EFFECTS OF EXPOSING ADULTS OF *AMBLYOMMA VARIEGATUM* TO NEEM CAKE EXTRACTS IN TRAPS BAITED WITH SEMIOCHEMICALS UNDER SEMI -LABORATORY CONDITIONS

A. Touré[1], R. Maranga[2], A. Hassanali[3], S. Kubasu[4], E. O. Osir[3] and E. Nyandat[3]
[1] Ministère de l'Elevage, Dakar, Sénégal
[2]Jomo Kenyatta University of Agriculture and Technology, Kenya
[3]International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya
[4]Kenyatta University, Kenya
Email : bellamaranga2010@yahoo.com

Abstract
The efficacy of a trap baited with an attractant blend comprising of attraction-aggregation-attachment pheromone (AAAP), 1-octen-3-ol and CO_2 and treated with Neem (*Azadirachta indica*) cake extracts (0.6% of azadirachtin) to attract and expose the ticks, *Amblyomma variegatum* (Fabricius) (Acari: Ixodidae) to the active constituents of the cake was evaluated in circular field plots.. Ticks were released at various distances from trap placed at the center of the plots. Ticks that arrived at the trap (and exposed to the extracts) and those in control plots were collected and their mortality was monitored in the laboratory over a three-week period. All concentrations of the neem extracts caused mortality of *A. variegatum* adults with highest mortality rate (97.8%) recorded in the concentration of 30 % of extracts. The mortality of the ticks was also dependent upon the time ticks were exposed to the extracts. The findings suggest the possibility of using semiochemicals-baited traps in combination with neem extracts for off-host control of these ticks in small-holder farms.

Key words: *Amblyomma variegatum*, traps, *Azadirachta indica*, semiochemicals

1.0 Introduction

Trapping of ticks in vegetation has been known for many years. Eads *et al.* (1982) and Gray (1985) demonstrated the attraction effect of CO_2 on *Dermacentor andersoni* and *Ixodes ricinus* in sampling experiments. Subsequently, (Schöni *et al.*, 1984) reported that *Amblyomma variegatum* female was attracted from 1 m by the attraction-aggregation-attachment pheromone (AAAP) and that ortho-nitrophenol induces orientation and dynamic aggregation while methyl salicylate and nonanoic acid, together with ortho-nitrophenol, induce mounting and clasping behaviour. Norval *et al.* (1989) showed in field experiments that ortho-nitrophenol is a long-range attractant used by the ticks to locate hosts and attached feeding males.

A combination of CO_2 and pheromones was later reported by Norval *et al.* (1989) to be attractive to *Amblyomma hebraeum*. They demonstrated that males and females were activated by carbon dioxide and attracted to AAAP in the field. Maranga *et al* (2003) reported the attraction of *Amblyomma variegatum* to carbon dioxide combined to AAAP up to 5 m. The findings of these workers paved way for studies in the attraction of ticks by CO_2 and pheromones traps for the control of ticks. The use of pheromone/acaricide mixtures to control *Amblyomma hebraeum* has been reported (Norval *et al.*, 1991). Maranga *et al*. (2005) also investigated the efficacy of fungi traps baited with AAAP for the control of *A. variegatum* under semi-laboratory conditions.

Neem derivatives are traditionally used by farmers in Asia and Africa to control insect pests of household, agricultural and medical importance (Kaaya, 2003) and could be useful in controlling ticks and therefore be incorporated in Integrated Tick Management.

Plants of the family Meliaceae have been evaluated against many tick species but most of the studies carried out so far targeted only *A. variegatum* larvae. Since the African 3-host ticks spend 95-97% of their life time in the vegetation (Punyua, 1992), an off-host control strategy using plants in baited traps would go along way in controlling ticks in the pastures. The aim of this study was to assess the effect of neem cake on adult *A. variegatum* attracted to traps baited with semiochemicals in an attempt to control the tick in vegetation.

2 Materials and methods

2.1 Study site

Field experiments were carried out at the Kenya Agriculture Research Institute, National Veterinary Research Centre, Muguga-KARI: latitude 1° 13' S, longitude 36° 18' E, altitude 2070 m (Obiri *et al*., 1994). The climate in Muguga is a modified equatorial type with a mean monthly temperature of 18°C. Mean annual rainfall is 1005 mm, distributed bimodally with peaks in April and October(Obiri *et al*., 1994).

2.2 Experimental plots

Twelve separate plots were prepared by measuring a circular plot of 8 m radius. The grass within each plot was cut to a height of 5cm (Maranga *et al.,* 2003). Each plot was marked from the centre using wooden pegs at intervals of 1 m in a straight line. The marking was done in straight lines prepared at 45^{o} interval all around the plot. A small circle of 10 cm radius was made at the centre of the plot and all the grass removed for placement of the trap. Each plot had a barrier of 5 m un-cleared grass surrounding it (Maranga *et al.*, 2003).

2.3 Traps

The traps used were prepared according to Maranga *et al*. (2006). In brief they consisted of a pheromone dispenser, dry ice container and a contamination chamber in which the Neem Cake Extract (NCE) was placed. The semiochemical/pheromone dispenser consisted of a Petri dish (9 cm diameter) on which a Whatman's filter paper (9cm diameter) fixed on the bottom side using laboratory parafilm. The contamination chamber consisted of an aluminum tray fixed to the cylindrical aluminum tube carrying the dry ice.

2.4 Preparation of the Neem Cake Extracts (NCE)

The Neem cake was obtained from the ICIPE Neem factory. 500 g of cake (0.6% Azadirachtin) was mixed with hexane to cover the neem cake and left to stand for 3 days. The mixture was then filtered using a fine sieve and the solvent evaporated in an evaporator at 60^{o}C until all the solvent was evaporated. The extract was then formulated as 10, 20 and 30% extracts using paraffin oil (Mwangi *et al.*, 1995b).

2.5 Semiochemicals/CO_2

The synthetic AAAP pheromone components consisting of ortho-nitrophenol, methyl salicylate and nonanoic acid were obtained from Sigma-Aldrich Company (Ltd), UK. The equivalent to the AAAP pheromone produced by one attached feeding *A. variegatum* male (0.2mg of ortho-nitrophenol, 0.1mg of methyl salicylate and 0.8mg nonanoic acid) (Schoni *et al*., 1984) was prepared by mixing 200mg of ortho-nitrophenol, 100mg of methyl salicylate and 800mg nonanoic acid in 1ml hexane. One microliter of this solution contained the three components in approximate amounts produced by one feeding male approximately 1.1mg of the pheromone. The source of CO_2 was dry ice obtained from Carbacid (Nairobi, Kenya) while 1-octen-3-ol was obtained from Sigma chemicals.

A blend of 2.2 mg of AAAP and 16 ng of 1-octen-3-ol (dissolved in dichloromethane) that attracted the highest number of *A. variegatum* ticks in previous studies (Toure, 2005) was used as the attractant. The blends were placed on Whatman's filter paper fixed on a Petri dish using laboratory parafilm (Maranga *et al.*, 2006).

2.6 Ticks

Two three-month old unfed adult male and female *A. variegatum* ticks were obtained from the animal rearing quarantine unit. The ticks were counted in batches of 10 with each batch consisting of five males and five females and then placed in separate vials. The ticks were marked with artists' paint (Rownwey Georgian oil paint diluted with linseed oil) with both males and females being marked on the lower quarter of the dorsal side using painters brush. Different batches of ticks were painted with different colours (Maranga *et al.,* 2003). The ticks were then returned to their respective vials and kept in darkness at relative humidity 75% and temperature 25±1^{o}C.

2.7 Experimental design and tick release

The semiochemical consisting of a mixture of 2.2mg of AAAP and 16ng of 1-octen-3-ol on filter paper fixed on a Petri-dish was placed on the upper portion of the trap. 500g dry ice was placed in the tube of the trap (Maranga *et al* 2003). 10% Neem cake extract was formulated using paraffin oil (Mwangi *et al*., 1995b) and 8 mls of the extract placed in the infecting tray of the trap. Two other doses one 8 mls of 20% neem cake extract and another consisting 30% of neem cake extract were similarly prepared and separately placed in different traps. The control consisted of 8mls liquid paraffin which was also placed in another trap. A trap was then placed at the centre of each of the twelve plots which had earlier been prepared. All experiments were set in triplicates.

Ticks were released as per the method of Maranga *et al.* (2003). In brief, adult male and female ticks marked with different colours were released from 1,2,3,4, and 8m downwind from the centre of the plot. Since the direction of the wind arrived within a range of 90° relative to the mean, the ticks were released from three different positions, one being the mean and the other two at ± 45° relative to the mean. The wind direction was monitored using a thin thread attached to a wooden stick of 1 m long at the centre of the plot. Tick movement was monitored from a down position. Ticks that arrived on all the traps were collected in separate vials and taken to laboratory where they were incubated at 28 ± 1°C and 75% relative humidity and their mortality monitored and recorded daily for 3 weeks. Each replicate was observed for four days using a fresh source of pheromone, CO_2 and neem cake extract.

2.8 Data analysis

Tick responses to various combined doses of AAAP, 1-octen-3-ol and CO_2 were recorded and entered on Microsoft Excel. Analysis of Variance (ANOVA) was carried out to test for differences in attraction due to doses, sex or their interaction on the data after square root transformation, using the Statistical Analysis System Software (SAS, 1988). A survival analysis was also performed and differences in the survival curves were analyzed by the Lifetest procedure (SAS)

and the significant level was based on Wilcoxon and Rank tests of equality over strata. Mean values were compared using the Student-Newman-Keuls test at a significance level of 0.05.

3.0 Results

The effectiveness of the traps in attracting ticks on the vegetation was assessed. The results showed strong attraction of *A. variegatum* to the blends of AAAP, 1-octen-3-ol and CO_2 ($p<0.0001$; $df = 63$; $F = 38.85$). The survival curves between the doses were compared and the rank tests for homogeneity indicated a significant difference between the doses ($p<0.0001$) for the Log-rank test and the Wilcoxon test. The log-rank test, which places more weight on larger survival times, was equally significant as the Wilcoxon test, which places more weight on early survival times. The mean survival time in days (Figure 1) showed that ticks exposed to the NCE concentrations (10% and 20%) lived significantly longer than those exposed to 30% NCE. This study also found that the acaricidal effect of the NCE against *A. variegatum* was concentration-dependent.

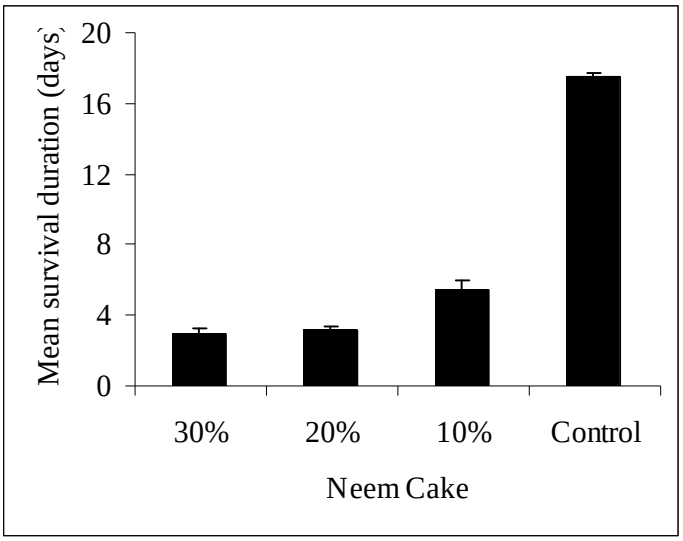

Figure 1: Mean (±SE) survival time of ticks

Higher concentration of NCE produced higher mortalities than the lower concentration and the control (Figure 2). The mortality of the ticks was also time-dependent (Figure 2), with mortalities decreasing from the fourth day. Higher mortality was observed in the first three days of exposure of the ticks to the NCE where 30%, 20% and 10% caused 83%, 79% and 66% of mortality, respectively, during this period.

Figure 2: Mean (±SE) percentage of mortality of A. variegatum ticks exposed to Neem Cake Extracts after 3 weeks

Figure 3 shows the mean survival duration (days) of the exposed female and male ticks which indicated no significant difference between the males and females ($p>0.05$).

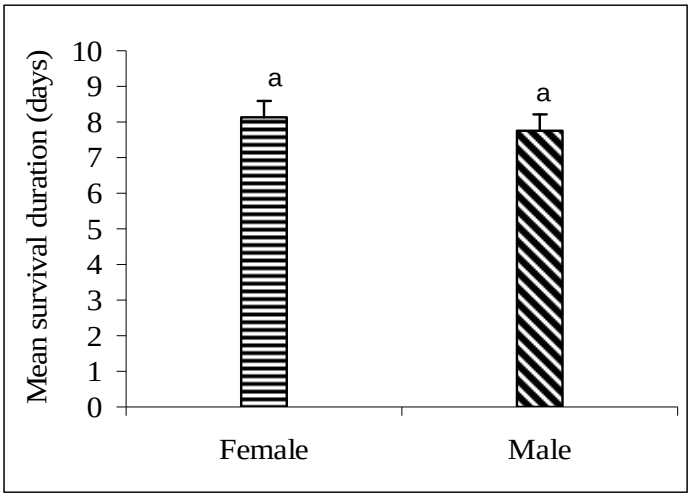

Figure 3: Mean (±SE) survival of male and female A. variegatum after 3-week exposure to Neem Cake Extracts. Means bearing the same letter are not significantly different to SNK test (P = 5%).

4.0 Discussion and conclusion

Azadirachtin is the predominant insecticidal active ingredient in the seeds, leaves, and other parts of the Neem tree (Mulla, 1999) and has been reported to have

acaricidal properties against *A. variegatum* and other tick species (Ndumu *et al.*, 1999; Ismail *et al.*, 2002; DiefAlla *et al.*, 2003). Long term mortality was expected mainly because of using adult *A. variegatum*, which are less sensitive than larvae and because of using diluted extracts. These results are similar to those of Ndumu *et al.* (1999) who reported that neem oil killed 100% of *A. variegatum* larvae within 48 hours. Similar findings were reported by Ismail *et al.*, (2002) who working on the 3-host tick *Rhipicephalus pulchellus* in Ethiopia, found that 40% neem oil was the most effective in achieving tick mortality which also recorded the highest toxicity compared to 10% neem oil. Investigating the effect of neem on *Hyalomma anatolicum*, Abdel-Shafy and Zayed (2002) used a commercial formulation of *A. indica*, neem Azal F, and obtained similar results. Most of the studies investigated only tick larvae which are more susceptible than adults.

The efficacy of neem on *Boophilus microplus* has also been reported (Mansingh and Williams, 1998). These workers investigated the crude ethanol extracts of the Neem leaves on engorged *B. microplus* and found an acaricidal index of 68% and concluded that Neem was very effective against this tick.
This study investigated the effect of different concentration of neem oil using a semiochemical baited trap as an attractant of adult *A. variegatum* and has recorded a similar trend of findings. It is therefore evident that neem extract has a high potential of controlling *A. variegatm* ticks and could be found particularly useful in integrated tick control programmes.

Acknowledgements
The authors thank the Ministry for Foreign Affairs of the Netherlands /DSO Programme for a fellowship through the ICIPE African Regional Postgraduate Programme in Insect Science (ARPPIS) to one of the authors. We acknowledge the support of Dr. Ochieng-Odero and Francis Onyango and the technical assistance of Mark Kimondo and Benjamin Omondi. We are also grateful to the National Veterinary Research Centre of Kenya (Muguga) for availing field facilities.

References
Abdel-Shafy S. and Zayed A. A. (2002). *In vitro* acaricidal effect of plant extract of Neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae). *Veterinary Parasitology,* **106**, pp. 89-96.

DiefAlla H. A., Azzam M. A., Hamdy I. H., Salah, M. K. (2003). Acaricidal effects cardiac glycosides, Azadirachta indica and Neem oil against the camel tick, *Hyalomma dromedarii* (Acari: Ixodidae). *Pest Management. Science,* **59**(11), pp. 1250-1254.

Eads R. B., Smith, G. C. and Maupin, G.O (1982). A CO_2 platform trap for taking adult *Dermacentor andersoni* (Acari: Ixodidae). *Proc. Entomol. Soc. Wash.,* **84,** pp. *342-348.*

Gray J. S. (1985). A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations. *Exp. Appl. Acarol.,* **1**, pp. 35-44.

Ismail M. H., Chitapa K. and Solomon G. (2002). Toxic effect of Ethiopian Neem Oil on larvae of cattle tick, *Rhipicephalus pulchellus* Gerstaeker. *Kasetsart J. (Nat. Sci.)* **36**, pp. 18-22.

Kaaya G. P. (2003). Prospects for innovative tick control methods in Africa. *Insect Sci. Applic.,* **23**(1), pp. 59-67.

Mansingh A. and Williams A. D. L. (1998). Pesticidal potential of tropical plants-II. Acaricidal activity of crude extracts of several Jamaican plants. *Insect Sci. Applic.,***18**(2), pp. 149-155.

Maranga R. O., Hassanali A. and Kaaya G. P. Mueke J. M. (2006). Performance of a prototype baited-trap in attracting and infecting the tick *Amblyomma variegatum* (Ixodidae) with the fungi *Beauveria bassiana* and *Metarhizium anisopliae* in field experiments. Experimental and Applied Acarology, **38(**2-3), pp. 211-218

Maranga, R.O., Hassanali, A and Kaaya, G.P. Mueke, J.M. 2005. Effects of combining the fungi *Beauveria bassiana* and *Metarhizium anisopliae,* on the mortality of *Amblyomma variegatum* (Ixodidae) in relation to seasonal changes. Mycopathologia Vol., **159(4)**, pp. 527-532.

Maranga R.O., Hassanali A and Kaaya G. P, Mueke J. M.(2003). Attraction of *Amblyomma variegatum* (ticks) to the attraction-aggregation-attachment-pheromone with or without carbon dioxide. Experimental and Applied Acarology ,**29**, pp. 121-130.

Mulla M. S. (1999). Activity and biological effects of Neem products against arthropods of medical and veterinary importance. *J. Am. Mosq. Control Assoc.,* **15(2)**, pp. *133-52.*

Mwangi E. N., Hassanali A., Essuman S., Nyandat E., Moreka L. and Kimondo M. (1995b). Repellent and acaricidal properties of *Ocimum suave* against *Rhipicephalus appendiculatus* ticks. *Exp. and App. Acarol.,* **19**, pp.11-18.

Ndumu P. A., George J. B. and Choudhury M. K. (1999). Toxicity of neem seed oil (Azadirachta indica) against the larvae of *Amblyomma variegatum* a three-host tick in cattle. *Phytother. Res.,* **13** (6), pp. 532-4.

Norval R. A. I., Butler J. F. and Yunker C. E. (1989). Use of carbon dioxide and natural or synthetic aggregation-attachment pheromone of the bont tick, *Amblyomma hebraeum*, to attract and trap unfed adults in the field. *Exp. App. Acarol.,* **7**, pp. 171-180.

Norval R. A. I., Yunker C. E., Duncan I. M. and Peter T. (1991). Pheromone/acaricide mixtures in the control of the tick *Amblyomma hebraeum*: Effects of acaricides on attraction and attachment. *Exp. & App. Acarol.,* **11**, pp. 233-240.

Obiri J. A. F., Giathi G. and Massawe, A. (1994). The effect of Cypress Aphid on *Cupressus lusitanica* Orchards in Kenya and Tanzania. *E. Afri. Agric. For. J. (1994),* pp. 227-234.

Punyua D.K. (1992). A review of the development and survival of ticks in tropical Africa. Insect Sci. Applic., **13,** pp. 537-544.

SAS Institute Inc. (1988). SAS/STAT User's guide, Release 6.03 Edition. SAS Institute Inc. Cary, NC.

Schmutterer H. (1990). Properties and potentials of natural pesticides from Neem tree, *Azadirachta indica. Annu. Rev. Entomol.,* **35**, pp. 271-297.

Schöni R., Hess E., Blum W. and Ramstein K. (1984). The aggregation-attachment pheromone of tropical bont tick *Amblyomma variegatum* Fabricius (Acari, Ixodidae): isolation, identification and action of its components. *J. Iinsect. Physiol.,* **30**, pp. 613-618.