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Full Length Article

Comparative Efficacies of *Curcuma longa*, *Citrullus colocynthis* and *Peganum harmala* against *Rhipicephalus microplus* through Modified Larval Immersion Test

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Abstract

The present experiment was conducted to evaluate the acaricidal activities of the aqueous – methanolic extracts of rhizome of *Curcuma longa*, fruit of *Citrullus colocynthis* and seed of *Peganum harmala*. Modified Larval Immersion test (syringe method) was used to evaluate the acaricidal activity of plant extracts in lab against *Rhipicephalus microplus*. Acaricidal activity of each plant was evaluated at two different exposure times (time-mortality experiment) i.e., 24 h post exposure and 6 day post exposure. The highest activity was recorded at 6th day after application of combined three plants at the dose rate of 50 mg/mL; whereas, lowest acaricidal efficacy was observed at 24 h with the dose rate of 3.125 mg/mL in case of individual effect of *P. harmala*. The herbal formulation is suitable for the poor farmers as a broad spectrum antiparasitic. The contents of the formulation are cheap and commonly available. The combination of plants may be recommended for use at farm level based on empirical evidence of its anti-parasitic activity. © 2015 Friends Science Publishers

Keywords: Acaricidal activity; *Curcuma longa; Citrullus colocynthis; Peganum harmala;* Crude aqueous-methanol extracts

Introduction

Ecto-parasite like ticks, mites and lice cause high economic losses due to effect on the skin and causes anemia by ingesting the blood of host (Jonsson, 2006; Abbas *et al*., 2014a). They also cause allergy, irritation and toxicosis (Niyonzema and Kiltz, 1986). Among ectoparasites, ticks play an important role in impairment of production, lowered weight gain (Pegram and Oosterwijk, 1990) and mortality (Niyonzema and Kiltz, 1986). These ecto-parasites also serve as vector for transmission of different diseases like anaplasmosis, babesiosis and theileriosis (Norval *et al.*, 1984). Ticks also act as carrier of certain pathogenic agents e.g. *Salmonella typhimurium, Pasturella multocida* and *Brucella abortus* that cause zoonosis (Jongejan and Uilenberg, 2004).

The prevalence of ticks in cattle ranges from 36 to 66.7% in Pakistan (Atif *et al.*, 2012). Control of parasitism largely depends upon the use of synthetic drugs. However, there is development of resistance in parasites against drugs (Rodriguez-Vivas *et al.*, 2006; Jabbar *et al.*, 2007; Miller *et al.*, 2007; Saeed *et al.*, 2007) and problem of potential residues of drugs in milk, meat and other animal products (Tarbin *et al.*, 2006). Thus, there is an urgent need for alternate parasitic control strategies to overcome the drawback associated with the use of synthetic drugs (Masood *et al*., 2013; Abbas *et al*., 2014b; Hamad *et al*., 2014). This may include phytotherapy (e.g., for ticks by Sindhu *et al.,* 2010), an important component of ethno-veterinary medicine. This paper describes anti-tick activity of some plants indigenous to the study area in Pakistan.

Materials and Methods

Selection of Plants

During survey, three of the screened plants, were selected for acaricidal activity, which includes were *Curcuma (C) longa* (rhizome) *Citrullus (Ct) colocynthis* (fruit) and *Peganum (P) harmala* (seed). Plants were selected from District Jhang of Pakistan and got authenticated from an expert in the Department of Botany, University of Agriculture, and Faisalabad Pakistan.

Extraction of Plant Materials

Plants were dried in dry and cold place to prepare methanolic aqueous extracts by following Tabassum *et al*. (2008). Briefly, dried powdered plant material was extracted with methanol-aqueous (70:30) solvent for about 72 h. After filtration, new solvents were added and the above said procedure was repeated for three times. Crude extract was obtained after the evaporation of solvent in rotary evaporator.

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Modified Larval Immersion Test

Rhipicephalis (R) microplus engorged female ticks were collected from infested cattle. Ticks were washed and then incubated for egg laying at 90% relative humidity (RH) and 27°C in incubator (Sindhu *et al.,* 2010).

Bioassay

Modified Larval Immersion test (syringe method) was used to investigate acaricidal effect of plant extracts against *R. microplus* under laboratory conditions. Approximately 200 eggs were placed in syringe and open end was closed with nylon gauze having double layer. For egg hatching syringes were incubated at 90% RH and 27°C. After 14 days of egg hatching, larvae were used for bioassay.

Stock solution of desired concentration (w/v) from each extract (Plant (P) 1, P2, P3, P1×2, P1×3, P2×3 and P1×2×3) was prepared in 0.2% solution of TritonX-100 and subsequently five dilutions of stock solution were prepared. Two ml of test solution was sucked inside the syringe (containing 12-14 day old larvae) and syringe was shaken for 30 sec to treat the larvae. After 30 sec, test solution was discarded and syringes were placed in fume hood for one hour for drying. After one hour, all the treated syringes were incubated at 27°C and 90% RH. Two sets of treatments were run in triplicate. One set was used to check mortality after 24 h of incubation and 2nd was used to check the mortality after 6 days of incubation.

Data Analyses

Data of syringe test were analyzed by probit analysis using Polo Plus (LeOra software, 2002) computer program.

Results

Anti-tick effect was observed in all the tests carried out in this study in dose dependent manner. Increasing concentrations of herbal extracts caused higher mortality of tick larvae as opposed to no or very less mortality in the control group. There was a significant reduction ($p<0.05$) in the number of ticks exposed to 50 mg/mL herbal extract compared with control. The combination of three selected plants (*C. longa, C. colocynthis* and *P. harmala*) at dose rate of 50 mg/mL showed the highest (100%) mortality; whereas, individual application of *P. harmala* @ 3.125 mg/mL showed the minimum mortality percentage.

The detailed result of various dose concentrations of different plants individually as well as in combination has been depicted in Fig. 1. Acaricidal efficacy of various concentrations of *C.longa*, *C.colocynthis*, *P.harmala* and their combinations were depicted in Fig. 2-6. After standardization of syringe test, preliminary screening of plant CEs was done against *R. microplus* by using at least five different concentrations of each CE. Initial screening of CE gives an idea about range of activity of CE against *R. microplus*. Plant extracts, which showed promising acaricidal effect against *R. microplus* were selected for evaluation of acaricidal activity following standard procedures i.e., bioassay for each plant was repeated at least for three times.

Acaricidal activity of each plant was evaluated at two different exposure times (time-mortality experiment) i.e., 24 h post exposure and 6 day post exposure. The highest activity was recorded at 6th day after application in case of three plants combination at the dose rate of 50 mg/mL, whereas lowest acaricidal efficacy was observed at 24 h with the dose rate of 3.125 mg/mL in case of individual effect of *P. harmala*.

Discussion

The *in vitro* bioassays used in this study for evaluation of acaricidal efficacy of herbal extract have been successfully carried out for initial screening of plants and their combinations. Modified larval immersion test/syringe test (Al-Rajhy *et al*., 2003; FAO, 2004; Miller *et al.*, 2007) by topical application were used for acaricidal efficacy.

Test used for investigation indicated anti-tick activity of the herbal extract (HE) alone and in combination. Mortality of tick larvae was higher ($p<0.05$) in combined herbal extract treated groups in comparisons with the individuals as well as control groups. Efficacy of extracts of plants on larvae has been investigated by other workers (Zaman *et al*., 2012) using various plants. However, variation has been recorded in the different concentrations of herbal extracts and time taken for exerting toxic effects on larvae in various studies. Dipeolu and Ndungu (1991) have reported anti-tick activity of *Nicotiana tabacum* leaves in Kenya. Larvae of *R. appendiculatus* were killed on the ears of calves within 24 h.

Exposure of *R. microplus* larvae to oleoresinous extract (oleoresin) from the copaiba tree, i.e. *Copaifera reticulata* resulted in larval mortality 24 h after treatment (Fernandes and Freitas, 2007; Ribeiro *et al.,* 2007) have demonstrated 11.7 to 14.7% with the hexane extract of *Calea serrata* (Ribeiro *et al*., 2007; Ferrarini *et al*., 2008) have demonstrated lethal effects of Limonene, limonene oxide and eight b-amino alcohol derivatives @ 10 and 2.5 mg/mL on larvae of *R. microplus,* respectively.

This can only be speculated that how herbal extract does gets in to the body of tick unless proved through experiments. However, the logical assumption would be that the phytochemicals of herbal extract get absorbed in the skin surrounding the site of tick attachment. The phytochemicals may thus interfere with the feeding of tick and/or cause their paralysis. Herbal extract was rubbed twice a day for six days on and around the sites of tick attachments; therefore, it should be sufficient time for absorption of phytochemicals in the skin in the concentrations to interfere with tick feeding and/or cause their paralysis.

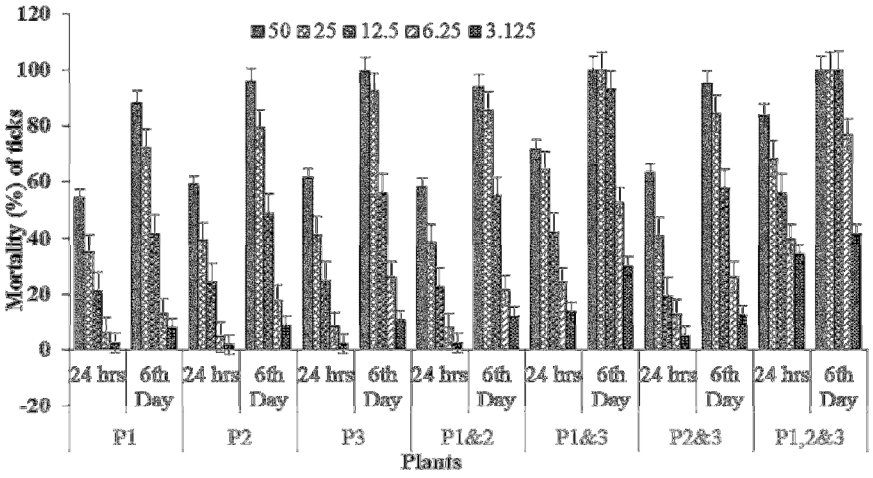

Fig. 1: Acaricidal efficacy of various dose concentrations of different plant extracts collected from district Jhang.

P1= *Curcuma longa;* P2= *Citrullus colocynthis* and P3= *Peganum harmala,* Dose concentrations (mg/ml) = 50, 25, 12.5, 6.25 and 3.125 (shown in the legend)

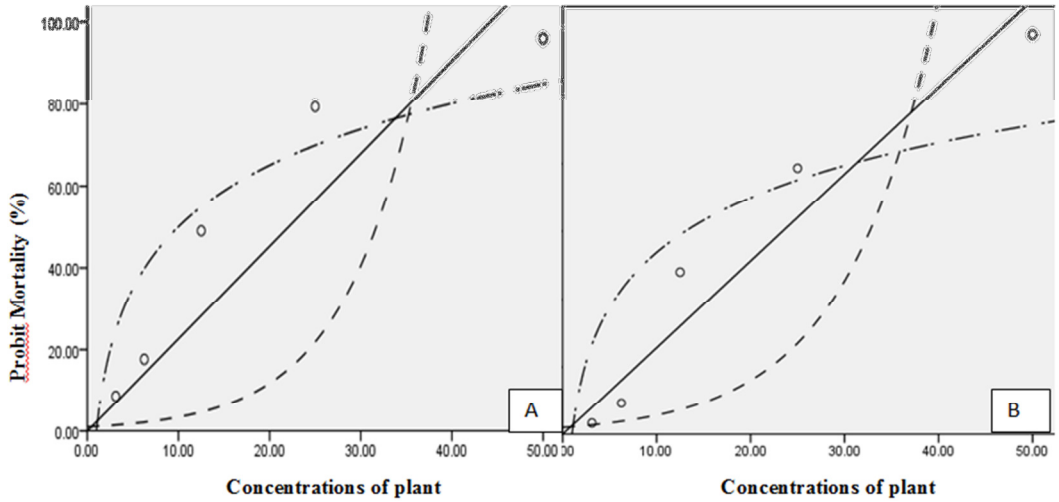

Fig. 2: Probit mortality x log concentration plot from *R. microplus* subjected to larval immersion test with aqueous methanol extract of *Curcuma longa*, diluted in 0.2% Triton X-100

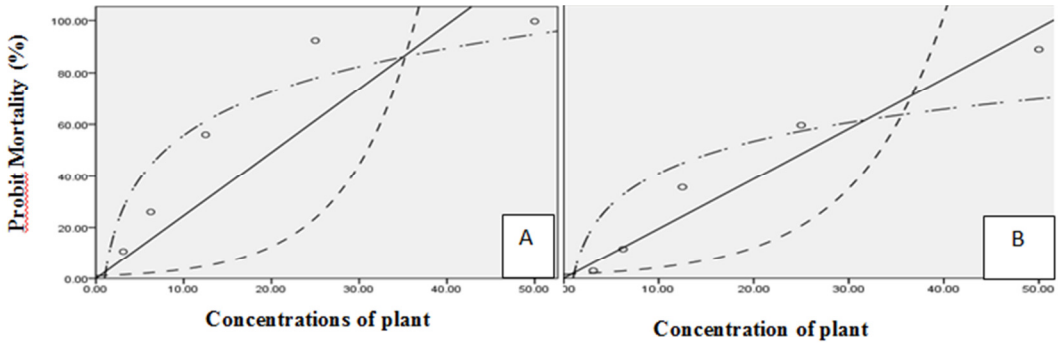

Fig. 3: Probit mortality x log concentration plot from *R. microplus* subjected to larval immersion test with aqueous ethanol extract of *Peganum harmala*, diluted in 0.2% Triton X-100

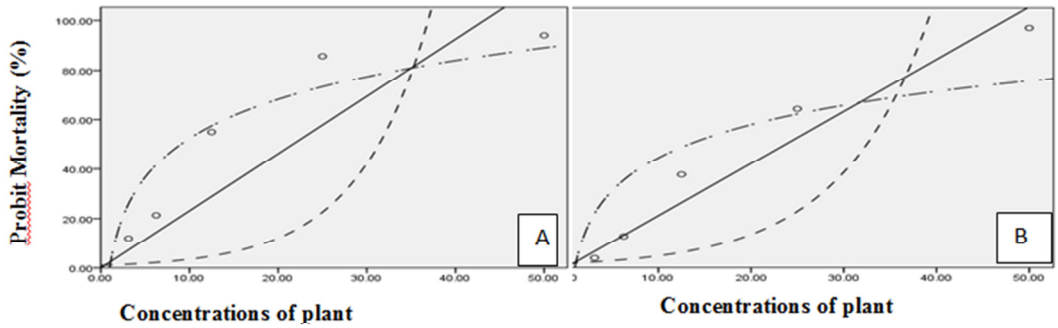

Fig. 4: Probit mortality x log concentration plot from R. Microplus subjected to larval immersion test with aqueous methanol extract of *Curcuma longa and Citrullus colocynthis*, diluted in 0.2% Triton X-100

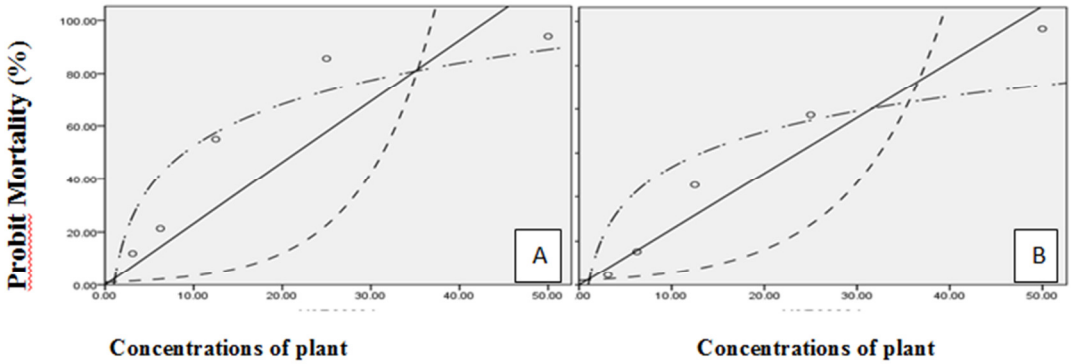

Fig. 5: Probit mortality x log concentration plot from R. Microplus subjected to larval immersion test with aqueous methanol extract of *Curcuma longa* and *Peganum harmala*, diluted in 0.2% Triton X-100

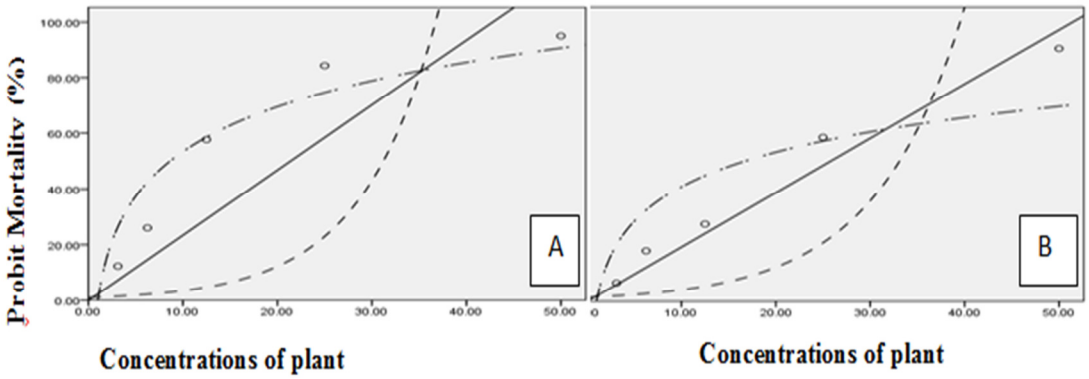

Fig. 6: Probit mortality x log concentration plot from R. Microplus subjected to larval immersion test with aqueous methanol extract of *Citrullus colocynthis and Peganum harmala*, diluted in 0.2% Triton X-100

Conclusion

Crude aqueous- methanol extracts (CAME) of the three plants and their combination (*C. longa, Ct. colocynthis,* and *P. harmala*) were found effective against (*R. microplus*) larvae of ticks. Optimum geo climate, which favors the growth of plants in Pakistan. Therefore, there is lot of opportunity in testing plants for its acaricidal effects.

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