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EVALUATION OF ANTI-TICK ACTIVITY OF *Lawsonia inermis* AND *Nicotiana tabacum* EXTRACTS AGAINST *Haemaphysalis bispinosa* (ACARI: IXODIDAE)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author SS is responsible for the study research, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author BE designed the study and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Purpose: Ticks (Acari: Ixodidae) are the heamatophages arthropods and an important disease causing ectoparasites of animals and Human. Ticks are primarily controlled by the application of chemical acaricides, though tick populations control has not been achieved adequately. The objective of this present study is to appraise the acaricidal and repellent efficiency of *Nicotiana tabacum* and *Lawsonia inermis* extracts using different solvents against *H. bispinosa*.

Methods: The leaves of *N. tabacum* as well as leaves and seeds of *L. inermis* were extracted by soxhlet apparatus with five solvents. The active compounds were analyzed by GC MS analysis. A stock solution of plant extracts was prepared in Dimethyl Sulpho Oxide and the subsequent concentrations of 5, 10.15, 20 and 25 mg/ml for acaricidal and 0.5, 1, 1.5,2, and 2.5 mg/ml for repellency bioassay were used as working concentration.

Findings: Among the tested extracts, the methanolic and ethanolic extract of both the plants showed the highest activity against ticks. The percent larval tick mortality of *N. tabacum* and *L. inermis* varied from 46.9 % to 96.5%, at higher concentration of 25 mg/ml.The repellencies observed in extracts of *N. tabacum* leaf ranged from the lowest 0.5 mg/ml (31.9%) to the highest 2.5 mg/ml (99%) concentration.

Conclusion: The results obtained from the study highlighted the ethanolic and methanolic extracts of *Nicotiana tabacum* and *Lawsonia inermis* plant products may be used as an alternative to synthetic anti-tick products.

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Keywords: *Haemaphysalis bispinosa*; *Lawsonia inermis*; *Nicotiana tabacum*; plants; ticks.

1. INTRODUCTION

Ticks (Acari: Ixodidae) are the obligate blood sucking arthropods and are important ectoparasites of animals of both domestic and wild life [1]. Ticks and tick-borne diseases (TTBDs) affect 75- 80% of the global cattle population, particularly in tropical and subtropical countries [2]. The world monetary losses due to TBDs have been estimated as US $14,000–18,000 million per year and in nationwide it was approximately US $ 498.7 million [3]. India is the fastest growing country and more than 70 percent population of India is earning from agricultural and animal husbandry sector and about 30.5 million people depend upon livestock for their livelihood. Ixodidae ticks are considered as the most economically important tick species parasitizing livestock, wild animals and humans in India [4], in which *Haemaphysalis bispinosa* is one among the important species that affect the health of cattle. These species of ticks cause major effect on the animal husbandry and productivity of cattle, veterinary health, diary production and quality of skin [2,5].

Control of ticks in livestock has primarily been brought about by the application of chemical acaricides and more recently by insect growth hormones [6]. Even recent technology developments in tick control strategies are ongoing, though tick populations control has not been achieved sufficiently [7]. Further, the enormous use of chemical acaricides leads to resistance, health hazards can harm the humans, animals and environment [8]. The amounts present in the environment have been shown to be toxic to some species of zooplankton and fish [9,10]. In humans, this repellent may cause insomnia, mood disturbances, impaired cognitive functions, seizures, toxic encephalopathy, and allergic reactions [11].

So there is an urgent need to develop an alternative plant based substances as acaricides to control Ixodidae ticks [12]. Botanical pesticides are biodegradable and their use as an acaricide is a practical sustainable alternative and reduces environmental contamination and human health hazards. Many plant species have been traditionally used to control ticks, but the efficacy of extracts of many of the plant species have not been investigated and validated in the laboratory. Validation of traditionally used plants in the laboratory would be a reliable source of anti-tick products and an important step in the strategy for development of plant based alternatives to chemical acaricides. The plants, *Lawsonia inermis* Linn (Family: Lythraceae) is a much branched glabrous shrub or small tree (2-6 m in height) and *Nicotiana tabacum* belongs to Solanaceae family commonly known as tobacco, is herbaceous plant. The plant *L. inermis* and *N. tabacum* has been reported high therapeutic value in the field of medicine including the insecticide and acaricidal effects [13,14]. In particular tobacco plants have been used as acaricides in past many years to control insect pests or parasites of medical importance.

The aim of the present study is to evaluate the acaricidal activity of *L.inermis* leaf and seed as well as *N.tabacum* leaf extract against *H.bispinosa* ticks. Here we extracted the plant parts with different polarity solvents by soxhlet extraction and evaluated their bioactivity against the *H.bispinoa* tick and identified the main chemical constituents of the effective extracts using gas chromatography-mass spectrometry.

2. MATERIALS AND METHODS

2.1 Tick Collection and Laboratory Maintenance

Blood fed adult *H.bispinosa* ticks were collected and identified by morphological identification key [15]. The collected ticks were placed in a plastic container (7 cm × 5 cm) capped with a piece of cotton cloth and tubes were placed in an incubator at 28 ±1°C and RH ≥ 80%for oviposition. The collected adults, freshly laid eggs and subsequent larva were used for acaricidal and repellency bioassay.

2.2 Plant Collection and Preparation

The healthy leaves and fresh seeds of *L.inermis* collect from different locations as well as the leaves of *N.tabacum* were purchased from a commercial supplier. The collected plant parts were washed with tap water to remove all the dust and shade dry at room temperature. The dried each plant parts were ground in electric grinder separately and it was stored in air tight container until further use.

2.3 Extraction by Soxhlet Apparatus and Yield Calculation

Five different solvents with different polarity such as distilled water ($D.H_2O$), 90% Ethanol (C_2H_5OH), 90.5% Methanol (CH_3OH), 99.5% Acetone (C3H6O), 99.8% Chloroform ($CHCl_3$) were used for the extraction according to the polarity. The powdered solid plant material (75–100 gm) was placed in a thimble made up of Whatman No.1 filter paper and

kept inside the soxhlet apparatus. Extraction for each plant materials was carried out in 9-16 hours at boiling temperature with 75 -85 cycles. After the complete evaporation of solvent, extract was stored in airtight glass vial and kept in 4^0C. The percentage yield of the plant extract in each solvent was calculated according to the formula: % Yield of the extract = Cx /C_Y X100 Cx = Plant material weight after extraction process C_Y= Plant material weight taken for extraction [16].

2.4 Preliminary Phytochemical Analysis

The extracted plant product's primary phytochemicals were identified using the procedures described by [17]. The phytochemical constituents such as alkaloids, flavonoids, tannins, terpenoids, steroids, saponins, and cardiac glycosides were tested.

2.5 Active Compound Identification by GC MS Analysis

The extract compound was analyzed by performed using a gas chromatograph coupled to a mass spectrometer (GC–MS) equipped with an auto-injector and a fused-silica capillarycolumn. Helium is used as the carrier gas at a flow rate of 1.2 ml/min. Injector and detector temperatures were set at 250°C and 280°C, respectively. Column temperature set to 60°C for 5 minutes, then gradually increased to 160°C at 4°C min- for 1 minute and finally increased to 270°C at 15°C for 1 minute min-1.

2.6 Egg Immersion Test (EIT)

150 eggs of *H. bispinosa* were placed in glass vials (5 cm x 2 cm) with filter paper at the bottom immersed with 1 ml of test solutions in different concentrations of 5,10,15,20 and 25 mg/ml. Control eggs were treated with 1% DMSO only. Three replicates were maintained for each treatment, and the experiment was conducted in an incubator temperature of 25 ±1°C and RH ≥ 80% and regularly observed until hatching began. The hatched larvae were separated every day from the unhatched eggs and observed for two more weeks before they were declared unhatched and dead. Percent ovicidal activity = Number of unhatched eggs/ Total number of eggs introduced ×100.

2.7 Larval Immersion Test (LIT)

One-week-old *H. bispinosa* larvae were immersed with different concentrations (5, 10, 15, 20 and 25 mg/ml) of test solutions. Each concentration was replicated 3 times (100 larvae/replicate) and control plates were treated with 1% DMSO. After treatment, the larvae were incubated at 25 ± 1°C, relative humidity of ≥ 80%. After 24 h, the dead larvae were counted for calculation of the mortality rate. The larvicidal activity was assessed by the following formula: Percent larvicidal activity = Number of dead larva / Total number of larva introduced × 100 [18].

2.8 Repellency Bioassay

A climbing repellency bioassay was adopted as previously described [19] to test for the repellent properties of extracts. Test and control Whatman No.1filter papers (2.5 cm × 2.5 cm) were treated with 5 ml of different concentrations (0.5, 1, 1.5,2, and 2.5 mg/ml) of sample solutions and repellency activity was reported. The control filter paper was impregnated with 1% DMSO and twenty adult ticks were introduced on a glass beaker for assay. The treated and control paper was placed in the middle of the glass rod. The ticks that were climbed on the upper part of the filter paper were considered not repelled, and those on the bottom of the filter paper, naked part of the apparatus, and on the base part were considered repelled. Each experiment was repeated three times. The repellent effect was calculated as percentage repellency according to the formula: Percentage repellency = 100–(Mean no. of ticks on test/mean no. of ticks on control) ×100 [20].

2.9 Statistical Analysis

Probit analysis (EPA 2006) was used to analyze the results of acaricidal and repellency assays percentage (LC_{50} & RC_{50}) with the calculation of Confidence Interval (CI) of the mean number of ticks dead or repelled by the treatment. Each replication was considered independently. Statistical significance on dose response with each concentration was determined by one-way analysis of variance (ANOVA). All significant levels are set at $P<0.05$. SPSS windows version IBM 20 was used for data analysis.

3. RESULTS AND DISCUSSION

With the aim of selecting potential acaricides, *L.inermis* and *N.tabacum* were screened for their toxic effects on egg, larva and adults of *H.bispinosa*. Among the tested extracts, the methanolic and ethanolic extract of both the plants showed the highest activity against ticks. The yield of two plants extraction is summarized in Fig. 1.The highest and lowest percentage yield obtained were 38.2% and 3.2% for *L. inermis ethanol* and *N. tabacum* chloroform leaf extract. The preliminary phytochemical constituents detected in the different

extracts are shown in Table 1. Phytochemical tests showed presence of alkaloids, saponins, glycosides, terpenoids and tannins in ethanolic extracts of *L. inermis* leaf and seed as well as alkaloid, terpenoids, flavonoids and tannins in *N. tabacum* ethanolic leaf extract.

The methanolic and ethanolic extract of both the plants showed the highest ovicidal activity ranged from 66.6% to 98.5%, at 25 mg/ml concentration. The percent larval tick mortality caused by the ethanol and methanol extracts of *N. tabacum* and *L. inermis* varied from 46.9% to 96.5%, at higher concentration of 25 mg/ml. When compared to tobacco extracts, there was only a low (46.9% to 53.5%) percentage mortality was showed by the *L.inermis* extracts. The probit analysis clearly indicates that the ethanolic and methanolic extract of *N. tabacum* leaf has the highest potential to kill the eggs of *H. bispinosa* with LC_{50} values of 1.47 & 0.68, followed by *L.inermis* seed extract with LC_{50} values of 1.33 & 1.25 and leaf extract with 0.95 & 1.17 LC_{50} values (Table 2). Analyzing the dose response, the LC_{50} and LC_{90} values of *N. tabacum* extract were ranged from 0.4 to 1.09 and 42 to 63.8 respectively and in *L. inermis* it range from 2.5 to 3.46 and 25.1 to 95.4 (Table 3). The homogeneity of variance was significant at all the analyses; also, the ANOVA was significant (*P* value <0.05).

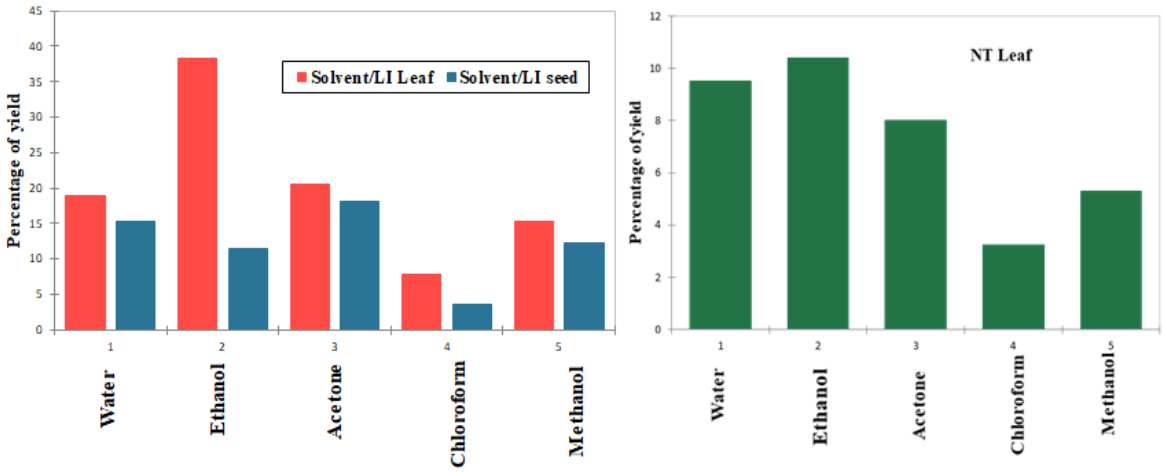

Fig. 1. Percentage extract yield of *L. inermis* (LI) and *N. tabacum* (NT) leaf and seed in water, ethanol, methanol acetone, and chloroform

Table 1. Preliminary phytochemical analysis of five solvent extracts of *L. inermis* and *N. tabacum*

Plant	Solvent	Alkaloid	Flavonoid	Saponin	Tannin	Glycoside	Terpenoid
L. inermis	Water	-	-	-	+	+	+
Leaf	Ethanol	+	-	+	+	+	+
	Acetone	+	+	+	+	-	-
	Chloroform	+	-	-	+	-	-
	Methanol	-	-	+	+	-	-
L. inermis	Water	-	-	+	+	-	-
seed	Ethanol	+	-	+	+	-	+
	Acetone	-	+	+	+	-	-
	Chloroform	-	+	-	+	-	-
	Methanol	-	-	+	+	-	-
N. tabacum	Water	+	-	+	-	-	-
leaf	Ethanol	+	+	+	+	-	+
	Acetone	+	+	+	-	-	+
	Chloroform	-	-	-	-	-	-
	Methanol	-	-	-	-	-	+

Table 2. Statistical analysis of ovicidal activity of *L. inermis* and *N. tabacum* against *H. bispinosa*

Extract	Solvent	Mortality % (Mean± SE) at 2.5 (mg/ml)	LC 50	LC90	R^2	df	P value	CI
L. inermis								
Leaf	Water	48.5±0.13	3.19	28	0.93	4	0.006	(0.08-0.23)
	Ethanol	71.0 ± 0.14	0.95	19.1	0.97	4	0.001	(0.44-0.76)
	Methanol	66.6 ± 0.15	1.17	17	0.97	4	0.001	(0.93-1.6)
	Acetone	36.8 ± 0.14	5.05	95.2	0.95	4	0.004	(0.72-1.71)
	Chloroform	46.4 ± 0.13	5.7	40.5	0.94	4	0.005	(0.37-0.96)
	Control	6.5± 0.51	-	-	-	-	-	-
L. inermis	Water	58.0 ±0.14	2.13	61	0.97	4	0.001	(1.14-2.12)
seed	Ethanol	81.9 ±0.13	1.33	44.2	0.96	4	0.002	(1.8-3.4)
	Methanol	83.0 ± 0.14	1.25	30.9	0.94	4	0.003	(1.7-3.9)
	Acetone	59.6 ±0.13	2.75	81.2	0.86	4	0.038	(1.15-3.08)
	Chloroform	58.5 ±0.14	2.24	22.3	0.97	4	0.001	(1.48-2.5)
	Control	4.5 ±0.43	-	-	-	-	-	-
N. tabacum	Water	93.7 ±0.18	0.54	37.1	0.94	4	0.005	(1.19-3.15)
Leaf	Ethanol	95.6 ±0.19	1.47	33.2	0.91	4	0.01	(1.03-3.68)
	Methanol	98.5 ±0.19	0.68	28.7	0.87	4	0.02	(0.71-4.21)
	Acetone	55.6 ±0.14	2.71	81	0.89	4	0.01	(0.41-1.89)
	Chloroform	92.4 ±0.18	0.90	18.3	0.98	4	0.000	(2.3-3.8)
	Control	4.4 ± 0.31	-	-	-	-	-	-

Table 3. Statistical analysis of larvicidal activity of *L. inermis* and *N. tabacum* against *H. bispinosa*

Extract	Solvent	Mortality % (Mean± SE) at 2.5 (mg/ml)	LC 50	LC90	R^2	df	P value	CI
L. inermis								
Leaf	Water	48.5 ±0.15	3.43	54	0.88	4	0.001	(0.32-1.58)
	Ethanol	46.9± 0.13	3.46	71. 6	0.99	4	0.000	(0.64-0.85)
	Methanol	56.3±0.13	2.5	51.6	0.88	4	0.01	(0.42-1.99)
	Acetone	31.9±0.13	8.2	48.7	0.98	4	0.001	(0.79-1.32)
	Chloroform	41.2±0.13	4.2	46.7	0.97	4	0.001	(0.72-1.25)
	Control	2.7± 0.05	-	-	-	-	-	-
L. inermis	Water	42.0±0.13	3.9	74.1	0.94	4	0.000	(1.04-1.41)
seed	Ethanol	51.9±0.15	3.01	25.1	0.79	4	0.04	(0.86-1.99)
	Methanol	53.5±0.15	2.7	95.4	0.79	4	0.04	(0.04-1.72)
	Acetone	46.9±0.13	3.4	25.1	0.92	4	0.008	(0.69-2.10)
	Chloroform	55.3±0.13	2.3	58.3	0.98	4	0.001	(1.25-2.09)
	Control	2.5 ±0.03	-	-	-	-	-	-
N. tabacum	Water	94.4 ±0.18	0.4	42	0.99	4	0.000	(1.7-2.39)
Leaf	Ethanol	97.6 ±0.19	0.65	57.1	0.60	4	0.122	(1.01-5.14)
	Methanol	95.9 ±0.19	0.56	63.8	0.75	4	0.054	(0.07-3.47)
	Acetone	75.5±0.14	1.09	60.2	0.92	4	0.009	(0.69-2.24)
	Chloroform	91.5 ±0.15	0.73	44.3	0.91	4	0.01	(0.97-3.43)
	Control	2.4 ± 0.01	-	-	-	-	-	-

The most active methanolic and ethanolic plant extracts were selected on the basis of their toxicity against ticks, for further analysis. The bioactive compounds were identified using GCMS analysis present in the ethanolic and methanolic extracts of *L. inermis* and *N. tabacum*. A total of 16 compounds were identified in *L .inermis*, with higher percentage of carotene (47.7%), tocopherol (42.2%), octadecadienoic acid (14.5%), linoleic acid (11.8%), hexadeconoic acid (11.1%), silicic acid (10.4%), Neophytadine (7.4%), and phytol (3.3%). In *N. tabacum*, a total of 20 compounds were identified with higher percentage of phytol (25.1%), phytol (19.3%), pyridine (15.3%), Neophytadiene (6.29%), stigmasta (4.9%), pyridiene (4.6%). and solanesol (3.3%) (Table 4).

The repellencies observed in extracts of *N. tabacum* leaf ranged from the lowest 0.5 mg/ml (31.9%) to the highest 2.5 mg/ml (99%) concentration. *L. inermis* extracts of leaf exhibit repellency ranged from 12.1% to 76% and repellencies observed in extracts of *L. inermis* seed ranged from 10.8% to 73.2% from 0.5mg/ml - 2.5mg/ml concentration. Tick repellency (> 90%) was found in both ethanolic and methanolic extracts at 2 mg/ml concentration producing an RC_{50} of 0.29 and 0.19 respectively. The effective Response concentration (RC_{50}) values of each extracts were shown in the Fig. 2.

In this study, the plant species such as *L. inermis* and *N. tabacum* were tested for the acaricidal activity against *H. bispinosa*. The results showed that ethanolic and methanolic leaf extracts of *N. tabacum* and *L. inermis* exhibited its toxicity against egg and larvae of *H. bispinosa*. Mortality increased with increases in concentration of extract. Among the identified bioactive phytochemicals terpenoids and alkaloids are potential insecticidal growth-inhibitors, and insecticidal activities. Earlier report suggested that flavonoids affect the reproductive functions of ticks. The neurotoxic properties of Alkaloid in the plant extracts were reported to cause mortality and inhibition of fecundity [21]. This finding corroborates with the findings of other researchers on *N. tabacum* against the cattle ticks *R. microplus* [22,23] and *R. appendiculatus* [24]. The acaricidal and repellent activity of the *N.tabacum* extracts in our study is consistent with results from other studies [21]. The tobacco leaf extract caused repellency even at the lower concentrations of 1mg/ml, 2mg/ml respectively. The presence of pyridine alkaloids, Neophytadiene, Phytol, Heptatriacotanol, and Solanesol in the extracts of *N.tabacum* may account, for the observed insecticidal and medicinal effects [25-27].

Table 4. Compounds identified from *L.inermis* and *N.tabacum* ethanolic and methanolic extract by GC MS analysis.

Lawsoniainermis			***Nicotianatabacum***		
Compound Name	**RT***	**%**	**Compound Name**	**RT***	**%**
Undecane	9.0	5.75	Decane	4.2	1.73
Neophytadiene	31.4	7.49	Pyridine	16.3	15.3
Hexadecanoic acid	33.6	9.81	Neophytadiene	33.2	9.82
12-cis-octadecadienoate	37.2	8.04	farnesyl acetone B	34.9	1.43
2-hexadecen-1-ol	37.6	17.9	1-Naphthalenepropanol	38.0	1.80
2,6,10,14,18-Pentamethyl-2	49.3	18.03	Sclareolide	38.2	1.1
2,6,10-trimethyl,14-ethylene	28.8	1.79	phytol isomer	38.	25.17
Phytol	35.2	3.34	Isolongifolol, methyl ether	39.7	2.38
Octadecatrienoic acid	36.4	22.55	1-Heptatriacotanol	40.7	3.37
psi.-Carotene	47.2	47.77	Lactaropallidin	40.7	1.27
Cyclopropanedecanoic acid	46.1	22.4	Octacosanol	45.6	1.5
alpha.-Tocopherol-.beta.-D-mannoside	47.1	42.2	Tetrapentacontane	48.7	2.72
Silicic acid	5.6	10.4	Squalene	50.3	2.17
Furanone,	18.0	7.2	Solanesol	50.6	3.37
Linoleic acid	38.5	11.81	Duvatriendiol	46.6	1.04
Vitamin E	49.1	59.73	Alloaromadendreneoxide	46.7	1.62
			Stigmasta	50.2	4.96
			1-Hexacosanol	47.5	0.77
			Tetrapentacontane	50.7	0.81
			gamma.-Sitosterol	52.5	1.78

** Retention time*

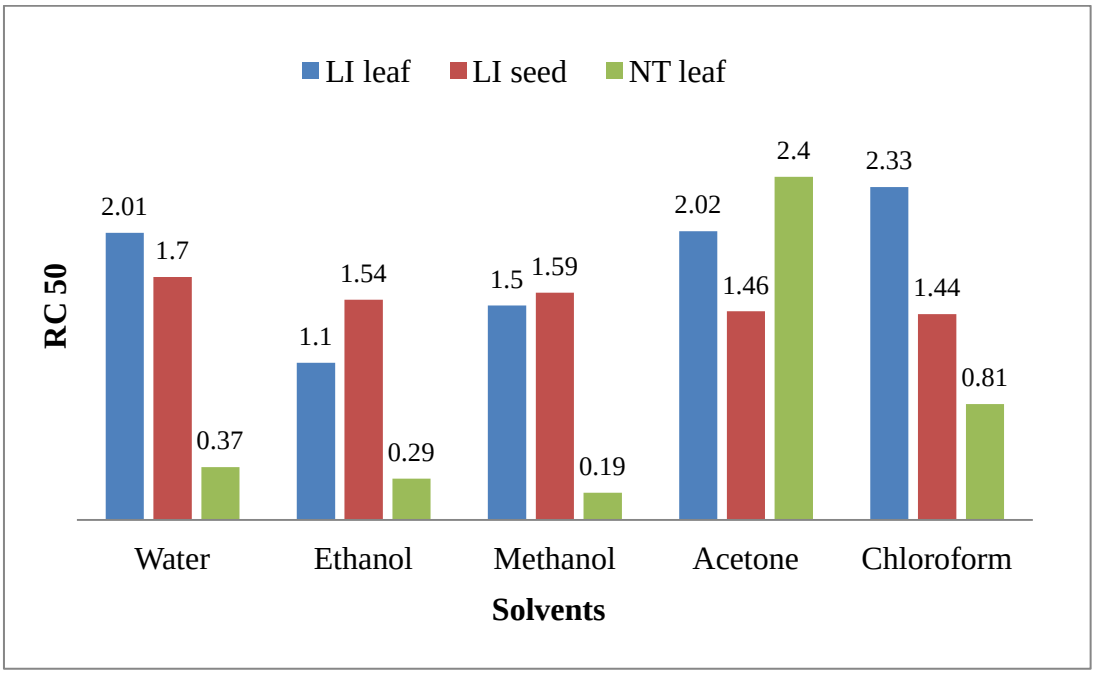

Fig. 2. RC50 values of *L. inermis* (LI) and *N. tabacum* (NT) leaf and seed against *H. bispinosa*

Our study also reported the acaricidal efficiency of *L.inermis* plants against *H.bispinosa* ticks. However the repellent efficiency of *L.inermis* is less toxic when compared with *N.tabacum*. The present study results are in agreed with earlier studies reported the insecticidal activity of *L. inermis* leaves that control cowpea weevil [28] as well as other insects pests [29]. The insecticidal activity of *L. inermis* leaves may attribute to its major constituents (eugenol, hexadecanoic acid, Phytol, α-terpineol and Etherphenylvinyl [30]. In *L.inermis*, the use of undecane as an anti-allergic and anti-inflammatory agent was reported by dabin et al , 2020. In adition presence of neophytadine and phytol is also reported in two plants, which is reported with insecticidal and other medicinal properties in previous studies [31,32].

4. CONCLUSION

The identification of compounds present in the study plants helps to reveal the repellent, insecticidal and growth inhibiting properties. The current results indicate that the extracts of *N. tabacum* were more effective in exhibiting the anti-tick activity against the *H.bispinosa* ticks tested. The present study also clearly establishes the acaricidal properties of the leaf and seed extract of *L.inermis*. Based on study results ethaolic and methanolic extracts of these plant may be used as a source of anti-tick agents. The results obtained from the study highlighted the use of plant products as an alternative to synthetic anti-tick products.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. De la Fuente J, Estrada-Pena A, Venzal JM, Kocan KM, Sonenshine DE. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Frontiers in Bioscience. 2008;1(13):6938-46.
2. De Castro JJ. Sustainable tick and tick borne disease control in livestock improvement in developing countries. Veterinary Parasitology. 1997;71(2-3):77-97.
3. Singh NK, Rath SS. Epidemiology of ixodid ticks in cattle population of various agro-climatic zones of Punjab, India. Asian Pacific Journal of Tropical Medicine. 2013;6(12):947-51.
4. Srikan Ghosh and Gaurav Nagar. Problem of ticks and tick-borne diseases in India with special emphasis on progress in tick control research. Journal of Vector-Borne Diseases. 2014;51:259–270.

5. Balasubramanian R, Yadav PD, Sahina S, ArathyNadh V. Distribution and prevalence of ticks on livestock population in the endemic area of Kyasanur forest disease in Western Ghats of Kerala. South India. Journal of Parasitic Diseases. 2019;43:256-262.
6. George E, Pound JM, Davey RB. Chemical control of ticks in cattle and the resistance of these parasites to acaricides. Parasitology. 2004;129(S1):53-66.
7. Tabanca N, Wang M, Avonto C, Chittiboyina AG, Parcher JF, Carroll JF, Kramer M, Khan IA.Bioactivity-guided investigation of germanium essential oils as natural tick repellants. Journal of Agricultural Food and Chemistry. 2013;61:4101–4107.
8. Elmhalli FH, Pålsson K, Orberg J, Jaenson TGT. Acaricidal effects of Corymbiacitriodora oil containing para-menthane-3,8-diol against nymphs of Ixodesricinus (Acari: Ixodidae). Experminat and Applied Acarology. 2009;48:251–262.
9. Kaloyanova F. Toxicity of selected groups of pesticides: Organophosfates. In: Editors. Toxicology of Pesticides. Copenhagen, Denmark: WHO. Health Aspects of Chemical Safety; Interim Document No. 9. 1982;133–144.
10. Seo J, Lee YG, Kim SD, Cha CJ, Ahn JH, Hur HG. Biodegradation of the insecticide N,N-diethyl-m-toluamide by fungi: identification and toxicity of metabolites. Archives of Environmental Contamination and Toxicology. 2005;48(3):323–328.
11. Qiu H, Jun HW, Mccall JW. Pharmacokinetics, formulation, and safety of insect repellent N,N-Diethyl-3-methylbenzamide (deet): A review. Journal of the American Mosquito Control Association. 1998;14(1):12–27.
12. Frances SP, Wirtz RA. Repellents: Past, present and future. Journal of the American Mosquito Control Association. 2005; 21:1–3.
13. Reddy KR. Folk medicine from Chittoor district Andhra Pradesh, India used in the treatment of jaundice. International Journal of Crude Drug Research.1988;26:137-140.
14. Sandra SW, Kacper P, Kaminski, Jean-Luc P, Patrice L, Anatoly M, et al Antiparasitic properties of leaf extracts derived from selected Nicotiana species and *Nicotiana tabacum* varieties. Food and Chemical Toxicology. 2019;132:110660.
15. Geevarghese G, Mishra AC. Haemaphysalis Ticks of India, 1st ed. London: Elsevier; 2011.
16. Dieu-Hien T, Dinh Hieu N, Nhat Thuy AT, Vo B, Tuong HD, Hoang CN. Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality; 2019; Available:https://doi.org/10.1155/2019/8178294
17. Kokate CK. Pharmacohnosy. 2001; 16th ed. Nirali Prakasham, Mumbai, India.
18. Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine. 2011;1(1):43-8.
19. Mawela KG, Luseba D, Magano S, Eloff JN. Repellent properties of Rothecaglabrum plant extracts against adults of *Rhipicephalus appendiculatus*. BMC Vet Res. 2019 Apr 27; 15(1):122.
20. Anuar AA, Yusof N. Methods of imparting mosquito repellent agents and the assessing mosquito repellency on textile. Fashion and Textiles.2016;3:12 .
21. Oyagbemi TO, Ashafa A, Adejinmi JO, Oguntibeju OO. Preliminary investigation of acaricidal activity of leaf extract of Nicotiana tabacum on dog tick *Rhipicephalus sanguineus*. Veterinary World. 2019;12(10): 1624-1629.
22. Castelblanco S, Sanabria O, Cruz Carillo A, Rodriguez Molano CE. Preliminary report of the ixodicidal effect of some plant extracts on ticks *Boophilus microplus*. Cubana de Plantas Medicinales. 2013;18:118-130.
23. dos Santos F, Albuquerque UP, Costa Junior LM, da Silva Lima A, Borba AL,scimento, Monteiro JM. An ethnopharmacological assessment of the use of plants against parasitic diseases in humans and animals. Journal of Ethanopharmacology. 2014;155:1332-1341.
24. Dipeolu OO, Ndungu JN. Acaricidal activity of kupetaba, a ground mixture of natural products, against *Rhipicephalus appendiculatus*. Veterinary Parasitology. 1991;38:327-338.
25. Moldoveanu and Serban. Evaluation of the Effect of Phytol on the Formation of PAHs in Cigarette Smoke. Beitragezur Tabak for schung. International/ Contributions to Tobacco Research. 2010;24:10-23.
26. Perumal GM; Prabhu K, Rao MRK, Janaki CS, Kalaivannan J, Kavimani M. The Gc Ms Analysis of Ethyl Acetate Extract of One Herbal Plant, Jatrophacurcus. NVEO-Nat. Nnatural Volatiles and Essential Oils Research. 2021 8:6347–6354.
27. Yan N, Liu Y, Gong D, Du Y, Zhang H, Zhang Z. Solanesol: A review of its resources,

derivatives, bioactivities, medicinal applications, and biosynthesis. Phytochemistry Reviews. 2015;14:403–417.
28. Chudasama JA, Sagarka NB, Sharma S. Deterrent effect of plant extracts against Callosobruchus maculatus on stored cowpea in Saurashtra (Gujarat, India). Journal of Applied and Natural Science. 2015;7(1): 18–191.
29. Kamal K, Biswas, Sharmin N, Rabbi M. Evaluation of insecticidal activity of *Lawsonia inermis* Linn. against the red flour beetle, *Tribolium castaneum* (Herbst). Natural products an Indian Journal. 2016;12(1): 008-011.
30. Kidanemariam T, Tesem T, Asressu K, Dekebo A. Chemical Investigation of *Lawsonia inermis* L. Leaves from Afar Region, Ethiopia. Oriental Journal of Chemistry. 2013;29:1129-1134.
31. Duke JA and Beckstrom-Sternberg SM. Dr. Duke's Phytochemical and Ethnobotanical Databases; 1994.
32. Giovanni B, Roman P, Ettore D, Nicolas D, Filippo M. Phytol, (E)-nerolidol and spathulenol from Stevia rebaudiana leaf essential oil as effective and eco-friendly botanical insecticides against Metopolophiumdirhodum, Industrial Crops and Products. 2020;155:112844.