Study on the acaricidal effects of *Azadirachta indica* and *Phytolacca dodecandra* on *Amblyomma* ticks in Ethiopia

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Abstract

A study was carried out to investigate the acaricidal effect of extracts of *Azadirachta indica* (neem) and *Phytolacca dodecandra* (locally known endod in Ethiopia) on *Amblyomma cohaerens* and *Amblyomma variegatum*. An adult stage of *A. cohaerens* was collected from east Wollega zone of the Oromia region of Ethiopia and the larval stage of *A. variegatum* was obtained from tick rearing unit of the National Animal Health Diagnostic and Investigation Centre (NAHDIC), Sebeta, Ethiopia. Neem seed was collected from Awash town of the Afar region. Berries of endod from Aklilu Lemma Institute of Pathobiology and commercial neem oil were obtained from India. The water extract of the two plants at doses of 18,750 ppm, 37,500ppm, 75,000 ppm, 150,000 ppm and 300,000 ppm were tested on Petri dish and using the immersion method. The result showed that, neem seed water extract produced mortality rate of 16.6% on adult stage of *A. cohaerens* at a dose of 300,000 ppm. The LD50 indicated a dose of 370,854.7 ppm and no statistically significant ($p>0.05$) difference was observed among the two methods of applications. The same extract in both methods of application produced a mortality rates of 8.3%, 16.6% and 41.6 % at doses of 75,000ppm, 150,000ppm and 300,000ppm respectively on *A.variegatum* and the probit analysis indicated LD50 of 366,64ppm. Endod extract did not produce any mortality at all doses tested on both species of ticks. Neem oil was also evaluated at a concentration of 20%, 40%, 60%, 80% and 100%. At 100% concentration, the oil caused 50% mortality on adult of *A. cohaerens* whereas 20% concentration resulted in 8.3% mortality rate. Likewise, 100%, 100% and 75% mortality rate on *A. variegtum* was observed at 100%, 80% and 20% concentrations of the oil respectively. Probit analysis indicated LD50 value of 11.7% concentration. The water extracts of both plants did not produce 100% efficacy while promising results were obtained by neem oil on larva of *A. variegatum* at high concentrations.

Keywords: Acaricidal effect, *Amblyomma variegatum, Amblyomma cohaerens, Azadirachta indica* (neem), *Phytolacca dodecandra* (endod)

Introduction

Ticks cause enormous economic losses to livestock all over the world and induce adverse effects on livestock hosts in several ways (Snelson, 1975). In Ethiopia ticks are the most important of all ectoparasits and the economic losses incurred on livestock particularly, cattle are enormous (Feseha Gebreab, 1983**)**. Ticks downgrade hides and skins, transmit protozoal, bacterial, rickettsial and viral diseases as well as reduce milk and wool production, reduce productivity and increase susceptibility to the other diseases **(**De Castro, 1997). Among *Amblyomma* species, *A. cohaerens* and *A. variegatum* are known to be widely distributed in Ethiopia (Morel, 1980, De Castro, 1994). The control of ticks and tick-borne diseases remain a challenge for many countries in tropical and subtropical regions including Ethiopia (Lodos *et al*., 2000). Even though, the use of acaricdes is still the basic procedure for controlling most ticks and ectoparasites, its continuous use created limitations among which the escalating cost for the resource poor farmers and the development of acaricde resistance strain of ticks. Nowadays various alternative methods have been developed to act in addition to the use of chemical acaricdes (Cuisance *et al*., 1994**).** Thus the use of botanical plants is among many other alternatives to be used for the control of ticks and other economically important ectoparasites of livestock. In this regard neem has been used for centuries for the control of household and agricultural pests. Recently neem was found to have an acaricdal effect to some cattle ticks, such as *Amblyomma cajennense* and *Boophilus micropilus* (Williams *et al*.,1996), *Hyalomma anatolicum excavatum* (Abdel-Shafi and Zayed, 2002), *Rhiphicephalus appendiculatus* and *Boophilus decoloratus* (Solomon *et al*., 2002) and *Rhiphicephalus pulchelus* (Ismael *et al*., 2002). More over the discovery of molluscicidal properties of *Phytolacca dodecandra* (endod) led to extensive subsequent studies, which revealed wide range properties against organisms and vectors. Its common medicinal uses include treatment of skin itching (ringworm), abortion, gonorrhea, leeches, intestinal worms, anthrax and rabies (Esser*et al.,* 2003). However, very limited or no studies were conducted of its efficacy on livestock ticks. Hence the objective of the present study was to evaluate the acaricidal effect of *Azadirachta indica* and *Phytolacca dodecandra* against adults of *A. cohaerens and* larvae of *A. variegatum*.

Materials and Methods

Study area and study design

The study was conducted in Faculty of Veterinary Medicine, Addis Ababa University using a randomized block study design. Differences between treatments were analyzed using the GLM-repeated measures analysis procedure of SAS (2002).

Ticks, plants and neem oil collection site

Adult *A. cohaerens* were collected from Wayou Tuka Woreda, East Wollega zone of the Oromia region, located 331 km, west of Addis Ababa. This Woreda is situated at an altitude between 1300 and 3140 meters above sea level. The annual temperature ranges from 12 ^{0}C to 32 ^{0}C and the average annual rainfall varies between 1250-1850mm. *A. variegatum* larvae were obtained from tick rearing unit of the acarology and entomology laboratory of the National Animal Health Diagnostic and Investigation Centre (NAHDIC), Sebeta. The neem seed was collected from Awash town of the Afar region located 220 km east of the capital Addis Ababa and *Phytolacca dodecandra* was obtained from Aklilu Lemma Institute of Pathobiology, Addis Ababa University while Indian commercial neem oil (100 % Azadirachtin) was brought from India (Ganga Pharmaceuticals LID Gangatat, Dhanvantari Marg. Virar (E) 401303, Mumbai, India).

Collection and identification of ticks and production of larva

A. cohaerens adult ticks were collected from cattle in the field by hand picking and transferred to clean glass test tubes (30 mm inner diameter x 205 mm long) plugged with cotton wool and transferred to the laboratory and identified according to taxonomic keys of Walker *et al*.;(2003). *A. variegatum* larvae were obtained from tick rearing unit of NAHDIC, Sebeta, Ethiopia; using technical methodologies described by Solomon Gebre and Kaaya (1998).

Plant collection, identification, preparation of water extracts

Seeds of *Azadirachta indica* and berries of *Phytolacca dodecandra* were collected and air dried at room temperature and later grinded and kept in amber colored bottle until processed. Aqueous extraction was made following the methodologies described by Tilahun *et al*.; (2003). Powder of *Phytolacca dodecandra* berries and neem seeds (each weighing 300 gram) were soaked sepa-

rately in 600 ml of clean tap water for 18 hours in a conical flask of 1000 ml. It was then filtered by using sieve and were reconstituted to one liter to form a stock solution of 1000 ml of 300,000 part per million (ppm). This stock solution was diluted with clean tap water to get different desired concentrations expressed in ppm. The concentrations prepared were 18,750 ppm, 75,000 ppm, 37,500 ppm, 150,000 ppm, and 300,000 ppm.

Preparation of different concentrations of neem oil and control solution

The commercial neem oil was diluted with distilled water to prepare different concentrations (20%, 40%, 60%, 80%, and 100%) using the methodologies described by Ismail *et al.,* (2002) and distilled water was used as a control.

In vitro test on petridish (exposure to treated surfaces)

The experimental procedure adopted by FAO (1984) was used with some modifications for this study. Six petri dishes (90 mm diameter) were marked serially. Whatman filter paper of the same diameter as the Petri dishes were impregnated with each of the concentrations 18,750 ppm, 75,000 ppm, 37,500 ppm, 150,000 ppm and 300,000 ppm of water extracts of both plants and neem oil at a concentration of 20%, 40%, 60%, 80%, 100% and placed on separate petridishes, no. 1 to 5. The last group of 6 petridishes impregnated with distilled water served as control. Twelve (12) live specimens of *A. cohaerens* adults were placed on the filter paper of each petridish and a similar filter paper impregnated with the same strength of the extract was placed on top of the ticks and examined every 4 hours for 24 hours. The dead ticks were checked by observing the movement and by pricking it with needle. This experiment was repeated thrice. The same procedure was also followed for *A. variegatum* larvae.

In vitro test by immersion

All the concentrations of the different solutions used in previous method (Exposure to treated surface) were also used in this experiment along with the same control solutions. The ticks were immersed in the universal bottle containing 30 ml of the same solution with different concentrations. After 10 minutes of exposure, the ticks were taken out, kept for a period of 24 to 34 hours on other clean Petri dishes and observed for any mortality if any. The entire experiment was repeated thrice. The procedure was used for both species of ticks separately.

Data analysis

The data was analyzed using a statistical soft ware package for social science (SPSS). Analysis of variance was done using an independent sample t- test whereas the mean mortality by different concentrations within the same plant product and between plants products were analyzed using One-way ANOVA and the LD_{50} value were computed statistical software package Stat plus 2006 professional 3.4.8 for windows. Difference b/n treatments were analyzed using the GLM repeated measures analysis procedure of SAS (2002).

Results

Neem water extract, by the petridish and immersion methods, only at dose of 300,000ppm produced mortality rate of 16.6% on *A. cohaerens* and no mortality was observed with the rest of the treatment concentrations and no statistically significant ($P>0.05$) difference was observed as compared with the control (Table 1).

Neem seed water extract by both application methods produced a mortality of 41.6% at 300,000ppm concentration on larvae of *A. variegatum*; while the least mortality 8.33% and 16.6% were recorded at 75,000ppm and 150,000ppm concentrations (Table 1) in which statistically significant ($P<0.0120$) variation was also observed between the highest concentration and the two least concentrations and the control. Mortality increased as the concentration of the plant preparation increased (Figure 1). *Phytolacca dodecandra* water extract on adult *A.cohaerens* and *A.variegatum* failed to cause any mortality in both application methods.

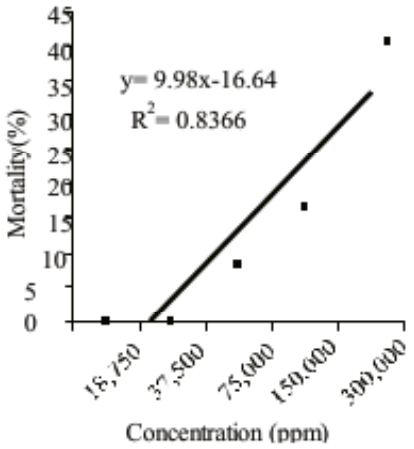

Figure 1.The regression line of the cumulative mortality of *A.variegatum* **larvae by water extract of neem**

Neem oil against adult *A. cohaerens* revealed highest mortality (50%) at 100% concentration. The least mortality (8.33%) was obtained at the least concentration (20%) where statistically significant (P<0.0247) difference was observed (Table 2). Mortality rate of 16.6%, 25% and 33.3% was recorded at 40%, 60% and 80% concentrations respectively. Mortality rate increased with increasing concentration of the oil (Figure 2). LD_{50} of 119.044 was recorded by probit analysis as presented in (Table 3).

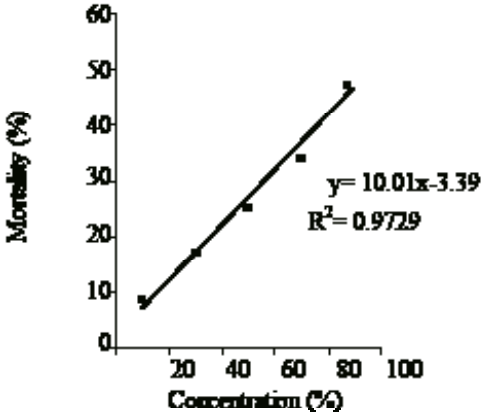

Figure 2.The regression line of the cumulative mortality of adult *A.cohaerens* **by neem oil**

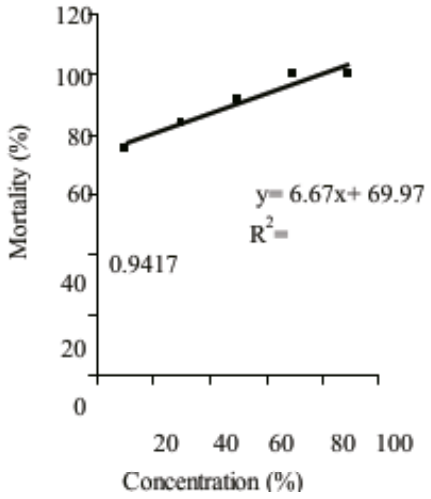

Figure 3.The regression line of the cumulative mortality of adult *A.variegatum* **by neem oil**

Table 1. Effect of water extracts of *Azadirachta indica* **(neem) seed and** *Phytolacca dodecandra* **(endod) on adult** *A. cohaerens* **and larvae of** *A. variegatum*

Plant extracts	Tick species	Appl. Methods	Control		Concentration															
					18,750ppm			37,500ppm			75,000ppm			150,000ppm			300,000ppm		
			Exp.	Dead	Exp.	Dead	%	Exp.	Dead	%	Exp.	Dead	%	Exp.	Dead	%	Exp.	Dead	%
Neem	AA A.c.	Pd.	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	2	16.6
Neem	AA A.c.	Imme	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	2	16.6
Endod	AA A.c.	Pd.	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0
Endod	AA A.c.	Imme	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0
Neem	Av. LL	Pd.	12	0	12	0	0	12	0	0	12	1	8.33	12	2	16.6	12	5	41.6
Neem	A.v. LL	Imme	12	0	12	0	0	12	0	0	12	1	8.33	12	2	16.6	12	5	41.6
Endod	A.v. LL	Pd.	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0
Endod	A.v. LL	Imme.	12	0	12	0	0	12	0	0	12	0	0	12	0	0	12	0	0

A.v = *Amblyomma variegatum*, A.c = *Amblyomma cohaerens*, Pd. = Petri dish, Imme. = Immersion, Exp = Exposed AA= Adult LL= Larvae

Table 2. Effect of *Azadirachta indica* **(neem) oil on adult of** *A. cohaerens* **and larvae of** *A. variegatum*

Plant extracts	Tick species	Appl Methods	Control		Concentration															
					20%			40%			60%			80%			100%		
			Exp	Dead	Exp.	Dead	%	Exp.	Dead	%	Exp.	Dead	%	Exp	Dead	%	Exp.	Dead	%
Neem oil	AA A.c	Pd	12	0	12	1	8.3	12	2	16.6	12	3	25	12	4	33.3	12	6	50
Neem oil	LL A.v.	Pd	12	0	12	9	75	12	10	83.3	12	11	91.6	12	12	100	12	12	100

A.v = *Amblyomma variegatum*, A.c = *Amblyomma cohaerens*, Pd. = Petri dish, Exp = Exposed AA= Adult LL= Larvae

Table 3.Probit analysis value of the effect of neem oil on adult *A. cohaerens*

Percentile	Probit (Y)	Log10(Dose)	Standard Error	Dose	Standard Error
1	2.673214667	0.879822054	1.470952227	7.582668227	112.0076141
5	3.35478856	1.230127505	0.910363828	16.98742316	68.05326161
10	3.718271243	1.416945056	0.616571129	26.11830904	50.85398974
20	4.158543283	1.643229685	0.286511782	43.97741371	31.16347504
25	4.32581086	1.729199445	0.198218068	53.60427732	25.32405719
30	4.47599813	1.806390517	0.184152877	64.0310343	27.97189658
40	4.747066732	1.945710421	0.321498725	88.24912761	71.46138178
50	5	2.075709389	0.509940375	119.0445146	174.1871458
60	5.252933268	2.205708356	0.710923475	160.5862499	397.0463345
70	5.52400187	2.34502826	0.931042893	221.3238723	931.1798876
75	5.67418914	2.422219332	1.054015924	264.3743591	1485.270231
80	5.841456717	2.508189092	1.191481765	322.2471552	2493.670109
90	6.281728757	2.734473721	1.554842551	542.5924181	9726.325078
95	6.64521144	2.921291272	1.85577816	834.2405041	29919.54954
99	7.326785333	3.271596723	2.421208036	1868.945869	246472.5285

The oil, against larvae of *A. variegatum* caused highst mortality (100%) at 100% and 80% concentrations whereas the least mortality (75%) was obtained at concentration of 20% as indicated in Table2. Mortality rate of 83.3% and 91.6% was observed at concentrations of 40% and 60% respectively and no mortality was observed in the control. Statistically highly significant ($P<0.0001$) difference was observed between all the treatment concentrations and the control whereas no statistically significant ($P<0.05$) difference was observed among all the treatment concentrations. In the experiment mortality increased as the concentration increases (Figure 3) and the probit analysis of the evaluated oil indicated LD_{50} value of 11.7163874% (Table 4).

Table 4.Probit analysis value of the effect of neem oil on larvae of *A. variegatum*

Percentile	Probit (Y)	Log10 (Dose)	Standard Error	Dose	Standard Error
1	2.67321467	0.060492422	2.0918702	1.149456187	71.00749279
5	3.35478856	0.35584921	1.685868925	2.269076877	55.01784551
10	3.71827124	0.513362693	1.469823797	3.261089315	48.04599222
20	4.15854328	0.704152477	1.208902672	5.060022837	40.77204248
25	4.32581086	0.776637082	1.110107779	5.979117414	38.29052958
30	4.47599813	0.841720017	1.021626549	6.945763908	36.17157458
40	4.74706673	0.9591863	0.862685951	9.103036838	32.55298217
50	5	1.068793723	0.71574202	11.71638739	29.31702899
60	5.25293327	1.178401145	0.571154972	15.07999318	26.06431669
70	5.52400187	1.295867428	0.421582054	19.76366247	22.34341417
75	5.67418914	1.360950363	0.343720201	22.95886231	20.12795653
80	5.84145672	1.433434968	0.266112649	27.12907389	17.68318788
90	6.28172876	1.624224752	0.205276948	42.09444151	20.64586903
95	6.64521144	1.781738235	0.351002297	60.49761241	54.39461018
99	7.32678533	2.077095023	0.72840197	119.4249376	308.3371855

Discussion

In the present study, the neem seed water extract at a concentration of 300,00 ppm produced 16.6% of mortality on adult *A. cohaerens* and this was not statistically significant (P>0.05) when compared with the rest of the treatment concentrations and the control. The low mortality rate might have been due to the lower level of the active ingredient obtained with the water extraction method. Ruskin (1992) indicated that, the active ingredient in neem is only slightly soluble in water and does not kill pests at high rate; meanwhile it repels and disrupts the growth and reproduction of pests. In addition, the difference in sensitivity might have lowered the mortality rate in both tick species according to the study conducted by Kaufmann (1989). The concentration used in this experiment had produced lowest mortality on adult *A. cohaerens,* so higher concentrations might be required to produce 50% of mortality as it has been revealed by the probit analysis.

The results in the present study has revealed a mortality rate of 41.6 % on larval stage of *A. variegatum* at a concentration of 300,000 ppm which was significantly (P<0.05) higher than the mortality produced by treatment group

of 18,750 ppm, 37,500 ppm, 75,000 ppm and the control. The lowest mortality rate (8.3%) was obtained at the least concentration (75,000ppm). The mortality increased as the dose increases and no statistically significant (P>0.05) difference was observed between the two application methods. This finding suggested that larvae are more sensitive than adult ticks.

In the present study, neem oil produced 50% mortality on larval stages of *A. cohaerens* at higher concentration (100%). Meanwhile this result was found to be lower than the findings of Solomon Gebre *et al.* (2002), who reported mortality rate of 57.4% on adult *A. variegatum*. This variation probably might be due to the difference in type of neem oil used and the sensitivity status of the target tick species. The least mortality (8.3%) obtained at a dose of 20% was significantly (P<0.0247) different than the mortality obtained at higher concentration (100%). This observation is in agreement with the findings of Abdel-Shafy and Zayed (2002) who reported positive correlation between mortality rate and concentration of neem oil extract in a study conducted on *Hyalomma anatolicum excavatum*.

In the current study neem oil produced high mortality (100%) at 100% and 80% of the concentrations on larvae of *A. variegatum*. This finding is in agreement with Solomon Gebre *et al.* (2002) who obtained 100% mortality at 100% and 75% of the concentration. This suggests that lower concentrations between 60% and 80% may have a probability of killing 100% of the larval population of both *Amblyomma* species. The respective mortality rate of 91.6%, 83.3% and 75% at concentrations of 60%, 40% and 20% in the present study is in contrast with Choudhury (2001), who reported 100% mortality in all concentrations on *Rhipicephalus sanguineus*. Similar results were reported also by Solomon Gebre *et al.* (2000) who observed very low mortality in all concentrations on adult *A.variegatum* and higher mortality on the immature stages. Neem seed water extract on both species of ticks produced low mortality even though better effect has been observed on larval stages of *A.variegatum*. Endod has not shown any mortality effect on adult and immature stages of both *Amblyomma* species however the neem seed oil has shown promising effect on larval stage of *A.variegatum* at 80% and 100% concentrations. As neem tree is common tree in all low land pastoral areas of Ethiopia efforts to make use of this botanical plant for acaricidal purpose for ticks and other economically important ectoparasites of livestock must continue in the future.

Conclusions and Recommendations

Neem seed water extract against adult *A. cohaerens* revealed low mortality (16.6%) at the highest concentration (300,000ppm) only. Neem seed water extract showed (41.6%) mortality at the higher concentration (300,000ppm) with LD_{50} of 366,644.08ppm for *Amblyomma variegatum* larvae and it showed an effect in killing more *A. variegatum* larvaee than adult *A. cohaerens* at similar concentrations. This study also revealed that *A. variegatum* larvae are more sensitive than *A. cohaerens* adult. *Phytolacca dodecandra* had no any effect on both studied ticks at the concentrations used. The immersion and exposure to treated surface of both species of ticks by the two plant water extracts do not show difference in mortalities. All concentrations of neem oil in *A. cohaerens* and *A. variegatum* caused mortality and the mortality increased as the concentration increases. The oil killed more larvae of *A. variegatum* than adult *A. cohaerens* and produced higher mortality in both species compared to the neem seed water extracts and endod water extracts at similar concentrations.

Based on above findings the following recommendations are forwarded;

Higher concentration of the water extracts of different parts of both plants with known level of active ingredient should be studied to see their effect on all developmental stages of both species. Neem oil extracted from indigenous Ethiopian plant should be tested in all developmental stages of the two species. Neem oil at concentrations of 80% and 100% can be used for the control of *A. variegatum* Larvae.

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