Comparison of the toxicity of three botanical extracts on the second nymph of the citrus mealybug *Planococcus citri* (Risso) under nursery and laboratory conditions

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Abstract

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Botanical insecticides play an important role in sustainable agriculture. The effect of Tondexir (pepper extract), Sirinol (garlic extract) and Palizin (eucalyptus extract) on Citrus Mealybug (CM) *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) and Coccinellid Predator (CP) *Cryptoleamus montrouzieri* was investigated. Citrus Mealybug was reared on Butternut pumpkin (2-3 kg). Factorial experimental design with random blocks with six replicates was used for each botanical insecticide under nursery conditions. Each toxin was tested separately and CM mortality was recorded at 24, 48, 72 and 96 hours after treatment. Tondexir (3000 ppm) resulted in the highest CM mortality (90.96±2.93%), followed by Palizin (3000 ppm) (89.16±1.92%) and Sirinol (3500 ppm) (87.11±1.11%) 96 h after treatment. Adult emergence was prevented by Tondexir (3000 ppm), Palizin (3000 ppm) and Sirinol (3500 ppm). Among three botanical insecticides, the least LC_{50} was obtained by Palizin with 811.297 ppm. There were no toxic effects due to the use of the above mentioned botanical pesticides on any of the Coccinellid Predator (CP) *Cryptoleamus montrazieri* Mulsant (Coleoptera: Coccinellidae) stages.

Keywords: Tondexir, Palizin, Sirinol, Citrus Mealybug (CM), *Planococcus citri*,Coccinellid Predator (CP), *Cryptoleamus montrazieri.*

Introduction

Citrus mealybug (CM), *Planococcus citri*, is one of the most important pests of citrus in the world. Keriokhin was the first person who identified this pest in Iran (16). Most of the research regarding the effect of pesticides on CM has been carried out in South Africa, Palestine, Egypt, India, America, Spain, USA and Japan (19). The use of synthetic insecticides has led to numerous problems unforeseen at the time of their introduction, in addition to the evolution of pesticides resistance in pest populations (10). The Coccinellid predator (CP) is one of the biological control agents for the control of CM and is inhibited by chemical pesticides (14). Many different pesticides such as Profenofos, Methidathion, Chlorpyrifos, fenpropathrin and Methomyl have been used to control CM (2) but are found harmful to coccinellid beetles (18).

The above mentioned negative effects of using conventional insecticidesgenerated a need to find alternative ways of controlling target pest species (23). Botanical insecticides can be an alternative to conventional pesticides as they are effective against pests, remain for a short period in the field, and are safe and cheap. Four Biological pesticides, Biofly (*Beauveria bassiana*), Biovar (*Beauveria bassiana*), Bioranza (*Metarhizium anisopliae*) and orange oil plus two chemical pesticides, Admiral (pyriproxyfen) and Cidial (phenthoate), were used against the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae). The highest mortality was obtained from Cidial treatment (3). Amongst various options, aqueous extract of neem seeds (kernels of *Azadirachta indica*), *Allium sativum*, *Lantana camara*, *Annona squamosa*, *carica papaya*, etc. were highly effective for the control of coffee mealy bugs (21).

The aim of this study was to investigate the effect of Tondexir (pepper extract), Sirinol (garlic extract) and Palizin (Eucalyptus extract) on Citrus Mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) in semi-field conditions.

Materials and Methods

The botanical insecticides used are listed in Table 1.
This research was done in semi-field condition in Randomized Complete Block Design in three replicate at Sari Agricultural University.

Rearing insects (*Planococcus citri*):
Semi field assay and experimental design: The toxicity of the three different botanical insecticides (Table 1) to CM was examined in the nursery at Sari Agricultural Sciences and Natural Resources University in 2010 & 2011. Seventy-two young trees (4 years) of the citrus variety Thomson Navel (*Citrus sinensis*) nursery within Sari Agricultural University and spray bioassay method were used. Experiments for each treatment were replicated four times, and distilled water was used as a control. At 24, 48, 72 and 96 hours post-treatment, the numbers of live and dead second instar nymphs in each replicate were counted in the laboratory under a stereomicroscope.

The rearing protocol followed was as described by Gerald-Robison (12) and Gharizadeh (13) with some modifications. Citrus Mealybug was reared on Butternut pumpkin *Cucurbita moschata* (2-3 kg) in plastic containers (25 cm diameter x 4 cm high) at 25 ± 3^{0}C and 16:8 hrs (dark:light) photoperiod in an incubator. The tops of the containers were covered by Vaseline oil to prevent CM escape and then these CM were used to infest young trees in the nursery.

Table 1. Botanical insecticides used in this research.

Common name	Trade name	Chemical group	Formulation	Dose (ml /1000 Lit water)	LD_{50} (mg/kg)	Company
Insecticidial gel (IG)	Sirinol	Garlic Extract in edible oil	EC	0- 3500/1000	> 5000	Kimia sabzavar Co.
Insecticidial soap (IS)	Palizin	Coconut soap 65%	SL	0-3000 /1000	> 5000	Kimia sabzavar Co.
Insecticidial emulsion (IE)	Tondexir	Hot red pepper extract in edible oil	EC	0-3000/1000	> 5000	Kimia sabzavar Co.
Fenpropathrin	Danitol	Pyrethroid	EC	400-800ppm	54.0 mg/kg	Bayer

Insecticides application

Five doses for each insecticide were chosen based on pilot experiments, plus a control.

Tondexir and Palizin were used with 0, 500, 1000, 1500, 2000 and 3000 ppm concentration, and Sirinol with 0, 1000, 1500, 2000, 2500 and 3500 ppm concentration. Insect mortality was recorded 24, 48, 72 and 96 hours after treatment.

Statistical analysis

The percentage of mortality was recorded from each treatment and any mortality in the control was corrected using Abbott's formula (1). Analysis of variance (ANOVA) was done using the spss program and the comparison of the means was done by Tukeys test (8). The LC_{50} and LC_{90} were calculated by polo+ program version 1 2002-2012 Leora software©.

Results

The analysis of variance (ANOVA) has shown that there were significant differences among different concentrations (factor A) and also among different times (factor B) and there were no significant differences for the interaction between dose and time (Table 2).

The results have also shown that the highest mortality of CM was obtained when using the 3000 ppm dose of Palizin and Tondexir (89.16±1.92% and 90.96±2.93% respective mortality) and at the 3500 ppm dose of Sirinol (87.11%±1.11% mortality). The lowest mortality was obtained with Palizin and Tondexir at the 500 ppm dose and by Sirinol at the 1000 ppm dose. There were significant differences among different doses of the above three toxins on *P. citri* (Table 3).

The highest mortality was obtained at 72 hrs and 96 hrs post-treatment, and there was no significant difference between 72 hrs and 96 hrs post-treatment when using Palizin and Sirinol. The highest mortality obtained with Tondexir was at 96 hrs post-treatment. The highest mortality for each of the toxins was obtained 96 hrs post-treatment (Table 4).

The effect of different concentrations of botanical insecticides on *P. citri* at different times post treatment is shown in Table 5. There were significant differences between different doses of all toxins and between the toxins and the control. The highest mortality was obtained at the 3000 ppm dose of Palizin and Tonexir, and at the 3500 ppm dose of Sirinol, 96 hrs post-treatment (Table 5).

Table 2. The analysis of variation (ANOVA) of the effect of three botanical insecticides on mortality rate of the second nymphs of *Planococcus citri*.

Source of variation	Df	Palizin	Sirinol	Tondexir
Block	2	58.55	05.315	09.105
Doses	5	5.10807 **	33.9616 **	18.11225 **
Times	3	52.988 **	11.1319 **	59.846 **
Doses ×Times	15	20.80	56.80	106.80
Error	46	13.47	16.52	07.70
Coefficient of variation	71	53.15	99.15	93.17

** Significant differences at P=0.01

Table 3. Effect of different concentrations of three botanical insecticides on mean mortality rate of *Planococcus citri.*

Dose (ppm)	Palizin	Tondexir	Dose(ppm)	Sirinol
0.0	24.73 e	27.91 e	0.00	35.13 d
500	38.27 d	41.66 d	1000	40.41 d
1000	52.63 c	53.74 c	1500	45.00 c
1500	58.47 b	64.13 b	2000	61.11 b
2000	89.16 a	90.96 a	2500	87.11 a
3000	1.80 f	1.87 f	3500	2.03 e

Means followed by the same letters are not significantly different at P=0.01

Table 4. *Planococcus citri* mortality rate as influenced by different doses of toxins observed at different periods after treatment.

Hours post treatment	Palizin	Sirinol	Tondexir
24	45.34 c	59.34 c	47.37 c
48	84.42 b	19.42 b	72.45 b
72	86.47 a	87.49 a	25.50 ab
96	59.51 a	94.53 a	21.53 a

Means followed by the same letters are not significantly different at P=0.01

Table 5. *Planococcus citri* mortality rate (%) means as influenced by different doses of toxins from botanical extracts observed at different times after treatment.

Dose (ppm)	Hours after treatment	Tondexir	Palizin	Dose (ppm)	Hours after treatment	Sirinol
0.0	24	11.66 gh	10.55 fg	0.0	24	20.00 fg
0.0	48	27.78 fg	21.11 eg	0.0	48	27.22 fg
0.0	72	32.22 eg	30.55 dg	0.0	72	43.89 cf
0.0	96	40.00 df	36.66 cg	0.0	96	49.44 bf
500	24	30.22 fg	31.55 dg	1000	24	28.89 eg
500	48	38.22 df	35.77 dg	1000	48	38.33 dg
500	72	45.77 df	40.88 bf	1000	72	44.44 cf
500	96	53.44 be	44.89 bf	1000	96	50.00 bf
1000	24	46.66 de	43.73 bf	1500	24	30.55 fg
1000	48	52.77 be	51.11 be	1500	48	44.44 cf
1000	72	57.77 bd	57.77 ae	1500	72	48.33 bf
1000	96	57.77 bd	58.33 ae	1500	96	56.66 af
1500	24	48.38 ef	42.77 bf	2000	24	47.22 bf
1500	48	61.55 bd	53.59 be	2000	48	55.55 af
1500	72	71.66 ac	62.77 ad	2000	72	68.33 ae
1500	96	75.00 ac	74.44 ac	2000	96	73.33 ad
2000	24	86.11 a	76.66 ab	2500	24	78.89 ac
2000	48	92.22 a	93.33 a	2500	48	85.55 ab
2000	72	92.22 a	93.33 a	2500	72	92.22 a
2000	96	92.20 a	93.33 a	2500	96	92.22 a
3000	24	1.87 h	1.87 g	3500	24	2.03 g
3000	48	1.87 h	1.87 g	3500	48	2.03 g
3000	72	1.87 h	1.87 g	3500	72	2.03 g
3000	96	1.87 h	1.87 g	3500	96	2.03 g

Means followed by the same letters are not significantly different at P=0.01

The LC_{50} of Palizin was 811. 297 ppm, followed by Tondexir (821.716 ppm) and Sirinol (1562.125 ppm) (Table 6). There was no toxicity effect of Tondexir and Palizin and Sirinol on all stages of the Coccinelllid Predator, 96 hrs post-treatment.

Table 6. The LC_{50} and LC_{90} of different toxins on the second instar nymphs of *Planococcus citri.*

LC (ppm)	Tondexir	Palizin	Sirinol	Phenpropathrin
LC_{50}	821.7	811.3	1562.1	414.9
LC_{90}	3452.9	3672.6	4937.7	1083.3

Discussion

This study is the first to quantitatively demonstrate that commercially available plant extract products vary in their efficacy against certain arthropod pests. The popularity of "organic" products and the general interest in sustainable crop production led to an extended interest in using "natural" products, such as plant extracts, for pest control.

The effect of these three botanical insecticides as a biological control of *P. citri* has been evaluated here for the first time. Many chemical insecticides have been used to control *P. citri;* methomyl, dimethoate and buprofezin the Insect Growth Regulators (IGR), which decreased the insect population, but they are harmful to natural enemies (9, 22).

This study showed that botanical insectcides are preferable for the control of this insect pest due to having fewer negative effects on natural enemies of the target pests than conventional chemical insecticides.The effect of botanical extracts on mealy bugs is slower than synthetic insecticides, but they are eco-friendly and comparatively non-toxic or less toxic to human beings and easily degradable (3, 21).

Tondexir, Sirinol and Palizin were effective for controlling *P. citri*, with Tondexir producing the highest mortality of citrus mealybug, at 3000 ppm. This result was similar to what was obtained by Mohamed *et al*. (20). The high mortality obtained by using Tondexir and Palizin at 3000 ppm, and with Sirinol at 3500 ppm were in agreement w3ith the result obtained earlier by Hollingsworth (15). These pesticides had no toxic effects on the coccinellids which is in agreement to what was obtained by Aida et *al*. (3). The same results were obtained earlier when using Palizin and Tondexir and *Bacillus thurigiensis* plus mineral oils against citrus leafminer (4, 5, 6, 7). Tondexir, Palizin and Sirinol are promising botanical insecticides and have the potential to be a significant component of an IPM program.

Several pest management tools must often be used simultaneously to deal with pest insect species. Natural plant extracts and biological control agents are two such tools that are used simultaneously because of their effectiveness, potential compatibility, and minimal risk to the environment and human health. Results of this study indicated that botanical pesticides conserve coccinellid predators better than the chemical pesticide fenpropathrin. Hence, it can be concluded that botanical pesticides were the most favorable insecticides for use in controlling *P. citri* because they kill the target pest but have no effect on natural enemies.

However, chemical toxins produced by plants may not only be detrimental to insect and mite pests (e. g. Palizin, Tonexir and Sirinol), but also to humans (11). For example, peppermint and rosemary oil have been shown to be effective against head louse, *Pediculus humanus capitis* De Geer (24) but contact dermatitis and occupational asthma have been attributed to exposure to rosemary oil (17).

The results of this study demonstrated that only a few selected plant-derived essential products available to consumers were effective in controlling specific arthropod pests. There was considerable variation in citrus mealybug mortality in response to the plant-derived essential oil products evaluated, which included products containing the same active ingredients. For example, the use of two canola oil products Garden Safe Houseplant and Garden Insect Spray (ready to use formulation) and Pyola, a canola oil concentrate, led to citrus mealybug mortality values of 74% and 50%, respectively.

Further research is needed to establish cultivation guidelines to ensure consistent quality of plant extract products. It is also important to assess phytotoxicity amongst commonly grown ornamental plants before introducing plant extract products to the marketplace and field testing for several years is needed.

الملخص

أحمدي، م وب. أميري بيشلي. 2015. مقارنة سمية ثلاثة مستخلصات نباتية على عذراء الطور الثاني لحشرة *Planococcus citri* (Risso) تحت ظروف المشتل والظروف المحبرية. مجلة وقاية النبات العربية، 33(1): 87-92.

تسهم مبيدات الحشرات النباتية بدور مهم في الزراعة المستدامة. تم بحث أثر توندكسيت (مستخلص الفليفلة)، سيرينول (مستخلص الثوم) وباليزين (مستخلص اليوكاليبتوس) في حشرة البق الدقيقي على الحمضيات/الموالح *Planococcus citri* (Risso) (متشابهة الأجنحة :Homoptera وفصيلة Pseudococcidae) والمفترس *Cryptoleamus montrouzieri*. تمت تربية المفترس على جوز اليقطين (2–3 كغ). استخدم تصميم التجارب العاملي مع قطع عشوائية وستة مكررات لكل مبيد جشري نباتي تحت ظروف المشتل. تم اختبار كل توكسين (سم) على انفراد وسجل نفوق بق الحمضيات/الموالح الدقيقي بعد 24، 48، 72 و96 ساعة من المعاملة. أدى استخدام توندكسير (3000 جزء بالمليون) إلى نسبة نفوق عالية (90.96±2.93%) تلاه باليزين (3000 جزء بالمليون) الذي حقق نسبة نفوق 89.16±1.92% وسيرينول (3500 جزء بالمليون) (87.11±1.11) وذلك بعد 96 ساعة من المعاملة. منع توندكسير (3000 جزء بالمليون) وباليزين (3000 جزء بالمليون) وسيرينول

(3500 جزء بالمليون) انبثاف البالغات. ومن بين المبيدات النباتية الثلاثة، تم الحصول على أقل LC_{50} مع الباليزين بتركيز 811.297 جزء بالمليون. لم تكن هناك آثار سمية من استحدام المبيدات المذكورة أعلاه لأي من مراحل المفترس الدعسوقي *Cryptoleamus montrazieri* Mulsant (Coleoptera: Coccinellidae).

كلمات مفتاحية: باليزين، بق الحمضيات/الموالح الدقيقي *Planococcus citri* ، تندكسير، مفترس الدعسوقة *Cryptoleamus montrazieri*، سيرينول.

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