

Toxic Effect of Ethiopian Neem Oil on Larvae of Cattle Tick, *Rhipicephalus pulchellus* Gerstaeker.

Ismail Mohamed Handule¹, Chitapa Ketavan¹ and Solomon Gebre²

ABSTRACT

This study was conducted to test susceptibilities of cattle tick, *Rhipicephalus pulchellus* Gerstaeker after exposure to neem oil. The ultimate goal was to determine the bioacaricidal activity of Ethiopian neem extract under laboratory conditions for the control of *R. pulchellus* larvae. The LT₅₀ values were determined for populations from each subsequent treatment by probit analysis and significant increases (chi-square test, $P > 0.01$) occurred from one concentration to the next. There was approximately a 5.4-fold increase in the LT₅₀ when concentration of 40% neem oil (LT₅₀ = 0.184) was compared to 10% neem oil (LT₅₀ = 1.01). In addition, serial concentrations of neem oil were applied and knock down concentration (KC₅₀) was obtained (KC₅₀ = 13.43). Baseline information from these experiments will serve as a guide for future studies on susceptibilities of tick, *R. pulchellus* populations.

Key words: neem oil, cattle tick, bioacaricidal activity

INTRODUCTION

Ticks are important ectoparasites of domestic animals and affects 800 millions cattle and sheep around the world. They affect their hosts by damaging their hides and skins, reducing their growth rates and milk production and transmitting diseases organisms (Sutherest and Wilson, 1986). *Rhipicephalus pulchellus*, a three host tick species, has been recorded from an extremely wide ranges of domestic and wild animal especially ungulates (Walker, 1995). It is the commonest tick on domestic livestock, particularly cattle, sheep, goat and camels in some parts of eastern Ethiopia and northern Somalia (Pegram, 1976; Pegram *et al.*, 1981). Approximate counts made *in situ* on individual cattle in Borana District, Sidamo Province, Ethiopia, was approximately 100 ticks per animal infestation. (Walker *et al.*, 2000). In Somali region of Ethiopia

where this research was conducted, 90% of the tick were often obtained from cattle and camels compared to another pests.

Rhipicephalus pulchellus has been associated with wide variety of pathogenic organisms affecting both animals and men i.e., anaplasmosis, brucellosis, anthrax. *Trypanosoma theleria*, a parasitic organism, was found in the tissues and haemolymph of 19 out of 258 *R. pulchellus* adults from cattles in some provinces of Ethiopia i.e., Negeli Borana, Sidamo Province, Ethiopia (Burgdorfer *et al.*, 1973). Nairobi sheep disease virus was first reported to transmit experimentally by Lewis (1949). Pellegrini (1950) subsequently demonstrated the potential of transstadial and transovarial transmission of the virus through tick.

Acaricidal spray has been the commonest method of tick control in Africa since 1890.

¹ Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

² National Animal Health Research Center, (NAHRC), Ethiopia.

However, chemical control may provoke many problems such as environmental pollution, development of resistant tick strains and more expensive costs (Dipeolu and Ndungu, 1991). In view of the above problems, there has been an increasing interest in searching for alternative sustainable control methods of tick control in recent years. These include biological control by means of pathogens (Kaaya *et al.*, 1995).

Biological insecticides have proved as one of effective control. Several plants extract have been used and introduced in many areas. Extract of neem tree (*Azadirachta indica* A. Juss), a native of Burma and arid regions of the Indian sub continent, have been traditionally used by farmers in Asia and Africa to control insect pests of household, agricultural and medical importance. It has been reported that neem extract provides broad-spectrum control of over 200 species of phytophagous insects (Ascher, 1993) and they remain less toxic to natural enemies of insect pests than to the pests themselves (Schumutter, 1990).

Neem extract comprises a compound with diverse behavioral and physiological effects on insects, including feeding, deterrence, inhibition of growth and oviposition (Saxena, 1989; Schumutter, 1990). Although neem has been used for centuries for the control of household and agricultural pests, very limited researches were conducted for the controlling of livestock ticks.

The objective of this study was to determine the bioacaricidal activity of Ethiopian neem extract under laboratory conditions for the control of *R. pulchellus* larvae which is considered as a major livestock tick species in Somali region of Ethiopia.

MATERIALS AND METHODS

Rearing tick colony

The engorged females of *Rhipicephalus pulchellus* were collected from cattle in Jigjiga clinics and Jigjiga slaughter house in Somali region, eastern of Ethiopia. They were reared in glass tubes

in the incubator at $24\pm2^{\circ}\text{C}$ and $70\pm5\%$ RH. Using the technique of Solomon and Kaaya (1998), the larvae were separated and kept in other tubes of further investigation.

Preparation of Ethiopian neem oil solution

Dry Ethiopian neem seed (*Azadirachta indica* A. Juss) were provided from Diredawa nursery center, 160 km east of Jigjiga. To obtain neem oil, neem seeds were crashed using the korner vegetable machine. The powder of neem seeds were squeezed between the metals, which neem oil was obtained for further experiment. The neem oil was then checked for percentage azadirachtin by High Pressure Liquid Chromatography (HPLC).

Solution of neem oil, diluted with distilled water, at different concentrations ranging from 10-40% were tested against the larvae of *R. pulchellus* by topical application. Solution at each concentration was pipetted at 0.5 ml and was applied onto 15 larvae of one month old, while distilled water was applied as a control treatment. There were three replicates for each concentration. The treated ticks larvae were dried on filter paper and transferred to the covered petridish kept at room temperature of $24\pm2^{\circ}\text{C}$ and $70\pm5\%$ RH. Their mortalities were continuously observed and recorded for a period of 24 hours. Ticks larvae which were unable to move at the end of each exposure period were considered as "dead". The mortality of tick was assessed and recorded at 30 min, 1 hr, 2 hr, 3 hr and 24 hours. The knockdown effects and LT_{50} values of each concentration were analysed by Probit Analysis.

RESULTS AND DISCUSSIONS

Toxic effect of Ethiopian neem oil on *Rhipicephalus pulchellus* larvae

Toxicity of Ethiopian neem oil against *R. pulchellus* was studied for the first time in Ethiopia. Dry neem seeds were crashed and the powder was squeezed between the two metals. From the total of

2 kg of dry neem seed, 3 ml of neem oil was obtained. Oil was then tested against one-month old of *R. pulchellus* larvae at the concentrations of 10, 20, 30 at 40%. Lethal Time-50 (LT₅₀) values for all four concentrations of neem oil are presented in Table 1.

LT₅₀ values were ranged from 0.184 to 1.01. The smallest LT₅₀ was obtained from neem oil concentration of 40% (0.184) whereas the highest was from neem at the concentration of 10% (1.01). No tick mortality was observed from the control during the investigation. Neem oil at 40% was the most effective and highest toxicity among the treated concentrations whereas neem at 10% showed the least toxicity (Table 1).

The slopes of regression line for test data from each neem concentration were computed, ranging from 0.504 to 2.36. The highest value was obtained from the neem concentration of 40% and the smallest slope was from the neem concentration of 10%. Small Chi square values indicated that the response of tick to neem oil in susceptibility test to follow completely a linear model (P>0.01).

This study implied that the acaricidal toxicity of Ethiopian neem oil varied directly with the concentration when tested against tick larvae. Higher concentration of neem oil produced high percent mortality than the lower concentration. However, neem oil at 30% was found to be the most effective and economically uses among the treated concentrations.

Ethiopian neem oil was investigated for the percentage of Azadirachtin with HPLC. Azadirachtins A and B at the level of 0.119% and 0.034% were detected from the sample of Ethiopian neem oil. Generally, Azadirachtin is non-toxic to tick when applied by topical application technique (Schmutter, 1990). It will produce toxicity effect only when ingested by the insects. Azadirachtin disrupts metamorphosis of insect and tick, hence they die without reproduction of new generation (Schmutter, 1990). However, the concentrations of Ethiopian neem oil used in this experiment were relatively high. All treated tick larvae were dead after exposing to 50% neem oil. Tick responded quickly to neem oil as indicative by the KC₅₀ (13.43) and KC₉₅ (78.91) values (Table 2).

Larval and nymphal stages of *R. pulchellus* can be easily controlled by using products of neem whereas the adult stage are not much sensitive to all employed dosages of the locally made neem products (KC₉₅ = 78.91%). It is strongly recommended that the minimum dose of Ethiopian neem oil applied on cattle in the field should not be less than 20%. To facilitate in control approach, the concentration can be as much as 30%, depending on each economic problem of the farmer.

The main objective for this experiment was to find the most simple and effective method for controlling cattle tick in Ethiopia. Neem oil seems to be possible in controlling *R. pulchellus* larval in Ethiopia. It is also known that, tick control using

Table 1 Acaricidal toxicity (LT₅₀) of ethiopian neem oil against *Rhipicephalus pulchellus* larvae by topical application.

Treatment neem oil (%)	N*	LT ₅₀ (%) (level of confidence = .95)	Slope ± SE	Chi square
10	45	1.01 (0.198-2.190)	0.504 ± 0.155	1.6511
20	45	0.29 (0.00-1.007)	0.452 ± 0.196	0.1224
30	45	0.21 (0.179-0.426)	0.57 ± 0.485	0.4978
40	45	0.184 (0.00-0.36)	2.36 ± 1.0772	0.4211

N* = number of tick larvae used in each treatment.

Table 2 Knockdown concentration (KC) values of neem oil against *Rhipicephalus pulchellus* larvae by topical application.

Knock down level	KC values	95% confidential limit
KC-20	5.432	2.948-7.986
KC-50	13.43	9.478-17.016
KC-95	78.91	62.88-110.98

only acaricides is not an achievable goal under socioeconomic circumstance of Africa where most owners of livestock in the area are poor. Therefore, attempts should be made to search for other effective control methods, the application of neem oil for controlling ticks seems to be one of the most effective and more suitable under this circumstance. Even though neem found abundance in Ethiopia and the method of application as acaricidal property is not complicate, knowledge of formulation or ready made neem oil for commercial is scarce. Further detailed research must be done to determine the most effective and specific formulation of neem against ticks by overcoming all the limitations.

CONCLUSION

The engorged females of *Rhipicephalus pulchellus* were collected from cattle in Jigjiga, eastern of Ethiopia. They were reared in glass tubes in the incubator at $24\pm2^\circ\text{C}$ and $70\pm5\%$ RH. Using the technique of Solomon and Kaaya (1998), the larvae were separated and kept in other tubes of further investigation.

Ethiopian neem seeds were crashed using the kornet vegetable machine. The powder of neem seeds were squeezed between the metals, which neem oil was obtained for further experiment and was checked for percentage azadirachtin by High Pressure Liquid Chromatography (HPLC).

Solution of neem oil, diluted with distilled water, at different concentrations ranging from 10-40% were tested against the larvae of *R. pulchellus* by topical application. Solution at each concentration

was pipetted at 0.5 ml and was applied onto 15 larvae of one month old, while distilled water was applied as a control treatment. Ticks mortalities were continuously observed and recorded for a period of 24 hours. The knockdown effects and LT_{50} values of each concentration were analysed by Probit Analysis.

LT_{50} values were ranged from 0.184 to 1.01. The smallest LT_{50} was obtained from neem oil concentration of 40% (0.184) whereas the highest was from neem at the concentration of 10% (1.01). Neem oil at 40% was the most effective and highest toxicity among the treated concentrations whereas neem at 10% showed the least toxicity.

All treated tick larvae were dead after exposing to 50% neem oil. Tick responded quickly to neem oil as indicative by the KC_{50} (13.43) and KC_{95} (78.91) values.

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It is strongly recommended that the minimum dose of Ethiopian neem oil applied on cattle in the field should not be less than 20%. To facilitate in control approach, the concentration can be as much as 30%, depending on each economic problem of the farmer.

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