Bangl. J. Vet. Med. (2005). 3 (2): 118–123

IN VITRO ACARICIDAL EFFECTS OF SOME INDIGENOUS PLANTS AGAINST *BOOPHILUS MICROPLUS* (ARACNIDA: IXODIDAE)

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ABSTRACT

In vitro efficacy of neem (*Azadirachta indica*), bishkatali (*Polygonum hydropiper*), ata (*Annona reticulata*), sharifa (*A. squamosa*) and durba ghas (*Cynodon dactylon*) against *Boophilus microplus* (tropical cattle tick) was tested during the period from July to December 2004 in the Department of Parasitology, Bangladesh Agricultural University, Mymensingh. To prepare the paste, aqueous and ethanol extracts, the leaves of neem, bishkatali, ata, sharifa and leaves along with stem of durba ghas were used. Three different types of preparation of plant materials such as paste, aqueous extracts and ethanol extracts were applied in three methods such as thin layer of paste, as spray and as impregnated filter paper (IFP). Extracts were used in 0.5%, 1% and 2% concentrations and the percent mortality of the ticks were recorded at 12, 24 and 72 hours after treatment. Among them, ethanol extract of ata at 2% concentration showed highest efficacy (100%) followed by aqueous extract of bishkatali (93.33%) at same concentrations and ethanol extract of ata (93.33%) at 1% concentration in spray on method. Among the various methods of application "spray on" method was found to be most effective followed by paste and impregnated filter paper. On the other hand, among the preparations ethanol extract was found to be more efficacious incase of ata plant only. From the study, it is revealed that ata and bishkatali have great acaricidal value against *B. microplus*.

Key words: Acaricidal effects, neem, bishkatali, ata, sharifa, durba ghas, *Boophilus microplus*

INTRODUCTION

Cattle tick, *Boophilus microplus* economically is the most damaging bovine ectoparasite in tropical and sub-tropical countries (Hoogstraal, 1956). They play an important role as vectors of many pathogens of animals and humans. *B. microplus* transmits the protozoa *Babesia bigemina, B. argentina, Anaplasma marginale*, the rickettsiae *Coxiella burnetti* and the spirochaetes *Borrelia theileri* in different parts of the world (Neitz, 1956). Babesiosis caused by *B. bigemina* and anaplasmosis caused by *A. marginale* are found in cattle in Bangladesh (Ahmed, 1976). Control measures of the *B. microplus* in tropical and sub-tropical countries, is usually practiced by regular acaricide applications (Fernandez and Cruz, 2000) where the use of chemical acaricides is main. Most of these acaricides are very expensive and not available everywhere, especially in rural areas. So, most of the farmers have no ability to use these acaricides to control tick infestations. The long-term use of these chemical compounds has harmful effects on human health, beneficial organisms and environment (Mansour *et al.*, 2004). Some synthetic compounds have residual effects and are accumulated in environment, which induce resistant strains of pests. The tropical cattle tick can also develop resistant strains against the synthetic acaricides (O' Sullvian and Green, 1971; Howell, 1977).

Bangladesh is plentiful with many plants, among them medicinal plants as a traditional system of therapy have been used from ancient periods of times to cure diseases of man and animals (Akhtar *et al.*, 2000). Medicinal plants are also used against ectoparasites like lice, ticks, mites etc in different countries.

The acaricidal effects of various plants have been studied in many countries of the world such as in India (Khudrathulla and Jagannath, 1998), USA (Mulla and Su, 1999), Egypt (Abdel-Shafy and Zayeed, 2002), Thailand (Chungsamarnyart and Jansawan, 2001; Chungsamarnyart *et al.*, 1992), Mexico (Muro *et al.*, 2003), Namibia (Kayaa, 2000) etc. But no research work has yet been conducted to study the acaricidal effects of indigenous plants of Bangladesh.

Considering the above points, this research work was conducted, to identify some plants having acaricidal efficacy against adult *B. microplus* and to establish a simple protocol for *in vitro* screening of some plants having acaricidal efficacy.

MATERIALS AND METHODS

The research work was conducted during the period from July to December 2004 in the Department of Parasitology, Bangladesh Agricultural University (BAU), Mymensingh.

Collection, identification and preservation of plant materials

The plant and plant materials used in this experiment were collected from BAU campus and its surrounding rural areas and brought to the Laboratory. The plant species were identified on the basis of their characteristic morphological features (Thakur, 2003).

Preparation of paste

Paste was prepared by pressing the fresh leaves with the help of pestle and mortar.

Processing of plant materials

After collection and bringing them to the laboratory all fresh leaves and stems were washed in running tap water and cut into small pieces. The plant materials were dried in the oven at 55-60^0 C to gain constant weight.

Preparation of dust of different plants

Dust of different parts of plants was prepared by pulverizing the dried leaves and stems with the help of a manual grinder, pestle and mortar. A 25 mm mesh (diameter) sieve was used to obtain fine dust and preserved them into airtight plastic container till their use in extract preparation.

Preparation of plant extract

To prepare aqueous and ethanol extracts, 10 g of dust of each plants was taken in each 500 ml beaker to which 100 ml of distilled water and ethanol was added respectively. Then the mixture was stirred for 30 minutes by a magnetic stirrer (6000 rpm) and left stand for next 24 hours. The mixture was then filtered through a fine cloth and again through filter paper (Whatman No. 1). The filtered materials were taken into a round bottom flask and then condensed by evaporation of solvent from filtrate in a water bath at 50 ^{0}C and 60 ^{0}C for ethanol and water respectively up to final volume appeared to 10 ml. The condensed extracts were preserved in tightly cork- labelled bottle and stored in a refrigerator until their screening for acaricidal property.

Preparation of solution of different concentration

For *in vitro* screening 0.5%, 1% and 2% solutions of each extract were prepared by adding 99.5 ml, 99 ml and 98 ml of distilled water or ethanol to 0.5 ml, 1 ml and 2 ml of the concerned stock solution respectively. The plant extract was pipetted into a 500 ml beaker (Pyrex, com) with the help of micropipette (Marek Ltd. 0.04 ml) and were mixed thoroughly with magnetic stirrer. All solutions were prepared just before trial.

Collection, identification and maintenance of ticks in the laboratory

Adult cattle ticks, *B. microplus* were collected by hand picking from infested cattle and were identified according to the keys given by Soulsby (1982), Urquhart *et al.* (1996). Ticks collected in the early morning were brought to the laboratory and treated with the plant extracts. When it was not possible to treat the freshly collected ticks then they were maintained in the laboratory for 12-24 h according to the standard procedure given by Anon. (1986).

Screening of paste

A thin layer of paste was made on the petridishes (4 inch.) with each plant materials and then 15 adult ticks were placed on the paste in each petridish. The petridishes were covered with fine cloth with the help of rubber band, and then each of the petridish was placed on another large petridish (6 inch). The space in between two petridishes was filled with moist cotton to prevent desiccation. Observations were made at 24, 48 and 72 h interval. During the study, death of the tick was confirmed by the absence of the movement of their legs, in some cases, the ticks were pricked by fine needle. Dead ticks showed no movement despite pricking. For control study, equal numbers of ticks were placed on blank but moist petridish. This petridish was also covered by moist cotton to protect from desiccation in same manner.

Screening of plant extract

For *in vitro* screening, extracts (aqueous and ethanol extracts) of different plants were applied in two methods such as spray on petridish and impregnated filter paper methods.

Spray on petridish

Different concentrations of aqueous and ethanol extract (0.5%, 1% and 2%) were sprayed on separate petridishes to make a even and thin layer of extract and 15 adult ticks were placed in each petridishes. The petridishes were covered in same manner. They were protected from desiccation by moist cotton in same way. For control study, in case of aqueous extract 15 adult ticks were placed on a petridish moist with water only. But in case of ethanol extract, 15 adult ticks were placed on each petridish sprayed with corresponding concentration of ethanol (0.5%, 1% and 2% ethanol). These petridishes were covered and protected in same manner, observation were made in same way. During the study, the efficacy of the plant materials was made in terms of the mortality of the treated ticks.

Impregnated filter paper (IFP)

Filter papers were impregnated with different concentrations of aqueous and ethanol extracts of plant materials separately. These filter papers were placed on separate petridishes and 15 adult ticks were placed on the filter paper of each petridish. For the control study in case of water extract 15 adult ticks were placed on filter paper soaked with water only but in case of ethanol extract 15 adult ticks were placed on filter paper soaked with different concentrations of ethanol (0.5%, 1% and 2% ethanol) separately. Coverings of petridishes and observation of ticks to detect efficacy were made in the same way.

RESULTS AND DISCUSSION

From the study it was revealed that ethanol extract showed the highest efficacy (100%) incase of ata plant only. In other plants, aqueous extract showed relatively higher efficacy followed by ethanol extract and paste (Table 1 & 2). Ethanol extract showed higher efficacy against gastrointestinal nematodes (Akhtar and Javed, 1991). Paste is a crude product, which possibly contained relatively lower concentration of active ingredients than the aqueous and ethanol extracts. Probably for this reason, paste of different plants was less efficacious.

Table 1. Acaricidal efficacy of freshly prepared pastes of different plants against adult *B. microplus*

Name of plants	Method of application	No. of dead ticks after 72 h (n =15)	Efficacy (%)
Neem	As a thin layer of paste on the petridish	09	60.00
Bishkatali	Do	12	80.00
Ata	Do	13	86.67
Sharifa	Do	09	60.00
Durba	Do	12	80.00
Control	On the petridish, moistened with water	00	–

n = Number of ticks used.

Among the methods applied, spray on method showed the highest (100%) efficacy followed by impregnated filter paper (86.67%) and paste (86.67%). These findings can not be compared due to paucity of relevant literature. However, it can be assumed that in case of thin layer of paste, probably active ingredients of plant did not get chance to come in proper contact with ticks, as it is a crude product. On the other hand, in impregnated filter paper method, the extract was almost absorbed by the filter paper and the ticks got minimum contact with active ingredients. But in spray on method, the ticks were properly moistened with the extract and thus they got proper contact with the active ingredients.

Table 2. Acaricidal efficacy of aqueous and ethanol extracts of different plants against adult *B. microplus*

Name of Plants	Methods of application	Concentration of extracts (%)	No. of dead ticks after 72 h (n =15)			Efficacy (%)	
			Treated		Control	Aqueous extract	Ethanol extract
			Aqueous extract	Ethanol extract			
Neem	Spray on	0.50	9	6	0	60	40
		1	12	9	0	80	60
		2	13	10	0	86.67	66.67
	IFP	0.5	6	3	0	40	20
		1	9	7	0	60	46.67
		2	12	9	0	80	60
Bishkatali	Spray on	0.5	12	9	0	80	60
		1	13	10	0	86.67	66.67
		2	14	12	0	93.33	80
	IFP	0.5	7	4	0	46.67	26.67
		1	10	8	0	66.67	53.33
		2	13	10	0	86.67	66.67
Ata	Spray on	0.5	6	13	0	40	86.67
		1	12	14	0	80	93.33
		2	13	15	0	86.67	100
	IFP	0.5	4	6	0	26.67	40
		1	9	9	0	60	60
		2	9	12	0	60	80
Sharifa	Spray on	0.5	6	9	0	40	60
		1	10	11	0	66.67	73.33
		2	12	13	0	86.67	86.67
	IFP	0.5	3	3	0	20	20
		1	6	6	0	40	40
		2	9	8	0	60	53.33
Durba	Spray on	0.5	6	3	0	40	20
		1	9	6	0	60	40
		2	12	9	0	80	60
	IFP	0.5	3	2	0	20	13.33
		1	7	5	0	46.67	33.33
		2	8	7	0	53.33	46.67

n = Number of ticks used, IFP = Impregnated filter paper.

Neem

It has been observed that aqueous extract of neem showed 86.67% efficacy *in vitro* on spray method at 2% concentration (Table 2). Abdel- Shafy and Zayeed (2002) studied the effects of neem seed oil against eggs, nymphs and adult, *Hyalomma anatolicum excavatum* and they recommended that 1.6% and 3.2% concentration might be used. Maske *et al.* (1995) reported that neem in association with *Cedrus deodara* and *Embelia ribs* showed 100% efficacy at 1:10 dilution against larvae, nymph and adult ticks. Probably, the active ingredients of neem plants having acaricidal efficacy are mostly water soluble.

Bishkatali

Aqueous extract of bishkatali showed highest efficacy (93.33%) at 2% concentration (Table 2). This finding can not be compared due to paucity of relevant literature because the acaricidal efficacy of this plant has yet not been studied. However, the insecticidal properties of this plant have been studied (Duke, 1990). Moreover, Ghani (2003) found that ethanol extract of the young shoots of this plant showed antibacterial activities. The present finding suggests that the active ingredients of this plant are mostly soluble in water.

Ata

Ethanol extract of ata showed 100% efficacy at 2% concentration when it is applied as spray (Table 2). No literature is available on the acaricidal efficacy of ata. However, other medicinal properties of ata have been well established. Their seed extract is toxic and is used as insecticide against lice (Ghani, 2003). Raja-Reddy and Sudarsanam (1987) also investigated the effects of seed, along with the unripe fruits, as a fine paste and applied on animals body infested with lice and other insects. On the other hand, aqueous extract of ata showed 86.67% efficacy at 2% concentration when used as spray (Table 2). This indicated that most of their active ingredients are soluble in alcohol. Paste of ata leaves showed 86.67% efficacy (Table 1). Therefore, paste of their leaves can be used as acaricide if *in vivo* trial gives satisfactory results as because preparation and application of paste are easier.

Sharifa

Both aqueous and ethanol extract of sharifa showed 86.67% efficacy at 2% concentration as spray (Table 2). Kalakumar *et al.* (2000) performed both *in vitro* and *in vivo* trials on the acaricidal activity of seed oil (*A. squamosa*) and they found that it was 100% efficacious against *B. microplus, H. anatolicum, Riphicephalus haemaphysaloides.* Chungsamarnyart *et al.* (1991) reported that crude extract of *A. squamosa* showed acute high acaricidal activity. Chungsamarnyart *et al.* (1988) also reported that ethanol extract of *A. squamosa* showed high *in vitro* larvicidal activity (90-100%) against *B. microplus* when used as thin film. From the present finding it may be concluded that aqueous extract may be used, as it is cost effective than the ethanol extract.

Durba ghas

Paste and aqueous extract (2% concentration) of durba ghas were found to be equally efficacious (80%) (Table 1 & 2). Literature concerning the acaricidal activity of durba is not available, but the medicinal value of durba is well established. Durba is used in dysentry, chronic diarrhoea, cystitis, nephritis etc (Ghani, 2003). Ethanol extract of durba showed 60% efficacy at 2% concentration as spray (Table 2). These data indicated that the active ingredients of durba are less soluble in alcohol.

In the present study, the acaricidal effect of some indigenous plants such as neem, bishkatali, ata, sharifa and durba ghas were studied preparing their paste, aqueous extract and ethanol extract by giving *in vitro* trial on tropical cattle ticks (*B. microplus*). The study has showed that ata and bishkatali have great acaricidal value. However, in this study, only the adult ticks were used. So, further study may be conducted to determine the acaricidal effects of these plants against other ticks and also against their various developmental stages (e.g. eggs, larvae and nymphs). Besides, *in vivo* trial may be given in the field condition to determine their acaricidal efficacy.

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