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Evaluation of Acaricidal Activity of *Nicotiana tabacum* extracts against *Rhipicephalus(Boophilus) microplus*

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Abstract: The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* is one of the most important ectoparasites of cattle. Traditional tick control is mainly based on the use of synthetic chemicals, However ticks are developing resistance to most of the available acaricides and also have many negative side effects. The aim of present study was to evaluate the acaricidal activity of extracts of *Nicotiana tabacum*. Acetone extract was prepared by cold maceration method. The acaricidal activity of acetone and aqueous leaf extracts of *Nicotiana tabacum* and deltamethrin were tested on fresh larvae using larval packet test (LPT). In the larval packet test, cent percent mortality was obtained at concentrations (ppm) of 400, 3000 and 6000 for the compounds deltamethrin, acetone and aqueous leaf extracts respectively. The LC_{50} and LC_{99} were highest for aquoeus leaf extract at 728.97 and 6094.438 ppm, respectively. The LC_{50} and LC_{90} were lowest for deltametrin at 57.11 and 1060.90 ppm, respectively. In conclusion, phytogenic compounds having the acaricidal activity and also eco-friendly.

Keywords: *Nicotiana tabacum,* acaricidal activity, *Rhipicephalus* (*Boophilus*) *microplus,* larval packet test.

Introduction

Ectoparasite infestation is one of the major problems affecting livestock industries in many parts of the world (FAO, 2004). Ticks are considered as one of the important and harmful blood sucking ectoparasites of livestock and human around the world after the mosquitoes (Zahir and Rahuman, 2012). The cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* is one of the most important ectoparasites of cattle and is widely distributed in tropical and subtropical regions including India. *Rhipicephalus* (*Boophilus*) *microplus* causes huge economic loss in cattle production by reducing weight gain, milk production and causing tick worry, blood loss, hide damage and injection of toxins. Further, indirectly they also involve in disease transmission like babesiosis and anaplasmosis. Most of the dairy and meat animals in India are affected by tick infestation leading to significant economic loss (Ghosh *et al*., 2006).

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In a recent estimate, the control cost of ticks and tick borne diseases in dairy sector has been estimated to the tune of $ 498.7 million per annum (Minjauw and McLeod, 2003) and is responsible for economic losses that range over hundreds of millions of dollars per year (Guerrero *et al*., 2006). According to an estimate, an annual shortfall of 3,000 million pieces of hides and skin is due to tick infestation (Mondal *et al*., 2013). Keeping in view the impact of ticks and tick borne diseases on the individual and national economics the developing world should focus on tick control on a priority basis (Bansal, 2005).

At present the use of synthetic chemicals is the backbone to control the tick infestations. However, ticks are developing resistance to most of the available acaricides and have many negative side effects, including residues in food and environmental contamination if these products are improperly used (Ghosh *et al*., 2013a). There is an urgent need to develop eco-friendly alternatives to chemicals. Alternatively, natural bioactive phytoacaricides are increasingly used for tick control as they have additional advantages such as low toxicity and more eco-friendly (Fernandes and Freitas, 2007). Natural products offer a cheap alternative to synthetic acaricides (Habeeb, 2010). 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). Moreover, these botanicals are found to contain a mixture of active substances which can delay or prevent the development of resistance to herbal products (Ghosh *et al*., 2015).

One of the commonly cited advantages that may result from the use of botanicals for tick control is their biodegradability (Parte *et al*., 2014).Some plant extracts like i.e. *Azadirachta indica*, *Annona squamosa* (Magadum *et al*., 2009), *Mangifera indica* (Srivastava *et al*., 2008*)*, *Nicotiana tabacum* (Zaman *et al.,* 2012*)* having the acaricidal activity.

***Nicotiana tabacum*/tobacco** (Family Solanaceae) popularly known as Pogaaku (Telugu)/ Hogesoppu (Kannada) is a stout, viscid annual herb growing upto 1-3 m in height. Traditionally it has been used to treat skin diseases, local infections, bronchitis, asthma and inflammation. In India, the leaves of tobacco plant have been used as sedative, antispasmodic, antiseptic, emectic and nacrotic. Tobacco leaves are rich in polyphenols besides containing vegetal oil, flavonoides, alkaloids and citric acid (Sharma *et al.,* 2016). In the current study attempts were made to know the acaricidal activity of *Nicotiana tabacum*.

Materials and methods

Preparation of *Nicotiana tabacum* leaf extracts

The leaves of *Nicotiana tabacum* was collected from fields of different parts of Andhra Pradesh and identified by the Department of Botany, Sri Venkateswara University, Tirupati. The leaves were washed thrice in water to remove dust and dried under shade. Ground into fine powder using blender.

The aqueous leaf extract was prepared as described earlier (Shankar *et al*., 2004). Fifty grams of leaf powder was mixed with 500 mL distilled water and boiled for about 30 min on a hot plate. The boiled solution was filtered using Whatman No.1 filter paper and clear aqueous extract was obtained. The extract was stored at 4°C until further use

Acetone extract (AE) of plant material under study was prepared by cold maceration method as previously described by Sindhu *et al.* (2012a). Prepared AEs were refrigerated till further use.

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Collection of ticks

Engorged adult ticks were collected from heavily infested cattle in and around Tirupati using forceps, with intact mouth parts, into a collection vial. These samples were immediately brought to the laboratory for identification and further use.

Identification of ticks

The collected ticks were processed as per the standard procedure for morphological confirmation of *Rhipicephalus* (*Boophilus*) *microplus*. The processed ticks were examined microscopically and identified as *Rhipicephalus (Boophilus) microplus* as per the descriptions of Alan Walker (1994).

Larval packet test (LPT)

The larval packet test was performed as per FAO (2004) to determine the *in-vitro* acaricidal activity of the test compounds. Engorged female ticks were obtained from the cattle in the study area, identified, cleaned, stored in a petri dish and maintained at 85-92% RH and 27.0 ±1^{o}C. The female ticks were examined daily until oviposition. The eggs were separated and allowed to hatch in glass vials with cotton plug and kept in optimal conditions. The obtained seed ticks were maintained at 27.0 ±1.0 ^{o}C and 85-92% RH for 14-21 days. The larvae aged between 14 to 21 days were subjected to larval packet test.

The dilutions for all the compounds were made in distilled water except for deltamethrin, which was diluted in olive oil with trichloroethylene (1:2).

Packets made of Whatman filter paper No. 1 (12 cm x 18 cm) were impregnated with 3mL of respective compounds and dried at room temperature for two hours. About 100 larvae were placed in acaricide impregnated filter paper packet and the top of the packet was sealed with aclamp. These larval packets were incubated at 27.0 ± 1.0 ^{o}C and 85-92% RH for 24 hours. Mortality of larvae was assessed after 24 h of exposure. Lethal concentrations of the compounds were determined using the live and dead count.

The percentage mortality in all of the experimental batches of larvae was corrected by applying Abbott's formula.

$$Corrected\ percent\ mortality = \frac{test\ mortality\ - \%control\ mortality}{100\ -\ \%control\ mortality} \times 100$$

Statistical Analysis

The mortality percentage from larval packet was subjected to probit analysis to calculate lethal concentration ($LC_{50}$and LC_{99}) for respective compounds using Statistical Package for Social Sciences (SPSS 19.0 V IBM, Illinois, Chicago). The lethal concentrations were expressed by 95% fiducial limits. The statistical difference between the lethal concentrations were compared with the absence of overlap of 95% fiducial limits. The level of significance was set at $p<0.05$.

Results and discussion

Acaricidal activity against *Rhipicephalus* (*Boophilus) microplus*

Larval packet test (LPT) was used to detect the activity of various compounds against *Rhipicephalus* (*Boophilus*) *microplus.*

The larval packet test was used in the present study, to determine the acaricidal activity against *Rhipicephalus* (*Boophilus*) *microplus* with various concentrations of deltamethrin; nicotianaaqueous leaf extract; acetone extracts of nicotiyana. A modified version of FAO (2004) larval packet test (LPT) was used to determine the deltamethrin activity to *R (B). microplus* larvae. The concentrations of deltamethrin varied from 25 to 400 ppm and the peak mortality (100%) were recorded at a concentration of 400 ppm. A total of six concentrations of acetone extracts viz., 187.5, 375, 750, 1500, 3000and 6000 ppm were used to test the compound activity. It was noted cent percent mortality was seen at 3000 ppm level. The effect of the crude nicotiyanaaqueous leaf extract against *R*(*B*). *microplus* was found to be 100 percent at 6000 ppm concentration. With the increase in concentration level the percent

mortality rate also increased. A total of six concentrations of acetone extracts viz., 187.5, 375, 750, 1500, 3000and 6000 ppm were used to test the compound activity.

The lethal concentrations of various compounds against fresh larvae (Table 1)

Table 1: Lethal concentrations of various compounds against fresh larvae

sl.no	compound	LC_{50}	LC_{99}	SD	SE	R^2	Slope	Intercept
1	Deltamethrin	57.11 (36.61-89.09)	1060.90 (680.05-1655.04)	0.595	0.099	0.9381	1.6807	2.0558
2	aqueous extract	728.971 (353.87-1501.65)	6094.438 (2958.51-NC)	0.937	0.160	0.9524	1.068	1.8828
3	Acetone extract	318. 805 (150.28-676.29)	2237.69 (1054.84-4746.91)	0.924	0.167	0.9844	1.082	2.3236

Values are inhibitory concentrations with 95% Fiducial Confidence Intervals in parenthesis

LC : Lethal concentration; SE = Standard error; SD = Standard deviation

Probit analysis using IBM SPSS 19.0 V

Deltamethrin showed significantly ($P<0.05$) lower LC_{50} compared to respective plant extracts The LC_{99} values also in similar manner, aqueous extract showed least acaricidal activity with significantly ($P<0.05$) higher LC_{99} values.

Now a days deltamethrin is the most commonly used syntheticpyrethroid against the tick infestations in India. *Rhipicephalus (Boophilus) microplus* are increasingly becoming resistant towards deltamethrin due to its wide spread usage. In the present study hundred percent larval mortality was obtained with deltamethrin at a concentration of 400 ppm. Highest resistance towards deltamethrin was reported from (Sharma *et al*., 2012) with less resistance (Jyothimol *et al*., 2014). But in the present study high resistance was observed for deltamethrin against *Rhipicephalus* (*Boophilus*) *microplus.* The reasons for the increased resistance might be due to its widespread usage, increase α/β esterase activity and target site insensitivity (mutation in para-sodium channel gene) (Kumar *et al*., 2013).

To over come these resistance problems, the leaf extracts of leaves of *Nicotiana tabacum*, were found to be alternative. It was selected based on their previous acaricidal activity reports, usage in traditional veterinary medicine.

N. tabacum was used in the ethno-veterinary practice as an anthelmintic (Iqbal et al., 2006), anti-inflammatory and anti-rheumatic agent (Nadkarni, 1976).

Dipeolu and Ndungu (1991) have demonstrated acaricidal efficacy mixture of *N. tabacum* leaves and a mineral salt, they founded the Larvae and nymphs of *R.appendiculatus* were

killed within 24 hours, and adult ticks were dead in vitro within 2-3 days. Zaman *et al*. (2012) assessed the efficacy of combined aqueous herbal extracts of *Azadirachtaindica* leaves, *Nicotiana tabacum* leaves, *Calotropisprocera* flowers and *Trachyspermumammi* seeds against the *Rhipicephalus (Boophilus) microplus* and reported that extract exhibited the lethal effects on egg laying and hatching.

Several other plant extracts were also shown to possess acaricidal activity against *Rhipicephalus (Boophilus) microplus*, viz., ethanolic extract of *Sapindussaponaria* (Fernandes *et al*., 2005). Srivastava *et al*. (2008) and Magadum *et al*. (2009) all these reports showed 80% efficacy against *Rhipicephalus (Boophilus) microplus* larvae.

Martinez- Velazquez *et al.* (2011) reported the acaricidal activity of essential oils extracted from cumin seeds (*Cuminumcyminum*), allspice berries (*Pimentadioica*) and basil leaves (*Ocimumbasilicum*) on *Rhipicephalus (Boophilus) microplus*. Similarly, Ribeiro*et al.* (2011) reported the acaricidal activity of essential oils of *Caleaserrata* extracts and prococene II against the larvae of *Rhipicephalus (Boophilus) microplus*. Gigliotie*t al*. (2011) reported that the efficacy of neem seed extracts on egg laying and larval hatching rates of adult *Rhipicephalus (Boophilus) microplus.* Several other plant extracts other than neem were also shown to possess acaricidal activity against *Rhipicephalus (Boophilus) microplus.* Ghosh *et al.* (2013b) reported that, the leaf extracts of *Ricinuscommunis* had the highest mortality at 10 percent concentration. In a study by Parte *et al*.(2014) demonstrated that the combination of aqueous extracts of *Azadirachtaindica, Mangiferaindica, Polyalthialongifolia, Annonasqamosa* and *Ficusbenghalensis* showed the 100 percent mortality as compared to a single plant extract against *Rhipicephalus (Boophilus) microplus* ticks

Conclusion

Plant extracts have the have potent acaricidal activity and herbal preparations also advisable due their eco-friendly nature. Phyto chemical analysis of tobacco revealed presence of phenols, alkaloids and saponins that have been known for various biological effects. However, further studies are needed to studies on the toxicity, these extracts evaluated in-vivo for their acaricidal activity before their therapeutic application in veterinary practice.

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