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ORIGINAL ARTICLE

An evaluation of the effect of botanical insecticide, palizin in comparison with chemical insecticide, imidacloprid on the black citrus aphid, *Toxoptera aurantii* Boyer de Fonscolombe and its natural enemy, *Aphidius colemani* Viereck

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Abstract

The black citrus aphid, *Toxoptera aurantii* Boyer de Fonscolombe (Hemiptera: Aphididae), an important pest of citrus species, feeds by sucking sap from plant leaves. It causes some leaf distortion and malformation of growing leaves and shoot tips. In this study, the effects of the botanical insecticide, palizin on *T. aurantii* and its parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Brachonidae) were compared with the chemical insecticide, imidacloprid. The compounds were evaluated at maximum recommended field concentrations: palizin 2,000 ppm, imidacloprid 500 ppm, water (as control) on adult aphids. Spraying was done with a 100-l engine Honda sprayer (GX120T1, 160T1, 200T) until run-off. The number of dead aphids was recorded 24, 48 and 72 hours after treatment. According to the results, statistically significant differences were found between treatments ($p \leq 0.05$). Twenty-four hours after treatment, imidacloprid was more effective than palizin in reducing the *T. aurantii* population. Palizin showed high efficiency (95% mortality) 72 hours after treatment. However, at that time, there was no statistically significant difference between the mean mortality percentages of imidacloprid and palizin. This shows that botanical insecticide, palizin can effectively control *T. aurantii*. Also, palizin caused low mortality (10.86%) on *A. colemani* while imidacloprid was more toxic (31.1% mortality). The results of the present study indicated that palizin can be used instead of chemical insecticide, imidacloprid in control programs of *T. aurantii*.

Key words: *Aphidius colemani*, imidacloprid, palizin, *Toxoptera aurantii*

Introduction

The black citrus aphid, *Toxoptera aurantii* Boyer de Fonscolombe (Hemiptera: Aphididae) is an important pest of *citrus* species and is found exclusively on the undersurface of flush leaves. The aphid feeds by sucking sap from plant leaves. This often curls and shrivels the leaves, deforming the plants (Metcalf and Flint 1962). Aphids cause extensive damage because they reduce the quality and the quantity of agricultural products (Wanga *et al*. 2015). Severe infestation leads to delayed recovery of the plant with the consequent effect of poor crop productivity (Bhathal *et al*. 1994). Occasional outbreaks of *T. aurantii* in citrus orchards have made this aphid an economically important species (Carver 1978). Chemical control (foliar insecticides) constitutes the most rapid and practical way to kill aphids because of its efficacy and the simplicity of application (Pike *et al*. 1993). The dependence on chemical pesticides results in several ecological and physiological problems for humans, animals and beneficial insects. Due to physiological similarities between pests and their natural enemies, insecticides usually cause mortality in both groups (Croft 1990). Therefore, knowledge of the effect of insecticides applied for the control of *T. aurantii* on its natural enemies such as parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Brachonidae) is essential to find a relatively safe compound. For the last few decades, due to a growing awareness of the harmful effects of pesticides, alternate methods of pest control with extremely safe pesticides are being advocated world over. There is a renewed interest in the use of botanical insecticides for crop protection (Sohail *et al*. 2012). There are many benefits of using botanical pesticides such as reduced environmental degradation, increased safety for farm workers, increased food safety, reduction in pesticide resistance and improved profitability of production (Erdogan *et al*. 2012). The majority of plant extracts are alkaloids and terpenoids which are now known to affect insects' behaviour, growth and development, reproduction, and survival (Warthen *et al.* 1990). The aim of this study was to investigate the effect of botanical insecticide, palizin on *T. aurantii* and its parasitoid, *A. colemani* compared with chemical insecticide, imidacloprid.

Materials and Methods

Chemical and botanical insecticides

Imidacloprid (35% SC) was obtained from the Ariashimi Corporation, Iran and the botanical insecticide palizin (coconut soap 65±5%) was provided by the Kimiasabzavar Co., Iran. The compounds were prepared at their maximum recommended field concentrations: imidacloprid 500 ppm and palizin 2,000 ppm.

Experiment on aphid and sampling

Citrus trees infested with *T. aurantii* were selected from a citrus orchard in Tonekabon city, Mazandaran province (northern Iran) and used for the experiment. Test was performed with complete randomized blocks with three replications (six trees for each treatment) and three treatments including imidacloprid, palizin and water (control). Spraying of trees was done using a 100l engine Honda sprayer (GX120T1, 160T1, 200T) until run-off. Sampling was carried out a day before doing the test and 24, 48 and 72 hours post-treatment. For sampling of each treatment, 30 leaves were randomly picked (10 leaves for each replication). The numbers of *T. aurantii* live adults on each leaf were counted in the laboratory. Aphids were considered to be dead if their bodies or appendages did not move when prodded with afine brush. The corrected efficacy percentage was calculated according to Henderson and Tilton's formula (Henderson and Tilton 1955):

$$\text{Corrected \%} = \left(1 - \frac{N \text{ in Co before treatment} \times N \text{ in T after treatment}}{N \text{ in Co after treatment} \times N \text{ in T before treatment}}\right) \times 100,$$

where: N = number of aphid population; T = treated; Co = control.

Bioassay on parasitoid

Citrus leaves with parasitoid pupae were immersed in the insecticide solutions or water (control) for 10 s (Abdel-Wali *et al*. 2007), with four replicates per treatment. The treated leaves were blotted dry on filter paper. Adult emergence in the control and all treatments was monitored daily. The numbers of emerging adults and mortality were recorded up to 7 days. Mortality percentages were corrected for untreated mortality according to the Abbott formula (Abbott 1925).

Statistical analysis

The analysis of data was performed using SPSS software and the comparison of means by Tukey's test ($p \leq 0.05$). For normalization the data obtained from the numbers of live adults of *T. aurantii* and mortality percentage data were transformed by sqrt and Arc sine √percentage, respectively. One-way analysis of variance was applied to analyze the percentage data.

Results

Mean numbers of live adults of *T. aurantii* one day before treatment and 24, 48 and 72 hours after treatment with imidacloprid and palizin are shown in Table 1. There were significant differences between treatments 24 ($F = 90.73$, $df = 2$, $p = 0.000$), 48 ($F = 83.45$, $df = 2$, $p = 0.000$) and 72 ($F = 305.93$, $df = 2$, $p = 0.000$) hours after treatment. Imidacloprid and palizin spraying decreased the number of *T. aurantii* adults compared with the control. After 24 h, imidacloprid was more effective than palizin in reducing the *T. aurantii* population. With palizin the survival of the aphids gradually decreased and reached its minimum 72 hours post--treatment. There were significant differences between imidacloprid and palizin treatments 24 and 48 hours post-treatment and therefore were categorized into different groups. In the 72 hours post-treatment samplings, there were no significant differences between imidacloprid and palizin treatments. Therefore, the two treatments were categorized into the same groups. The corrected mean percentage of mortality of *T. aurantii* adults, which were subjected to imidacloprid and

Table 1. Mean numbers of live *Toxoptera aurantii* adults one day before test and 24, 48 and 72 hours post-treatment with imidacloprid and palizin

Treatments	1 DBT	24 HAT	48 HAT	72 HAT
Control	43.56±3.1	36±1.8 a	47.61±3.3 a	51.84±3.1 a
Imidacloprid	46.24±2.4	6.25±1.6 c	14.44±2.03 b	2.25±0.4 b
Palizin	51.84±3.5	20.25±1.2 b	4.41±1.1 c	3.61±0.6 b

DBT – days before treatment; HAT – hours after treatment
Means±SEM in columns followed by different letters differ significantly (p ≤ 0.05)

Table 2. Effect of imidacloprid and palizin on *Aphidius colemani* pupae (mean±SEM)

Treatments	Concentration [ppm]	Number of tested pupae	Mortality [%]
Control	–	60	3.3±0.25 a
Imidacloprid	500	92	31.1±0.47 b
Palizin	2,000	52	10.86±0.28 a

Means in a column with the same letter are not significantly different (p ≤ 0.05) according to Tukey´s tests

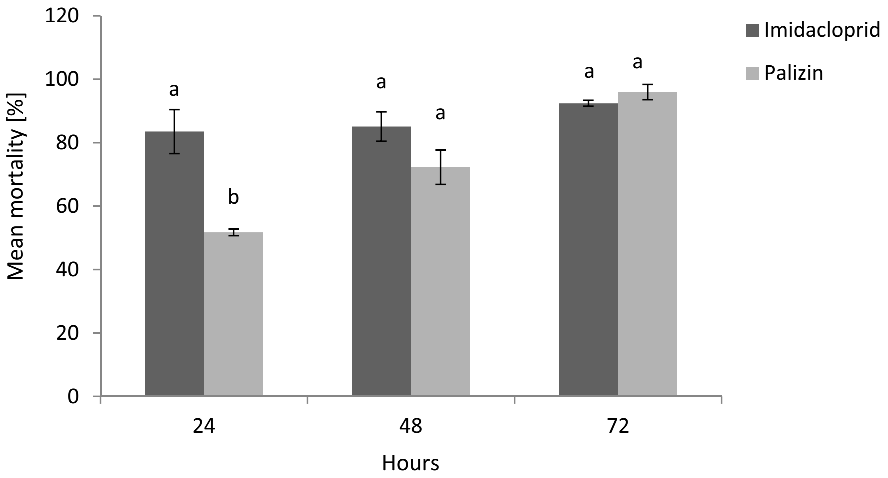

Fig. 1. Mean (±SEM) mortality percentages of *Toxoptera aurantii* adults at different times post--treatment (24, 48 and 72 hours). Means±SEM in each hour with the same letter are not significantly different

palizin, is shown in Figure 1. A comparison of mean percentages of mortality showed that there were significant differences between treatments 24 hours post--treatment. At that time, imidacloprid caused more than 80% mortality while palizin had 51% mortality. Forty-eight hours post-treatment, increased mortality of *T. aurantii* treated by palizin was observed. Palizin showed high efficiency (95% mortality) 72 hours after treatment. However, both insecticides caused more than 90% mortality of *T. aurantii* adults 72 hours after treatment and there were no significant differences between treatments.

The effect of insecticides on *A. colemani* pupae is presented in Table 2. Both imidacloprid and palizin affected adult emergence from pupae. There was a significant difference between treatments (p ≤ 0.05). The adult emergence from treated pupae with imidacloprid was less than from pupae treated with palizin.

Discussion

The results of this study clearly demonstrated that the *T. aurantii* population was affected significantly 24 hours after exposure to imidacloprid. It shows that this insecticide acts faster than palizin. Imidacloprid was very effective against aphid in cotton (Shivanna *et al.* 2011).

Also, good control of sucking insects with imidacloprid has been observed (Saleem and Khan 2001; Khattak *et al.* 2004). Imidacloprid (1,000 ppm) caused high mortality of the pomegranate aphid, *Aphis punicae* Passerini (Hemiptera: Aphididae) (Rouhani *et al.* 2013).

It has been reported that synthetic chemical compounds are unsafe methods in controlling pests since they are one of the major sources of environmental pollution. They cause chronic disease in humans and are harmful for most living organisms (Helmy *et al.* 2012). Imidacloprid (500 ppm) proved to be the most effective insecticide in reducing the cotton aphid population. The insecticide reduced the population of the predators, *Coccinella undecimpunctata* Linnaeus (Coleoptera: Coccinellidae) and *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), which are classified as harmfuland moderately harmful, respectively (Gaber *et al.* 2015). Also, 100% mortality of *A. gossypii* Glover (Hemiptera: Aphididae) was obtained using chlorpyrifos (500 ppm) when aphids were introduced to plants on the same day of spraying. But the insecticide was severely harmful to the parasitoid *A. colemani* adults and pupae (Irshaid and Hasan 2011). As a response to this situation, research on biocide is essential (Pirali-Kheirabadi and Silva 2010). Extracts of plant origin containing insecticidal properties are considered comparatively safe for the environment and public health. It has been reported that over 2,000 plant species belonging to about 170 natural families are known to have insecticidal properties (Delvin and Zettel 1999). Several plants have been found to contain bioactive compounds with a variety of biological actions against insects, including repellent, antifeedant, anti-ovipositional, toxic, chemosterilant, and growth regulatory activities (Sertkaya *et al.* 2010). Botanical insecticides have long been recommended as attractive alternatives to synthetic chemical insecticides for pest management because they pose little threat to the environment or to human health (Isman 2006). In recent years, many studies have also been conducted on the activities of plant extracts and essential oils against aphids (Lai and You 2010; Erdogan and Yildirim 2016). When the effect of *Ammi visnaga* (Algerian aromatic plant, Apiacae) on *T. aurantii* was investigated a maximum of mortality (98.33%) was obtained with the highest dose (30,000 ng/aphids) after 24 h. Also, cumulative mortality of the treated aphids occurred on the second day with a rate of 100% (Fairouz *et al.* 2016). Tobacco extract at 2% caused the highest mortality of 98% of *T. aurantii*, neem extract at 2% ranked second with a mortality of 68% and garlic extract at 2% caused 66% mortality. The botanical pesticides showed high efficacy against aphids. Spraying with tobacco extract effectively controlled aphid populations followed by neem extract and garlic extract (Sohail *et al.* 2012). The plant extracts, *Xanthium strumarium* Linnaeus (Asterales: Asteraceae), *Tanacetum parthenium* Linnaeus (Asterales: Asteraceae) were tested on adults and nymphs of green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). The results showed mortality of more than 80% with the highest concentration (12%) (Erdogan and Yildirim 2016). Pavela (2009) reported that extracts derived from pyrethrum (Asterales: Asteraceae) gave a 100% mortality rate of *M. persicae* after 12 days of treatment. The extract obtained from *Dendropanax morbifera* Léveille (Apiales: Araliaceae) and *Ficus carica* Linnaeus (Rosales: Moraceae) decreased the reproductive rate by 100% of *A. gossypii* 24 h after treatment (Kim *et al.* 2005). In this research, the effects of botanical insecticide, palizin was compared with chemical insecticide, imidacloprid against *T. aurantii*. Palizin is coconut soap (65±5%) manufactured by Kimiasabzavar Company, Iran. It is recommended for the control of some pests especially sucking insects. Experiments have proven that insecticidal soap can reduce plant pest populations and provide effective control (Moore *et al.* 1979). Insecticidal soaps can cause high mortality rates in a variety of soft bodied insect pests such as aphids, whiteflies, leaf hoppers, thrips, and scale insects (Baniameri 2008).

The insecticidal properties of palizin against aphids were investigated by some researchers (Farazmand *et al.* 2012; Ketabi *et al.* 2014). In the present study, palizin caused 95% mortality of *T. aurantii* 72 hours post-treatment. This shows that palizin has insecticidal properties against *T. aurantii* and can be an effective control. Kabiri and Amiri-Besheli (2012) reported that palizin provides a physical and chemical barrier against insect pests and shows considerable potential for effective control of them in certain agricultural crops. Palizin (2,500 ppm) caused a 90.6% reduction of the *A. gossypii* population in greenhouse cucumber (Baniameri 2008). The insecticide at 2,500 ppm had 84.93±0.65% mortality on the second nymph of pistachio psyllids, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) 72 hours post-treatment (Kabiri and Amiri-Besheli 2012). Sheibani and Hassani (2014) reported 93.33±1.06% mortality of pistachio psyllid 14 days after treatment by palizin (2,500 ppm). The researchers concluded that the botanical insecticide is suitable for integrated pest management of the common pistachio psyllid. Also, the results of Ahmadi *et al.* (2012) showed that palizin treatment (3,000 ppm) on the citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae) caused 89.1% mortality.

It has been reported that insecticides can have a detrimental effect on natural enemies (Gholamzadeh-Chitgar and Ghadamyari 2012; Gholamzadeh *et al.* 2012, 2015). Therefore selective application of insecticides can reduce their adverse effects on natural enemies (Galvan *et al*. 2005). In this study, palizin had low mortality on *A. colemani* while imidacloprid was confirmed to be a toxic insecticide. Some studies have revealed a harmful effect of imidacloprid on parasitoids (Golmohammadi 2015). Imidacloprid was the most toxic insecticide for green peach aphid parasitoid, *A. matricariae* Haliday (Hymenoptera: Braconidae) (Abdel-wali *et al*. 2007). Araya *et al.* (2005) reported that 24 hours after application, imidacloprid was toxic to *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) adults. It has been reported that palizin is nontoxic to humans and is relatively harmless to natural enemies (Kabiri and Amiri-Besheli 2012). For example, palizin (2,500 ppm) had a slightly harmful effect on the adult pistachio psyllid parasitic wasp, *Psyllaephagus pistaciae* Ferrière (Hymenoptera: Encyrtidae) and a harmless effect on the pupae. Also, the insecticide caused low mortality (8.68%) of the fourth instar larvae of the coccinellid predator, *Oenopia conglobata* Linnaeus (Coleoptera: Coccinellidae) (Kabiri and Amiri-Besheli 2012).

The results of the present study proved the insecticidal activity of palizin against *T. aurantii*. No statistically significant difference was found when mortality was compared with the chemical insecticide, imidacloprid. A similar result was obtained by Baniameri (2008) when palizin was compared with organophosphorous insecticide, oxydemetonmethyl (Metasystox). The researcher recommended the use of palizin insecticidal soap as a safe alternative to synthetic pesticides in controlling aphids in greenhouses.

Conclusions

The present research is the first study on the effects of botanical insecticide, palizin against *T. aurantii*. Palizin caused excellent control of the aphid having low mortality on its parasitoid, *A. colemani*. So, palizin can be used instead of chemical insecticide, imidacloprid in the control programs of *T. aurantii.* However, further testing is necessary to evaluate the effects of palizin on the parasitoid in order to obtain more accurate information on its compatibility in an integrated pest management program with *A. colemani* to control *T. aurantii*.

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