

Full Length Research Paper

Effect of topical application of neem seed (*Azadiracta indica*) extract on sheep infested with *Amblyomma variegatum*

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Accepted 18 June, 2007

The effect of topical application of neem seed extract on sheep infested with tick *Amblyomma variegatum* was examined for acaricidal and repellent activity. The seed extract showed acaricidal activity at 5.0, 2.5 and 1.0% concentration *in vitro*. The extract had no acaricidal activity *in vivo*. However, it showed a repellent activity against ticks at 5.0 and 2.5% concentration. The activity of the extract seems to be concentration dependent. The extract appears to have some potentials as a repellent or antifeedant and could provide an inexpensive integrated pest management resource for farmers.

Key words: Acaricidal, repellent, ticks.

INTRODUCTION

Amblyomma variegatum is found in the tropics and subtropics. It is endemic in sub Saharan savannahs in many countries in Africa including Northern parts of Nigeria. *A. variegatum* is a hard tick that feeds on a number of domestic animals including cattle, sheep, goats, horses and dogs. The bite is severe and painful and may result in septic wounds, abscesses, inflammation on the teats of cows and significant damage to the skin may reduce its market value (Wilson and Bram, 2003). Ticks are responsible for the maintenance and propagation of a great number of human and livestock diseases throughout the world (CAB International, 1989). They are known to serve as vectors of responsible for the maintenance and propagation of a great number of human and livestock diseases throughout the world. The losses in domestic animals caused by these diseases and the direct action of ticks themselves make tick infection an important phenomenon to the livestock owner. Ticks are known to serve as vectors of *Cowdria ruminantium* (cause of heart water), *Ehrlichia bovis* (cause of bovine Ehrlichiosis) and *Theileria mutans* (cause of benign theileriosis in cattle).

Bites from ticks cause skin damage, and feeding adults predispose livestock to dermatophilosis (Hall, 1982). Bab-

esia infections of sheep, equine and dogs results in unthriftiness and toxicosis (Gatenby, 1991; Camus and Barre, 1992).

Losses as a result have been estimated to be in the range of US \$7 billion annually (McCosker, 1979). Ticks control measures include keeping chickens (tick predators) with livestock, applying zero grazing, removal of vegetation harboring the ticks and application of acaricides. The chemicals are applied as sprays, dips, manual application, impregnations into the ear tags and neck or tail bands and in pour-on or spots as preparation (CAB International, 1989; ITDG and IIRR, 1996). The chemical preparations contain compounds like arsenicals, chlorinated hydrocarbons, amitraz and pyrethroids (F.A.O., 1984; Natal et al., 2005). Acaricide usage poses a health risk; there have been reports of increased residues in meat and the development of resistance (Soulsby, 1982; Natal et al., 2005). Some of the acaricides are toxic and misapplication can deal a serious consequence to the farmer in terms of death to the livestock within hours of application (CAB International, 1989; F.A.O., 1984).

To the rural farmer, the technical know how for the application of acaricides is not available, and the chemicals are expensive and toxic. Hence a need exist to research for cheaper, formidable and easily available plant alternative that are ecologically safe and do not leach residues into the environment. *Azadiracta indica* commonly known

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Table 1. *In vitro* acaricidal effect of neem seed extract on ticks (*Amblyomma variegatum*).

Time (h)	Concentration of extract (%)					F (Steladone) 0.045
	A 5.0	B 2.5	C 1.0	D 0.5	E 0.05	
0	13*	13	13	13	13	13
1	9	9	10	11	12	0
3	7	7	8	11	12	0
6	3	3	5	8	10	0
24	1	1	1	5	9	0
>24	0	0	0	4	8	0

*Numbers indicate ticks alive. Initial number of ticks per group is 13.

as neem is an evergreen tree that grows throughout the tropics and sub tropics (Oliver, 1959; Gupta, 1993; Sofowora, 1993). The medicinal properties of the leaf, fruit, bark and seed extract have been therapeutically used to control respiratory disorder, constipation, rheumatism, skin infections, and applied as insect repellent and sometimes used as pesticide (Schmutter, 1990; Sofowora, 1993; Isma, 1997). The present study examines the effect of topical application of neem seed extract on sheep infested with tick *A. variegatum*.

MATERIALS AND METHODS

Seed collection

Ripe seeds of *A. indica* (neem plant) were collected from grounds of College of Agriculture and Animal Science, Ahmadu Bello University Mando Kaduna. The fruits were depulped washed, cleaned and dried in a shaded area to avoid it getting moldy.

Preparation of seed extract

500 g of the dried neem seeds were finely pulverized using a blender. The powdered neem was soaked in 10 liters of distilled water for 12 h, and then filtered using a muslin cloth and whatman filter paper. The filtrate was used for the experiment.

Tick collection

A total of 156 adult male and female ticks (*A. variegatum*) were obtained from a heavily infested farm in Kaduna in the month of July 2005. The farm keeps sheep and goats.

Experimental animals used

A total of 28 healthy sheep consisting of male and female with weights ranging between 20 – 35 kg aged between 2 – 3 years were kept in a clean pen and supplied with fresh water *ad libitum* and fed with pasture, concentrate, hay and salt lick. They were acclimatized for five days prior to the experiment.

In vitro acaricidal effect of the neem extract

A total of 78 ticks each consisting of adult male and female ticks (*A. variegatum*) were placed in six groups (A, B, C, D, E and F) of 13 each, in Petri dishes, with perforated lids. The bottom of group A, B, C, D, and E Petri dishes was covered with filter paper soaked in

various concentrations of the extract; 5.0, 2.5, 1.0, 0.5, and 0.05%, respectively. Group F was the control; distilled water was used. Ticks were observed for reduction in activity (shown by reduced motility) to outright death using a magnifying hand lens over a period of time.

In vivo effect of neem seed extract on tick infested sheep

A total of 20 tick infested sheep were grouped (A, B, C and D) into groups of five each. The groups were bathed with the seed extract at 5.0, 2.5, 1.25% and steladone at 0.048%, respectively. They were fed and watered *ad libitum*. At 3 h interval they were examined for detached or dead ticks.

Repellent effect of neem seed extract

A total of 20 tick free adult sheep were grouped into (A, B, C and D) in groups of five and labeled with ear tags. The tags A, B, C and D were bathed with neem seed extract at concentrations of 5.0, 2.5, 1.0%, and acaricide (steladone) at 0.048%, respectively. They were fed and watered and allowed to move freely in a pen heavily infested with ticks. The animals were checked after every two days, and thereafter, every day for two weeks for the presence of ticks.

RESULTS

In vitro acaricidal effect of neem seed extract

The *in vitro* acaricidal effect of the seed extract on the ticks is shown (Table 1). At 0 h all the groups (A - F) had 13 ticks alive. After one hour Groups A, B, C, D, and E had 9, 9, 10, 11, and 12 ticks alive, respectively. At 6 h groups A, B, C, D, E and F had 3, 3, 5, 8 and 10 ticks alive, respectively. After one day (>24 h) group D and E had 4 and 8 ticks, respectively.

In vivo effect of neem seed extract on tick infested sheep

After three hours of application of seed extracts on the groups (A, B and C), the ticks were detached from the animals in groups A and B and were found far away on the walls of the pen, while Group C still had some ticks attached. The sheep's showed no sign of irritation as a

Table 2. Repellant effect of neem seed extract on *Amblyomma variegatum*. Concentration of extract (%).

Time (Days)	Concentration of extract (%)			D (Steladone) 0.045
	A 5.0	B 2.5	C 1.25	
2	0*	0	1	0
4	0	0	1	0
6	0	0	1	0
10	0	0	1	0
14	0	0	1	0

*Numbers indicate ticks found on Sheep. Initial number of ticks per group is 5.

result of application of the seed extract. At the end of 15 h of observation, the ticks were still found in Group C. One of the ticks was found dead at the end of the experimental observation.

Repellant effect of seed extract on ticks infested sheep

The repellant effect of neem seed extract on *A. variegatum* (Table 2) reveals that after 2 days a tick was found on group C only. After 10 days observation Group C still had one tick. At the end of two weeks observation the tick was still found on the sheep in Group C. The other groups did not reveal any sign of infestation of ticks during the experimental period.

DISCUSSION

In vitro effect of neem seed extracts in *A. variegatum* revealed that the acaricidal effect of the extracts appears to be concentration dependent as well as time dependent. At the end of one hour a range of 69 - 92% of the ticks were alive at 5-0.05% concentration respectively, while at the end of 24 h a range of 7 - 59% were remaining alive. Concentrations of 5, 2.5, and 1.0% were effective in eliminating the ticks *in vitro*. The mechanism by which the extracts act is not known for now. Kurmar and Parmar (1966) reported that the seed extracts contain salannin and azadirachtin which had activity on insects. Schmutterer (1990) reported that neem-based products are medium to broad-spectrum pesticides for phytophagous insects. The acaricide (stelladone) synthetic used was a pure compound as such only a little concentration (0.048%) was needed to eliminate the ticks. The extract could contain a complex mixture of chemicals which need further purification to separate the active ingredient to make it more potent.

In vivo effect of neem seed extract on tick infested sheep showed that the neem seed extract did not show any acaricidal property (killing the ticks) at various concentrations. It, however, could repel the ticks at higher

concentrations due to the presence of azadirachtin it might contain. This is in agreement with Gupta (1993) who reported that seed extract might not actually kill insects but deter them. The chemical steladone, however, within 3 h killed the ticks found on the sheep.

Repellant effect of neem seed on *A. variegatum*

The seed extracts at concentration of 5.0 and 2.5% was able to repel ticks from infesting the sheep while the 1.25% concentration did not repel the tick as they were found in the sheep in groups C. The result agrees with the work of previous authors (Kraus et al., 1985; Broughton et al., 1986; Schmutterer, 1990) who reported that the seed extract contain a chemical, azadirachtin, that had a deterrent, antiovipositional, antifeedant, growth disrupting and fitness reducing properties on insects. The results reveal that neem seed extract could be a great potential as a repellant of tick rather than acriacidal. We however, are speculating that the active ingredient could be the azadirachtin eliciting the repellant activity. We are currently carrying out a phytochemical screening of the extract in our ongoing research.

Conclusion

The result of this study though is preliminary; shows that name seed extract could provide an inexpensive integrated pest management resource for farmers. As the plant is found almost everywhere and farmers could easily use the seeds to prevent tick infestation especially just before rains.

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