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EFFICIENCY OF NATURAL EXTRACT, *Aaloe barbadensis* GAINST TWO SPECIES OF PHYTOPHAGOUS MITES

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ABSTRACT

Three different extracts of Aloe barbadensis Miller leaves were evaluated for acaricidal activity against adult female of mites, Tetranychusurticae and Cenopalpuspulcher by spray bioassay. At 72 h after treatment the acetone extract showed the strongest acaricidal activity with LC_{50} value of 105 ppm while, the LC_{50} values for ethanol and water extracts were 322 and 366 ppm for T. urticae, respectively. LC_{50} values were 80, 289 and 320 ppm at the same order for extracts against C. pulcher. The acetone extract was fractionated using GC-MS analysis to twenty-three fractions. Using LC_{50} of acetone extract a reduction in longevity of adult females of both species occurredas 14.45 and 12.37 days for T. urticae and C. pulcher, respectively. While, control treatment was 20.27 and 16.18 days for T.urticae and C. pulcher, respectively. Acetone extract caused significant reduction on fecundity. Deterrent index were 43.61 and 31.29 % for T.urticae and C. pulcher, respectively. The obtained results indicated that A.barbadensis has a great potential as a botanical acaricide for T. urticae and C. pulcher control.

Conclusively, from these results of the present study show great potential and must be more advanced for developing Aloe extracting based mite control products.

Keywords: *Aloe barbadensis*, *Cenopalpuspulcher*, *Tetranychusurticae*

INTRODUCTION

Mites belonging tothe families, tetranychidae and tenuipalpidae (Acari: Prostigmata) can be regarded as some of the most destructive of the plant feeding species. The tenuipalpidae species are known as flat mites or false spider mites and worldwide in distribution. Tenuipalpidae species are phytophagous and damage plants by sucking on the epidermal cells. Most of the species can cause economic damage to cultivated and ornamental plants (Ripka, 1998; Didem and Sultan 2010). *C. pulcher* is considered one of the major pest followed this family.The tetranychidae, *T. urticae* is a phytophagous pest that infests over 100 crop species including beans,

cucumbers, eggplants, tomatoes, and cucurbits grown in field and greenhouse throughout the world (Cakmak*et al*., 2005). For the past several decades, the control of mites has depended mainly on application of pesticides. The extensive use of pesticides has led to rapid development of resistance in this mite. Besides the extensive and long-term use of chemical pesticides have serious adverse effects on beneficial organisms, humans and the environment. Therefore, identification and development of effective, anti-resistance, safe and eco-friendly non chemical control alternatives for mites are needed. Use of natural compounds from plant extracts has been suggested as a viable source of alternative treatments for insect and mite control because many substances of such compounds have novel modes of action, no or low toxicity to non-target organisms and mammals, and are less harmful to the environment (Schmutterer, 1997). Numerous plant extracts have been reported to have different biological activities against insects and mites including repellence, feeding and oviposition deterrence, toxicity, and growth regulatory activity (Singh and Saratchandra, 2005). Moreover, plant based pesticides often contain a mixture of active substances, which can delay or prevent resistance development (Wang *et al*., 2007). *A. barbadensis* is one of Liliaceae family. *A. barbadensis*'s biological activity is widely accepted and it is used for various medical, cosmetic and nutraceutical purposes. Compared to the benefits of Aloe to humans, relatively little is known about its insecticidal and/or acaricidal activities.

Therefore, the objectives of this study were to assess acaricidal activity of *A. barbadensis* against the mite pests and to isolate active components in an effort to gain information on developing new Aloe based pesticides for mite control that are effective and safe.

MATERIALS AND METHODS

Tested animals

The stock culture started with females collected from eggplantand navel orangefrom a farm in Sharkia Governorate. The eggplant leaves (*Solanummelongena*) infested by the two-spotted spider mites, *T. urticae* and navel orange leaves**,** *Citrus sinensis* infested by *C. pulcher*, were collected and placed in paper bags. Samples were transferred immediately to the laboratory. The mass culture was initiated by transferred individuals of females and males using a camel's hair brush placed in petri-dishes about10 cm diameter, which provided with untreated fresh leaves discs of mulberry (*Morus alba* L.) for *T. urticae* and discs of *Citrus sinensis* for *C. pulcher* about 3 cm diameter placed on a pad of cotton wool, fully saturated with water as a source of moisture and to prevent mite escaping. Newly laid eggs

were obtained by releasing the adult females on fresh and clean mulberry and navel orange leaf discs overnight and removing all the adult females at the next day. After eggs hatching, the newly larvae were placed on fresh leaf discs in prepared petri-dished as mentioned above. The old leaf discs were removed after one day and mites were fed on fresh leaf discs, wherever, it was necessary about 4-6 changes, to complete lifecycle of the experimental mites. All colonies were kept in an incubator at 28± 2°C and 65± 5% R.H. The population density of mites in each colony was kept by providing of fresh host plants.

Plant material collection and extraction

The plant of *Aloe barbadensis* (leaves) was collected from Zagazig district. The leaves of *Aloe barbadensis* were air dried and crushed to small pieces using mortar and pestle then powdered in an electric grinder. Twenty grams of powdered plant materials were mixed with 100ml of various solvents (distilled water, ethanol and acetone solution). The extracts preparations were done as previously described by Alade and Irobi (1993). Phytochemical components were analyzed qualitatively. The GC-MS analysis of the *A. barbadensis* was performed.

Bioassay tests:

1. ***Effects on adult females of mite****:*

The spray technique was applied to test the efficacy of the different extracts on females of *T. urticae* and *C. pulcher*. Eight replicates of mulberry leaf for *T. urticae* treated and navel orange for *C. pulcher* were done, each disc about 3 cm diameter was gently sprayed with serial of concentrations of each experimented extract. Ten adult females at the same age of laboratory reared mite colony were individually transferred by means of a camel hair brush to treated leaf discs of mulberry and navel orange. For conserving leaf discs fresh, water moist cotton pad below the leaf disc equipped each petri-dish. For each concentration, total numbers of 80 adult females were tested for each mite. Also, 8 petri-dishes equipped with the same number of adult mite females on water treated leaf discs were used as control. All petri-dishes were held at the same conditions of 28 ± 2 ºC and relative humidity of 65 ± 5 % R.H. These techniques were made according to Ebeling (1960). Mites treated with different extracts were examined daily and mortality % was calculated. The efficiency of different plant extracts was measured by comparing the tested extract with the most effective extract by using the equation of Sun (1950).

$$\text{Toxicity index} = \frac{\text{LC50 of the most effective one}}{\text{LC50 of the tested extract}} \times 100$$

2. ***Latent effect of LC_{50} of some extracts on longevity and fecundity of T. urticae and C. pulcher females:***

Eight adult individuals of *T. urticae* and *C. pulcher* were transferred to mulberry and navel orange leaf discs about 3 cm diameter, respectively. Treatments were replicated 10 times for each species, *T. urticae* and *C. pulcher*. The spray technique was applied as mentioned above. The live individuals from each species were observed then the longevity and fecundity of *T. urticae* and *C. pulcher* were recorded. Deterrent indices for adult females were calculated according to Lundgren (1975) formula as follow:

$$\text{Deterrent index } \% = \frac{B - A}{B + A} \times 100$$

A: Number of eggs in treatment, B: Number of eggs in control.

Statistical analysis:

Data were analyzed with one-way ANOVA followed by Duncan, (1955) multiple range tests at $P < 0.05$ using Costat.

RESULTS

Table (1) cleared that the acetone extract was the most effective against adult females of *T. urticae*, while water extract was the least effective. LC_{50} for tested extracts can be arranged as the following descending order: acetone, ethanol and water extracts since LC_{50} values for them were 105, 322 and 366 ppm, respectively. On the other hand, C. *pulcher* was more sensitive for the extracts compared with *T. urticae*, where LC_{50} values were 80, 289 and 320 ppm, respectively. for the same previous order. According to the toxicity index at LC_{50} the most potent extract was acetone (100%) for both mites, while the least one was water (28.68 and 25.00%) for both mites.

Table 1. Comparative toxicity of three extracts against the two species of mites after 72 hr.

Extracts	LC_{50} (ppm)		Toxicity index %		Confidence limits for LC_{50}			
	T. urticae	*C. pulcher*	*T. urticae*	*C. pulcher*	*T.urticae*		*C. pulcher*	
					Upper	Lower	Upper	Lower
Acetone	105	80	100	100	117	96	94	69
Ethanol	322	289	32.61	27.68	346	309	302	265
Water	366	320	28.68	25.00	382	347	336	311

The acetone extract shortened the longevity and reduced the fecundity of adult females of *T. urticae and C. pulcher*. Results in Table (2)

revealed that, the pre-oviposition period when used acetone extract were 1.63 and 1.96 days for *T. urticae and C. pulcher*, respectively. Compared with 1.41 and 1.84 days for the control. On the other hand, oviposition period was 10.21 and 8.52 days for the previous order compared with 16.56 and 12.11 days for the control. Longevity of individuals was 14.45 and 12.37 days for *T. urticae and C. pulcher* compared with 20.27 and 16.18 days for the control. Concerning, the total number of eggs was 21.17 and 20.74 eggs for *T. urticae and C. pulcher*and 53.92 and 39.63 eggs for control. The acetone extract caused deterrent index % 43.61 and 31.29 for *T. urticae and C. pulcher*, respectively.

Table 2. Effect of LC_{50} for acetone extract on longevity and fecundity of two species mites'females.

Mite species	Pre-oviposition	Oviposition	Post-oviposition	Longevity (days)	Fecundity	Deterrent index, %
T. urticae	1.63	10.21	2.61	14.45b	21.17b	43.61
Control	1.41	16.56	2.30	20.27a	53.92a	-
C. pulcher	1.96	8.52	1.89	12.37b	20.74b	31.29
Control	1.84	12.11	2.23	16.18a	39.63a	-

Means in columns followed by the same letters are not significantly different at p=5% according to Duncan's multiple range test (Duncan, 1955)

Table (3) indicated that the GC-MS analysis, 23 bioactive phytochemical compounds were identified in the acetone extract of *Aloe barbadensis*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula.

DISCUSSION

Plant based acaricides have long been recommended as alternatives to synthetic chemical acaricides for pest control because these chemicals pose little threat to the environment and/or to human health (Isman, 2006). Pipernonaline and piperoctadecalidine, two alkaloids isolated from *Piper longum* L., were also potent against *T. urticae* (Park *et al*., 2002). This study investigates the contact acaricidal, repellent, fumigant, and oviposition inhibition activities of the acetone extract of *A. vera*L. leaf against *Tetranychuscinn abarinus.* The aloe acetone extract was found to have good contact acaricidal activity against the cinnabar of female adult mites. Through the toxicity regression line of the aloe acetone extract against female carmine spider mites, the LC_{50} values to *T. cinnabarinus* were found to be 0.836 and 0.167 mg/mL for 48 and 72 h, respectively. (Wei *et al*., 2011) reported LC_{50} values of 0.614 and 0.099 mg/mL for 48 and 72 h, respectively. The main modes of action of the extract against adult mites' females were contact and repellent, and preferable effects were observed on adult mites. These results indicate that *A. vera* L. extract contains acaricidal and repellent

Table 3: Phytocomponents identified in the plant sample extract

	RT/min.	Name of the Compound	Molecular Formula
1	16.05	1-Tetradecyne	$C_{14}H_{26}$
2	17.67	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$
3	18.70	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
4	18.93	Hexadecanoic acid, ethyl ester	$C_{18}H_{40}O$
5	21.07	Phytol	$C_{20}H_4O$
6	21.85	Oleic Acid	$C_{18}H_{34}O_2$
7	22.06	9,12,15- Octadecatrienoic acid methyl ester, (ZZZ)	$C_{19}H_{32}O_2$
8	24.13	Oxalic acid, allylpentadecyl ester	$C_{20}H_{36}O_4$
9	25.73	Oxalic acid, allylhexadecyl ester	$C_{21}H_{38}O_4$
10	27.11	9-Ocatadecenal	$C_{18}H_{34}O$
11	28.48	1-Octadecyne	$C_{18}H_{34}$
12	28.77	Sulfurous acid, hexyl pentadecyl ester	$C_{21}H_{44}O_3S$
13	30.21	1-lodo-2-methylundecane	$C_{12}H_{25}$
14	31.60	Eicoane	$C_{20}H_{42}$
15	31.90	Squalene	$C_{30}H_{50}$
16	32.95	Octadecane, 2-methyl-	$C_{19}H_{40}$
17	34.26	Nonadecane, 2-methyl	$C_{20}H_{42}$
18	36.09	Vitamin E	$C_{29}H_{50}O_2$
19	36.80	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$
20	37.38	9, 12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$
21	38.25	Tetracontane, 3, 5, 24-trimethyl-	$C_{43}H_{88}$
22	38.78	-Sitosterol	$C_{29}H_{50}O$
23	40.28	Lupeol	$C_{30}H_{50}O$

RT: Retention time/minute

bioactive components that may be useful in future control of the phytophagous mites (Zhang *et al*., 2013). In this study there were found some compounds which isolated from Aloe extract caused mortality percent for genus Tetranychus, these compounds were Lupeol (Wang *et al*., 2012), Oliec acid (Eleawa, 2007) Sulfurous acid, 9,12-Octadecadieoic acid (Z, Z) (Lucie, *et al*. 2013). We forced our efforts to search alternative methods for controlling mite based on Aloe because the plants are readily available around the world, they have been used extensively for medical, nutritional and cosmetical purposes and they pose a minimal threat to humans and the environment.

Conclusively, from these results of the present study show great potential and must be more advanced for developing Aloe extracting based mite control products.

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فاعلية المستخلص الطبيعى *Aloe barbadensis* ضد نوعين من الأكاروسات نباتية التغذية

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تم تقييم ثلاث مستخلصات مختلفة من أوراق نبات الصبار (***Aloe barbadensis***) ضد إناث نوعين من الحلم النباتي *T.urticae* و *C.pulcher* بطريقه الرش. أظهر المستخلص الاسيتونى فاعليه عاليه كمبيد أكاروسى حيث بلغت قيمه التركيز النصف قاتل ١٠٥ جزء في المليون بينما كانت قيمه التركيز النصف

قاتل للمستخلص الايثانولى والمائي ٣٢٢ و ٣٦٦ جزء فى المليون على التوالي ضد إناث الحلم العنكبوتى *T.urticae* بينما كان النوع*C.pulcher*أكثر حساسية حيث بلغت قيمه التركيز النصف قاتل ٨٠ و ٢٨٩ و ٣٢٠ جزء فى المليون وذلك باستخدام المستخلص الاسيتونى والايثانولى والمائي على التوالي وحيث أن المستخلص الاسيتونى أكثر المستخلصات النباتية فاعليه تم فصل مكوناته باستخدام جهاز التحليل الكروماتوجرافى الغازي حيث تم فصل ٢٣ مكون.

- تم دراسة التأثير النصف قاتل للمستخلص الاسيتونى على بيولوجي الحلم النباتي *T.urticae*و *C.pulcher* حيث انخفضت مده معيشة إناث الحلم لكلا النوعين حيث بلغت مده معيشه اناث النوع *T.urticae* ١٤.٤٥ يوم و ١٢.٣٧ يوم للنوع *C.pulcher* مقارنه بالكنترول ٢٠.٢٧ و ١٦.١٨ يوم على نفس الترتب السابق.

كان للمستخلص الاسيتونى تأثير معنوي على خفض الخصوبة وكميه البيض الموضوعة لإناث الحلم النباتى حيث بلغت نسبه اعاقه وضع البيض ٤٣.٦١ و ٣١.٢٩ ٪ للنوعين *T.urticae* و *C.pulcher* على التوالي.

التوصية: أظهرت النتائج المتحصل عليها ان المستخلص النباتي من (***Aloe barbadensis***) ذو كفاءة عاليه كمبيد اكاروسى ضد نوعى الحلم.