The Pharma Innovation Journal 2023; 12(6): 4138-4141

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(6): 4138-4141

www.thepharmajournal.com
Received: 18-03-2023
Accepted: 26-04-2023

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In vitro evaluation of acaricidal activity of different plant extracts against *Rhipicephalus* (Boophilus) *microplus*

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DOI: https://doi.org/10.22271/tpi.2023.v12.i6av.20967

Abstract
Ticks and tick born diseases affect the economics of dairy and farm animals. Continuous use of acaricides leads to development of acaricide resistance in the tick population, in order to overcome resistance, newer alternatives like herbal extracts can be used as acaricides. The following study is designed to evaluate the acaricidal efficacy of *Azadirachta indica, Calotropis gigantea* and *Ricinus communis* against *R.* (Boophilus) *microplus* tick. Aqueous plant extracts were prepared from dried plant material and these were evaluated using two different bioassays like adult immersion test and larval packet test. At 80 mg/ml concentration *A. indica* showed lowest LC_{50} and LC_{99} values when compared to *R. communis* and *C. gigantea.* In conclusion *A. indica* is relatively more efficient in acaricidal activity than *R. communis* followed by *C. gigantea*.

Keywords: *Azadirachta indica, Calotropis gigantea, Ricinus communis, R.* (Boophilus) *microplus*

Introduction
Ticks are one of the important ectoparasites of domestic animals and are ranked second to mosquitoes in their vector potentiality. Economic losses from tick and tick borne diseases are attributed to the loss of production and mortaliy (Lenka *et al.*, 2016) [11]. *R.* (Boophilus) *microplus* is a one host tick which transmits diseases like anaplasmosis and babesisosis. For control of ticks, synthetic chemicals *viz.*, organophosphates (OP) and pyrethroids (SP) are used continuously without maintaining any specific dose regime which inturn leads to acaricide resistance (Ghosh *et al.*, 2015) [5]. Medicinal plants has phytochemical constituents which are effective ticks. The usage of medicinal plants is advantageous because they are readily available and cheaper than the modern residues. *A. indica* commonly called as neem which belongs to family Meliaceae and the phytochemical constituents *viz.*, nimbolinin, nimbin, nimbidin, nimbidol, salannin and quercetin present in neem are active against many pathogens (Srivastava *et al*., 2020) [23]. Medicinal properties of these plant includes antimicrobial, antibacterial, antiviral, anti-inflammatory, antipyretic, antiseptic, fungicidal, insecticidal, larvicidal and spermicidal activity (Gupta *et al.,* 2017) [6]. Acaricidal activity of the plant was reported by (Avinash *et al*., 2017) [20].

C. gigantea is a giant milkweed belongs to Asclepiadaceae family.The plant has medicinal properties like analgesic activity (Pathak and Argal 2007) [15], antipyretic, CNS activity, anti-inflammatory, antioxidant, antimicrobial, anti-tumor, antifungal activity (Singh *et al.*, 2014) [21]. Acaricidal activity of *C. gigantea* has been identified (Marimuthu *et al.,* 2013) [12]. Phytochemical constituents in flower extract are alkaloids, anthraquinones, flavonoids, quinones, sterols, tannins, phenol and proteins are present in the flower extract (Rajamohan *et al.,* 2014) [17].

R.communis belongs to Euphorbiaceae family popularly known as castor plant. It is fast growing perennial shrub grown commonly in India. Phytochemical constituents in leaves include ricinine, N-dimethylricinine, glycoside, kaempferol, quercetin, monoterpenoids, gallic acid, rutin and ellagic acid. Medicinal properties of the plant included antioxidant, anti nociceptive, anti asthamatic, anti histamine, hepatoprotective,anti-inflammatory, antidiabetic, antimicrobial, wound healing property, antiulcer, molluscidal, insecticidal, larvicidal, purgative, laxative (Jena and Gupta 2012) [9], anti implantation and bone regeneration activity (Rana *et al.,* 2012) [18].

2. Materials

2.1 Herbs collection

The leaves of *A. Indica* and *R. communis*, flowers of *C. gigantea* were collected from Tirupati.

2.2 Preparation of plant extract

The collected plant material like leaves and flowers are removed from the stalk and washed twice with tap water and distilled water. Then the plant material was dried under shade, grounded, sieved and stored for further use. Plant extract of 10.0 percent concentration was prepared using distilled water by boiling method. The boiled solution was cooled and then filterd through four layers of muslin cloth and finally with Whatman No.1 filter paper and thus collected extract was allowed in dry in evaporator. The weights of the dried crude extracts were determined and a yield percentage was calculated for each plant material.

2.3 Collection and identification of ticks

Engorged ticks were collected from cattle farms and nearby villages in and around Tirupati. The ticks were collected using blunt end forceps and care has been taken to avoid decapitation of ticks. Thus collected ticks were processed using standard procedure and identified microscopically as *R.* (Boophilus) *microplus.*

2.4 Bio assay methods

Acaricidal activity was performed using two tests – Adult Immersion Test (AIT), Larval Packet Test (LPT) as per (FAO 2004 with slight modifications)

2.4.1 Adult Immersion Test (AIT)

For each concentration of test compound 10 ticks were used, each tick was weighed and dipped in test compound for about 2 minutes and the ticks were dried and taped to petridish by placing ventral side of ticks towards the petridish. Thus prepared petriplates were maintained at 27±1°C and 80 to 90 percent RH and they are observed for adult mortality, egg mass weight, percent oviposition inhibition after 14 days.

Reproductive Index (R.I) was calculated by

$$\text{Reproductive index (R. I.)} = \frac{\text{Total egg mass weight}}{\text{Total female weight}}$$

The percent oviposition inhibition was calculated by

$$\text{Percent oviposition inhibition} = \frac{\text{R.I ofcontrol - R.I of treatment}}{\text{R.I of control}}$$

2.4.2Larval Packet Test (LPT)

Engorged *R.* (Boophilus) *microplus* female ticks were placed in test tubes plugged with cotton and maintained at 80 to 90 percent RH and 27±1°C for oviposition. Thus laid egg mass were separated and placed in separate test tubes and kept under optimal conditions for the eggs to hatch. Thus obtained seed ticks were maintained at 80 to 90 percent RH and 27±1°C. Seed ticks aged between 14-21 days were subjected to larval packet test.

Filter papers was impregnated with 3 ml of each concentration of test compound and then air dried for about 1-2 hrs. About 100 larvae of 14-21 days age were transferred in to packets prepared by impregnated and dried filter paper and maintained them at 80 to 90 percent RH and 27±1°C. Mortality of larvae was checked after 24 hrs. The lethal concentrations of the prepared compounds were judged using the live and dead larval counts

2.5 Data analysis

Lethal concentrations (LC_{50} and LC_{99}) and inhibitory concentrations (IC_{50} and IC_{99}) for various tested compounds was done using software Statistical Package for Social Sciences (IBM SPSS 22.V, Illionois, Chicago). The lethal concentrations, reproductive efficacy percent and oviposition inhibition were expressed at 95percent of fiducial limits. *Post hoc* analysis was done using Duncan tests. The mean and standard error values were calculated in Microsoft Excel 2007. The level of significance $p<0.05$ was considered as statistically significant.

3. Results

The highest oviposition inhibition of 87 percent was observed for both *A. indica* and *R. communis* at 80 mg/ml concentration. (Table 1)

4. Discussion

Ticks are the major limiting factors to cattle industry in many tropical and subtropical areas of the world. *R.* (Boophilus) *microplus* is a one host tick and is one of widely distributed tick species in India.The direct losses include decrease in hide quality, tick worry, anaemia, injection of toxin whereas indirect losses include transmission of diseases like babesisosis and anaplasmosis (Pradeep *et al*., 2012) [16]. Direct tick affects leads to losses in leather industry amounts to shortfall of 3000 million pieces of hides and skin per year. The cost of management of ticks and tick borne diseases in India is roughly estimated as 2000 crore rupees per annum (Minijauw and Leod 2003) [13]. Introduction of exotic cattle by crossbreeding to native breeds leads to increases tick menace in intensive farming (Shyma *et al*., 2013) [19]. It is therefore imperative that tick infestation in livestock should be effectively controlled to avoid economic looses and to maintain health and productivity of animals.

Use of acaricides is most common method adopted by owners to control ticks. Continuous and indiscriminate use of acaricides leads to development of tick resistance, environmental and human health concerns (FAO 2004). Among the synthetic pyrethroids deltamethrin and cypermethrin is most commonly used acaricides. Deltamethrin resistance in cattle tick *R.* (Boophilus) *microplus* is observed widely in different parts of India (Shyma *et al.,* 2013 [19], Kumar *et al.,* 2017 [10], Singh *et al.,* 2019 [22], Amrutha *et al.,* 2021) [1]. These problems substantiate the need for the development of novel selective control alternatives.

Table 1: Acaricidal activity of plant extracts

***A. indica* Conc**	**10**	**20**	**40**	**60**	**80**
Percent AM	16.3	36.6	53.3	70	83.3
Percent OI	08	36	62	74	87
LC_{50}	**31.33 (27.206-35.885)**				
LC_{99}	**443.19 (288.49-825.48)**				
Percent LM	31.6	41	54.3	70.3	81.6
***C. gigantean* Conc**	10	20	40	60	80
Percent AM	6.6	16.6	30	43.3	60
Percent OI	29	41	57	65	71
LC_{50}	**67.49 (57.02-84.44)**				
LC_{99}	**1149.93 (609.944-3121.57)**				
Percent LM	11.6	29.3	36.3	45	53.6
***R. comunis* Conc**	5	20	40	60	80
Percent AM	13.3	23.3	40	56.6	66.6
Percent OI	24	40	51	62	87
LC_{50}	**49.241 (42.01-59.47)**				
LC_{99}	**1076.54 (572.99-2877.41)**				
Percent LM	22	40.6	49.3	53.6	68

Table 1: Acaricidal activity of plant extracts conc-concentration mg/ml, percent AM, percent adult mortality, percent OI percent oviposition inhibition, LC_{50}- lethal concentration 50, LC_{99}- lethal concentration 99, R^2-coefficient of determination, percent LM- percentage larval mortality, mg/ml- milligram per milliliter.

Medicinal plants are important for healing of human and animal diseases due to the existence of certain specific substances known as phytochemicals. They are not nutritious chemical compounds they are naturally found in medicinal plants which results in defense mechanisms and protection against various diseases (Nostro *et al*., 2000) [14]. There are many advantages by using plant extracts and they are associated with lower environmental, food contamination, slower development of acaricidal resistance and lower toxicity to animals and humans (Jain *et al*., 2020) [8].

Different concentrations of *A. indica* plant extract were used to test acaricidal activity using LPT and AIT against *R.* (Boophilus) *microplus*. It was noticed that neem leaf extract exhibited 81.0 percent mortality of larvae, 70.0 percent of adult mortality and 87.0 percent of oviposition inhibition percentage at 80 mg/ml concentration and LC_{50} and LC_{99} values obtained at 31.33 and 443.19 mg/ml, respectively. The results were in comparable with Dalei (2018) [3] who reported that 75.66 percent of larvae, 86.66 of percent adult mortality and 92 of percent oviposition inhibition, respectively at concentration of 100 mg/ml. The results are less agreement with the results of Shyma *et al.* (2014) [20] who reported 55.2 percent of larval, 33.33 percent of adult mortality and 20.73 of percent oviposition inhibition at 100 mg/ml concentration.

In present investigation five different concentrations of *C. gigantea* plant extract was used for testing acaricidal activity using LPT and AIT against *R.* (Boophilus) *microplus*. It was noted that *C. gigantea* flower extract exhibited significant acaricidal activity at 80 mg/ml concentration showed 53.66 percent of larval and 60 percent of adult mortality, 71 percent of oviposition inhibition. The results were in comparable with Shyma *et al.*(2014) [20] reported that *C. gigantea* at 100 mg/ml concentration showed 63.2 percent of larval and 66.67 of percent adult mortality, 71.43 of percent oviposition inhibition rate, similarly, Dalei (2018) [3] who reported that 60.33 percent of larval and 56 percent of adult mortality, 66.144 percent of oviposition inhibition and Marimuthu *et al.*(2013) [12] reported that at 50 mg/L concentration 94 percent mortality of *R.* (Boophilus) *microplus* larvae.

Five different concentrations of *R. communis* plant extract was used to test the acaricidal activity against *R.* (Boophilus) *microplus* using LPT and AIT and the results noted that at 80 mg/ml showed 66.6 percent of adult mortality, 86.66 percent of oviposition inhibition rate. The results were in accordance with Sudha Rani *et al.* (2018) [24] reported that at 100 mg/ml concentration 100 percent of mortality of larvae and 68.79 percent of adult mortality, 73.15 percent of oviposition inhibition rate and Islam *et al*. (2018) [7] who reported that 90 percent ofadult mortality at 3 percent concentration.

Conclusion

Among the plant extracts *A. indica* showed lowest lethal concentration values followed by *R. communis* and then *C. gigantea.* Whereas *A. indica* and *R. communis* plant extractsshowed similar oviposition inhibition values at their highest concentration. Plant extracts like *A. indica, C. gigantea, R. communis* used in this report were having significant acaricidal property which can be used as alternative for chemical acaricides

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