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The potential of neem products for control of economically-important African ticks

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ABSTRACT

The potential of Neem oil, two Neem oil-based liquid formulations and powdered Neem seed mixed with diet ingested by goats were evaluated for their effects on *Amblyomma variegatum* ticks. The test materials at high concentrations *in vitro* induced significant mortality of immature stages of *A. variegatum* and to a lesser degree of adults. Mortality increased with the increasing concentration of the Neem products as well as azadirachtin content. Application of Neem compounds on rabbit skin inhibited attachment of all instars of *A. variegatum* for 2-4 days. Thereafter, ticks that attached took longer to feed than controls that were fed on peanut oil treated rabbits. This phenomenon gradually disappeared with increasing period post Neem application. Fewer numbers of all instars of *A. variegatum* were able to attach on rabbits treated with the 3 Neem products. However this inhibitory effect disappeared by day 10 post Neem application. Neem oil significantly reduced hatchability of eggs of *R. appendiculatus* and *B. decoloratus* and the effect was observed to be concentration dependent. *A. variegatum* ticks that were allowed to engorge on goats feeding on a diet containing powdered Neem seed in 3:1 or 2:1 proportions exhibited a significant reduction in their ability to attach and engorge. Feeding, molting and overall development periods were also significantly prolonged. Mortalities were also higher in all tick instars and fecundity was significantly reduced. All these effects were concentration dependent. Spraying 25% Neem oil on de-ticked Zebu cattle grazing in tick-infested pastures *(A. variegatum, R. appendiculatus and B. decoloratus*) significantly reduced the number of immature and adult ticks attaching on cattle for a period of 4 to 5 days.

Keywords: *Azadirachta indica*, azadirachtin, cattle, ticks control, Neem, *Rhipicephalus appendiculatus, Amblyomma variegatum, Boophilus decoloratus.*

INTRODUCTION

Ticks are important ecto-parasites of domestic animals, affecting about 800 million cattle and a similar number of sheep around the world. They ravage the hosts by damaging their hides, reducing growth rate and milk production, transmitting disease organisms, which are often lethal, and by causing paralysis and injuries which lead to secondary infections (Sutherst and Wilson, 1986). Worldwide, economic losses caused by ticks and tick-borne diseases (T&TBDs) in cattle alone have been estimated at $13.9 - 18.7 billion annually (de Castro, 1997). Since their introduction in South Africa around 1890, acaricides have been the commonest method of tick control in Africa, leading to many problems, such as environmental pollution, development of resistant tick strains, and escalating costs (Dipeolu and Ndungu, 1991; Kaaya, 1992). Annual costs of importing acaricides run into millions of dollars in African countries. In Australia and South Africa, resistance to benzene hexachloride in ticks developed as rapidly as within 18 months after its introduction in the market, whereas in South Africa, resistance to DDT and toxaphene developed after 5 and 4 years, respectively (Wharton and Roulston, 1970; Kaaya, 1992). In view of above problems, interest has grown in searching for sustainable, alternative tick control methods (Kaaya, 2003). These include biological control with pathogens

(Mwangi et al., 1995; Kaaya et al., 1996; Kaaya and Hassan, 2000; Maranga et al., 2005; Maranga et al., 2006) and tick parasitoids (Mwangi et al., 1994b; Mwangi et al., 1997), and the use of anti-tick plants (Dipeolu and Ndungu, 1991; Malonza et al., 1992; Hassan et al., 1994; Mwangi et al., 1994a; Kaaya et al., 1995; Kaaya, 2000).

Products derived from Neem, *Azadirachta indica* A. Juss., a tree native to Burma and arid regions of the Indian subcontinent have traditionally been used by farmers in Asia and Africa to control insect pests of household, agricultural, and medical importance (Saxena, 1989; Schmutterer, 1990). Neem products affect ~500 species of phytophagous and other noxious pests (Schmutterer and Singh, 1995), but they have little or negligible side effects on pests' natural enemies and other non-target organisms (Schmutterer, 1995). Neem derivatives comprise a complex array of novel compounds with diverse behavioral and physiological effects, such as repellence, feeding and oviposition deterrence, inhibition of growth and development, mating disruption, reduced fecundity and hatchability, thereby lowering pests' overall fitness (Saxena, 1989; Schmutterer, 1990).

Although neem has been used for centuries for the control of household and agricultural pests, its potential in the control of livestock ticks has not been adequately investigated (Rice, 1993). We therefore evaluated the efficacy of four Neem seed products for the control of *Rhipicephalus appendiculatus* Neumann*, Amblyomma variegatum* Fabricius, and *Boophilus decoloratus* Koch under laboratory and semi-field conditions.

MATERIALS AND METHODS

Neem products

Fresh neem seeds, collected from ripe fruits harvested from 30-year old Neem trees growing at Garissa, Northeastern Kenya, were cleaned and dried in shade to 12-14% moisture content. Whole seeds were manually pounded using a large steel mortar-and-pestle to produce a fine Neem seed powder (NSP). Neem oil (NO) was obtained by cold-pressing whole seeds in a single-screw vegetable oil expeller (IBG Monforts, GmbH & Co., Mönchengladbach, Germany). The azadirachtin-A (henceforth aza) content of NSP and NO, determined by high-pressure liquid chromatography at the International Centre of Insect Physiology and Ecology (ICIPE) Natural Products Chemistry Laboratory was found to be ~5000 ppm and ~850 ppm, respectively. NO and peanut oil (PO) were emulsified to various concentrations in water by using 1% Tween-80. Other test materials were two Neem oil-based formulations: Formulation # 1 with ~300 ppm of aza and formulation # 2 with ~5000 ppm of aza, which were supplied by Saroc Ltd., Nairobi, Kenya.

Ticks

Amblyomma variegatum, Rhipicephalus appendiculatus and *Boophilus decoloratus* were reared separately on New Zealand white rabbits kept in metal cages at ICIPE, as described by Dossa et al. (1996) and Solomon and Kaaya (1998). The rabbits were given rabbit pellets and water *ad libitum.*

Effects of Neem treatment on *A. variegatum (in vitro)*

Unfed as well as fed (engorged) larvae in batches of 100 each, nymphs in batches of 50 each, and adults in batches of 10 each were dipped in vials containing 10, 25, 50, 75, or 100% NO, formulation # 1, or formulation # 2. Control, ticks were dipped in undiluted PO. At 10 minutes after treatment, the ticks were taken out, dried on Whatman No.1 filter paper, and then transferred to glass Petri dishes to record tick mortality, if any. Each treatment was replicated five times.

Anti-feedant effect of Neem formulations

To determine the anti-feedant effect of Neem products on ticks, undiluted NO or formulation # 2 were applied on the right ear, and undiluted PO (control) on the left ear of test rabbits using a fine brush as explained earlier. Rabbits were then exposed to 200 larvae, 100 nymphs, and 50 adult female *A.variegatum* per ear on the first day of treatment, while others were exposed to ticks on days 2, 3, 4, 5, 6, 7, 8, and 9 after Neem application. One day after each exposure, ticks that failed to attach were discarded and the duration (days) which the attached ticks took to engorge fully and to detach was recorded.

Deterrence of tick attachment by Neem products

The procedure was similar to that explained earlier for anti-feedant effect, except that in this experiment, the total numbers of unfed instars of *A. variegatum* able to attach on Neem-treated rabbit ears when exposed on various days post-Neem application were recorded for a continuous period of 10 days. In this experiment, 2 Neem products, namely, undiluted NO and formulation # 2 were used. Undiluted PO was used as control.

Effect of NO on hatchability of eggs

Five batches of 100 eggs of *R. appendiculatus* and *B. decoloratus*, respectively, were placed on filter papers (Whatman No.1) wetted with undiluted NO (850 ppm aza), undiluted PO (control) or 25% NO (diluted with PO), placed in glass vials and incubated at 28°C and 85% Rh till larval emergence was complete (30days) after which the numbers of emerged larvae were recorded.

Host-mediated effect of Neem on *A. variegatum*

Using the ear bag method (Bailey,1960; Solomon and Kaaya,1998), Individual 12- to 14-month-old, male white 'Galla' goats, each weighing 30 - 40 kg, obtained locally, were infested with 200 tick larvae, 100 nymphs, or 25 adult males and 25 adult females per ear. They were kept individually in pens (1 x 1m) and provided with Lucerne and water *ad libitum* daily morning and evening and released for grazing for 2 to 3 h every other afternoon. For protection against *A. variegatum*, goats in three groups of four each were treated as follows: Group #1 goats were fed on rabbit pellets mixed with NSP in 2:1 proportion; Group #2 goats were fed on rabbit pellets mixed with NSP in 3:1 proportion, while goats in Group #3 (control) were fed on pellets only at 20g/ 10kg body weight. The goats were shaved on ears and on the back and infested with 200 larvae, 100 nymphs, and 25 adult males and 25 adult females of *A. variegatum*, as described by Solomon and Kaaya (1998).

To determine host-mediated effects of Neem on *A. variegatum*, the number of ticks attached, the duration for engorgement, engorgement weight, molting period, and tick mortality were recorded for various developmental stages. To determine the effect on fecundity, engorged females in batches of 10 each, randomly selected from the daily collection from each animal, were kept in individual glass vials (75 x 25 mm) in an incubator at 28°C and 85% RH. They were allowed to lay eggs for 20 d, after which the eggs were counted. Eggs from a similar number of ticks were allowed to hatch into larvae and egg hatchability determined based on the number of larvae that emerged.

Effect of NO on tick attachment on cattle in pastures

Ten Zebu cattle grazing in tick-infested pastures at Muhaka, coastal Kenya, were completely de-ticked on the ears, forehead, dewlap, and the entire ventral side, including udders and then divided into 2 groups of five. Cattle in one group were then sprayed with 25% NO (25ml NO+74 ml water +1ml Tween-80) using a hand sprayer, while those in the second group (control) were sprayed with 25% PO (25ml PO+74 ml water +1ml Tween-80). All cattle were then allowed to graze and the attached tick larvae, nymphs, and adults, regardless of their species (mostly *R. appendiculastus, A. variegatum and B. decoloratus*) were counted and recorded daily for 5 consecutive days.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch (REGW) multiple range test to determine differences between experimental and control groups (SAS, 1987).

RESULTS

Effect of Neem treatment *(in vitro)*: Application of NO or NO-based formulations caused mortality of unfed as well as engorged larvae, nymphs, and adult females of *A. variegatum* (Table 1). Immature stages were more susceptible than adults. NO and formulation # 2 were more effective than formulation # 1 against unfed larvae and nymphs. The higher the concentration of NO, NO-based formulations and aza-content, the higher was the tick mortality.

Anti-feedant effect of Neem compounds: During the initial 2-4 days after the application of undiluted NO or formulation # 2 on rabbit ears, all tick instars were unable to attach and feed. Thereafter, ticks were able to attach and the initially long engorgement periods gradually decreased with increasing time post-Neem application. PO had no anti-feedant effect on ticks (Table 2).

Deterrence of tick attachment by Neem products: Application of pure NO or formulation # 2 completely protected rabbits from attachment by all instars of *A. variegatum* for 2-4 days. Thereafter, the numbers of ticks attaching increased progressively with increasing time post-Neem application .No inhibition of tick attachment occurred in the control rabbits treated with PO (Table 3).

Effect of NO on hatchability of eggs: Compared to peanut oil (control), Neem oil significantly reduced the hatchability of eggs of *R. appendiculatus* and *B. decoloratus*. The reduction was higher in the eggs that were treated with undiluted (100%) than in 25% Neem oil (Table 4).

Host-mediated effect on *A. variegatum*: Administration of the diet mixed with NSP to goats significantly reduced attachment of larvae, nymphs,

Table - 1: Mortality of unfed and engorged larvae, nymphs, and adults of *A. variegatum* after dipping for 10 minutes in Neem oil or Neem formulations of varying concentrations *in vitro.* Controls were treated with PO.

	Tick mortality (%) after 24 h					
		Unfed			Engorged	
Concentration (%)	**Larva**	**Nymph**	**Adult**	**Larva**	**Nymph**	**Adult**
Neem oil (850 ppm aza)						
10	68.6c	54.2c	12.4d	68.2c	13.6e	5.6d
25	83.4b	78.6b	36.8c	85.4b	38.3d	16.4c
50	100.0a	90.4a	47.6b	98.5a	56.3c	38.5b
75	100.0a	100.0a	63.6a	100.0a	79.8b	51.2a
100	100.0a	100.0a	66.4a	100.0a	100.0a	57.4a
0 (control)	2.8d	1.7d	0.0e	1.8d	1.6f	0.0e
Formulation # 1 (300 ppm aza)						
10	47.8b	45.6c	1.5d	80.4b	6.2de	3.4d
25	55.3b	74.3b	16.8c	85.6b	21.3c	14.8c
50	100.0a	87.6a	31.4b	100.0a	72.3b	38.5b
75	100.0a	100.0a	48.2a	100.0a	78.5b	46.5b
100	100.0a	100.0a	52.8a	100.0a	89.6a	67.5a
0 (control)	1.7c	1.1d	0.0d	1.7c	0.5e	0.0e
Formulation # 2 (5000 ppm aza)						
10	74.3b	68.6b	5.4d	88.7a	12.4c	2.6d
25	98.4a	93.4a	16.8c	94.5a	38.8b	16.1c
50	100.0a	100.0a	36.4b	100.0a	86.3a	41.7b
75	100.0a	100.0a	55.1a	100.0a	88.2a	67.4a
100	100.0a	100.0a	61.3a	100.0a	94.6a	73.6a
0 (control)	2.0c	0.6c	0.0e	1.8b	0.0d	0.0e

For a particular neem material tested, means followed by the same letter within a column are not significantly different (*P < 0.05;* REGW Multiple Range Test).

Table - 2: Engorgment periods (days) of *A. variegatum* larvae, nymphs, and female adults attached to rabbits ears on various days after treatment of the ears with undiluted Neem oil or with formulation # 2. Controls were treated with undiluted PO.

	Days of tick exposure after treatment with Neem									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Larva										
Neem oil	0.0a	0.0a	0.0a	0.0a	9.3b	8.7b	8.1b	7.7b	7.4b	7.0b
(Control)	8.3a	7.6a	8.1a	7.7a	7.5a	7.1a	8.2a	7.6a	7.5a	8.1a
Nymph										
Neem oil	0.0a	0.0a	0.0a	10.4c	10.2c	9.5c	9.5c	8.2b	7.8b	7.5b
(Control)	8.2a	7.8a	7.5a	7.3a	8.1a	7.6a	7.5a	8.1a	7.6a	7.9a
Adult (Female)										
Neem oil	0.0a	0.0a	13.7e	12.2d	11.8d	10.3c	9.5c	8.5b	7.8b	7.7b
(Control)	7.7a	7.9a	7.6a	7.6a	8.5a	8.2a	8.1a	7.4a	7.8a	7.5a
Larva										
Formulation # 2	0.0a	0.0a	0.0a	0.0a	8.6b	8.5b	8.2b	7.9b	7.7b	7.6b
(Control)	8.1a	7.8a	7.5a	8.2a	7.1a	7.3a	7.6a	7.5a	7.9a	8.1a
Nymph										
Formulation # 2	0.0a	0.0a	0.0a	9.7c	8.4b	8.2b	7.9b	7.6b	7.6b	7.4b
(Control)	8.3a	8.6a	7.8a	7.5a	8.1a	7.9a	7.6a	8.3a	7.7a	8.4a
Adult (Female)										
Formulation # 2	0.0a	0.0a	12.4d	11.8d	10.3c	9.7c	7.6b	7.4b	7.3b	6.8b
(Control)	7.5a	8.2a	7.8a	7.4a	8.1a	7.9a	7.6a	8.3a	7.5a	7.8a

Means followed by the same letter within a row are not significantly different ($P < 0.05$; REGW Multiple Range Test).

and adults (Table 5). Engorgement period was also prolonged and the engorgement weight significantly reduced. Larval-nymphal and nymphal-adult development periods were significantly prolonged on goats fed the diet containing NSP. In contrast to negligible larval mortality in the control, 74% and 86% larvae died on goats fed a diet containing NSP in 3:1 and 2: 1 proportion, respectively (Table 5). Likewise, nymphal mortality on goats fed on diet containing NSP in 3:1 and 2:1 proportion was 58% and 61%, respectively. Adult mortality on goats fed NSP-mixed diet was low. Tick fecundity as well as egg hatchability significantly declined on goats fed on diet containing NSP.

Effect of Neem oil on tick attachment on cattle in pasture**:** Compared with de-ticked cattle treated with PO (control), de-ticked cattle sprayed with 25% NO had much fewer larvae and nymphs attached up to 5 days after application and fewer adults up to 4 days after application (Fig.1).

DISCUSSION

The sensitivity of ticks to Neem products reportedly differs from one tick species to another. For instance, in the U.S., Kaufffman (1988) reported that in *Amblyomma americanum* (Linnaeus), one ecdysteroid system was insensitive to azadirachtin.

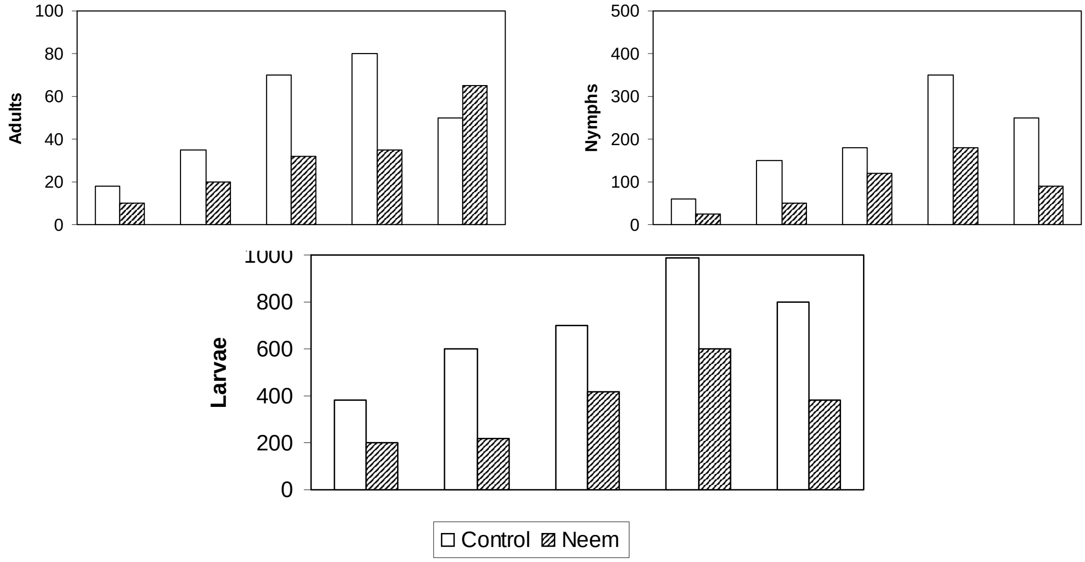

Fig. - 1: Number of ticks on cattle sprayed with 25% Neem oil and on controls (25% peanut oil) and grazed in tick-infested pastures for 5 days. Means of ticks from 5 cattle are presented.

However, in Australia, Rice (1993) reported that a monthly spray application of ethanolic azadirachtins at 3000 ppm or weekly bathing in an aqueous azadirachtin-rich solution gave good control of the bush tick *Ixodes holocyclus* Neum. and the cattle tick *Boophilus microplus* (Canestrini), but was not very effective against the brown dog tick *Rhipicephalus sanguineus* (Tointon).

In the present study, Neem oil and two azadirachtin-rich formulations showed acaricidal activity against all developmental stages of *A. variegatum,* especially the immature stages. The mortality increased with increasing concentrations of the Neem products as well as the azadirachtin content of the compounds. Application of Neem products on rabbit skin inhibited tick attachment and prolonged feeding periods. At higher concentrations, the residual effect of NO and aza-rich formulations were found to persist 3 to 4 days in the laboratory. But even application of diluted (25%) NO reduced for 4 to 5 days the number of ticks attached on cattle grazing in tick-infested pastures. Thus, spray application of Neem materials to livestock grazing in tick-infested pastures could reduce tick infestation. Also, administering to goats a diet containing NSP affected tick behavioral and physiological responses, such as tick attachment, engorgement, reproduction, growth and development, thereby impairing their overall fitness.

Azadirachtin, the major bio-active principal in Neem is known to affect arthropod chemoreceptors, deterring feeding and oviposition and the overall fitness through deleterious effects on meiosis, muscle and gut physiology and biological rhythms (Jacobson, 1989; Mordue (Luntz) and Blackwell, 1993). Its primary mode of action, however, is through the inhibition of release of pro-thoracicotropic hormones and allatotropins. The blockage of these morphogenic peptide hormones is reported to impair ecdysteroid and juvenile hormone titer in hemolymph, thereby affecting molting, metamorphosis and reproduction (Mordue (Luntz) and Blackwell, 1993).

The reduction in engorgement weights results in small size ticks which will produce fewer eggs and a small progeny (Solomon and Kaaya, 1998). The overall effect of Neem products on ticks is therefore likely to adversely affect tick populations and thereby reduce the damage to livestock caused by ticks.

Table - 3: Numbers of larvae, nymphs, and adults of *A. variegatum* able to attach on rabbits when exposed on various days after application of undiluted Neem oil or formulation #2. Controls were treated with undiluted PO.

	Days of tick exposure to Neem treated rabbit ears.									
	1	2	3	4	5	6	7	8	9	10
Larvae										
Neem oil	0a	0a	0a	0a	47b	85c	93d	96d	98d	96d
(Control)	97a	96a	96a	98a	98a	93a	95a	98a	95a	95a
Nymphs										
Neem oil	0a	0a	0a	22b	71c	88d	94e	96e	98e	98e
(Control)	96a	100a	95a	99a	100a	99a	100a	100a	99a	99a
Adults (female)										
Neem oil	0a	0a	0a	52b	68c	85d	98e	97e	94e	96e
(Control)	100a	99a	94a	99a	100a	97a	100a	97a	100a	100a
Adults (male)										
Neem oil	0a	0a	26b	39c	72d	81e	88e	97f	93f	98f
(Control)	97a	97a	98a	100a	100a	98a	100a	100a	95a	95a
Larvae										
Formulation # 2	0a	0a	0a	0a	88b	93c	97c	99c	99c	95c
(Control)	99a	98a	98a	100a	97a	98a	99a	98a	100a	99a
Nymphs										
Formulation # 2	0a	0a	0a	46b	79c	87d	95e	99e	95e	98e
(Control)	99a	99a	100a	100a	98a	99a	99a	100a	99a	99a
Adults (female)										
Formulation # 2	0a	0a	0a	59b	78c	92d	96d	99d	99d	100d
(Control)	100a	100a	99a	97a	100a	98a	100a	100a	99a	96a
Adults (male)										
Formulation # 2	0a	0a	29b	36b	65c	76d	85e	95e	99e	98e
(Control)	97a	100a	98a	99a	100a	98a	100a	100a	99a	98a

Means followed by the same letter within a row are not significantly different ($P < 0.05$; REGW Multiple Range Test).

Although Neem products lack an immediate 'knock-down effect,' which livestock keepers are accustomed to associating with acaricides, Neem has diverse and subtle effects on ticks, rendering their use ideal in integrated tick management. Furthermore, Theileriosis is a cattle disease of great economic importance in Africa (Mukhebi, 1992). Since *Theileria* parasites are transmitted transstadially (i.e. infection picked by the larva is transmitted by the nymph and that picked by the nymph is transmitted by the adult stage) (Norval et al, 1992), factors reducing tick molting may also affect transmission of *Theileria* parasites. Thus, besides reducing tick populations, Neem is likely to reduce transmission of *Theileria* parasites to cattle by reducing or stopping molting in larvae

Table - 4: Hatchability of *R. appendiculatus* and *B. decoloratus* eggs treated with Neem oil at varying concentration. Control treated with undiluted peanut oil (PO)

Concentration	Egg hatch (%) R*. appendiculatus*	*B. decoloratus*
Neem oil 25%	17a	46a
Peanut oil (control)	92b	95b
Neem oil 100%	10a	18a
Peanut oil (control)	94b	97b

For a given concentration, means followed by the same letter within a column are not significantly different ($P < 0.05$); REGW Multiple Range Test).

Table - 5: Biological fitness of *A. variegatum* maintained on goats fed on rabbit pellets mixed with powdered Neem seed (NSP) in varying proportions

Parameter	Developmental stage	Proportion of diet to NSP 2:1	3:1	1:0
Tick attachment (%)	Larva	41.6a	67.9b	93.6c
	Nymph	58.5a	74.6b	98.6c
	Adult	78.9a	81.4a	97.4b
Engorgement period (d)	Larva	21a	14b	7c
	Nymph	29a	21b	11b
	Adult (female)	34a	22b	13c
Engorgement weight (mg)	Larva	1.1a	2.3b	4.1c
	Nymph	36.0a	43.0b	51.0c
	Adult	1060.0a	1430.0b	2830.0c
Development period (d)	Larva-nymph	29a	19a	15b
	Nymph-adult	31a	26a	16b
Mortality (%)	Larva	86.8a	73.6b	1.3c
	Nymph	61.4a	57.8a	0.0b
	Adult (female)	4.3a	3.9a	0.1b
	Adult (male)	2.3a	2.1a	0.2b
Fecundity (eggs/tick x 10^3)	Adult female	6.45a	8.16a	11.40b
Hatchability (%)	Egg	37.6a	51.0b	94.6c

For a given parameter, means followed by the same letter within a row are not significantly different ($P < 0.05$; REGW Multiple Range Test).

and nymphs, a phenomenon that warrants further studies.

The Neem tree is widespread in many African countries. Neem seed from which NO is extracted is a renewable natural resource with multiple uses. Compared with chemical acaricides, the use of low-costing NO, aza-rich formulations or NSP for tick management could be a boon to cash-strapped livestock farmers in Africa. In addition, relative safety of Neem materials to warm-blooded animals and other ecologically-important non-target organisms (Jacobson 1989; Schmutterer, 1995) renders the use of Neem highly attractive for tick management. More persistent Neem formulations could further improve their effectiveness in tick management.

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