J. of Plant Protection and Pathology, Vol. 1 (1): 9 -19, 2010

REPELLENCY AND TOXICITY EFFECT OF PLANT EXTRACT FROM *Francoeria crispa* (Forssk) AGAINST *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae)

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ABSTRACT

Laboratory bioassays were conducted to evaluate the activity of plant extract *Francoeria crispa* (Forssk) (Family Compositae) against the citrus brown mite, *Eutetranychus orientalis* (Klein). Ethyl acetate was tested for preparing the crude extract of *F. crispa*. The extract was tested for its toxicity against eggs and adult females of the mite pest *E. orientalis*. Ethyl acetate extract of *F. crispa* was affected the behavior, toxicity and fecundity of females under laboratory conditions. The extract had similar toxic effects on egg stage and adult females of *E. orientalis* (LC_{50} = 0.00050 g / ml), respectively. Leaf discs treated with increasing concentrations of ethyl acetate extract of *F. crispa* showed a high percentage of repellency (97.45%), respectively. Treated females with LC_{50} concentration of ethyl acetate extract showed a higher remarkable percentage of mortality as well as a reduction in the total number of eggs laid during 7 days. Ten isolated fractions of ethyl acetate crude extract from *F. crispa* were detected. Results clearly indicate that the isolate number (10) was the most toxic isolate on eggs and females of *E. orientalis* (LC_{50} =0.00014 and 0.000125 g / ml), respectively.

Keywords: Acari, *Eutetranychus orientalis*, *Francoeria crispa*, Plant extract, Tetranychidae,

INTRODUCTION

In recent years, the plant extracts have received much attention as resources of potentially useful bioactive compounds. Particular emphasis has been placed on their antimicrobial, antifungal, antitumor and insecticidal action as well as on their action on the central nervous system (Gerasimos *et al.*, 1997). Few studies have been done on such plants having acaricidal influences are assumed to be acceptable because they are alterable in nature (Schauer and Schmutter, 1981; Amer *et al.*, 1989; Dimetry *et al.*, 1990; Hussein *et al.*, 2006). Previous work has been done by El-Gengaihi *et al.*, 1999 as well as Dimetry *et al.*, 2000 & 2003 showed the activity of some medicinal plant such as *Glossostemon bruguieri*, *Curcuma longa*, *Nicandra physaloides* and *Dodonaea viscose* were plant extracts against adult females of the pest *Tetranychus urticae* Koch (Acari: Tetranychidae). Recently, Hussein *et al.*, (2006) demonstrated that extracts of leaves and fruits of the plant *Capparis aegyptica* L. prepared from various solvents were affected the behavior, toxicity and fecundity of *T. urticae* females. Also, Shi *et al.*, (2006) indicated that extracts of an annual herbaceous plant, *Kochia scoparia* (L.) had both contact and systematic toxicity to *T. urticae*, *Tetranychus cinnabrinus* (Boisdaval) and *Tetranychus viennensis* Zacher (all Acari: Tetranychidae). Research has been done by Amer *et al.*, (1993), indicated

that the orange peel oil showed a remarkable deterrent effect with *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae) than *T. urticae*. Studies on plant extracts having acaricidal influences on the citrus brown mite *E. orientalis* are few. Among these plants, the essential oils from the family Labiatae such as *Majorana hortensis* Moench, *Rosmarinus officinalis* L., *Ocimum basilicum* L. and *Lavandula officinalis* Chaix (Amer *et al.*, 2001; Refaat *et al.*, 2002). *Francoeria crispa* (Forssk) (Family Compositae) is wildly grown plant in South and middle Sinai of Egypt. Its perennial bushy desert plant, often growing in cushion-shape, more branched near the lower part of the plant. The plant is used under the name of (El-Kayswam) in folk medicine for various purposes. *Francoeria crispa* has been a subject of interest to some investigators from the phytochemical point of view and in particular its terpenes content (Soliman *et al.*, 2003). They stated that the analysis of the crude extract of *F. crispa* characterize by presence of high concentration of total terpene compounds (89.776%), of which monoterpens accounted for 36.622% from the total. Soliman *et al.*, (2003) and *Amer et al.*, (2005) discussed the insecticidal and acaricidal activity of the crude extract from *F. crispa* on two serious pests, *Spoodoptera littoralis* (Boisd.) larvae (Lepidpoptera: Noctuidae) as well as eggs and females of the spider mite *T. urticae*.

Studies were implemented to determine the potential acaricidal activity of *F. crispa* against *E. orientalis* in the laboratory. The objectives were to 1) test crude extract of *F. crispa* for acaricidal activity and repellency, 2) test the various fractionations from ethyl acetate crude extract for acaricidal and ovicidal activity and 3) identification and determination of the main constituents of the most effective isolate on eggs and females of *E. orientalis*.

MATERIALS AND METHODS

Maintenance of mite stock cultures

The stock culture of the citrus brown mite, *E. orientalis*, was obtained from cultures maintained on sweet potato in the laboratory at National Research Centre, Cairo. The mites were reared in a controlled climate room at 25-27 C° and 65 ± 5% R.H.

Plant Material

The wild plant, *F. crispa* was collected from different areas in Middle and Southern Sinai. Plants were air dried under natural laboratory conditions for one week. Dried plants were ground using an electric mill, sieved and kept for extraction.

Extraction procedure

Preparation of the crude extract

Plant extract was prepared according to the method adopted by Su and Horvat (1981) with some modifications. Two hundred grams of powdered plant materials was extracted with organic solvent. Ethyl acetate was used, where the dried plant material was soaked in 100 ml. solvent at rate of 5 ml / gm. and kept for 48-hr. under laboratory conditions. The mixture was mechanically shaken for 8 hr. The extract was then filtered over anhydrous sodium sulphate and ethyl acetate was evaporated under reduced pressure

using a rotary evaporator at 40- 50 C° for dryness. The resulting crude extract was weighted and kept in a deep freezer till evaluation.

Toxicity effects of ethyl acetate crude extract of *F. crispa* to egg and female stages of *E. orientalis*

Ten females of *E. orientalis* were transferred to lower surface of raspberry leaf discs 5 cm in diameter and placed in Petri-dishes 10 cm in diameter containing saturated cotton wool, then left for oviposition 24 h. and removed thereafter. The accumulated eggs (0-24 h old) were sprayed with different concentrations of the extract using glass atomizer. The concentrations used are 0.0005, 0.00017, 0.000056 and 0.000019 g./ ml. Each concentration was replicated 5 times (20 eggs / replicate). The number of unhatched eggs was recorded after one week of treatment. A similar number of untreated eggs were included as a control. Adult females of the same age were sprayed with different concentrations from the extract. Females were confined on raspberry clean leaves (5 cm in dia.), while the upper surface were placed in Petri-dishes 10 cm in diameter containing cotton pad saturated with water. Mortality was recorded 48 hours after application. Five concentrations were tested and each concentration had 5 replicates (20 females / replicate). In every treatment, a control was included.

Repellency and oviposition deterrence test procedure for adult females of *E. orientalis*

Raspberry leaf discs (5 cm dia.) were placed with lower surfaces up in Petri dish 10 cm in diameter, containing moist cotton wool. One half of each disc was treated separately with different concentration (LC_{50} and less concentration), while the other half served as a control. Ten adult females of *E. orientalis* were placed in the center of each leaf. Each treatment comprised 5 replicates and each replicate contained 10 females. Orientation of the female's *E. orientalis* on treated and control discs was recorded after 4, 8, 16 and 24 hrs. after treatment. {Repellency = mites which had left the treated discs was considered as repelled}. The numbers of eggs laid on each half were recorded after 24 h.

Effects of LC_{50} concentration of *F. crispa* on fecundity and mortality of *E. orientalis* females

Newly emerged females were sprayed with LC_{50} of the crude extract using glass atomizer, and then transferred singly on clean raspberry leaf discs in Petri dishes. Twenty replicate leaf discs were used per each extract and similar number of untreated females was used as a control. The mortality and fecundity of females were recorded for 7 days. Reduction in the total number of *E. orientalis* eggs was calculated.

Fractionations of ethyl acetate crude extract of *F. crispa*

Column was used to separate the isolated fraction of ethyl acetate crude extract from *F. crispa* (Soliman *et al.*, 2003). A column of 2.3 cm in diameter X 50 cm in length, with 20 cm height silica gel and a 2 cm anhydrous sodium sulphate was used for each fraction. The fractions were collected every 10 ml. A sample of ethyl acetate extracts was directly applied on the column top and then eluted with the following solvent mixture: hexane / ethyl acetate 90: 10 – 80: 20 – 70: 30 – 60: 40 – 50: 50 – 40: 60 – 30: 70 –

20: 80 –and 10: 90% and methanol 100%. The fractions were collected and the solvent was evaporated till dryness and then tested.

Toxicity effects of various fractions of crude extract from *F. crispa* to eggs and females of *E. orientalis*

The collected fractions were tested for its toxicity against eggs and females of E. orientalis (treatment as described above).

Chemical analysis and identification of the most promising fraction of *F. crispa* tested above

The chemical composition of *F. crispa* was determined by (GCMS) gas chromatography- mass spectrometry technique, GC-MS finnigan mat SSQ 7000 Digital DEC 3000. work station: Digital DEC 3000. ionization mode Elven 70. Column: DB-5 capillary column 30 m in length, 0.32 mm i.d and 0.25 um thicknesses. Carrier gas: Helium at 13 psi. Temperature-programming initial column temperature was set at 50 °C for 3 min. then the temperature increased at 7 °C / min to reach 250 °C, and hold for 10 min. at 250 °C, the injector temperature was 200 °C and the injected volume was 1 ul. Transition- line and ion source temperatures were 250 °C and 150 °C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m / z 40 to m / z 350. ionization energy was set at 70 eV.

Identification was based on the comparison with the MS computer library (NIST- Software Package, Finnigan) and on the respective retention indices. The separated components were identified by matching with the National Institute of Standards and Technology (NIST) mass spectral library data with those of the published data by Adams (1995).

Statistical analysis and toxicity lines

1. Corrected mortality counts according to Abbott's formula (1925).
2. Mortality curves were drawn on Probit logarithmic graph paper according to the method developed by (Finney, 1971).
3. The repellency was calculated according to (Lwande *et al.*, 1985).
4. Biological data were analyzed statistically by t- test.
5. The oviposition deterrent indices (ODI) as defined by Lundgren (1975).

RESULTS

Toxicity effects of ethyl acetate crude extract of *F. crispa* to eggs and females of *E. orientalis*

Figure (1) shows the relation between the percentage of mortality and concentrations of ethyl acetate crude extract from *F. crispa* on eggs and females of *E. orientalis*. The data obtained in Figure (1) shows that the LC_{50} value of ethyl acetate crude extracts from *F. crispa* were similar in eggs and females of *E. orientalis* (LC_{50} = 0.0005 g / ml). The LC_{90} = of eggs and females was different (LC_{90} = 0.0049 and 0.021 g / ml. respectively).

Repellency and oviposition deterrence for adult females of *E. orientalis*

Table 1 show that females preferred to feed and deposit their eggs on untreated section. The repellency varied between (97.45 – 76.49 %). The average number of eggs laid by females *E. orientalis* after 24 h. of treatment varied according to the tested concentrations (Table 1)..

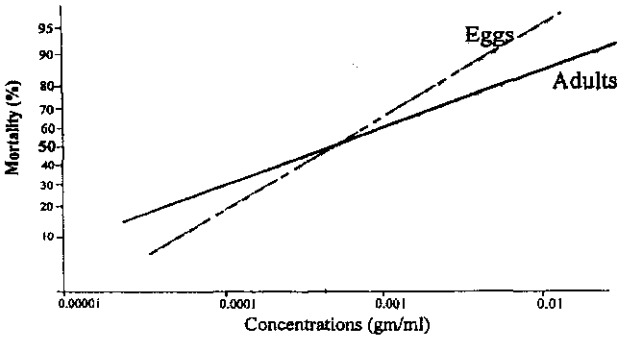

Fig. 1: Toxicity of ethyl acetate crude extracts of *Francoeria crispa* against eggs and adult females of *Eutetranychus orientalis*

Table (1): Relative percentage distribution and oviposition of *Eutetranychus orientalis* on treated leaf discs with *Francoeria crispa* crude extract at various concentration

g. / ml Concentration	% of mites distributed on treated plant after				ODI	No. of eggs deposited / ♀ after 24 hr.		% Repellency
	4 hr.	8 hr.	16 hr.	24 hr.		Treated	Control	
0.0005	5.43	6.32	8.25	9.00	95.03	0.08	3.14	97.45
0.00017	10.31	13.13	17.89	22.22	78.41	0.38	3.14	87.90
0.000056	21.05	23.16	23.16	28.42	64.85	0.58	2.72	78.68
0.000019	21.65	25.51	27.00	30.61	61.93	0.67	2.85	76.49

Effects of LC_{50} concentration of ethyl acetate crude extract from *F crispa* on fecundity and mortality of *E. orientalis*

Highly significant reduction in the total numbers of eggs laid by *E. orientalis* was recorded during 7 days period (Table 2). Percentage mortality after 7 days reached 100% when using LC_{50} concentration of ethyl acetate crude extract from *F. crispa*.

Toxicity of detected fractions of *F. crispa* extract to eggs and females of *E. orientalis*

The data presented in Figs (2 and 3) shows that the isolate number 10 was the most toxic fraction on females and eggs of *E. orientalis*, respectively. The corresponding values of LC_{50} were: 0.000125 and 0.00014 g / ml., while values of LC_{90} were: 0.00039 and0.0005 g/ml., respectively.

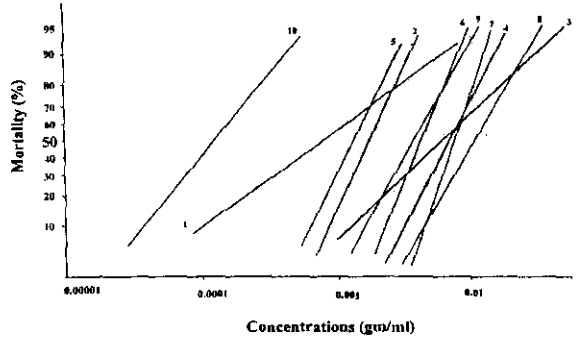

Fig. 2: Toxicity of various fractions (1-10) isolated from ethyl acetate crude extract of *Francoeria crispa* against adult females of *Eutetranychus orientalis*

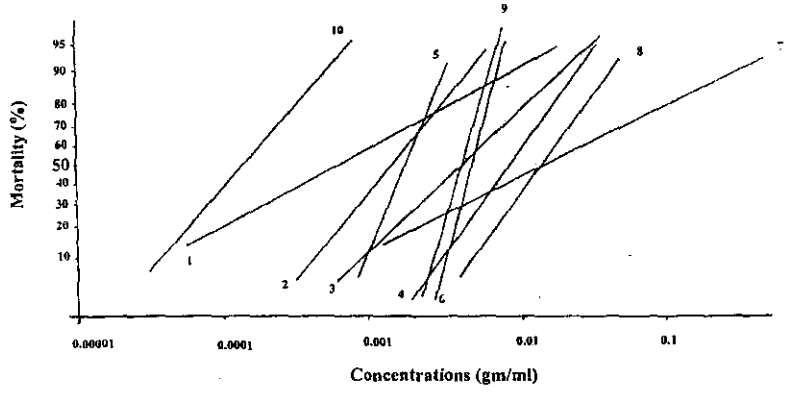

Fig. 3: Toxicity of various fractions (1-10) isolated from ethyl acetate crude extract of *Francoeria crispa* against eggs of *Eutetranychus orientalis*

Table (2) : Effect of LC_{50} concentration of the crude extract of *Francoeria crispa* on the fecundity and mortality of *Eutetranychus orientalis*

Treatment	No. of eggs / female / 7 days (mean ± S.E.)	% Reduction in no. of eggs / female / 7 days	% Mortality of females after 7 days	% Hatchability
LC_{50}	2.0 ± 0.16**	75	100	83.82
Control	14 ± 0.36	-	6.67	97.14

t (statistical value) = 30.716
** Highly significant

Chemical constituents of the main component of the tenth fraction from the ethyl acetate crude extract of *F. crispa*

The quantitative and qualitative analysis of the fraction no. 10 is presented in Table (3). Twenty four components were positively identified. The analysis showed that the isolate no. 10 was mainly characterized by high concentrations of total terpene compounds (75.45%) (Table 3). Six fatty acids amounted for 14.16% of the tenth isolate, which were identified as shown in Table (3). A. hydrocarbone was also identified in this fraction extract, the main of which is pentatricontene with area percent 8.83%.

Table (3): Chemical constituents of the main components of the (fraction no. 10) from the ethyl acetate crude extracts of *Francoria crispa*

Peak no.	Component	Area %	Peak no.	Component	Area %
	Terpenes			**Fatty acids**	
1	Champhor	3.49	11	Tetradecanoic acid	1.98
2	Pipertone	9.09	12	Pentadecanoic acid, 14-methyl, methyl ester	0.72
3	Carbamic acid, acetyl-, ethyle ester	2.93	13	Hexadecanoic acid (Palmitic acid)	4.19
4	1,3-Cyclopentadiene 5, 5-dimethyl ethyl	3.42	14	9, 12-Octadecanoic acid-methyl ester	2.18
5	2- Propenoic acid, 3-phenyl, ethyl ester	15.23	15	Ethyl linoleolate	3.54
6	∂- Cedrene	3.36	17	13-Octadecanoic acid, methyl ester	1.55
7	∂- Cedrol	4.66		**Total**	**14.16**
8	Δ- Cadinene	0.77			
9	Cinnamic acid ethyl ester	17.4			
10	Aminosalicylic acid	0.68		**Hydrocarbone**	
16	Phytol	0.58			
18	Estra- 1, 3, 5- (10), 16-tetraen-3-01	2.09	24	Pentatricontene	8.83
19	8- Hexadecene	0.59		**Unknown**	**1.56**
20	Hexadecane, 1-Chlorotricosane	4.09			
21	Cycloisolongifolene, 9, 10-dehydro	0.84			
22	Retinal	2.79			
23	∞- Necrodol	3.44			
	Total	75.45			

DISCUSSION

The citrus brown mite *E. orientalis* is a common spider mite and main a serious pest in citrus orchards in Egypt. The mite generally feed underneath the leaves and cause graying of the leaves due to mesophyll collapse and yellowing. Necrotic spots occur in the advanced stages of the leaf damage. To develop a botanical miticide, the supply of source plant is obviously crucial, and ecological important and toxicity to mammals and predacious mites are other factors that need be considered. The wide distribution of this plant in Middle and Southern Sinai should enable easy collection and propagation if it is used for the production of botanical insecticides and miticides (Soliman *et al.*, 2003; Amer *et al.*, 2005). Significantly, our results are the second to demonstrate acaricidal activity of *F crispa*.

GC-MS analysis of the ethyl acetate crude extract of *F. crispa* was reported by Soliman *et al.* (2003). They found that forty six compounds (comprising 96.52% of the crude extract) were identified, and the crude extract was mainly characterized by high concentration of total terpene compounds (89.778%). Amer *et al.* (1993) demonstrated that the orange peel oil showed a deterrent effect, with *E. orientalis*, whereas the essential lemon grass oil and above one were toxic to females and eggs of both tetranychid mites *T. urticae* and *E. orientalis*.

In the present study, a higher percentage of repellency was recorded at the Lc_{50} concentration (0.0005 grm. / ml; also a lower frequency of oviposition occurred following contact with leaf discs treated with the extract. These results would indicate rejection in response to a contact stimulus and this is in coincided with data obtained by Renwick and Radke (1982) that used cabbage extract in deterring oviposition by the cabbage lopper *Trichoplusia ni*. Similarly, research has been done by Momen *et al.* (2001), Amer *et al.* (2001) and Refaat *et al.* (2002) revealed that the essential oil of (mint, peppermint, rosemary, sweet marjoram, sweet basil and French lavender) deterred females of *T. urticae* and *E. orientalis* from settlement and feeding on treated parts.

Females of *E. orientalis* treated with Lc_{50} of the crude extract of *F. crispa* showed a high significant reduction in the number of eggs laid over 7 days. The reasons explaining this phenomenon may be it is due to direct influence of the substance on the female ovaries or it is also possible, that after contact application of any substance on the cuticle of the pest, the production of pheromones is disturbed.

Refaat *et al.* (2002) demonstrated that a higher percentage of *E. orientalis* mortality was recorded for both essential oil *O. basilicum* and *L.* officinalis than *T. urticae*. Also, Amer *et al.* (2005) reported that the adult females of *T. urticae* were sensitive to crude ethyl acetate extract of *F. crispa* than the eggs. (Lc_{50} = 0.04 and 0.05 gm / ml), respectively. They also demonstrated that the crude extract of *F. crispa* has high sterility effect on females of *T. urticae* (86.34%).

The mode of action of the acaricides in *F. crispa* is unknown, but the extract exhibited both contact and systemic toxicity to tetranychid mites, *T. urtica* and *E. orientalis* (Amer *et al.*, 2005; present study). However, though

many plants in the world possess insecticidal properties, only a few have demonstrated acaricidal activity (Prakash and Rao, 1997). Using active components from several plants in a miticide preparation could potentially increase efficacy and reduce the potential for development of resistance in mite population.

CONCLUSION

In integrated control programmed, much information is needed on the effect of the crude extract and its fractions on predatory mites of these pests (*T. urticae* and *E. orientalis*), so a careful choice of plant extract should be made to harm predator or parasite as little as possible. The work presented here based on laboratory conditions, however, care should be taken in translating results of laboratory to glass house or to open field.

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التأثير الطارد والسام لمستخلص نبات القيسوم (فراكوريا كريسبا) على اكاروس الموالح البني (ايوتيترانيكس اورينتاليس)

أميرة عبد المنعم عبد الخالق ، سناء عبد الرحمن عبدالله عامر و فاتن ممدوح مؤمن
قسم افات ووقاية النبات - المركز القومي للبحوث – الدقي – القاهرة

تم اختبار مستخلص الاثيل أسيتات لنبات القيسوم لدراسه تأثيره على عنكبوت الموالح البني *Eutetranychus orientalis* ومعرفة تأثير المستخلص الأولي لنبات القيسوم و تم اختبار سمية المستخلص على بيض و اناث الآفة *E. orientalis* .

أظهرت النتائج أن لمستخلص القيسوم الاثيلي تأثير على سلوك وخصوبة الاناث تحت الظروف المعملية ، و كذلك كان للمستخلص سمية مماثلة على كل من بيض واناث *E. orientalis* وكان التركيز النصفي القاتل من المبيد (Lc_{50} = 0.00050) .

أثبتت النائج أنه عند معاملة الأقراص النباتية بتركيزات متزايدة من التركيز النصفي القاتل (Lc_{50})من المبيد أظهر تأثير طارد عالي (97.45 %) . وأظهرت كذلك أن الاناث المعاملة بتركيز (Lc_{50}) من مستخلص اثيل اسيتات القيسوم أظهرت أعلى نسبة موت و أقل عدد من البيض الوضوع خلال سبعة أيام .

تم فصل عشرة مركبات من مستخلص أسيتات القيسوم وأظهرت النتائج أن المركب العاشر كان أكثر المركبات سمية على بيض واناث *E. orientalis* وكان التركيز النصفي القاتل من المبيد (Lc_{50} = 0.00014 and 0.000125 g / ml.) على التوالي .

قام بتحكيم البحث
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