Examination of the acaricidal effect of a set of colombian native plants-derived extracts against *Tetranychus urticae* Koch under laboratory conditions

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ABSTRACT

The spider mite, *Tetranychus urticae* Koch, is one of the main pests of greenhouse cut flowers. Different alternative strategies have been pursued for the management of this pest to avoid the excessive use of chemical products of high toxicological levels. Within these strategies is included the use of ethanolic and oily extracts from plants belonging to various plant families exhibiting acaricidal activity. For this reason, the objective of this work was the selection and evaluation of some botanical extracts from native plants in Colombia that can be used to manage *T. urticae*. Thus, as part of our research on bioacaricides, the direct effect of five plant extracts on the mortality and fecundity of the egg, larva, nymph and adult stages of the mite and the chemical composition of each extract was additionally determined by high performance liquid chromatography coupled with mass spectrometry (LC/MS). It was found that the test extracts showed a low efficiency on eggs, larvae and nymphs of *T. urticae*, since mortalities did not reach 50%. However, in the case of adults, the extracts of *Cnidoscolus aconitifoluis*, *Copaifera oficinalis* and *Anadenanthera peregrina* could be suggested as promissory extracts for the management of adults of *T. urticae*, since they caused mortalities greater than 60% and reduced female fecundity. The main secondary metabolites identified were flavonoids, whose presence in test extracts would be associated with the acaricidal potentiality referred to.

Keywords: spider mite, plants extracts, secondary metabolites, mortality, fecundity.

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INTRODUCTION

The rose is a main ornamental plant traded in the world. In Colombia there are approximately 7,266 hectares planted with flower crops, placing the country as the second largest exporter worldwide, generating 98,641 direct jobs and 83,533 indirect jobs. The cut rose occupies about 29.69% of the area sown in Colombia (Asocolflores, 2013).

One of the main pests affecting the rose crops is the red spider mite *Tetranychus urticae* Koch (Prostigmata: Tetranychidae) (Hoog, 2001; Larson, 1992; Asocolflores, 2013). This phytophagous mite presents a wide range of host plants (Santamaría *et al.,* 2002) distributed throughout the world (Vélez, 1997; Santamaría *et al.,* 2002). These arachnids can cause a uniform discoloration in the leaves because they suck the sap, eventually leading to whitening or yellowing and drying them. When the mite population is very high, they produce a spider web that covers the infested areas and extends from leaf to leaf, until covering the whole plant (Helley Sabelis, 1985).

Because acaricides have been used as the main control method to regulate populations of *T. urticae*, this pest has generated resistance to these products (Helley Sabelis, 1985). Due to the negative effects associated with the use of chemicals, such as environmental and commodities contamination and resistance to insecticides, the use of biological controllers such as the predators *P. persimilis* and *N. californicus* (Acari : Phytoseiidae) (Skirvin *et al.,* 2002; Oliveira *et al.*, 2007),

entomopathogenic microorgamisms and botanical extracts have increased (Alma *et al.*, 2007; Begume *et al.*, 2007; Pérez *et al.*, 2007). The use of plant extracts can be a reasonable alternative strategy for pest management, since the presence of multiple active compounds can reduce the probability of resistance (Koul and Walia, 2009), and they can be considered environmentally safer since many of them exhibit high biodegradability, breaking-down into harmless compounds after a few hours or days (Khater, 2012, Rahman *et al.,* 2016). In addition, they could be compatible with biological controllers, the main reason being they are involved in Integrated Pest Management (IPM) (Pérez *et al.*, 2007; Charleston *et al*., 2006).

This fact can be rationalized through the chemistry of naturally-occurring plant products. Thus, the analysis and search for new sources of chemical agents useful for the treatment of different pests and diseases affecting commercial crops remains as an important issue. In this sense, the botanical families Lauraceae, Rutaceae, Myristicaceae, Fabaceae, and Piperaceae have a large number of species and are present in different locations and habitats worldwide. Their members possess a broad range of secondary metabolites, which have been studied for search for bioactives of diverse nature and biological uses identified originally in traditional medicine (Garcez *et al*., 2009). Thus, as part of our research on bioacaricides, the main aim of this work was to determine the effect of ethanol extracts of five native plants of Colombia on the mortality of eggs, larvae, nymphs and adults of *T. urticae*, as well as its effect on female fecundity of this phytophagous.

MATERIALS AND METHODS

Biological material

The collection of plants used for the preparation of the ethanolic extracts was carried out in the Municipality of Agua Azul (Casanare, Colombia). These plants were identified with the collaboration of the Herbario Nacional Colombiano which belongs to the Instituto de Ciencias Naturales in Colombia. A voucher specimen of each accession was then deposited. Hence, the plants used for the present work were identified as *Cnidoscolus aconitifolius* (COL389957), *Hymenaea courbaril* (COL493607), *Bowdichia virgilioides* (COL551433), *Copaifera officinalis* (COL393135) and *Anadenanthera peregrina* (COL296745) are locally called "a*rnica*", "*algarrobo*", "*alcornoque*", "*aceite*" and "*yopo*", respectively.

The different stages of development of *T. urticae* used in the experiments were obtained from mites maintained under greenhouse conditions (25±15ºC and 73.0 ± 0.22%) in the Nueva Granada Campus (Cundinamarca, Colombia). Cohorts of eggs, larvae, nymphs and adults of the same age were obtained and employed for the evaluation of mortality and oviposition induced by the extracts.

Extraction of plant material

The collected plant material was subsequently dried, ground and subjected to extraction by maceration (for 7 days) using 96% ethanol with daily solvent removal. The mixture was concentrated under reduced pressure using a rotaevaporator affording the crude extracts.

High performance liquid chromatography – mass spectrometry analysis

The ethanol extracts were analyzed by high performance liquid chromatography coupled with mass spectrometry (LC-MS) using a Shimadzu LCMS2020 system consisting of a separation module equipped with a Detector with Photodiode Array (DPA), ionization by electrospray and a mass detector with a quadrupole analyzer. The separation of the components of each extract was carried out using a standard Premier C-18 column (4.6 mm x 150 mm, 5 µm). The flow was 0.7 mL/min, and the mobile phases were carried out with 0.005% Trifluoroacetic Acid (TFA) and Acetonitrile (ACN). 10 uL of each extract (2.5 mg / mL in absolute ethanol) was injected into the LC/MS system monitoring at wavelengths between 270 to 330 nm. The mass spectrometry method reported by Timóteo *et al.* (2014), Proestos *et al.* (2006) and Fraser *et al.* (2014) was used with the following modifications: a positive and

negative scan was performed as ionization, with an acquisition time of 2-33 min, scan spectrum at a mass interval of 50-800 m/z, scanning speed of 1667 u/sec, nebulizer gas flow of 1.5 L/min, with interface temperature of 350°C, DL temperature of 350°C, block temperature of 450°C, and drying gas flow of 9 L/s.

Bioassay

Experiments were organized within a completely randomized design for the evaluation of the effect of the ethanol extracts under laboratory conditions (19 ± 0.2 ° C temperature and 60 ± 2 % relative humidity) at MU Nueva Granada, Colombia. Treatments comprised an absolute (no application), a relative (70% ethanol), and a positive (Sunfire® as commercial acaricide containing 24% chlorfenapyr as active ingredient) controls and five unfractionated ethanolic extracts of leaves of *Cnidoscolus aconitifolius*, *Hymenaea courbaril*, *Bowdichia virgilioides*, *Copaifera officinalis* and seeds of *Anadenanthera peregrina.* Each assay was performed using 3 repetitions and 3 replications over the time. All test extracts and positive control were used at the same concentration (0.06 % w/v) in this bioassay.

Experimental unit consisted of a 6-cm Petri dish. A 3-cm bean leaf disk was placed inside experimental unit with moistened cotton surrounded. Twenty individuals (eggs, larvae, nymphs or adults) were positioned onto the underside of the bean leaf. Once the extracts were applied on each experimental unit, they were subsequently sealed with stretch film and located into a breeding room (20.5 ± 1ºC and 58.6 ± 3%) to complete the data registration.

Application of ethanol extracts was made as follows: the bean leaf disk was first immersed in the dilution of the respective extract for 15 minutes. The disk was then exposed to a soft air stream to eliminate the excess of moisture and placed inside the Petri dish. Additionally, a direct application of the extracts and controls solutions was carried out on mites at corresponding developmental stage (egg, larva, nymph or adult) located into the experimental unit. This direct application was carried out using an airbrush at a height of 20 cm above the experimental unit, calibrated at 96 drops/cm^2 and a pressure of 20-30 PSI. Registered variables were fecundity of *T. urticae* females as well as mortality of eggs, larvae, phytophagous nymphs until these mites passed to the next stage of development (24, 48, 96 hrs respectively), or when adults in the absolute control mortality reached a maximum of 10% (96 hrs).

Data Analysis

The average daily mortality from three replicates in the last evaluation day was corrected over time by means of Abbott's formula (Abbott, 1925). Fertility *per capita* at 24, 48, and 96 hrs and mortality of the phytophagous stages [such as eggs (24 hrs), larvae (48 hrs), nymphs (96 hrs) and adults (96 hours)] were transformed with the function of arcsine, and analyzed via ANOVA and Tukey's multiple comparison test. Normality of residuals was tested with the Shapiro-Wilk test. All analyses were done with the statistical language R 3.4.3 (R Core Team, 2017).

RESULTS AND DISCUSSION

Positive control showed significantly higher mortalities than these of relative control on all stages (P<0.05) (Table 1). This outcome indicates that results from this bioassay are reliable and it can be considered accurate. Thus, crude botanical extracts were prepared using 96% ethanol as primary extracting solvent due to their well-known capacity to solubilize and extract most of bioactive secondary metabolites. Accordingly, a single concentration was used to test the ethanol extracts on mites as an unbiased screening parameter for all botanicals. This dose has been considered in several studies as a mean value within the concentration range used for some common biopesticides (Biswas, 2013). Evaluation of different extracts showed variable effects on the mortality and the fecundity of *T. urticae* at test concentration. Mortality generated by the test extracts on *T. urticae* egg stage did not exceed 29% (Table 1), it being the highest corrected mortalities obtained for extracts of leaves of *C. aconitifolius* and seeds of *A. peregrina* whereas mortalities for larvae were found to

Table 1. Corrected mortality of different stages of *T. urticae* exposed to ethanolic extracts under laboratory conditions (19 ± 0.2 ° C and 60 ± 2%).

Treatment	Corrected Mortality (%)[a]			
	Eggs	**Larvae**	**Nymphs**	**Adults**
Relative Control	6.67±1.92 d	0.00±0.00 e	0.00±0.00 e	20.83±2.20 d
Positive Control	70.90±2.93 a	91.85±3.29 a	91.11±2.56 a	98.33±0.83 a
C. aconitifolius	23.81±1.53 b	35.56±2.22 b	42.22±2.72 b	83.33±2.04 b
H. courbaril	1.48±0.98 d	35.56±3.14 b	29.63±1.61 c	59.44±4.44 c
B. virgilioides	1.48±0.97 d	13.89±2.86 cd	16.30±1.27 d	64.44±5.68 c
C. officinalis	16.35±1.58 c	20.00±2.94 c	20.74±2.06 d	61.11±1.62 c
A. peregrina	28.33±0.83 b	8.89±1.64 de	6.67±1.64 e	60.56±2.56 c

[a]Values are expressed as percentages (%) ± standard error. Treatments followed by the same letter indicates that there were no significant statistical differences between them with the Tukey test (α=0.05).

be 35.5% caused by *C. aconitifolius* and *H. courbaril*. On the other hand, mortalities for nymphs did not exceed 43%, it being the highest corrected mortality induced by *C. aconitifolius* extract, which also exhibited the highest mortality for *T. urticae* females. Hence, results suggested none of test extracts can induce mortality above 50% (Table 1) on immature stages of *T. urticae*, which indicates these extracts could not be considered effective for the management of eggs, larvae and nymphs in accordance with that reported by Shi *et al.* (2008). However, all unfractionated test extracts generated mortalities on adults higher than 50%, and *C. aconitifolius* extract can be considered as a promissory management strategy for *T. urticae* adults, since the mortality generated by this extract at 96 hours after its application was greater than 80%.

Particularly, some crude extracts also affected the fecundity at different levels (Table 2). The largest reduction in the number of eggs were induced by *C. aconitifolius*, *A. peregrina* and *C. oficinalis* at 24, 48, 72 and 96 hours after contact with the evaluated extracts. The oviposition of individuals exposed to leaf extracts of *H. courbaril* and *B. virgilioides* was not significantly different from that observed in the relative control. According to this fact,

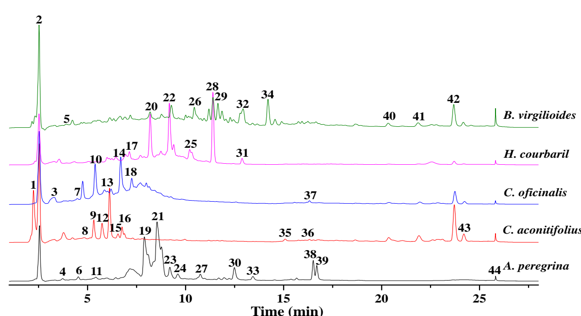
Table 2. Effect of ethanolic extracts on fecundity of females of *T. urticae* under laboratory conditions (19 ± 0.2 ° C and 60 ± 2%).

Treatment	Fertility per capita (eggs / day / female)[a]			
	24 hrs	48 hrs	72 hrs	96 hrs
Relative Control	0.31±0.09 a	0.49±0.10 a	0.76±0.08 a	1.03±0.06 a
Positive Control	0..02±0.01 c	0.03±0.01 c	0.05±0.02 e	0.09±0.02 e
Cnidoscolus aconitifolius	0.06±0.02 bc	0.09±0.03 c	0.20±0.06 de	0.35±0.06 d
Hymenaea courbaril	0.36±0.12 a	0.55±0.12 a	0.57±0.12 b	0.88±0.08 b
Bowdichia virgilioides	0.30±0.09 a	0.45±0.09 a	0.52±0.11bc	1.09±0.04 a
Copaifera officinalis	0.12±0.03 b	0.27±0.04 b	0.38±0.05 cd	0.57±0.04 c
Anadenanthera peregrina	0.08±0.01 bc	0.10±0.02 c	0.11±0.02 e	0.58±0.02 c

[a]Values are expressed as eggs / day / female ± standard error; Treatments followed by the same letter indicate that there were no significant statistical differences between them in the Tukey test (α=0.05).

They do not generate the expected negative sublethal effect consisting of fecundity reduction, and do not agree with previous studies suggesting application of most plant extracts on *T. urticae* can generate reduction in the amount of eggs laid by females (Soto *et al.,* 2011).

Fig. 1. LC-MS-derived profiles of test plant extracts.



LC/MS-derived profiles of ethanolic crude extracts (Fig. 1) showed 44 main phytoconstituents, which were tentatively identified at level three according to their mass spectra data (Table 3). Most of these compounds were presented only in one extract, but some compounds were common in several extracts (Fig. 1). The main kind of compounds detected among identified metabolites was related to flavonoids, which agrees to that reported for several plants (Neha and Jyoti, 2013). Flavonoids play an important role in protecting plants from herbivory (Harborne, 2000) and can affect phytophagous in several ways: reductions in palatability and/or digestibility, or acting directly as toxins (Mierziak, 2014).

The three main compounds (1, 2, 13) for the leaf extract of *C. aconitifolius* are mainly flavonoids, followed by two xanthones (9, 12). Biological activity of xanthones on insects has been previously reported, having as a dose dependent repellant-attactant activity, such as xanthones extracted from *Swertia densifolia* on *Apis florea* (Naik *et al.*, 2007). Compound 2 (i.e., gramine) was presented in common for *C. officinalis* and *A. peregrina* extracts. The effects of this indole-containing alkaloid, naturally occurring in several plants, have been already documented. Gramine (2) decreased survival of aphids *Rhopalosiphum maidis* and *Schizaphis graminum,* associated with feeding deterrent activity (Corcuera, 1984). In addition to this alkaloid, other major compounds such as flavonoids were identified in this *A. peregrina* extract (isoorientin, leucopelargonidin and prosogerin D), diterpene (mimosaside C y B) and terpene (prosopidione) (Table 3). The three major compounds in *B. virgilioides* leaf extract are represented by ormosanine (alkaloid), licoagrone (phenylpropanoid) and lotusine B (amide). The present results can be considered as the basis of the development of new control alternatives focused on controlling *T. urticae* populations using most acaricidal extracts. Completion of future studies is therefore encouraged in order to demonstrate and validate their effectiveness in the control of *T. urticae* in field. Moreover, caution must be exercised regarding the generalization of the results herein exposed, since the direct comparison to the results obtained in other conditions can be different, since physiological and behavioral aspects of plants (impacting on extracts composition) and insects (impacting on susceptibility) would be involved and these facts would modify the magnitude of observed effect.

In conclusion, results suggest *C. aconitifolius* extract could be considered as a promising control for adults of *T. urticae* as reported by Escalante-Erosa *et al.* (2004), since the flavonoids naturally-occurred in this plant generate a repellent effect, and according to the results exposed in Table 1, it could be a control method for adults of this phytophagous mite. Furthermore, the putative identification of secondary metabolites existing in test extracts represents important information about the rationalization of the exhibited miticidal activity on the phytophagous *T. urticae* by these botanicals to be included in further studies, which are however required to exploit these findings for development of management protocols for *T. urticae* control in commercial greenhouses, in order to contribute to the reduction of very toxic synthetic pesticides.

Table 3. Chemical composition (peak annotation)of the ethanol extracts.

R_t^a (min)	Name	ct[b]	Molecular Formula	*m/z* $[M+H]^+$	Plant samples[c]				
					B.v	*H.c*	*C.o*	*C.a*	*A.p*
2.24	Hispidulin sulphate	f	$C_{16}H_{12}O_9S$	381.03				x	
2.49	Ormosanine	a	$C_{20}H_{35}N_3$	318.29	x				
2.53	Gramine	a	$C_{11}H_{14}N_2$	175.12			x		x
2.53	Tricin	f	$C_{17}H_{14}O_7$	331.08		x			
2.54	Eucalyptin	f	$C_{19}H_{18}O_5$	327.12				x	
3.33	Dihydroxyflavone	f	$C_{15}H_{10}O_4$	255.06			x		
3.76	*N,N*-dimethyltryptamine	a	$C_{12}H_{16}N_2$	189.13					x
4.22	Bowdichine	a	$C_{31}H_{43}N_3O_6$	554.32	x				
4.53	*N*-methyltryptamine	a	$C_{11}H_{14}N_2$	175.12					x
4.74	Myricitrin	f	$C_{21}H_{20}O_{12}$	465.10			x		
4.89	Polyanxanthone C	x	$C_{28}H_{32}O_4$	433.24				x	
5.36	Cadensin G	x	$C_{24}H_{20}O_{10}$	469.11				x	
5.38	Copaiferolic acid	d	$C_{20}H_{32}O_3$	321.24			x		
5.42	Mimosine	a	$C_8H_{10}N_2O_4$	199.07					x
5.79	Parvixanthone D	x	$C_{24}H_{24}O_7$	425.16				x	
6.18	(*epi*)Gallocatechin di-*O*-gallate	f	$C_{29}H_{22}O_{15}$	611.10				x	
6.69	Copalic acid	d	$C_{20}H_{32}O_2$	305.24			x		
6.81	(epi)Catechin di-*O*-gallate	f	$C_{29}H_{22}O_{14}$	595.11				x	
6.91	Fraxetin	co	$C_{10}H_8O_5$	209.04				x	
7.13	Cinaroside	f	$C_{21}H_{20}O_{11}$	449.10		x			
7.14	Erysovine	a	$C_{18}H_{21}NO_3$	300.15					x
7.26	Kaurenoic acid	d	$C_{20}H_{30}O_2$	303.23			x		
7.89	Isoorientin	f	$C_{21}H_{20}O_{11}$	449.10					x
7.98	Brazilin	b	$C_{16}H_{14}O_5$	287.09			x		
8.17	Licoagroside F	f	$C_{21}H_{24}O_{10}$	437.14	x				
8.19	Afzelin	f	$C_{21}H_{20}O_{10}$	433.11		x			
8.54	Leucopelargonidin	f	$C_{16}H_{12}O_4$	269.08					x
9.17	Methoxyhydnocarpin	f	$C_{26}H_{22}O_{10}$	495.12		x			
9.20	Prosogerin D	f	$C_{19}H_{18}O_7$	359.11					x
9.27	Liquiritigenin diglucoside	f	$C_{27}H_{32}O_{14}$	581.18	x				
9.60	Isovitexin	f	$C_{21}H_{20}O_{10}$	433.11					x
10.21	Protosappanin E-2	p	$C_{32}H_{26}O_{11}$	587.15		x			
10.46	Afrormosin malonylglucoside	f	$C_{26}H_{26}O_{13}$	547.14	x				
10.77	Dalbergin	co	$C_{16}H_{12}O_4$	269.08					x
11.39	MethoxydehydroIsoliquiritigenin	f	$C_{16}H_{12}O_4$	269.08		x			
11.40	Afrormosin	f	$C_{17}H_{14}O_5$	299.09	x				
11.63	Licoisoflavone B	f	$C_{20}H_{16}O_6$	353.10	x				
12.49	Anadanthoside	f	$C_{20}H_{22}O_9$	405.11					x
12.87	Pentahydroxychalcone	f	$C_{15}H_{12}O_6$	289.07		x			
12.91	Trihydroxyxanthone	x	$C_{15}H_{10}O_5$	271.06	x				
13.42	Prosogerin A	f	$C_{17}H_{12}O_6$	313.07					x
14.21	Licoagrone	ph	$C_{45}H_{42}O_{10}$	743.28	x				
14.63	Acutifolin D	f	$C_{20}H_{24}O_5$	345.17				x	
15.13	Hamaudol	c	$C_{15}H_{16}O_5$	277.11				x	
16.32	Neocaesalpin D	d	$C_{24}H_{32}O_7$	433.22			x		
16.51	Mimosaside C	d	$C_{28}H_{48}O_6$	481.35					x
16.69	Mimosaside B	d	$C_{30}H_{50}O_7$	523.36					x
20.38	Sucutinirane C	d	$C_{22}H_{28}O_3$	341.21	x				
20.57	Moreollic acid	x	$C_{34}H_{40}O_9$	593.28				x	
21.83	Sucutinirane A	d	$C_{22}H_{32}O_5$	376.22	x				
23.67	Lotusine B	am	$C_{36}H_{49}N_5O_5$	632.38	x				
23.74	Friedelanone	tr	$C_{30}H_{50}O$	427.39		x	x		
23.91	Tiegusanin f	l	$C_{39}H_{38}O_{11}$	683.25				x	
24.25	Pulcherrimin E	d	$C_{36}H_{38}O_{10}$	631.25			x		
25.81	Prosopidione	t	$C_{13}H_{20}O_2$	209.15					x

[a]R_t = retention time; [b]Ct = Compound type: flavonoid (f), phenolic (p), alkaloid (a), coumarin (co), terpene (t), diterpene (d), lignan (l), phenylpropanoid (ph), xanthone (x), amide (am), chromone (c), triterpene (tr), benzopyran (b); [c]Plant-derived extracts which compound is presented: *Bowdichia virgilioides* (Bv), *Hymenaea courbaril* (Hc), *Copaifera officinalis* (Co), *Cnidoscolus aconitifolius* (Ca) *Anadenanthera peregrina* (Ap).

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