. While, ingestion of rotenone produce effects ranging from mild gastric irritation to vomiting. Inhalation of high doses of rotenone can cause increased respiration followed by depression and convulsions. In humans, the compound also causes mild rashes and in rabbits severe eye irritant. Approximately 20% of oral rotenone is eliminated from body systems within 24 hours, suggesting the toxic effect may not last long. However, animal may be in danger if they show vomiting after rotenone ingestion. Inhaled is even more toxic than the ingested rotenone. Toxicity as the result of inhalation of plant materials containing rotenone is dependent on the particle size. The smaller the plant particle size, the easier for the material to enter deep into the lungs.

Chronic toxicity of rotenone can manifest as growth retardation and vomiting. However, this effect is debatable because rats fed diets containing rotenone at doses of as high as 2.5 mg/kg body weight for 2 years did not show ill-effect (National

Research Council 1983). On the other hand, dogs fed rotenone for six months at doses 10 mg/kg/day showed reduced food consumption that lead to loss of body weight. It has been proposed that high doses of rotenone can cause gastrointestinal lesions and chronic bleeding (National Research Council 1983).

The toxic effect of Citronella has not been conclusively demonstrated. Citronella has also been claimed to have carcinogenic properties. Citronella contains methyleugenol and this compound was suggested to cause tumour in mice if given orally via stomach tube (Johnson *et al,* 2000). Others showed large doses of components of citronella oil fed to rodents five times per week for two years did not cause cancer (National Pesticide Information Center). However, because of the possibility that citronella can occasionally be carcinogenic, handling of the compound must be with extreme care. To minimise the adverse effect of citronella, handlers should wear gloves and masks to avoid direct contact and inhalation of the plant products. However, there is need more extensive studies to determine the safety of citronella oil for consumption or therapeutic use in animals and humans.

6.0 Conclusion

The study showed tuba root water extract, citronella oil and the tuba root extract-citronella oil combination 100 and 50% concentrations cause 100% tick mortality. Thus tuba root water extract and citronella oil are effective in the control of tick infestation in Red Jungle Fowl.

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