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Toxicity of Palizin©, Mospilan© and Consult© on *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae), *Oenopia conglobata* L. (Coleoptera: Coccinellidae) and *Psyllaephagus pistaciae* Ferrière (Hymenoptera: Encyrtidae)

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Abstract: The common pistachio psyllid *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is one of the most important pests of pistachio trees. The effect of three concentrations of Palizin© (eucalyptus extract) on *A. pistaciae* (nymphs), cocinellid predator (CP) *Oenopia conglobata* L (Coleoptera: Coccinellidae) (1st, 4th larvae and adults) and *Psyllaephagus pistaciae* Ferrière (Hymenoptera: Encyrtidae) (pupae and adults) was tested. The toxicity of this toxin was compared with two chemical insecticides, Consult© and Mospilan©. Topical bioassay was achieved in laboratory conditions. In order to investigate the mummy stage of the parasitic wasp (PW), completely random design experiment was used in the field in 2010 and 2011. The results of these experiments have shown that the high doses of Palizin (2500ppm) has 84.93±0.65 % mortality and the LC_{50} for Palizin was 750.825 ppm 72 h post treatment on the pistachio psyllid nymphs and the chemical pesticide, Mospilan, had more mortality than Palizin on all stages of the two above natural enemies. Howvere, Palizin had low mortality on above stage of PW and CP. The results have shown that Palizin had a slightly harmful effect on the adult of PW while having a harmless effect on other stages, which is cconsistent with IOBC categories, the standard for the effects of pesticides on natural enemies.

Key words: Pistchio psyllid · Botanical insecticides · Parasitic Wasp · *Psylluphagus* · Coccinellid Predator *Oenopia*

INTRODUCTION

The use of biopesticides towards phytophagous insects has increased in recent years, particularly in cropping systems that rely on natural enemies as a major component of integrated pest management [1, 2]. Use of these natural compounds instead of conventional insecticides can reduce environmental pollution, preserve non-target organisms and avert insecticide-induced pest resurgence that resulting from insecticide resistance.

The using of botanical pesticides in sustainable agriculture dates back at least two millennia in ancient China, Greece, Egypt and India [3,4]. The documented use of botanicals extends back more than 150 years in Europe and North America, pre-dating discoveries of the major classes of synthetic chemical insecticides (*e.g.* organochlorines, organophosphates, carbamates and pyrethroids) in the mid-1930s to 1950s. However, overuse of synthetic insecticides has led to numerous problems unpredicted such as acute and chronic toxicity of applicators, farm workers and even consumers; destruction of fish, birds and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening to human and environmental health; and the evolution of resistance to pesticides in pest populations [5]. These changes in the environmentappeared to heighten the impetus for the discovery and development of alternative pest management products including insecticides derived from plants. Indeed, the scientific literature of the past 25 years described hundreds of isolated plant secondary metabolites that showed feeding deterrent or toxic effects to insects in laboratory bioassays. Botanical insecticides have been the subject of several recent volumes [6].

Biochemical or microbial biopesticides are an important group of naturally-occurring, often slow-acting crop protection products that are usually safer to humans and the environment than conventional pesticides and with minimal residual effects. Biochemical pesticides may include plant-derived pesticides (botanicals) that can

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interfere with the growth, feeding, or reproduction of pests or insect pheromones applied for mating disruption, monitoring or attract-and-kill strategies [7].

Pistachios are an important agricultural product and have historical cultural significance [8]. The common pistachio psyllid *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae) is a major pest in all pistachio orchards and feeds on different parts of the plant and fruit, causing damage to the pistachio and hence, decreasing the yield. [9].

This insect is controlled by chemical insecticides but consequently the overuse of these pesticides has had an adverse effect on the natural enemies and the environment. Also, the target pest has developed resistance to the insecticides and has increased in number. This has resulted in farmers being very interested in using natural enemies. One of the most common is the Cocinellid Predator (CP) *Oenopia conglobata* L (Coleoptera: coccinellidae), [10]. The CP is one of the most prevalent predators of this pest in pistachio orchards and is active in all pistachio orchards in Kerman Province (a province in south-east Iran) throughout the year. The adults and larvae of CP feed on the eggs and nymphs of the pistachio psyllid. Another natural enemy of common psyllid pistachio is the Parasitic Wasp (PW) *Psyllaephagus pistaciaee* Ferrière (Hymenoptera: Encyrtidae). This wasp controls the pistachio psyllid in all pistachio orchards in Iran, sometimes decreasing the psyllid population up to 80% in some years [11].

The increasing price of pistachios has resulted in the economic threshold of the pest to decrease and this has led most growers to use chemical pesticides to control the pistachio pest. Consequently, because of the high doses and overuse of pesticides, the pest has become resistant to the pesticides, so the doses of the chemicals has increased and the number of pesticide applications has also increased each year.

As a result of this increase in pesticide dose and application frequency, it has become necessary to use less harmful biological pesticides, at low doses and to apply them at appropriate times, such as when pest numbers reach a point that are above an acceptable level [12]. Botanical and microbial pesticides can control pests more effectively and are less harmful for the environment, people and also for non-target organisms [13].

Plant extracts and phytochemicals have long been a subject of research in an effort to develop alternatives to the conventional insecticides and in the present paper we focused on the effect of the botanical insecticides Palizin© with insecticidal activities on the pistachio psyllid *A. pistaciae* and the larvae and adult of its two natural enemies, the Coccinellid Predator (CP) *O. conglobata* and the Parasitic Wasp (PW) *P. pistaciae*. The effects of this pesticide were compared with two chemical pesticides, Mospolin© and Consult© on PW and CP.

MATERIALS AND METHODS

The pesticides used are shown in Table 1.

Effect of Palizin© on Pistachio Psyllid Nymphs: Psyllid nymphs were collected from unsprayed pistachio orchards near Rafsanjan city, Kerman Province. A leaf-dip assay was used to measure pesticide effects; a pilot was used to determine the maximum and minimum pesticide doses for the main experiments. Five concentrations were considered after preliminary test (250, 355, 525, 758, 1112, 1660 and 2500 ppm). Water was used as the control group. The bioassay involved leaf disc (2cm diameter) being dipped in the toxin solution for 15 seconds and then being allowed to dry. After drying, the leaf was placed upside down in an agar plate (0.8 % agar solution). Thirty psyllids were then released into the petri-dish and the petri-dish placed in a germinator (22±1°C, 60±5 % relative humidity (RH) and 16:8, D: L photoperiod conditions). Psyllid mortality was recorded at 24, 48 and 72 h post-treatments). If there was any mortality in the control it was corrected by Abbott's formula [14].

Table 1: The pesticides used in experiments

Common name	Trade name	Chemical group	Active ingredient	Formulation	Dose	LD_{50} mg/kg body weight	Company
Insecticidil gel(IG)	Palizin	Biorational insecticide	coconut fatty acid soap, eucalyptus and mint extracts	WS (water soluble)	2500, 1250 and 833cc/1000 L water	> 5000	Kimia sabzavar Co.
Acetamiprid	Mospilan	Neonicotinoid	-	SP20%	250 g/1000 L water	146-217	Trustschen Co.
Hexaflumuron	Consult	benzoy lurea	-	EC10%	500cc/1000 L water	> 5000	Arman sabz adineh Co.

Collection and Rearing of *Oenopia conglobata*: The coccinellid *O. conglobata* was collected from unsprayed orchards in the urban area of Rafsanjan city. Beetles were collected by aspirator and then placed into a plastic box for transport. Fresh leaves with psyllids were collected to feed the coccinellid predators. The insect box was checked daily and the leaves that had coccinellid eggs were then transferred to a new box and kept at 22°C. The eggs were kept until the first instar appeared and these were used for the larval bioassay. A number of individuals were kept until they became 2-3 old days that used in the adult stage bioassay.

Collection of *P. pistaciae*: The parasitic wasp was collected from unsprayed orchards in the urban area of Rafsanjan city where parasitised psyllid nymphs were abundant.Leaves and parasitic wasps were collected and then transported to the lab in plastic bags. They were then transferred into a cylindrical glass with one side covered with cotton netting so that when the adult parasitic wasps emerged, they could be separated with an aspirator and used in the bioassay.

The Effect of the Pesticides on 1st and 4th Larvae and Adults of *O. conglobata*: Three concentrations of the recommended field dose (2500 ppm manufacturer's instructions), half of the field dose (1250 ppm) and 1/3 field dose (833 pmm) of Palizin were used. The field doses of Mospilan (250g/1000 L water) and Consult (500 ppm) were used.. All these concentrations were dissolved in acetone. First instar larvae were anaesthetised by placing them into a 4°C fridge for 2 minutes, 1.0 ìL of solution of each toxin was put into the first segment of the thorax (via microinjection). Acetone was used for the control.

After each treatment, the larvae were transferred to a new Petri-dish with a leaf with psyllid nymphs and maintained at 25°C ±2°C and relative humidity 65%±2%. The numbers of live and dead insects were counted at 24, 48 and 72 h post treatment. The same treatments were repeated for fourth instar larvae and adult coccinellids. These experiments were repeated four times.

The Effect of the Pesticides on the Adults of *P. pistaciae*: The adults of the parasitic wasp were used for the bioassay one day post emergence. They were transferred by aspirator to a small glass tube and put in fridge at 4°C for 2 minutes for anesthetising. Ten insects were used per dose. Toxins were dissolved in acetone and 1.0 µL was then put in the first segment of the thorax of the parasitic wasp (via microinjection). Acetone was used for the control. After treatment wasps were put into 5cm × 1cm glass tubes individually.

Adult wasps were fed with pure honey on paper, 3cm × 0.2 cm. Each piece of paper was put into a glass tube with one wasp in each tube. Ten wasps were used for each concentration of each toxin. After each assay the glass tubes were transferred to the incubator and maintained at 25°C and 75 % RH and the numbers of live and dead insects were counted at 24, 48 and 72 h post treatment. Any mortality in the control was corrected with Abbott's Formula [14].

Field Experiment: The effect of the pesticides on mummy *P. pistaciae* (larvae and pupae inside the psyllid nymph) One non-sprayed pistachio orchard was selected where PW was active. The orchard was divided into four sections randomly and each section was sprayed with the following toxin: 1. Sirinol© with 2500cc in 1000L water; 2. Mospilan© with 250g in 1000L water; 3. Consult© 500cc in 100L of water; 4. Control was water. Each treatment was sprayed with a 100 litre sprayer. The morning following treatment application, 100 nymphs of PW larvae and pupae were collected. One month after collection, the PW emerged from the pupa, the number of alive PW was counted and the percentage of mortality was corrected with Abbott's Formula [14]. This experiment was repeated two times (2010, 2011).

Experiment 1: this experiment was carried out during August 2010 in Jafarabad Village in Rafsanjan. The trees were in rows and were approximately 18 years old. The application was carried out between 6.00 am – 8.30am while the weather was mild (*i.e.* no wind).

Experiment 2: this experiment was carried out during September 2011 in Akbarabad village in Rafsanjan. The trees were in rows and were approximately 18 years old. The application was carried out between 6.00 am - 8.30 am while the weather was calm (*i.e.* no wind).

Statistical Analyses: According to the principles of the IOBC (International Organisation for Biological Control), four evaluation categories consist of Hassan references [(% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25% mortality or reduction in beneficial insects), 2 = slightly harmful (25- 50% mortality or reduction in beneficial insects), 3 = moderately harmful (51-75% mortality or reduction in beneficial insects) and

4 = harmful (>75% mortality or reduction in beneficial insects) [15]. The mortality of PW and CP were counted and mean numbers per leaf were calculated and statistically evaluated using univariate ANOVA (SPSS 11.0). The LC_{50} and LC_{90} were calculated by polo+ program version 1 copyright© 2002-2012 Leora software.

RESULTS

The Effect of Palizin© on the Nymphs of the Pistachio Psyllid under Laboratory Conditions: The effect of Palizin© has shown that there were significant differences between different doses and also between different times of post treatments on the psyllid (Table 2) (p<1%). The highest mortality was obtained using 2500 ppm (84.93 ± 0.65 %) and lowest mortality was obtained using 250 ppm (19.01 ± 0.89 % mortality) 72 h post treatment. Mortality was categorised in different classes using a Tukey's test (Table 2). The LC_{50} and LC_{90} were 750.825 and 3734 ppm 72 h post treatment, respectively (Table 3).

The Effect of Pesticides on the Adults of *Oenopia conglobata*: The results showed that the 2500 ppm dose of Palizin© gave 11.6% mortality and at 1250 ppm and 833 ppm gave 6.66% and 0 % mortality, respectively. Mospilan© resulted in 39.99% mortality and Consult© in 8.56% mortality. There were significant differences among different groups and different doses of Palizin© and with two chemical pesticides (p<5%). These were categorised in two different groups 24 h post-treatment, consistent with IOBC Categories which is used for the effect of toxin on the beneficial insect. The results of the 2500 and 1250 doses were the same as 24 h post treatment, however, the 833 ppm dose resulted in 3.33% mortality, Mospilan in 46.66% mortality and Consult in 16.90% mortality. There were significant differences among different doses of Palizin as well as Mospilan and Consult and they were categorised in separate groups 48 h post-treatment consistent with IOBC Category. The results from the 2500 and 1250 doses were the same as the 24 h post-treatment but Mospilan showed the highest mortality with 57.49% and mortality with Consult was 16.90% 72 h post-treatment. Consistent with IOBC categories, the three doses of Palizin and Consult were categorised in the harmless toxins and Mospilan was in the slightly harmful category to the adult of the Coccinellid Predator toxin category (Table 4 and Fig. 1).

Table 2: Mean % mortality of the second nymph of pistachio psyllids by the different doses of Palizin© consistent with Tukey`s test

Hours post treatment	Control	250 ppm	355 ppm	525 ppm	758 ppm	1122 ppm	1660 ppm	2500 ppm
24	3.2±0.89	6.41±0.24	11.58±0.85	11.58±0.85	14.32±0.49	17.28±0.65	20.74±1.28	28.14±1.54
48	0.65±5.67	11.85±0.85	25.43±1.07	25.43±1.07	35.30±1.78	1.48±40	0.42±46.66	58.51±1.13
72	0.89±8.39 a*	19.01±0.89 b	28.39±0.89 c	38.27±0.65 d	50.12±0.65 e	62.46±1.07 f	71.60±0.65 g	84.93±0.65 h

*Different letters indicate significant differences (p<5%)

Table 3. The LC_{50} and LC_{90} of the Palizin 72 h post treatment

	Number of insects	LC (ppm)	X^2 (K Squire)	Upper confidential intervals (ppm)	lower confidential intervals(ppm)
LC_{50}	405	750.825	2.03	798.694	705.667
LC_{90}	405	3734	2.03	4384	3253

Table 4: Comparison of the means of the % mortality in different times of post-treatment on the adults of *Oenopia congelobata*

Name of pesticides	Doses/1000 L H_2O	24 h post treatment	48 h post treatment	72 h post treatment
Palizin	2500 ppm	$11.66\ ^{1}$**c*e	$11.66\ ^{1}$ce	11.66^{1}ce
"	1250 ppm	$6.66\ ^{1}$df	6.66^{1}df	6.66^{1}df
"	833 ppm	0^{1}	3.33^{1}df	$6.49\ ^{1}$df
Mospilan	250 g	39.99^{2} b	46.66^{2}b	57.49^{2} a
Consult	500 ppm	8.56^{1}cf	16.90^{1}c	16.90^{1}c

*different letters indicate significant differences (p<5%)

** According to the principles of IOBC, four evaluation categories (% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25%), 2 = slightly harmful (25- 50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan, 1994).

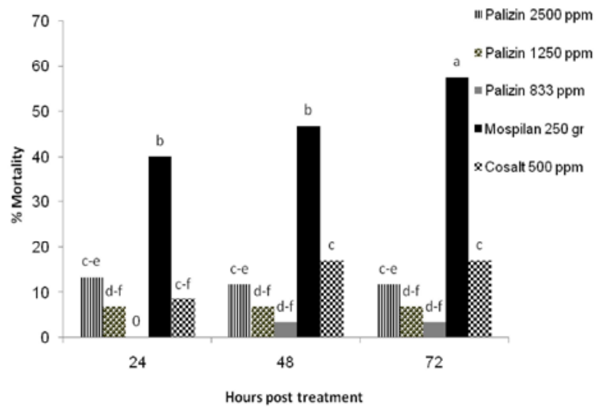

Fig. 1: Percentage mortality of the adult cocinellid predator *O. conglobata* sprayed by Mospilan, Consult and different doses of Palizin

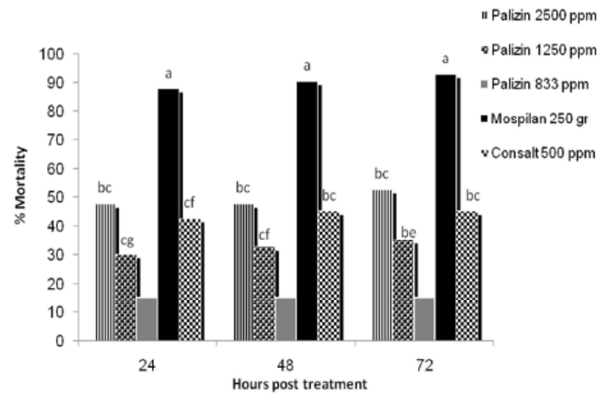

Fig. 2: Percentage mortality of the adult Parasitic Wasp *P. pistaciae* sprayed by Mospilan, Consult and different doses of Palizin at 24, 48 and 72 hrs post treatment

Table 5: Comparison of the means of % mortality at different times of post-treatment on the adults of *Psyllaephagus picataciae*

Name of pesticides	Doses/1000 litre H_2O	24 h post- treatment	48 h post-treatment	72 h post- treatment
Palizin	2500 ppm	47.50 ^{2}**bc*	47.50^{2} bc	52.50^{2}bc
"	1250 ppm	30^{2}cg	32.50^{2}cf	35^{2} be
"	833 ppm	15^{1}fh	15^{1}fh	15^{1}fh
Mospilan	250 g	87.50^{3}a	90^{3}a	92.50^{3}a
Consult	500 ppm	42.50^{2}cf	45^{2}bc	45^{2}bc

* different letters indicate significant differences (p<5%)

** According to the principles of IOBC, four evaluation categories (% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25%), 2 = slightly harmful (25- 50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan, 1994).

The Effect of the Pesticides on the Adult *Psyllaephagus pistaciae*: The results showed that the highest mortality was obtained with Mospilan with 87.5% and Palizin at 2500, 1250 and 833 ppm concentrations with 47.5%, 30% and 15% mortality, respectively. Consult resulted in 42.5% mortality (500 ppm) 24 h post-treatment and the analyses of variance (ANOVA) showed that there were significant differences among different doses of Palizin as well as Mospilan and Consult and they were categorised into two different groups 24 h post-treatment consistent with IOBC categories (p<5%). The mortality of the highest dose of Palizin (2500 ppm) and the lowest dose (833 ppm) were the same as 24 h post treatment; however, the 1250 ppm had 32.5 % mortality and Monspilan and Consult have shown 90% and 45% mortality, respectively 48 h post-treatment. The ANOVA showed that the three doses of Palizin and Mospilan were significantly different and hence, were categorised in separate groups. However, there were no significant differences between Palizin at 2500 ppm and Consult (500 ppm) and were in the same group consistent with IOBC Categories. Palizin had 52.5% mortality and 35% mortality at 2500 ppm and at 1250 ppm, respectively; however, the mortality at 833 ppm was the same as 24 h post-treatment, respectively and the highest mortality was obtained with Mospilan with 92.5 %. The two doses (2000 ppm, 1250 ppm) of Palizin and Consult was slightly harmful to beneficial insects and the low dose of Palizin (833 ppm) was harmless, whereas Mospilan was shown to be moderately harmful, which was consistent with the IOB categories (Table 5 and Fig. 2).

The Effects of the Pesticides on the First Instar Larvae of The Coccinellid Predator: The results showed that Mospilan was very effective with 86.54% mortality; Consult had 17.26% mortality and Palizin had 8.68% mortality, 3.51% and 0% mortality 24 h post-treatment at 2500 ppm, 1250 ppm and 833 ppm concentrations. The ANOVA showed that there were significant differences among different doses of Palizin as well as two chemical pesticides and they were categorised into different groups consistent with IOBC Categories 24 h post-treatment. Mortality with Palazin at the 2500 ppm dose increased up to 18.02%, mortality with Mospilan increased up to 91.30% and mortality with Consult increased to 24.16%.

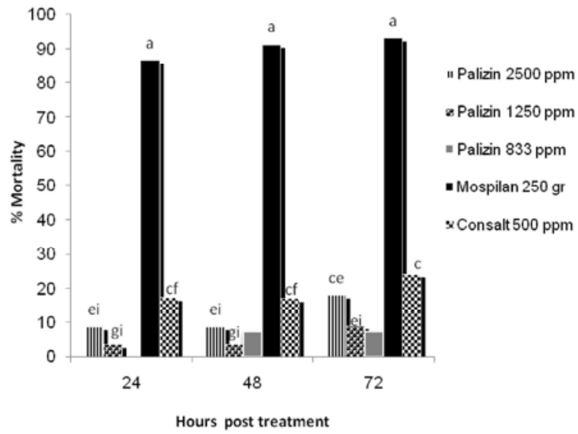

Fig. 3: Percentage Mortality of the first instar larvae of the coccinellid predator *O. conglobata* sprayed by Mospilan, Consult and different doses of Palizin at 24, 48 and 72 hpost-treatment

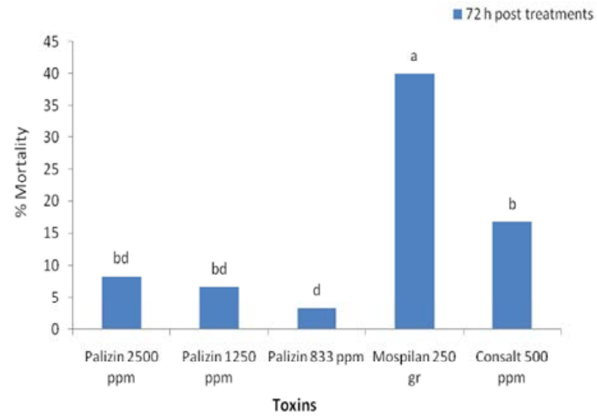

Fig. 4: Percentage Mortality of the fourth instar larvae of the coccinellid predator *O. conglobata* sprayed by Mospilan, Consult and different doses of Palizin at 72 h post-treatment

Table 6: Comparison of the means of % mortality at different times post-treatment on the first instar larvae of the coccinellid predator

Name of pesticides	Doses/1000 L H_2O	24 h post-treatment	48 h post- treatment	72 h post- treatment
Palizin	2500 ppm	8.68 ^{1**}ei*	8.68 ^{1}ei	18.02^{1}ce
Palizin	1250 ppm	3.51^{1}gi	3.51^{1}gi	8.93^{1}ei
Palizin	833 ppm	0^{1}i	0^{1}i	7.27^{1}ei
Mospilan	250 g	86.54^{3}a	86.54^{3}a	91.30^{3}a
Consult	500 ppm	17.26^{1}cf	17.26^{1}cf	24.16^{1}c

* Different letters indicate significant differences (p<5%)

** According to the principles of IOBC, four evaluation categories (% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25%), 2 = slightly harmful (25- 50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan 1994).

Table 7: Comparison of the means of % mortality 72 h post-treatment on the fourth instar larvae of the coccinellid predator

Name of pesticides	Doses/1000 L H_2O	72 h post-treatment
Palizin	2500 ppm	8.32 ^{1**}bd*
Palizin	1250 ppm	6.66 ^{1}bd
Palizin	833 ppm	3.33 ^{1}d
Mospilan	250 g	39.99 ^{2}a
Consult	500 ppm	16.90 ^{1}b

*The different letter indicate significant differences (p<5%)

** According to the principles of IOBC, four evaluation categories (% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25%), 2 = slightly harmful
(25-50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan, 1994).

The ANOVA indicated there were significant differences among different doses of Palizin as well as two chemical pesticides 48 h post-treatment. The lowest % mortality was obtained with Palizin at the 833 ppm dose with 7.27%; however, the highest % mortality was obtained with Mospilan with 93.09% whereas the highest dose of Palizin (2500 ppm) had only 23.04% mortality and Consult had 36.18% mortality 72 h post-treatment; the ANOVA showed there were significant differences among different doses of Palizin as well as two chemical pesticides. They were categorised into different groups consistent with IOBC Categories 72 h post-treatment. All doses of Palizin was harmless toxin, consistent with IOBC categories; however, Consult was categorised as slightly harmful and Mospilan was categorised as moderately harmful toxin on the first instar larvae of the Coccinellid Predator (Table 6 and Fig. 3).

The Effects of the Pesticides on the Fourth Instar Larvae of The Coccinellid Predator: The results showed that the highest mortality on the fourth instar larvae of CP was obtained with Mospilan with 39.99%, followed by Consult with 16.90% mortality and thirdly with Palizin with 8.32% mortality at a concentration of 2500 ppm, 72 h post-treatment; the lowest % mortality was obtained with Palizin with 3.33% at 833 ppm 72 h post-treatment.

Table 8: Mean comparison of % efficacy of the larvae and pupae of the Parasitic Wasp using different toxins and at different times

	250 g Mospilan		500 ppm Consult		2500 ppm Palizin		Control	
Replicate	No. of PW emerged	% efficacy of toxin	No. of PW emerged	% efficacy of toxin	No. of PW emerged	% efficacy of toxin	No. of PW emerged	% efficacy of toxin
1	33	8.33	35	2.77	29	19.44	36	-
2	33	23.25	43	0	34	20.93	43	-
% mean efficacy	-	15.79*[1]	-	1.38[1]	-	20.18[1]		

** According to the principles of IOBC, four evaluation categories (% mortality or reduction in beneficial capacity) were used: 1 = harmless (< 25%), 2 = slightly harmful (25- 50%), 3 = moderately harmful (51-75%) and 4 = harmful (>75%) (Hassan, 1994).

All doses of Palizin and Consult were shown to be harmless to beneficial insects and Mospilan was slightly harmful 72 h post-treatment (Table 7and Fig. 4), consistent with the IOBC category.

The Effects of the Pesticides on the Larvae and the Pupae of the Pw in the Psyllid Nymph: The results of the field experiment showed that Palizin had the highest mortality with 20.18% followed by Mospilin with 15.79% mortality and thirdly Consult with 1.38% mortality on the larvae and pupae of the PW during 2010 and 2011. All pesticides examined (Palizin, Mospilan and Consult) were categorised as harmless on the parasitized mummies stage of the PW in the psyllid nymph (Table 8), consistent with the IOBC category.

DISCUSSION

This is the first report about the effect of Palizin on the pistachio psyllid. This research showed that Palizin is a botanical pesticide that can control the pistachio psyllid effectively [16], however, the efficacy of Palizin had not been investigated on the natural enemies of this pest up to now and this research showed its effect on its natural enemies for the first time.

The results showed that the effect of Mospilan on the Parasitic Wasp *P. pistaciae*, one of the most important natural enemies of the pistachio psyllid, is high (causing 92.5% mortality of PW) compared with the high dose of Palizin (2500 ppm) causing only 52.5% mortality of PW. The effect of Mospilan was 57.9% mortality of the CP, which is one of the important predators of the pistachio psyllid; however, the high dose of Palizin (2500 ppm) had a low effect on this predator (mortality was only 11.66%). Conclusively, Palizin is very promising botanical pesticides against the pistachio psyllid.

Other advantages of Palizin are that pests are unlikely to develop resistance to it, it has no phytotoxic effects, it lasts longer than most insecticides on plants when it does not rain or there is no excessive dew, is non-toxic to humans and is relatively harmless to natural enemies. Additionally, it is dissolvable, forming a suspension in water, thereby making it easy to apply using conventional spray equipment. It may eventually reduce the number of applications of conventional insecticides.

It was observed that the adult psyllids landed on the treated leaves and appeared to try to escape from treated leaves by moving around and testing the leaf surface, although their mouthparts could penetrate through the Palizin barrier on the surface into leaf tissue. These results indicated that the activity of the Palizin may be related to interference with tactile perception of the host plants.

Palizin provides a physical and chemical barrier against insect pests and shows considerable potential for effective control of insect pests in certain agricultural crops. Palizin can also be mixed with other biorational insecticides such as mineral oil, botanical oils and essential oils to enhance their efficacy. However, because of the importance of a continuous coverage of plant material with Palizin, better application methods and perhaps frequent applications will be required to cover newly expanding foliage.

It is possible that the psyllid nymphs in our experiments ingested Palizin while feeding on the phloem of the treated plants or that Palizin may have systemic activity or it is assumed that when the psyllid came into contact with Palizin on top or under the leaf, the toxin may penetrate the insect body from the moth part (this is the same as ingestion) or from the tarsus of the legs.

We could not find any published data concerning the effect of Palizin on pistachio psyllid. However, Mospilan is a novel and highly active insecticide with broad-spectrum efficacy against sucking and biting insects. Moreover, this compound is systemic and is regarded as having low acute toxicity to mammals. Its mode of action is through interference with the nicotinergic acetycholine receptor and is active via contact and ingestion [17] but it is highly effective against two most important natural enemies of the pistachio psyllids. Also, Mospilan and Consult did not provide adequate protection of pistachio psyllid damage when it was applied, probably because of its relatively short residual effectiveness, the appearance of resistant populations, or because of its high toxicity to parasitoids and predators [18].

Currently, the most common control practices against pistachio psyllid are based on the use of various insecticides [19]. The lack of significant differences in the mean numbers of pistachio psyllid nymphs between Palizin, Mospilan and Consult indicates that Palizin is a suitable alternative to insecticides. However, the Mospilan and Consult treatments did not control the pistachio psyllid when they were applied mid-season, whereas Palizin residues suppressed pistachio psyllid after three applications. Moreover, Palizin seems ideal for pistachio psyllid control in the east Mediterranean region. The dry summer minimizes Palizin wash-off and the limited shoot growth after mid-May prevents pistachio psyllids from infesting newly uncovered leaves.

Throughout the two seasons of field trials, Palizin-sprayed foliage was checked for phytotoxic effects (*e.g.* leaf burn, leaf drop), but no adverse effects were found even after four applications. In contrast, Palizin prolonged pistachio foliage half-life (unpublished observation). From a pest management perspective, the current study provided a good understanding of Palizin efficacy under pistachio orchard conditions and revealed its potential for use against pistachio psyllid infestations. Moreover, the widespread adoption of Palizin is unlikely to select resistance since this technology provides a physical mechanism of preventing pest attack rather than a toxic chemical selection pressure [20]. The most important pistachio psyllid endoparasite *P. pistaciae* [21] was detected by the presence of parasitised psyllid nymphs, mummies and other beneficial insects, like ladybeetles and common green lacewing, were often observed in the experimental orchard. Nevertheless, these promising results should stimulate further investigations of Palizin applications to control another pistachio psyllid, *Agonoscena targionii* (give taxonomic detail here) in pistachio orchards in different geographical regions.

The present data indicate potential for Palizin as a repellent for landing and a barrier to oviposition and it may prove to be an economically viable and environmentally sound component of an integrated approach for control of pistachio psyllids and related pests, giving growers an environmentally friendly and sustainable alternative to chemical pesticides.

The use of biopesticides that are compatible with biological controls currently in place are desirable in crops such as citrus that often cover large acreages. Palizin, known for its selectivity, has been shown to effectively control other phloem-feeding insects including several species of Aphididae and Citrus leafminers[22, [23, 24, 25, 26] and at least one species of Psyllidae, the pistachio psyllid, *A. targionii* [16]. Our results indicate that Palizin can be applied at very low concentrations to effectively manage developing psyllid populations. This botanical insecticide has been found to be relatively non-toxic to beneficial insects and should be safe to apply in the presence of natural enemies found in pistachios, particularly at concentrations required to control psyllid nymphs. Furthermore, no phytotoxicity was observed in any of the plant tissues used in these experiments. Palizin biopesticides may therefore be well suited for inclusion in pistachio integrated pest management programs. Since low concentrations of Palizin have been demonstrated to be effective against *Pistachio psyllid* and other important pistachio pests, field trials are warranted to determine appropriate methods of application and efficacy in commercial settings. The results from this study emphasis the need further to evaluate the efficacy of Palizin in large-scale field studies for management of pistachio psyllid in Iran and in other places where this insect pest occurs.

REFERENCES

1. Tengerdy, R.P. and G. Szakacs, 1998. Prospectives in agrobiotechnology. J. Biotechnol., 66: 91-99.
2. Rausell, C., A.C. Martinez-Ramirez, I. Garcia-Robeles and M.D. Real, 2000. A binding site for *Bacillus thuringiensis* Cry1Ab toxin is lost during larval development in two forest pests. Appl. Environ. Microbiol., 66: 1553-1538.
3. Thacker, J.M.R., 2002. An Introduction to Arthropod Pest Control. Cambridge, UK: Cambridge univ. Press. pp: 343.
4. Ware, G.W., 1883. Pesticides. Theory and Application. San Francisco: Freeman press. pp: 308.
5. Perry, A.S., I. Yamamoto, I. Ishaaya and R.Y. Perry, 1998. Insecticides in Agriculture and Environment: Retrospects and Prospects.Berlin: Springer-Verlag. pp: 261.
6. Regnault-Roger, C., B.J.R. Philog`ene and C. Vincent, eds. 2005. Biopesticides of Plant Origin.Paris: Lavoisier. pp: 313.
7. Copping, L.G. and J.J. Menn, 2000. Biopesticides: A Review of their Action, Applications and Efficacy Pest Manag. Sci., 56: 651-676.

8. Radjabi, G., 1989. Insects attacking rosaceous fruit Trees in Iran. Vol. III. Publication of Plant Pest & Diseases Research Institute, Tehran.
9. Mehrnejad, M.R., 2001. The current status of pistachio pests in Iran. Cahiers Options Méditerranéennes, 56: 315-322.
10. Hodek, I., 1973. Biology of the coccinelidae. Academic Publishing House of the Czechoslovak Academy of science, Prague. pp: 260.
11. Mehrnejad, M.R., 2002. Bionomics of the common pistachio psylla, *Agonoscena pistaciaee*, in Iran. Acta Horticul., 591: 535-539.
12. Samih, M.A., A. Alizadeh and R. Saberi Riseh, 2005. Pistachio pests and diseases in Iran and their IPM. Organization of Jihad-e-University, Tehran. pp: 301.
13. Matthews, G.A., 1999. Pesticides, IPM and training. Phytoparasitica, 27: 253-256.
14. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
15. Hassan, S.A., 1994. Results of the sixth joint pesticide testing program of the IOBC/WPRS – working group "Pesticide and beneficial organisms". Entomophaga, 39(1): 107-119.
16. Kabiri, M., B. Amiri-Besheli and M. Basirat, 2011. The effect of Tondexir, Sirinol and Palizin on nymph of the common psyiid of pistachio *Agonoscena pistaciaee* on lab and field conditions the conference of the national sustainable agriculture 20-23 November 2011 Veramin, Tehran, Iran.
17. Tomizawa, M. and J.E. Casida, 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu. Rev. Entomol., 48: 339-364.
18. Suh, C.P.C., D.B. Orr and J.W. Van Duyn, 2000. Effect of insecticides on *Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) preimaginal development and adult survival. J. Econ. Entomol., 93: 577-583.
19. Lababidi, M.S. and C.P. Zebitz, 1995. Preliminary study on the pistachio psyllid (*Agonoscena targionii*) (Licht) (Psyllidae: Homoptera) and its associated natural enemies in some regions of Syria. Arab J. Plant Prot. 13: 62-68.
20. Souliotis, C., D. Markoyiannaki-Printziou and F. Lefkaditis, 2002. The problems and prospects of integrated control of *Agonoscena pistaciaee* Burck. and Laut. (Hom., Sternorrhyncha) in Greece. J. Appl. Entomol., 126: 384-388.
21. Mazor, M. and A. Erez, 2004. Processed kaolin protects fruit from Mediterranean fruit fly infestation. Crop Prot. 23: 47-51.
22. Saur, G., 2005. Efficacy of kaolin particle film and selected synthetic insecticides against pistachio psyllid *Agonoscena targionii* (Homoptera: Psyllidae) infestation. Crop Protection, 24(8): 711-717.
23. Amiri Besheli, B., 2007. Efficacy of *Bacillus thuringiensis* and Mineral Oil Against *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae). International Journal of Agricuture and Biology, 9: 893-896.
24. Amiri Besheli, B., 2008. Efficacy of *Bacillus thuringiensis*, Mineral Oil, Insecticidal Emulsion and Insecticidal Gel Against *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). Plant Protection Science, 44(2): 68-73.
25. Amiri Besheli, B., 2009. Toxicity evaluation of Tracer, Palizin, Sirinol, Runner and Tondexir with and without mineral oils on *Phylocnistis citrella* Stainton African. Journal of Biotechnology, 8(14): 3382-3386.
26. Amiri Besheli, B., 2010. Efficacy of chlorpyrifos-methyl, methoxyfenozide, spinosad, insecticidal gel, insecticidal soap and mineral oil on citrus leafminer. Journal of Food, Agriculture & Environment (JFAE) 8(2): 668-671.