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A Laboratory Study on The Toxicity of Some Insecticides Against The California Red Scale, *Aonidiella aurantii* on Mandarin Leaves in Qena Governorate, Egypt

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ABSTRACT

Citrus trees are an important strategic crop in Egypt grown in large areas with high productivity. As a result of climatic changes during this period, it was attacked by several pests causing heavy losses, whereas, California red scale (*Aonidiella aurantii*) is one of the most challenging pests to control in citrus orchards. In this laboratory study, we evaluated the toxic effect of nine selected insecticides, namely Daikon-lambada (10% WP), Aceta gold (20% WP), Acetampride, Malatox (57% EC), KZ oil (95% EC) + Malatox (57% EC), Metarhizium anisopliae (2.5% WP), IGR (0.5% EC), KZ (95% EC) + IGR (0.5% EC), KZ oil (95% EC) and Clean Touch (5% WP) against *A. aurantii* on mandarin leaves. It can be concluded that adult numbers have systematically decreased over time after spraying up to two weeks of treatment. The four insecticides KZ oil + Admiral,KZ oil + Malatox, KZ oil and Malatox were the most effective insecticides with a lethal effect against adult insects while increasing the from one day to two weeks after treatment. The highest mean reduction percentages of *A. aurantii* at adult stage (71.65, 58.75, 58.45 and 53.30 %) were recorded as result of applying the insecticides. The highest mean reduction percentages of pre-adult insects 66.31, 61.77, and 52.11% were recorded with KZ oil + Admiral, KZ oil, and KZ oil + Malatox, respectively. The study suggested introducing tank mixtures of KZ oil + Admiral, KZ oil, and KZ oil + Malatox, to minimize the development of pesticide resistance, it should prepare the management program on alternate insecticide combinations after determine the optimal timing and targeting vulnerable life stages to enhance sustainable California Red Scale management.

KEYWORDS: California Red Scale (CRS), Citrus, Toxicity, Pesticides, Mixtures.

1. INTRODUCTION

Citrus fruits come second after grapes in terms of fruit production in the world and are among the most important fruits in Egypt. Citrus fruits in Egypt are considered one of the most important crops exported to most countries of the world, as approximately 2.3 million tons of citrus fruits are exported annually, according to the Egyptian Agricultural Export Council (AEC), (Sultan 2020**;** Spreen et al., 2020). *A. aurantii* is considered one of the most dangerous and economically important insect pests that infect citrus trees in the Mediterranean region and most countries of the world (Vacas et al., 2015; Mansour et al., 2017).Infestation by this insect initially leads to yellowing of the leaves. As the infestation increases in severity, the leaves begin to wilt and fall off. Fruits may fall due to being heavily covered with California red scale (CRS) insects, and branches and small twigs may die. In severe cases, young trees die. The damage increases during hot weather due to water stress. Fruits that were infected during early growth show noticeable holes where the insects were, even after their death. The scales remain firmly attached to the surface of the fruit and are difficult to remove, thus reducing the marketing value of the infected fruit, (Cass et al., 2020; Just et al., 2020). It also causes the growth of black mold as a result of the insect secreting a sugary substance that helps the fungus grow, which hinders the process of photosynthesis in trees and the production of small fruits (Mohammed et al., 2020). The use of pesticides over the past decades has proven the importance of the effect of pesticide mixtures on the California red scale (CRS) insect and reducing and controlling pest numbers. In the laboratory study, it was possible to reduce the dose of organophosphate insecticides without affecting their effectiveness against California red scale insects. Insecticides, which are widely used in general due to its high effectiveness is chlorpyrifos and has an effective effect in reducing the pest (Walker et al., 1991 Garcerá et al., 2011; Legrand et al., 2017). Effect of different insecticides on the population of *A. aurantii* (CRS) on orange trees the tested insecticides reduced the population of the treated insect stages as a function of the treatment stage, mode of action of the insecticide and time after treatment. Among the tested insecticides, Kz oil was the most effective in reducing the population of the treated insect through the smothering effect (Rezk et al., 2021; Singh et al., 2023). Climate change affects citrus productivity due to increased insect pests and causes a decrease in yield and fruit quality, which causes restrictions on citrus exports (Fares et al., 2017). Therefore, it is necessary to investigate the insect pests and diseases of citrus trees, which cause damage to the fruits and to choose effective and tangible control methods when applied in the short and long term and whether these methods respect the export specifications of citrus fruits as a result of the application of chemical pesticides (Grafton-Cardwell et al., 2005; Lahlali et al., 2021). Chemical control is the most commonly used method against California red scale (CRS) pests. However, repeated applications of non-specific insecticides can lead to failure of control programs and pose a risk to beneficial arthropods (Desneux et al., 2007; Levitin and Cohen 1998; Planes et al., 2013; Mansour et al., 2018).

California red scale (*Aonidiella aurantii*) is one of the most challenging pests to control in citrus orchards due to its rapid reproduction and high Infestation Levels, waxy protective cover, Scales can infest leaves, twigs, and fruit, making thorough coverage with sprays challenging, Disruption of natural enemies often results in red scale outbreaks, and citrus-growing Conditions, favor the development of red scale populations Therefore, the aim of the present study was to evaluate the efficacy of nine selected insecticides to determine the most effective insecticides against adult and pre-adult insects. The results of such research serve as a basis for developing control programs that include sequence of insecticides to slow the development of resistance in California red scale (CRS).

2. MATERIALS AND METHODS

2.1. Treated insect:

The toxicological study was carried out to evaluate the toxicity effects of different chemical and non-chemical compounds against pre-adult and adult stages of the California Red Scale (CRS), *A. aurantii* on mandarin leaves, under laboratory conditions in the Plant Protection Department, Agricultural Research

Station of Al-Marashda, Qena Governorate, Egypt.

2.2. Tested Insecticides:

Nine commercial insecticides belong to different chemical groups were tested for their lethality against the (nymphs) of the *A. aurantii* on mandarin trees through laboratory conditions. The common name, trade name, type of formulation and the tested rate for each insecticide is shown in Table 1. The infested mandarin leaves were collected randomly from infested farm in Qena governorate and kept in paper bags then transferred to laboratory; five leaves from each trees were examined and the number of alive individuals of the insect was counted before applying treatment and after one day, 3 days, one week and 2 weeks of the treatment (Madgwick and Kanitz 2023). So, the present experiment was laid out in split-plot design. The different time of post treatments (pre-dipped, one day after treatment (AT) two days AT, one week AT, and two weeks AT) were as main plots. While, the sub-plots were represented by ten insecticide treatments, the infested leaves were dipped in the tested pesticides treatments, whereas the control leaves dipped in water only and the leaves were left for dryness in air**.** Under a microscope, the collected leaves were investigated. The percentage of population reduction was calculated according the following equation (Mohamed and Ibrahim 20220):

$$\% \text{ Reduction} = \frac{\text{pre treat} - \text{post treat}}{\text{pre treat.count}} \times 100$$

2.3. Data analysis:

According to Piepho et al., 2024, the average percentage of adjusted insect mortality for each insecticide and control was computed. Data were statistically analysed using ANOVA test and the means were compared using the least significant difference (L.S.D.) test at probability level of ≤ 0.05 for the comparison according to Gomez and Gomez (1983).

Table 1. Insecticides and treatments used in the study

No.	Common name	Trade name	Tested rate
1	KZ oil	KZ oil 95% EC	1.5 l/100 liter water
2	Touch clean	Touch Clean 5% WP Emametin-benzoate	100 g/100 liter water
3	Daikon lambada	Daikon-lambada 10% WP	250 g/100 liter water
4	Aceta gold	Aceta gold 20% WP Acetampride	100 g/ 100 liter water
5	Malatox	Malatox 57% EC Malathion	500 cm^3/ 100 liter water
6	KZ oil+ Malatox	KZ oil 95% EC + Malatox	1.5 l/100 liter + 500 cm^3/ 100 liter
7	Biomita	Metarhizium anisopliae WP 2.5%	250 g/ 100 liter water
8	Admiral EC	IGR EC 0.5%	50 cm^3/ 100 liter water
9	KZ oil + Admiral EC	KZ 95% EC + Admiral EC	1.5 l/ 100 liter+0.5 l/ 100 liter
10	Control (sprayed with water)		

3. RESULTS AND DISCUSIONS

3.1. Effect on adult insects:

The tested pesticides were treated adult insect stage according to Minster of Agriculture Protocols. These effects are shown in Table 2 and Figure 1 that the adult population decreased significantly over time after dipping up to two weeks of treatment. As shown, the adult population differed significantly with the pesticides tested. The treatment of KZ oil + Admiral, KZ oil + Malatox, KZ oil , and Malatox were the most effective insecticides with a lethal effect against adult insects as the time in systemic order increased from one day to two weeks after treatment. These effective insecticides have led to a drastic reduction in the treated population from 37.0 to 6.0, from 41.0 to 11.0%, from 40.0 to 13.0 and from 38.0 to 14.0 %in the case of adult stage, respectively. On the

Table 2. Effect of different insecticides in controlling of *A. aurantii* (adult) population at one day, 3 days, one week and two weeks after treatment

Time post treatment (A)	Insecticides (B)										
	KZ oil	Touch clean	Daikon lambada	Aceta gold	Malatox	KZ oil + Malatox	Biomita	Admiral (IGR)	KZ oil + Admiral	Control	M
Pre-dipped	40.0	35.0	37.0	32.0	38.0	41.0	37.0	41.0	37.0	38.0	37.6
One day	21.0	28.0	24.0	19.0	23.0	24.0	30.0	33.0	18.0	35.0	25.5
3 days	18.0	26.0	21.0	17.0	18.0	18.0	27.0	30.0	10.0	33.0	21.8
1week	14.0	24.0	19.0	15.0	16.0	15.0	25.0	25.0	8.0	31.0	19.2
2 weeks	13.0	22.0	17.0	13.0	14.0	11.0	24.0	22.0	6.0	29.0	17.1
M	21.2	27	23.6	19.2	21.8	21.8	28.6	30.2	15.8	33.2	
LSD 5%				A=2.9		B=7.1		AB=15.257			

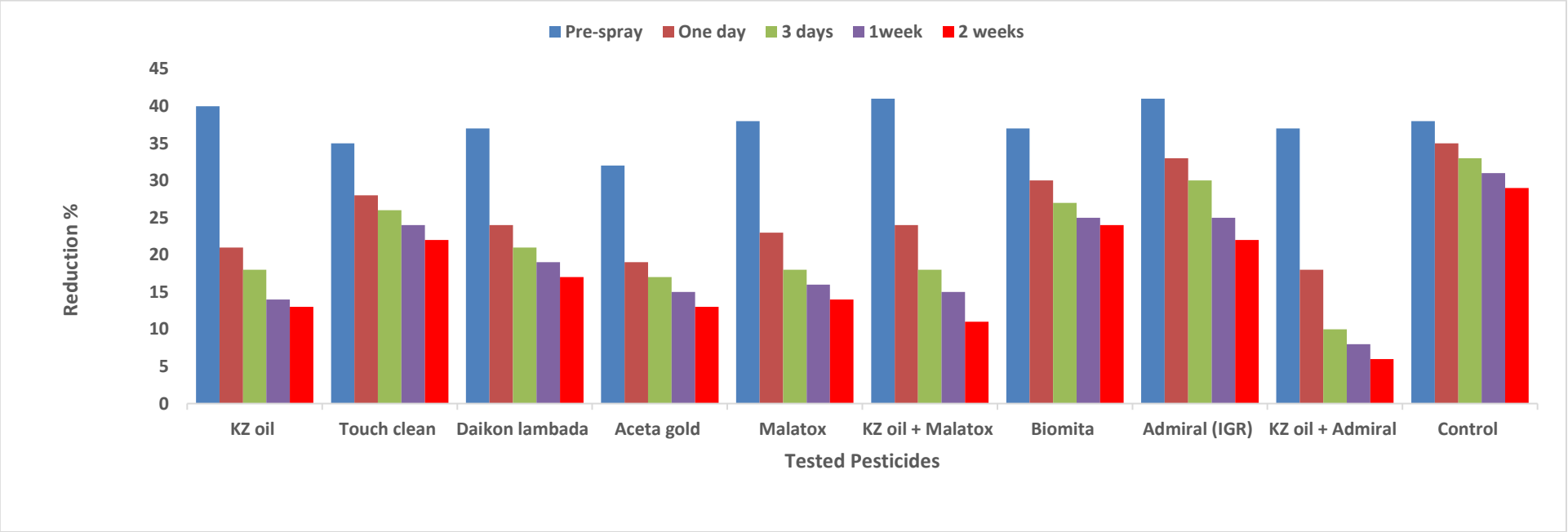

Figure 1. Effect of different insecticides in controlling of *A. aurantii* (adult) population at one day, 3 days, one week and two weeks after treatment

reduced for the adult population from 38.0 to 6.0, 37.0 to 24.0, and 35.0 to 22.0% respectively. The effect of the interaction between the post-treatment time and the insecticide showed that the application of the insecticides KZ oil + Admiral, KZ oil, KZ oil + Malatox and Malatox, respectively. Meanwhile, the lowest average decreases were observed with untreated insects (15.80%), Biomita insecticides (28.38%) and Touch clean (31.02%). However, KZ oil + Admiral, followed by KZ oil, and KZ oil + Malatox were the most effective insecticides against adult of the California Red Scale insect, that resulting in the highest ratios of insect reduction after one day, three days, and one week of treatment. KZ oil + Admiral pesticides, followed by KZ oil + Malatox and KZ oil were the most effective in controlling adult insects, resulting in the highest decreases of 83.8, 73.2 and 67.5 % after two weeks of treatment, respectively. On the other side, the insecticide Biomita, followed by Touch clean had the least effect on reduction ratios of 35.1 and 37.0 % after two weeks of treatment, respectively. These findings have been agreed with Rezk et al., (2021) and Planes et al., (2013), who studied the effect of certain insecticides on the California Red Scale on orange trees and found that the insecticides tested reduced the number of treated insects. They stated that among the pesticides tested, Kz oil was the most effective, reducing the treated population through the choking effect. the insecticide KZ oil + Admiral for two weeks, followed by the same insecticide for one week led to a greater decrease in the number of adults compared to other treatments. These obtained results agreed with Rezk et al., (2021), who indicated that among the insecticides tested on *A. aurantii* , Kz oil was the most effective, reducing the treated population in the systemic order through the choking effect. At the same time, Pyriproxyphen (Admiral) appeared to be low effective in reducing the adult population due to the way the insect growth regulator works. These pesticides are known as integrated pest management compatible with little or no effect against California's natural, red-crusted enemies, and low mammalian and human toxicity. Singh et al., (2023) suggested an allergy of *A. aurantii* to Kz oil. Also, Mansour et al., (2018) concluded the pathological findings of Kz oil against *A. aurantii* at 2, 4, and 6 weeks post-treatment on ballade orange trees.

3.2. Effect on the reduction percentage of adult insects:

As shown in Table 3 and Figure 2, the highest average rates of decline of *A. aurantii* of adult stage (71.65, 58.75, 58.45 and 53.30%) were recorded as a result of the application of other hand, the lowest activities achieved from Biomet and Touch clean treatments that recorded the largest number of adults alive after three days, one week and two weeks of treatment. These effective treatments were

Table 3. Effect of different insecticides in reduction (%) of *A. aurantii* (adult) population at one day, 3 days, one week and two weeks after treatment

Insecticides	Time post treatment					
	Pre-dipped	One day	3 days	1 week	2 weeks	M
KZ oil	-	47.5	55.0	65.0	67.5	58.75
Touch clean	-	20.0	35.7	31.4	37.0	31.02
Daikon lambada	-	35.1	43.0	48.6	50.1	44.20
Aceta gold	-	40.6	46.9	53.0	59.4	49.98
Malatox	-	39.5	52.6	57.9	63.2	53.30
KZ oil + Malatox	-	41.5	56.1	63.0	73.2	58.45
Biomita	-	18.9	27.0	32.4	35.1	28.38
Admiral (IGR)	-	19.5	26.0	39.0	46.3	32.70
KZ oil + Admiral	-	51.4	73.0	78.4	83.8	71.65
Control	-	7.89	13.2	18.4	23.7	15.80

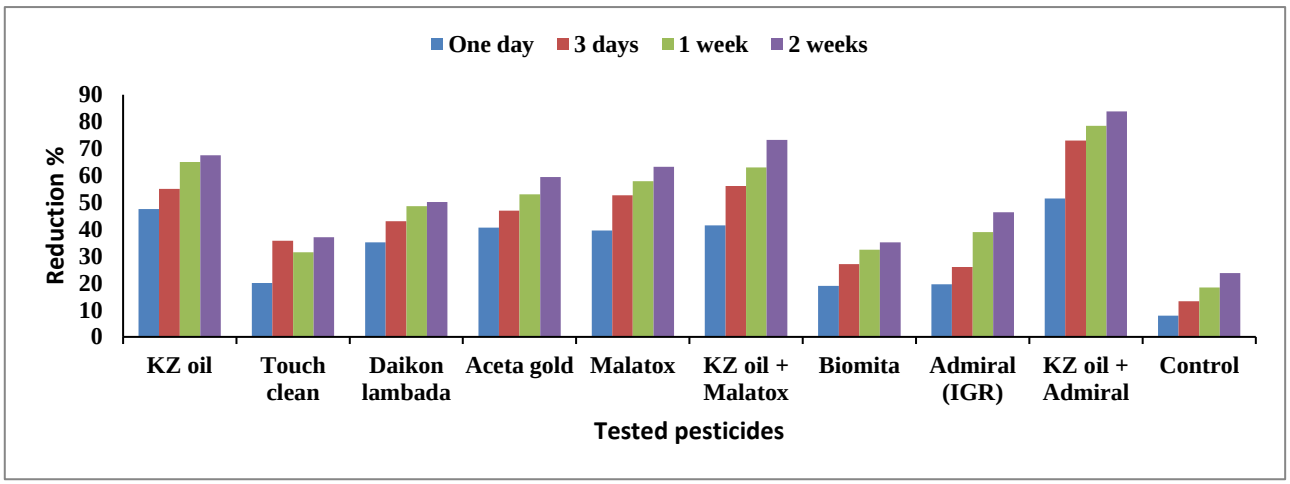

Figure 2. Effect of different insecticides in reduction (%) of California red scale, *A. aurantii* (adult) population at one day, 3 days, one week and two weeks after treatment

3.3. The effect on the pre-adult insects:

The effects of post-treatment time and pesticides were significant on the pre-pubertal population as shown in Table 4 and Figure 3. Among the tested treatments, the insecticides KZ oil + Admiral, KZ oil + Malatox, KZ oil, and Malatox were the most effective insecticides and harshly decreased the treated pre-adult populations from 122.0 to 27.60%, from 120.0 to 30.40, from 140.0 to 36.40%, and from 132.0 to 42.50%, respectively. On the other side, the insecticides Biomita, Touch clean, and Admiral had the least impact on the pre-adult insects. These insecticides decreased the pre-adult populations from 115.0 to 63.9%, from 135.0 to 70.60%, and from 110.0 to 53.40%, respectively, after two weeks of treatment. The effect of the interaction between post-treatment time and pesticides showed that the application of the insecticide KZ oil + Admiral for two weeks, followed by the same insecticide for one week led to a greater decrease in the number of pre-adult compared to other treatments. It could be concluded that the effect of insecticide for controlling the *A. aurantii* depend on the time of the control. Our results agreed with those of Rezk et al. (2021). Garcerá et al. (2011) studied the influence of some insecticides in controlling the pre-adult California red scale showed that the tested insecticides can be arranged according to their effects as follows, Sulfoxaflor, Pyriproxyfen, Azadirachtin, Acetamiprid and Kz oil, respectively.

3.4. The effect on the reduction percentage of pre-adult insects:

Results in Table 5 showed the reduction percentages of pre-adult of California red scale as affected by different insecticides under laboratory conditions. The highest mean reduction percentages 66.31, 61.77, and 52.11% were recorded with insecticides KZ oil + Admiral, KZ oil, and KZ oil + Malatox, respectively. Meanwhile, the lowest reduction percentages were 36.42 and 37.47 % in pre-adult insects due to apply Acela gold and Biomita insecticides, respectively. However, insecticides KZ oil and KZ oil + Admiral were the most effective ones in controlling the pre-adult insect, as it led to the highest reduction percentages of 49.64 and 46.31 % after one day of treatment, respectively. Insecticides KZ oil + Admiral, KZ oil, and Admiral were the most effective ones in controlling the pre-adult insect, as it resulted in the highest reduction percentages after three days of treatment. KZ oil + Admiral, followed by KZ oil and KZ oil + Malatox were the most effective insecticides in controlling the pre-adult insect, as it resulted in the highest reduction percentages of 75, 68.71 and 66.25 % after one week of treatment, respectively. Moreover, insecticides KZ oil + Admiral, KZ oil + Malatox and KZ oil and Malatox were the most effective ones in controlling the pre-adult insect, as it led to the highest reduction percentages of 77.38, 74.67, 74.0 and 67.80 % after two weeks of treatment, respectively. Our results supported by Walker et al., (1991 Garcerá et al., (2011); Legrand et al., (2017) they evaluated the effect

Table 4. Effect of different insecticides in controlling of *A. aurantii* (pre-adult) population at one day, 3 days, one week and two weeks after treatment

Time post treatment (A)	Insecticides (B)										
	KZ oil	Touch clean	Daikon lambada	Aceta gold	Malatox	KZ oil + Malatox	Biomita	Admiral (IGR)	KZ oil + Admiral	Control	M
Pre-dipped	140.0	135.0	128.0	130.0	132.0	120.0	115.0	110.0	122.0	140.0	127.2
One day	70.5	101.3	109.3	117.8	110.6	93.7	81.3	82.4	65.5	130.7	96.3
3 days	63.4	81.5	83.1	90.3	85.7	65.3	75.3	56.2	40.8	115.3	75.7
1week	43.8	76.5	55.9	70.6	60.3	40.5	67.2	54.9	30.5	95.9	59.6
2 weeks	36.4	70.6	53.8	51.9	42.5	30.4	63.9	53.4	27.6	88.9	51.9
M	70.8	93.0	86.0	92.1	86.2	70.0	80.5	71.4	57.3	114.2	
LSD 5%				A=1.2		B=7.4		AB=15.8			

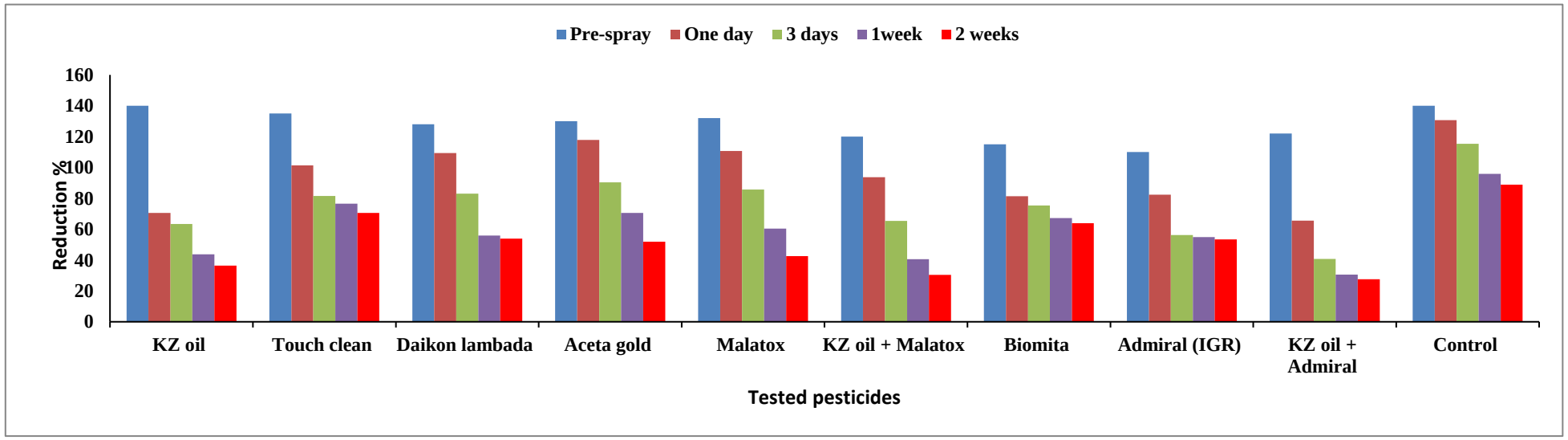

Figure 3. Effect of different insecticides in controlling of California red scale, *A. aurantii* (pre-adult) population at one day, 3 days, one week and two weeks after treatment

Table 5. Effect of different insecticides in reduction (%) of California red scale, *A. aurantii* (pre-adult) population at one day, 3 days, one week and two weeks after treatment

Insecticides	Time post treatment					
	Pre-dipped	One day	3 days	1week	2 weeks	M
KZ oil	-	49.64	54.71	68.71	74.00	61.77
Touch clean	-	24.96	39.63	43.33	47.70	38.91
Daikon lambada	-	14.60	35.08	56.33	57.97	41.00
Aceta gold	-	9.38	30.54	45.69	60.08	36.42
Malatox	-	16.21	35.08	54.32	67.80	43.35
KZ oil + Malatox	-	21.92	45.58	66.25	74.67	52.11
Biomita	-	29.30	34.52	41.57	44.43	37.46
Admiral (IGR)	-	25.09	48.91	50.09	51.45	43.89
KZ oil + Admiral	-	46.31	66.56	75.00	77.38	66.31
Control	-	6.64	17.64	31.50	36.50	23.07

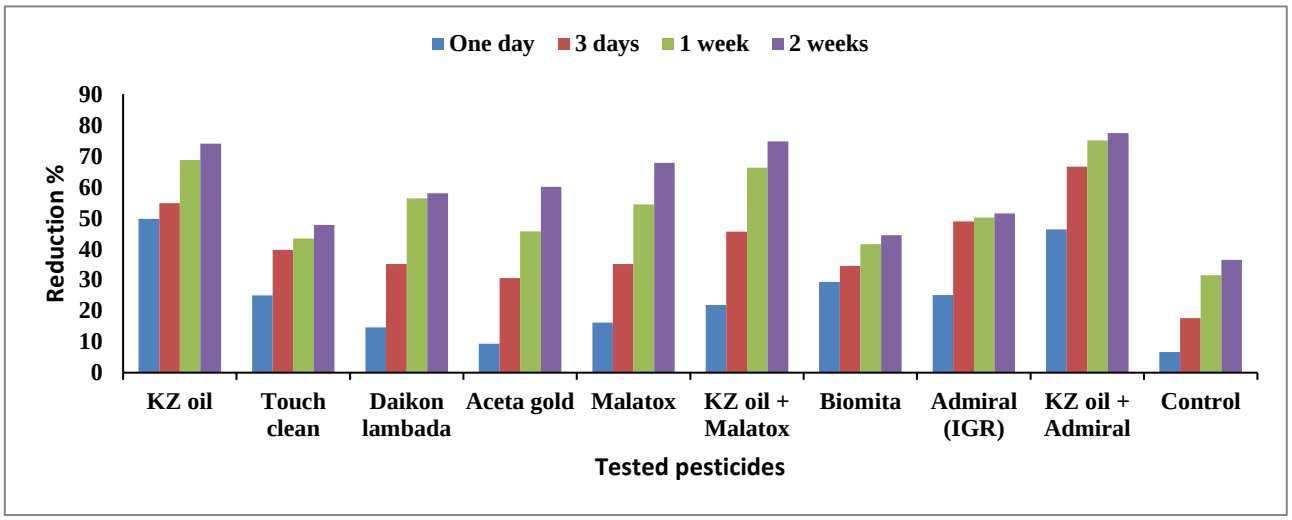

Figure 4. Effect of different insecticides in reduction (%) of California red scale, *A. aurantii* (pre-adult) population at one day, 3 days, one week and two weeks after treatment.

of certain insecticides in controlling the California red scale pointed out that some of the tested insecticides had a positive toxic effect on the insect. It can be concluded that there were some insecticides among the tested ones as KZ oil + Admiral, KZ oil, and KZ oil + Malatox that had a highly toxic effect on the insect, whether adult or pre-adult. These insecticides were effective in increasing the reduction percentage in the number of insects treated with them.

4. CONCLUSION

At the conclusion of this study, it can be concluded that the pesticide mixtures used succeeded in reducing the numbers of California red treated stages (adult, crawler, nymphs) as well as against the total population in different ways according to their different modes of operation. The combined use of KZ oil with Admiral or Malatox proved to be highly effective in controlling *Aonidiella aurantii* infestations, particularly under the serious conditions of climate change. These treatments achieved significant reductions in both adult and pre-adult stages, with prolonged effectiveness lasting up to two weeks after application in integrated pest management (IPM) by reducing environmental risks and preserving agricultural productivity. Insecticide mixtures are one way to delay the development of insecticide resistance. The effect of oil pesticides is negligible against natural enemies and low toxicity to mammals and humans. It has also been classified as non-carcinogenic, which encourages us to emphasize its use to control California red scale (CRS)

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الملخص العربي

دراسة معملية عن سمية بعض المبيدات الحشرية ضد الحشرة القشرية الحمراء الكاليفورنية، *Aonidiella aurantii* التي تصيب أوراق اليوسفي بمحافظة قنا، مصر.

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تعد أشجار الموالح من المحاصيل الاستراتيجية الهامة في مصر، حيث تزرع في مساحات واسعة وتتمتع بإنتاجية عالية. ونتيجة للتغيرات المناخية خلال هذه الفترة، تعرضت لهجوم من الحشرة القشرية الحمراء الكاليفورنية، مما تسبب في خسائر فادحة في المحاصيل والأشجار. في هذه الدراسة المعملية، قمنا بتقييم التأثير السام لتسعة مبيدات حشرية مختارة، وهي: Daikon-lambada (10% WP), Aceta gold (20% WP) Acetampride, Malatox (57% EC), KZ oil (95% EC) + Malatox (57% EC), Metarhizium anisopliae (2.5% WP), IGR (0.5% EC), KZ (95% EC) + IGR (0.5% EC), (KZ oil (95% EC) and Clean Touch (5% WP ، ضد *A.aurantii* على أوراق اليوسفي. ويمكن الاستنتاج أن أعداد البالغين انخفضت بشكل منهجي بمرور الوقت بعد رش ما يصل إلى أسبوعين من المعاملة. كانت المبيدات الحشرية الأربعة KZ oil + Admiral و KZ oil + Malatox و KZ oil و Malatox هي المبيدات الحشرية الأكثر فعالية مع تأثير قاتل ضد الحشرات البالغة مع زيادة في مدة المعاملة من يوم واحد إلى أسبوعين. تم تسجيل أعلى متوسط نسب انخفاض لـ *A. aurantii* في مرحلة الحشرة الكاملة (٧١,٦٥ و ٥٨,٧٥ و ٥٨,٤٥ و ٥٣,٣٠٪) نتيجة لتطبيق المبيدات الحشرية. تم تسجيل أعلى متوسط نسب انخفاض للحشرات قبل البلوغ ٦٦,٣١ و ٦١,٧٧ و ٥٢,١١٪ باستخدام المبيدات الحشرية KZ oil + Admiral و KZ oil و KZ oil + Malatox على التوالي. وقد أظهرت مخاليط هذه المبيدات الحشرية نتائج جيدة في مكافحة حشرة كاليفورنيا الحمراء.

الكلمات المفتاحية: الحشرة القشرية الحمراء الكاليفورنية (CRS) ، الموالح، السمية، مخاليط المبيدات الحشرية.