Original Research

Comparative Efficacy of Herbal (Lemon Grass Oil and Litsea Grass oil) and Synthetic (Deltamithrin, Fipronil, Amitraz) Agents against *Rhipicephalus microplus* Infestation in Mithun (*Bos frontalis*)

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Abstract

Ticks are important ectoparasites which results in economic loss in livestock industry. Therefore, the present study was undertaken with aim to study the comparative efficacy of some herbal extracts i.e. oil of Lemon grass (Cymbopogon citratus) and Litsea grass (Litsea cubeba) and three synthetic acaricides viz. Deltamethrin, Fipronil, and Amritraz against the Rhipicephalus microplus tick infestation in mithun. Among the chemical drugs, Fipronil showed 100% efficacy followed by Amitraz (98.38%) and Deltamithrin (93.28%). The lemon grass oil on 12 days of application resulted in very high efficacy (98.00 %) comparable to synthetic acaricides (Fipronil, Amitraz and Deltamithrin), however, Litsea grass oil did not showed significant efficacy on day 12 post application. However, the Adult Immersion Test (AIT) showed dose dependent acaricidal efficacy for Deltamethrin, Fipronil and Amritraz at final concentration of undiluted, 0.40%, 0.20 %, 0.10 % and 0.05% while among the herbal acaricides in 100 % concentration, lemon grass oil showed comparable efficacy with deltamethrin (12.5 W/V) while Listea grass oil did not showed better effects. Author concludes that lemon grass oil has potential acaricidal activity and may be recommended for holistic management of nymphal stages of tick infestation and can be proved as an alternative to synthetic acaricides without adversely affecting health and environment.

Key words: Herbal, Ticks, Efficacy, Ectoparasites, Mithun, Acaricidal

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Introduction

Ticks are among the most competent and versatile vectors of pathogens and are second to mosquitoes as vectors of a number of human pathogens, like viruses, bacteria, rickettsia, spirochetes and the most important vector of pathogens affecting cattle worldwide (Ghosh and Nagar, 2014 and Kakati *et al.*, 2015). Ticks represent a serious problem worldwide among livestocks leading to huge losses by transmitting diseases. *Rhipicephalus microplus also* referred as Asian Blue Tick that parasitises a variety of livestock species in tropical and subtropical regions (Klafke *et al.*, 2006). Initially, this tick was geographically limited to Asian continent later it has been reported from Mexico, Central and South America, Africa, Madagascar, Australia, and Taiwan (Labruna *et al.*, 2009). The *R. microplus* mainly infests cattle, deer and buffalo, but it can also be found on horses, goats, sheep, donkeys, dogs, pigs and some wild mammals (Kahn and Line, 2003). R. *microplus* has been found potential vectors and reservoirs *Babesia bovis*, *Anaplasma phagocytophilum*, *Anaplasma ovis*, *Ehrlichia canis*, *Ehrlichia ewingii* and *Ehrlichia muris* (Matysiak *et al.*, 2016).

Mithun (*Bos frontalis*), unique ruminant species geographically limited to North-Eastern Hilly region of India and particularly to the states of Arunachal Pradesh, Nagaland, Manipur and Mizoram. Traditionally mithun being reared under forest ecosystem make the animal prone to ecotoparatic infestation. However, the limited reports of different tick infestation are available in mithun (Chamuah *et al.*, 2012; Chamuah *et al*., 2016). Management of tick infestation in mithun with different acaricides has been attempted by various workers from time to time (Chamuah *et al*., 2014). The emergence of resistance is a constant challenge for control of *R. microplus* (Morel *et al.*, 2017) and development of new acaricides is a long and expensive process, which reinforces the need for alternative approaches to control ticks infestations (Graf *et al.*, 2004). In past few years, the studies on herbal acaricides have gain momentum worldwide in attempt to overcome the increasing resistant to chemical acaricides (Magadum *et al.*, 2009; Chagas *et al.*, 2011).

Keeping in view the rapid development of resistance, the present study was designed with aim to investigate the acaricidal in-vitro efficacy of Lemon grass oil and Litsea grass oil in comparison to commercial synthetic acaricides against *R. microplus* infestation in mithun.

Materials and Methods

A total of 24 numbers of *R. microplus* infested mithuns were divided into six groups. The groupings were done randomly irrespective of age and sex of the animals. Each group was treated separately with Deltrin (deltamethrin emulsifiable concentrate-1.25% W/V, ARIANS BIOPHARMA), Nayflee (Fipronil 0.25% W/V, Intas Pharmaceutical Ltd., Ahmedabad), Extick (Amritraz dip concentrate liquid 12.5% W/V Vet Mankind, New Delhi) in undiluted form. Lemon grass *(Cymbopogon citratus)* and Litsea (*Litsea cubeba*) were collected locally from Medziphema, Dimapur, Nagaland and essential oil were prepared by steam

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distillation (Boukhatem *et al.*, 2014). Four tick infested animals were maintained without any treatment as the control group. The herbal extracts were prepared as per conventional procedure. Efficacy of various treatments was analyzed at 0th, 3rd, 6th, 9th and 12th days of treatment. The efficacy of all the drugs was expressed by per cent reduction of nymphal stages of ticks per square inch body surface area of the affected animals (Ong'are *et al.* 1983).

Adult Immersion Test

Briefly, the Adult Immersion Test (AIT) was conducted as per the protocol described by Drummond *et al.*, 1973. A total of 4 ticks for each groups were immersed in pertidish having 10mL of chemical acaricides (Deltamethrin, Fipronil, Amitraz) in different concentrations of namely, undiluted, 0.40 %, 0.20 %, 0.10 % and 0.05 %. The ticks were exposed to 100 % concentration of Litsea grass oil and Lemon grass oil. The control group ticks were immersed in water. The ticks were observed for cessation of pedal reflex on pricking of needle and time of cessation of movement was recorded.

Statistical Analysis

The experimental data generated were analyzed by adopting repeated measures GLM procedure using the statistical software program SPSS (version 17.0 for Windows; SPSS, Chicago, III., U.S.A.). A one-way analysis of variance (ANOVA) was used for comparison of means according to Duncan's multiple range tests (Duncan, 1995). The effects were considered to be significant at 5% level.

Results

The findings of the study of efficacy of certain herbal and commercial synthetic acaricides against *R. microplus* infestation in mithun has been presented in Table 1.

Table 1: Efficacy of acaricides against tick infestation in mithun

Groups	Treated with	No. of Nymph/Tick Present on Day of Treatment				
		0 day	3rd day	6th day	9th day	12th day
I	Deltamithrin	20.25 ^{ab} ±1.45	11.5 ^{c} ±1.50 (43.20%)	3.75 ^{ab} ±0.75 (81.48%)	1.75 ^{a} ±0.48 (91.35%)	1.25 ^{a} ±0.48 (93.82%)
II	Fipronil (Nayflee)	16.5 ^{a} ±1.56	0.00 ^{a} ±0.00	0.0 ^{a} ±0.00	0.0 ^{a} ±0.0	0.0 ^{a} ±0.0
III	Amitraz (Extick)	15.5 ^{a} ±3.22	5.50 ^{b} ± 1.56 (64.51%)	1.0 ^{a} ±0.41 (93.54%)	0.25 ^{a} ±0.25 (98.38%)	0.25 ^{a} ±0.29 98.38%
IV	Lemon grass oil	25.5 ^{b} ±2.32	13.5 ^{cd} ±1.71 (47.05%)	6.5±0.87^{b} (74.5%)	1.75±1.18^{a} (93.13%)	0.5±0.50 ^{a} (98%)
V	Listea oil	20.25 ^{ab} ±2.56	16.75 ^{de} ±2.75 (17.25%)	13.50 ^{c} ±2.53 (33.33%)	13.25 ^{b}±2.66 (34.56%)	13.0 ^{b} ±1.78 (35.8%)
VI	Control group	21.75 ^{ab} ±0.75	21.5 ^{e} ±1.32	20.75 ^{d} ±1.25	21.0 ^{c} ±0.91	21.25 ^{c} ±0.63
P value		0.045	<0.001	<0.001	<0.001	<0.001

^{abcde} *superscript differ significantly (P<0.05) between the rows*

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The Fipronil (Nayflee) have shown 100 % efficacy on the third day of application and onwards whereas Amritraz (Extick) Deltrin and lemon grass oil have shown significant increase in efficacy on day 12 in comparison to day 0. The Litsea extract did not show significant efficacy in terms of reduction of nymphal stages of ticks on day 12 in comparison to day 0. No variation in the control group animals in terms of reduction of nymphal stages of tick was observed in the course of the study. The findings of AIT revealed the dose dependent increase in acaricidal efficacy (Table 2). Among, undiluted commercial preparations, deltamethrin showed highest efficacy followed by lemon grass oil, Amitraz and Fipronil.

Table 2: Dose dependent response of Adult Immersion Test (AIT) to various commercial and herbal acaricidal preparations against *R. microplus*

Final Concentration	Deltamethrin 1.25% W/V	Amritraj 12.5% W/V	Fipronil 0.25% W/V	Litsea grass oil (100 %)	Lemon grass oil (100 %)
Undiluted	4 minutes	29 minutes	36 minutes	68 minutes	7 minutes
0.40%	70 minutes	67 minutes	72 minutes		
0.20%	83 minutes	94 minutes	92 minutes		
0.10%	87 minutes	113 minutes	99 minutes		
0.05%	91 minutes	142 minutes	115 minutes		

Discussion

Ticks represent a serious problem worldwide among livestocks leading to huge losses by transmitting diseases. The emergence of resistance to synthetic acaricidal agents are a constant challenge for control of *R. microplus*. In present study for per cent reduction of nymphal stages of ticks per square inch body surface area of the affected animals, Fipronil showed 100% efficacy followed by Amitraz (98.38%) and Deltamithrin (93.28%). However, on AIT commercial acaricides have shown the dose dependent acaricidal efficacy which was recorded highest in Deltamethrin followed by Amritraz and Fipronil at final concentration of undiluted, 0.40%, 0.20 %, 0.10 % and 0.05%. In other studies on the efficacies of cypermethrin and deltamethrin on the *R. microplus* populations varied from 48.33% to 70.5% and 61.22% to 76.84%, respectively (Brito *et al.*, 2011). Another study using cis-cypermethrin (0.01%) + DDVP (0.11%), coumaphos (0.05%), deltamethrin (0.0025%), amitraz (0.025%) and cypermethrin (0.015%) on R. microplus showed that eggs treated with high cis-cypermethrin and cypermethrin has eclosion inhibitions of 72.1% and 67.3%, respectively, whereas coumaphos was much less effective (only 11.7%). Except for coumaphos, which resulted in 63.3% and 80.0% mortality of nymphs and unfed females, respectively, all compounds tested killed 100% of all tick stages to which they were exposed (Bicalho *et al.*, 2001).

However the studies suggests that synthetic acaricidal agents fail to overcome the problem and selective treatment and culling do not represent feasible methods to control *R. microplus* infestation on cattle (Morel *et al.*, 2017). The resistance of *R. microplus* to organophosphates (chlorpyrifos, coumaphos and diazinon),

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pyrethroids (flumethrin, deltamethrin and cypermethrin), phenylpyrazole (fipronil), amitraz and ivermectin has been documented (Sharma *et al.*, 2012; Rodríguez-Vivas *et al.*, 2014, and Shyma *et al.*, 2015). Herbal acaricides may be a potential substitute for synthetic acaricides currently used against tick. The present study showed that lemon grass oil on 12 days of application resulted in very high efficacy comparable to synthetic acaricides (Fipronil, Amitraz and Deltamithrin). Further, the lemon grass oil has also showed the comparable acaricidal efficacy on AIT. In various studies the lemon grass oil has been proved to have anti-bacterial, anti-inflammatory and anti-fungal properties (Naik *et al.*, 2010; Boukhatem *et al.*, 2014), however, the acaricidal efficacy has not been studied.

In a study of efficacy of extracts from leaf, bark, and seed of *Azadirachta indica*, leaf and seed of *Prunus persica*, bark of *Mangifera indica*, and leaf of *Psidium guajava* against *Boophilus microplus*, the *A. indica* seed extract have shown 80 % efficacy after 5 h of treatment and caused the significant reduction ($P < 0.01$) in the reproductive index of ticks in comparison to control. The efficacy of the neem seed extracts was also found comparable with the commonly used synthetic pyrethroids against *B. microplus* (Srivastava *et al.*, 2008). Pirali-Kheirabadi and Teixeira da Silva (2010) studied *Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick *Rhiphicephalus annulatus* control. Pirali-Kheirabadi and Teixeira da Silva (2011) also reported *In vitro* efficacy of the acaricidal properties of *Artemisia annua* and *Zataria multiflora* essential oils to control cattle ticks. Chagas *et al*. (2011) reported *In vitro* efficacy of *Artemisia annua* extract against *Rhiphicephalus microplus. In vitro* efficacy of ethanolic extracts of *Ageratum conyzoides* and *Artemisia absinthium* was also assessed on *Rhipicephalus microplus* using adult immersion test (AIT). The *A. absinthium* was to have better acaricidal properties than *A. conyzoides* againts R. microplus (Praveen *et al.*, 2014).

The 100% acaricidal activity of *Annona squamosa* and 60% activity of neem oil against ticks of genera *Boophilus microplus* and *R. haemaphysalis* were reported by Kalakumar *et al.* (2000). However, Magadum *et al.* (2009) had reported 70.8% efficacy of *A. squamosa* against *R. microplus* during *In-vitro* trial. While, Kumar *et al.* (2005) had reported that a 50:50 mixture spray of neem + karanj oil was effective on 97.95 per cent of ticks from natural infection in cows on day 15 post application suggesting that formulation is safe alternative to control ticks in field conditions. In a comprehensive study by Ghosh *et al*. (2013), 95% ethanolic extract of *Ricinus communis* extract significantly affected the mortality rate of ticks with an additional effect on reproductive physiology by inhibiting oviposition. Chamuah *et al.* (2014) has also recorded efficacy of crude extract of neem at high concentration and crude extract of tobacco at low concentration against *R. microplus* in naturally infested mithun. The leaves of tobacco (*Nicotina tabacum*) was also found effective against *R. haemaphysaloides (*Choudhury *et al.*, 2004).

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Conclusion

The author concludes that lemon grass oil could be used as a potential substitute to synthetic acaricides due to natural origin, safe and environment friendly. Further, the repetitive and long term application of lemon grass oil did not pose any problem to development resistance which occurs commonly with synthetic agents.

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