SUSCEPTIBILITY OF APHID PARASITOID *Aphelinus mali* (Haldeman) TO DIFFERENT ACTIVE INGREDIENTS COMMONLY USED IN ORGANIC APPLE ORCHARDS IN TRENTINO (NORTH ITALY)

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ABSTRACT

Aphelinus mali (Haldeman) is the most important biological control agent of woolly apple aphid (WAA) *Eriosoma lanigerum* (Hausmann), a serious pest of apples. The amount of products used in apple orchards could compromise the effectiveness of *A. mali* on its biological control. The rate of parasitism reaches high levels (80-90%) in late summer when part of the damage has already been caused. In Trentino region (Italy) the first peak of flight of *A. mali* is in the post-flowering period, although limited in size and is essential for an exponential flight increase and the consequent culmination of the parasitisation ratio in summer, generally at the end of July. It is essential to avoid pesticides toxic to *A. mali* after flowering but also other active substances that can reduce *A. mali* activity. For these reasons, we tested several plant protection products to evaluate potential compatibility in integrated and organic pest management programs. The direct (topical sprays in Potter's Tower) and residual (leaf residue) effect of insecticides and fungicides on the adult stage of this parasitoid were investigated under laboratory conditions. The active ingredients used in trials were deltamethrin (as a chemical referent), azadirachtin, sulphur and Lime Sulphur. The products were tested at recommended field rates. A treatment with water was used as control in both experimental trials. The insects' mortality rate on direct effect in Potter's Tower was different only for the deltamethrin at different control timing. Deltamethrin caused the highest mortality also in residual effect than the other products. After 3 and 7 days from the treatment no product were caused mortality.

Key words: Aphelinus mali, susceptibility, organic apple orchards, treatment, beneficials

1 INTRODUCTION

In the last few years, one of the most worrying pests for apple production in the Southern Alps, in Trentino region (Italy), is the woolly apple aphid (WAA), *Eriosoma lanigerum* Hausmann (Hemiptera: Aphididae). This pest is one of the economically important and most widely distributed pests of apple, *Malus domestica* (Borkh.). A native of North America (Baker, 1915), it now occurs throughout the apple growing

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countries of the world (Eastop, 1966). Both nymphs and adults cause damage to apples by feeding on the roots and stems, particularly the tender places on the trunk and branches, new lateral growth and areas with damage caused by mechanical injury (Asante, 1995). Infestation of the aphid on apple roots and shoots has been shown to reduce survival of nursery trees and to weaken mature apple trees, leading to the loss of vitality and poor quantitative and qualitative yields caused also by the honeydew drips on fruit (Sherbakoff & McClintock, 1935; Klimstra & Rock, 1985). A great control of the WAA population is made by the parasitoid *Aphelinus mali* Haldeman. In the climatic conditions of northern Italy, the cool spring temperatures support the activity of the host over that of the parasitoid, making the parasitisation process slower (Bonnemaison, 1965; Evenhuis, 1958; Mueller *et al*, 1992; Trimble *et al.*, 1990). The rate of parasitism reaches high levels (80-90%) in late summer when part of the damage has already been caused. In Trentino the first peak of flight of *A. mali* is in the post-flowering period, although limited in size, is essential for an exponential flight increase and the consequent culmination of the parasitisation ratio in summer, generally at the end of July (Chiesa *et al.*, 2019). For this reason, it is important to avoid pesticides toxic to *A. mali* after flowering (Blommers, 1991) but also other active substances that can reduce *A. mali* activity. Some plant protect product such as lime sulphur and sulphur, mainly knowns for their fungicidal action (Abbott, 1945; Tweedy, 1967; Smilanick & Sorenson , 2001; Holb *et al.*, 2003; Montag *et al.*, 2005; Navarro & Méndez, 2014) can caused effect also as an acaricide and insecticide (Guerra, 1985; Penteado, 2000; Guirado, 2001; Soto-Giraldo *et al.*, 2013; Cabrera-Marulanda *et al.*, 2018; Di *et al.*, 2016; Civolani *et al.*, 2023) and are also accepted by most organic certifiers (FederBio, Italian input list). Furthermore, many conventional broad-spectrum insecticides are extremely toxic to non-target organisms, especially natural enemies. Parasitic Hymenoptera often are far more susceptible to insecticides than their hosts (Baker *et al.*, 1995). Therefore, the effects of pesticides and fungicides commonly used in organic and integrated apple orchards in Trentino on the parasitoid, *A. mali*, were examined under controlled conditions.

2 MATERIAL AND METHODS

2.1 Insects

Shoots with parasitized WAA mummies were collected from uncultivated apple orchards (cv. Fuji) in Trentino. The infested shoots were positioned in an insect rearing cage to guarantee emerging adults of parasitoids. The newly emerged females were removed using an aspirator, kept inside small glass tubes and used, on the same day, in the bioassays. Collections were made at different times during summer as parasitoids became available.

2.2 Insecticides and Fungicides

The complete list of products tested are presented in Table 1.

Table 1: List of products, active ingredients and field dose applicated on trials.

Product	Active ingredient	Manufacturer	Conc. ingredient	Form.	Field rate (g or mL/hL)
Decis® Evo	Deltamethrin	Bayer	25 g/L	EW	50 mL
Oikos®	Azadirachtin	Sipcam	26 g/L	EC	100 mL
Polisolfuro di calcio Polisenio®	Lime Sulphur	Polisenio	380 g/L	L	1200 g
Thiopron®	Sulphur	UPL	825 g/L	SC	500 mL

Deltamethrin was used as a referent chemical active ingredient and water as a control treatment.

2.3 Residual effects (leaf residues)

A common experimental protocol was followed for all the toxicity tests. Five apple plants per each product were sprayed following recommended field rates. Controls were sprayed with water only. The products were applied using a cap back sprayer until dripping. The treated leaves were air dried for about 1 h. A treated and dried shoot was placed in a plastic box (9 cm diameter) and provisioned with honey drops. The parasitoids to be tested were transferred to the plastic box where they were exposed to 3-4 treated leaves. All adults were monitored to determine the survival rate at each treatment at 2, 24 and 48. The initial bioassay conducted immediately after spraying is designated as 0 d post spray (T+0). To measure residual effects of the insecticides, new sets of shoots were excised from the potted plants at 3 and 7 days after spraying (named T+3 and T+7 respectively) and presented to new sets of test insects. Each experiment had a control check consisting of parasitoids exposed to untreated shoots. Each plastic box with shoot and parasitoids were covered with gauze to guarantee an adequate aeration. All boxes were kept in a controlled temperature environment [24 ± 2ºC, 70 ± 5% RH, 12:12 (L:D) h]. Treatments were arranged with 5 replicates, each replicate consisting of 10 insects.

2.4 Direct spraying effects (topical sprays)

The chemicals tested for their effects on *A. mali* were applied at locally-registered field rates diluted with distilled water (Nel *et al.,* 1999) using a Potter's spray tower (Potter, 1952). The newly emerged adults of *A. mali* were placed into plastic Petri dishes (5.5 cm diameter x 1.0 cm) in groups of five for topical spray treatments. The tower was calibrated to deliver the smallest possible droplet size to achieve complete coverage of the treated area. The adults in control groups (n = 25) were sprayed with 3 ml of distilled water and in treatment groups (n = 25) were sprayed with 3 ml aqueous solution of the chemicals. Following these treatments, individual adults were immediately positioned for 1 hour for air drying and after were isolated in an individual plastic box and provisioned with honey drops. All adults were monitored to determine the survival rate at each treatment at 2, 24 and 48 hours.

2.5 Data analysis

The mortality value was corrected for control mortality using Abbott's formula (Abbott, 1925). To establish the degree of statistical correlation between the different products and the mortality frequency, the odds ratio (OR, R epitools package (95%)) was used. All the products were compared respect with the control (untreated). If the value of OR=1 the parameter under examination (product) is considered to have no influence on the mortality. If the OR >1 it is considered a factor implicated in the onset of the mortality; the other hand, an OR<1 the factor analysed is considered a protective factor.

3 RESULTS

Results were reported in following tables.

3.1 Residual effects

Table 2: Laboratory results on resudual effect, the control's mortality in residual effects trials was always lower than 10%.

Active ingredient	Timing	Corrected percentage mortality (%)		
		2 hours	24 hours	48 hours
Deltamethrin	T+0	2	22	30
Azadirachtin		0	0	2
Lime Sulphur		0	4	8
Sulphur		0	0	8
Deltamethrin	T+3	0	6	10
Azadirachtin		2	4	4
Lime Sulphur		0	2	2
Sulphur		6	10	16
Deltamethrin	T+7	0	8	8
Azadirachtin		0	0	2
Lime Sulphur		2	2	4
Sulphur		0	4	6

3.2 Direct spraying effects (topical sprays)

Table 3: Laboratory results on resudual effect, the control's mortality in direct effects trials was always lower than 5%.

Active ingredient	**Corrected percentage mortality (%)**		
	2 hours	**2 hours**	**48 hours**
Deltamethrin	100	100	100
Azadirachtin	4	12	16
Lime Sulphur	4	16	16
Sulphur	2	2	18

The Figure 1 showed the results obtained with the statistical test Odd Ratio (OR).
At the timing T+0 was showed the higher OR differences between treatment and the control. Deltamethrin was found to be implicated in the A. mali mortality sill at 2 hours after the direct treatment and after 24 for residual effect. Also, the Sulphur with direct application was found to be implicated in A. mali mortality after 48 hours.
At the same time in residual trials, Sulphur showed a OR value >1 for this reason is considered a factor implicated in the onset of the mortality.
The residual effect at 7 days from the treatment (T+7) no products were implicated in the *A. mali* mortality (OR=0).

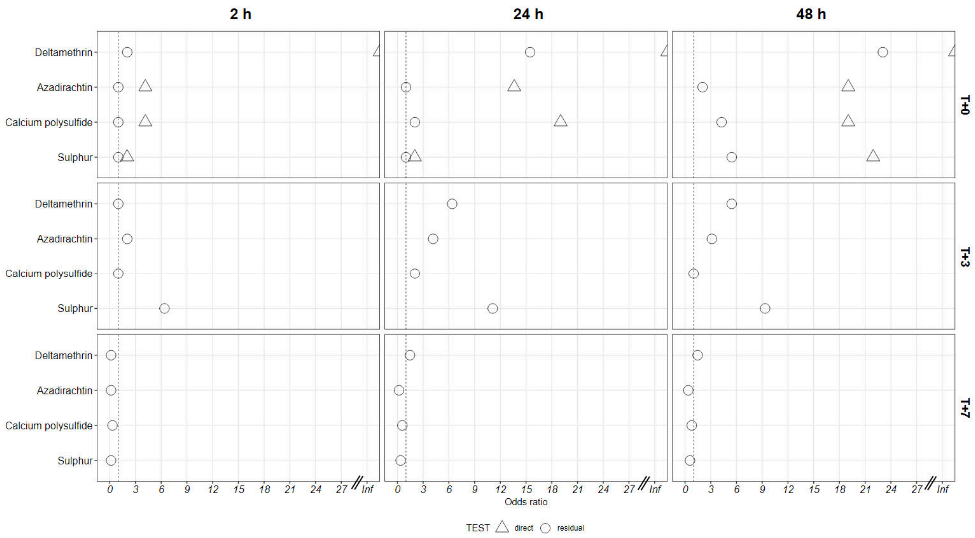

Figure 1: Odds Ratio: OR=1 the parameter under examination (product) is considered to have no influence on the mortality; OR >1 it is considered a factor implicated in the onset of the mortality; OR<1 the factor analysed is considered a protective factor.

Table 3: Odds Ratio statistical test, OR value [min and max] and p value only for the thesis with statistical differences.

Timing	Test	Thesis	2 h		24 h		48	
			OR [min-max]	p	OR [min-max]	p	OR [min-max]	p
T+0	Direct	Delta-methrin	Inf [1-Inf]	**0.000**	Inf [1-Inf]	**0.000**	Inf [1-Inf]	**0.000**
	Resi-dual	Delta-methrin	2.04 [0.07-62.23]	0.5	15.47 [1.93-124.30]	**0.001**	23.06 [2.92-182.21]	**0.000**
	Direct	Azadira-chtin	4.17 [0.18-94.76]	0.247	13.64 [0.74-251.15]	**0.013**	19.05 [1.06-341.50]	**0.003**
	Direct	Lime Sulphur	4.17 [0.18-94.76]	0.247	19.05 [1.06-341.50]	**0.003**	19.05 [1.06-341.50]	**0.003**
	Direct	Sulphur	2.04 [0.07-62.23]	0.5	2.04 [0.07-62.23]	0.5	21.95 [1.24-390.09]	**0.001**
T+3	Resi-dual	Sulphur	6.38 [0.31-130.83]	0.121	11.11 [0.59-209.10]	**0.028**	9.33 [1.12-77.70]	**0.017**

4 DISCUSSION AND CONCLUSION

The insects' mortality rate on direct effect in Potter's Tower was different only for the Deltamethrin at different control timing. Even Abbar *et al.,* (2012) showed higher mortality on chemical referent Deltamethrin. Deltamethrin caused the highest mortality in residual effect than the other products, with an 30% of mortality. After 3 and 7 days from the treatment no product were caused mortality.

Sulphur showed a certain residual implication on *A. mali* mortality with time-limited effect. In fact, different study (Tacoli *et al.*, 2020; Gesraha & Ebeid, 2021; Della Nora Cardoso *et al.*, 2021) have shown that Sulphur treatment can caused an insect mortality. No fungicide treatments showed any detrimental effect. Schneider (1958) and Bradley *et al*., (1997) also found most fungicides, except wettable sulphur, to be benign to *A. mali.* Lime sulphur is the most commonly used pesticide for disease control in organic apple production (Tate *et al.* 2000). Furthermore, sulphur compounds are known to be insecticidal and suppress insect populations. Even Rogers *et al.,* (2011) has demonstrated that lime sulphur residues on apple leaves do not appear to be toxic to *A. mali*.

In conclusion, all the product commonly utilized in organic apple orchard not caused *A. mali* adults mortality, showing high selectivity.

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