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Effect of herbal acaricides on hatchability of eggs of deltamethrin and amitraz resistant cattle tick *Rhipicephalus microplus*

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Abstract
For getting quick control of tick infestations, chemical or synthetic acaricides are being used continuously in uncontrolled and indiscriminate way. This led to development of Acaricide resistance, pollution of ecosystem, residues in animal products and toxicity in animals and farmers. For assessment of an alternative, two phyto Acaricide oils *viz.,* Neem (*Azadirachta indica*) and Karanj (*Pongamia pinnata*) were evaluated to find out efficacy on the off host stage *i.e.* eggs. The phyto-acaricides individually and in combination (1:1) were tested on 200 eggs each in six replicates and kept in desiccators for 15 days. The hatchability rate reduction (HR %) was observed to be highest (71.20 %) with neem oil (group-I) followed by neem+ karanj oil combination (group-III, 56.20%). The eggs from group-II (Karanj oil) showed the lowest hatchability rate reduction (52%). The hatchability rate (H) in group-IV (Control) was 97.92±0.30 percent. This indicates that the neem oil is individually the most potent herbal Acaricide as compared to Karanj oil, while its combination with Karanj oil increased the efficacy marginally by 8.75 percent than the karanj oil.

Keywords: Cattle tick, *Rhipicephalus microplus, Azadirachta indica,* Hatchability, Neem Oil, Karanj oil, phyto Acaricide, Herbal Acaricide

Introduction
The ectoparasites (arthropods) of livestock are having great economic significance on three important points *viz.*, (1) direct losses from their painful bites, haematophagus habbits, annoyance, worries and psychological disturbances produced during the act of biting and feeding, (2) diseases they transmit, and (3) expenditure incurred for their control [1]. Among the ectoparasites (arthropods) infesting livestock, ticks are one of the most damaging pest and potent vectors for transmission of various bacterial, viral, rickettsial, and protozoan diseases of animals and man.

The cattle tick *Rhipicephalus microplus* came up as the most harmful pest, leading to animal stress, reduced growth rate and poor performance, in addition to higher production costs due to the expenditure incurred on constant anti-parasitic treatments [2, 3]. Since the climatic conditions are favourable to their survival and development [4], increasing control costs with synthetic acaricides. The economic impact is of approximately a $ 275.7 million a year due to transmission of tick-borne diseases and cost of tick control [1, 5]. In India, almost all the livestock species suffer from tick infestations and in India alone the cost of TTBDs in animals has been estimated to the tune of US$ 498.7 million (more than 2000 crore) per annum [6].

Chemical acaricides represent the main line of anti-tick defence in domestic animals, but increasing concerns regarding development of Acaricide resistance [7-11], residues in food and environmental safety. These drawbacks of chemical acaricides enhanced the development of effective, non-chemical, herbal alternatives including essential oils for control of ticks [12, 13] and physical method of Caulking process involving burning of tick eggs in the cattle shed [14] for the annihilation of breeding places.

Several sporadic reports on tick infestation patterns of dairy animals from Maharashtra state has been reported earlier [15-20]. However, very few reports on effects of herbal acaricides on the egg stage of *R. microplus* are available. Considering possible role of Neem and Karanj oil as part of integrated tick control programme, present *in vitro* study was undertaken for the assessment of the potential of two commercially available phyto-acaricides against the eggs of *R. microplus.*

Materials and Methods

Collection of Ticks

Deltamethrin (RF-19.75) and amitraz (RF-2.06) resistant fully engorged live dropped adult female ticks of *R. microplus,* [21] were collected from cattle sheds of local farmers at Udgir, Dist. Latur, Maharashtra state along with detail information about the Acaricide used, and frequency of treatment and post application efficacy of acaricides. The ticks were collected in specially designed insect breeding plastic dishes / jars (Himedia Laboratories Pvt. Ltd. India) bearing the mesh window ventilator which allow air and moisture exchange. These insect dishes / jars kept in plastic basket and transported to the Entomology Laboratory, Department of Veterinary Parasitology, Bombay Veterinary College, Mumbai. The collected ticks were cleaned with distilled water, transferred to fresh vials with proper labels and kept at 28 ºC and 85 percent relative humidity for oviposition [8].

Herbal Acaricide preparation

The commercially available oil extracts of Neem (*Azadirachta indica*) and Karanj (*Pongamia pinnata*) oil (M/s. Shree Akshar Pharma Ltd.) were used as the stock solution of herbal acaricides. For the experimental bioassay, the working solution was prepared in 100 ml of soap water as emulsifier @ 2g per liter of water by mixing 3 ml each Neem oil and Karanj oil and 1.5 ml of each Neem oil + Karanj oil for its combination respectively. The solution was mixed well by using vertex primarily and vertexed prior to its every use and tested on the eggs of untreated females of *R. microplus*.

Egg Hatch Assay (EHA)

Egg hatch assay was conducted according to the method of Ribeiro *et al.* [22] with minor modifications. Two hundred embryonated eggs of untreated female of *R. microplus* were placed in each glass vial of all four groups *viz.*, Group-I (Neem oil), Group-II (Karanj oil), Group-III (Neem + Karanj oil) and Group-IV (Control-2% soap water) and immersed for 2 min in 1 ml of the test solution. Subsequently, the solution was decanted and after evaporation of the solvent, all vials were covered with a muslin cloth. Eggs were incubated at 28 ± 1 ºC and 85 ± 5% relative humidity for 15 days, until hatching was completed. The eggs in the control group were treated with distilled water. The hatched larvae and unhatched eggs were counted after the 14 days of incubation period. Each treatment replicated 6 times and the following parameters were compared:

1. Hatchability Rate % (H) :determined by counting the number of hatched larvae divided by the total number of incubated eggs with respect to remaining unhatched eggs in a representative sample from the dishes, as described by Amaral [23], with some modifications.
2. The hatchability reduction rate % (HR): determined by comparing the number of hatched larvae in treated groups (HT) in relation to the control group (HC).

Hatching rate reduction / Inhibition of Hatchability: $\% HR = \frac{HC - HT}{HC} \times 100$

Statistical analysis

The data was analyzed by using completely randomized design (CRD) by using Web Based Agricultural Statistics Software Package (WASP2.0, ICAR, Goa) and the effects of various herbal acaricides on hatchability was assessed with the help of inhibition of hatching (HR%).

Results and Discussion

Table 1: Effect of different herbal oils on hatchability of eggs of untreated females of cattle tick *Rhipicephalus microplus.*

Herbal acaricides used	No of eggs / Replicate (r=3)	Treated eggs of untreated females		
		No. of eggs hatched	% Hatched	% Hatchability Reduction
Neem Oil	200	165	**27.50^{c}**	71.20
Karanj Oil	200	272	45.33^{b}	52.53
Neem Oil + Karanj Oil	200	251	41.83^{b}	56.20
Control	200	573	95.50^{a}	
	SE±23.41, Coefficient of Variation = 4.605, Treatments found Significant at 1% and 5% level of significance, CD (0.01) = 13.256 CD (0.05) = 9.111, r= no. of replicates. Similar superscripts indicate the values statistically at par.			

As a result of herbal acaricide treatment, the hatchability rate reduction (HR %) was observed to be highest (71.20 %) in group-I followed by group-II (56.20%). The eggs from group-II showed the lowest hatchability rate reduction or inhibition of hatchability (52.53%) as compared to the control group. The hatchability rate (H) in group-IV (Control) was 95.50 percent. The inhibition of hatchability (HR %) was highest in group-I and the difference is statistically significant with other two groups. (Table.1, Fig 1). Though the HR of group-III was numerically higher than that of group-II, the values are statistically par. This indicates that the neem oil is individually the most potent herbal Acaricide as compared to karanj oil as well as its combination with Karanj oil. Its combination with karanj oil increased the efficacy marginally by 3.67 percent. The increase in the percent inhibition of hatchability is indicative of reduced viability of eggs in the treated group, thus greatly affecting the regeneration of ticks.

The hatchability of only 17-65 percent was observed by Narladkar and Shivpuje [24] which is in agreement with the observations of the present study. Bisen *et al.*, [25] reported 75.27 percent and 21.29 percent hatchability with Neem oil and Neem + Karanj oil in combination respectively which supports the current study as far as the neem oil is concerned. However, the HR recorded in combination group in the present study was higher. This difference may be attributed the source of Karanj extract used and the concentration of the active principals in it. The higher efficacy (100%) on hatchability was reported by Kumar *et al.* [26] with *Nicotiana tobacum*, *Annona Squamosa*, *Nerium oleander* and *Datura stramonium.* While Abdel-Shafy and Zayed [27] reported maximum hatchability reduction of 16.66 percent (60% in relation to 72 % in control group) with neem against eggs of *Hyalomma* spp. The higher efficacies reported by later both the authors may be attributed to the variation in either herbs or the tick species used for the experiment.

Irrespective of the level of efficacy, trend of inhibition of hatchability observed in the present study was following the foot prints of Bisen *et al.* [25]. However, the difference in the

level of efficacy observed in this study and both the other studies [25, 28] may be due to the difference in the eggs chosen for hatchability inhibition bioassay. It has already been proved that the neem extract inhibits the oviposition rate, thus the reproduction [29]. The female ticks were not treated prior to experiment in the present study while Bisen *et al.* [25] observed the hatchability of eggs of female ticks treated with neem oil.

Few eggs of group-I and III were observed dark reddish after 6-7 days and after 15 days of incubation these eggs became blackish and were unable to hatch. The shape and architecture of the egg shell was also found to alter as they were shrunken and the shape looked like rugby ball with a longitudinal groove. These findings also are in agreement with those of Rao *et al.* [28].

The observations of the present study are definitely encouraging and hopeful towards its use as a part of integrated pest management module (IPM) as the present study is of unique nature and the herbal acaricides were evaluated against known resistant isolate of *R. microplus* from Maharashtra state. Though the results of the present study are comparable with observations of earlier authors [24-30], Acaricide resistance status of the ticks used by them for the experiment was unknown.

The high efficacy of Karanj (*Pongamia glabra*) oil alone than the mixture of neem (*Azadirachta indica*) and Karanj (*Pongamia glabra*) was reported [25, 30]. In the contrast to this observation, the mixture of neem (*Azadirachta indica*) and Karanj (*Pongamia pinnata*) was observed more efficacious in present study than Karanj (*Pongamia pinnata*) alone. The efficacy of the mixture was marginally high than karanj oil alone and 18.67 percent less than neem (*Azadirachta indica*) alone. In the present study two herbs were evaluated and it yielded moderate to high and hopeful results to the extent of 52.53 to 71.20 percent reduction in egg hatchabilty. The findings of the present study were well supported by the observations of Ajith Kumar *et al*., [31] mono herbal products were found more efficacious as compared to polyherbal products.

A number of natural compounds of plants may interfere with the biological processes of insects interrupting their development [32]. It is well known that phyto-acaricides are usually less toxic to mammals, have no residual effect [33]. There is less chance of development of resistance in tick populations compared to synthetic or chemical acaricides, as herbal formulations contains number of active principles which may have synergistic action. Neem extract includes about 135 compounds *viz.*, alkaloids, lavonoids**,** triterpenoids, phenolic compounds, carotenoids, steroids and ketones, very complex structure azadirachtin. Other compounds that have a biologaical activity are salannin, volatile oils, meliantriol and nimbin2 [34]. Karanjin is the main active ingredient of Karanj oil also includes other alkaloids *viz.*, demethoxy-kanugin, gamatay, glabrin, glabrosaponin, kaempferol, kanjone, kanugin, karangin, neoglobin, pinnatin, pongamol, pongapin, quercetin, saponin, b-sitosterol, and tannin.

Conclusion

The results of the present study are unique as it was conducted on tick species known to be resistant to deltamethrin and amitraz. It revealed a state of moderate to high effectiveness of mixture of neem (*Azadirachta indica*) and Karanj (*Pongamia pinnata*) and individual neem (*Azadirachta indica*) against the egg stage of resistant isolate of *R. microplus* and suggests need of further study by using higher concentrations of these herbal oils and shows a ray of hope as an option for the effective control of egg stages of acaricide resistant ticks specifically apart from the host at their breeding places in the animal sheds. The data generated may be useful in effective control of deltamethrin and amitraz resistant isolate of *R. microplus* off the host stages and may hence lead to lesser number of infective stages available in the environment and thus may contribute largely to control of resistant isolates of ticks and may play down the menace of acaricide resistance.

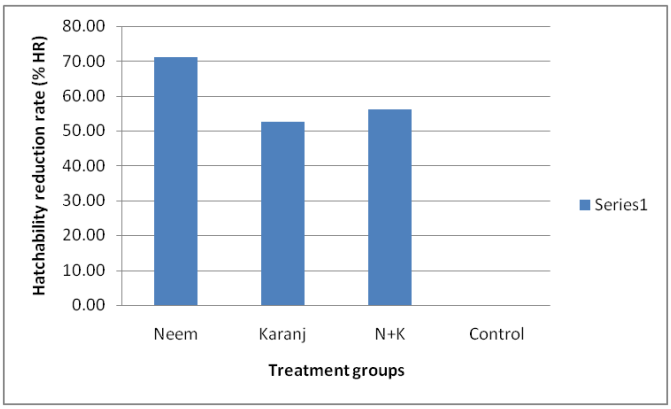

Fig 1: Showing the percent Hatchability Rate Reduction (% HR) of eggs of untreated females of cattle tick *Rhipicephalus microplus* after treatment with herbal acaricides.

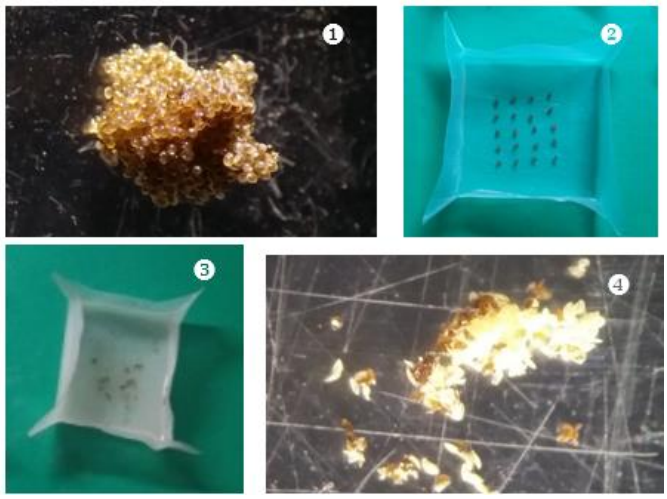

Fig 2: Fresh eggs collected from untreated females, 2. 200 tick eggs counted under stereozoom microscope for each experimental group. 3. Eggs immersed in herbal solutions. 4. Some eggs failed to hatch

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