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IN VITRO EVALUATION FOR ACARICIDAL EFFICACY OF *MELIA AZEDARACH* AND *EUPATORIUM ADENOPHORUM* AGAINST *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* TICKS OF GOATS

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ABSTRACT

In vitro acaricidal activity of biopesticides prepared from *Melia azedarach* and *Eupatorium adenophorum* was evaluated against adult engorged females of *Rhipicephalus* (*Boophilus*) *microplus* of goats at concentrations of 5, 10 and 20% using adult immersion test and larval packet test. A dose-dependent larval mortality response (82, 42 to 90, 46%) was recorded in all the concentrations of *M. azedarach*. Significant (P<0.05) inhibition of oviposition (IO) in adult females at concentration of 20% suggested negative effect of *M. azedarach* on the reproductive physiology of ticks. *E. adenophorum* significantly (P<0.05) affected the oviposition and mortality rates of the tick larvae in a dose-dependent manner with highest mortality at 20% concentration. The results indicated potential use of biopesticides formulated from *M. azedarach* and *E. adenophorum* in developing sustainable strategy for integrated tick management and a step towards organic chevon production.

Key words: Adult immersion test, *Eupatorium adenophorum,* Goat, Larval packet test, *Melia azedarach, Rhipicephalus* (*Boophilus*) *microplus*

Ticks and tick-borne diseases (TTBDs) of livestock are major biological constraints for the growth of livestock farming causing significant reduction in income due to severe losses in the production of meat, milk, leather and in many cases death of the affected animals (Hurtado and Giraldo-Rios, 2018). *Rhipicephalus (Boophilus) microplus* tick is widely distributed among the cattle and goat population of India (Ghosh and Nagar, 2014). Fatal diseases of sheep and goats like babesiosis, anaplasmosis, theileriosis, eperythrozoonosis are all tick-borne diseases. In the past few decades, prevalence of vectors-cum-pests and vector-borne diseases in livestock is on high rising trend due to the effects of global warming, resistance to acaricides, wrong animal husbandry practices and poor nutritional status (Narlakdkar, 2018). This poses major public and animal health problems that essentially require the strategic tick control methods. At present, tick control in India is based on large scale use of synthetic acaricides of three major classes, namely organophosphates, carbamates, and pyrethroids. Rampant use of synthetic acaricides has led to the emergence of acaricidal resistance in tick population (Ghosh et al., 2015; Shyma et al., 2015). Therefore, there is need to formulate strategies to minimize the development, progression and impact of resistance.

Compounds of plant origin have been used in the recent past against all the stages (adult, nymph and larva) of economically important tick species with encouraging results (Nathan et al., 2006). *Melia azedarach* commonly known as dharek/bread/neem tree has been investigated extensively for its potential acaricidal activity (Williams, 1993; Singh and Williams, 1998; Borges et al., 2003). *Eupatorium adenophorum* (mistflower/kaali basuuti) is a perennial herbaceous shrub with acaricidal properties and found in subtropical Himalayas. A potent acaricidal activity (100%) of ethanol thermal circumfluence extract from *E. adenophorum* has been reported against *Psoroptes cuniculi* and *Sarcoptes scabei in vitro* (Nong et al., 2011).

The biopesticides named *Dharekastra* and *Eupatorium ark*, formulated from *M. azedarach* and *E. adenophorum,* respectively are commonly used against crop pests like aphids, pea leaf miner in the organically cultivated crops in Himachal Pradesh (Sharma et al., 2015a, b). These biopesticides are formulated in cow dung and cow urine. Cow-dung harbours high density of microorganisms that are directly involved in stabilization processes leading to biodegradation of xenobiotic compounds (Khan and Manchur, 2015) and high on antibacterial properties making it an effective choice to use as an insect repellent or a pesticide alternative (Dhama et al., 2005). Cow urine is a powerful and natural pesticide as it can effectively reduce the harmful effects of chemical pesticides on human beings (Dhama et al., 2005; Kaphle and Bastakoti, 2016). Hence, the present study was conducted to assess the acaricidal efficacy of these biopesticides against *R.* (*B.*) *microplus* tick of Gaddi goats.

MATERIALS AND METHODS

The study was conducted at Dr G.C. Negi College of Veterinary and Animal Sciences, Palampur (Himachal Pradesh), during February to April, 2019. A total of 85 fully engorged dropped off adult female *R. (B.) microplus* ticks were collected from the Gaddi Goat Farms of Himachal Pradesh. The ticks were thoroughly washed and dried on filter paper followed by their morphological characterization (Miranpuri, 1979). *Dharekastra* and *Eupatorium ark* were prepared by adding 5 kg *M. azedarach* / *E. adenophorum* leaves to 5 kg cow dung and 5 litre cow urine from indigenous hill cow. As the concentration of 10% was the most potent and effective concentration against aphids (Sharma et al., 2015), the working concentrations of the *Dharekastra* and *Eupatorium* ark were prepared in distilled water at concentrations of 5, 10 and 20% to conduct *in vitro* based bioassays.

The adult immersion test (AIT) was performed as described by Drummond et al. (1973) and Sharma et al. (2012). Briefly, the ticks were weighed and assigned randomly to groups (three ticks per group). The different groups of ticks were dipped in different concentrations (5, 10 and 20%) of respective biopesticide (*Eupatorium* ark and *Dharekastra*) by placing them directly into containers and stirred with glass rod before and after adding ticks. After 2 min, the biopesticide was poured off through a sieve and the ticks were transferred to the filter paper for drying and kept separately in glass tubes and sealed with muslin cloth. For each concentration, four replications of three tick (n=12) were maintained. Simultaneously, ticks in the control group were treated with distilled water and four replications were maintained. The treated ticks were placed in desiccators maintained at a temperature of 28±2°C, relative humidity of 85±5% and a photoperiod of 0:24 L:D for oviposition in BOD incubator. The ticks which did not oviposit even after 14 days were considered as dead. After 14 days, the ticks were discarded and the eggs produced by the ticks in each group were weighed. The observations were recorded for % mortality, recorded up to 14 days post treatment when normal ticks completed egg laying, the egg masses laid by the live ticks, the reproductive index (RI) - a ratio of average weight of eggs laid and average weight of live ticks and % inhibition of oviposition (IO %) = {(RI of control ticks - RI of treated Ticks) / (RI of control ticks) x 100.

Larval packet test (LPT) was employed to evaluate the acaricidal efficacy of *Eupatorium ark* and Dharekastra against larva of engorged female ticks of *R. (B.) microplus* as per FAO (2004). Briefly, the larvae were allowed to rest unfed for 14 to 21 days following hatchability prior to their use. The larvae were exposed to the acaricides in filter paper envelopes (7×7cm) containing micropores to allow proper ventilation. Filter papers were moistened with solutions containing different concentrations of acaricides (5, 10 and 20%) and allowed to dry for at least 30 min in incubator at 37°C. Following treatment of each envelop for insertion of larval ticks, the packets were resealed with a tape with its identification mark. The packets were then incubated at temperature of 28±2°C, relative humidity 85±5% and a photoperiod of 0:24 (L:D) for 24 h. The larvae in the control group were treated with distilled water. Four replications were made for each concentration. Control packets were opened first and examined for larval mortality. Then other packets were opened in order of increasing the concentration of the acaricides. The number of live and dead larvae was counted and per cent mortality of larva was calculated.

The data were subjected to analysis of variance. Dose response data were analyzed by probit method (Finney, 1962) using Graph Pad Prism 4 software. The lethal concentrations (LC_{50} and LC_{95}) were determined by applying regression equation analysis to the probit transformed data of mortality.

RESULTS AND DISCUSSION

Significant (P<0.05) dose-dependent reduction in egg weight mass (36.2 mg to 10.5 mg) and reproductive index (0.21 to 0.06) in adult female engorged ticks of *R.* (*B.*) *microplus* at 20% concentration of *Dharekastra* was recorded (Table 1). There was significant (P<0.05) reduction in oviposition. A dose-dependent mortality was recorded in ticks treated with different concentrations of Dharekastra whereas no mortality was seen in the control group. Significant (P<0.05) dose-dependent reduction in egg weight (from 66.33 to 29.00 mg) and reproductive index (from 0.39 to 0.17) of adult female engorged ticks of *R. (B.) microplus* was recorded with *Eupatorium ark* (Table1). *Eupatorium ark* adversely affected the egg laying capacity of *R. (B.) microplus* ticks in dose-dependent manner with significant increase in reduction of oviposition (-22.99 to 46.19%). Significant dose-dependent increase in larval mortality (0 to 22%) suggested a high larvicidal activity of *Eupatorium ark.*

Table 1. Mean (±S.E.) mortality, tick / egg weight, reproductive index and inhibition of oviposition in *R. (B.) microplus* adults exposed to *Dharekastra* and *Eupatorium ark*

Character	Concentration (%) of *Dharekastra*				Concentration (%) of *Eupatorium ark*			
	Control	5	10	20	Control	5	10	20
Mortality (%) on AIT	0[a]	0[a]	11.11 ±0.11[b]	22.00 ±0.46[c]	0[a]	0[a]	11.00 ±0.11[b]	22.00 ±0.15[c]
Mortality (%) on LPT	0[a]	82.42 ±6.32[b]	82.59 ±6.25[b]	90.46 ±3.07[c]	0[a]	62.35 ±2.55[b]	72.90 ±6.62[c]	84.34 ±4.64[c]
Live tick weight (mg)	183.66 ±18.31	163.88 ±16.69	190.44 ±22.64	172.44 ±22.02	143.66 ±18.31	166.44 ±16.21	172.77 ±15.72	166.33 ±20.31
Egg weight (mg)	36.20 ±0.99[a]	38.10 ±5.31[b]	36.10 ±5.20[a]	10.50 ±2.71[d]	46.55 ±6.58	66.33 ±1.11	50.50 ±4.92	29.00 ±3.40
Reproductive index	0.25[a]	0.21[b]	0.19[c]	0.06[d]	0.32[a]	0.39[b]	0.29[c]	0.17[d]
Inhibition of oviposition (%)	-	12.33[a]	20.37[b]	74.89[c]	-	- 22.99[a]	9.79[b]	46.19[c]
LC_{50} (95% Cl) on LPT	15.64 (15.05-16.26)				27.38 (26.12-28.70)			
LC_{95} (95% Cl) on LPT	24.24 (22.35-26.29)				77.65 (22.35-26.29)			

**Values with different superscripts are significant (P<0.05)*

The results revealed very low efficacy against female engorged ticks. However, larval packet test revealed significant (P<0.05) concentration-dependent increase in larval mortality (Table1). *Dharekastra* was found 82.42 to 90.46% efficacious against the larval stages. The LC_{50} and LC_{95} values of 15.64 and 24.24% with 81% goodness of fit (R^2) for mortality data indicated a high larvicidal activity of *Dharekastra* (Fig.1). It was found that *Eupatorium ark* was 84.34% efficacious against the larval stages (Fig. 2). The LC_{50} and LC_{95} values of 36.05 and 154.84% with 94% goodness of fit (R^2) mortality data indicated a moderate larvicidal activity of *Eupatorium ark*.

Similarly, hexachloroform extract of ripe fruit of *M. azedarach* showed good efficacy on larvae mortality, and less level of efficacy against adult females of *Boophilus microplus* (Borges et al., 2003). The fruit of *M. azedarach* was as effective as azadirachtin in inhibiting the oviposition and embryogenesis of *R.* (*B.) microplus* (Singh and Williams, 1998). The leaves of *M. azedarach* had strong larvicidal action on *Aedes aegypti* larvae (Wandscheer et al., 2004; Coria et al., 2008) whereas leaves and seeds showed strong larvicidal, pupicidal, adulticidal and ovipositional activity against *Anopheles* sp. mosquitoes (Nathan et al., 2006). The acaricidal and insecticidal properties could be attributed to the presence of number of organic molecules having insecticidal properties i.e. terpenoids, flavonoids, steroids, acids, anthraquinones, alkaloids, saponins, tannins in *M. azedarach* fruit and leaves (Rishi and Singh, 2003).

Eupatorium contains active principles in the form of flavonoids, terpenoids, pyrrolizidine alkaloids, phenyl propanoids, quinonoids that has wide range of pharmacological activities i.e. antibacterial, antifungal, cytotoxic and insecticidal. The biological extract of the *Eupatorium* contains caffeic acid derivatives, polysaccharides and tannins that possess potent acaricidal action against the hard ticks (Nong et al., 2011). Stigmasterol obtained from *Eupatorium* was found toxic to rabbit ear mite *Psoroptes cuniculi* (Nong et al., 2013). Ethanolic extracts of *Eupatorium* possess anti-plasmodium activity because of eupafolin and sesquiterpen lactones (Hensel et al., 2011).

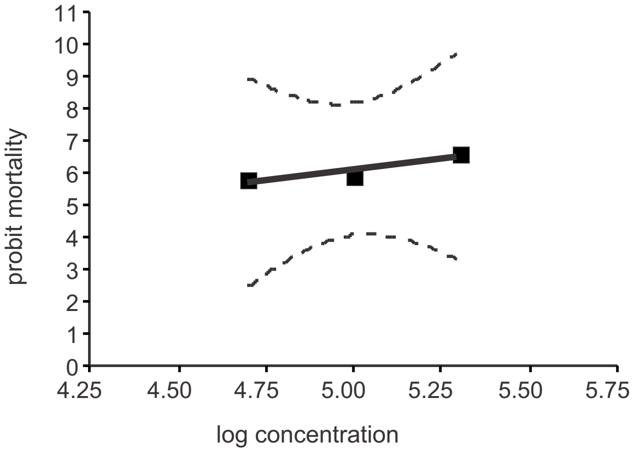

Fig. 1. Dose mortality curve of R.(B.) *microplus* against Dharekastra by larval packet test

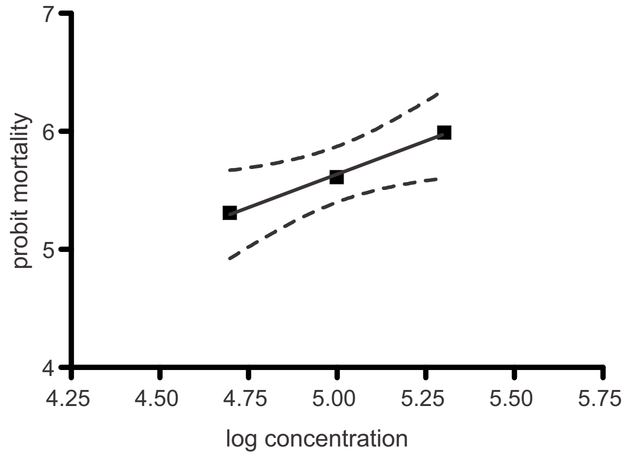

Fig. 2. Dose mortality curve of *R (B.) microplus* against *Eupatorium* ark by larval packet test

Thus, out of two biopesticides tested, *Dharekastra* had better oviposition limiting and larvicidal action against *R. (B.) microplus* ticks of goats. *Dharekastra* can be utilised as the one of the promising and economical botanical alternatives to the synthetic compounds used against ectoparasites of goats. Further research should be taken up to determine the *in vivo* efficacy, mechanism of action and on the most potent active ingredient against ticks.

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