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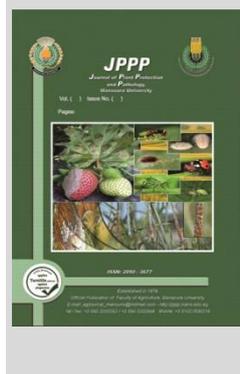
Laboratory and Semi - Field Evaluation and Effect of Clove Essential - Oil against *Two - Spotted Spider - Mite Tetranychus urticae*, Koch. (Acari:Tetranychidae)

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ABSTRACT

The Effects of clove, *Syzygium aromaticum* essential-oil against spider-mite *Tetranychus urticae*, Koch was estimated in lab and semi-field conditions. Treated adult-females conducted by different concentrations of clove essential oil (eugenol) concentrations using spraying method for 1, 3 and 7 days. Mortality was measured upon treatment with three concentrations of 0.25, 0.50 and 1.0% V/V. Female mortality increasing with increasing concentrations and LC₅₀ value was calculated. Moreover, all the tested concentrations of the essential oil were significant. The effect of LC₅₀ of eugenol on the neuronal-enzymes acetylcholine-esterase "AChE" and alkaline-phosphatase "ALP" of spider-mites were investigated on 24 hours and 3 days post-treatment. The obtained data resulted the activity of "AChE" and "ALP" showed decreasing after treatment on 24 hours by clove-oil while, "ALP" showed increasing on 3-days post-treatment. The evaluation of clove essential oil effect in semi-field condition on "*T. urticae*", results indicated that it gave satisfying results whereas 92 % population reduction respectively. Finally, we can conclude that clove essential oil was achieved good results to control this spider mites.

Keywords: *Tetranychus urticae* clove oil, acetylcholine esterase, alkaline phosphatase, semi-field.

INTRODUCTION

Most of foods and ornamentals crops losses contributed to infest by two-spotted spider-mite "*Tetranychus urticae*" Koch., and this mites are classified as a dangerous polyphagous-species attacks major of economical agricultural-crops, Jepson, *et. al.*, (1975) and Helle, & Sabelis (1985).

The classical acari-cides with repeated applications usually used in population control against spider-mite "*T. urticae*" Anonymous, (2003), and the biological-control-systems with repeated applications were very effective and it was used a long time ago in spider-mite suppression, Lee (1990), even though the results of resistance and development sometimes, Lee and Yoo (1971) and Song *et. al.* (1995). Therefore, effective modern alternatives that are not harmful to the environment must be found to replace classic-pesticides and have the ability to suppress this pest.

The current work the treated extraction of essential-oils extracted from aromatic-plants was assessment as a alternatives friendly-conditions to replace for classic-pesticides. Commercially, the tested essential-oils used in five-main aspects.. e.g. aromatic-scents and flavors, In the field of pharmaceutical, pesti-cides and acari-cides Kim *et. al.* (2004), also they discovered the recent the essential-oils used in many fields and more attention as antifungal; antitumor; antimicrobial; insecticidal and acaricidal, De Souza *et. al.* (2005) and Han *et. al.* (2010).

The "Acetylcholinesterase" responsible to transport of "Cholinesterase" in the nervous-system, Srivastava and Singh (2015).

In current work were conducted to evaluate the effect of "eugenol" against two spotted spider-mite "*T. urticae*".

MATERIALS AND METHODS

Mite Rearing:

In this study, spider-mite "*T. urticae*" as a original-colony was obtained and supply from Dept. of Acarology-lab at Plant-Protection-Research-Institute, Agric. Res. Center, Dokki, Giza Governorate. The rearing of mites was continues as a experimental-mite at "25±1°C" throughout the lon time (many years) and protects it by insecticides or any chemical contaminations. Tested spider-mites was located on detached-cucumber-leaves with lower-surface up wards was located on moist-cotton-wool-pads in fiber dishes, 20cm diameter. To prevent the mite escape, the cotton-pads were moistened every day. The used of cucumber-leaves replaced by fresh-one when required in time to time, Hassan (2003).

Study of toxicity on eugenol properties:

Has been obtained of "Eugenol" from recognized-distributor, El-Gomhouria Pharmaceutical company in Cairo.

Bioassay:

Twenty of same age of adult-females "*T. urticae*" transmitted on cucumber-leaf-disc, diameter 5cm., to tested of clover-oil toxicity against spider-mites. Treatments were replicated three times. The disc surfaces carrying the individuals of the same age were sprayed with three concentrations of eugenol. Percentage of mortality was determined daily after treatment till the seventh day. Data obtained (%mortality) were corrected according-to "Abbott's formula" Abbott, (1925). The values of (LC₂₅, LC₅₀, LC₉₀ and slope) calculated according-to Finney, (1971), by using of (Ldp-line-software) Bakr (2000).

Biochemical studies:

Eugenol effect (LC₅₀) was determined on neuronal-enzymes to determine physiological-impacts against "*T.*

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urticae” Acetylcholine-esterase “AchE” and Alkaline-phosphatase “ALP” were calculated after treatment on one and three-days comparison with control.

Preparation of samples:

Treated-mites were prepared after one and three-days after treatments to describe according to “Amin, 1998”. The treated-mites were homogenized in distilled-water 50mg/1ml., and the homogenates were centrifuged in a refrigerated-centrifuge with “8000/r.p.m.” for time “15min” at temperature degree “2°C”. Deposit and supernatant were discarded; it’s called with enzyme-extract which can-be stored at 50°C for one-week at-least without appreciable-losses activity.

Acetylcholine-esterase “AchE” determination:

Activity measured of Acetylcholine-esterase “AchE” were estimated according-to Simpson-method, (Simpson *et. al.* 1964), by using the substrate Acetylcholine-bromide “AchBr”.

Activity determination of Alkaline-phosphatase “ALP”:

Determination of acids and alkaline-phosphatases estimated according-to Powell and Smith method (Powell and Smith, 1954).

Statistics:

The obtained data calculated statistically and analyzed by “ANOVA” and the least-significant-differences “LSD” at P<0.05, with Costat-program, (Cohort 2005). The Henderson-Tilton’s formula were used for estimate the pesticide-efficacy “Ef%”.

$$Ef\% = \left[1 - \left(\frac{n \text{ in co before treatment} \times n \text{ in T after treatment}}{n \text{ in co after treatment} \times n \text{ in T before treatment}} \right) \right] \times 100$$

Where

n = the number of living mites

t = treated

co = control

Semi field experiments:

Cucumber (*Cucumis sativus* L.) plants were used as the host plant in the laboratory. Cucumber were prepared for planting in pots in open space and then transferred to the laboratory. Tested-plants isolated from each-others to stop mites touching and drift. Plants were infested by experimental mites (20 female/plant). After mite’s number reached to 3:4 in one inch, the toxicity of three different concentrations of clove-oil were examined. In the control distilled-water used and each-treatment was repeated four-times, and 10-leaves were taken from each-replicate and mite’s-numbers “immature and adults” counted by use of “stereomicroscope” before-treatments and at 1, 3, 7-days after-treatment.

RESULTS AND DISCUSSION

Laboratory evaluation of clove essential oil against *T. urticae* showed that, mortality was high at 24 hrs. and three days after treatment . No effect on mortality percentage was observed after seven days post-treatment. No mortality was noticed in control. The calculated LC₅₀ after 7 days of treatment was 0.203 % V/V results illustrated in Fig (1) and Table (1), results showed that %mortality of adult-females “*T. urticae*” increased with concentrations increasing when treated the clove essential oil .

Effect on the neuronal enzymes.

The Acetylcholine-esterase “AchE” and the Alkaline-phosphatase “ALP” are influential and decisive on nervous-tissues, Srivastava, & Singh, (2015). Data in Table (2), showed that, LC₅₀ effects on “*Syzygium aromaticum*” .

The essential-oil on Acetylcholine-esterase “AchE” and Alkaline-phosphatase “ALP” data obtained reported that, levels decreased for the Acetylcholine-esterase “AchE” with significant at three-days after treatments when compared-with the control, but, the levels of Alkaline-phosphatase “ALP” were increasing again. Throughout previous obtained data cleared that, the clove-essential-oil caused discouragements for Acetylcholine-esterase “AchE” and highly increasing for Alkaline-phosphatase “ALP”.

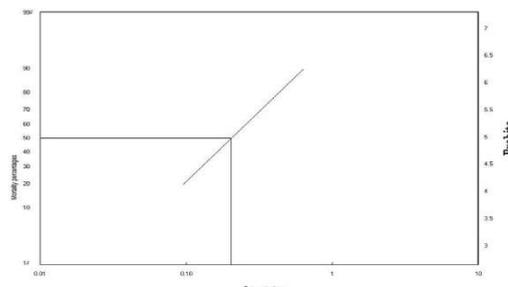


Fig. 1. Toxicity line for clove essential oil against *Tetranychus urticae* adult females.

Table 1. Toxicity of clove-essential-oil on adult-females of “*Tetranychus urticae*”.

Clove-essential-oil concentrations	Mortality%	LC ₅₀ %	LC ₉₀ %	Slope
0.25	61.6			
0.5	80	0.203	0.6386	2.5745
1	98			

Enzymes effects on nervous-tissues.

Acetylcholine-esterase “AchE” and Alkaline-phosphatase “ALP” were influential and decisive as a neuronal-enzymes, Srivastava, & Singh, (2015). Data presented in Table (2), cleared that, the LC₅₀ effects on *Syzygium aromaticum*.

The essential-oil on Acetylcholine-esterase “AchE” and Alkaline-phosphatase “ALP” data obtained reported that, levels decreased for the Acetylcholine-esterase “AchE” with significant at three-days after treatments when compared-with the control, but, the levels of Alkaline-phosphatase “ALP” were increasing again. Throughout previous obtained data assured that, the “eugenol” caused discouragements for Acetylcholine-esterase “AchE” and highly increasing for Alkaline-phosphatase “ALP”.

Table 2. LC₅₀ effects of clove-essential-oil on neuronal-enzymes against “*T. urticae*” after different periods

Hours	AchE. means±SD	ALP.means±SD
Cont.	57.9± 1.95 ^a	14.94±0.36 ^{ab}
24	36.36±2.50 ^c	14.52±0.47 ^b
72	50.63±1.40 ^b	17.32±2.05 ^a
L.S.D.	3.99	2.46

The means followed-by same letter “s” in same the column doesn’t significantly differs at P<0.05.

AchE= Acetylcholine esterase. ALP= Alkaline phosphatase

Effect of clove oil on *T. urticae* population

Results in Table (3) showed that, the percentages of reduction in the two-spotted spider mites population on cucumber seedlings after different intervals following treatment with three concentrations of clove oil. It is clearly shown that the percentage of reduction increased as clove oil concentration increased at each investigation date. The percentage of reduction after 1,3and7 days from treatment with 0 .25, 0.5 and 1% of clove oil were 39, 52 and 66.5%; 62.9, 72.8 and 81.2 %; 72.66, 76.2 and 92.9 %, respectively.

Table 3. Effect of clove oil against *T. urticae* population on cucumber under semi-field conditions

Conc. V/V	Pre-treatment	1 day		3 day		7 day	
		No.	Reduction%	No.	Reduction%	No.	Reduction%
0.25%	272	177	39	157	52	230	66.5
0.50%	236	93	62.9	78	72.8	112	81.2
1%	283	78	72.66	82	76.2	51	92.9
Control	230	244		279		580	

From the achieved results, this material could be used in integrated pest management programs after successful work under field conditions is reached.

Isman, (2000), demonstrated that, the insecticides based-on plant-essential-oils or their constituents illustrated that, the effects on major pests of stored-products, domestic-pests, blood-feeding-pests, some soft-bodied-agricultural-pests, and several plant-pathogenic-fungi are accountable about of diseases for pre&post-harvest.

Barua Somnath *et al.* (2015), mentioned that, the mortalities% of "*Oligonychus coffeae*" was different according-to "concentrations" and "exposure-time-duration" for spider-mites after oil-applications. The deposition-eggs-rates of spider-mites on treated-leaf-surfaces and the viable-eggs-rates were significant decreasing. In-addition-to, some tested-concentrations for clove-oil were proven effect on adult-mites.

The acaricidal characteristics illustrated that, the "eugenol" and "eugenol-analogues" illustrated Confirmation-promise and leads-to newly-developments of alternative-topical-acaricides to scabies-control, Pasay *et al.* (2010).

Abo-El-Saad *et al.* (2011), reported that, the oil-extracted from clove-buds were highly-effect on "*Tribolium castaneum*".

In spite of, the best realizations at the pure-constituent-levels along with relationships of structure-activities are very vital-to "*S. aromaticum*" develop as a oil-fumigant or oil-repellent-agent on insect-pests of stored-products.

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تقييم معلمي وشبه حقتي للزيت الأساسي بالقرنفل وتأثيره على الحلم العنكبوتي ذي البقعتين - Two-Spotted Spider-mite *Tetranychus urticae*, Koch.

رانيا حسن محمود حسن و ايناس مصطفى قطب قاسم

مركز البحوث الزراعية ، معهد بحوث وقاية النباتات ، دقي ، جيزة ، مصر

تمت دراسة تأثير زيت القرنفل العطري ضد الحلم العنكبوتي ذي البقعتين تحت الظروف المعملية وشبه الحقلية. حيث تمت معاملة الاناث البالغة بتركيزات مختلفة من زيت القرنفل العطري (الاووجينول) باستخدام طريقة الرش لمدة 1، 3، 7 ايام وقد تم قياس نسب الموت بعد المعاملة بالتركيزات 0.25، 0.50، 1.0 و 1.5 ججم/ججم. ولوحظ تزايد معدل نسب الموت لاناث الحلم العنكبوتي ذي البقعتين مع زيادة التركيزات و تم حساب قيمة التركيز النصفى المميت (LC₅₀). علاوة على ذلك، فان جميع التركيزات المختبرة من الزيت العطري كانت معنوية. و تمت دراسة تأثير LC₅₀ للزيت العطري على انزيمات الانسجة العصبية استيل كولينستريز (AChE) و الفوسفاتيز القلوي (ALP) للحلم بعد 24 ساعة و 3 ايام من المعاملة. و اظهرت النتائج انخفاض نشاط الانزيمين بعد 24 ساعة من المعاملة بينما ارتفع انزيم (ALP) بعد 3 ايام من المعاملة. وقد تم تقييم فعالية زيت القرنفل ضد الحلم العنكبوتي تحت الظروف شبه الحقلية، و اظهرت النتائج انه اعطى نتائج مرضية حيث انخفض التعداد للحلم بنسبة 92% تقريبا. و اخيرا، يمكننا ان نستنتج ان الزيت الاساسي للقرنفل قد حقق نتائج جيدة في المكافحة.