

**ACARICIDAL POTENTIAL AND REPRODUCTIVE IMPACT
OF MARINE ALGA, *Laurencia obtusa* COMPOUNDS AGAINST
Tetranychus urticae (Koch) and, *Panonychus ulmi* (Koch)**

**Eman S.Swelam and Samia EL-Kabbany
Department of Economic Entomology & Pesticides, Faculty of
Agriculture, Cairo University, Egypt**

ABSTRACT

The red alga *Laurencia obtusa* (Huds), has long been known as a reliable source of secondary metabolites. The toxicity of dichloromethane extract and its fractions against *Tetranychus urticae* (Koch) and *Panonychus ulmi* (Koch) spider mites was assessed. Bioassay efficacy guided to isolate two halogenated terpene and one nonterpenoid compounds. The latent effects of three compounds were also investigated. The results showed that the crude extract and its three fractions were active against both mites. It was found that *T. urticae* was more sensitive than *P. ulmi* to the isolated fractions of *L. obtusa* and to the reference acaricide dicofol. The number of progeny and the longevity of the exposed adults of both mites were used as criteria for the deleterious effects of the fractions. It was observed that the three fractions showed highly effect on the hatchability of eggs. Significant variations were recorded on the reduction in total numbers of eggs/female against the two mites.

Key words: *Marine alga, Laurencia obtusa, bioactivities, mites*

INTRODUCTION

Intensive research is currently being conducted on some biologically active compounds as natural sources of pesticides. However, natural products providing the raw material required yielding the end products in a few steps of a synthetic route. The increasing interest in the possible application of secondary metabolites to pest management has directed the investigation toward research for new sources of biologically active natural products with low mammalian toxicity, low persistence in the environment and to avoid the development of resistance of the pest (**Park et al., 2000**). In the last few decades, benefits of marine algae, including the use of seaweeds as fertilizers and soil conditioners, the biologically active compounds in seaweed extracts and the compounds from marine algae with activity against agricultural pests have been reviewed (**Blunden and Gorden 1989, Jolivet et al., 1991**).

To date, research focused on isolating insecticidal prototype leads from marine origin has resulted in the report of about 40 active compounds (nor 5% from algae). In an attempt to summarize these compounds and their activity margin, they have been categorized into six classes of chemical structures (**El Sayed et al., 1997**)

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most important mite owing to its wide distribution all over the world on different plant hosts. It is also one of the most destructive pests of many crops. Their economic importance due to their feeding on leaves, buds and fruits, causing direct injury to plants, while others phytophagous mites may transmit plant diseases, causing great damage to agricultural crops. Mites population increase in different parts of Egypt and with wide range of infesting most agriculture

plant. Chemical control of mites has been extensively practiced in Egypt for control mite's population (El Kady *et al.*, 2007).

T. urticae control in Egypt has been based almost exclusively on pesticides. Many acaricides have been registered to control *T. urticae* in Egypt. A major problem in controlling spider mites is their ability to rapidly develop resistance to acaricides. As a result of their high reproductive potential and short life cycle, combined with 10 or 12 pesticide applications per season in vegetable greenhouses, enhanced the development of resistance in spider mites.

One of the major acarine pest species of deciduous fruit trees like apple and plum is the European red mite, *Panonychus ulmi* Koch (Acari: Tetranychidae). Feeding damage by *P. ulmi* results in leaf bronzing followed by an early defoliation and a reduction in yield, which is usually combated by the application of acaricides (Thiel and Nauen, 2006). The European red mite *P. ulmi* (Koch) was the only serious, widespread mite pest in apple orchards (Hardman *et al.*, 2007).

The main objective of this work was to investigate the potency of *Laurencia obtusa* (crude extract and its three fractions) as an acaricide against two mites, *T. urticae* (Koch) and *P. ulmi* (Koch). One of the major questions to be answered was whether any relationship existed between the biological activity and the chemical structure of three compounds, isolated from the red alga *L. obtusa*.

MATERIALS AND METHODS

1-Algae collection

The red alga *L. obtusa* was collected from red sea and identified by the staff from the Institute of Marine Science, (Algae Biology), Hurghada, Egypt. The collected alga was washed with tap water to remove the sand and salt residue from the outer surface, then kept in a dark room under ambient temperature to dryness. The dried alga was ground and stored in a black sack until the biological and chemical experiments.

2-Test mites

The two mites' cultures were laboratory strains which obtained from Institute for plant Pathology, department of Entomology and Plant Protection, Bonn University. The mites were reared under 25±2°C, 12 hrs light and 65±5% relative humidity Two spotted spider mite, *Tetranychus urticae* (Koch) and Red spider mite, *Panonychus ulmi* (Koch).

3-Acaricide

One commercial formulation of acaricide used (as standard reference) in bioassays was dicofol, 4-chloro- \square -(4-chlorophenyl)- \square -(trichloromethyl) benzenemethanol (Kelthane 50% WP, scientific office Dow AgroSciences).

4-Preparation of alga extract

The biologically active fractions were previously isolated and identified by Swelam, 2003. The dry algal ground tissue (3 kg) was exhaustively extracted with dichloromethane (DCM) at the ratio 1:4 weight/volume and then with methanol to afford 94.829 g of dichloromethane-soluble material. Vacuum-liquid chromatography (vlc) was used to fractionate the crude extract over silica gel, using petroleum ether containing increasing proportions of ethyl acetate (5%) as eluent, afforded 21 fractions each of 40 ml. Thin Layer Chromatography (TLC) and H¹ NMR analysis was carried to identify these fractions and bioassay indicated fractions 2, 5, and 6 to be further interest.

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Therefore, further studies were carried out by HPLC in separation of fraction 2, over normal phase silica with petroleum ether as eluent yielded one sesquiterpenes. As well as fraction 5, in 10% acetone: petroleum ether as eluent yielded one nonterpenoid C₁₅ acetogenins and fraction 6 was an isomer of compound which was isolated from fraction 5.

5-Bioassay

-Toxicity test

Different concentrations of *L. obtusa* crude extract or fractions were prepared in acetone. The leaves were treated with the test alga extract by dipping for 30 second, then allowing drying at ambient temperature. The control leaves were treated with acetone. The treated leaf disk was put on an agar surface (instead of wet cotton) in a Petri dish, and then ten new emerged females were transferred on the disk. All the treatments and control (ten replicates) were incubated for 4 days at 25°C and the mortality was recorded daily.

Newly emerged females were transferred singly to treated leaf disk according to the method mentioned to test the toxicity. Each experiment consisted of 50 replicates and followed to record some biological parameters such as pre and oviposition periods, female longevity, no. of egg/female, total no. of eggs/female and no. of unhatched eggs.

6-Statistical analysis

The Probit analysis program of EPA version 1.5 was used to analyze the data from the bioassay experiments. All the toxicity lines were graphed by the Microcal origin software version 5.

RESULTS AND DISCUSSION

Biological Activities of *L.obtusa* extract against *T. Urtica*

The toxicity of different extracts (dichloromethane and methanol) of *L.obtusa* to the adult females of *T. urtica* and *P. ulmi* was tested. The most efficient extract was found to be dichloromethane. Since the dichloromethane extract of *L.obtusa* yielded the most toxic crude extract, it was subjected to further investigations. It is known that natural crude extract contains many compounds and if the most active ones could be isolated, the effect would be magnified. The dichloromethane extract of *L.obtusa* which showed relatively higher activity, was fractionated using the thin layer chromatographic technique according to **Swelam, 2003** method.

Among the 21 fractions obtained from DCM crude extract, 3 fractions were found effective against two mites, *T. urticae* (Table, 1) and *P. ulmi*. (Table, 3).

It is worth noting that the efficacy of the *L.obtusa* extract may be varied by the methods of application. The leave dipping technique was used to test the toxicity of the crude extract and its fractions against both of *T.Urticae* and *P. ulmi*. Mortality of the DCM increased steadily and maximum mortality reached at 96 hr after treatment indicating that the red alga contains slow acting toxic components lethal to mites. **Amer et al. (1994)** found similar results after 96 hr after treatment, when studied the toxicity of the different crude extracts of the brown marine alga (*Petalonia fascia*) against the *T.urticae* The toxicity of *L.obtusa* DCM crude extract and the 3 most biologically active isolated fractions (2, 5 and 6) against *T.urticae* is shown in table (1). The most potent toxicity was found in isolated fractions from fraction 5 (LC₅₀ 0.016 %) and fraction 2 (LC₅₀ 0.030 %) showed moderately

toxicity but, fraction 6 (LC₅₀ 0.057 %) showed weak toxicity (table, 1). When all the tested compounds were considered, the synthetic acaricide, dicofol ranked first followed by isolated fraction 5, fraction 2 and then fraction 6, respectively. The present data demonstrated that all extracts were less effective against the tested mites at LC₅₀ than the synthetic acaricide (dicofol). The toxicity indexes of the fractions were 46.25, 24.6 and 12.98 for fractions 5, 2 and 6, respectively incomparable with dicofol. The relative potency was in ascending order as follows: fraction 5 > fraction 2 > fraction 6.

Table (1): Bioactivity values, slope, relative potency and toxicity index of dichloromethane extract and three isolated fractions of *L.obtusa* and dicofol against *T. urticae*

Tested material.	LC ₅₀ % (95 % C L)	Chi-Square		LC ₉₀	Slope	Relative potency *	Toxicity index ** %
		Cal.	Tab.				
Fraction No. 5	0.016 (0.013-0.02)	2.879	9.488	0.073	1.968	8.188	46.25
Fraction No. 2	0.030 (0.024-0.036)	3.003	7.815	0.086	2.761	4.366	24.6
Fraction No. 6	0.057 (0.04-0.097)	1.80	7.815	0.308	1.754	2.298	12.98
Crude extract	0.131 (0.103-0.16)	4.359	11.07	0.392	2.690	1.0	5.649
Dicofol	0.0074 (0.006-0.009)	2.097	7.815	0.0245	2.482	17.7	100.0

LC₅₀ and LC₉₀: Concentrations needed to kill 50% and 90% of exposed female, respectively
C L: confidence limits

Cal. and Tab.: calculated and tabular Chi-square for heterogeneity at 0.05 level.

* obtained by comparing the potency of the crude extract to the tested material at the level of the LC₅₀ values

** Obtained by comparing the efficiency of the tested material at the level of LC₅₀ to the dicofol

This finding was agreed with the results obtained by **San-Martin *et al.* (1991)** who found that violacene (the major component isolated from the alga *Plocamium cartilagineum*, identified by comparison of its physical and spectroscopic properties) showed the most potent acaricidal activity among the compounds tested, mainly against *T. urticae*.

In the present study, it was obvious that treatment with *L. obtusa* fractions influenced the biotic and reproductive potential of *T. urticae*, somewhat as mite growth regulator compounds, when the *T. urticae* were feed on bean leaves containing different fractions of DCM extract of *L.obtusa* for 4 days. There was a wide variability in responses of *T urticae* ranged from slightly effect in female longevity with fraction 6 (25.9 ± 0.34 days) to a pronounce decrease with fraction 5 (13.8 ± 0.29 days), while the average longevity of females was 26.5 days in the control (Table 2).

The duration of the pre-oviposition period for ovipositing females increased from 0.1 days in control to 2.5 and 1.6 days in fraction 2, 5 respectively. Where as the oviposition period decreased from 9.2 to 7.7 days fraction 5.

The number of eggs/female was highly significantly decreased. It recorded as 0.1 ± 0.10 and 0.4 ± 0.16 eggs/female for fraction no. 2 and 5, respectively, compared to the control 2.0 ± 0.33 eggs/female. Reduction in

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fecundity of *T. urticae* (Total no. of eggs/female) suggests that fraction 2 and 5, which was isolate from the, DCM extract of *L. obtusa* might cause either hormonal imbalance or partial damage to ovarian tissues of *T. urticae* resulting in an inhibitory action on oviposition further studies are needed to shed more light on the effect.

Table (2): Latent effects of dichloromethane extract of *L. obtusa* and its three isolated fractions against *T. urticae*

Treatments	Biological aspect						
	Periods (in days) of			No. of eggs/female after 24 h Mean ± SE	Total No. of eggs/female Mean ± SE	No. of unhatched eggs Mean ± SE	Hatchability %
	Pre Oviposition Mean ± SE	Oviposition Mean ± SE	Female longevity Mean ± SE				
Frac. 5	1.6 ± 0.37**	7.7 ± 0.30**	13.8 ± 0.29**	0.4 ± 0.16**	18.3 ± 0.83**	2.4 ± 0.37**	86.9
Frac. 2	2.5 ± 0.30**	8.3 ± 0.26**	18.0 ± 0.51**	0.1 ± 0.10**	16.5 ± 0.76**	5.0 ± 0.49**	69.7
Frac. 6	0.8 ± 0.24*	9.1 ± 0.23	25.9 ± 0.34	1.5 ± 0.16	30.9 ± 0.64**	1.0 ± 0.33*	96.7
Control	0.1 ± 0.10	9.2 ± 0.13	26.5 ± 0.26	2.0 ± 0.33	39.3 ± 0.77	0.2 ± 0.13	99.4

These data are in agreement with obtained by Ali and Sayed (2006), who mentioned that the acetone extract of the brown marine alga *Sargasium spirifolium* decreased the longevity of the adults and the fecundity nymphs of the *T.urticae*

Factor which, in principle, could have contributed in reducing rate of oviposition may be absence, retardation, or abnormalities in the ovarian development as suggested by **Toppozada et al., (1966), Emara et al., (2002) and Singh, (2003)** both also mentioned that reduction in deposition rate of mature eggs and reduced egg fertility could result from lack of fertilization or disturbed embryogenesis.

It was observed that the three fractions showed much ovicidal potential. Highly significant variations were recorded on the reduction in total No.of eggs/female (Table, 2).

On the other hand, the number of unhatched eggs increased from 1.0 ± 0.33, 2.4 ± 0.37 and 5.0 ± 0.49, respectively by fraction 6, fraction 2 and fraction 5.

Biological Activities of *L. obtusa* extract against *P. ulmi*

Data in table (3) show that the three fractions had also effect on the *P. ulmi*. Fraction 2 and fraction 5 are similar in toxicity with the LC₅₀ values 0.030 and 0.031% respectively, whereas fraction 6 is at least two times less active against *P. ulmi*. with the LC₅₀ value 0.074%.

The comparison between the effect of the fractions and the classical acaricide, dicofol showed that the toxicity index of fraction 2 was 30, fraction 5 was 29.03 and fraction 6 was 12.16 of the dicofol toxicity (Table, 3).

Table (3): Bioactivity values, slope, relative potency and toxicity index of dichloromethane extract and three isolated fractions and dicofol against *P. ulmi*

Tested material.	LC ₅₀ % (95 % C L)	Chi-Square		LC ₉₀	Slope	Relative potency*	Toxicity index ** %
		Cal.	Tab.				
Fraction No. 5	0.031 (0.023-0.042)	0.383	5.991	0.175	1.69	5.5	29.03
Fraction No. 2	0.030 (0.022-0.041)	0.345	5.991	0.182	1.64	5.6	30.0
Fraction No. 6	0.074 (0.051-0.122)	0.037	5.991	0.508	1.52	2.3	12.16
Crude extract	0.169 (0.128-0.230)	1.184	5.991	0.492	2.76	1.0	5.33
Dicofol	0.0090 (0.007-0.011)	0.886	7.815	0.0326	2.30	18.8	100.0

LC₅₀ and LC₉₀: Concentrations needed to kill 50% and 90% of exposed female, respectively
C L: confidence limits

Cal. and Tab.: calculated and tabular Chi-square for heterogeneity at 0.05 level.

* obtained by comparing the potency of the crude extract to the tested material at the level of the LC₅₀ values

** obtained by comparing the efficiency of the tested material at the level of LC₅₀ to the dicofol

By comparing the biological activity of the different isolated fractions of *L. obtusa* against the *T. urticae* and *P. ulmi*, it was found that, *T. urticae* was more sensitive than *P. ulmi* to the isolated fractions of *L. obtusa* and also to the reference acaricide dicofol. This may be due to the structure-activity relation among these isolated and the pest species (**San-Martin et al., 1991**).

Data in table (4) show that the three fractions had also a high significant effect on oviposition period and longevity of the females as it gave a remarkable decrease in the duration of oviposition period and longevity, whereas the pre-oviposition period for those females was not significantly affected in case of fraction 6. In contrast, females offered discs treated with fraction 2 and fraction 5 caused significant prolongations in the pre-oviposition period.

Moreover, the fecundity of the *P. ulmi* adult females exposed to treated discs were highly and significantly reduced. The most efficient fractions were found to be fraction 2, the adult female deposited 8.2 ± 0.35 eggs. Average number of the eggs deposited for the other fractions was 13.7 ± 0.36 and 27.6 ± 0.33 for fraction 5 and fraction 6, respectively and 31.2 ± 0.48 eggs/female for the control. The hatchability of the *P. ulmi* eggs is also confirmed in our study, the highly significant reduction were observed for the three fractions against the *P. ulmi*

Table (4): Latent effects of the dichloromethane extract of *L. obtusa* and its three isolated fractions against *P. ulmi*

Treatments	Biological aspect						
	Periods (in days) of			No. of eggs/female after 24 h Mean ± SE	Total No. of eggs/female Mean ± SE	No. of unhatched eggs Mean ± SE	Hatchability %
	Pre Oviposition Mean ± SE	Oviposition Mean ± SE	Female longevity Mean ± SE				
Frac. 5	0.7 ± 0.26*	7.1 ± 0.23**	10.5 ± 0.16**	0.5 ± 0.16**	13.7 ± 0.36**	4.1 ± 0.23**	70.1
Frac. 2	1.5 ± 0.16**	7.6 ± 0.22**	10.9 ± 0.31**	0.3 ± 0.15**	8.2 ± 0.35**	4.3 ± 0.21**	47.5
Frac. 6	0.2 ± 0.13	9.5 ± 0.22**	17.8 ± 0.32**	1.3 ± 0.21	27.6 ± 0.33**	1.7 ± 0.3**	93.8
Control	0.1 ± 0.10	13.5 ± 0.26	19.8 ± 0.24	1.7 ± 0.26	31.2 ± 0.48	0.2 ± 0.13	99.3

In general, it should be mentioned that the different fractions of *L. obtusa* tested proved to cause complete or partial sterility to *T.urticae* and *P. ulmi* adult females. Pre-oviposition period also was disturbed

Characterization of Biologically Active compounds

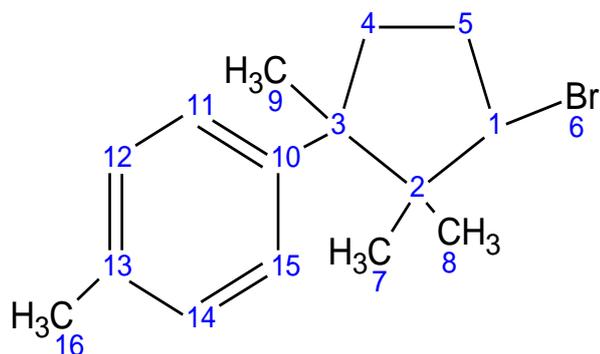
Identification details of the compounds which isolated from the biologically active fractions as shown in Fig. (1). have been published previously **Swelam, (2003)**.

Waraszkiewicz et al. 1976 and Watanabe et al (1989) isolated a number of nonterpenoid C₁₅ acetogenins from red algae of the genus *Laurencia*, which possessed a strong insecticidal activity against mosquito larvae (*Culex pipiens pallens*), and were identified as Z-laureatin and Z-isolaureatin. They reported that the toxicity of the isolated compounds related to the terminal triple bond and the cis- double bond. Moreover the C₈-C₁₂ double bond is an E-configuration in the trans-isomers which makes the trans isomers less polar than cis isomers. This finding may explain that the trans isomers (fraction 5) exhibited toxicity more than cis isomers (fraction 6).

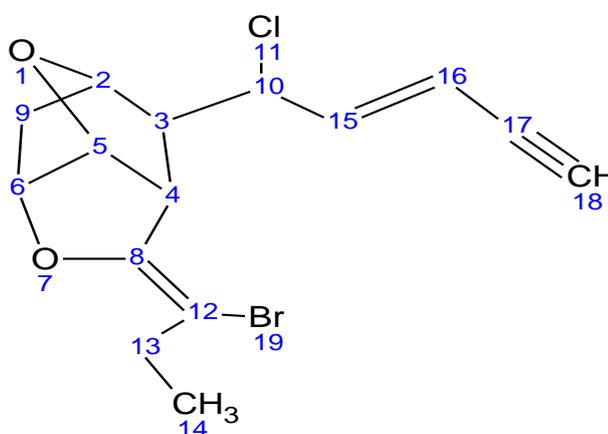
It is known for some plant-derived terpenoids that they can act as feeding and growth inhibitors of mites (**Klocke and Kubo, 1982; Murray et al., 1999; Isman, 2000**).

It was reported in literature that the presence of CH₃, O, Cl, Br substituent groups at carbon atoms are also responsible for the biological activity. In addition compound which containing both an aldehyde and a methyl group was also proved to be responsible for an antifeedant toxic effect (**Morimota et al., 2002**).

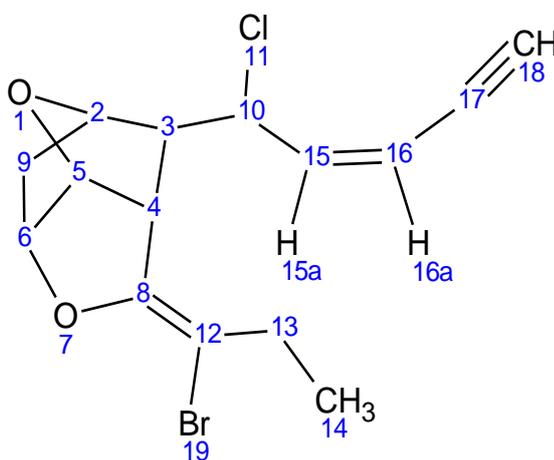
Nonterpenoid structure was isolated from the DCM fraction 5, could explain the more growth inhibition effect and could act as IGR. **Bede et al., (1999)** reported that the sesquiterpenoids and nonterpenoid regulate the developmental process, such as metamorphosis and reproduction.



Major compound in fraction 2 (halogenated terpene)



Major compound in fraction 5 (nonterpenoid C₁₅ acetogenins)



Major compound in fraction 6 (nonterpenoid C₁₅ acetogenins)

Fig. (1): Chemical structures of the major compounds from isolated fractions

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الكفاءة الإبادية والتأثير على التكاثر لمركبات الطحلب البحري *Laurencia obtusa* ضد كل من

العنكبوت ذو البقعتين *Tetranychus urticae*

والعنكبوت الأحمر الأوروبي *Panonychus ulmi*

إيمان سعيد سويلم ، سامية القباني

قسم الحشرات الإقتصادية والمبيدات- كلية الزراعة- جامعة القاهرة- الجيزة- مصر.

تهدف الدراسة الحالية الى بحث الأثر السام لـ ٢١ قطعة من مستخلص داي كلوروميثان للطحلب البحري *Laurencia obtusa* ضد كل من العنكبوت ذو البقعتين *Tetranychus urticae* والعنكبوت الأحمر الأوروبي *Panonychus ulmi*. وتبين ان كل من القطفات ٢، ٥ و ٦ اكثر تأثيرا على كل من العنكبوت ذو البقعتين والعنكبوت الأحمر الأوروبي. كما أوضحت المقارنة بين قيم الـ LC₅₀ للقطفات ٢، ٥ و ٦ ان القطفة ٥ أكثر تأثيرا من القطفة ٢ والقطفة ٦ ضد كل من الافتين. حيث كانت قيم الـ LC₅₀ للقطفات ٥، ٢ و ٦ (0.016 %) ، (0.030 %) و (0.057%) على التوالي ضد العنكبوت ذو البقعتين بينما كانت قيم الـ LC₅₀ للقطفات ٥، ٢ و ٦ (0.031%)، (0.031%) و (0.074%) على التوالي ضد العنكبوت الأحمر الأوروبي. وقد اظهرت النتائج ان العنكبوت ذو البقعتين اكثر حساسية للقطفات الثلاث مقارنة بالعنكبوت الأحمر الأوروبي. وبناء على هذه التأثيرات الحيوية أمكن فصل مركبين من التربينات الهالوجينية ومركب آخر غير تربيني. أوضحت الدراسات البيولوجية للمستخلص الخام وكذلك القطفات الثلاثة المفصولة منه، ان له تأثير متأخر على كل من الافتين. لقد أوضحت هذه الدراسات أن القطفات الثلاثة لهم تأثير على خفض نسبة الفقس، كما سجلت اختلافات معنوية في خفض عدد البيض الموضوع للأنتى الواحدة، وعمر الأنتى وفترة وضع البيض وفترة ما قبل وضع البيض.