RESEARCH ARTICLE

In-Vitro Evaluation of the Acaricidal Efficacy of Polyherbal Aqueous Formulation against *Rhipicephalus microplus*

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Abstract

A total of 140 fully engorged adult female ticks were included for the present study. Out of this, 20 ticks were used for performing larval packet test (LPT) and remaining 120 ticks were used in adult immersion test (AIT). Polyherbal aqueous formulation consisting of paste of 10 gm each of Garlic pearls (*Allium sativum*), Tobacco leaves (*Nicotiana tabacum*), Tulsi leaves (*Ocimum tenuiflorum*), Lantana camara leaves (*Shrub verbena*), Sweet flag rhizome (*Acorus calamus*), and 20 gm each of Neem leaves (*Azadirachta indica*) and Turmeric powder (*Curcuma longa*) was used as acaricidal agent in the present study. The paste was dissolved in one liter of water and strained. In AIT, polyherbal formulation exhibited notable inhibition of oviposition and adult tick mortality. Furthermore, In LPT, it demonstrated increased effectiveness on R. *microplus* larvae from the Mhow region, resulting in a higher mean percent mortality. These findings highlight the promising potential of the polyherbal formulation as a potent acaricidal agent in tick control strategies.

Key words: Adult immersion test, Larval packet test, Polyherbal spray, *Rhipicephalus microplus.*

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Introduction

Ticks are considered as one of the important and harmful blood sucking ectoparasites of livestock and human around the world after the mosquitoes. The prevalence of ticks and tick-borne diseases particularly in the wet seasons (Keyyu *et al.,* 2003) is an important restraint. The use of synthetic active ingredients belonging to organochlorines, organophosphates, carbamates, synthetic pyrethroids and more recently the insect growth regulators (George *et al.,* 2004) are common among cattle owners, albeit these have environmental, human health hazard issues and led to selection for resistance, resulting in serious problems in many countries.

Among the natural products, plant extracts and essential oils have been shown to have significant activity against economically important tick species (Ribeiro *et al.,* 2011) to reduce the environmental and financial impact of synthetic acaricidal substances, which have demonstrated their ability to repel ectoparasites, in addition to causing their death by direct effects on the respiratory system, as well as by the denaturation of their external structure. Present study was designed with the objective to study the *in vitro* acaricidal efficacy of polyherbal aqueous formulation against *Rhipicephalus microplus*.

Materials and Methods

The study was conducted in Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Mhow, NDVSU (MP, India). Fully engorged adult female ticks (n=140) were collected from cattle sheds in and around Mhow.

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Polyherbal Aqueous Formulation

The following ingredients were mixed together in the grinder: 10 gm each of Garlic pearls (*Allium sativum*), Tobacco dried leaves (*Nicotiana tabacum*), Tulsi green leaves (*Ocimum tenuiflorum*), Lantana camara green leaves (*Shrub verbena*), Sweet flag rhizome dried (*Acorus calamus*) and 20 gm each of Neem green leaves (*Azadirachta indica*) and Turmeric powder (*Curcuma longa)*. These ingredients were grind and dissolved in one liter of water. Then solution was strained for determination of efficacy of different concentrations of polyherbal formulation.

Tick Bioassay

In Vitro Evaluation

Collected ticks were washed in tap water and were dried on an absorbent paper. Out of 140 fully engorged adult female ticks collected, 20 ticks were separated and held individually at 28±1 °C and 85±5 % relative humidity in labeled glass bottle with the mouth covered by muslin cloth for oviposition. The eggs were allowed to hatch to larvae in 14 days under similar conditions of incubation. The 10-12 day old larvae were used for performing larval packet test (LPT). The remaining 120 ticks were divided into six groups each of 20 ticks and were treated with polyherbal aqueous formulation at different concentration, *viz.*, 0.0 (control), 6.25, 12.5, 25.0, 50.0 and 100 %. For each concentration 5 ticks and such 4 replicates were used.

Adult Immersion Test (AIT)

The AIT was conducted according to FAO (1984) guidelines (Fig. 1). All the petri dishes with treated ticks were kept at room temperature for 24 h. After 24 h, ticks were transferred to glass vials covered with muslin cloth and kept in desiccators having 85±2 % relative humidity and placed in BOD incubator at 28±2 °C. These ticks were observed for oviposition and death up to 14 days.

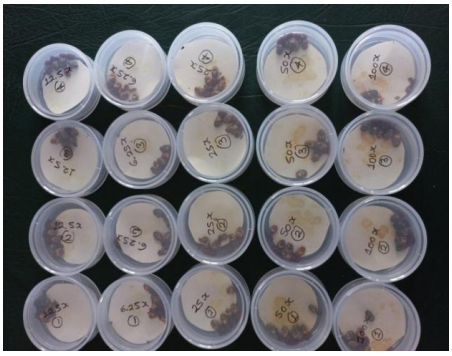

Fig. 1: Ticks after dipping in different concentrations of polyherbal formulation for AIT

The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded and compared with control. The eggs were incubated at the same condition, and the percentages of hatched eggs were estimated. The indexes of egg laying and percentage inhibition of fecundity were calculated using formulae given by Goncalves *et al.* (2007).

Larval Packet Test (LPT)

The LPT was also conducted according to FAO (1984) guidelines. After the insertion of approximately 100-150 larvae (10-12 days old), the top of each packet was sealed with adhesive tape (Fig. 2, 3), and the packets were placed in desiccators in a BOD incubator maintained at 28±1 °C and 85±5 % RH. The packets were removed after 24 h, and larval mortality was calculated. Control group was treated with 0.6 mL of distilled water, similar to the above mentioned procedure.

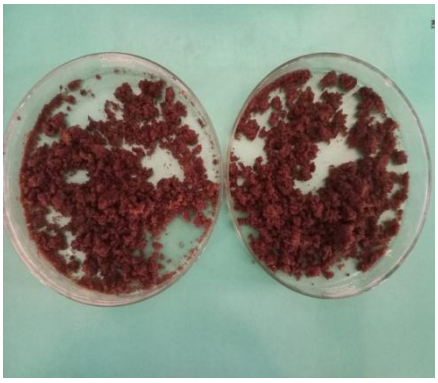

Fig. 2: Eggs for "Larval Packet Test"

Fig. 3: Larvae for "Larval Packet Test

Statistical Analysis

Dose-response data were analyzed by Probit method using GraphPad Prism 4 software. The lethal concentrations for 50 % (LC50) values of various extracts were determined applying regression equation analysis to the Probit transformed data of mortality.

Results and Discussion

In Vitro Acaricidal Efficacy of Polyherbal Formulation against *R. microplus*

Adult Immersion Test

The per cent adult tick mortality caused by polyherbal formulation at 6.25, 12.5, 25, 50 and 100 % concentrations were recorded as 5, 10, 15, 35 and 55%, respectively. The average egg mass weight was recorded 14 days post-treatment (DPT). The mean RI (Reproductive Index) values at above concentrations were significantly lower as compared to control. The per cent IO (Inhibition of Oviposition) of polyherbal formulation at 100 % concentration was significantly higher as compared to 6.25%, 12.5%, 25 % and 50% concentration. At 6.25 % concentration, 100% hatchability was recorded, whereas at 100 % concentration, 40% hatchability was recorded (Table 1). Similar findings were also observed by Srivastava *et al.* (2008) using *Azadirachta indica* seed extract. Magadum *et al.* (2009) reported that *Azadirachta indica* extracts were more effective than *Annona*

Table 1: Efficacy of different concentrations of polyherbal formulation against *R. microplus* by adult immersion test

Concentration (%)	Av. Tick wt. ±SE (mg)	Av. Egg mass ±SE (mg)	RI ± SE	% IO ± SE	% Adult mortality	% Hatch-ability
6.25	657.75±28.72	307.5^{a}±28.68	0.465$^{a\pm}$0.02	0.21^{a}± 0.05	5	100
12.5	653.5±9.21	302.5^{a}± 19.31	0.462^{a}± 0.03	0.22^{a}± 0.03	10	80
25.0	672.25±23.49	297.5^{a}± 23.58	0.440^{a}± 0.28	0.23^{a}± 0.04	15	75
50.0	652 ±49.36	277.5^{a}±10.30	0.429^{a}± 0.02	0.25^{b}± 0.06	35	65
100	701.75±42.44	292.5^{a}± 37.05	0.412^{a}± 0.03	0.28^{c}± 0.05	55	40
Control	707.50±4.76	412.75^{b}±20.30	0.583^{b}±0.030	0	0	100

RI- Reproductive index, IO- Inhibition of oviposition,
Means bearing different superscripts (a,b) within the columns indicate significant difference ($p<0.05$).

squamosa extracts, but Broglio-Micheletti *et al.* (2010) opined that seed extract (hexanic fraction) and emulsified oil of *Azadirachta indica* can be used as an adjuvant instead of acaricides on cattle ticks by testing adult immersion test. Aboelhadid *et al.* (2013) indicated that at concentrations above 5%, garlic and onion oils are having acaricidal effect. Adenubi *et al.* (2018) studied acaricidal efficacy of *Azadirachta indica* and other herbs using *in vitro* anti-tick assays, and observed 90-100% acaricidal and larvicidal efficacy. Nasreen *et al.* (2020) studied the efficacy of *Allium sativum* and *Cannabis sativa* extract against *Rhipicephalus microplus* ticks using the adult immersion and the larval packet tests. Ticks were dramatically reduced after 96 h of treatment with a 45 % solution of both herbal extracts. De Almeida *et al.* (2021) showed acaricidal efficacy of dehydrated garlic extract concentration (0.1%, 0.5%, and 1%) against *Rhiphicephalus microplus*. Similar results were also revealed by Giglioti *et al.* (2011) and Zaman *et al.* (2012).

Status of Polyherbal Formulation

Goodness of fit (R^2) and LC50 (95% CI) against polyherbal formulation are represented in Table 2. These were 0.973 and 93.63, respectively. Veeramani *et al.* (2014) studied acaricidal activity of different crude solvent extracts of leaves of *Ocimum basilicum* and *Spilanthes acmella* against *Rhipicephalus microplus*. They observed 70% and 100% mortality of ticks at 6% and 10% chloroform extract of *O. basilicum* when compared to control. The LC50 and LC90 values of the chloroform extract of leaves of *O. basilicum* treatment on the ticks after 24 h were observed as 5.46% and 7.69%.

Table 2: R^2 and LC50 (95% confidence limit) value of polyherbal formulation against *R. microplus*

Concentrations (%)	Log concentration	Probit mortality	R^2	LC50
6.25	0.796	3.36	0.973	93.63
12.5	1.097	3.72		
25	1.398	3.96		
50	1.699	4.61		
100	2.000	5.13		

Larval Packet Test

Mean per cent mortality caused by polyherbal formulation in LPT at 100 % concentration was significantly higher than 6.25 %, 12.5%, 25%, 50% and control (Table 3). Similar findings were also observed by Giglioti *et al.* (2011), Avinash *et al.* (2017) with acetone and aqueous leaf extracts of *Nicotiana tabacum*. The LC50 and LC99 were highest for aqueous leaf extract at 728.97 and 6094.438 ppm, respectively. Martinez-Velazquez *et al.* (2011) used LPT to investigate the acaricidal activity of essential oils of various leaves and seeds and stated that Cumin had a substantial toxicological effect, killing *R. microplus* larvae at all tested doses. Zaman *et al.* (2012), Nasreen *et al.* (2020) and De Sousa *et al.* (2020) studied anti-tick efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* and other, against all the developmental stages of *Rhipicephalus microplus* using adult immersion test, larval packet test and ear bag method. The extracts exhibited lethal effects on egg laying, hatching and total larval mortality.

Table 3: Mean per cent mortality of larvae (*R. microplus*) in Larval packet test by polyherbal formulation

Larval mortality (%)	Conc. of polyherbal formulation (%)					
	6.25	12.5	25	50	100	Control
Mean ± SE	25.25^{a} ± 1.65	43.25^{b} ± 4.55	56^{c} ± 6.01	72.5^{d} ± 2.25	87^{e} ± 2.82	0

Means bearing different superscripts ($p<0.05$) between the columns shows significant difference

Conclusion

The findings of this study revealed that in AIT, polyherbal formulation was more efficient in bringing up high per cent inhibition of oviposition and per cent adult tick mortality. Polyherbal formulation was also more effective on *R. microplus* larvae from the Mhow Tehsil in LPT, with a higher mean percent mortality. The present findings highlighted the promising potential of the polyherbal formulation as a potent acaricidal agent in tick control strategies.

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