Full Length Research Paper

Evaluating the effect of some botanical insecticides on the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae)

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Accepted 23 May, 2012

Planococcus citri (Risso) (Homoptera: Pseudococcidae), is one of the key pests of citrus. The use of chemical pesticides for a long time can cause many problems such as pesticide resistance, as well as having an adverse effect on the environment. The use of chemical pesticides needs to be replaced with non-chemical control methods. The effects of tondexir (pepper extract) and palizin (eucalyptus extract) using five doses (500, 1000, 1500, 2000 and 3000 ppm) and sirinol (garlic extract) with five doses (1000, 1500, 2000, 2500 and 3500 ppm) on citrus mealybug was investigated. The effect of barter (a botanical synergist) using a single dose (1000 ppm) being added to tondexir and palizin at three doses (500, 1000 and 1500 ppm) added to sirinol at four doses (1000, 1500, 2000 and 2500) on citrus mealybug was recorded after 24, 48, 72 and 96 h post-treatments. Analysis of variance showed that the highest mortality with 3000 ppm doses of tondexir and palizin was 90/60 \pm 2/93 and 89/16 \pm 1/92% with sirinol (3500 ppm) with 87.11 \pm 1.11% mortality, respectively. However, the highest mortality by using barter plus tondexir and palizin (1500 ppm) was 94.44 \pm 2.59 and 86.66 \pm 3.6% and barter plus sirinol (2500 ppm) was 83.33 \pm 3.6% mortality, respectively. There were significant differences between the two experiments (p < 1%).

Key words: Palizin, sirinol, tondexir, bartar synergist, Planococcus citri.

INTRODUCTION

Citrus mealybug (CM) is a polyphagus pest of citrus orchards and nurseries (Rung et al., 2009). It has a wide host range of different citrus species and other fruit trees in subtropical and tropical areas. The nymph stage and adult female cause damage to the trees (Goldasteh et al., 2009). Aheer et al. (2009) indicated that the insecticides profenofos, methidathion, chlorpyrifos and methomyl were effective against CM after seven days post-treatment. Lo et al. (2009) showed that a single application of profenofos was more effective than a single application of buprofezin (ApplaudTM). However, buprofezin has less effect on coccinellid beetle. Mansour et al. (2010) showed that imidacloprid, methidathion and spirotetramat (Movento® 150 OD) were effective against

CM but also that they affected other predator insects. Chemical insecticides can have adverse effects on the environment and human health and can induce insecticide resistance in the target pest species and kill beneficial insects (Damavandian and Hashemi, 2005).

Plant extracts were used here as an alternative to chemical insecticides, to control CM and since the second and third instar nymph is covered with whitecottony wax and has a fringe of elongated waxy filaments that extends about the periphery of the body consequently, it is quite difficult to control CM (Saboori et al., 2003). Draga (2005) showed that the combination of mineral oil with the insecticides parathion, methidathion, fenoxycarb and imidacloprid can increase the toxicity on CM. Duyn and Murphey (1971) used a combination of mineral oil with some toxins (that is, etion, parathion, dimethoate, endosulfan and dieldrin) and concluded that ethion and parathion were most effective in controlling

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S/N	Common name	Traditional name	Chemical group	Formulation	Dose (ml/1000 L water)	LD₅₀ (mg/kg)	Company
1	Insecticidial gel (IG)	Sirinol	Garlic extract in edible oil	EC	0-3500/1000	>5000	Kimia sabzavar company
2	Insecticidial soap (IS)	Palizin	Coconut soap 65%	SL	0-3000/1000	>5000	Kimia sabzavar company
3	Insecticidial emulsion (IE)	Tondexir	Hot red pepper extract in edible oil	EC	0-3000/1000	>5000	Kimia sabzavar company
4	Synergist	BarTar	Non-ionic surfactant	SL	500 ppm	>5000	Kimia sabzavar company

CM. The aim of this study was to investigate the effect of different concentrations of the botanical insecticides palizin, sirinol and tonexir, with or without barter as a synergist on citrus mealybug (CM) in laboratory conditions during 2010 and 2011.

MATERIALS AND METHODS

The botanical insecticides used are listed in Table 1.

Rearing insects (Planococcus citri and Cryptoleamus montrazieri)

The rearing protocol was followed as described by Robison (1972) and Gharizadeh (2002) with some modification. Citrus mealybug was reared on butternut pumpkin *Cucurbita moschata* (2 to 3 kg weight) in plastic containers (25 cm diameter and 4 cm high) at $25 \pm 3^{\circ}$ C and 16:8 (d:I) photoperiod in an incubator. The tops of the containers were covered by vaseline oil to prevent CM from escaping. There were high numbers of CM after two months.

Five doses for each insecticide were chosen based on pilot experiments and plus control. Three replicates were used and each replicate had two Petri-dishes with 15 insects in each. The bottom of each petri-dish was covered with moisture filter paper (Whatman No. 1) and one citrus leaf on top of the paper. Fifteen insects were released onto each leaf and then each leaf was sprayed with toxin by A Potter precision laboratory spray tower (Burkard Scientific Limited, Uxbridge, United Kingdom) and spray pressure was adjusted between 54 and 80 kPa. Tondexir and palizin were used with 0, 500, 1000, 1500, 2000 and 3000 ppm concentrations (a_1 , a_2 , a_3 , a_4 , a_5 and a_6) and sirinol with 0, 1000, 1500, 2000, 2500 and 3500 ppm dose (a_1 , a_2 , a_3 , a_4 , a_5 and a_6). Mortality was recorded after 24, 48, 72 and 96 h post-treatment. Barter as a synergist (1000 ppm) was added to the tondexir and palizin, with four doses (0, 500, 1000 and 1500 ppm) (a_1 , a_2 , a_3 and a_4), and sirinol also with four doses (0, 1000, 1500, 2000 and 2500 ppm) (a_1 , a_2 , a_3 and a_4).

Statistical analysis

The percentage of the mortality was recorded and the mortality was corrected with Abbot formula (Abbott, 1925). The analysis of variance (ANOVA) was done by SPSS program and the comparison of mean was done by Tukey's test (Ark, 1983).

RESULTS

The analysis of variance (ANOVA) with no synergist has shown that there were significant differences among different concentrations (factor A) and also among different times (factor B) (Table 2). The ANOVA with the synergist (bartar) has shown that there were also significant differences among different doses (factor A) and that there were significant differences among different times (factor B) for palizin, tondexir and sirinol at 1% probability (p < 1%). The interaction between factor A and B was also significantly different at 5% probability (p < 5%) (Table 3). With

no synergist, the highest mortality on CM was obtained with a 3000 ppm dose of palizin and tondexir, and a 3500 ppm dose of sirinol. The lowest mortality was obtained with a 500 ppm dose of palizin and tondexir, and a 1000 ppm dose of sirinol (Table 4). With synergist, whereby the synergist was added to the insecticides, mortality increased. Palizin and tondexir, at a 500 ppm dose, gave mortality of 52.49 ± 3.89 and 56.66 ±0.96%, respectively and with sirinol, at a 1000 ppm dose; mortality was 44.99 ± 4.19%. When tondexir and palizin were used at the highest dose (1500 ppm), mortality was 94.44 ± 2.93 and 86.66 \pm 1.92%, respectively, and with sirinol at 2500 ppm, the mortality was 83.33 ± 1.11% (Table 5).

There was significant difference between the 24 and 48 h post-treatments; but no significant difference between the 72 and 96 h posttreatments (Table 6). The highest mortality was obtained after 96 h post-treatment. In the second experiment, with the synergist, there were significant differences between the 24 and 48 h post-treatments (Table 6). The highest mortality was obtained at the 72 h post-treatment with palizin, tondexir and sirinol (Table 7).

DISCUSSION

This is the first research about the effect of

S.O.V	df	Palizin	Sirinol	Tonexir
Block	2	55.58	315.05	
Doses	5	10807.5**	9616.33**	11255.18**
Times	3	988.52**	1319.11**	846.59**
Doses × times	15	80.20	80.56	80.106
Error	46	47.13	52.16	70.07
CV ²	71	15.53	15.99	17.93

Table 2. An ANOVA of the mortality (%) of some botanical insecticides on P. citri.

**Significantly differences (p < 1%). ¹Sources of variance; ²Coefficient of variation.

Table 3. An ANOVA of the mortality (%) of some botanical insecticides plus bartar as synergist on P. citri.

S.O.V	Df	Palizin	Sirinol	Tonexir
Block	2	790.47	1957.83	2214.92
Doses	5	10921.98**	10590.86**	12596.07**
Times	3	525.69**	481.63**	306.00**
Doses x times	15	44.420*	76.59*	89.83*
Error	46	73.65	89.42	39.45
CV	71	14.93	17.07	10.03

**Significant differences (p < 1%); *Significant differences (p < 5%).

Table 4. Comparisons of means of mortality (%) of the different doses of toxins on P. citri.

Dose	Palizin	Sirinol	Tonexir
a ₁	24.73±1.005 ^e *	35.13±3.09 ^d	27.91±0.96 ^e
a ₂	38.27±1.97 ^d	40.41±2.85 ^{cd}	41.66±2.98 ^d
a3	52.63±0.33 ^c	45±1.46 ^c	53.74±3.18 ^c
a4	58.47±2.42 ^b	61.11±1.11 ^b	64.13±1.66 ^b
a_5	89.16±1.92 ^a	87.11±11.1 ^a	90.96±2.93 ^a
a ₆	1.8 ± 0.00^{f}	2.03±0.00 ^e	1.87±0.00 ^f

*Different letters indicate significant differences.

Table 5. Comparison means of the mortality (%) of different doses of toxins plus bartar as synergist on P. citri.

Dose	Palizin	Sirinol	Tonexir
a ₁	52.49±2.10 ^b *	44.99±3.59 [°]	56.66±2.47 [°]
a ₂	56.66±3.24 ^b	50.55±3.44 ^c	66.38±3.17 ^b
a ₃	78.33±3.26 ^a	65.83±3.29 ^b	83.88±2.59 ^a
a4	-	79.44±3.60 ^a	-
a_5	-	-	-
a_6	0.00 ^c	0.27±0.12 ^d	0.73 0.05 ^d

*Different letters indicate significant differences.

botanical insecticides such as palizin, sirinol and tondexir with or without a synergist (bartar) against citrus mealybug *P. citri* in laboratory conditions. It was shown that when the synergist (bartar) was added to the above toxins, the percentage mortality was increased and the mortality with or without bartar was dose related. The resultant mortality, using the synergist with a 500 ppm dose of palizin and tonexir, was the same as the resultant

Time (h) Palizin Sirinol Tondexir 24 34.45^c* 34.59^c 37.47^c 45.72^b 42.84^b 42.19^b 48 50.25^{ab} 72 47.86^a 49.87^a 53.21^a 96 51.59^a 53.94^a

Table 6. Comparison of means of mortality (%) of the different toxins and different time intervals on *P. citri.*

*Different letters indicate significant differences.

Table 7. Comparisons of mean of mortality (%) of the different toxins plus synergist and different time intervals on *P. citri.*

Time (h)	Palizin	Sirinol	Tondexir
24	49.38 ^b *	45.78 ^b	56.97 ^b
48	59.93 ^a	54.86 ^a	61.97 ^{ab}
72	60.30 ^a	55.97 ^a	65.67 ^a
96	60.30	57.08 ^a	65.67 ^a

*Different letters indicate significant differences.

mortality using a 1500 ppm dose of palizin and tondexir alone, and that the mortality using 1500 ppm palizin and tondexir plus bartar was the same as 3000 ppm palizin and tondexir alone, and the mortality with 1000 ppm of the tondexir and palizin plus bartar was the same as 2000 ppm of the above toxins alone. The same result was obtained with palizin and tondexir and *Bacillus thurigiensis* plus mineral oil (as synergist) against citrus leafminer (Amiri, 2007, 2008, 2009, 2010).

Different insecticides have been used againstCM, and all of them can kill CM very easily but they raised problems for the environment. Many pesticides have been used to control CM in northern Iran including azinphos-methyl (guthion), malathion, ethion, etrimfos (Ekamet^R), methidathion and buprofezin (Applaud^{1M}), and most of them were combined with mineral oils and some with acaricides activity such as tetradifon (Tedion V-18), neoron (bromopropylate 25% EC) and APOLLO® SC (clofentezine) was added to them to kill mites as well as (Bodenheimer, Methidathion insects 1951). and imidacloprid were used against CM with the second pesticide being more effective than the first one, while not harming natural enemies (Walton and Pringle, 2004). However, when mineral oils were added to methidathion, the efficacy on CM was increased (Kerns, 2004). Mohamed and Husseini (2006) showed the effect of Bacillus thuringiensis (Bt) on CM, concluding that Bt was effective on CM but that there was no effect of Bt on predator insects. Bentley and Martin (2002) tested methomyl, dimethoate, buprofezin and phenpropetrin on the first nymphal instar of CM and concluded that phenpropatrin, dimethoate and methomyil, Insect growth regulators (IGR) were effective on CM, and that methomyl was also effective on CM. They also concluded

that methomyl and dimethoate can kill CM quickly. Hoffmann (2009) indicated that imidacloprid and dimethoate were effective on CM. Lo et al. (2009) showed that prothiofos and buprofezin (ApplaudTM) (250, 500, 1000 ppm) were effective on CM, and that a single application of prothiofos was more effective than two applications of buprofezin (ApplaudTM). They also demonstrated that there were no significant differences between a single application of prithiofos and two applications of buprofezin (ApplaudTM) on CM. It was shown that buprofezin (ApplaudTM) had contact toxicity on CM and no efficacy on adult CM. Manjri (2006) indicated that azadracthin (2 ml/L) and methomyl (1 g/L) were effective on CM, which was confirmed by our results.

Our results show that the botanical insecticides sirinol, palizin and tondexir were effective on CM. Draga (2005) showed that the addition of mineral oil to parathion, methidathion, phenoxicarb and imidacloprid can increase the toxicity of these toxins on *P. citri*. Howard (1989) showed that mineral oil alone cannot control the P. citri but that in combination with dimethoate and chlorpyrifos, can increase the toxicity of the two toxins. Ethion, parathion, demeton-s-methyl (Meta-Systox^r), dimethoate, endosulfan (Thiodan^R) and dieldrin, plus mineral oil, was tested with ethion and parathion being the most effective combination with a mineral oil against P. citri (Duyn and Murphey, 1971). High mortality was obtained after 72 h post-treatment in the first experiment but in the second experiment (with the synergist), the high mortality were obtained after 48 h post-treatment. It can be concluded that with the addition of the synergist (bartar), the concentration of the toxin can be decreased without affecting the mortality. The time of effectiveness is also decreased due to the presence of the synergist. These results are promising for future research in that, other synergists could be potentially combined with botanical toxins to obtain greater mortality.

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