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ORIGINAL ARTICLE

Larvicidal efficacy of certain phytoextracts of *Citrullus colocynthis* fruits against *Rhipicephalus (Boophilus) microplus*

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ABSTRACT
The acaricidal activity of petroleum ether, hexane and methanol extracts of *Citrullus colocynthis* fruits was assessed against the larvae of *Rhipicephalus (Boophilus) microplus* using larval packet test (LPT), independently. The results obtained indicated that the crude petroleum ether, hexane and methanol extracts of *C. colocynthis* were effective against the larvae of *R. microplus* and methanol extract was found the most potent with LC_{50} 19.84 and LC_{90} 96.25 ppm followed by hexane extract with LC_{50} 35.44 and LC_{90}86.59 ppm and petroleum ether extract with LC_{50} 39.48 and LC_{90} 155.56 ppm. The methanolic extract of *C. colocynthis* possesses promising acaricidal property against *R. microplus* than other extracts. It is an efficient alternative for the management controlling of *R. microplus larvae.*
KEYWORDS: Larvicidal, *Rhipicephalus (Boophilus) microplus*, *Citrullus colocynthis*, Phytoextracts, Efficacy.

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INTRODUCTION

Ticks act as the enervating parasites and a vector of an extent variety of the diseases by transmitting the major pathological constraints (*Anaplasma marginale*, *Arbovirus, Babesia bovis, B. bigemina* etc.) to the veterinary and human health. Tick borne diseases ranked fourth among the major infections of livestock and are the most important vector borne diseases of livestock [1]. The cattle tick, *Rhipicephalus (Boophilus) microplus* is the economically important ectoparasite mainly threat to the domestic animals by infestation of direct effect on their production, quality of skin, blood etc. and indirect related to transmission of rocky mountain spotted fever, relapsing fever, tularemia, meningo encephalitis, Colorado tick fever, crimeancongo hemorrhagic fever, babesiosis [2]. In addition, *R. microplus* population is highly affecting heavy economic losses in terms of high mortality and morbidity rate which reduced production of milk, meat and affect the quality of livestock production. The issue necessitates the promotion of an alternative tick control strategy. Highly potent chemical substances such as cypermethrin, deltamethrin (Pyrethroid), amitraz, organophosphates and vaccines like ivermectin have been used to eliminate ectoparasites and play an important role in tick control [3]. However, the indiscriminate use of these chemical acaricides has some drawbacks *viz.* development of resistance, environmental pollution and residues in meat, milk, skin and natural toxicity [4]. An eco-friendly and economically viable alternative is needed urgently to decrease the implication of synthetic pesticides. Natural products of plant origin with insecticidal properties have been tried in the recent years to control the variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of tick control agents. There is paucity of information on the acaricidal efficacy, persistence, and required rate of application of plant products, particularly when sub-optimal dosages are likely to result in tick resistance. The use of indigenous plants for pest control by farmers is cost-effective, environment friendly and locally available [5]. However, their use needs to be optimized since different chemotypes of

some pesticidal plant species occur which can lead to highly variable efficacy [6,7], safe handling should be promoted widely since there is no adequate information about the safety is available. Natural products are generally preferred due to their less harmful nature to non-target organisms and to their innate biodegradability. [8] reported that some pesticidal plants show acute mammalian toxicity to animals but these were at very high dosages so typical use by farmers is unlikely to expose them to toxic levels. Many studies on plant extracts against tick larvae have been conducted around the world. [9] evaluated the seed extracts of *Achyranthes aspera, Antisomeles malabarica, Gloriosa superb, Psidium guajava, Ricinus cumminis* and *Solanum trilobatum* tested against the larvae of cattle tick *Rhipicephalus microplus* and the results suggest that the leaf ethyl acetate extract of *A. aspera*, leaf acetone and chloroform extract of *A. malabarica* shows higher Efficacy against ticks in comparison to other ectoparasites. [10] observed the tick controlling efficacy of extracts from *Petiveria alliacea* against the cattle tick *R. (Boophilus) microplus* and methanolic extract of stem and leaves of *P. alliacea* showed 100% mortality of target organism. [11] worked on herbal drug for controlling ectoparasites and evaluated the invitro efficacy on *Rhipicephalus microplus* of extracts from *Cymbopogan martini* and *Cymbopogan schoenanthus* leaf essential oil and *Piper tuberculatum* leaf crude extract and similar synthesized substance. [12] studied plants extracts for eco-friendly development of phyto-acaricides for chemical resistant tick infestation in animals. [13] recorded activity of petroleum ether extract of leaves of *Tetrastigma leucostaphylum* against cattle tick, *Rhipicephalus (Boophilus) annulatus*. [14] reviewed the chemical composition of *C. colocynthis* and showed the seed kernels of the plants contain good amount sources of amino acids such as tryptophan, argentine and methionine and also reported that all parts of the plants like fruit and leaf were found to highly possess flavonoids and flavones. *Citrullus colocynthis* (L.) Schrad a medicinal plant belonging to the family Cucurbitaceae is commonly called 'bitter apple' or 'wild water melon' [15], in Hindi as 'Indrayan' and in Sanskrit as 'Indravaruni' [16]. The plant is found throughout India both wild and cultivated. It is also indigenous in the West Asia, tropical Africa and Mediterranean regions [16]. The plant has been utilized in traditional medicine as an abortifacient [17, 18]. The plant also possesses anti-inflammatory [19], anticandidal and antibacterial [20], antioxidant [21], analgesic [22], hypoglycemic [23], hypolipidemic [24], antialopecia [25], antidiabetic [26] and antifertility [27] properties. Therefore, the purpose of this study was to investigate the acaricidal activities of different extracts of *C. colocynthis* fruits against the Indian cattle tick *R. microplus* under the laboratory conditions.

MATERIAL AND METHODS

Rearing of ticks

Engorged female ticks, *Rhipicephalus (Boophilus) microplus* were collected from different naturally infested cattle sheds (Gaushala's animals) from Kalindi Vihar of Agra, (Uttar Pradesh). The collected ticks were transported into the laboratory and were identified by using the identification key [28]. After proper identification, the ticks were rinsed in distilled water twice to remove dust and other foreign particles. These engorged females were weighed and kept into the tick rearing glass tubes covered with muslin cloth with the help of elastic rubbers and were kept in glass desiccators contained 10% KOH solution and kept it into BOD incubator at 70-80% relative humidity and 27±2^{o}C temperature.

Collection of plant material and preparation of extracts

Fruits of *C. colocynthis* were collected from the different localities of Agra. The separated fruits from the plant were washed in running tap water and chopped by knife and left them for dried in shade at room temperature. Completely dried chopped fruits were grinded to make course powder independently in mixer and kept into air tight glass containers. The powdered plant material was packed into thimble of the soxhlets extractor and subjected to extraction independently using petroleum ether, hexane and methanol, subsequently upto 72 hours or till the solvent in the siphon tube of an extractor becomes colourless for the complete extraction. The completion of the extraction was confirmed by taking the solvent from the thimble and evaporated to check the absence of residue. The extracts were taken out, filtered and distilled to concentrate to get the syrupy consistency in Rotary Vacuum Evaporator. The extracts were kept in airtight pre-weighed glass containers into refrigerator to avoid loss of volatile principles [29].

Larval Packet test (LPT):

The refrigerated above extracts were used to prepare their stock solutions. Petroleum ether (0.5gm/50ml), hexane (0.5 gm/50ml) and methanol extracts (0.5gm/ 50 ml) were dissolved in ethanol to prepare 10,000 ppm stock solutions, independently. The stock solutions were further diluted in distilled water to make the series of desired six concentrations independently in increasing order. The experiments were conducted in triplicates along with control and highest amount of alcohol used in preparation of test concentration, as well as in control.

The modified LPT was used to test the acaricidal activity of different crude extracts of fruits from *C. colocynthis* against *R. (B.) microplus* larvae [30]. Tween-20 was diluted in distilled water at 2% concentration and used to prepare stock solution in desired concentrations (20, 40, 60, 80, 100 and 120 ppm) along with the control group.7–14 days old tick larvae were used in this study. Hatching vials with the highest larval eclosion rate (90–100%) were selected and placed in the centre of a petri dish that was subsequently filled with water and soap, which prevented their escape. The diluted plant extract (3 ml) was transferred to petri dishes (60mm x 15mm in diameter) and 300–500 larvae were placed between two Whatman No. 1 papers and immersed for 10 min. Approximately 100-150 larvae were picked with a no. 4 paintbrush and gently transferred to clean filter paper packets. The opening of the envelopes (treated and control with larval ticks) was folded with metallic clip, with its identification mark (tested solution and concentration) on the outside. The packets were placed in an incubator at 27± 2^0 and 75 ±5% relative humidity and observed after 24 hours. The envelopes were opened after 24 hour of post-treatment and observed using a stereoscopy. The number of live larvae, mortality and any toxicological effects observed were recorded. Larvae that were unable to walk forward were considered dead.

STATSTICAL ANALYSIS

The mortality data observed in LIT, were corrected by using Abbott's formula [31] to remove the factors other than the extract tested. The corrected data were subjected to calculate the LC_{50} and LC_{90} values of dose response by Probit Analysis [32] along with other statistical parameters at 95 percent confidence level with upper and lower fiducial limits, chi-square test and standard error by using the software developed by [33].

$$\text{Corrected \% mortality} = \frac{\text{\% Mortality in treatment - \% Mortality in control}}{\text{100 - \% Mortality in control}} \times 100$$

RESULTS

The acaricidal property of petroleum ether, hexane and methanol extracts was evaluated against the larvae of *R. microplus* and the results are depicted in table 1 and Figure 1, 2. Table1 revealed that extract of petroleum ether showed least efficacy LC_{50} 39.48 ppm with upper and lower fiducial limits 42.52 ppm and 36.42 ppm and LC_{90} 155.65ppm with upper and lower fiducial limits 175.19 and l 136.11p pm. Hexane extract showed moderate activity exhibit LC_{50} were 35.44ppm with upper and lower fiducial limit 37.45 and 33.4 ppm and LC_{90} 86.59 with upper and lower fiducial limits 92.13 and 81.05ppm respectively. Methanol extract showed highly effective LC_{50} were 19.84 ppm with upper and lower fiducial limits is 21.59 and 18.09 ppm and LC_{90} 96.25 with upper and lower fiducial limits 112.02 and 80.48 ppm, respectively. The relative toxicity showed that methanol extract was the 20.9 times most efficient followed by hexane extract was 5.0 times more effective than petroleum ether extract.

Table 1-Relative larvicidal activity of *Citrullus colocynthis* (fruits) extract against *R. microplus*

Solvents	Experiment Period (Hr)	Chi-Square **(χ2)**	Regression equation	LC_{50} ±SE UL-LL (ppm)	Relative toxicity	LC_{90}±SE UL- LL (ppm)	Relative toxicity
Petroleum ether	24	27.7212	Y=0.58+2.15	39.48±1.55 42.52-36.42	1.0	155.65±9.69 175.19-136.11	1.0
Hexane	24	21.764	Y=3.41+3.30	35.44±1.03 37.45-33.4	5.0	86.59±2.83 92.13-81.05	83.0
Methanol	24	64.49	Y=0.71±1.87	19.84±0.89 21.59-18.09	20.9	96.25±8.04 112.02-80.48	63.2

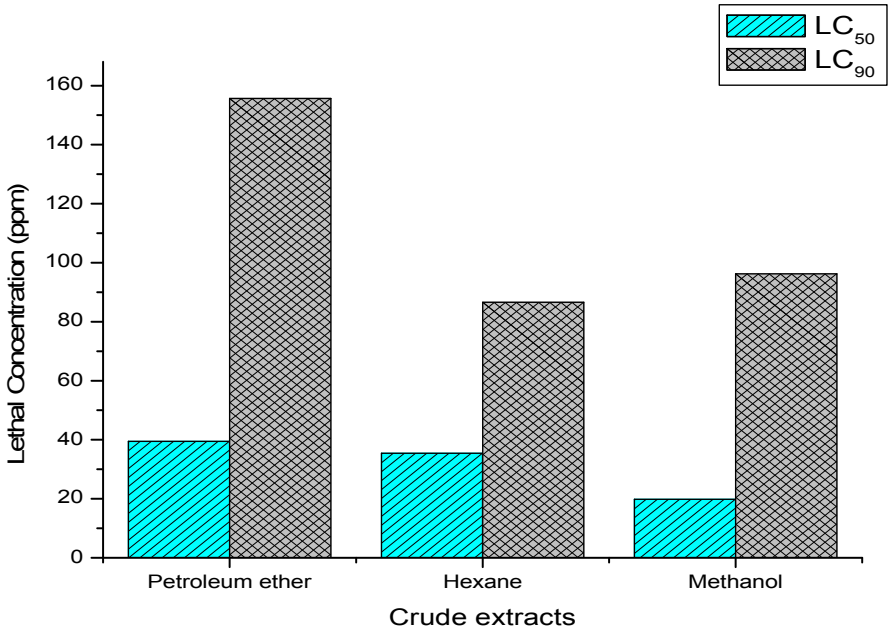

Figure 1: Relative toxicity of petroleum ether, hexane and methanol extracts of*Citrullus colocynthis* fruits against *Rhipicephalus (Boophilus) microplus*

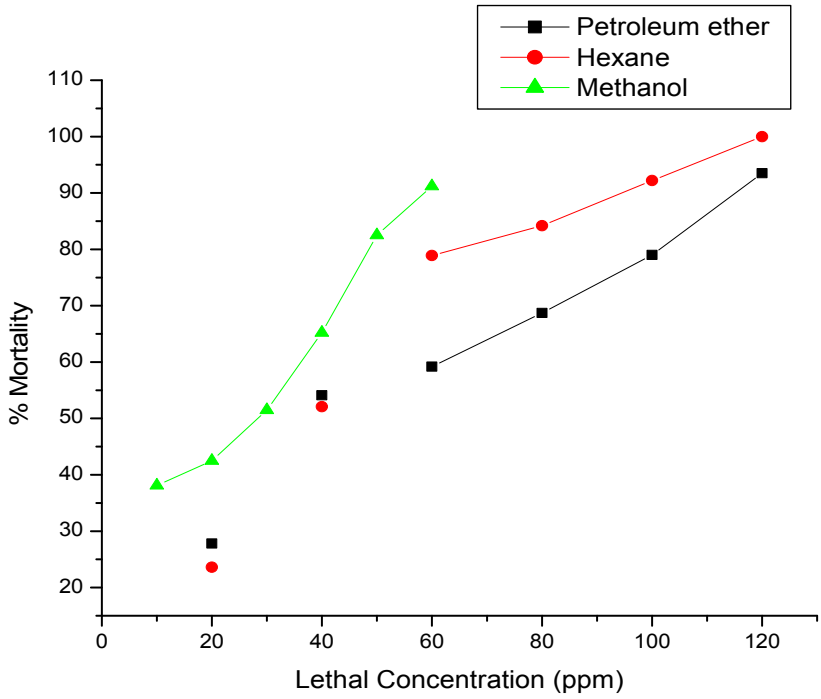

Figure 2: Dose–effect relationship of Citrullus colocynthis extracts against the larval stage *Rhipicephalus (Boophilus) microplus*

DISCUSSION

The use of natural products mainly botanical acaricides for the control of ticks has been the focused in many countries, principally to with stand the noticeable increasing frequency of acaricides resistant tick strains. Acaricidal resistance has been documented as a result of limiting properties of various plant extracts. [34], worked on the hexane extract *of Calea serrata Less. (Asteraceae)* and observed LC_{50} value 0.28 µ/ml hat demonstrates the efficient larvicidal continuous uses or abuses of pesticides. This situation has encouraged efforts that should be undertaken to present the emergence of acaricides resistant ticks of veterinary importance. The present study showed that the promising property of *Citrullus colocynthis*

fruit extracts against *Rhipicephalus (Boophilus) microplus.* The petroleum ether extract exhibited low efficacy. However, methanol extracts possessed a strong larvicidal activity with LC_{50} 19.84 ppm after 24 hours of exposure against the target organism. There is sufficient available literature demonstrating the acaricidal activities of plant extracts by different workers has been reported. The results were consistent with the previous reports on the population potential against larvae of *R. microplus*. [35] worked on extracts of *Hyptissu aveolens* and *Ocimum sanctum* against African dog tick (*Rhipicephalus sanguinneus*) and observed after 24 hr with LC_{50} 175.00, 81.25 and 225.00 ppm for chloroform, methanol and hexane, respectively. These result shows methanol extract was more toxic than other tested extracts. These results are consonance with the antecedent reports using *R. (B). microplus* as model.[36] investigated the acaricidal activities of *Annona squamosal*, *Gloriosa superb* against cattle tick (*Haemaphysalis bispinosa*) and results demonstrates that the hexane leaf extract of *Annona squamosa* induced LC_{50} value of 145.39 ppm, while the acetone and methanolic extracts of *Gloriosa superb* leaf possessed LC_{50} value of 419.83 and 225.57 ppm, respectively and hexane extract exhibited more potent than other extracts tested.[10] investigated acaricidal activity of crude extract of stem and leaves of *Petiveria alliasea* and methanol extract shows 100% mortality in LIT. [37] studied the ethanolic extract of *Jatropha curcas* against *Rhipicephalus (Boophilus) annulatus* at the different concentration (50-100 mg/ml) and reported considerably blocked the hatchability of eggs into larvae when compared it with control. [38] studied the ethanolic extract activity of leaves (*Annona squamosa*) against the *Hyalomma anatolicum* , reported that mortality rate increased with the increase of concentration LC_{50} and LC_{90} were1.366% (13660 ppm) and 10.170% (101700 ppm). In the case of AIT, 100% mortality was observed at the concentration of 15% (150000 ppm) and 7.5% (75000 ppm) and the egg laying inhibition of 60.365, 62.282, 81.224 and 94.117%.

In the present study, the methanolic extract of *C. colocynthis* fruit showed higher efficacy against the larvae at very low doses which demonstrates the potential of the plant as biopesticide and may be considered as an alternative sustainable to commercial acaricides. The results reported here open the possibility of future investigation of efficacy on the adulticidal properties and need to promote their acaricidal activities against the other stages of *R. microplus.*

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