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Dilpreet Kaur
Dept. of Zoology, Govt. Degree College, Hata, Kushinagar, Uttar Pradesh, India

Kamal Jaiswal
Dept. of Applied Animal Sciences, B.B. Ambedkar University, Lucknow, Uttar Pradesh, India

Suman Mishra
Dept. of Applied Animal Sciences, B.B. Ambedkar University, Lucknow, Uttar Pradesh, India

Correspondence
Suman Mishra
Dept. of Applied Animal Sciences, B.B. Ambedkar University, Lucknow, Uttar Pradesh, India

Evaluation of Anti-Tick Activity of *Lantana Camara*: A Preliminary Study

Dilpreet Kaur, Kamal Jaiswal and Suman Mishra

Abstract
The present study deals with the evaluation of anti-tick property of *Lantana camara*. The leaves of the plant were dried, powdered and extracted in organic solvents and evaluated by using standard toxicity bioassay. The plant extract in organic solvent extracts showed highest mortality at 5000 ppm i.e 70%± 0.84, 88%±0.34 and 90% ± 0.24 at 5000 ppm in chloroform, ethyl acetate and methanol extracts. The results of bioassay indicated that all the plants showed variable toxicity against ticks in different extracts. The differences among the percentage mortality of ticks in different extract were found to be non-significant (P>0.05). It may be concluded that the plant has potency to develop as a substitute of synthetic acaricides and thus can help in reducing the cost of tick control and loss due to development of resistance by the use of conventional acaricides.

Keywords: Ticks, Acaricides, Lantana camara, Anti-tick etc.

1. Introduction
Control of tick infestations has primarily been brought about by the application of acaricides. Different groups of chemicals *viz.*, synthetic pyrethroids (SP), organophosphates (OP), formamidines and macrocyclic lactones are used widely [1]. However, widespread use of these chemicals causes serious ecological problems. In addition, the use of chemicals to control ticks on cattle usually generates hundreds of gallons of residues (3-4 liters of solution per animal) which are often discarded indiscriminately, leading to soil and water contamination [2]. These chemicals kill non-target organisms and threaten human health due to the toxic residues in milk and meat [3]. Another most serious problem of intensive use of these chemical acaricides is the development of resistant tick populations which causes failure of the chemical-based tick control program [4]. These limitations have forced researchers worldwide to look at alternative measures for controlling tick infestations.

Plant extracts, used in ethnoveterinary medicine represent a cheaper and easily accessible method for tick control which is usually preferred by most rural farmers when financial constraints restrict the use of modern western medicines [5]. Many of the India's resource poor farmers fail to dip their cattle regularly due to increased cost of the synthetic acaricides used in tick control. Natural products may offer these farmers a cheaper alternative to synthetic acaricides. The livestock holders in rural areas have experimented on the use of plants over many years. There is also a potential for the use of plants to develop sources of acaricides to reduce the cost of tick control [1]. The loss is estimated at the rate Rs. 2000 crores per year [6, 7], therefore, it is worthwhile to look for alternative methods of tick control.

Although many plant species have been traditionally used to control ticks, 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. Botanical pesticides with minimal mammalian toxicity, minimal impact on pollinators and natural enemies, minimal environmental impact, cost effective and easy availability have many advantages over synthetic chemicals [8].

Several researchers have conducted studies on efficacy of plant extracts against ticks. The botanical pesticides had varied effects against ticks such as reducing tick feeding, molting, fecundity and viability of eggs, as also being toxic to nematodes and mites; and additionally having antifungal, antivirus and antibacterial properties against pathogens [8].

Lantana camara Linn. (Verbenaceae) is a hardy, evergreen, straggling shrub with characteristic odour. It ranks among the top invasive weeds on the earth. It is a perennial shrub found growing up to 2000 m altitude in tropical, sub-tropical and temperate parts of the world. The plant is spread widely over Himachal Pradesh, Uttarakhand, Uttar Pradesh and north-eastern States of India.

All parts of this plant have been used for several ailments throughout the world since ancient times. Traditionally *Lantana camara* is considered antiseptic, antispasmodic, carminative and diaphoretic [9].

Keeping in view the dearth of knowledge on evaluation of indigenous/traditionally used plants with anti-tick activity, the present study is designed to evaluate the acaricidal effect of *Lantana camara* plant. The plant has been identified through survey and/or literature review for being traditionally used by locals.

2. Materials and Methods

2.1 Collection of Plants/Plant part(s)

Lantana camara Linn. (Family: Verbenaceae) is a hardy, evergreen, straggling shrub with characteristic odour. The selection of plant was based on survey done in some villages in U.P. and extensive literature survey and review

2.2 Test Organism

Rhipicephalus (Boophilus) microplus, the most common cattle tick found in India was commonly prevalent in the study area. Therefore, the adult ticks of *Rhipicephalus (Boophilus) microplus* were used for *in-vitro* evaluation of anti-tick activity of the plant / plant's part extracts.

2.3 Processing of Plant Material (s)

The plant materials were dried in shade at room temperature and subsequently pounded into powder using pestle and mortar or sometimes electronic blender. Further, to remove the ungrounded parts of the material, the powder was sieved out with the help of muslin cloth. After that the powders were stored in well labelled air tight containers and kept in a cool place.

2.4 Preparation of Plant Extract

Plants extract was prepared by using organic solvents like chloroform, ethyl acetate and methanol. Plant material was dissolved in solvent and macerated for three days. After that the mixture was filtered out using Whatman No. 1 filter paper. The filtrate was kept in water bath for evaporation at 40^0 C and then dried at room temperature. The dried extracts were stored in properly sealed tubes in refrigerator at 4^0 C and used as and when required.

2.5 Preparation of Test Solutions (Concentration)

A stock solution of 10,000 ppm was prepared in 1% Di methyl sulpho oxide (DMSO). Before commencing the experiment, different concentrations: 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm were prepared..

2.6 Preparation of Control

A control of 1% DMSO solution was prepared for the experiments. Mortality of the ticks (if any) was observed and percentage mortality was calculated.

2.7 Collection of Ticks

Adult ticks of *Rhipicephalus (Boophilus) microplus* were collected from cattle owned by rural people/livestock holders of Lucknow, Uttar Pradesh, India. The ticks were stored in vials covered with muslin cloth, and maintained at 25^0C, in B.O.D incubator.

2.8 Methodology of Toxicity Bioassay

The toxicity bioassay as described by Food and Agricultural Organization (FAO) was adopted for investigations of anti-tick properties of the plant extract [10]. As per the protocol a series of envelops were prepared by using Watman filter paper No. 1. The filter paper was treated uniformly with each concentration of extracts on the internal surfaces. The filter paper was folded to form a packet, and 10 ticks were kept in each packet and sealed with adhesive tape. These closed packets were kept in BOD incubator maintained at 27±2º C and 85–92% RH. Alive and dead ticks were counted after 24 hr duration of exposure time and percentage mortality was calculated in comparison to the control. Five replicates of each concentration of plant extract were performed.

2.9 Calculation and Formulae

Percentage mortality was calculated after required time exposure as per the experiment. Mean percentage mortality was calculated for the five replicates by the following formula:

$$(\%)\ \text{Percentage Mortality} = \frac{\text{No. of dead ticks}}{\text{Total No. of ticks}}\ \text{X100}$$

2.10 Probit Analysis

Probit analysis method is adopted in order to evaluate the response outcomes from bioassays [11]. LC 50 of the plant extracts was calculated to assess toxicity of the plant/ plant part(s) extracts.

3. Results and Discussion

3.1 Toxicity Bioassay

The anti-tick activity of *Lantana camara* was evaluated by using the leaf extracts in different organic solvent *viz*., chloroform, ethyl acetate and methanol by the toxicity bioassay, as earlier described [10]. The ticks were exposed to different concentrations (1000ppm, 2000 ppm, 3000ppm, 4000 ppm and 5000 ppm) of plant extracts in each solvent upto 24 hr. The number of dead and live ticks was recorded after 24 hr and mean percentage mortality was calculated. The results are depicted in Table 1.

Table 1: Percentage Mortality and LC 50 of different organic extracts of *Lantana camara* leaf

Solvent used in extract preparation	1000 ppm	2000 ppm	3000 ppm	4000 ppm	5000 ppm	LC 50 (ppm)
Chloroform	41%± 0.74	50%± 0.62	59%± 1.6	65%± 1.69	70% ± 0.84	1776.2332
Ethyl acetate	45%± 1.9	53%± 0.77	65%± 1.22	72%± 0.22	88% ± 0.34	1464.7719
Methanol	46%± 0.38	59%± 1.97	69%± 1.42	79%± 0.22	90% ± 0.24	1314.4074

Control (1% DMSO solution) percentage mortality= 5.5%±1.5

The plant extract in organic solvent extracts showed highest mortality at 5000 ppm i.e 70%± 0.84, 88%±0.34 and 90% ± 0.24 at 5000 ppm in chloroform, ethyl acetate and methanol extracts. The results of probit analysis are shown in Figure 1. The LC 50 values of different organic extract was calculated with the help of regression equation and shown in Table 1. The differences among the percentage mortality of ticks in different extract were found to be non-significant (P>0.05).

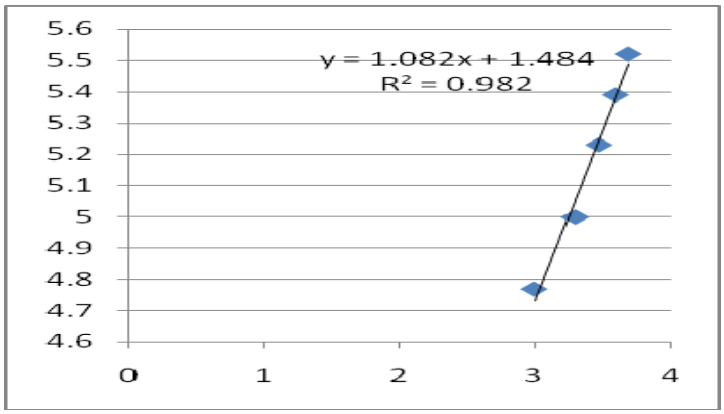
Extracts of *Lantana* leaves have shown strong insecticidal and antimicrobial activity in numerous experiments. Several

reports show that, *Lantana camara* leaf extracts exhibit antimicrobial, fungicidal, insecticidal and nematicidal activity [12-13]. The leaves of this plant were used as an antitumor, antibacterial, and antihypertensive agent [14], and roots for the treatment of malaria, rheumatism, and skin rashes [15]. Several tri-terpenoids, flavonoids, alkaloids, and glycosides isolated from this plant are known to exert diverse biological activities [16]. Extract from the leaves of *L. camara* possessed larvicidal activity [17] while extract from flowers of the plant showed repellent activity against mosquitoes [18]-[19].

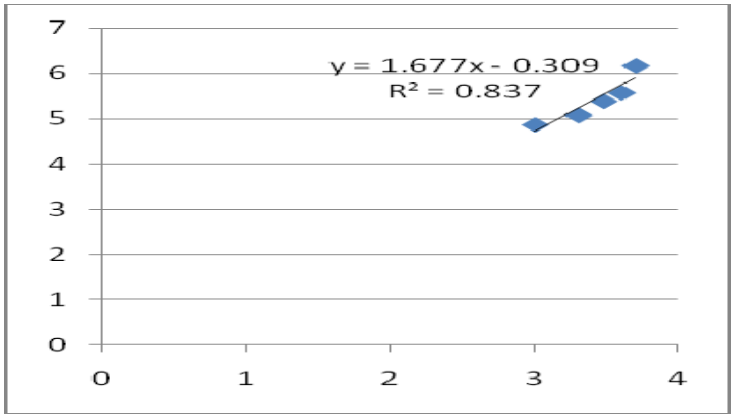
The adulticidal activity of essential oil from the leaves of *L. camara* is investigated against different mosquito species and it is found to have the potency to repel or kill the mosquitoes [18]. Acaricidal property of *Lantana* have been discussed by [8], [20, 21]. Toxic and repellent effect of *Lantana camara* has also been reported by [22, 25].

Conclusion

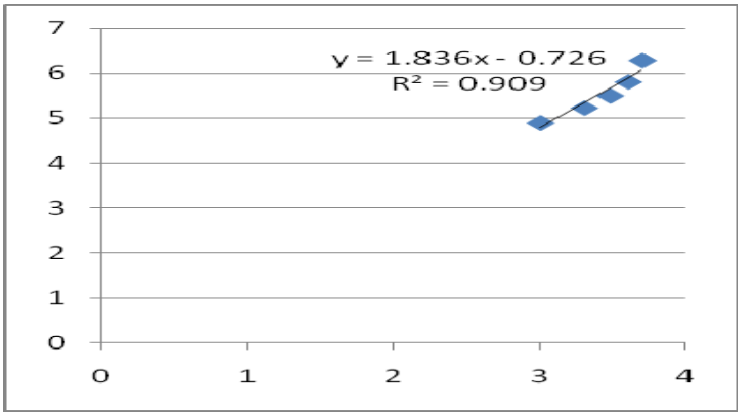
The present study demonstrated that the leaf extract of *Lantana camara* possesses anti-tick activity in a concentration-dependent manner with higher concentration of extracts proved more effective. It may be a source of anti-tick agents. However, further studies are needed to further elucidate the efficacy of the identified compounds. The results obtained from the study further strengthen the widely held view that plant products can be used as alternative to synthetic anti-tick products or along with them under the integrated management system.



Chloroform Extract



Ethyl Acetate Extract



Methanol Extract

Fig 1: Probit mortality x log concentration plots from *Rhipicephalus (Boophilus) microplus* submitted to toxicity bioassay with different extracts of *Lantana.*

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