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ACARICIDAL ACTIVITY OF ESSENTIAL OIL OF LEMONGRASS, *Chymbopogon citratus* (DC.) STAPF AGAINST *Tetranychus urticae* KOCH

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ABSTRACT

The chemical constituents of the essential oil of lemongrass, *Chymbopogon citratus* that collected from Sharquia Governorate, Egypt, were determined by GC-MS analysis. Geranial and neral were the basic constituents in the essential oil that recorded (48.692 and 34.137%, respectively). Moreover, the acaricidal and repellent activity of the essential oil were evaluated against *T. urticae* under laboratory conditions. According to LC_{50} and LC_{90}, the lemongrass oil was more toxic against adults of *T. urticae* using spraying method than leaf dip technique method (direct feeding). Moreover, all the tested concentrations of the essential oil significantly reduced hatchability percentages of *T. urticae* eggs than control, that recorded (85.33 ± 3.72, 77.33 ± 3.76, 57.33 ± 3.59 and 33.33 ± 3.01%) for (0.25, 0.50, 1.00 and 2.00 %, respectively). Control gave 96.00 ± 0.00%. Also, the higher used concentration of lemongrass oil, recorded the highest significant repellency percentages against adults of *T. urticae* (79.96 ± 3.44%). While the lower one gave the least significant decrease (46.32 ± 2.61%).

Keywords: *Chymbopogon citratus*, lemongrass, chemical constituents, essential oil, *Tetranychus urticae*

INTRODUCTION

Two spotted spider mite, *Tetranychus urticae* Koch, is one of the most important and highly polyphagous pest of wide range of field crops (Zhang, 2003). The greatest problem with this mite is its ability to rapidly acquired resistance to pesticides (Cranham and Helle, 1985).

Insect Pest Management (IPM) has to face up to the economic and ecological consequences of the use of pest control measures. Sixty years of sustained struggle against harmful insect synthetic insecticides, that produced perverse secondary effects (mammalian toxicity, insect resistance and ecological hazards). The diversification of the approach inherent in IPM is necessary for better environmental protection. Among the alternative strategies, the use of plants insecticidal allelochemicals appears to be promising (Roger, 1997). Aromatic plants and their essential oils are among the most efficient botanicals, which obtained through stream distillation of herbs and medicinal plants (Yatagai, 1997). Most of these oils are environmentally nonresistant and nontoxic to humans (with some exceptions) (Cockayne and Gawkrodger, 1997) and wildlife (Kumar *et al*., 2000). Their activities are manifold. They induce topical toxicity and fumigant as well as repellent or ovicidal effects.

Lemongrass, *Chymbopogon citratus* is a plant in the grass family that contains 1 to 2% essential oil on a dry basis (Carlson *et al*., 2001). The chemical composition of lemongrass essential oil can vary widely as a function of genetic diversity, habitat and agronomic treatment of the culture (Ferrua *et al*., 1994). Thus, in this study, the chemical composition, acricidal and repellent activities of lemongrass, *Chymbopogon citratus* essential oil against *T. urticae* were assessed.

MATERIALS AND METHODS

Plant materials and isolation of essential oil:

Essential oil was extracted from the leaves of lemongrass, *Chymbopogon citratus* Stapf that collected from Sharquia Governorate, Egypt. The essential oil was extracted by steam distillation for 4-6 h, using a clevenger-type apparatus where 300 gm of plant materials in 300 ml of water subjected to hydro distillation (Marcus and Lichtenstein, 1979 and Weaver *et al*., 1994). The oil was separated, dried over anhydrous sodium sulfate and stored in dark glass bottles at 4 ºC in the refrigerator until used. The isolated oil has a pale yellow color and strong odor.

GC-MS analysis of essential oil:

The constituents and identification of oil constituent's analysis were performed using a Hewlett Packard gas chromatography coupled to mass spectrometry (GC-MS analysis) in National Research Center, Cairo, Egypt according to the method of Likens and Nickerson (1966) and Benhard *et al.* (1983).

Rearing technique:

Samples of mulberry, *Morus alba* L. leaves heavily infested with *T. urticae* were collected from Zagazig district, Sharquia Governorate. Pure culture of *T. urticae* was initiated by transferring males and females using a fine hairbrush to fresh discs of mulberry leaves in Petri-dishes (10 cm in diameter). Each leaf was put on a pad of cotton saturated with water as a source of moisture and to prevent mite escaping, under laboratory conditions of 27 ± 2 ºC and 75 ± 5% R. H.

Acaricidal activity of essential oil of lemongrass, *C. citratus* against adult females of *T. urticae* using two different methods:

To evaluate the toxic effects of *C. citratus* essential oil on the adult females of *T. urticae*, two different methods were used, leaf dip technique (feeding on treated leaves) and spraying technique. Four tested different concentrations of *C. citratus* oil (0.25, 0.50, 1.00 and 2.00 %) were used in both methods that prepared using ethyl alcohol (95%) as solvent. Each concentration and control were replicated 5 times using fifteen adult females for each replicate. Control disks were prepared in both methods using ethyl alcohol.

Fifteen females of *T. urticae* were confined on the lower surfaces of mulberry leaf discs (3 cm. in diameter) which were dipped in tested concentrations of each treatment for 10 seconds using the leaf-dip technique method as described by Dittrich (1962). In the second method, the tested concentrations were sprayed on both mulberry leaves and adults of *T. urticae.* Mortality was calculated after 24hr post treatment. Mortality was corrected according to Abbott's formula (1925) and statistically analyzed by

Finney (1971), dose-mortality data (LC) were evaluated using log-probit software program Ldp Line® model "Ehabsoft" (Bakr, 2000). The toxic index of each compound was determined according to Sun (1950).

Acaricidal effects of essential oil of lemongrass on eggs of *T. urticae*:

The toxic effects on the egg stage was studied by confining four females that transferred to each leaf disc of mulberry plant using fine hairbrush (2.5 cm. in diameter) for 24 hr to deposit eggs on the lower surface of leaf discs, and then removed. These discs with deposited eggs (24 hrs. old) were dipped in the same precedent concentrations for 10 seconds and the excess solution was dried off by filter paper. Each concentration and control were replicated 3 times (50 eggs per each). Control was prepared using ethyl alcohol. Eggs were kept under laboratory conditions and hatchability of eggs on each concentration was recorded till 6 days post treatment.

Repellent effects of essential oil of *C. citrates* on adult females of *T. urticae*:

The repellency effect of *C. citratus* oil was estimated against *T. urticae* according to the method of Sawires (1992). Mulberry leaves were cut into discs (5 cm. in diameter) of symmetrical portion along the midrib obtained per each disc. On half portion of the disc was dipped in tested concentration and the other half was dipped in ethyl alcohol (95%) as solvent.

The treated discs were left to dry and put on moistened cotton wool in Petri-dishes. The adult females were transferred on the mid rib of each disc. The mites left move freely across the two portions of the disc then counted after 24, 48 and 72 hr. Four discs were used as replicates, the repellency percentages were computed according to El-Halawany *et al*. (1986).

Statistical analysis:

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) (Snedecor & Cochran 1980). Data were subjected to statistical analyses using a software package CoStat® Statistical Software (2005) a product of Cohort Software, Monterey, California.

RESULTS AND DISCUSSION

Chemical constituents of essential oil of lemongrass, *Chymbopogon citratus*:

Data in Table (1) indicated that the basic constituents of lemongrass, *C. citratus* oil were geranial (citral a) and Neral (citral b) which represented (48.692 and 34.137%, repectively). Followed by myrcene (13.365%), linalool (1.459%), limonene (1.128%), geraniol (0.770%) and finally, geranyl acetate (0.449%).

Table (1): Essential oil constituents extracted from *C. citratus*.

Essential oil composition	Relative % abundance	Retention Time (RT)
Myrcene	13.365	3.619
Limonene	1.128	5.240
Geraniol	0.770	6.107
Linalool	1.459	6.389
Neral	34.137	7.527
Geranial	48.692	8.013
Geranyl acetate	0.449	9.496

Acaricidal activity of *C. citratus* essential oil against *T. urtica*e (Koch):

According to LC_{50} and LC_{90} values, using the essential oil in the spraying method was more toxic than leaf-dip technique method (feeding on treated leaves) against *T. urticae* adults which recorded (0.4313 and 2.6520 %, respectively) comparing to (1.0252 and 4.2343 %) for the second method, respectively, (Table, 2).

Table (2): Acaricidal activity of lemongrass essential oil against *T.urticae* adults.

Tested methods	LC_{50} % (Lower-upper)	LC_{90} % (Lower-upper)	Slope	Toxicity index at LC_{50}
Spraying	0.4313 (0.2944 - 0.5639)	2.6520 (1.7092 – 6.2243	0.295	100
Leaf-dip technique	1.0252 (0.7721 - 1.4909)	4.2343 (2.7719 – 8.8774)	0.313	42.07

Acaricidal effects of the essential oil on the hatchability percentages of *T. urticae*:

All the tested concentrations of *C. citratus* oil significantly reduced the hatchability percentages of *T. urticae* eggs than control, (Table, 3).

Table (3): Effect of lemongrass essential oil on the hatchability percentage of *T. urticae.*

Tested concentrations (%)	Hatchability% (Average ± S. E.)	LC50 % (Lower-upper)	LC90 % (Lower-upper)	Slope
0.25	85.33 ± 3.72 **b**	1.2999 (1.0057 -1.8807)	6.2690 (4.2648 -1.04445)	0.201
0.50	77.33 ± 3.76 **b**			
1.00	57.33 ± 3.95 **c**			
2.00	33.33 ± 3.01 **d**			
Control	96.00 ± 0.00 **a**			
LSD $_{0.05}$	10.214			

Generally, the higher the concentration the lesser the hatchability percentages, that recorded (85.33 ± 3.72, 77.33 ± 3.76, 57.33 ± 3.95 and 33.33 ± 3.01) for (0.25, 0.50, 1.00 and 2.00 % concentrations), respectively. Control gave 96.00 ± 0.00% hatchability. In addition, the values of LC_{50} and LC_{90} were recorded 1.2999 and 6.2690 % respectively.

Repellent effects of the essential oil of lemongrass against *T. urticae*:

Results obtained in Fig. (1) showed that the repellency percentages of different tested concentrations of *C. citratus* oil on the adult females of *T. urticae*, it decreased gradually by time elapsed. The higher repellency percentages were observed after 24 hr of treatment that ranged between 91.30 ± 5.61 at the concentration 2.00 % to 57.14 ± 3.57 at 0.25 %, whereas, ranged between (83.72 ± 4.82 to 48.48 ± 2.89%), respectively after 48 hrs. post treatment. The higher tested concentration the highest repellency (64.87 ± 3.49%) after 72 hrs of treatment followed desendingly by the remaining tested concentrations that gave (52.94 ± 2.84, 61.11 4.83 and 33.33 ± 2.03%), respectively. The average repellency percentages were recorded (79.96 ± 3.44, 69.66 ± 4.25, 67.11 ± 3.40 and 46.32 ± 2.61%) for concentrations 2.00, 1.00, 0.50 and 0.25 %, respectively.

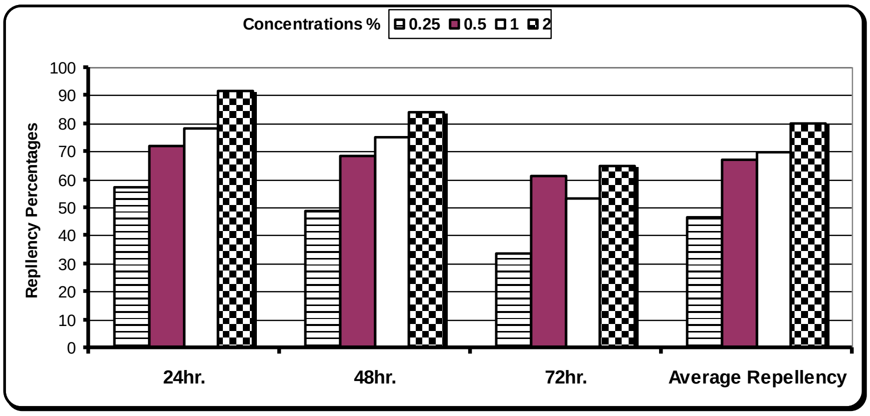

Fig. (1): Repellent effects of lemongrass essential oil against adult females of *T. urticae.*

Independently of the origin place, the predominant compound (30 to 93.74%) of the lemongrass essential oil obtained is the citral (mixture of the aldehydes neral and geranial, with general predominance of this last one) (Negrelle and Gomes, 2007) that responsible for the lemon smell which characterizes the species (Saito and Scramin, 2000). Furthermore, myrcene is a characteristic compound of lemongrass (Miyazaki *et al.*, 1970). The amounts of this compound are highly variable (2 to 25.30%), whereas limonene is one of the most frequent monoterpenes, which isolated in concentrations between (0.3 and 5%) (Cicogna Junior *et al*., 1986 – 1987 and Chisowa *et al*., 1998).

The essential oil abundant in lemongrass is very well known by their bactericideal and fungicidal properties (Guenther, 1950). According to Onawunmi *et al.* (1984), the geranial and neral compounds existent in the essential oil also present positive antimicrobian effect and the myrcene reinforces this effect when mixed to one of such compounds. Consequently, the toxic effect of *C. citratus* essential oil against *T. urticae* was undoubtedly due to citral (geranial and neral).

Based on LC_{50} and LC_{90}, the essential oil was more toxic against adults of *T. urticae* using spraying method than leaf dip technique method. In the spraying method, adults of *T. urticae* vulnerable to not only feeding on treated leaves but also to direct contact with lemongrass, *Chymbopogon citratus* essential oil that penetrate through its cuticle, thus increase the toxicity more than leaf dip technique. In another study, Sim *et al*. (2006) examined both direct contact and vapor phase toxicity of 44 plant essential oils, including rosemary against almond moth, *C. cautella* larvae. They found rosemary oil very effective as both contact and fumigant toxicant. Additionally, Miresmailli and Isman (2006) found that rosemary oil was toxic to *T. urticae* as a contact toxicant. Also Choi *et al*. (2004) reported that, among the 53 essential oils of carway seed, citronella, lemon, eucalyptus, pennyroyal and peppermint were found to be highly toxic to both mite species, *T. urticae* and *P. persimilis*.

As a general trend, all the tested concentrations of the lemongrass oil caused significant reduction in the hatchability percentages of *T. urticae* eggs than control.

The marked decline in egg hatchability may be due to diffuse of oil vapors into eggs and affected the physiological and biochemical process associated with embryonic development (Raja *et al*., 2001). These findings corroborate the observations recorded for peppermint and citronella oil vapors on *Earias vittella* eggs (Marimuthu *et al*., 1997). Recently, the toxic and oviposition deterring activities of three essential oils from *Laurus nobilis, Myrtus communis* and *Artemisia absinthum* were studied by (Topuz and Erler, 2007) against the carmine spider mite, *T. cinnabarnius* under laboratory conditions. All three essential oil were found to be toxic to adults and eggs of the mite, but to variable degrees.

Generally, all the used concentrations of *C. citratus* oil found repellence to *T. urticae* than the control at all inspected times. Comparison among treatments revealed significant difference between the higher concentration and other treatments. Minimal repellent effect was observed for the lowest concentration as it expressed significantly lower repellency than the rest treatments. Leal and Unchida (1998) identified geranial as the repellent active compound of *C. citratus* against mosquitoes.

According to the result of Mansour *et al*., (1986), different concentrations of acetonic solutions of the essential oils from 14 species of Labiatae oils caused mortality and induced repellency in adult females of carmine spider mite, *T. cinnabarinus*. Miresmailli and Isman (2006) reported that rosemary oil repels two spotted spider mite, *T. urticae* and can affect oviposition behavior. Among the plant families with promising essential oils used as repellents, *Cymbopogon* spp., *Ocimum* spp. and *Eucalyptus* spp. to control insects and arthropods (Nerio *et al*., 2010).

In conclusion, natural miticide of essential oil extracted from *C. citratus* is a desirable alternative to synthetic miticides because they have low toxicity on mammals, little environmental effect and wide public acceptance. The experimental results suggest that the essential oil has the potential for use in the control of *T. urticae*. The lemongrass essential oil could be considered as potential alternatives for synthetic miticides as their structures could lead to the development of new classes of miticideal compounds. However, further studies need to be conducted to evaluate the mode of action and cost-effiecincy of the essential oil on wide range of pests.

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النشاط الابادى الأكاروسى للزيت العطرى لحشيشة الليمون على الحلم العنكبوتى ذو البقعتين

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تم تحديد المكونات الكيمائية للزيت العطرى الطيار لحشيشة الليمون و التى تم تجميعها من محافظة الشرقية بجمهورية مصر العربية وذلك باستخدام تحليل جى سي ماس و كان مركبى الجيرانيال و النيرال هما المكونان الأساسيان لهذا الزيت و سجلوا نسبة تواجد 48.692 و 34.137٪ على الترتيب، كما تم تقييم التأثير الأبادى و الطارد لهذا الزيت على اكاروس الحلم العنكبوتى ذو البقعتين تحت الظروف المعملية و طبقاً لقيمة التركيزين القاتلين لـ 50 و 90٪ من التعداد كان الزيت العطرى لحشيشة الليمون أكثر سمية على الأفراد الكاملة من العنكبوت وذلك باستخدام طريقة الرش عن طريقة التغذية المباشرة للأفراد، علاوة على ذلك فقد أحدثت كل التركيزات المستخدمة خفضاً معنوياً فى نسب فقس بيض العنكبوت عن المقارنة حيث سجلوا (3.72±85.33 و33 .77±3.76 و 57.33±3.59 و 33.33±3.01٪)على الترتيب فى حين سجلت المقارنة 96.00%. و لقد أعطى أعلى تركيز مستخدم من الزيت العطرى لحشيشة الليمون اعلى زيادة معنوية فى نسب الطرد على الأفراد الكاملة للحلم العنكبوتى (79.96± 3.44 ٪)بينما سجل اقل تركيز مستخدم اقل نسبة خفض معنوى (46.32± 2.61٪)

قام بتحكيم البحث

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