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Efficacy and residues of selected insecticides for control of cotton aphid (*Aphis gossypii*) and mealybug (*Planococcus citri*) in pomegranates

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Abstract. Mealybug (*Planococcus citri* Risso) and aphids (*Aphis gossypii* Glover and *Aphis punicae* Passerini) are the most important pests of pomegranate production in Alicante (Spain). Due to the minor crop status of pomegranates, registered pest control chemicals for this crop are scarce. Experimental field efficacy of new conventional insecticides and alternative sprays against these sucking pests was assessed. Experiments to deal with these pests were carried out in randomized block design with three replications during 2009-2010, in two experimental pomegranate orchards in the Southeastern region of Spain. Other management practices and natural enemies of these pests were identified and quantified during the crop period. Results of the experiments suggest that the population of cotton aphid was resistant to Pirimicarb. New generation insecticides (Imidacloprid, Flonicamid, Acetamiprid) were more effective in controlling aphids. Moderately effective control can be achieved with soap, citrus oil and other tolerance exempt products, or some combination of these, but more than two applications may be necessary. Pesticide residue levels in ripening fruit after treatments were analysed.

Keywords. Pest management – Residue levels decline – Natural enemies.

I – Introduction

Pomegranate (*Punica granatum* L.) is one of the most important fruit crop in the south of Alicante (Southern Spain) where more than 90% of national production originates. Pomegranate pests and their management strategies differ greatly, depending on climate, countries and cultivars. Whereas pomegranate trees are attacked by more than 90 species of insects in India (Balikai *et al.*, 2011), in Spain most of these species have not been recorded. Literature on pomegranate pests in Spain is not extensive, but it is known that infestation by sucking pests like aphids and mealy bugs is more common in pomegranates in this Mediterranean area (Toledo *et al.*, 2000). Aphid infestation occurs after shooting, during flowering and fruiting stages of the crop, thereby reducing the vigour of the plant through the excretion of honeydew on the leaves and the development of sooty mould which covers the surface of leaves and fruits.

The available pesticides for use on pomegranates are expanding in other non UE countries. Because of the minor crop status of pomegranates, pesticides authorized and registered available for pest control on pomegranates in Spain are very scarce. Nowadays, Pirimicarb is the only conventional insecticide authorized for aphids control in Spain. Experimental trials with new active ingredients registered in other fruits can show their efficacy and perhaps their use can be extended to pomegranates. New products are being introduced into the market, many of which are safer to humans and the environment than older pesticides. The purpose is to ensure that producers of minor crops have an adequate range of pest control products (both traditional pesticides and biopesticides) in order to competitively produce safe and wholesome agricultural products. Natural enemies can contribute to the reduction of pest populations, but their use was not sufficient to clean up all present aphids and prevent fruit damage in experimental trials in Spain (Bartual *et al.*, 2010). The level of pesticide residues in fresh fruit is considered an

important internal quality parameter. In the case of fruit destined for export, the maximum residue level (MRL) standards for the destination country should be met.

The aim of the present study was to evaluate natural enemies, and the efficacy and residue levels of new generation insecticides and registration exempt products for control of the most important sucking pests of pomegranates in Spain.

II – Material and methods

Studies were conducted during 2009-2010 on mature pomegranate groves located in Elche and Albaterra (Alicante). Aphids and natural enemies were identified and quantified weekly during the crop period. In the experimental orchards, adult trees of 'Mollar de Elche', the most common cultivar in Spain, were sprayed with eleven insecticides in order to study their field efficacy. The pesticides selected for the current study include compounds from various pesticide classes which are authorized for citrus or other fruits in Spain. Aphid control treatments included five insecticides, Acetamiprid, Flonicamid, Imidacloprid, Pimetrozina and Pirimicarb (04/27/2010, Elche plot); of which only Pirimicarb has been approved for use on pomegranates in Spain until now, and three alternative products (04/29/2010, Albaterra plot), neem extract, citrus extract and potassic soap; the last two products from MRL regulations. Mealybug control treatments (07/12/2010, Elche plot) included three insecticides (Chlorpyrifos, Chlorpyrifos-methyl and Fenoxicab). A randomised block design with 12 trees per treatment and three replicates was performed. Each experimental unit had three rows, with 4 trees per row, with at least one unsprayed bay between plots. A motorised mist was used to apply from 2 litres (for aphid control) to 3 litres (for mealybug control) of spray per tree (1000-1500 litres/ha) respectively, so that the spray on the fruit was at the point of runoff. A control with only water spray was maintained for comparison. At all application times fruit were dry at the start of spraying, there was no wind, and temperatures were between 18 and 23°C. The number of nymphs and adult aphids was counted from twenty terminals shoots, twenty flowers and twenty pieces of fruit in six trees per treatment. No rain fell for at least 2 days after application for all trials. An analysis of variance of angular transformed values was used to determine significance levels of mortality between treatments.

Eight fruit per plot were sampled for analysis of residue remaining on the fruit. For decline residue levels analysis of Chlorpyrifos, Chlorpyrifos-methyl and Fenoxicab, residue levels were calculated at 0, 7, 14, 21 and 28 days after spraying (09/04/2010). Evaluation of insecticide residue levels in ripening fruit was performed by Agri-Food Laboratory of 'Conselleria de Agricultura' (CAPA), using established and validated methods. Multi-residue analysis employed high performance liquid chromatography (HPLC/MS/MS) for Acetamiprid, Flonicamid, Fenoxicab, Imidacloprid, and Pimetrozina, and gas chromatography with mass spectrometric detection was used (GC/MS/MS) for Pirimicarb, Chlorpyrifos, and Chlorpyrifos-methyl. Instrumental limits of quantification (LOQ) for these active ingredients are 0.01.

III – Results and discussion

1. Aphids

Adults and nymphs of *Aphis gossypii* and *Aphis punicae*, were monitored during the assay, as well as a few individuals of *Aphis spiraecola*. The peak activity of pomegranate aphids was observed during the second fortnight of April. At first, *Aphis gossypii* appears in shoots and floral buds and causes different kinds of damage during April, whereas *Aphis punicae* appears in May and June and affects flowers and fruits. Aphidiids (Aphidiidae) parasitoids were recorded within the samples of *Aphis gossypii* and *Aphis punicae*. Other natural enemies of pests monitored were large lady beetles and *Scymnus* spp. (Coccinellidae), syrphid maggots (Syrphidae), green

lacewings (Chrysopidae) and *Aphidoletes aphidimyza* (Cecidomyiidae). Biocontrol contributed to reduce the pest populations but it was not sufficient to clean up all present aphids, and fruit damage was recorded in some untreated control areas. Ants aggravated this situation because they protected the aphids from their natural enemies.

At the Elche experimental plot, all insecticide treatments gave significantly ($P < 0.05$) higher levels of protection as compared to untreated fruit (Table 1). Higher efficacy was shown by Imidacloprid, Acetamiprid and Flonicamid at 7, 14 and 21 days after spraying (04/27/2010).

Table 1. Efficacy of different treatments on pomegranate aphids, *Aphis punicae* Passerini and *Aphis gossypii* Glover in Elche plot. Application date 04/27/2010

Treatment	Active ingredient	Concentration	Dose per 100 litres	Per cent reduction in aphid population			% fruit damaged by stains or sooty mould
				DAT 7	DAT 14	DAT 21	
T1	Pirimicarb	50 %	60 ml	64.12b	26.25b	27.52b	70
T2	Imidacloprid	20 %	60 ml	97.32a	88.75a	57.54a	4
T3	Acetamiprid	20 %	30 g	95.41a	79.58a	67.11a	8
T4	Pimetrozina	50%	40 g	63.33b	24.59b	6.32bc	72
T5	Flonicamid	50 %	13 g	98.25a	82.53a	50.33ab	11
T6	Untreated control	---	---	3.11c	2.52c	5.22c	90

Evaluation dates: 4 May (7 DAT), 11 May (14 DAT), and 18 May (21 DAT). Angular transformed values were used to determine significance levels of mortality between treatments. Means followed by same letter within columns are not significantly different (95%, test LSD).

At the Albaterra experimental plot (04/29/2010 with exempt products), moderately effective control was achieved with soap, citrus oil and neem, however a citrus extract and soap combination showed significantly different results, reaching more than 68% efficacy (Table 2). The relative importance of major variables such as sunlight, humidity, temperature or water pH range, may influence these results.

Table 2. Efficacy of different treatments on pomegranate aphids, *Aphis punicae* Passerini and *Aphis gossypii* Glover in Albaterra plot. Application date 04/29/2010

Treatment	Product treatment	Conc.	Dose per 100 litres	Per cent reduction in aphid population			% fruit damaged by stains or sooty mould
				DAT 7	DAT 14	DAT 21	
T7	Potassic Soap	ND	200 ml	24.32 bc	41.25 b	9.52 cd	48
T8	Citrus extract	ND	300 ml	36.15 b	52.74 b	36.34 bc	58
T9	Neem oil (azadirachtin)	3.2 %	100 ml	27.85 bc	53.49 b	13.33 cd	86
T10	Citrus extract + Soap	ND	300+200 ml	68.20 a	71.21 a	74.14 a	17
T11	Untreated control	---	---	16.52 c	20.88 c	7.41 d	92

Evaluation dates: 6 May (7 DAT), 13 May (14 DAT), and 20 May (21 DAT). Angular transformed values were used to determine significance levels of mortality between treatments. Means followed by same letter within columns are not significantly different (95%, test LSD).

The current results suggest that the combination of citrus extract and soap works more effectively than the use of each of them separately against the aphids, but more than two

applications may be necessary. Pesticide residues were not detected (Table 3) in fruit after harvest (10/07/2010).

Table 3. Pesticide residue levels (mg/kg) in harvested pomegranates (10/07/2010)

Insecticides	Classification	Dosage (g ai ha ⁻¹)	MRLs in EU	Residue (mg.kg ⁻¹)
Neem extract	Azadirachtin	32	0.01 [†]	<LOQ
Acetamiprid	Nicotinoids	60	0.01 [†]	<LOQ
Fonicamid	Pyridine	65	0.05 [†]	<LOQ
Imidacloprid	Nicotinoids	120	1	<LOQ
Pirimicarb	Carbamate	300	1	<LOQ
Pymetrozine	Not Established	200	0.02 [†]	<LOQ

[†]Indicates lower limit of analytical determination. Pesticides Web Version - EU MRLs (08/19/2011) Instrumental limit of quantification (LOQ) is 0.01. Treatment 04/27/2010.

2. Mealybug

The number of mealybug infested fruitS following application of pesticides was not significantly different from the untreated fruit 7 days after application (data not shown) due to the low level of the pest population in some experimental units. This occurrence confirms the recommendation made in Integrated Pest Management, that treatments should be applied only on those areas of the orchard with incidence of mealybug if they are well defined.

Insecticide residue decay levels are presented for each trial in Table 4. For the three formulations sprayed for mealy bug control, the dissipation of methyl-chlorpyrifos in pomegranates was faster than Chlorpyrifos and Fenoxicarb. The residues were long lasting, with a slow degradation rate. On ripening day (10/07/2010), only Fenoxicarb residues were detected in the marketable fruit. No samples contained residues that exceeded the MRLs set by the European Union (Table 4).

Table 4. Pesticide residue levels (mg/kg) in pomegranates at 0, 7, 14, 21 and 28 days after spraying (09/04/2010)

Insecticides	Dosage (g ai ha ⁻¹)	MRLs in EU	Residue (mg.kg ⁻¹)				
			DAT 0	DAT 7	DAT 14	DAT 21	DAT 28
Chlorpyrifos	1440	0.05 [†]	0.20	0.06	0.02	<LOQ	<LOQ
Chlorpyrifos-methyl	1344	0.05 [†]	0.17	0.02	<LOQ	<LOQ	<LOQ
Fenoxycarb	150	0.05 [†]	0.11	0.04	0.03	0.03	0.02

[†]Indicates lower limit of analytical determination. Pesticides Web Version - EU MRLs (08/19/2011). Instrumental limit of quantification (LOQ) is 0.01.

IV – Conclusions

The results obtained in this trial have given us information about the natural enemies of aphids found in the SE Spanish pomegranate orchards. However, the untreated trial areas showed an elevated percentage of fruit damage from aphids and mealybugs, which could necessitate the use of phytosanitary treatments, especially those which respect auxiliary fauna.

To increase the availability of pest control tools to pomegranate growers, it seems appropriate to authorize some of the newer generation products used in this trial (Imidacloprid, Flonicamid, Acetamiprid) which have proven more effective than Pirimicarb. Significantly effective results have been obtained with the combination of citrus extract and potassium soap. This application could be useful in organic orchards or to obtain lower fruit residues in the event that more than one pest control treatment is required. Among the traditional products used against mealybug, metal clorpirifos demonstrated a more rapid dissipation curve.

New pest control assays must be performed in pomegranate. Priority must be given to the use of biological control methods, alternative pesticides and newer active ingredients especially those which respect beneficiary insects.

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