Tropical and Subtropical Agroecosystems, 12 (2010): 417 - 422

Tropical and Subtropical Agroecosystems

SHORT NOTE [NOTA CORTA]

SCREENING OF THE ACARICIDAL EFFICACY OF PHYTOCHEMICAL EXTRACTS ON THE CATTLE TICK *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae) BY LARVAL IMMERSION TEST

[EVALUACION DE LA EFICACIA ACARICIDA DE EXTRACTOS FITOQUIMICOS CONTRA LA GARRAPATA BOVINA *Rhipicephalus* (*Boophilus*) *microplus* (Acari: ixodidae) MEDIANTE LA PRUEBA DE INMERSION LARVAL]

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SUMMARY

The objective of the study was to determine the acaricidal efficacy of selected native plants from Yucatán, Mexico on acaricide resistant larvae of *Rhipicephalus* (*Boophilus*) *microplus*. Methanolic extracts from roots, leaves, stems, and stem barks of 15 plants were tested using the modified larval immersion test. A final concentration of 10% (100 mg/ml) of plant crude-extract was used. The percentage mortality from different plants and extracts were: *Petiveria alliacea* leaves (95.7±2.9 %) and stems (99.2±0.5 %)*; Diospyros anisandra* leaves (87.9±8.6 %) and stem bark (98.8±1.0 %); *Havardia albicans* leaves (93.0±12.0 %), *Caesalpinia gaumeri* (90.1±4.8 %) and *Capraria biflora* (86.6±9.9 %), stems of *Solanum tridinamum* (98.0±1.7 %) and *Solanum erianthum* (97.8±1.8 %), stem bark of *Bursera simaruba* (99.1±0.7 %) and *Casservia corymbosa* (99.5±0.5 %)*;* and the root of *Ocimum micrantun* (87.0±3.2 %). We concluded that plants from Yucatan, Mexico showed a high acaricidal efficacy that could be used to control *R.* (*B.*) *microplus* acaricide resistant larvae. *P. alliacea*, *Havardia albicans* and *Caesalpinia gaumeri* were of the most encouraging plants to be used as an acaricide. Further studies are needed to evaluate these plants on adult ticks (*in vivo* conditions) and to identify the active compound(s) on *R.* (*B.*) *microplus.*

Key words: Acaricidal efficacy, plant crude-extracts, *Petiveria alliacea*, *Diospyros anisandra*, *Havardia albicans*, *Caesalpinia gaumeri.*

RESUMEN

Se determinó la eficacia acaricida de 15 plantas nativas de Yucatán, México, empleando larvas de *Rhipicephalus* (*Boophilus*) *microplus* resistentes a acaricidas, mediante el uso de la prueba de inmersion larval. Se evaluaron extractos metanolicos de raíces, hojas, tallos y cortezas a una concentración del 10% (100mg/ml). Las mortalidades obtenidas fueron: *Petiveria alliacea*, 95.7±2.9 y 99.2±0.5 % hojas y tallos respectivamente*; Diospyros anisandra* 87.9±8.6 y 98.8±1.0 % hojas y corteza respectivamente; hojas de *Havardia albicans* (93.0±12.0 %), *Caesalpinia gaumeri* (90.1±4.8 %) y *Capraria biflora* (86.6±9.9 %), tallos de *Solanum tridinamum* (98.0±1.7 %) y *Solanum erianthum* (97.8±1.8 %), corteza de *Bursera simaruba* (99.1±0.7 %) y *Casseaeria corymbosa* (99.5±0.5 %)*;* y la raíz de *Ocimum micrantun* (87.0±3.2 %). Se concluyó que las plantas evaluadas tuvieron una alta eficacia acaricida y podrían ser empleadas en el control de larvas de *R.* (*B.*) *microplus* resistentes a acaricidas. *P. alliacea*, *Havardia albicans* y *Caesalpinia gaumeri* fueron las plantas con mejores resultados. Se requieren studios adicionales para evaluar las plantas en garrapatas adultas y en condiciones *in vivo*, así como la identificación de los compuestos activos.

Palabras clave: Eficacia acaricida, extractos crudo metanolicos, *Petiveria alliacea*, *Diospyros anisandra*, *Havardia albicans*, *Caesalpinia gaumeri.*

INTRODUCTION

Rhipicephalus (*Boophilus*) *microplus* (Acari: ixodidae) is an endemic ectoparasite of cattle in tropical and subtropical regions of the world, causing major economic losses to cattle producers directly through feeding on cattle and indirectly by transmitting several blood borne diseases pathogens to the host (*Babesia bovis, Babesia bigemina* and *Anaplasma marginale*) (Solorio *et al*., 1999; Rodriguez-Vivas *et al*., 2004).

Chemical acaricides such as synthetic pyrethroids (SP), organophosphates (OP) and amitraz (Am) have played a pivotal role in the control of *R.* (*B.*) *microplus*. However, as a consequence of intensive use of chemicals this tick specie has developed resistance to the major classes of acaricides in different countries. In Mexico, tick resistance to acaricide is recognized in several states mainly in the Mexican tropics (Rodriguez-Vivas *et al*., 2007). Development of resistance to commercial acaricides by *R.* (*B.*) *microplus* has stimulated the search for new control strategies.

Commercial acaricides are usually toxic to humans and leave residues in the environment. However, acaricide from plants are usually of low toxicity to mammals, water soluble, producing non-residual effects and have a low incidence of developing resistant strains (Chungsamarnyart *et al*., 1988, 1991). The study of the native flora from the Peninsula of Yucatan, Mexico has shown to be an important source of active metabolites against ticks. Some studies related to the flora of the Yucatan peninsula are based on botanical and ethnobotanical knowledge, and only few of them have been studied phytochemically or evaluated for their biological activity (Sánchez Medina *et al*, 2001a, 2001b). However, there are not studies in Yucatan, Mexico of native plants with acaricide activity. Therefore, the objective of the present study was to evaluate the acaricidal efficacy of 45 plant crude-extracts on *R.* (*B.*) *microplus* larvae resistant to OP, SP and Am acaricides.

MATERIAL AND METHODS

Study background

The study was conducted in Yucatan, Mexico, located between 19º 30' and 21º 35' north latitude and 90º 24' west longitude of the Greenwich meridian. Climate is generally sub-humid tropical with two seasons: rainy (June to October) and dry (November to May). The monthly maximum temperature varies from 35 ºC to 40 ºC (mean 26.6 ºC). The relative humidity (RH) varies from 65 to 100% (mean 80%) and the annual rainfall varies from 415 mm to 1290 mm depending on the area (INEGI, 1996).

Biological material management and sampling

Plants: Plants were selected by searching in the literature from journals, books, computer database surveys, and ethnobotanic interviews on plants with acaricide or insecticide properties (Mendieta and Del Amo, 1981; Durán *et al*., 1998). A list of 15 plants were collected in the Yucatan state in the period of February-October 2007; voucher specimens were authenticated and deposited at the herbarium Centro de Investigación Científica de Yucatán (CICY) under the following code numbers: *Petiveria alliacea* L (Phytolaccaceae, MMendez 1417); *Diospyros anisandra* S.F Blake (Ebenaceae, MMendez 1438); *Havardia albicans (*Kunth) Britton & Rose (Fabaceae, MMendez 1429); *Caesalpinia gaumeri* Greenm. (Fabaceae, MMendez 1435); *Capraria biflora* L. (Scrophulariaceae, MMendez 1410); *Solanum tridynamum* Dunal. (Solanaceae, MMendez 1434); *Solanum erianthum* D. Don. (Solanaceae, MMendez 1425); *Bursera simaruba* L. Sarg. (Burseraceae, MMendez 1437); *Spondias purpurea* L. (Anacardiaceae, MMendez 1443); *Ocimum micranthum* Willd. (Labiatae, MMendez 1433); *Parthenium hysterophorus* L. (Asteraceae, MMendez 1431); *Casearia corymbosa* Kunth. (Flacurtiaceae, MMendez 1448); *Clusia flava* Jacq. (Clusiaceae, MMendez 1436); *Sapindus saponaria* L. (Sapindaceae, MMendez 1444) and *Tabebuia guayacan* (Seem.) (Bignoniaceae, MMendez 1449).

Roots, leaves, stems (for bushes) and stem barks (for trees) from each plant collected (15 plants) were dried at 40 °C for 72 h and ground in a mill with a 5 mm diameter mesh. The ground material was immersed in 100% methanol (absolute methanol) for 72 h (100ml of methanol and 83g of ground material). The methanolic extraction was filtered and evaporated at 45 ºC by a vacuum rotational evaporator. The 45 plant crude-extracts obtained (three extracts/plant) were transferred to glass vials and kept at 4 ^{o}C until use (Borges-Argáez *et al*., 2007).

Engorged females ticks from a farm on which ticks were previously diagnosed resistant to three acaricide families (SP, OP and Am) (Rodriguez-Vivas *et al*., 2006), 100-200 *R. (B.) microplus* were collected from at least 20 cattle. The engorged females were placed into small plastic boxes with air holes and transported to the Parasitology laboratory at the College of Veterinary Sciences (FMVZ-UADY). Upon arrival, engorged adult females were placed on Petri dishes and incubated at laboratory conditions, at 27±1.5 °C and a relative humidity of 85%-86% (Cen *et al*., 1998). After oviposition, eggs were transferred into 10 ml glass vials with a cotton cap. Hatching of larvae occurred approximately 30 days after collection from engorged females. Larvae of 7-14 day-old were used for bioassays.

Bioassays

The modified larval immersion test (Soberanes *et al*., 2002) was used to test the acaricide efficacy of 45 plant crude-extracts against *R. (B.) microplus* larvae. Tween-20 was diluted in distilled water at 2% concentration and it was used to dilute plant crude-extracts (100mg/ml final concentration) (Rosado-Aguilar *et al*., 2008). The diluted plant crude-extract (3 ml) was transferred in Petri dishes (60 x 15 mm in diameter), and ~300-500 larvae were placed between two Whatman No. 1 papers and immersed for 10 minutes. Three replicates from each plant crude-extract and a control containing Tween-20 (2%) were used. Approximately 100 larvae from the treated and control were transferred to clean filter paper packets, and kept for 48 h in an incubator (27±1.5°C, 80-90% relative humidity). The numbers of live and dead larvae were recorded. The mortality (LM) was calculated using the corrected mortality (CM) formula (Abbott, 1925).

$$CM = \frac{\text{\% Treated LM} - \text{\% Controls LM}}{100 - \text{\% Controls LM}} \times 100$$

Acaricidal efficacy was classified according to the corrected mortalities as follows: High (H) efficacy (86-100% mortality); relatively high (RH) efficacy (71-85% mortality); moderate (M) efficacy (56-70% mortality); low (L) efficacy (31-55% mortality); and non significant (NS) efficacy (0-30% mortality) (Chungsamarnyart *et al*., 1991).

RESULTS

The results of screening the acaricidal efficacy of 45 plant crude-extracts showed that 12 extracts belonged to the high activity group, 7 extracts to the relatively high activity group, 2 extracts to the moderate activity group,9 extracts to the low activity group and 15 extracts belonged to the non significant activity group (Table 1). The high acaricide activity extracts were the leaves of *Petiveria alliacea* (95.7±2.9%), *Havardia albicans* (93.0±12.0%), *Caesalpinia gaumeri* (90.1±4.8%) *Diospyros anisandra* (87.9±8.6%), , and *Capraria biflora* (86.6±9.9 %); the stems of *Petiveria alliacea* (99.2±0.5%), *Solanum tridynamum* (98.0±1.7%) and *Solanum erianthum* (97.8±1.8%)*;* the stem barks of *Casearia corymbosa* (99.5±0.5%), *Bursera simaruba* (99.1±0.7%) and *Diospyros anisandra* (98.8±1.0%) and roots of *Ocimum micranthum* (87.0±3.2%).

DISCUSSION

In the last two decades, plants extract have been widely used against phytophagous pests and mosquitoes (Balandrin *et al*., 1995; Chavan and Nikam, 1998), because some of them are selective and have little or no harmful effects on non-target organism (Valladares *et al*., 2003). Additionally, some plant extracts or phytochemicals are found to be highly effective against insecticide-resistant insect pests (Lindquist *et al*., 1990; Ahn *et al*., 1997). They must be applied to hiding places or host animals in the same way as other conventional acaricides (Kim *et al*., 2004).

This study was performed to evaluate 45 plant crude-extracts against larvae from the cattle tick, *R.* (*B.*) *microplus* resistant to OP, SP and Am. The results from bioassay on *R.* (*B.*) *microplus* larvae, indicated that 12 plant crude-extracts (belonging to 10 plants) have high (86.0-99.5%) acaricidal efficacy. From this plant group, four showed higher efficacy *P. alliacea*, *D. anisandra*, *H. albicans* and *C. gaumeri.* However, Borges-Argaez *et al.* (2007) reported that *D. anisandra* is toxic to animals when used topically.

In the present study leaves of *P. alliacea*, *H. albicans* and *C. gaumeri* showed >90% efficacy to control *R.* (*B.*) *microplus* larvae. Leaves are abundant material in these plants and can be regenerated in a short period of time. In this study, leaves were the plant component that showed highest efficacy. This result is in agreement with Chungsamarnyart *et al.* (1991) who evaluated 151 plants and found that leaves were the more active component. Valladares *et al.* (2003) mentioned that leaves are more exposed to insect and as a response produce higher concentrations of metabolites with potential insecticidal effect. From the 15 studied plants, *Petiveria alliacea* is the only one that has been previously studied for acaricidal efficacy. Lyndon *et al.* (1999) has revealed a potential acaricidal efficacy on *R.* (*B.*) *microplus* adults due to an active compound from the root (Dibenzil-trisulphide). Root of *P. alliacea* only showed moderate efficacy (59.6%); however, crude-extract from leaves (95.7%) and stems (99.2%) showed higher efficacy on larvae. These finding showed that *P. alliacea*, *H. albicans* and *C. gaumeri* were of the most encouraging plants to be used as an acaricide. Further studies are needed to evaluate these plants on adult ticks and *in vivo* conditions.

Table 1. Acaricidal efficacy of 15 plant crude-extracts on *Rhipicephalus* (*Boophilus*) *microplus* larvae.

	Corrected mortality (Mean, %)			
Scientific name (and mayan name)	Leaves	Stems	Stem bark	Roots
Petiveria alliacea L. (Paiché)	95.7±2.9 (H)	99.2±0.5 (H)	ND	59.6±7.1 (M)
*Havardia albicans (*Kunth) Britton & Rose (Chukum)	93.0±12.0 (H)	ND	74.8±29.9 (RH)	24.0±15.7 (NS)
Caesalpinia gaumeri Greenm. (Kitinché)	90.1±4.8 (H)	ND	13.4±2.8 (NS)	14.5±3.4 (NS)
Diospyros anisandra S.F Blake (Kakal Ché)	87.9±8.6 (H)	ND	98.8±1.0 (H)	52.2±14.8 (L)
Capraria biflora L. (Chech kitam)	86.6±9.9 (H)	78.8±10.5 (RH)	ND	62.1±23.0 (M)
Solanum tridynamum Dunal *(*Put balam)	80.7±8.2 (RH)	98.0±1.7 (H)	ND	32.1±17.6 (L)
Bursera simaruba L. Sarg. (Chacá)	73.7±8.1 (RH)	ND	99.1±0.7 (H)	13.2±4.2 (L)
Solanum erianthum D. Don (U kuch)	72.6±8.5 (RH)	97.8±1.8 (H)	ND	39.7±9.1 (L)
Spondias purpurea L. (Chi´abal)	33.1±13.2 (L)	ND	40.6±10.7 (L)	11.6±3.5 (L)
Ocimum micranthum Willd. (X-kakaltún)	31.8±11.7 (L)	22.2±7.3 (NS)	ND	87.0±3.2 (H)
Parthenium hysterophorus L. (Chemisa)	15.2±5.7 (NS)	04.8±1.3 (NS)	ND	84.7±19.2 (RH)
Casearia corymbosa Kunth (Xi´imché)	11.0± 2.3 (NS)	ND	99.5±0.4 (H)	72.7±10.2 (RH)
Clusia flava Jacq. (Chunuup)	08.9±5.1 (NS)	ND	44.9±10.1 (L)	26.1±4.4 (NS)
Sapindus saponaria L. (Ts´ibuul)	1.5±0.3 (NS)	ND	5.5± 1.3 (NS)	0 (NS)
Tabebuia guayacan (Seem.) Hemsl. (Jajawché)	0 (NS)	ND	20.3±6.3 (NS)	14.9±2.4 (NS)

Mean (%±SD) of corrected mortality of 3 replications.
Activity classification (mean % of mortality of tick larvae at 48 h); H (high), mortality 86-100%; RH (relatively high), mortality 71-85%; M (moderate), mortality 56-70%; L (low), mortality 31-55%; and NS (non significant activity), mortality 0-30%.
ND: Not Determined.

Plant crude-extracts of *Diospyros mollis* (leaves), *Caesalpinia pulcherrima* (flowers) and *Ocimum sanctum* (leaves-stems) were studied by Chungsamarnyart *et al*. (1988, 1991) and found that *O. sanctum* is the only crude-extract that showed high acaricidal efficacy on *R.* (*B.*) *microplus* larvae (88.9%). In our study, plants belonging to the same genus were used, and high acaricidal efficacy was found (leaves of *D. anisandra* 87.9%, stem bark of *D. anisandra*, leaves of *C. gaumeri* 90.1%, roots of *O. micrantun* 87.0%). On the other hand, *Capraria biflora*, *Solanum tridynamum*, *Solanum erianthum*, *Bursera simaruba* and *Casearia corymbosa* showed high acaricidal efficacy in stems, stem bark and roots; however, the availability of these parts of the plant is reduced.

Acaricidal efficacy of *Diospyros anisandra*, *Havardia albicans, Caesalpinia gaumeri, Capraria biflora, Solanum tridinamum*, *Solanum erianthum, Bursera simaruba, Casearia corymbosa* and *Ocimum micranthum* have not been reported. Therefore, these plants are interesting to further bioassay on adults of *R.* (*B.*) *microplus*. The fractionation, purification and identification of the active compound should be carried out on the high acaricidal plant extracts. Further studies are in progress in order to identify these active acaricidal compounds. Likewise, the efficacy of the extracts on ticks in other life stages and *in vivo* conditions need to be evaluated.

CONCLUSIONS

It is concluded that In Yucatan State there are plants with high acaricidal efficacy that could be used in order to control *R. (B.) microplus* resistant larvae. This is the first report for acaricidal efficacy of *D. anisandra, H. albicans, C. gaumeri, C. biflora, S. tridynamum, S. erianthum, B. simaruba, C. corymbosa* and *O. micranthum*. *P. alliacea, H. albicans* and *C. gaumeri* were of the most encouraging plants to be used as an acaricide.

ACKNOWLEDGEMENTS

The authors are grateful to Fundación Produce Yucatán A.C. for their financial support to the present work (clave 31-2007-0552). We are very grateful to CONACYT for supporting Alberto Rosado Aguilar in his first year of the PhD degree at FMVZ-UADY. Special thanks to the technicians Mirbella Caceres, Alfredo Dorantes and Elena Coello for their support during sample collection and vegetal material processing.

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Submitted May 12, 2009 – Accepted August 28, 2009
Revised received November 03, 2009