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Contrasting effects of ethanolic extracts of leaf and flower of *Chromolaena odorata* against *Rhipicephalus (Boophilus) annulatus*

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ABSTRACT

In the present study, ethanolic extracts of leaves and flowers of *Chromolaena odorata* were compared at different dilutions (6.2 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL and 100 mg/mL) for their efficacy against ticks. Per cent adult mortality, inhibition of fecundity and hatching of laid ova were studied. Leaf extract did not cause any adult tick mortality. The negative values for the per cent inhibition of fecundity observed with the leaf extract indicated that it promoted egg laying. On the contrary, the ethanolic extract of flowers at 10% concentration caused 62% adult tick mortality and 80%inhibition of fecundity. However, both extracts did not produce any change in the hatching of the laid ova by treated ticks.

Key words: *Chromolaena odorata*, Egg production, Fecundity, Flower, Leaf, Mortality, *Rhipicephalus* (*Boophilus*) *annulatus*

Many plant extracts with promising acaricidal properties are found in literature (Ribeiro *et al.* 2008, Magadum *et al.* 2009, Fernadez–Salas *et al.* 2011, Pirali-Kheirabadi *et al*. 2011, Juliet *et al*. 2012, Ravindran *et al*. 2012, Sunil *et al*. 2013). Herbal extract with the property of eclosion blocking was also reported (Ravindran *et al*. 2011).

Chromolaena odorata, a weed, belonging to the family Asteraceae is largely exploited by traditional herbalists for its biological activities. Fresh leaves and extract of *C. odorata* are used in traditional herbal treatment in developing countries for burns, soft tissue wounds and skin infections (Phan *et al*. 2001). Previous reports on phytochemical analysis of the ethanolic extract of the aerial parts of *C. odorata* revealed the presence of steroids, triterpenes, alkaloids, flavanoids, tannins, diterpenes, glycosides, lactone and saponins. Phan *et al.* (2001) reported the presence of high amount of phenolic compounds in crude ethanolic fresh leaf extract of *C. odorata* responsible for the haemostatic, antioxidant and anti-inflammatory effects. The high content of phenolic compounds in the chloroform extract of *C. odorata* leaves were capable of inhibiting and quenching free radicals to terminate the radical chain reactions (Rao *et al*. 2010). Flavanoids were found linked with the analgesic, anti-inflammatory and antipyretic activity of ethanolic extract of *C. odorata* (Owoyele *et al*. 2008).

Panda *et al*. (2010) reported the anthelmintic and antimicrobial activities of the leaves of the plant. Anthelmintic property of ethanolic extract of the whole plant was reportedly mainly due to the presence of polyphenolic compounds and tannins (Patel *et al*. 2010). Bouda *et al.* (2001) studied the insecticidal effect of essential oil of *C. odorata* leaves on the maize grain weevil, *Sitophilus zeamis*. Matur and Davou (2007) reported larvicidal property of leaf extract of *C. odorata* against *Simulium*.

Available records did not reveal any acaricidal activity of this plant especially against ticks. *Rhipicephalus (Boophilus) annulatus* is reported as the commonest tick species in south India (Jagannath *et al*. 1979, Rajamohanan 1982, Koshy *et al*. 1982, Singh *et al.* 2000). Hence, in the present study, ethanolic extract of leaves and flowers of *C. odorata* (Linn) were investigated for their effects against adult female *R. (B.) annulatus* ticks.

MATERIALS AND METHODS

Plant material: The plant materials were collected from Pookode, Vythiri taluk of Wayanad district, Kerala. The

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plant was identified by a botanist. The voucher specimen was deposited at Calicut University herbarium (CALI, Accession number: 6629), Calicut, Kerala.

Preparation of plant extract: The leaves and flowers of the plant were dried separately in shade at room temperature. Dried plant parts were finely pulverized using a grinder. The powdered plant material (100 g) was used for ethanolic extraction in a soxhlet extraction apparatus attached with rotary vacuum evaporator. Solvent was completely removed by drying at room temperature. Required quantity of the extract was weighed and dissolved in methanol for making 5 different dilutions, viz. 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.2 mg/mL.

Ticks: Fully engorged adult *R. (B.) annulatus* female ticks were collected from infested animals, washed with water and dried using tissue paper. These ticks were used for adult immersion test.

Adult immersion test (AIT): Various dilutions (6.2–100 mg/mL) of ethanolic extracts of *C. odorata* were tested using adult immersion test (Drummond *et al*. 1973, Food and Agricultural Organization 2004). In the experiment, 288 ticks were divided into 4 replicates of 6 numbers of ticks for each dilution of the extract. Group of 6 ticks was weighed prior to the experiment and they were immersed for 2 min in the respective dilution (10 mL) in a 50 mL beaker with gentle agitations. Methanol was used as control. Ticks were recovered from the solution, dried using tissue paper towels and placed in separate plastic specimen tubes (25 × 50 mm). The tubes were incubated at 28°C and 80% relative humidity in a BOD incubator.

Adult tick mortality: The specimen tubes were observed. The ticks died within 15 days after treatment were identified. The per cent adult tick mortality was calculated in comparison to the control.

Per cent inhibition of fecundity and hatching: The eggs laid by the ticks were collected, weighed and observed at the same condition of incubation for the next 30 days for visual estimation of hatching. Ticks under different treatments were compared with that of the control.

The percentage inhibition of fecundity was calculated as follows:

Index of fecundity/egg laying (IE) = weight of eggs laid (mg) / weight of females (mg)

Percentage inhibition of fecundity (IF) = [IE (control group) - IE (treated group)] × 100 / IE (control group)

Statistical analysis: All the data were expressed as the mean±SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. Duncan's test was used for post-hoc analysis. A value of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The efficacy of extracts against adult *R. (B.) annulatus* females was assessed by measuring per cent adult mortality,

Table 1. Effects of ethanolic extract of leaves of *C. odorata* against *R. (B.) annulatus*

Acaricide	Mean ticks weight per replicate ±SEM(g)	Mean % adult mortality within 15 days±SEM	Mean eggs mass per replicate± SEM (g)	Index of fecundity ±SEM	Percentage inhibition of fecundity (%)	Hatching % (visual)
Methanol (control)	1.0970±0.0009^a	0±0^a	0.3858±0.014^a	0.3517±0.013^a	0	100
6.2 mg/mL	1.0949±0.0011^a	0±0^a	0.4326±0.023^a	0.3952±0.021^a	–9.3	100
12.5 mg/mL	1.0962±0.0004^a	0±0^a	0.4313±0.028^a	0.3943±0.025^a	–10.52	100
25 mg/mL	1.0974±0.0033^a	0±0^a	0.4321±0.027^a	0.3939±0.026^a	–12	100
50 mg/mL	1.0938±0.0004^a	0±0^a	0.4261±0.013^a	0.3887±0.012^a	–12.11	100
100 mg/mL	1.0945±0.0008^a	0±0^a	0.4209±0.023^a	0.3844±0.020^a	–12.37	100

N=4, Values are mean±SEM, means bearing different superscripts a, b or c ($P<0.05$), indicate significant difference when compared with the control and recommended concentration of ethanolic extract of *C. odorata* leaf.

Table 2. Effects of ethanolic extract of flowers of *C. odorata* against *R. (B.) annulatus*

Acaricide	Mean ticks weight per replicate ±SEM(g)	Mean% adult mortality within 15 days±SEM	Mean eggs mass per replicate± SEM (g)	Index of fecundity ±SEM	Percentage inhibition of fecundity (%)	Hatching % (visual)
Methanol (control)	1.2155±0.001^a	0±0^a	0.3978±0.015^c	0.3273±0.012^c	0	100
6.2 mg/mL	1.2144±0.0.001^a	0±0^a	0.2484±0.014^b	0.2046±0.011^b	37.49	100
12.5 mg/mL	1.2148±0.0009^a	0±0^a	0.2148±0.045^b	0.1769±0.037^b	45.95	100
25 mg/mL	1.2150±0.001^a	0±0^a	0.1935±0.026^b	0.1593±0.021^b	51.33	100
50 mg/mL	1.2156±0.001^a	4.165±4.16^a	0.1763±0.018^b	0.1451±0.015^b	55.67	100
100 mg/mL	1.2150±0.001^a	62.49±7.97^b	0.076±0.037^a	0.063±0.031^a	80.66	80

N=4, values are mean±SEM, means bearing different superscripts a, b or c ($P<0.05$), indicate significant difference when compared with the control and recommended concentration of *C. odorata* flower.

Table 3. Dose response data of ethanolic extract of flowers of *C. odorata* against *R. (B.) annulatus*

Variables	Slope±SE	R^2	P value	LC_{50}
Mortality	1.703±0.7947	0.6047	0.1216	19%
Egg mass	–0.02285±0.009109	0.6113	0.0662	
IF	–0.01880±0.007489	0.6118	0.0660	
% IF	5.744±2.288	0.6117	0.0660	

Table 4. The pattern of mortality of *R. (B.) annulatus* after treatment with various dilutions of ethanolic extract of flowers of *C. odorata*

Ethanolic extract of flowers of *C. odorata*	Number of ticks	Day 1	Day 5	Day 7	Day 15	Total mortality
100 mg/mL	24	0	0	9	6	15
50 mg/mL	24	0	0	1	0	1
25 mg/ mL	24	0	0	0	0	0
12.5 mg/mL	24	0	0	0	0	0
6.2 mg/mL	24	0	0	0	0	0

inhibition of fecundity and hatching rate. The ethanolic extract of leaves of *C. odorata* did not produce any adult tick mortality (Table 1).

Surprisingly, the survived ticks exhibited a higher egg laying response. The percentage of inhibition of egg laying observed in AIT was of negative value; indicating the increase in egg mass produced by the extract treated ticks compared to untreated ones. However, hatchability of these eggs was not affected.

In contrast, the ethanolic extract of flowers of *C. odorata* produced 62.49% mortality when ticks were treated at 10% concentration (Tables 2, 3).

The mortality (Table 4) observed with ethanolic extract of flowers of *C. odorata* was not an immediate process as it occurred during 7 to 15 days after treatment. The inhibition of fecundity ranging from 37 to 80% was observed in the extract treated ticks. The hatching of the laid ova was not much affected with flower extract too.

In recent years, use of environment-friendly and biodegradable natural insecticides of plant origin has received importance for vector control (Thomas *et al*. 1994). The toxic phytochemicals that primarily constitute the secondary metabolites synthesized as a result of aberrant biosynthetic pathway affect the nerve axon and synapses, muscles, respiration and behaviour of insects (Klocke 1989). Though many plants possess insecticidal properties, only few demonstrated ideal acaricidal activity (Prakash and Rao 1997).

C. odorata, a common weed, is abundant in the south Indian state of Kerala. The present study reports that acaricidal activity of the plant lies with the ethanolic extract of flowers. N-oxide of 5 pyrrolizidine alkaloids (PAs): 7 and 9 angeloyl retronecines, intermedine, rinderine and 3´acetyl rinderine were previously reported from *C. odorata* (Biller *et al*. 1994)**.** Their highest concentration occur in roots and mature flower heads, while leaves and stems are almost devoid of alkaloids and no pyrrolizidine alkaloids are present in nectar (Biller *et al.* 1994). Thus, the acaricidal activity observed in the flower extract may be attributed to the pyrrolizidine alkaloids present in the flowers. However, there were reports on the acquisition of pyrrolizidine alkaloids from *C. odorata* by grasshoppers of genus *Zonocerus,* improving their population dynamics (Fischer and Boppre 1997). Pyrrolizidine alkaloid of *Senecio jacobaea* (Macel and Vrieling 2003) and henanthroindolizidine alkaloid of *Tylophora tanakae* (Honda *et al.* 2008) were reported for their oviposition stimulatory activity in cinnabar moth and danaid butterfly, respectively**.** Hence, the 'cidal' effects of the flower extract could not be conclusively attributed to the pyrrolizidine alkaloids. Several studies revealed that the terpenoids (Riberio *et al*. 2007, Magadum *et al*. 2009**)** and tannins (Fernadez-Salas *et al.* 2011) present in various plant extracts contributed to the acaricidal effects against *R. (B.) microplus*.

In the present study, the ethanolic extract of leaves of the plant recorded absence of any acaricidal activity. However, it promoted the development of acarines. The negative value of inhibition of fecundity indicated its property of stimulation for oviposition. Increase in egg mass production in the treated female ticks compared to the control (untreated) ticks observed in this study could be attributed to the activity of volatile essential oil obtained from *C. odorata,* which can increase activity of prostaglandin-H synthase, the enzyme which catalyzes the biotransformation of arachidonic acid to prostaglandin G_2 (PGG_2), thereby increasing cyclooxygenase (COX) function (Bedi *et al.* 2010). The prostaglandin biosynthesis in insects is roughly similar to the biosynthetic pathways known from mammals (Stanley 2006). The COX pathway involves three enzymatic steps. In the first step, arachidonic acid is converted to unstable PGG_2 which is catalysed by Prostaglandin–H synthase. Later, a hydroperoxidase reduces PGG_2 to more stable PGH_2. The PGH_2 serve as the substrate for biosynthesis of PGs, including PGE_2, thromboxane B_2, PGD_2, PGI_2 and $PGF_2\alpha$. The presence of PGE_2 in *R. (B.) annulatus* (Higgs et al. 1976), PGE_2 and $PGF_2\alpha$ in *Hyalomma anatolicum* (Shemesh *et al.* 1979), PGE_2 and 6-keto $PGF_1\alpha$ (a stable metabolic product of PGI_2) in *Ixodes dammini* (Reibeiro *et al*. 1988) and PGE_2, $PGF_2\alpha$, PGD_2 and PGA_2 / PGB_2 in *Amblyomma americanum* (Ribeiro *et al.* 1992, Bowman *et al*. 1995, Pedibhotla *et al*. 1995, 1997)

were reported earlier. Eicosanoids play a major role in increased egg laying behavior in some species of insects and that they may act at the cellular level for influencing ovarian protein uptake in many species (Stanley 2006). Medeiros *et al.* (2002) showed that eicosanoids modulate ovarian uptake of a specific yolk protein, *Rhodnius* haeme binding protein (RHBP). PGE_2 also plays a very important role in peristaltic activity of oviduct to transport the oocytes to the genital aperture (Stanley 2006).

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