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Research Paper

Evaluation of four organically-acceptable insecticides against mealy aphids of the *Hyalopterus pruni* complex in almond orchard

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Abstract. Four organically-farming allowed insecticides, Neem extract (Oleorgan at 30%), Rotenone extract (Rotorgan at 5%) and two products of Potassium salt of fatty acid (Kabon at 50% and Biosoap at 49%), were field tested for the control of mealy aphids *Hyalopterus pruni* complex (Homoptera: Aphidoidea) in almond orchard in 2008 and 2009 in Tunisia. The experiment was arranged in a randomized block design consisting of 5 replications each consisted of 2 trees. The substances were applied three times in 2008 (on 8 April, on 14 April and on 6 May) and twice in 2009 (on 27 March and 10 April). In 2008, a significant decrease in densities of aphids per leaf (small larva, medium larvae and apterous adults), 6 days after the first treatment compared with control. These densities remain low during 5 weeks thereafter. The effectiveness of these products was confirmed in 2009 trial in comparison with control, however, the highest aphid population suppression was found with the application of Neem followed by Rotenone, while the lowest population suppression was found with Kabon and Biosoap.

Keywords: Botanical insecticides, *Hyalopterus pruni* species complex, almond, organic agriculture.

INTRODUCTION

Almond, *Prunus amygdalus* is one of the most widely cultivated stone fruits in Tunisia. Under organic agriculture, the species occupies the third place after olive tree and date palm in term of acreages (CTAB, 2013). Many species of insect pests attack almond particularly bark beetles and aphids. The most damaging aphid species attacking almond in the Mediterranean region are: *Myzus persicae*, *Brachycaudus amygdalinus*, *Hyalopterus amygdali* and *Hyalopterus pruni* species complex (Barbagallo et al., 2007).

The mealy aphids of the *Hyalopterus pruni* complex are according to Basky and Szalay-Marsz, (1987) composed of two morphologically undistinguished species *Hyalopterus pruni* (Geoffroy) and *Hyalopterus amygdali* (Blanchard). The main primary hosts of *H. pruni* are plum, *Prunus domestica* L., and apricot, *Prunus armeniaca* L., while peach, *Prunus persica* L., and almond, *Prunus dulcis* (Mill.), are colonized by *H. amygdali.* Jerraya (1997) cited *H. pruni* as the main aphid species attacking almond and peach in the north of Tunisia. We refer here as mealy aphids of the *Hyalopterus pruni* complex either *H. pruni* or *H. amygdali* attacking almond because we cannot differentiate species morphologically. The mealy aphids of the *Hyalopterus pruni* complex produce a bisexual generation on various *Prunus* species (Rosaceae) (primary hosts) in autumn, which alternates with several unisexual (all females) generations produced parthenogenetically on *Prunus* species in spring and on secondary hosts, such as *Phragmites communis* Trin. and *Arundo donax* L. (Poaceae) during summer. The plum mealy aphid, *Hyalopterus pruni complex* forms colonies on the lower leaf surface, on twigs and even on

Figure 1. Colonies of *Hyalopterus pruni* complex on the underside of almond leaves (Jammel locality April 3, 2007).

Figure 2. Colonies of *Hyalopterus pruni* complex on the branch of almond (Jammel locality April 10, 2007).

branches (Figures 1 and 2) feeding on sap and cellular juice. This species produces honeydew on which sooty-mould grows. As a consequence the physiological processes (photosynthesis, transpiration, respiration) are seriously affected due to premature leaf drop.

This study was conducted to evaluate some eco-friendly substances/chemicals against the mealy plum aphid *Hylaopterus pruni* species complex. Thus, Neem and Rotenone extracts along with Insecticidal Soaps and light mineral oils which are known for their use against aphids (Perring et al., 1999; Edelson et al., 2002), as alternatives to chemicals insecticides in a certified organic

Table 1. Characteristics of the tested products and doses used.

Product names	Active ingredients	Doses used	Companies
Kabon	Potassium soap (50%)	400 ml/100 L water	Atlantica Agricola (Spain)
Biosoap	Potassium salt of fatty acid (49%)	250 ml/100 L water	Koppert
Oleargan	Neem extract (30%)	100 ml/100 L water	Atlantica Agricola (Spain)
Rotorgan	Rotenone extract (5%)	250 ml/100 L water	Atlantica Agricola (Spain)

almond orchard were tested.

MATERIALS AND METHODS

The study was conducted in a certified organic almond belonging to « Centre de formation Agricole de Jammel » in the centre-East of Tunisia (35°38' latitude North, 10°41'longitude East) at an elevation about 20 m above sea level. The area is characterized by a typical Mediterranean climate with hot dry summers and mild winters with an annual rainfall of about 350 mm. Rains are primarily between November and April. A randomized block design was used with five replications and an untreated control. Each treatment includes 2 neighboring trees. Sprays were undertaken in the year 2008 on April 8, April 14 and May 6 and on March 27 and 10 April in 2009. The active ingredients, commercial names and doses of bio-insecticides are presented in Table 1. All treatments were applied with an 18-L backpack sprayer. Every tree received on average 2 L of solution.

Sampling method

Before the first spray, 100 leaves were sampled from about 20 almond trees, transported to the laboratory in plastic bags for further assessment.

After sprays, two almond twigs (10 to 20 cm in length) were sampled per cardinal point (North, South, East, West and Center) for a total of 20 twigs per treatment. Leaves were examined in the laboratory using a stereomicroscope to count the aphid morphs presents (young larvae, medium larvae and apterous adults).

Statistical analysis

The aphid infestation scores were analyzed by a one way analysis of variance (ANOVA) using the Minitab Version 13.0 statistical package. The least significant difference was calculated when values were significantly different ($P < 0.05$).

RESULTS

In both study years, the aphid populations of *H. pruni* from late March to May culminating in April are presented in Figure 3.

Trials conducted in 2008

Just before sprays, the mean number of live aphids(small larvae, medium larvae and apterous adults) per leaf is the same for all treatments (28 aphids/leaf; Figure 1). Six days after the first spray (14 April 2008), the densities of aphid per leaf show significant difference between treated plots and control for all biological stages (small larvae, medium larvae and wingless adults) (One way ANOVA $P < 0.05$, Table 2). The population of aphid in the control plots is two to three-fold more important (Table 2).

Seven days after the second spray and 14 days after the first spray, the aphid population in the control plots remains high compared with plots treated with bio-insecticides particularly small and medium larvae (ANOVA, 1 factor $P < 0.05$, Table 3).

The same difference was observed 14 days following the second bio-insecticide sprays (28 April) and 22 days after (ANOVA, 1 factor $P<0.05$, Tables 4 and 5). However, after the third spray there was no significant difference on aphid densities between treated plots and untreated plots (Table 6).

Overall, there were significant decreases in *H. pruni* species complex densities (small larva, medium larvae and apterous adults) in treated plots for about 5 weeks following sprays. Globally, the tested products show similar efficacy with a minor advantage of Neem extract (Tables 4 to 6).

Trial conducted in 2009

Three days before the first spray (on March 24, 2009), the mean number of aphid (larvae and apterous adults) was 5.17 (Figure 3). Four days following the first spray (on 31 March 2009), the density of aphid did not significantly varies ($F_{4,20} = 1.70$ $P = 0.18$) showing variations in which plots treated with Kabon were less infested (Table 7a); this situation changed thereafter where infestation increases drastically almost 1 moth after sprays (Table 6).

The effectiveness of these products was confirmed in 2009 trial in comparison with control, however, the highest aphid population suppression was found with the

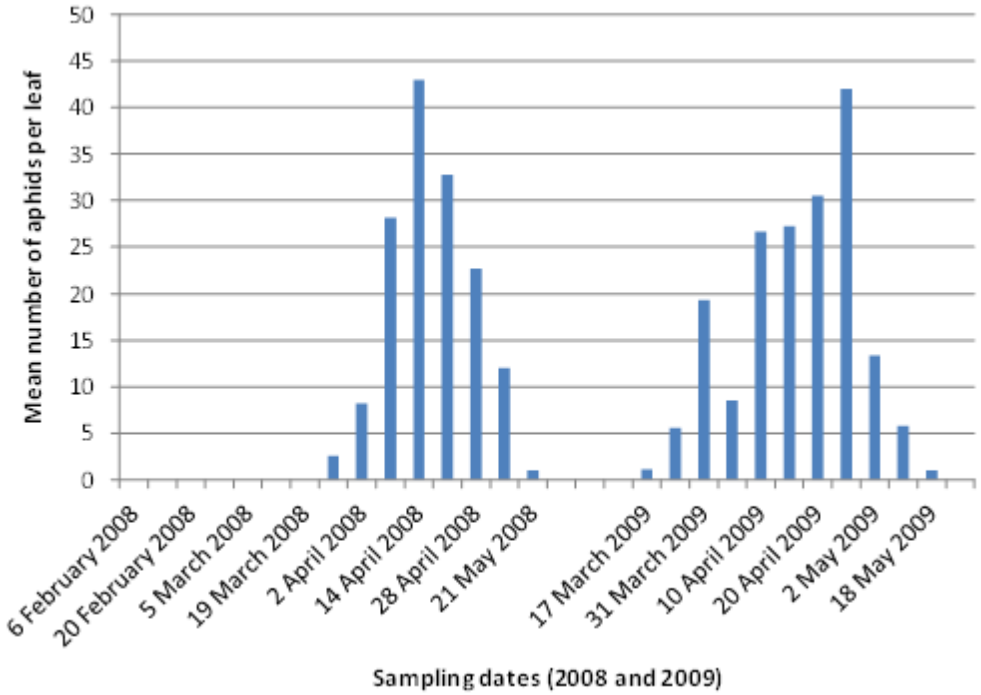

Figure 3. *Hyalopterus pruni* species complex population dynamics in almond orchard in 2008 and 2009. Mean of 100 leaves sampled in the orchard.

Table 2. Mean number of aphids (small larvae, medium larvae and apterous adults) 6 days following the first spray in 2008.

Products	S. larvae*	M. larvae*	Apterous adults*	Total*
Neem	7.5^{b}	3.62^{b}	0.87^{b}	11.99^{b}
Rotenone	6.4^{b}	2.80^{b}	0.86^{b}	10.06^{b}
Biosoap	7.8^{b}	4.82^{b}	1.22^{b}	13.84^{b}
Kabon	10.5^{b}	3.11^{b}	0.53^{b}	14.14^{b}
Control	19.6^{a}	9.38^{a}	2.16^{a}	31.14^{a}

Small larvae $F_{4,75} = 5.5$; $P < 0.05$. Medium larvae $F_{4,75} = 8.3$; $P < 0.05$. Apterous adults $F_{4,75} = 7.35$; $P < 0.05$. Total $F_{4,75} = 6.5$; $P < 0.05$. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

Table 3. Mean number of aphids (small larvae, medium larvae and apterous adults), 14 days following the first spray and 7 days after the second spray in 2008.

Products	S. larvae*	M. larvae*	Apterous adults*	Total*
Neem	0.59^{a}	0.70^{a}	0.17^{a}	1.46^{a}
Rotenone	1.03^{a}	1.27^{a}	0.38^{a}	2.58^{a}
Biosoap	1.05^{a}	2.10^{a}	0.46^{a}	3.61^{a}
Kabon	1.74^{a}	0.79^{a}	0.47^{a}	3^{a}
Control	4.47^{b}	6.3^{b}	1.79^{b}	12.56^{b}

Small larvae $F_{4,75} = 5.5$; $P < 0.05$ Medium larvae $F_{4,75} = 29.9$; $P < 0.05$. Apterous adults $F_{4,75} = 8.26$; $P < 0.05$. Total $F_{4,75} = 11.5$; $P < 0.05$. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

application of Neem extract (Oleorgan) followed by Rotenone (Rotorgan), while the lowest population suppression was found with Kabon and Biosoap (Table 7a). Ten days after the second spray (10DAT2) there is a significant difference on aphid infestation between treated and control plots, in which plots treated with Neem extract

Table 4. Mean number of aphids (small larvae, medium larvae and apterous adults) 14 days following the second spray in 2008.

Products	S. larvae*	M. larvae*	Apterous adults*	Total*
Neem	4.91	4.6[a]	0.72[a]	10.23[a]
Rotenone	1.77[a]	2.0[a]	0.77[a]	4.54[a]
Biosoap	2.42[a]	3.90[a]	0.99[a]	7.31[a]
Kabon	1.95[a]	1.0[a]	0.31[a]	3.26[a]
Control	8.02[b]	7.03[b]	1.1[a]	16.15[b]

Small larvae $F_{4,75} = 2.76$; $P<0.05$ Medium larvae $F_{4,75} = 12.45$; $P < 0.05$ Apterous adults $F_{4,75} = 2.30$; $P= 0.32$; Total $F_{4,75} = 11.5$; $P < 0.05$. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

Table 5. Mean number of aphids (small larvae, medium larvae and apterous adults) 22 days following the second spray in 2008.

Products	S. larvae*	M. larvae*	Apterous adults*	Total*
Neem	4.91[ab]	4.6[a]	0.73[a]	10.24[a]
Rotenone	1.44[a]	1.7[a]	0.68[a]	3.82[a]
Biosoap	2.42[a]	4.0[a]	1.04[a]	7.46[a]
Kabon	1.91[a]	1[a]	0.31[a]	3.22[a]
Control	8.02[b]	9.7[b]	0.48[a]	18.2[b]

Small larvae $F_{4,75} = 29.85$; $P < 0.05$. Medium larvae $F_{4,75} = 17.36$; $P < 0.05$. Apterous adults $F_{4,75} = 1.26$; $P = 0.11$ Total $F_{4,75} = 10.5$; $P < 0.05$. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

Table 6. Mean number of aphids (small larvae, medium larvae and apterous adults), 8 days following the third spray in 2008.

Products	S. larvae*	M. larvae*	Apterous adults*	Total*
Neem	0.13[a]	0.01[a]	0.01[a]	0.15[a]
Rotenone	0.06[a]	0.03[a]	0.01[a]	0.1[a]
Biosoap	0[a]	0[a]	0[a]	0[a]
Kabon	0[a]	0[a]	0[a]	0[a]
Control	0.5[a]	0[a]	0[a]	0.5[a]

Small larvae $F_{4,75} = 0.81$; $P = 0.52$ Medium larvae $F_{4,75} = 0.85$; $P = 0.49$ Apterous adults $F_{4,75} = 1.29$; $P = 0.28$ Total $F_{4,75} = 0.94$; $P = 0.69$. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

harbored the low aphid densities. Twenty four days after the second spray Neem, Biosoap and Rotenone plots were less infested than control or Kabon treated plots (Table 7b).

DISCUSSION

Our results show good efficacy of plant extracts particularly Neem and Rotenone in the reduction of aphid infestation in Almond orchards. Potassium salts performed less than Neem or Rotenone. Also, Diarisso et al. (2005) reported that spraying extracts from Neem seed (*Azadirachta indica*) could effectively control aphids in Sorghum. Moreover, the transmission of viruses by aphids may be reduced by Neem applications. Transmission of potato virus Y to sweet pepper by the green peach aphid, *Myzus persicae* (Sulzer), was inhibited by foliar applications of 1.0 or 2.0% Neem seed oil to infected source plants or to uninfected recipient plants (Lowery et al., 1997). Ochieng and Nderitu (2011) tested two soft products (Neem extract and dish washing soap teepol) and hard product (Karaté) to control the peach aphid, *Myzus persicae* in garden peas and demonstrated the effectiveness of Neem and “teepol” suggesting their possible use since they are friendly for

Table 7a. Mean number of aphid per leaf (larvae and apterous adults), days after treatments (DAT).

Products	4DAT1* (1)	7DAT1* (2)	14DAT1* (3)	10DAT2* (4)
Neem	8.22[a]	7.64[a]	18.12[a]	4.22[a]
Rotenone	14.32[a]	9.72[a]	17.70[a]	22.82[b]
Biosoap	5.60[a]	9.56[a]	35.44[a]	23.56[b]
Kabon	1.96[a]	8.16[a]	41.06[a]	40.42[b]
Control	7.40[a]	7.48[a]	19[a]	45.32[b]
Statistical test	$F_{4,20}$ = 1.70 P = 0.189	$F_{4,20}$ = 0.06 P = 0.99	$F_{4,20}$ = 0.89 P = 0.49	$F_{4,20}$ = 3 P = 0.041

(1) On 31 March 2009; (2) On 7 April 2009; (3)On 10 April 2009 (7DAT2); (4)On 17 April 2009.*Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

Table 7b. Mean number of aphid per leaf (larvae and apterous adults), days after sprays (DAT).

Product	13DAT2*(5)	20DAT2*(6)	24DAT2*(7)	33DAT2*(8)
Neem	24.28[a]	35.40[a]	9.24[a]	0.08[a]
Rotenone	37.04[a]	20.82[a]	3.94[a]	1.82[a]
Biosoap	30.78[a]	58.18[a]	3.60[a]	0.36[a]
Kabon	31.18[a]	25.84[a]	18.26[ab]	0.90[a]
Control	29.42[a]	69.86[a]	31.7[b]	0.94[a]
Statistical test	$F_{4,20}$ = 0.37 P = 0.82	$F_{4,20}$ = 2.66 P = 0.06	$F_{4,20}$ = 3.89 P = 0.015	$F_{4,20}$ = 1.03 P = 0.49

(5) On 20 April 2009; (6) On 27 April 2009; (7) On 2 May 2009; (8) On 11 May 2009. *Means in the same column with the same letter were not significantly different (Test F followed by the Least Significant Difference Test).

aphid parasitoids.

Azadirachtin, a tetranortriterpenoid isolated from the seeds of Neem tree, *Azadirachta indica* (Meliaceae), acts as an antifeedant and inhibits the growth and the development of several insects (Meisner et al., 1981; Raffa, 1987). The compound is effective against several insects (Mordue and Blackwell, 1993). Rotenone has been reported to be an excellent insecticide against a wide range of insect pests. Davidson (1930) found that rotenone was a toxic and effective contact insecticide against several species of whiteflies, aphids, caterpillars and mites. However the use of rotenone is limited to organic food production. In California (USA), about 200 kg are used annually, mostly on lettuce and tomato crops (Isman, 2006). Rotenone is widely used in organic olive farming for the control of *Bactrocera oleae* (FAO, 2010).

Insecticide soaps (Kabon, Biosoap) are made from potassium salts of various fatty acids killing insects that are hit by sprays (Nielsen, 1990). They are classified as least toxic chemicals since they have non-persistent residues.

We did not evaluate the activity of aphid natural enemies (particularly lady birds, lacewings and the parasitoid *Aphidius transcaspicus*) in the orchard before and after bio-insecticide applications, but we consider that their toxicity is lower compared with broad spectrum insecticides. Nevertheless, while organically-approved insecticides may have a low toxicity to beneficial insects, they are not necessarily completely benign. Lo and Blank (1992) tested mineral oils on the predation of scale insects by the steel blue ladybird *Halmus chalybeus.* Although no ladybirds were killed, the oil did disrupt their feeding.

Several natural enemies attack *Hyalopterus pruni*, including the eleven spotted lady bird *Coccinella undecimpunctata*. We noticed that the population of this ladybird was numerous at the trial site in May, but was still scarce in April when aphid damage was already apparent and abundant (Figure 3). Increases in the population of lady birds and aphids were not sufficiently well synchronized to prevent aphid outbreaks. Alternatives to chemical insecticides, neem, rotenone oils or soaps and despite of their lower toxicity can be effective if they were more compatible with natural enemies. An organic insecticide cannot be fully recommended for use against *H. pruni* until its compatibility

with natural enemies such as eleven-spotted ladybirds and the parasitoid *A. transcaspicus*.

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