

## Research Article

# Lower doses of acetamiprid reduce the intensity of *Aphis pomi* (Hem., Aphididae) and benefit their dominant predator *Hippodamia variegata*

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**Abstract:** A few species of aphids are widespread and economically important in Iran's apple orchards. In this study, we looked for economical, environmentally friendly, and efficient management of aphids with particular emphasis on green apple aphid (GAA) *Aphis pomi* (de Geer). We aimed to avoid early-season pesticide applications, avoiding specific applications for aphid control and managing them via applications done against codling moth (CM), *Cydia pomonella* L., choosing suitable insecticide and dose to minimize side effects on prevalent natural enemies. Thus, CM was monitored by pheromone traps from mid-March in an apple orchard of the Agricultural Research Station of the University of Tabriz to determine the time of application based on degree days accumulated from a biofix. The number of GAA, rosy apple aphid (RAA), *Dysaphis plantaginea* Passerini, and their natural enemies were counted separately. It is known that GAA is the predominant aphid, and two species of ladybirds, *Coccinella septempunctata* (L.), and *Hippodamia variegata* (Goeze), are dominant species of the region. Acetamiprid was chosen as an effective insecticide against both CM and aphids. The lethal effects of this compound were studied on different stages of *H. variegata* and the last instar GAA. The orchard was divided into four plots, and each plot was assigned to a treatment including control, label dose (LD), ½ LD, and ¼ LD. Although LD killed 10% more GAA than ¼ LD, the damage intensity was 12-16% higher in the former. Considering economic benefits and reducing side effects on natural enemies, we recommend using ¼ LD of acetamiprid.

**Keywords:** timing, integrated pest management, side effects, natural enemies

## Introduction

Apple is a luxury, economical, and nutritious crop. Iran is among the top 10 apple-producing countries (FAO, 2018). There is a long list of pests that attack this valuable crop throughout the world. Northwestern provinces are the main

apple-producing areas in Iran. The most important pests of apple orchards in this region are the codling moth, *Cydia pomonella* L. (CM) (Lep., Tortricidae), and, several species of aphids, including green apple aphid (GAA), *Aphis pomi* de Geer (Hem., Aphididae), rosy apple aphid (RAA), *Dysaphis plantaginea*

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Passerini (Hem., Aphididae), woolly apple aphid (WAA), *Eriosoma lanigerum* Hausmann (Hem., Pemphigidae) and two-spotted spider mite *Tetranychus urticae* Koch (Acari, Tetranychidae) (Radjabi, 1986; Esmaili, 1991).

Aphids are important species among various pests that attack apple trees because they can cause direct and indirect damage. Moreover, they cause quantitative and qualitative injuries (Blackman and Eastop, 2000). Aphids uptake plant sap, deplete their energy reservoirs, and can cause direct damage in this way. They also reduce photosynthesis in host plants by destroying chloroplasts and produce signs such as chlorosis in leaves (Opfer and McGrath, 2013). Aphids can stop plant growth by removing nutrients and disrupting photosynthesis. They can also transmit plant viruses and cause considerable indirect damage in this way (Blackman and Eastop, 2006, 2007; Shah *et al.*, 2015). Leaf deformity and gall-making by salivary secretions, sticky honeydew secretions on which molds can grow and prevent light utilization by leaves, and physiological disorders are other qualitative damages caused by aphids (Ghosh and Basu, 1995; Alston *et al.*, 2010b). A high level of infestation causes a reduction in the number and area of leaves; as a result, the growth of young trees stops (Madahi and Sahragard 2012; van Emden and Harrington 2017). Death of seedlings and young trees is also expected (Radjabi, 1986). Therefore, heavy injuries and yield losses are observed (Davies *et al.*, 2004; Singh *et al.*, 2004). More than 15 species of aphids attack apple trees, among which, *A. pomi*, *D. plantaginea* and *E. lanigerum* are the most serious (Alston *et al.*, 2010b; Milenkovic *et al.*, 2013). A pre-blossom treatment is very effective against this aphid. Spraying insecticide is justified when 1-2% of branches are infested. However, due to a slower growth rate, *A. pomi* does not cause considerable damage during the flowering stage, and an 8-12% infestation level is considered as its action threshold (van Emden and Harrington, 2017). This gives a chance to tolerate injuries by GAA and allows spraying to coincide with the sprays

for the key pest, codling moth, *C. pomonella* (Radjabi, 1986).

Fortunately, aphids have many natural enemies including parasitoids and predators. Predators are more effective than parasitoids in controlling aphid populations. Among the key natural enemies of apple aphids, ladybirds (Coccinellidae), lacewings (Neuroptera, Chrysopidae), and hoverflies (Dip., Syrphidae) are more important (Radjabi, 1986). This rich fauna of natural enemies provides a high potential for integration in IPM programs that need to be conserved.

Considering the high importance of aphids in apple orchards and the presence of numerous perennial pests such as codling moth, chemical control of the pest complex is unavoidable (Radjabi, 1986). Numerous compounds are used against different pests in apple orchards. Some insecticides are effective against both key pests and sap feeders. Unfortunately, these treatments have undesirable effects on natural enemies (Sarita Gaur, 2007). Acetamiprid is effective against both codling moth and aphids (Milosevic *et al.*, 2012; Vukovic *et al.*, 2016); and is recommended against them in Iran.

On the other hand, aphids have a high capacity for developing resistance to insecticides due to their high growth rate and numerous overlapping generations (Dixon, 1987). The compatibility of insecticides with biocontrol agents is important for pest managers and researchers (Specos *et al.*, 2010). Investigators search for pesticides selective for natural enemies (Francis *et al.*, 2001; Boszic *et al.*, 2002; Khan and Alhewairini, 2019). Low doses often open a selectivity window for natural enemies and can be used to their benefit (van Emden and Peakal, 1996).

In this study, we focused on green apple aphid (GAA) and one of its key natural enemies in the region *Hippodamia variegata* (Goeze) (Col., Coccinellidae). The aims of this study were: 1. To avoid specific applications against aphids; 2. To time applications to control codling moth and aphids by a common application; 3. To find a probable selectivity of acetamiprid (a recommended compound

against both codling moth and GAA) to benefit *H. variegata* by doing laboratory bioassays; and 4. To assess the possible advantage of reduced field doses of the insecticide for the benefit of natural enemies.

## Materials and Methods

### Site of the study

This study was carried out in Khalatpooshan Research Station of the University of Tabriz, located east of Tabriz. The geographical position of the orchard was determined using GPS model Garmin Oregon 650. The coordinates of the place were UTM zone 38 N 622565 4210195 and 1586 m ASL. The area of the orchard was 2132 m<sup>2</sup>. The study was carried out from April 2017 to August 2018.

### Insecticides

Three insecticides were used to spray the apple orchard. Acetamiprid (Mospilan 20 WP, Golsam Co., Gorgan, Iran) was used against the first generation of *C. pomonella* and aphids. Fenprothrin (Danitol® 10 EC, Ariashimi Co., Zahedan, Iran) was used against the second generation of *C. pomonella* and mites. Diflubenzuron (Dimilin® 25 WP, Golsam Co., Gorgan, Iran) was used against the third generation of *C. pomonella*.

### Monitoring of aphids

Monitoring began at bud swell in mid-March. Ten branch tips (15 cm long, seven-leaf) were examined by a handy lens to observe eggs of both green and rosy apple aphids. The soil around the trunk was dug to look for overwintering individuals of wooly apple aphid. After bud break, inspection of branch tips (20 cm each) was continued to find nymphs and adults.

### Monitoring of *Cydia pomonella*

Two delta sticky pheromone traps (Russell IPM-SPI, England), spaced 10 m apart, were placed 1.5 m above ground. Sticky surfaces were renewed once, and pheromone baits were used twice a month. The traps were inspected twice a week at regular intervals. The plants'

phenological stage was also recorded as Flickinger's phenology system (cited by Radjabi, 1986). The temperature was recorded using a max-min thermometer. Whenever, three sequential samples caught male moths and the sunset temperature was > 16 °C, it was regarded as biofix, and temperatures above the threshold of 10 °C, were summed (Assadi *et al.*, 2009; Ranjbar-Aghdam, 2009). Effective temperatures (E) were calculated by Alston *et al.* (2010a):

$$E = \frac{T_{max} + T_{min}}{2} - 10$$

### Field studies

The mentioned orchard was divided into four equal plots. Each plot was assigned for treatment as follows: 1) control (C) (no insecticide application, just spraying of water to exclude the effect of physical pressure of spray droplets), 2) recommended dose (Label dose) (LD), 3) half of the recommended dose (½ LD), 4) one-fourth of the recommended dose (¼ LD).

Spraying was done using a 20L backpack sprayer. Since the aphids' damage was tolerable (no leaf curling signs occurred), no specific treatment was done against the aphids, and a single application was carried out against CM, *C. pomonella*, and aphids. Fenprothrin was sprayed against both CM and mites in the second generation; and diflubenzuron against CM alone in the third generation. There was no need to control aphids in the next two generations due to the negligible number of aphids.

### Bioassays

1) *Aphis pomi* is a monoecious species in Iran (Radjabi, 1986). Hence, one can rear them only on apple trees. Therefore, seedlings of apples were grown in plastic pots (25cm height, 22 cm diameter). Orchard soil, perlite, and coco peat were mixed at a ratio of 2:1:1. The seedlings were planted at a depth of 15 cm in pots. They were rinsed once a week and kept in a greenhouse at 27 ± 2 °C, 60 ± 5% RH, and 16: 8 h L: D photoperiod in Department of Plant Protection, University of Tabriz. As the seedlings grew, they were infested with adult GAA from orchards on five-leaf branches. Cloth

nets confined the pots and then, 100 adults GAA were transferred on leaves. The adults were removed after 24 h, and a cohort of first instar nymphs remained on leaves.

2) Nine days later, when the nymphs reached the fourth instar, they were removed and used in bioassays. The range of concentration that could cause 20-80% mortality was determined by a preliminary test. The main experiments consisted of five concentrations equally spaced on the logarithmic scale within the mentioned ranges. Distilled water was used to dilute the insecticides, and Tween 80® (Merck, Darmstadt, Germany) was used as a surfactant. The control consisted of distilled water + Tween 80®. The fifth leaves were dipped in the insecticide solution and dried at room temperature. Then, they were transferred to Petri dishes (6cm diameter) and exposed to 4<sup>th</sup> instar nymphs. The petioles were wrapped in wet cotton wool to prevent wilting of the leaves. This experiment was repeated three times on different days with 20 insects per concentration in each experiment. Mortality was recorded 24 hours later. The insects that were not able to stand up were considered dead.

3) *Hippodamia variegata*: to rear the ladybirds, chrysanthemum aphid, *Macrosiphoniella sanborni* (Gillette) (Hemiptera: Aphididae) was used as the prey. The aphids were collected from *Chrysanthemum* plants on the landscape of Tabriz city and transferred to *chrysanthemum* plants in the greenhouse. The chrysanthemum plants were confined with net cloth to prevent infestation of uninfested plants and avoid infestation by other insects or mites. Apple leaves bearing different stages of *M. sanborni* were exposed to ladybirds within plastic containers (35 × 25 × 15 cm) equipped with a rectangular hole (10 × 20 cm on lid) covered by a fine mesh. These containers were kept in the greenhouse at 27 ± 2 °C, 60 ± 5% RH, and 16: 8 h L: D photoperiod. Ladybird eggs were collected daily and three days later when the eggs hatched, first instar nymphs of the same age (0-24h old) were removed to do bioassays. About four weeks later, < 24h-old 4<sup>th</sup>

instar larvae were used for bioassays. A preliminary experiment was done as previously explained, and the range of concentrations for the main experiment was determined based on the range of 20-80% mortality. Five concentrations with logarithmic intervals as well as a control were considered for bioassay. The range of concentrations of acetamiprid tested against 4<sup>th</sup> instar *A. pomi*, 1<sup>st</sup> and 4<sup>th</sup> instar *H. variegata* were 5-150, 15-120, and 50-500 mg ai/l, respectively. This experiment was repeated three times on different days with 20 insects per concentration in each experiment.

### Data analysis

Mortality data were corrected using Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality} = \frac{\text{mortality in treatment} - \text{mortality in control}}{100 - \text{mortality in control}} \times 100$$

Dose-response curves were drawn and probit analysis was done by SPSS ver. 22.

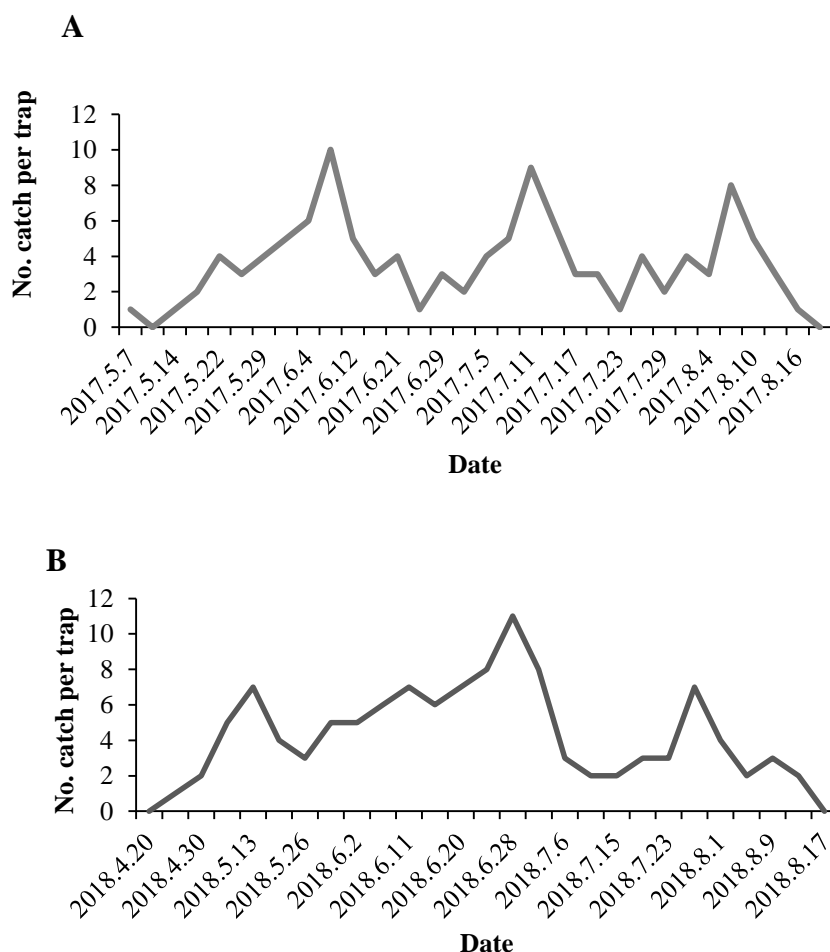
### Results

#### Monitoring *C. pomonella*

In both years, three peaks were observed in pheromone traps (Fig. 1). The peak of the first generation occurred on June 6 2017 and May 11 2018. Biofix was determined to be May 11 2017 and April 20 2018. The date of spraying and number of degree-days accumulated from the biofix are presented in Table 1.

#### Biological events of apple aphids hinged to apple phenology

All phenological events of apple trees in 2018 occurred 10-15 days earlier than in 2017 (Fig. 2). Inspecting apple trees began in mid-March and continued throughout the growing season. Overwintering eggs of GAA were observed at the silver tip stage in both years. The nymphs of GAA were observed at the green tip stage. The presence of RAA was documented about one month later, on May 12 2017 and April 28 2018, respectively, which coincided with petal fall. Therefore, both aphid species' seasonal activity followed the apple trees' phenology.



**Figure 1** Fluctuation of catches of male *Cydia pomonella* moths by pheromone traps in apple orchards of Khalatpoushan Research Station of the University of Tabriz in A) 2017 and B) 2018.

**Table 1** Time of different events and corresponding degree-days accumulated from biofix in apple orchards of Khalatpoushan Research Station of the University of Tabriz in 2017 and 2018.

Event	2017		2018	
	Date	Day-Degree accumulated	Date	Day-Degree accumulated
Biofix	May 11	0	April 20	0
First spray	June 15	238	May 28	184
Second spray	July 17	697	July 4	650
Third Spray	August 11	1191	August 2	1174

### Population fluctuations of GAA and RAA

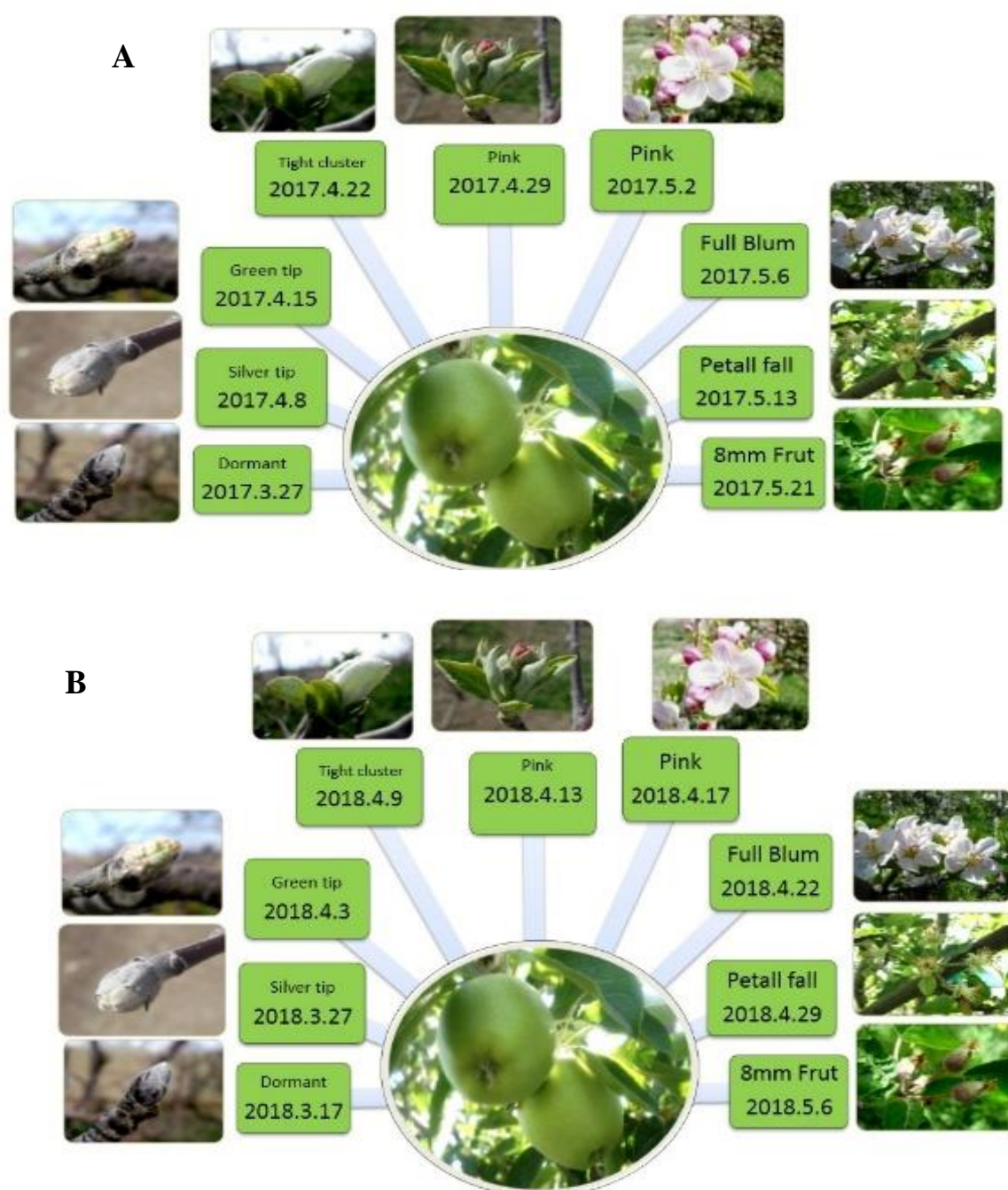
#### i) Before spraying

The abundance of both aphid species increased until spraying time. In 2017, between May 12 and June 11, an approximately linear increase with some fluctuations was observed (Fig. 3). Abundance of RAA increased more rapidly than

GAA and exceeded that of the GAA after mid-May. The trend line slope showed the population growth rate of RAA to be twice as high as that of GAA. Abundance of both species finally approached a plateau that was determined to be 40 and 70 aphids per a seven-leaf 15cm, branch for GAA and RAA, respectively. In 2018, both

aphids began to increase exponentially and then reached a plateau of 40 and 60 aphids per the same sample unit (Fig. 3). Similar to 2017, the

abundance of GAA was initially above that of the RAA, but finally was exceeded by RAA after April 24 2018.



**Figure 2** Phenological events of apple trees in apple orchards of Khalatpoushan Research Station of the University of Tabriz in A) 2017 and B) 2018.



## ii) After spraying

The abundance of the RAA declined to zero after spraying, and there was no evidence of further increase in either year. In 2017, after the first application of acetamiprid on June 15 at doses equivalent to 0 (control),  $\frac{1}{4}$  LD,  $\frac{1}{2}$  LD, and LD, GAA abundance declined to 63.3, 17.5, 10.0, and 5.8% of that of the control before spraying, respectively. In other words, 36.7, 82.5, 90.0, and 94.2% of mortality occurred due to the treatment. Mortality in control was probably due to the mechanical pressure of water droplets sprayed on them. Label dose showed an 11.7% advantage over  $\frac{1}{4}$  LD and 4.2% over the  $\frac{1}{2}$  LD. In all treatments including the control, the subsequent increase in the number of aphids was obvious (Fig. 4). The subsequent increase of GAA abundance was steeper at higher doses of the insecticide, and abundance in higher dose plots exceeded that of the lower doses; and finally reached a maximum of 29-32 aphids per above mentioned sample unit (7-leaf, 15 cm long branch) on July 6; and continued with negligible fluctuations for 10 days. After July 16, the declining trend of abundance began and continued until August 10, until it reached zero. The areas under the curves were calculated as 1182.5, 1050.8, 975.3, and 903.6 aphid-days for control, LD,  $\frac{1}{2}$  LD, and  $\frac{1}{4}$  LD plots respectively. These values may reflect the damage intensity (persistence of aphids on leaves during the time). Therefore, we can conclude that using insecticide had an advantage of 12.5, 21.2, and 30.9% over the control (no action) in LD,  $\frac{1}{2}$  LD, and  $\frac{1}{4}$  LD plots respectively. Moreover, although LD killed 11.7% more aphids than  $\frac{1}{4}$  LD, it caused 16.3% more injury in the long run. This may reveal the advantage of the lower doses compared with LD. The difference between  $\frac{1}{4}$  LD and  $\frac{1}{2}$  LD was small, and only a 7.9% advantage was noticed for  $\frac{1}{4}$  LD. The treatment with  $\frac{1}{2}$  LD also had a 7.7% advantage over LD.

The results obtained in 2018 revealed that LD resulted in the highest mortality i. e. 89.0%. Two other concentrations caused

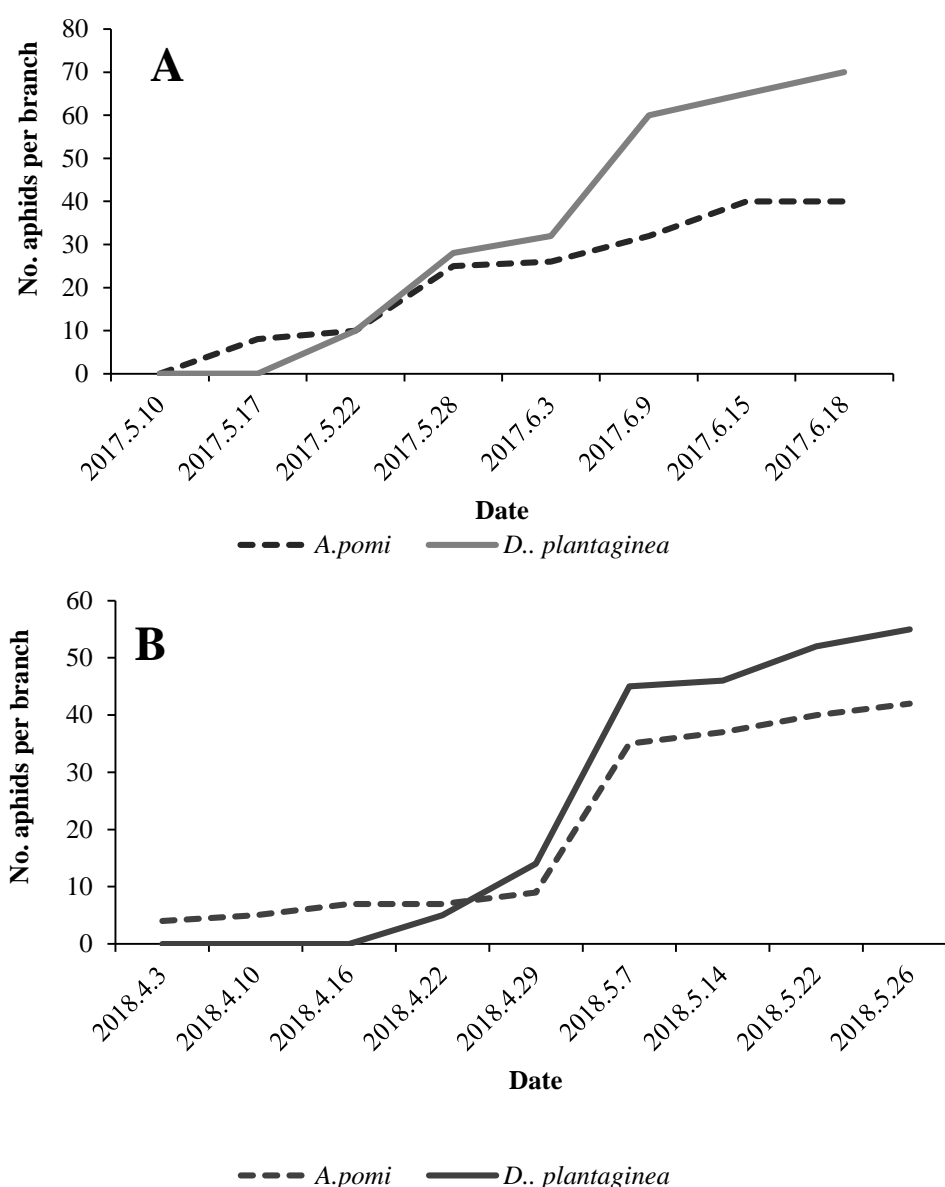
similar mortalities (80.4 and 80.6%, respectively). In addition, the physical pressure of the sprayed water caused 41.2% mortality in control. After spraying, the population of the aphid fluctuated between 25 and 33 with an average of 30 aphids per mentioned unit of habitat in control. This situation continued from spraying on May 24 until June 21; and then declined (Fig. 4). In insecticide-treated plots, the GAA population increased gradually to reach the equilibrium of 30-32 aphids per sample unit until June 21. Abundance had a similar declining trend and reached zero in all treatments from June 21 to July 30. Under-curve areas were 1470.8, 1235.0, 1142.6, and 1104.6 aphid-days for control, LD,  $\frac{1}{2}$  LD, and  $\frac{1}{4}$  LD, respectively. Although LD caused 8.5% higher mortality compared with both  $\frac{1}{2}$  and  $\frac{1}{4}$  LD, however, regarding damage intensity (persistence of damage on leaves),  $\frac{1}{2}$  LD, and  $\frac{1}{4}$  LD had 8.1 and 11.8% advantages over LD, respectively. No considerable advantage was observed in  $\frac{1}{4}$  LD over  $\frac{1}{2}$  LD (only a 3.4% lower damage intensity). Insecticide application had 19.1, 28.7 and 33.2% advantages over control plots in LD,  $\frac{1}{2}$  LD, and  $\frac{1}{4}$  LD treated plots, respectively.

## Selectivity of acetamiprid to the benefit of *Hippodamia variegata*

Field observations showed that only two species of ladybirds, namely *Coccinella septempunctata* and *Hippodamia variegata* were frequent enough to be considered in integrated pest management of apple aphids. The second species was considered for bioassay experiments. The summary results of the probit analysis are shown in Table 2. As can be seen, the 4<sup>th</sup> instar nymphs of *A. pomi* were more sensitive to acetamiprid than both stages of its natural enemy, *H. variegata*. However, at higher concentrations of acetamiprid, the situation was reversed, and LC<sub>90</sub> of the 1<sup>st</sup> instar *H. variegata* was lower than that of *A. pomi*. To increase mortality from 50% to 90%, one needs to use 5, 6.8, and 16 times more insecticide against the 1<sup>st</sup> and 4<sup>th</sup> instar larvae of the ladybird, and 4<sup>th</sup> instar nymph of the aphid,

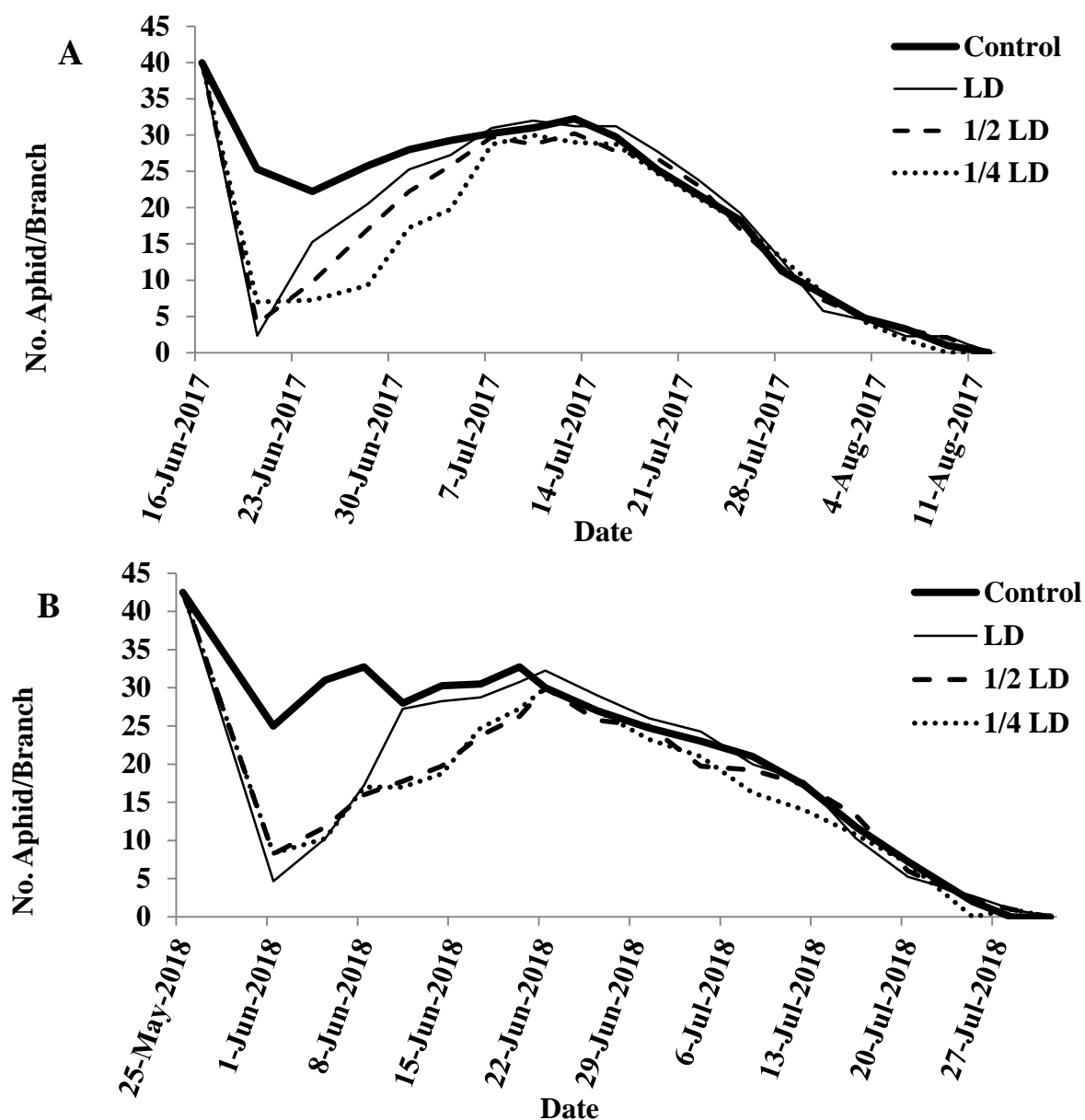
respectively. This means that acetamiprid was selective for the 1st instar larvae of *H. variegata*, but only at doses below 58.4 mg ai/l, at which mortality falls below 59.4%. These are the point coordinates where the two dose-response lines intersect. The dose-response line of the fourth instar larvae of *H. variegata* also intersects that

of the fourth instar nymph of *A. pomi* at a point with the coordinates of 2678.08 mg ai/l, and a mortality rate of 97.8%. This is because the slope of the probit line was steeper for *H. variegata* than for *A. pomi*. The lines of both stages of *H. variegata* were parallel and did not intersect (Fig. 5).



**Figure 3** Population fluctuation of apple aphids, *Aphis pomi* and *Dysaphis plantaginea* before spraying apple orchards of Khalatpoushan Research Station of the University of Tabriz in A) 2017 and B) 2018.

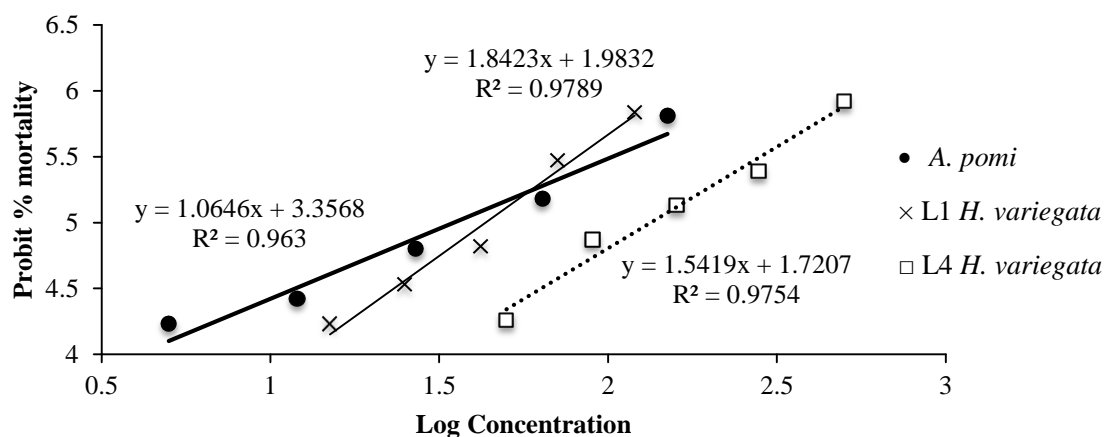




**Figure 4** Population fluctuations of *Aphis pomi* after spraying with acetamiprid in apple orchard of Khalatpoushan Research Station of the University of Tabriz in A) 2017 and B) 2018.

**Table 2** Summary of probit analysis of mortality of 4<sup>th</sup> instar nymphs of *Aphis pomi*, and 1<sup>st</sup> and 4<sup>th</sup> instar larvae of *Hippodamia variegata* at different concentrations of acetamiprid.

Insect stage	Slope $\pm$ SE	LC <sub>50</sub> (mg ai/l) (95% CI)	LC <sub>90</sub> (mg ai/l) (95% CI)	df	$\chi^2$
<i>H. variegata</i> L1	1.87 $\pm$ 0.252	43.66 (36.08-53.08)	211.48 (145.27-392.13)	3	1.191 ns
<i>H. variegata</i> L4	1.50 $\pm$ 0.225	133.26 (102.9-167.9)	956.97 (602.21-2153.54)	3	1.299 ns
<i>A. pomi</i> L4	1.081 $\pm$ 0.0154	35.63 (25.83-50.99)	545.81 (270.20-1812.0)	3	2.208 ns



**Figure 5** Dose-response lines of 1<sup>st</sup> and 4<sup>th</sup> instar larvae of *Hippodamia variegata* and 4<sup>th</sup> instar nymph of *Aphis pomi* treated with different concentrations of acetamiprid.

## Discussion

It was documented that three aphid species attacked apple trees in the Khalatpoushan region, among which GAA was more prevalent. It appeared sooner than the other species and disappeared after them. The duration of GAA activity was three months or longer, while RAA was observed for 1-2 months and disappeared earlier in June. This situation resembles Mediterranean countries like Greece (Perkidis *et al.*, 2008), Tunisia (Ben Halima Kamel and Ben Hamouda, 2005; Mdellet and Ben Halima Kamel, 2015), and Algeria (Laamari *et al.*, 2010). WAA, *Eriosoma lanigerum* had a local distribution. We observed it only on one tree in both years. Dominant natural enemies in the region were two species of ladybirds *C. septempunctata* and *H. variegata*. In addition, three species of hoverflies, namely *Syrphus ribesii* (L.), *Scaeva albomaculata* (Macquart), and *Scaeva pyrastris* (L.) were also collected. These species were also collected in Tunisia (Mdellet and Ben Halima Kamel, 2015). Ladybirds, syrphids, cecidomyiids, and chrysopids are prevalent predators of aphids including apple aphids worldwide (Gontijo *et al.*, 2012).

This study focused on GAA, *Aphis pomi*, and predatory ladybird *H. variegata* to approach

selectivity by choosing appropriate doses. Our main goals were avoiding early season spraying and specific applications against aphids, and obtaining possible selectivity via lower doses. Fortunately, the results revealed that we can achieve these goals by choosing a relevant strategy. We avoided early season applications and delayed spraying of insecticides up to the peak of the codling moth flights. We could control aphid populations without suffering considerable aphid damage and leaf-curling symptoms. Codling moth is the central focus of apple pest management programs in most apple orchards worldwide (Radjab, 1986; Thaler *et al.*, 2008). Aphids are early-season pests and often farmers prefer to intervene as soon as they observe them on branches and leaves. They often can be found before the flowering, of apple trees, and many early-season natural enemies such as ladybirds, can be affected by insecticide applications (James *et al.*, 2001; James, 2002). Avoiding early season applications can effectively conserve natural enemies and is highly recommended (Radjab, 1986; Dreistadt, 2016; Dib *et al.*, 2016). Our results showed a one-month interval between the appearance of aphids on apple trees and the time for taking control measures against codling moth. Because the weather is cold in spring in the region, aphid populations grow slowly and are tolerable

without early-season spraying. We must choose an effective insecticide for both groups to achieve a common control of aphids (GAA and RAA) and codling moth. Acetamiprid is a recommended insecticide against aphids and codling moth. Using acetamiprid, we could achieve the second goal of simultaneously controlling the key pest *Cydia pomonella* and aphids; we could also avoid specific measures against sap-feeding aphids. Choosing bio-rational insecticides, reducing the number of applications by delaying application, and using as low doses as possible are highly emphasized measures of pest management programs (Veres et al., 2013; Uyttenbroeck et al., 2016). In this study, a bioassay was done on the effect of dose on the selectivity of acetamiprid to the benefit of natural enemies on both GAA and one of the two most prevalent predators *H. variegata*.

Two stages of the ladybird and only one of the aphid were chosen. This was because we tried to show the dose's effect on the pest's most resistant stage and the predator's most sensitive stage. Our results showed that the most resistant stage of aphids was still more sensitive to acetamiprid than a more sensitive stage of the natural enemy, say the 1<sup>st</sup> instar larvae of *H. variegata* at lower doses. At higher doses (above LD<sub>50</sub>), first instar *H. variegata* mortality increased more rapidly. It exceeded that of the GAA, although last instar larvae of *H. variegata* were still more resistant to acetamiprid. It seems that choosing a higher dose had an undesirable effect on the younger stages of the ladybird and could change the natural enemy-to-pest ratio in favor of the pest. This is only the case if earlier stages of the predator coincide with later stages of aphids. Earlier stages of aphids are expected to be more sensitive to insecticide because toxicity is often dependent on an organism's weight (Takeuchi and Endo, 2012). Generally, choosing lower doses of insecticides than displayed on the label is recommended because of resistance management and selectivity purposes (Begg et al., 2017; Pretty et al., 2018). This will open a selectivity window for natural enemies to control pests with lower mortality on non-target natural enemies (van Emden and

Peakal, 1996), which was the case in this study. Although more developed stages of the ladybird were more resistant to the insecticide irrespective of the applied dose, higher doses may have undesirable effects on younger stages. It seems that choosing higher doses will lead to the elimination of earlier stages of both organisms. Because populations usually consist of a larger number of earlier stages, and a lower number of older ones (Ebert, 1999), higher doses will eventually destroy a larger number of ladybirds. Although this is true for aphids, aphids often recover more rapidly and enhance their abundance quickly (Madahi and Sahragard, 2012). Therefore, using a lower dose is strongly recommended based on the results of the present study.

Field evaluations also revealed that although lower doses killed fewer aphids, the subsequent increase in aphid abundance occurred more slowly at lower doses. It is probably so because fewer survivors at higher doses are under lower competition pressure. This finally led to a lower damage intensity of GAA at lower doses. Unfortunately, due to the low and unpredictable abundance of ladybirds, no natural enemy per pest ratio is evaluated before or after insecticide application. However, based on the bioassay results, improvement of this ratio to the benefit of ladybirds is expected. Therefore, using lower doses of acetamiprid is recommendable. Youn et al. (2003) reported 100% mortality of eggs and 1<sup>st</sup> to 4<sup>th</sup> instar larvae of *Harmonia axyridis* (Pallas) treated with LD level of acetamiprid. Using the lower dose also has another advantage of delaying resistance to insecticide (Rosaiah, 2001). Although the advantage of ¼ LD over ½ LD was minor regarding damage intensity, it would certainly be more economical, particularly in larger areas. We recommend that apple growers avoid early season application against apple aphids in the region, and use ¼ LD for control as late as 200 DD accumulated from the biofix for the first generation of the codling moth. There is no need for action against aphids in the next generations of codling moth, and only the mites may be controlled in the second generation.

### Conflict of interests

The authors declare no conflict of interest.

### Authors' Contribution

A. M conducted experiments and field studies, S. I suggested the objective of the study, analyzed the data, designed the experiments, and wrote the MS. M. J. H advised the bioassay experiments, edited the MS draft, and H. L. identified the species and edited the MS.

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## انتخابیت وابسته به دز استامیپرید در برابر *Hippodamia variegata*، شکارگر غالب شته سبز سیب (*Aphis pomi* (Hem., Aphididae) در باغات سیب شمال غرب ایران

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**چکیده:** چندین گونه از شته‌ها پراکنش گسترده دارند و از آفات مهم سیب در باغات ایران می‌باشند. در این بررسی ما به جستجوی روشی اقتصادی، زیست سازگار و مؤثر علیه این آفات با تأکید بر شته سبز سیب *Aphis pomi* (de Geer) پرداختیم. هدف ما خودداری از سمپاشی اول فصل توسط آفتکش‌ها، خودداری از سمپاشی اختصاصی برای کنترل شته‌ها و تلاش برای مدیریت آن‌ها از طریق سمپاشی کرم سیب *Cydia pomonella* L و انتخاب حشره‌کش و دز مناسب برای به حداقل رساندن اثرات جانبی روی دشمنان طبیعی غالب بود. بدین‌ترتیب، کرم سیب با استفاده از تله‌های فرومونی از اوایل نوروز در باغات سیب ایستگاه تحقیقاتی دانشگاه تبریز پایش شد تا زمان سمپاشی براساس درجه-روزهای انباشته شده از زمان بیوفیکس تعیین شود. تعداد شته‌ها و دشمنان طبیعی آن‌ها جداگانه شمارش گردید. معلوم شد که شته سبز سیب گونه‌ی غالب شته و دو گونه از کفشدوزک‌ها *Coccinella septempunctata* (L.) و *Hippodamia variegata* (Goeze) گونه‌های غالب دشمنان طبیعی منطقه می‌باشند. استامی‌پرید به‌عنوان حشره‌کش مؤثر علیه کرم سیب و شته‌ها انتخاب شد. اثرات کشندگی این ترکیب روی مراحل مختلف *H. variegata* و سن آخر شته سبز مطالعه شد. باغ به چهار قسمت مساوی تقسیم شد و هر یک به یکی از چهار تیمار شاهد، دز برچسب، نصف و ربع دز برچسب تخصیص داده شد. هر چند دز برچسب ۱۰٪ بیش از یک چهارم دز برچسب تلفات ایجاد کرد، ولی شدت خسارت در دز کامل ۱۲ تا ۱۶٪ بیشتر بود. با در نظر گرفتن سود اقتصادی و کاستن از اثرات جانبی، ما استفاده از ربع دز برچسب استامی‌پرید را توصیه می‌نماییم.

**واژگان کلیدی:** زمان‌بندی، مدیریت تلفیقی آفات، اثرات جانبی، دشمنان طبیعی