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Biochemical effects of two kinds of mineral oils and an IGR on adult female mealybug *Ferrisia virgata* (Cockerell)

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ABSTRACT

A study was conducted to offer a preliminary understanding of the role played by intensively used field controlling agents, two mineral oils, namely Alboleum and Super Misrona oils as well as an IGR, Admiral (pyriproxyfen) with the recommended doses upon adult female mealybug *Ferrisia virgata* (Pseudococcidae: Homoptera) as an arbitrary model for mealybugs. The present work points out to the importance of the delayed effect of these different compounds. The highest increase in the total protein content was achieved by Admiral reached 29.2% while Super Misrona caused the greatest enhancement in the total carbohydrates concentration (72.93%). On the other hand, Alboleum caused a highly significant inhibition in the activity of glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminases (GPT) reached 82.03 and 22.10 %, respectively. These biochemical changes were intensively discussed. Among most of the tested biochemical parameters, Admiral showed the strongest delayed effect.

Key words: Biochemical, mineral oils, IGR, mealybug

INTRODUCTION

A wide range of mealybug species is infesting many kinds of citrus orchards and ornamental plants (Hammon and William 1984 and Helmy *et al.*, 2002) causing damages for leaves such as yellowish appearance, dryness and a noticeable reduction in the yield. Moreover, the continuous discharge of honeydew characteristic for mealybugs opens the gate for the black sooty mould to grow (Hanafi, 1976). In Egypt, the efficacy of mineral oils as well as other chemicals like insect growth regulators (IGRs) against citrus pests has been dealt by many authors (Korashy, 1998; El-Imary *et al.*, 1999; Hemida *et al.*, 2005). For a long time, all previous studies were limited to measure the toxicity of these spraying agents. In deed, no available studies were focusing upon the effect of such intensively used chemicals upon the mealybugs bodies. Thus, the present study -to the best of our knowledge- is the first trial to deal with the biochemical effect the conventionally spraying compounds in the Egyptian fauna on mealybugs. We used the adult female mealybug, *Ferrisia virgata* (Homoptera: Pseudococcidae) as our arbitrary experimental insect sprayed with mayonnaise and miscible oils namely, Alboleum and Super Misrona, respectively as well as an IGR, pyriproxyfen (Admiral).

On the other hand, maintenance of the balanced amino acid pool in insects is the result of various biochemical reactions carried out by a group of enzymes called amino acid transaminases (Meister, 1957) glutamic oxaloacetic transaminases (GOT) and glutamic pyruvic transaminases (GPT) are the key enzymes in the formation of non essential amino acids, in the metabolism of nitrogen waste, gluconeogenesis and correlated with protein anabolism and catabolism (Mordue and Goldsworthy, 1973). Moreover, transaminases especially GPT acts as a catalytic agent in carbohydrates metabolism (Katuma *et al.*, 1968).

Thus, the present study is a pioneer attempt aiming to answer two questions: (1) Do the previously mentioned compounds have an effect-whether stimulatory or inhibitory-upon the main mealybug metabolites? And (2) Can these

compounds play a role in a regulatory transaminases dealing with the protein and carbohydrate metabolism namely, glutamic oxaloacetic and glutamic pyruvic transaminases (GOT and GPT), respectively.

MATERIALS AND METHODS

Chemicals:

1. Mineral oils

Mineral oils used with recommended doses in the Egyptian fields being diluted with distilled water.

A. Alboleum (Mayonaise oil) (85%EC): the tested rate was 2.5%.

B. Super Misrona (miscible oil) (95%): the tested rate was 1.5%.

2. IGR: Admiral (pyriproxyfen) 10% EC: 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy) propyl ether.

Insects:

F. virgata mealybugs were found infesting leaves, branches and tree trunks of Sesban tree, *Sesbania aculata* L. in Giza governorate with no history of insecticidal exposure. They were collected by taking the heavily infested areas and only newly hatched adult females were selected and carefully removed from leaves with a fine paint brush to allow settling upon medium sized sprouting potato tubers (about 30 individuals per tuber). Infested tubers were kept at room temperature under laboratory conditions, 25±2°C temperature, 60-70% RH and 14: 10 L: D photoperiod. Each compound was used to spray tubers (2 ml /tuber) and the control ones were sprayed with the used diluting agent, dist. water. At the needed time intervals, survived individuals (about 10⁄interval) were kept frozen at -20 °C in a small polyethylene tubes for future biochemical analysis.

Biochemical analysis:

1. Tissue preparation

The whole body was homogenized in dist. water (1 gm of tissue in 1 ml of dist. water), using hand glass homogenizer on ice jacket. The homogenate was centrifuged at 3500 rpm for 10 min.at 4 °C and the supernatant was frozen till use.

2. Detection and evaluation of the main metabolites

Total proteins and carbohydrates were determined in the entire body homogenate according to Bradford 1976 and Singh and Sinha (1977) methods, respectively.

3.Detection and evaluation of transaminases activity

The levels of GOT and GPT were determined according to Harold (1975).

Statistical analysis

Data were subjected to statistical analysis using analysis of variance two ways ANOVA (Snedecor & Cochran, 1967) and the least significant difference (LSD) test was used for mean separation at $P \leq 0.01$.

RESULTS AND DISCUSSION

The main metabolites and the titer of transaminases were studied during the adult female mealybug, *F. virgata*. Tracing these biochemical parameters in the normal untreated adult female was necessary to determine whether they are stored or used during her life span and give us an oriented background to study the effect of different insecticidal agents. The quantity of body proteins was much more than carbohydrates (Fig.1). Total proteins were 15.91, 16.80, 20.80, and 23.23 mg/g.b. wt at 4, 6, 8 and 10 days for adult female mealybug, respectively. Total carbohydrates were 6.66, 10.94, 12.90 and 11.16 mg/g.b. wt. at the same previously mentioned periods.

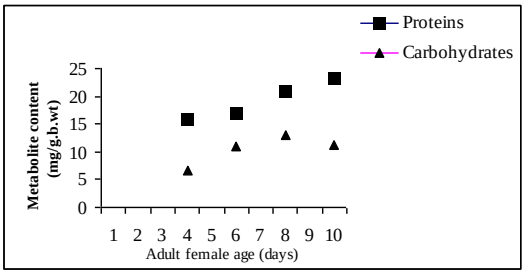


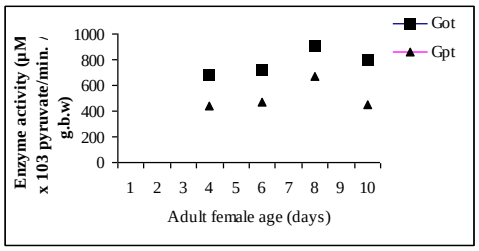
Fig. 1. The main metabolite during the development of adult female , *F. vifgata*.

It seems that the total proteins and carbohydrates accumulated during the adult stage, so the older insects have more proteins and carbohydrates than the newer ones.

Titer of transaminases were slightly fluctuated the adult life (Fig.2). GOT activity was 683µx1000/g.b.wt. for 4 days old adult female mealybug and began to increase reaching to 916 µx 1000/g.b.wt. for 8 days old insect, then the titer declined at the 10^{th} day (796

µx1000/g.b.wt.). The same trend was observed for GPT activity. In general the titer of GOT was more than GPT.

Fig. 2. The titer of transaminases during the development of adult female, *F. vifgata*.



Concerning the biochemical effect of the used spraying agents in the present study, the effect of Alboleum, Super Misrona and Admiral on adult female mealybug, *F. virgata* on total protein and carbohydrates contents and their related enzymes (GOT and GPT) was studied. The present study includes measuring the early and delayed action of these treatments at 4th and 10th day post treatment, respectively.

Total proteins were reduced insignificantly after 4 days of treatment of Alboleum oil (Table 1) while a significant decrease by 30.65% after10 days of treatment compared to the untreated larvae. A non significant decrease in the total proteins in case of treatment of Super Misrona oil was found in both 4 and 10 days post treatment compared to the untreated insects.

Table 1. Total protein concenteration after treatment of adult female *F. virgata* using different spraying agents

Total protein concenteration expressed in µg/mg tissue	Concentration	Concentration % to control	Concentration	Concentration % to control
Days post treatment	4		10	
Alboleum oil	14.70 ±10.26 ^{f}	-7.60	16.13 ± 0.41 ^{ef}	-30.56
Super Misrona oil	15.2± 0.26 ^{f}	-4.46	22.63 ± 1.09 $_{ab}$	-2.58
Admiral	20.56 ± 0.26 ^{cd}	29.22	21.16 ± 1.05 ^{bcd}	-8.91
Control	15.91 ± 0.62 ^{ef}	_	23.23 ± 0.64 ^{g}	_

*Means followed by the same letter are not significantly different at 1 % level.

Different trend was observed due to Admiral as it significantly increased in the early treatment reached to 29.22% but after 10 days post treatment, it was reduced significantly by 8.91% relative to the control. This points out to the delayed effect caused by Admiral. It was reported that pyriproxyfen did not affect the protein band pattern in treated insects, although it affected the amount of protein concentrations (Aribi *et al.*, 2006). Also it was reported that fenoxycarb and pyriproxyfen induced an inhibition of larval haemolymph protein synthesis in *Locusta migratoria* (De Kort, and Koopmanshap, 1991) and *B. mori* (Monconduit, and Mauchamp, 1998). It has been shown that different insects exposed to various stresses may decrease the amount of total protein as in silkworm haemolymph (Etebari and Matindoost, 2004). This could be due to the break down of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. So, protein depletion in tissues seems to constitute a physiological mechanism and may play a role in compensatory mechanisms under insecticidal stress, to provide intermediates to the Krebs cycle, by retaining free amino acid content in haemolymph (Nath *et al.*, 1997).This explaination may verify our findings as the used chemicals in the present study decreases the total protein contents after 10 days post treatment while the normal adult female tend to accumulate proteins in the first 10 days in her normal adult life span (Fig.1). Alboleum oil increased the carbohydrates level significantly in the early record (4 days post treatment) being 54.35% relative the control (Table 2).

Table 2. Total carbohydrate concenteration after treatment of adult female *F. virgata* using different spraying agents

Total carbohydrate concenteration expressed in µg/mg tissue	Concentration	Concentration % to control	Concentration	Concentration % to control
Days post treatment	4		10	
Alboleum oil	10.28 ± 0.26^{f}	54.35	10.93 ± 0.11 ^{e}	-2.06
Super Misrona oil	7.13 ± 0.14 ^{h}	7.06	19.3 ± 0.43 ^{g}	72.93
Admiral	6.9 ± 0.1^{h}	3.60	7.8 ± 0.1 ^{g}	-30.11
Control	666 ± 0.152 ^{h}	_	11.16± 0.15 ^{c}	–

*Means followed by the same letter are not significantly different at 1 % level.

On the other hand, a slight non significant decrease was achieved in the delayed treatment after 10 days post treatment reached only 2.06% compared to

the control. Total carbohydrates were elevated significantly at only 10 days post treatment Super Misrona as a delayed effect reached 72.93% relative the control while a non significant increase was detected early after 4 days of treatment being only 7.06% compared to the control. In contrast, the effect of Admiral appeared to cause a highly significant reduction in carbohydrates reached to 30.11% compared to the control after 10 days of treatment although it caused a nonsignificant increase in this metabolite in the early treatment i.e. 4 days post treatment. This again points out to its delayed effect. This may be attributed to the slower mode of action of IGR (Anwar and Abdel-Mageed, 2005). Similar Trend was reported by Abdel-Hafez *et al*. (1988) in case of cotton leaf worm, *Spodoptera littoralis* treated with a number of IGRs.

It seems that the accumulation of carbohydrates caused by the mineral oils used during the present work, might resulted from the enhancement of carbohydrates metabolism or from preventing building tissue needs carbohydrates like cuticle. Results of the present study showed also that the total carbohydrates were more affected than proteins as it increased significantly either in early or delayed effect in most cases of the present work. We have to mention that, very little is known about the dynamics of carbohydrate utilization mealybugs as it ejects continuously the honey dew regardless to the physiological state it passes through. Only Super Misrona showed an increase in both early and latent treatment. Similarly, some insects like *Chrysocoris stolli* showed an increase in the carbohydrate contents after being treated with different IGRs (Saha *et al*., 1986).

The growth regulator used in the present study, Admiral (pyriproxyfen) seemed to have a delayed adverse effect (10 days post treatment) on both total proteins and carbohydrates in contrast to the earlier records i.e. 4th day after treatment which showed an increase in these metabolites.

Although it was established long ago that IGRs were mostly applied on immature stages, but they were used on adult stage as well (Weaver *et al,* 2008). IGRs are known to play a key role in reproduction such as stimulating the synthesis and uptake of vitellogenesis in females (Wyatt and Davy, 1996). Accordingly, Admiral would be expected to prevent egg production and would change proteins and enzymes of their synthesis dramatically after the treatment which is the time for egg production. Surprisingly, the present results show that proteins increased significantly during this period in the treated females relative the control i.e. Admiral seems to be not acting by suppressing protein synthesis necessary for egg production. Similar conclusion was proven by Cloyed (2003) who found that there was no consistent pattern on egg production of the adult female citrus mealybug, *Planococcus citri* after its treatment with different IGRs at different rates. He also reported that there is no effect of IGRs on egg production.

In the present study, GOT was greatly affected by Alboleum oil with a highly significant reduction starts by 49.78% after 4 days post treatment then it continues to be reduced dramatically reaching to 82.03% in the 10th day after treatment relative to the control (Table 3).

Table 3. GOT activity after treatment of adult female *F. virgata* using different spraying agents

GOT (µ x 10^3 / g.b.wt)	Activity	Activity % to control	Activity	Activity % to control
Days post treatment	4		10	
Alboleum oil	343 ±11.5 ^{g}	-49.78	14.3 ±20.8 ^{i}	-82.03
Super Misrona oil	783± 20.8 ^{cd}	14.64	11.56 ± 15 ^{b}	45.23
Admiral	756 ±20.8 ^{d}	10.69	596 ± 15 ^{f}	-25.13
Control	683 ±15 ^{e}	_	796 ± 11.5 ^{cd}	_

*Means followed by the same letter are not significantly different at 1 % level.

In contrast, Super Misrona had an enhanced effect and caused an elevation in the GOT activity after 4 and 10 days post treatment being increased by 14.64 and 45.23%, respectively compared to the control. Admiral caused also a significant enhancement of GOT achieved after 4 days post treatment reached to 10.69% relative to the control but its effect decreased dramatically after 10 days of

treatment being decreased by 25.13% compared to the untreated insect body homogenate. these data disagree with Zera and Zhao (2004) who reported that the application of Juvenile hormone analogue, methoprene on cricket, *Gryllus Wrmus*, showed a significant decrease in transaminase.

GOT activity depends on insect species, strain, developmental stage, age, tissue and type of the chemical treatment (Saha *et al.*, 1986; Tabassum, 1994; Tabassum *et al*., 1994, 1998; Abdel-Ghaffar and Ghoneim, 2007; Bakr *et al.*, 2007; Al-Dali. 2008).

GPT activity was greatly disturbed during this study (Table 4). Alboleum oil caused a significant increase on the 4th day post treatment by 21.82% but the activity was dramatically suppressed at the 10th day by 22.10% compared to the control. Similar trend was achieved by Super Misrona oil as it increased- insignificantly- after 4 days of treatment by 5.9% then its activity decreased significantly by 15.66% compared to the control. Admiral caused a slight non significant decrease 4 days post treatment followed by a significant increase reached to 15.02% relative to the control.

Table 4. GPT activity after treatment of adult female *F. virgata* using different spraying agents

GPT (μ x 10^3 / g.b.wt)	Activity	Activity % to control	Activity	Activity % to control
Days post treatment	4		10	
Alboleum oil	563 ± 15 ^{d}	21.82	363 ± 20 ^{a}	-22.10
Super Misrona oil	466 ± 20 ^{e}	5.90	393 ± 5.7 ^{gh}	-15.66
Admiral	436 ± 15 ^{efg}	-0.91	396 ± 25 ^{fgh}	-15.02
Control	440 ± 26 ^{ef}	_	466 ± 5.7 ^{e}	_

*Means followed by the same letter are not significantly different at 1 % level.

The general trend of suppressing Got activity in particular 10 days post treatment (except for Super Misrona oil),to a great extent, agreed with those effects reported for other insect species after treatment with different botanical and IGRs, such as *T. castaneum* (Tabassum, 1994; Tabassum *et al*., 1994), *Alphitobium diaperinus* (Tufail, 1991).This inhibitory effect observed in the present study , may be due to difficulty in the formation of dissociable enzyme-inhibtor complexes, which reduce the specific enzyme activity (Dragomirescu *et al.,* 1979).On the other hand, the increase in GOT activity due treatment by Super Misrona oil present in this study may suggests the mobilization of amino acids during the insecticidal stress exerted by certain toxic components to meet the energy demands (Zeba and Khan, 1995).

The general latent decrease of GPT activity in our study may be explained similar to Abulyazid *et al.,* (2005) who concluded that the changes in transaminases activities might be correlated with protein metabolism. The results in the present work support these suggestions in case of treatment by Alboleum and Admiral but it don't coincide with the treatment of Super Misrona oil. This is may be due to the fact that the interrelationships between protein synthesis and transaminases levels was confused by the hormonal control of protein synthesis and neurosecretory hormones which involved in the regulation of transaminases levels (Abulyazid *et al.*, 2005). However, the varying effects of the present plant extracts on the GPT activity in decreasing or increasing levels may be due to the effect on the synthesis or functional levels may be due to the effect of synthesis or functional levels of these enzymes directly or indirectly by altering the cytomprphology of these cells (Nath, 2000), or to the neuron secretory hormonal pattern (Salah *et al*., 2002).

On the other hand, it seems that age has a major role in the response of these enzymes in different insects treated with various agents (Chen, 1966; Bakr *et al.*, 2002).

However, the inhibition of GPT activity may have a serious effect on the insect because transaminases (in particular, GPT) is an important components of oxidative metabolism of praline which in certain insects is utilized during the initial periods of flight (Mostafa, 1993). Also this inhibited activity was possible because pyruvate is the precursor of Krebs cycle compounds, concerned with mitochondrial oxidation phenomenon and ATP production (Azmi *et al*., 1998).

The transaminases are the important components of amino acid catabolism; which is mainly involved in transferring an amino group from one amino acid to another keto acid. The GOT and GPT serve as a strategic link between the carbohydrate and protein metabolism and are known to be altered during various physiological and pathological conditions (Etebari and Mirohoesieni, 2005). Accordingly, the disturbances in GPT and GOT concentrations will be closely related to metabolism of proteins and amino acids. Thus it will disrupt many physiological functions and ultimately lead to death of these adults, in other way control them.

It's not known the exact mode of mineral oils but the effect of Alboleum oil on protein metabolism was more pronounced in all studied measures here than Super Misrona oil. This effect appears to increase after several days post treatment suggesting that mineral oils have a secondary effect. These findings coincide with Hemeida *et al.,* (2005) who found that Alboleum oil gave excellent results against the soft scale *Saissetia coffeae* at different tested rates.

The present work points out to the importance of the delayed effect of the experimental compounds on the adult female mealybugs. On the bases of our minor on no back ground , our results up recently could not easily interpreted and the exact role of these agents needs further researches to ascertain the exact mode of action of such environmentally safe compounds rather than synthetic harmful ones. We hope also that the biosynthetic pathway of such compounds on mealybugs will be elucidated in the near future.

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ARABIC SUMMARY

التأثيرات الكيموحيوية لنوعين من الزيوت المعدنية ومنظم للنمو الحشرى علي الانثى البالغة لحشرة البق الدقيقى *فيريزيا فرجاتا*

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تمت هذه الدراسة بهدف الفهم المبدئي للدور الذي تلعبة عوامل المكافحة الكيماوية المستخدمة حاليا بكثرة بالحقل وهم نوعين من الزيوت المعدنية (ألالبوليوم و سوبر مصرونا) ومنظم نمو (ادميرال) بالجرعات الحقلية الموصى بها للأنثى البالغة لحشرة *فيريزيا فرجاتا* (متجانسة الأجنحة: سودوكوكسيدى) كنموذج مختار للحشرات القشرية وقد أظهرت هذة الدراسة التأثير القوى المتأخر لهذة المركبات.وقد سجلت الدراسة أن أعلي تركيز للبروتين نتيجة رش الادميرال وصل الى ٢٩,٢% بينما تسبب الزيت المعدنى سوبر مصرونا فى أعلى زيادة فى تركيز الكربوهيدرات (٧٢,٩٣%) .من ناحية أخرى،عند استخدام زيت الالبوليوم تبين أنة قُداحدث انخفاضا ملحوظا فى نشاط الانزيمات الناقلة للامين مثل انزيم الجلوتاميك اوكسالواستيك ترانزامينيز (GOT) الذى انخفض بنسبة ٨٢,٠٣% بينما انخفض نشاط انزيم الجلوتاميك بيروفيك ترانزامينيز (GPT) الى ٢٢,١% ،ناقشت الدراسة اسباب هذة التغيرات الكيموحيوية لهذة المركبات و قد أوضحت النتائج أن الادميرال كان صاحب أكبر تأثير فى معظم المعاييرالكيموحيوية المستخدمة فى هذة الدراسة.