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Assessment of the acaricidal activity of several plant extracts on the phytophagous mite *Tetranychus urticae* (Tetranychidae) in Tunisian citrus orchards

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Abstract

To develop sustainable pest control in Tunisian citrus orchards, the present work aimed to evaluate the toxicity of 31 plant extracts obtained from Tunisia and two synthetic acaricides (spirodiclofen and fenbutatin oxide) on the phytophagous mite species *Tetranychus urticae* (Koch). Field experiments showed that the extracts of seven plant species (*Haplophyllum tuberculatum, Deverra scoparia, Mentha pulegium, Chrysanthemum coronarium, Hertia cheirifolia, Citrus aurantium* and *Santolina africana*) are effective and the population density of *T. urticae* was reduced at 0.30, 0.36, 0.37, 0.46, 0.48, 0.50, and 0.53 mites per leaf respectively for more than 21 days compared with the untreated control (3.7 mites per leaf). They also showed a comparable activity to classical synthetic acaricides (0.50 mites per leaf for spirodiclofen and 0.53 mites per leaf for fenbutatin oxide). The evaluation of the potential of biologically active plant volatiles against *T. urticae* might provide a new approach to the development of natural acaricides to be used both in biological and integrated pest management strategies for controlling two-spotted spider mites in Tunisian citrus orchards.

Keywords: Tunisia, plant extracts, acaricidal activity, *Tetranychus urticae*, essential oil, distillates.

Introduction

The two-spotted spider mite *Tetranychus urticae* Koch, is one of the most important pests of fruit, vegetable and ornamental plants worldwide (JOHNSON & LYON, 1991). The economic impact of this mite has increased recently in Tunisia, mainly because of its resistance to acaricides, which hampers pest control in citrus nurseries (LEBDI & DHOUIBI, 2002). The main problem with the development of pesticide resistance and the resurgence of mite populations is the use of non-selective synthetic pesticides that also eliminate natural enemies such as predatory mites and spiders (CRANHAM & HELLE, 1985). Spider mites have developed a resistance to more than 80 acaricides in more than 60 countries (ANONYME, 2005). For these reasons, essential oils are realistic alternatives to synthetic acaricides because of their selectivity, biodegradability and few side effects on non-target organisms and the environment (HAY & WATERMAN, 1993; SINGH & UPADHYYAY, 1993; ISMAN, 2000, 2001; CHIASSON *et al.*, 2001; BASTA & SPOONER-HART, 2002; RASIKARI *et al.*, 2005). They can be applied to field and greenhouse crops in the same manner as current acaricides. They also contain numerous secondary metabolites that deter attack from insect and generalist herbivores (HARBORNE, 1988) and provide an alternative for resistance management because some plant phytochemical preparations can be highly effective against insecticide-resistant pests (LINDQUISTL *et al.*, 1990; SCHMUTTERER, 1992; AHN *et al.*, 1997; YI *et al.*, 2007).

Both BOYD & ALVERSON (2000) and CHIASSON *et al.* (2004) have investigated the use of plant extracts as alternative acaricides for adult *Tetranychus urticae* with promising results. They reported the repellent effect of garlic extracts against *Tetranychus urticae*. The oil toxic effect of *Chenopodium ambrosioides* was assessed on *T. urticae* and *Panonychus citri* (CHIASSON *et al.*, 2004). Other experiments showed the acaricidal efficacy of neem (*Azadirachta indica*) against *Tarsonemus latus* and revealed that the population growth rate became negative when mites were exposed to plants treated with this extract (VENZON *et al.*, 2008). Other studies pointed out that the essential oil of *Satureja hortensis* L., *Ocimum basilicum* L. and *Thymus vulgaris* L. were effective as fumigants against *Bemisia tabaci* and *Tetranychus urticae* (ASLAN *et al.*, 2004).

In this study, our aim was to characterise the plant extracts of 31 plant species in citrus orchards in Tunisia and analyse their potential acaricidal effects against *T. urticae* compared with that of spirodiclofen and fenbutatin oxide, two commercial acaricides.

Materials and Methods

Selected plants and extraction technique

Thirty-one aromatic and medicinal plant species, representing seventeen families were collected from different Tunisian localities, South (Saddine, Mednine), South-West (Téjrouine), North-West (Kef), North-East (Hammamet, Mraissa, Tunis) during the years of 2006 to 2008 (Table1). The selection of plant species was based on previous work but also on the use of plant products in traditional medicine in Tunisia (BEN HAJ JILANI *et al.*, 2007). The fresh material was free of any pre-harvest chemical treatment (organic products). These samples were freshly harvested and sorted for uniformity and absence of defects before being stored at -2 °C until analysis.

Extracts were obtained from fresh material (Table1) by hydro-distillation (essential oils and distillates) during three hours using a Clevenger-type apparatus. A few drops of adjuvant were added (Agral 90) for gluing the extracts at the leaf surface.

Toxicity test

Extracts obtained by hydro-distillation were first diluted 100x in ethanol then diluted again 100x in water.

Field experiment for *T. urticae* control were established in a three hectares lemon (var. Lunari) orchard situated at Birbouragba, Tunisia where an infection of *T. urticae* developed naturally. The orchard was divided into 34 plots, each one containing 4 plants. Two untreated rows separated each plots. Plant extracts and insecticide were applied using a knapsack sprayer equipped with a solid-cone nozzle that operated at a pressure of 2-3 bar. Special attention was paid to ensure spraying underneath the leaves. Each treatment was applied to 3 replicates. Spirodiclofen and Fenbutatin oxide were used as a reference to be compared between with the treatments using plant extracts.

Sampling took place on 4 occasions: 3, 7, 14 and 21 days after the application. At each sampling, one hundred leaves were taken randomly from the four different plants in each plot. Leaves were immediately examined in the laboratory under a binocular microscope to assess the density of mite species. Leaves were also observed for phytotoxicity symptoms caused by acaricides or plant extracts.

Data analysis

A three way Anova with Newman-Keuls test of variance, and proc GLM of the SAS institute inc. including Scheffe's and Tukey's tests were used to compare all treatments with control. Tests were performed using Graph Pad Prism version 5.01 for windows, Graph Pad Software (San Diego, California, USA). All tests were applied under two-tailed hypotheses and the significance level P was set at 0.05.

Results

Essential oil yields

The yield of essential oil in the current study varied greatly from a minimum of 0.01% for *A. sativum*, to a maximum of 0.5% for *D. scoparia*. Nine plant extracts did not yield essential oils (Table 2).

Table1: List of plant species tested for their acaricidal activity, plant part used for extraction, site and date of samplings.

Plant family	Scientific name	Plant organ	Sampled site	Sampling date
Anacardiaceae	*Pistacia lentiscus*	Aerial part	Saddine	June 2007
	Cotinus coggyra	Aerial part	Tunis	March 2008
Apiaceae	*Daucus carota*	Aerial part	Hammamet	October 2006
	Deverra scoparia	Aerial part	Téjrouine	May 2008
Asteraceae	*Hertia cheirifolia*	Aerial part	Saddine	February 2008
	Seriphidium herba-album	Aerial part	Saddine	June 2007
	Chrysantemum coronarium	Flowers	Tunis	March 2008
	Santolina africana	Aerial part	Téjrouine	Avril 2007
Cupressaceae	*Juniperus phoenica*	Green female cones	Maraissa	March 2008
Fabaceae	*Acacia cyanophylla*	flowers	Tunis	January 2008
	Sophora secundiflora	Pods and seeds	Tunis	March 2008
Geraniaceae	*Pelargonium graveolens*	Aerial part	Hammamet	March 2008
Globulariaceae	*Globularia alypum*	leaves	Saddine	June 2007
Lamiaceae	*Salvia officinalis*	Aerial part	Tunis	March 2008
	Thymbra capitata	Aerial part	Saddine	June 2007
	Rosmarinus officinalis	Aerial part	Saddine	June 2007
	Mentha pulegium	Aerial part	Saddine	June 2007
	Lavandula officinalis	Aerial part	Saddine	June 2007
Lauraceae	*Laurus nobilis*	leaves	Hammamet	January 2007
Liliaceae	*Allium sativum*	bulbs	Hammamet	March 2008
	Allium cepa	bulbs	Hammamet	March 2008
Meliaceae	*Melia azedarach*	fruit	Tunis	January 2007
Myrtaceae	*Eucalyptus gomphocephala*	leaves	Saddine	September 2006
	Myrtus communis	Aerial part	Saddine	June 2007
Papaveraceae	*Papaver rhoeas*	Aerial part	Saddine	June 2007
	Lantana camara	Aerial part	Tunis	June 2007
Rutaceae	*Ruta chalepensis*	Aerial part	Saddine	June 2007
	Citrus aurantium	leaves	Tunis	March 2008
	Haplophyllum tuberculatum	Aerial part	Mednine	Septembre 2007
Urticaceae	*Urtica pilulufera*	Aerial part	Saddine	October 2006
Zygophyllaceae	*Peganum harmala*	Aerial part	Saddine	June 2007

Field experiments

Colonization patterns of phytophagous mites on Lunari variety (control plots)

Control population density of *T. urticae* at each sampling date is shown in Figure 1. It appears that phytophagous mites occurred at negligible densities from January to May. Then the population grew to a high level (four mites per leaf) on Lunari varieties until beginning of July. A slight decline was recorded from mid-July, followed by a second peak in mid-August, with an average of 5 *T. urticae* mites per leaf. Finally, at the end of August to end of September, mite populations dropped nearly to zero. A few individuals of phytophagous mites were still recorded in the beginning of October (Fig.1).

Toxicity tests on field

The acaricidal effects in citrus orchards of the 31 plants extracts is summarized in Table 4 and 5.

According to the ANOVA test, while the effect of plots and exposure time interactions of extracts on *T. urticae* were not significantly different ($P= 0.5593$), the effect of treatment and exposure duration interactions were very significant at $P<0.01$ (Table 3). All extracts have a significant toxic effect on *T. urticae* compared to control ($P<0.001$) (Table 3). In term of mortality, three groups are distinguishable (Table 4). In the first group, the number of mites recorded per 100 leaves never overpasses 60 individuals after 21 days. We find in this group three endemic plants from North Africa *Hertia cheirifolia, Santolina africana, Deverra scoparia*, the last one producing the higher level of mortality among these plants.

Table 2. Essential oil yields according to plant species

Plant species	Fresch weight (g)	Volume oil obtained (ml)	% yield
Pistacia lentiscus	1500	1	0.06
Cotinus coggyra	3000	0	0
Peganum harmala	1500	0	0
Juniperus phoenicea	3500	2.5	0.07
Globularia alypum	1500	0	0
Laurus nobilis	800	2	0.25
Melia azedarach	526	0	0
Pelargonium graveolens	450	1.5	0.33
Urtica pilulifera	700	0	0
Daucus carota	423	0	0
Deverra scoparia	1166	6	0.51
Hertia cheirifolia	1705	2	0.11
Seriphidium herba-album	3614	10	0.28
Chrysantemum coronarium	7300	5	0.07
Santolina africana	500	1	0.02
Papaver rhoeas	359	0	0
Lantana camara	1732	0	0
Allium sativum	9000	1	0.01
Allium cepa	9000	1.2	0.013
Acacia cyanophylla	12000	0.5	0.0041
Sophora secundiflora	8500	0	0
Eucalyptus gomphocephala	5300	2	0.037
Myrtus communis	6200	3	0.048
Ruta chalepensis	3000	0.5	0.016
Haplophyllum tuberculatum	2000	2.5	0.1
Citrus aurantium	1500	6	0.4
Salvia officinalis	1250	5.5	0.45
Thymbra capitata	7339	35	0.48
Rosmarinus officinalis	4203	15	0.36
Mentha pulegium	3325	6	0.18
Lavandula officinalis	2000	2.5	0.20

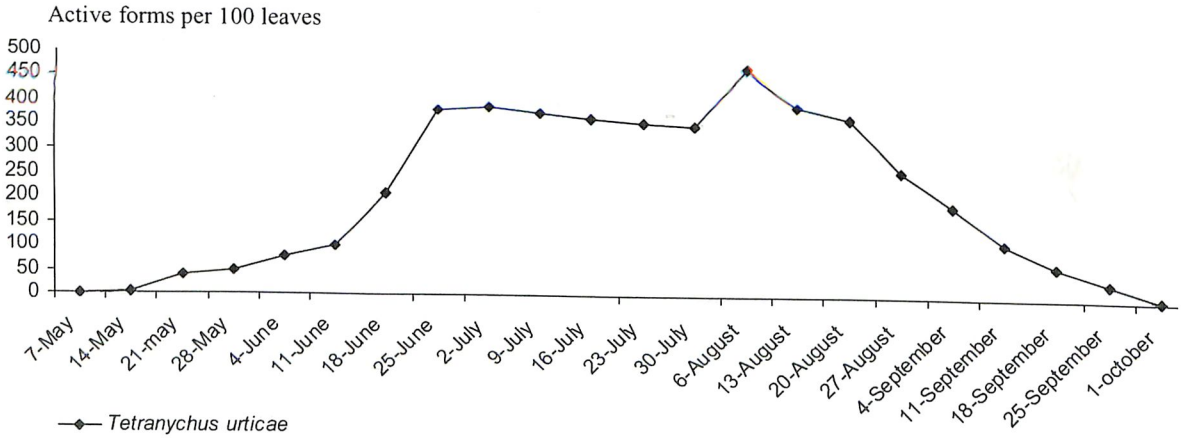

Fig. 1. Population dynamics of *T. urticae* on Lunari variety in control plots during 2008.

Table 3. Acaricidal effects in citrus orchards in function of different interactions (treatments, Plots, and exposure duration)

Source	Mean square	F value	Pr>F
Treatments	43899.791	136.95	<0.001
Plots	1066.232	3.33	0.0370
Exposure time	760723.799	2373.11	<0.001
Treatment*Plots	320.702	1.00	0.4856
Treatment* Exposure time	12472.936	38.91	<0.001
Plots* exposure time	272.417	0.85	0.5593

The second group showed number of mites per 100 leaves after 21 days ranging from 97 to 244.67. In the third group, more than 300 individuals per 100 leaves recorded that indicates a quasi-absence of activity (Table 4). In general, the acaricidal activity was relatively enhanced with adjuvant (Agral 90) and exposure times for all extracts (Table 3). During the experiments, any phytotoxic effects of extracts were noted.

Discussion and Conclusion

To develop sustainable pest control in Tunisian citrus orchards, we have evaluate the toxicity of 31 plant extracts obtained from Tunisia and two synthetic acaricides (spirodiclofen and fenbutatin oxide) on one phytophagous mite specie: *T. urticae.* Altogether, 31 extracts (essential oils and distillates) from 31 plants were tested in field trials. Some plants are endemic to North African Sahara such as *D.* scoparia, *S. africana* and *H. cheirifolia* (ALAPETITE 1981; OBERPRIEDER, 2002; DJERIDANE *et al.*, 2007), some can be found throughout the mediterranean circumference such as *Thymbra capitata, Rosmarinus officinalis, S. herba-album, Pelargonium graveolens* (ALAPETITE, 1981).

Contact acaricidal activity of plant extracts have been well demonstrated against mites (ISMAN, 2000, 2001; MIRESMAILLI & ISMAN 2006). Differences of toxicity between extracts and plant species were recorded in field experiment. All selected extracts caused mortality statistically similar to that obtained with synthetic products Spirodiclofen and Fenbutatin oxide except extracts from *P. rhoeas, R. chalepensis, L. camara P. harmala, S. secundiflora, E. ghomphocephala* and *L. nobilis* that did not cause significant *T. urticae* adult mortality in comparison to the control after 21 days after treatment.

Efficiency of essential oils

The yield of essential oil varied considerably depending upon plant species. Maximum yields of 0.5% were achieved with the essential oil of *D. scoparia.* Nine plant extracts do not contain essential oils at all. This quite poor yield limits the large scale application possibility of some of the plant extract and further development will be needed to implement these applications and to look toward a more efficient method of oil extraction and plant culture.

Here we show that the most effective of extracts are *S. africana, C. aurantium, C. coronarium, H. cheirifolia M. pulegium D. scoparia and H. tuberculatum*, the number of mites recorded per 100 leaves never overpasses 60 individuals after 21 days compared to two synthetic acaricides. Three of these plant are endemic to north Africa (POTTIER ALAPETITE, 1981; LE FLOC'K, 1983; OBERPRIEDER, 2002; DJERIDANE *et al.*, 2008).

Other studies showed the acaricidal properties of essential oils. PHANANJAY *et al.* (2005) showed the effect of some rhizome oil on *T. urticae.* MANSOUR *et al.* (1986) pointed out that besides the toxicity, the residues of essential oils of some Labiatae species are repellent and strongly reduce the fecundity of *T. cinnabarinus* females. Others studies indicate that pure rosemary oil and Eco/Trol (rosemary oil based pesticide) cause complete mortality of spider mites *Tetranychus urticae* at concentrations that are not phytotoxic to the host plant and that did not cause any mortality in *Phytoseiilus persimilis* (Phytoseiidae) neither affected their eggs (MIRESMAILLI & ISMAN 2006). Some of the previous studies (TUNC & SAHINKAYA, 1998; ISMAN *et al.*, 2001) have been reported that acaricidal effects of plant essential oils are related to their chemical compositions. Essential oils can affect animals from different orders including mites. For instance, ANTHONY *et al.* (2008) showed the biocidal properties of the essential oil of *H. tuberculatum* and *Plectranthus cylindraceus* against *Meloidogyne javanica* (Nematoda).

Efficiency of distillates

Our work is the first testing the efficiency of distillates on *T. urticae.* One main technical difficulty is the quantification of the molecules in the distillates. Here we show that the most effective are *H. tuberculatum* and *H. cheirifolia*, the latter is endemic in North Africa.

Among 31 plants tested as acaricides against the phytophagous mite *T. urticae* (Boisduval) in field trials, the most toxic were: *H. tuberculatum, D. scoparia, M. pulegium, C. coronarium, H. cheirifolia, C. aurantium* and *S. africana*. Three of these highly effective plants are endemic to North Africa (see Table 4). In vivo experiments, the acaricidal activity was relatively enhanced with adjuvant Agral 90 and exposure time for all extracts: distillates or essential oils. However, except *C. coronarium* and *M. pulegium*, this is the first study demonstrating that *D. scoparia, H. tuberculatum, H. cheirifolia, C. aurantium, S. africana*, have acaricidal activity against *T. urticae*. Toxicity effect of *D. scoparia* and *H. tuberculatum* may be explained by higher alpha-pinene and sabinene contents (MASOUDI *et al.*, 2004). According to MIRESMAILLI *et al.* (2006), the major chemical components of essential oils with acaricidal activity have been identified as 1,8 cineole, alpha-pinene, and myrcene. Difference in activity between plant species is that clearly related to difference in chemical composition and proportion of main components presents in extracts (KOUNINKI *et al.*, 2007). Our results suggest that these seven plants may have great potential for effective managements of *T. urticae*. Furthermore, plant distillates are widely available and some are relatively inexpensive, as

Table 4. Tests toxicity bioassays of extracts and reference products to *T. urticae* on field (results after 1 application, small letters indicate significant differences at $p<0.05$ and capital letters D and E indicate type of extract (distillate and essential oil) (bold writing indicate endemic plant from North Africa)

Active forms per 100 leaf				
Day 3 (02 July 2008)			**Day 7 (06 July 2008)**	
Extracts	Mean value ± standard deviation		Extracts	Mean value ± standard deviation
P. rhoeas D	409.67±13.05	a	*D. carota D*	392.67±8.02	a
Control	399±12	ab	*Control*	390±1053	a
C. coggyra D	394±2.88	ab	*S. secundiphlora D*	385.33±7.37	ab
D. corota D	390±4.36	ab	*C. coggyra D*	378.67±13.86	ab
P. lentiscus D	385±6.11	ab	*L. camara D*	378±19.15	ab
L. camara D	385.67±16.62	ab	*P. harmala D*	376.67±11.59	ab
P. graveolens E	385.67±6.11	ab	*G. alypum D*	371±6.24	abc
G. alypum D	378.33±14.15	abc	*U. pilulifera D*	366.33±13.20	abc
L. officinalis E	376.67±15.044	abc	*T. capitata E*	358±30.11	abcd
M. communis E	366±7	abcd	*J. phoenicea E*	357.67±21.38	abcd
J. phoenicea E	362.67±21.54	abcd	*P. rhoeas D*	356±7.54	abcd
C. aurantium E	357.33±10.06	abcd	*L. officinalis E*	331.67±30.55	abcde
T. capitata E	356.67±9.60	abcd	*C. aurantium E*	330.33±3.21	abcde
S. secundiflora D	356.67±7.50	abcd	*S. herba-album E*	322.33±22.03	abcde
H. cheirifolia D	354±8.18	abcd	*R. officinalis E*	280±19	bcdef
R. officinalis E	350±46.35	abcd	*A.cynophylla D*	269±23.45	cdefg
A. cyanophylla D	348±8.54	abcd	*R. chalepensis E*	269±17.57	cdefg
E. ghomphocephala E	347.33±16.62	abcd	*A. sativum D*	268±25.23	cdefg
S. herba-album E	345.67±17.03	abcd	*M. communis E*	266.67±17.78	cdefg
P. harmala D	345.67±10.01	abcd	*S. officinalis E*	260.33±11.59	defg
U. pilulifera D	333.67±15.27	abcd	*A. cepa D*	259.33±20.42	defg
M. pulegium E	329.67±8.08	abcde	*S. africana E*	259±27.05	defg
S. africana E	317.33±17.12	abcdef	*H. cheirifolia D*	257±7.54	defgh
S. officinalis E	313±38.74	abcdefg	*M. pulegium E*	247±8.54	efgh
A. cepa H	303.33±28.50	bcdefg	*P. graveolensD*	239.33±24.98	efgh
D. scoparia E	281±15.09	cdefg	*P.lentiscus D*	239.33±24.98	efgh
C. coronarium E	279±17.34	cdefg	*C. coronarium E*	231.67±17.61	efgh
A. sativum D	273±15.71	defg	*Spirodiclofen*	188.67±17.21	fghi
Spirodiclofen	230±18.02	efgh	*Fenbutatin oxide*	183±17.34	fghi
Fenbutatin oxide	228±18.52	fgh	*D. scoparia E*	166.67±19.55	ghij
H. tuberculatum D	215.67±16.28	gh	*H. tuberculatum D*	151.33±13.31	hij
L. nobilis D	166.67±16.50	hi	*E. ghomphocephalaE*	108.33±14.57	ij
M.azedarach D	139±17.05	hi	*L. nobilis E*	93.67±7.02	ij
R. chalepensis E	96.67±13.86	i	*M. azedarach D*	77±14.42	j

Active forms per 100 leaf				
Day 14 (13 July 2008)			Day 21 (19 July 2008)	
Extracts	Mean value ± standard deviation		Extracts	Mean value ± standard deviation
Control	388.67±18.82	a	*Control*	379.33±16.92 a
P. harmala D	388±18.68	ab	*P. rhoeas D*	379.33±17.24 a
G. alypum D	387.33±13.50	ab	*R. chalepensis E*	367.33±29.67 a
P. rhoeas D	377±8	abc	*L. camara D*	358.67±11.26 a
D. carota D	369±12.34	abcd	*P.harmala D*	357.67±10.59 a
S. secundiflora D	349.33±5.85	abcde	*S. secundiflora D*	357.33±11.23 a
L. camara D	345±20.66	abcde	*E. ghomphocephala E*	355±12.48 a
R. chalepensis E	331±30.80	abcdef	*L. nobilis E*	350.67±44.04 a
T. capitata E	288.33±20.30	abcdef	*M. azedarach D*	341±16.70 ab
U. pilulifera D	284±17.57	abcdefgh	*S. herba-album E*	328.67±24.41 ab
C. aurantium E	282.33±12.66	bcdefgh	*D. carota H*	322.33±37.16 ab
C. coggyra D	273.67±24.58	cdefgh	*G. alypum D*	232.33±60.92 bc
L. officinalis E	270.67±15.56	defgh	*T. capitata E*	180.67±14.57 cd
R. officinalis E	267.67±19.50	defgh	*J. phoenicea E*	174.33±10.50 cd
S. herba-album E	262.33±25.54	efghi	*C. coggyra D*	162.67±16.62 cde
A. cyanophylla D	250±15.39	efghij	*A. cyanophylla D*	128.33±32 cdef
M. azedarach D	238.67±20.74	fghijk	*M. communis E*	126±6.55 cdef
L. nobilis E	237.33±24.94	fghijk	*L. officinalis E*	117.33±17.24 cdef
J. phoenicea E	232.67±11.37	fghijk	*U. pilulifera D*	115.33±8.08 cdef
E. ghomphocephala E	227.67±22.67	fghijkl	*A. cepa D*	112.33±8.32 def
A. cepa D	187±11	ghijklm	*P. lentiscus D*	106.67±12.50 def
S. officinalis E	184.33±7.63	ghijklm	*P. graveolens E*	106.67±12.50 def
P. lentiscus D	180±14.10	hijklm	*R. officinalis E*	106±10.14 def
P. graveolens E	180±14.10	hijklm	*A. sativum D*	105.33±7.02 def
A. sativum D	179±16.52	hijklm	*S. officinalis E*	97±4 def
H. cheirifolia D	160.67±15.53	ijklm	*Fenbutatin oxyde*	53.67±8.60 ef
M. communis E	155.33±7.02	jklm	***S. africana E***	53.33±9.29 ef
M. pulegium E	151±29.05	jklm	*C. aurantium E*	50.67±8.50 ef
Spirodiclofen	142.67±18.71	klm	*Spirodiclofen*	50.33±5.50 ef
Fenbutatin oxide	139.33±14.97	klm	***H. cheirifolia D***	48.33±5.68 ef
C. coronarium E	122±18.15.39	lm	*C. coronarium E*	46.67±7.76 ef
S. africana E	119±18.73	m	*M. pulegium E*	37.67±9.60 f
D. scoparia E	95.67±12.34	m	***D. scoparia E***	36.67±7.09 f
H. tuberculatum D	85.33±8.50	m	*H. tuberculatium D*	30.67±5.50 f

compared with essential oils and distillates. In that context, *D. scoparia* and *H. tuberculatum* need further studies from an economical point of view. Distillates are especially interesting as they are not very expensive. Moreover, their extraction is very easy even for farmers having no laboratory materials and does not require high quantity of plants on the contrary to essential oils.

Another further study is also necessary to determine the toxicity of these extracts on predatory mites and on other economically important pests in citrus orchards where pest management depends on chemical applications, which is causing environmental pollution and resistance in pest population (LAMIRI *et al.*, 2001).

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A new myrmecophilous *Allochernes* from ant nests in the high altitude of the eastern Spanish Pyrenees (Arachnida: Pseudoscorpiones: Chernetidae)

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Abstract

Allochernes struyvei sp.n., a new myrmecophilous pseudoscorpion from the Spanish Pyrenees, is described.

Keywords : Pseudoscorpion, *Allochernes struyvei* sp.n., myrmecophilous, Pyrenees, Spain

Introduction

Some species of the pseudoscorpion genus *Allochernes* BEIER occur occasionally with ants (BEIER, 1963), other species of this genus have only been found in the nests of a particular ant species (HENDERICKX & VETS, 2003) and seem restricted to this host.

During collection trips in May and September 2009 an unidentified pseudoscorpion was found in heaps of the ant *Formica paralugubris* SEIFERT, 1996 near Setcases, Spain by Tim STRUYVE (Muizen, Belgium), who was searching for Myrmecophilous Staphylinidae. The pseudoscorpions were kindly donated to the author and the new species is described in this publication.

Material and methods

All specimens were hand captured by sifting material from the ant-heaps and fixed in 70% ethanol.

Microscopical examination was performed with a Leitz microscope and optics, measurements with a Zeiss calibration grid. A FEI Quanta-200 was used for scanning electron microscopy.