

EFFECT OF SOME NATURAL COMPOUNDS ON *AONIDIELLA AURANTII* (MASKELL) (HEMIPTERA; COCCOIDEA: DIASPIDIDAE) AND ITS PARASITOID *COMPERIELLA LEMNISCATA* COMPERE AND ANNECKE (HYMENOPTERA: ENCYRTIDAE) ON CITRUS IN EGYPT

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Abstract

Aonidiella aurantii (Maskell) (Hemiptera; Coccoidea: Diaspididae) is a serious pest on various economic crops in Egypt. The main injury caused by this insect is the ingestion of plant sap. Severely infested plants grow poorly, may drop their leaves prematurely and can suffer dieback of twigs and branches. The encyrtid parasitoid, *Comperiella lemniscata* Compere and Annecke (Hymenoptera: Encyrtidae) has been recorded as an effective parasitoid of armored scale insects in various parts of the world, including Egypt. The present paper describes some laboratory and field tests on the effect of KZ oil, Neemex, Sulphur and Actalic compounds on *A. aurantii* and its parasitoid. In the laboratory tests, five concentrations of KZ oil, Neemex, Sulphur and Actalic were used; twenty infested leaves of citrus were dipped in each concentration for five seconds. Leaves were divided into five replicates. Five leaves were dipped in clean water as untreated controls. The cultures was maintained at room temperature about 25±1°C and 65±1% relative humidity. After 24 hours, the live *A. aurantii* and parasitoids were counted.. The results suggested that the potency of KZ oil, Neemex, Sulphur and Actalic varied greatly between compounds and concentrations. Also the results suggest that when adult female and nymphal *A. aurantii* and the adult parasitoid were treated with the these chemicals , the percentage mortality ranged from 74.0-95% with KZ oil, 45.0-89.4% with Neemex, 57.2-93.8% with Sulphur and 82.5-100% with Actalic respectively. In field tests, two fields were selected. The first had a heavy infestation of *A. aurantii* and the second had abundant parasitoids. The fields were treated twice, in 2008 and 2009, and samples were collected after 3, 7 and 15 days post-treatment. Neemex and Sulphur gave moderate effects against the nymphs and adult female of *A. aurantii*, with average mortalities of 60 & 57% due to Neemex and 64 & 61 % due to Sulphur after 15 days in the two years. The parasitised scales had percent mortalities of 55 & 59 % respectively after 15 days. On the other hand, Actalic gave high efficacy against all targets, with 95 & 96% and 95 & 98% mortalities after 15 days against *A. aurantii* in the 2 years, while the mortality of the parasitised scales was 84 & 81% after 15 days in the 2 years, respectively.

INTRODUCTION

Red scale *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) is a serious pest on different economic crops (Claps *et al.* 2001) and the red scale occurs on host plants belonging to at least 77 plant families (Borchsenius, 1966). In Egypt, Abd-Rabou (2009) reported *A. aurantii* attacked six host plants. This pest inserts its mouth parts deep into plant tissue and sucks sap from parenchyma cells. Saliva injected as the scales feed is very toxic to the leaves, twigs, branches and fruit of citrus trees. The leaves develop a characteristic yellow spot under and around each female scale. Prolonged infestation may cause leaf drop and defoliation and dieback of twigs and eventually large branches. Maturing fruit can become completely encrusted with scales, developing scales form prominent pits on young fruit which are still evident when the fruit matures. Such fruit tend to dry out and fall off. Even the trunk can become heavily infested (Bedford, 1998). The encyrtid parasitoid, *Comperiella lemniscata* Compere and Annecke (Hymenoptera: Encyrtidae) was recorded in Egypt for the first time in Egypt associated with the armored scale insect, *A.aurantii* in Giza by Abd-Rabou and Attia.(2006). The parasitoid, *C. lemniscata* recorded as an effective parasitoid of armored scale insects (Abd-Rabou, 2006 and Abd-Rabou and Attia. 2006).

Effect of insecticides and biopesticides on the red scale, *A. aurantii* and its natural enemies was studied by many workers, Catling (1971),Nigam (1971), Ofek *et al.* (1997), Phillips *et al.* (1983), Uygun and Sekeroglu (1984), Zuniga (1985), Morse and Bellows (1986), Grafton-Cardwell and Reagan (1995), Krishnamoorthy and Rajagopal (1995 and 1998), Liotta and Mineo (2008) and Reecha, *et al.* (2009).

The present paper describes some laboratory and field tests on the effect of KZ oil, Neemex, Sulphur and Actalic compounds on *A. aurantii* and its parasitoid, *C. lemniscata*.

MATERIALS AND METHODS

1. Laboratory experiments:

1.1. Mass rearing of *Aonidiella aurantii* and its parasitoid, *Comperiella lemniscata* :

A. aurantii was reared on citrus seedling under laboratory conditions (23±2°C and 60±5% R.H.) . Culture of the parasitoid, *C.lemniscata* was started that emerged from the collected specimens from the field and the colony was reared on *A. aurantii* feeding on citrus seedling. The laboratory conditions were 25-27 C° and 65-70 R.H.

1. 2. Tested commercial formulation :

The following four compounds were tested:

- a. KZ oil
- b. Neemex, a botanical extract containing 1% Azadirachtin a (10 g/liter) from the neem tree, *Azadirachta indica* (Meliaceae), applied at a rate of 2 ml/liter of water.
- c. Micronized Sulphur 85% It was applied at a rate of 2 gm /liter of water.
- d. Actalic a chemical pesticide was applied at a rate of 3 ml/ liter of water.

1. 3. Tested methods

The laboratory experiments were carried out in the Laboratory of Plant Protection Research Institute, ARC, Dokki, Giza. The method of indirect exposure was used to evaluate the effect of the four compounds on the *A. aurantii* and its parasitoid, *C. lemniscata* throughout the present investigation. Five concentrations of KZ oil, Neemex, Micronized Sulphur and Actalic were used; twenty infested leaves of citrus were dipped in each concentration for five seconds. Leaves were divided into five replicates. Five leaves were dipped in clean water as untreated check (control). The leaves were transferred to clean wide plastic dishes, which were then covered with muslin cloth held in position by rubber bands. After 24 hours the alive of *A. aurantii* and its parasitoid, *C. lemniscata* were counted.

1. 4. Statistical analysis

In laboratory tests, the mortality percentages were calculated and corrected for natural mortalities by Abbott's formula (1925). The corrected percent mortalities were statistically computed according to Finney (1971) and plotted on probit analysis paper. The tested compounds were compared for their efficiency on parasitoid and prey according to their LC_{50} , LC_{90} and slopes of the toxicity lines.

2. Field experiments:

The experiments were carried out to evaluate of the four compounds (KZ oil, Neemex, Sulphur and Actalic) on *A. aurantii* and its parasitoid, *C. lemniscata* on citrus were carried out at Beni-Suef Governorate. When the numbers of *A. aurantii* and its parasitoid, *C. lemniscata* were high during the two seasons, 2008 and 2009.

2.1. The experiments comprised 4 compounds:

- a. KZ oil
- b. Neemex, a botanical extract containing 1% Azadirachtin a (10 g/liter) from the neem tree, *Azadirachta indica* (Meliaceae), applied at a rate of 2 ml/liter of water.
- c. Micronized Sulphur 85%+ Super Misrona 95% EC, a local mineral oil, containing 95% paraffinic oil w/w and 5% inert ingredients, unsulfonated residue content reached 92%. It was applied at a rate of 2 gm + 20ml/liter of water.
- d. Actalic a chemical pesticide was applied at a rate of 3 ml/ liter of water.

Each treatment conducted in 1/4 Fadden. One quarter of Fadden was also used as an untreated check (control). Spraying was applied at the rate of per plant which was accomplished by the use of sprayer of 600 liter capacity. Pre-spraying counts were made just before spraying. The post spraying counts were made after 3, 7 and 15 days from application. Random samples of 30 leaves were picked up from each replicate. A total number of 60 infested leaves for each treatment thus examined. By means of a stereoscopic microscope insect was inspected.

2.2. Statistical analysis

The percent reduction of infestation was statistically calculated according to the equation of (Henderson and Tilton 1955).

$$\% \text{ mortality} = 100 \left[1 - \frac{\text{Ta} \times \text{Cb}}{\text{Tb} \times \text{Ca}} \right]$$

Where:

Ta = Post treatment insect counts

Cb = Untreated insect count before treatment

Tb = Pretreatment counts

Ca = Untreated insect count after treatment.

RESULTS AND DISCUSSION

1. Laboratory Experiments:

1.1. On nymphs and adult of *A. aurantii*:

Data presented in Table (1) showed the potency of KZ oil, Neemex, Sulphur and Actalic on nymphs and adult of *A. aurantii* under laboratory conditions.

Tabulated data indicate that the potency of KZ oil, Neemex, Sulphur and Actalic was varied tremendously due to compounds. As a general trend, data proved that at any of the compounds the higher the concentration, the higher was the rate of mortality was obtained and vice versa.

According to the obtained data (Table 1), different mortality percentages were recorded when *A. aurantii* were treated with Neemex, Sulphur, KZ oil and Actalic, the mortality percentages of *A. aurantii* nymphs ranged from 52.9-89.4, 63.4-93.8, 80.4-95%, and 86.3- 100% respectively.

When compare between the effects of Neemex, Sulphur, KZ oil, and Actalic it was found that LC₅₀ were (0.545, 0.27, 0.101 and 0.095), respectively (Table, 2). On

base of the LC_{90} values, it was found Neemex, Sulphur, KZ oil, and Actalic, were (6.153, 3.45, 0.921 and 0.743), respectively (Table, 2).

According to the obtained data (Table 3), different mortality percentages were recorded when *A. aurantii* were treated with Neemex, Sulphur, KZ oil and Actalic, the mortality percentages of *A. aurantii* adults ranged from 45.0-71.3, 57.2-85.1, 74.0 – 90.2%, and 85.1-95.1% respectively.

When compare between the effects of Neemex, Sulphur, KZ oil, and Actalic it was found LC_{50} were (0.657, 0.37, 0.152 and 0.120), respectively (Table, 4). On base of the LC_{90} values, it was found Neemex, Sulphur, KZ oil, and Actalic, were (7.100, 4.60, 1.104 and 0.940), respectively (Table, 4).

The slope of line is useful to known the homogeneity of stages of nymphs and adult of *A. aurantii* population, which reared under laboratory conditions to effect of different compounds. When the population of scale insect is similar in homogeneity or the degree of resistant meaning the slope is big or increase in regression.

Table 1. Mortality percentages of Neemex, Sulphur, KZ oil and Actalic on *A. aurantii* nymphs.

Compound	Conc. (ml/L.W.)				
	0.5	1	2	3	4
Neemex	52.9	58.3	70.7	81.0	89.4
Sulphur	63.4	73.1	83.2	87.3	93.8
KZ oil	80.4	89.8	90.4	92.1	95
Actalic	86.3	95.2	97.3	99.0	100

Table 2. LC values of Neemex, Sulphur, KZ oil and Actalic on *A. aurantii* nymphs.

Compound	LC_{50}	LC_{90}	Slope
Neemex	0.545	6.153	1.23
Sulphur	0.27	3.45	1.144
KZ oil	0.101	0.921	1.287
Actalic	0.095	0.743	1.443

Table 3. Mortality percentages of Neemex, Sulphur, KZ oil and Actalic on *A. aurantii* adults.

Compound	Conc. (ml/L.W.)				
	0.5	1	2	3	4
Neemex	45.0	50.0	55.3	68.3	71.3
Sulphur	57.2	64.3	74.5	78.2	85.1
KZ oil	74.0	76.7	81.3	85.4	90.2
Actalic	82.5	88.0	86.5	90.0	95.1

Table 4. LC values of Neemex, Sulphur, KZ oil and Actalic on *A. aurantii* adults.

Compound	LC ₅₀	LC ₉₀	Slope
Neemex	0.657	7.100	1.30
Sulphur	0.37	4.60	1.210
KZ oil	0.152	1.104	1.320
Actalic	0.120	0.940	1.511

2.1.2. On *A. aurantii* parasitoid:

Data presented in Table (5) showed the potency of Neemex, Sulphur, KZ oil, and Actalic on *C. lemniscata* under laboratory conditions. Tabulated data indicate that the potency of Neemex, Sulphur, KZ oil, and Actalic was varied tremendously due to compounds. As a general trend, data proved that at any of the compounds the higher the concentration, the higher rate of mortality was recorded and vice versa.

According to the obtained data (Table 5), different mortality percentages on *A. aurantii* parasitoid, *C. lemniscata* treated with Neemex, Sulphur, KZ oil, and Actalic, the mortality percentages ranged from 56.1-83.5, 60.0-91.5, 75.1-96.8 and 86.5-100%, respectively. When compare between the effects of Neemex, Sulphur, KZ oil, and Actalic it was found LC₅₀ were (0.37, 0.288, 0.113 and 0.057), respectively (Table, 6). On base of the LC₉₀ values, it was found Neemex, Sulphur, KZ oil, and Actalic, were (16.180, 4.91, 0.544 and 0.787), respectively (Table, 6). Data in Table (6) show that the slope of armoured scale population in Neemex, Sulphur, KZ oil, and Actalic, they gave (0.780, 1.040, 0.990 and 1.120), respectively.

Table 5. Mortality percentages of Neemex, Sulphur, KZ oil and Actalic on the parasitoid, *C. lemniscata* associated of *A. aurantii*

Compound	Conc. (ml/L.W.)				
	0.5	1	2	3	4
Neemex	56.1	60.2	69.7	73.4	83.5
Sulphur	60.0	73.5	79.5	82.7	91.5
KZ oil	75.1	80.6	85.4	91.2	96.8
Actalic	86.5	93.0	94.5	100	100

Table 6. LC values Neemex, Sulphur, KZ oil and Actalic on the parasitoid, *C. lemniscata* associated of *A. aurantii*

Compound	LC ₅₀	LC ₉₀	Slope
Neemex	0.37	16.180	0.780
Sulphur	0.288	4.91	1.040
KZ oil	0.113	0.544	0.990
Actalic	0.057	0.787	1.120

2. Field experiments:

The experiments were carried out to evaluate of the four compounds (KZ oil, Neemex, Sulphur and Actalic) on *A. aurantii* and its parasitoid, *C. lemniscata* on citrus were carried out at Beni-Suef Governorate. When the numbers of *A. aurantii* and its parasitoid, *C. lemniscata* were high during the two seasons, 2008 and 2009.

2.1. The first season (2008):

In the first season (2008), the pre-spraying counts of adults and nymphs of *A. aurantii* were 1575-1933 and 3105-3554 / 30 leaves, respectively and the numbers of *C. lemniscata* were 460-600 /30 leaves ,respectively (Table, 7). Results in Table (8) indicate that in first year (2008), the two compounds Neemex and Sulphur gave moderate effect against adults and nymphs of *A. aurantii*, 55 & 59% and 59 & 67% reduction, respectively against adults and nymphs after 15 days. They also showed moderate toxic effect against *C. lemniscata* 51 and 55 %reduction for Neemex and Sulphur, respectively. On the other hand, KZ oil and Acatalic gave high efficacy against all targets. Adult and nymphs of *A. aurantii* were reduced by 79 &95 and 88-96% respectively. Reduction in *C. lemniscata*. was78 and 84% respectively. after 15 days from application.

2.2. The second season (2009):

In the second season (2009), the pre-spraying counts of adults and nymphs of *A. aurantii* were 1530-1935 and 2790-3555/ 30 leaves, respectively and the numbers of *C. lemniscata* were 480-540 /30 leaves ,respectively (Table,9). Results in Table (10) indicate that in first year (2009), the two compounds Neemex and Sulphur gave moderate effect against adults and nymphs of *A. aurantii*, 59 & 63% and 61 & 61% reduction, respectively against adults and nymphs after 15 days. They also showed moderate toxic effect against *C. lemniscata* 59 and 63 %reduction for Neemex and Sulphur, respectively. On the other hand, KZ oil and Acatalic gave high efficacy against all targets. Adult and nymphs of *A. aurantii* were reduced by 75 &95 and 85-98% respectively. Reduction in *C. lemniscata*. was 77 and 81% respectively. after 15 days from application.

The present work indicated that Neemex gave moderate effect against adults and nymphs of *A. aurantii*. In India, Krishnamoorthy and Rajagopal (1998) said neem oil (2%) when applied once, gave only initial control of the scale. The population increased after 14 days of treatment. All insecticides (Monocrotophos (0.08%), dimethoate (0.07%), chlorpyrifos (0.10%)) except neem oil were highly toxic to the natural enemies. Levels of chlorpyrifos were safe to natural enemies after 7 days of treatment. The botanical insecticides, neem oil and sulfur were safer to natural enemies of *A. aurantii* (Krishnamoorthy and Rajagopal,1995).

The result here observed that Mineral oil KZ was the superior compound on controlling the pest. While Ofek *et al.* (1997) in Australia confirmed our results. They stated that careful monitoring of pest populations and control measures using mineral oils according to infestation levels and appearance of the different development stages led to a drastic reduction in the pest population. But in case of natural enemies our work including the toxic effect of oil on the parasitoid, *C. lemniscata*. While the same authors, the population of natural enemies increased (10 natural enemies including, *Comperiella bifasciata*) and the restoration of the biological equilibrium. narrow-range oil was not effective against *A. aurantii* when applied to 3rd-instar nymphs in March and was only moderately effective when applied to high densities of 1st- and 2nd-generation crawlers in May and July, resp. Chlorpyrifos reduced densities of the encyrtid *C. bifasciata* the parasitoid of *A.aurantii*. (Grafton-Cardwell and Reagan, 1995). Uygun and Sekeroglu (1984) in Turkey stated that carefully timed mineral oil applications in winter and early summer kept populations of *A. aurantii* at a low level.

Table 7. Average numbers of *Aonidiella aurantii* and its parasitoid /30 leaves on citrus when treated with four different compounds during 2008 season.

Treatment	Rate /L.W.	Pre spraying count			Post spraying count								
					3 days			7 days			15 days		
		A.	N.	P.	A.	N.	P.	A.	N.	P.	A.	N.	P.
Neemex	2ml/liter	1755	3240	460	1350	2205	300	900	1575	200	765	1170	140
Sulphur	2gm/liter	1800	3465	640	1215	1890	400	945	1440	240	720	990	180
Acatolic	3ml/liter	1575	3105	600	810	1350	360	360	630	160	70	110	60
KZ oil	1.5ml/liter	1702	3120	522	1270	1659	275	769	908	188	335	320	72
Control		1933	3554	540	2475	3870	520	2205	3780	420	1890	3150	340

Table 8. Percent reduction reduction of *A. aurantii* /30 leaves on citrus were carried out at Beni-Suef when treated with four different compounds during 2008 season

Treatment	Rate /L.W.	Post spraying count								
		3 days			7 days			15 days		
		A.	N.	P.	A.	N.	P.	A.	N.	P.
Neemex	2ml/liter	39	29	32	54	48	74	55	59	51
Sulphur	2gm/liter	47	49	35	53	60	51	59	67	55
KZ oil	3ml/liter	43	51	45	60	72	62	79	88	78
Acatolic	1.5ml/liter	58	61	37	79	81	70	95	96	84

Table 9. Average numbers of *Aonidiella aurantii* and its parasitoid /30 leaves on citrus when treated with four different compounds during 2009 season.

Treatment	Rate /L.W.	Pre spraying count			Post spraying count								
		A.	N.	P.	3 days			7 days			15 days		
					A.	N.	P.	A.	N.	P.	A.	N.	P.
Neemex	2ml/liter	1530	2790	480	1170	1935	240	855	1395	160	585	855	160
Sulphur	2gm/liter	1575	3555	540	1170	2205	300	855	1665	180	540	1080	160
Acatolic	3ml/liter	1935	3375	520	990	1575	240	495	675	100	90	45	40
KZ oil	1.5ml/liter	1805	3227	492	930	1850	215	670	890	105	425	370	45
Control		1755	3240	540	1800	3015	400	1890	2790	300	1665	2565	220

Table 10. Percent reduction reduction of *A. aurantii* /30 leaves on citrus were carried out Beni-Suef when treated with four different compounds during 2009 season

Treatment	Rate /L.W.	Post spraying count								
		3 days			7 days			15 days		
		A.	N.	P.	A.	N.	P.	A.	N.	P.
Neemex	2ml/liter	25	25	32	48	41	40	59	61	59
Sulphur	2gm/liter	27	33	25	49	45	40	63	61	63
KZ oil	3ml/liter	49	38	41	65	67	61	75	85	77
Acatolic	1.5ml/liter	50	49	37	76	76	65	95	98	81

Phillips *et al.* (1983) tested Cygon (dimethoate) at 6 pt/acre on Lemon trees, infested with *A. aurantii* and with measurable populations of its natural enemies and the results indicated the population were rapidly decimated by compound. Concentration-mortality regressions in the laboratory tests on *A. aurantii* infested citrus were quantified of the insecticides and showed toxicities (at the LD50) in the order chlorpyrifos > carbaryl > dimethoate > acephate > parathion > formetanate > methidathion to *A. melinus* and carbaryl > acephate > parathion (Morse and Bellows, 1986). In Swaziland, when dangerous populations of *A. aurantii* developed in midsummer (when natural enemies were least effective), a mixture of parathion and dimethoate, or summer oil emulsion, was applied in February-March (Catling, 1971). Nigam (1971) stated that *A. aurantii* attacks mulberry in the Darjeeling district of West Bengal (India) and controlled chemically by 0.05% parathion (Folidol) .

Liotta and Mineo (2008) concluded that the replacement of white oils by organophosphorous compounds is not justified providing that the control is directed against larvae and young females of *A. aurantii* In Italy. Under field condition, chlorpyrifos (0.04%) and thiamethoxam (0.025%) and dimethoate (0.03%) sprays showed significant reduction in the scale population, *A.aurantii* in India (53.3, 53.2 and 49.7%, respectively) (Reecha, *et al.* 2009).

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تأثير بعض المركبات الطبيعية على الحشرة القشرية الحمراء و الطفيل المتخصص عليها كومبيريلامنيسكاتا في مصر

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تعتبر الحشرة القشرية الحمراء من أهم الآفات التي تصيب العديد من المحاصيل الاقتصادية وأن الضرر الرئيسي لهذه الآفة يتمثل في أمتصاص العصارة. وان طفيل كومبيريلامنيسكاتا من الطفيليات الهامة في مكافحة الحشرات القشرية المسلحة. ولذا فان هذا العمل يتضمن دراسات معملية و حقلية على تأثير بعض المركبات الطبيعية (زيت. KZ و النيمكس و الكبريت) و المركبات الكيميائية (والأكتليك) على الحشرة القشرية الحمراء و الطفيل المتخصص عليها كومبيريلامنيسكاتا وقد أتضح من النتائج المعملية ان نسب الموت بزيت KZ على الحشرة القشرية الحمراء و الطفيل المتخصص عليها كومبيريلامنيسكاتا تراوحت بين 74-95 و بالنسبة للنيمكس تراوحت بين 45.0-89.4%. اما الكبريت فكانت 57.2-93.8% وأخيرا أكتليك وكانت . أما بالنسبة للدراسات الحقلية و التي أجريت عامي 2008-2009 فان النيمكس و الكبريت اعطيا نتائج متوسطة على الحوريات والحشرة الكاملة للحشرة القشرية الحمراء مسجلا معدلات موت قدرها 57% & 60 للنيمكس و معدلات موت قدرها 61 & 64 للكبريت بعد خمسة عشر يوما في سنتي الدراسة على الترتيب. أما تأثير المركبين السابقين على الطفيل فقد كانت معدلات الموت % 59 & 55 على الترتيب. على الجانب الآخر فان مركب الأكتليك سجل نسب موت قدرها 96% & 95 و 95 & 98% في عامي 2008-2009 على الحوريات و الحشرة الكاملة للحشرة القشرية الحمراء في حين أعطى معدلات موت 81% & 84 بعد 15 يوما من معاملة طفيليات خلال عامي الدراسة على الترتيب..