Efficacy of some insecticides on Mortality and Enzyme Activity of Egyptian mealybug (*Icerya aegyptiaca* Douglas) Attacking *Lantana camara* L. in Alexandria, Egypt.

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Abstract : Nine chemicals [four IGRs insecticides [Lufenuron (match®); Chromafenozide (Acro®); Pyriproxyfen (Admiral®) and RH- 2485 (Runner®)]; Kz (light mineral oil) and four binary combinations] were evaluated on their effect on *Lantana camara* shrubs against Egyptian mealybug *Icerya aegyptiaca* (Douglas) (Hemiptera : Monophlebidae) at El-Nozha Public Garden, Alexandria Governorate. Mortalities were recorded at zero, one, two, three, four and eight weeks. Mealybugs from treated *Lantana camara* shrubs had been collected after 24, 48, 72 and 96 hours respectively to investigate the effect of tested chemicals on Alkaline phosphatase (AlKP), Alanine transferase (ALT) and Aspartate transferase (AST) enzymes activity. There were no significant differences among chemical combinations, which caused more reduction effect than single treatments. Hence affected enzyme activity by the same trend. It could be concluded that Kz oil enhanced the toxicity effect of IGRs.

Keywords: Insecticides, *Icerya aegyptiaca* Douglas, Mortality, Enzyme Activity, *Lantana camara* L.

1.Introduction

Evergreen shrubs such as *Lantana camara* are fundamental for designing public gardens, which are considered as unique green areas in Alexandria that can be used for recreation all year round. These trees and shrubs are infested by several insects, which need to be controlled safely as we can. (Abdel Fattah Rasha, 2009).

Lantana camara has spread from it's native Central and South America to around 50 different countries, where it has become an invasive species. It spread from the Americas into the rest of the world when it was brought back to Europe by Dutch explorers and cultivated widely, soon spreading into Asia and Oceania, where it established itself as a notorious weed. It can also cause problems if it invades agricultural areas as a result of its toxicity to livestock as well as its ability to form dense thickets which if left unchecked can greatly reduce the productivity of farm land. (Day, *et al.,* 2003 and Abdul-Rassoul, *et al., 2015*).

Lantana camara shrubs are attacked by many scale insects such as Egyptian mealybug *Icerya aegyptiaca* (Douglas) which cause serious damage and yield reduction. These insect pests are usually controlled by chemical insecticides or biological means (Abo-Shanab, 2012 and Abo-Shanab *et aI.,* 2002).

Extensive uses of chemical toxicants for pest control caused many problems, such as acute and chronic human and animal toxicity, development of insect resistance to chemicals and environmental pollution. So we need alternative effective and environmental safe insecticides such as IGRs and mineral oils (Abdel Salam, 1993; MARL, 1997).

Insect growth regulators (IGRs) disrupt insect development in three ways: (1) Juvenile hormone (JH), (2) Precocenes (3) Chitin synthesis inhibitors. IGRs show good effect against scale insects and white flies on cotton, citrus, fruit shrubs and vegetables. Their effects have been observed in embryonic; larvae and nymphal development on metamorphosis, reproduction (in both males and females), behavior and several forms of diapauses. (Ware, 2000). It also affects development, maturation and survival of the immature (Pawar *et al*., 1995). Pyriproxyfen (IGR insecticide) is environmentally safe and non-toxic to animals and human where the mode of action of pyriproxyfen revelled that it mimics the action of natural insect juvenile hormone, (Palma and Meola, 1993; Dhadialla, *et al.,* 1998). Insect treated with pyriproxyfen cannot reproduce normally (Shiotsuki *et al.,* 1999).

Local sprays of mineral oils are used for years against scale insects, mealy bugs, thrips, aphids and mites on different crops and fruit shrubs, (Moursi, 1996; El-Deeb *et al*., 2002; Abo-Shanab, 2012). Oil sprays are used most commonly in horticulture to control scale insects and mites. As stated by Abo-Shanab (2005); Micks and Berlin, (1970) and El Sebae *et al*., (1976) that resistance was not recorded for mineral oils which still have the advantage of being effective to resistant strains.

The importance of phosphatase enzymes in the different developing insect stages had led to several studies.

Alkaline phosphatase is located in cells which are the most active in the synthesis of fibrous proteins and may be correlated to the gradual growth and development of the imaginal tissues that overlap with histolysis of the larval tissues. Maintenance of the balanced "amino acid pool" in insects resulting from various biochemical reactions is usually carried out by a group of enzymes called amino transferases (Meister, 1955). Such reactions are mainly responsible for the degradation and biosynthesis of amino acids, linking the glucose and protein metabolism and synthesis of certain specific compounds

The amino transferases, especially Alanine transferases (ALT) and Aspartate transferases (AST) are two components of oxidative metabolism of proline, which is utilized in certain insects during the initial periods of heights (Bursell, 1965). It also acts as a catalytic agent in the metabolism of carbohydrate (Katunuma *et al.*, 1968).
The aim of the present work is to evaluate the field toxicity of some IGRs, Kz oil and their combinations on the Egyptian mealybug *Icerya aegyptiaca* (Douglas) (Hemiptera : Monophlebidae). Also the study was directed to throw the light on the effect of these chemicals on the activity of Alkaline phosphatase, (AlKP) Alanine transferase (AST) and Aspartate transferase (ALT).

2.Materials and Methods

Table (1). Tested chemicals

Trade name	Rate % apply	Common name	Chemical name	Produced by
			I- IGRs	
Match 5% (wlv)	0.2	Lufenuron	(RS)- 1- (2,5- dichloro - 4 - (1,1 2,3,3-hexafluoro-prpoxyl) -phenyl)-3-(2,6-difluoro benzoyl) - Urea	Novartis Co. (Syngenta)
Admiral 10% E.C.	0.05	Pyriproxyfen	4-phenoxyphenyl *(RS)*- 2- (2- pyridyloxy) propyl ether	Sumitomo Chemical Co
Runner 24% S.C.	0.05	RH- 2485	N- t- butyl-N'-(3,5- dimethyl benzoyl) -3- methoxy - 2- methyl benzohydrazide	Rohm & Haas European Region
Acro E.C.	0.2	Chromafenozide	2'-t- butyl- 5- methyl- 2'-(3,5-xyloyl) chroman - 6-carbohydrazide	Nippon Kayaku Co
			Il-Mineral Oil	
Kz oil 95% E.C.	1.5		Kafr El-Ziat Pesticides and Chemicals Co.	
			Ill – The binary Mixtures	
			Kz + (Lufenuron or Pyriproxyfen or RH- 2485 or Chromafenozide) at 1 : 1	

2.1.Tested Insect :

Egyptian mealybug *Icerya aegyptiaca* (Douglas) (Hemiptera : Monophlebidae)

2.2.Field Experiment:

The field experiment was conducted in an orchard of *Lantana camara* shrubs (15 years old) at El-Nozha Public Garden, Alexandria Governorate.
Treatments as well as control treatment were replicated five times and randomly distributed over 50 shrubs (same age, height and size). Knapsack sprayer used for the application. Forty leaves from each treatment were selected, pre-treatment and five periods post treatment (one, two, three, four and eight weeks). Numbers of living individuals (adults and nymphs) were counted immediately, before and after spraying. To evaluate the efficiency of the tested chemicals, percentage of reduction was calculated according to (Henderson and Tilton, 1955). Statistical analysis of variance and L.S.D value for comparing the mean effects of each treatment were adopted according to (Snedecor, 1961).

2.3.Biochemical studies:

Samples of treated and untreated leaves were collected after 24, 48, 72 and 96 hours after application. Alive insect stages were collected and kept in a deep freezer (0^oC) until the biochemical assay. (Moursi *et al.*, 2010)

2.4.Sample Preparation and assays :

One gram of collected stages was placed in clean vials and homogenized in one ml distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 5000 r.p.m for 30 minutes at 4^oC and the supernatants were used directly as an enzyme source.

- Alkaline-phosphatase activity was spectrophotometrically determined according to the method of (Powell and Smith, 1954).
- Alanine transferases (ALT) and aspartate transferases (AST) activities were determined according to the method of Reitman and Frankle (1957).

3.Results and Discussion

3.1.Field Experiment:

Figure (1) illustrates the reduction effect of tested treatments against *Icerya aegyptiaca* (Douglas) after one, two, three, four and eight weeks of treatments application. The data indicated that there were no significant differences among tested binary mixtures of IGRs (lufenuron, chromafenozide, pyriproxyfen and RH- 2485) with Kz oil and they could be arranged in the descending order as follows : Kz + lufenuron > Kz + RH- 2485 > Kz + chromafenozide > Kz + admeral where they caused

reduction effect as 90.96, 87.44, 87.19 and 84.46%, respectively. There were no significant differences among chromafenozide, pyriproxyfen and RH-2485 when applied as a single treatment where they caused reduction effect as 77.28, 74.6 and 73.94%, respectively. However, IGR lufenuron which caused a reduced effect as 69.48% differ significantly from the other treatments. Kz oil alone did not differ significantly with it's binary mixtures with chromafenozide, admeral and RH- 2485.

Lufenuron had the lowest effect when applied alone but it was the best one when mixed with Kz oil. These findings may be resulted as an increasing of spreading and/or insect cuticle easily penetration which caused by Kz oil in the mixture. IGRs alone caused a reduction effect that is increased with time up to 3 weeks while Kz oil and it's binary mixtures were increased up to 8 weeks of application.

These results are in agreement with finding of Abdel Hafez *et al*., (1993); El-Kordy *et al*., (1995); El-Deeb (1999) and Abo-Shanab, (2005). They stated that pyriproxyfen could be considered as inhibiting agents for protein synthesis.

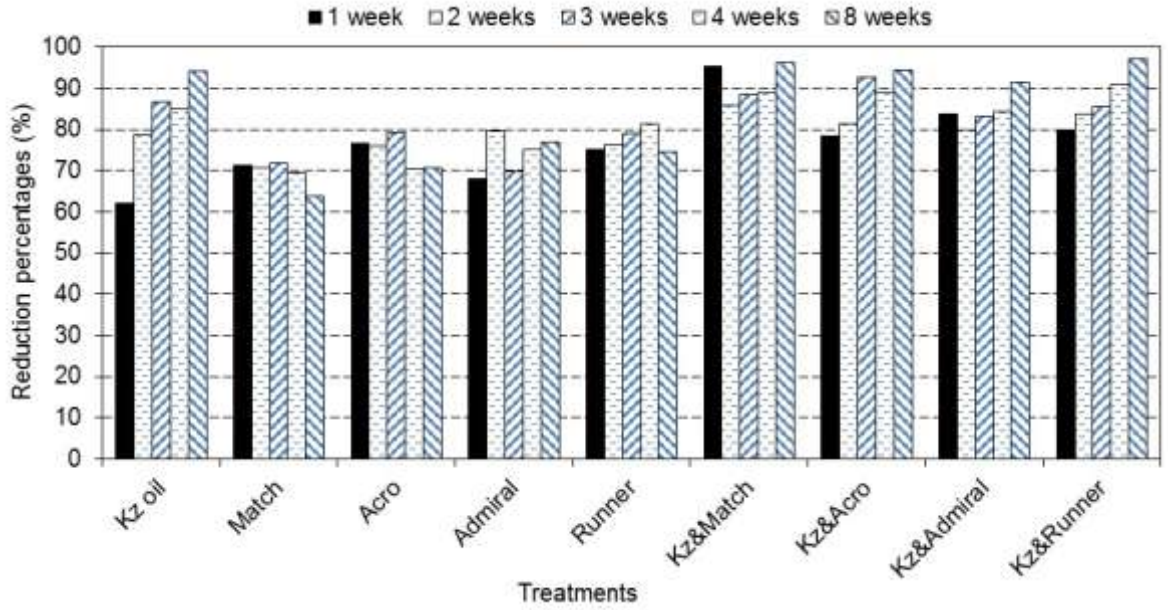

Fig. (1): Reduction effect of IGRs, Kz oil and binary mixtures against mealybug *Icerya aegyptiaca* (Douglas) attacked *Lantana camara* shrubs in Alexandria Governorate.

3.2.Biochemical studies (Enzyme activities assay) :

Data presented in Figures (2, 3 and 4) illustrate the effects of the tested chemicals against enzyme activity of Aspartate transferase (AST), Alanine transferase (ALT) and alkaline phosphatase (AlKP) of Egyptian mealy bug *Icerya aegyptiaca* (Douglas) infested *Lantana camara* shrubs in Alexandria Governorate after 24, 48, 72 and 96 hours of the application.

Generally, treatments increased the tested enzymes inhibition (*in vivo*) as a function of time (24, 48, 72 and 96 hours). The mixture of Kz oil and pyriproxyfen was the most effective one in *in vivo* inhibition of Aspartate transferase (AST) without significant differences with the other tested mixtures and so Kz oil alone where they caused 100, 95.2, 91.52, 89.6 and 87.65% for Kz + pyriproxyfen, Kz + RH- 2485, Kz + chromafenozide, Kz oil and Kz + lufenuron, respectively (Fig, 2).

There were no significant differences among the tested IGRs when used lonely except lufenuron, which showed the least inhibition effect (37.8%) after showing little enzyme activation after 24h. of treatment (-6.79%).

RH- 2485 and mixtures of Kz oil with tested IGRs inhibited AST enzyme activity without significant differences where they caused inhibition percentages of 100, 100, 99.71, 97.26 and 96.7 by Kz + RH- 2485, Kz + Lufenuron, RH- 2485, Kz + Pyriproxyfen and Kz + Chromafenozide, respectively.

Chromafenozide caused least inhibition effect (79.6%) without significant differences with Kz oil (89.4%), Lufenuron (86.86%) and Pyriproxyfen (83.8%). These results are not in agreement with finding of Said, (1998) who stated that IGRs increased the ALT and AST activity in larvae of *A. ipsilon*. Abdel Hafez *et al*., (1993) found that the changes in ALT and AST activities were in harmony with the changes in protein and free amino acids. Also Zidan *et al*., (1996), El-Deeb (2004) and Abo-Shanab (2005) mentioned that the inhibition of AST by IGRs reached its greatest effect within 24h.

Data showed in Figure (4) illustrate that there were no significant differences among RH- 2485 (100%), Kz + Chromafenozide (98.02%), Kz + RH- 2485 (95.64%), Kz oil (93.61%) and Kz + Pyriproxyfen (89.1%). Pyriproxyfen caused the least inhibition effect (71.48%) without significant differences with Chromafenozide (72.67%) and so there were no significant differences between Kz + Lufenuron (88.02%) and Lufenuron (86.12%).

It can be concluded that the use of insect growth regulator (IGRs) and their mixtures with mineral oils instead of conventional hazardous insecticides were efficient as inhibitors for insect enzymes such as AlKP, ALT and

AST and this may reduce the environmental pollution and hazard effects on human health. The obtained results are in agree with Moursi *et al*., 2010.

Our data supported that IGRs are effective when applied in very minute quantities and apparently have no undesirable effects on human and wildlife. Consequently, IGRs, when used with precision may play an important role in future insect pest management programs especially when mixed with mineral oils.

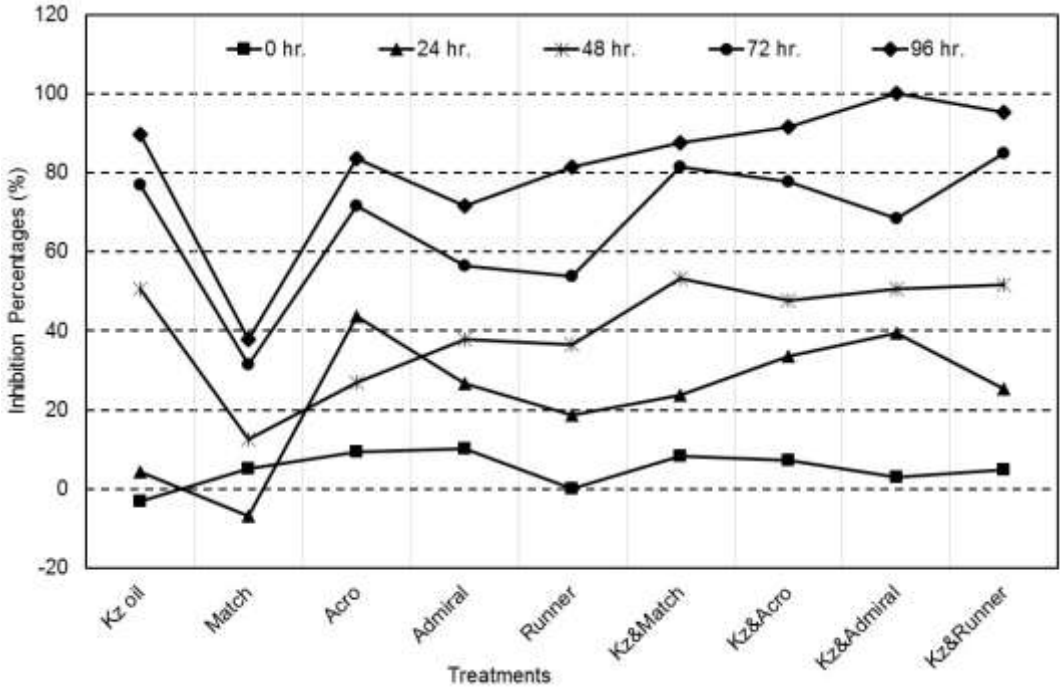

Fig. (2) : Effect of IGRs, Kz oil and binary mixtures on Aspartate transferase (AST) enzyme activity of mealybug *Icerya aegyptiaca*.

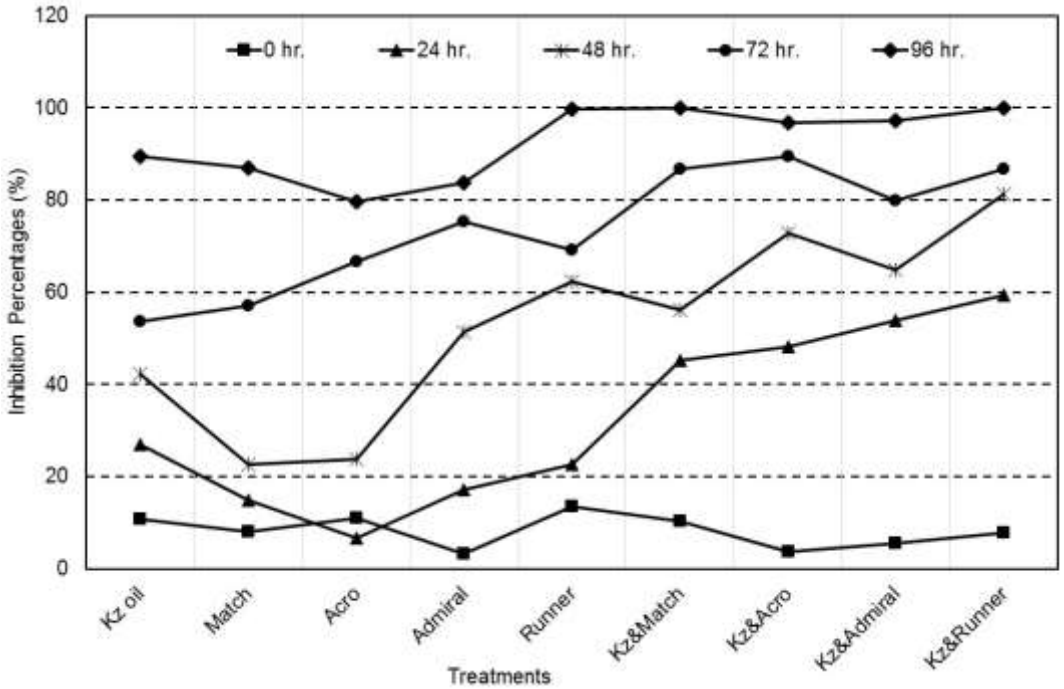

Fig. (3) : Effect of IGRs, Kz oil and binary mixtures on Alanine transferase (ALT) enzyme activity of mealybug *Icerya aegyptiaca*.

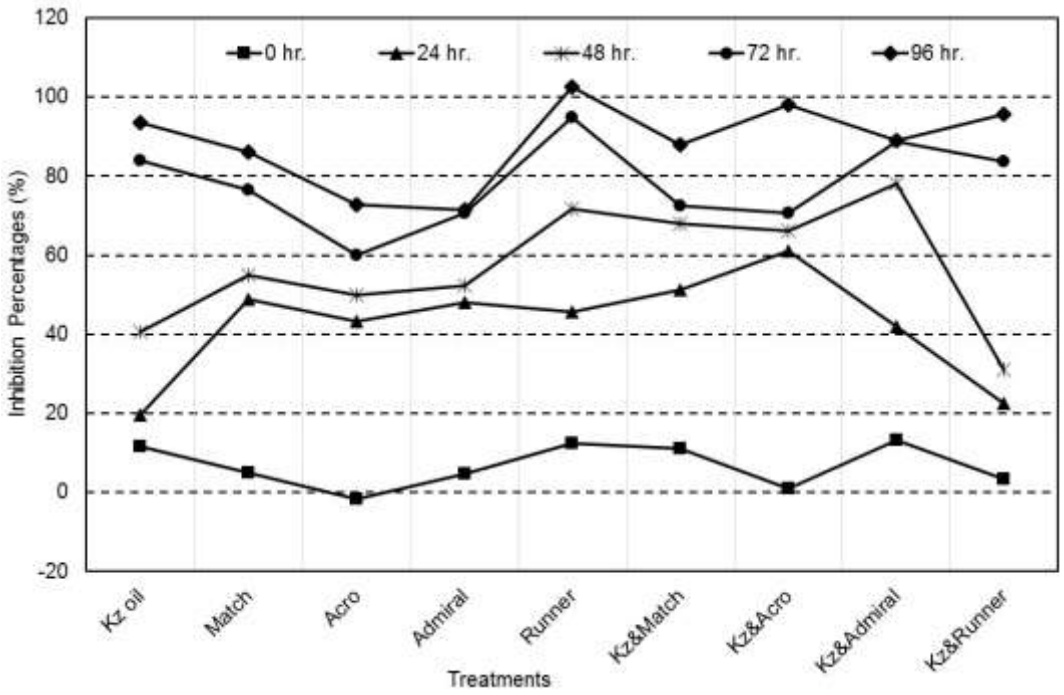

Fig. (4) : Effect of IGRs, Kz oil and binary mixtures on alkalinephosphatase (AlKP) enzyme activity of mealybug *Icerya aegyptiaca*.

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الملخص العربى

كفاءة بعض المبيدات على عدد الوفيات ونشاط بعض الانزيمات لحشرة البق الدقيقى المصرى التى تصيب شجيرات الزينة (لانتاتا كامارا) فى محافظة الاسكندرية ، مصر

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تم تقييم تأثير خمسة منظمات نمو حشرية (ماتش – أكرو – أدميرال – أرإتش٢٤٨٥ - رونر) والزيت المعدنى (كزد) ومخاليط منظمات النمو مع الزيت المعدنى على تعداد حشرة البق الدقيقى المصرى التى اصابت شجيرات الزينة (لانتاتا كامارا) فى حديقة النزهة العامة بالاسكندرية حيث سجلت النتائج قبل المعاملة وبعد ١ ، ٢ ، ٣ ، ٤ ، ٨ اسابيع.

تم جمع عينات حشرات كاملة من على الشجيرات المعاملة بعد ٢٤ ، ٤٨ ، ٧٢ ، ٩٦ ساعة لدراسة تأثير المعاملات المطبقة على نشاط انزيمات الألكالاين فوسفاتيز (AlKP)، إسبرتيت ترانسفيريز (AST) ، ألانين ترانسفيريز (ALT).

أظهرت النتائج ان الخلائط اعطت نسبة خفض فى تعداد الحشرة ونسبة تثبيط لمجموعة الانزيمات المختبرة اكبر من معاملات منظمات النمو الحشرية او الزيت المعدنى عند تطبيقهم بصورة فردية مما يوضح ان الزيت المعدنى (كزد) ينشط عمل منظمات النمو الحشرية. ولم تظهر اى فروق معنوية بين تأثيرات مجموعة الخلائط المختبرة وكذلك المعاملات الفردية.