Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Evaluation of systemic effects of four plant extracts compared with two systemic pesticides, acetamiprid and pirimicarb through leaf spraying against *Brevicoryne brassicae* L. (Hemiptera: Aphididae)

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Vol. 58, No. 3: 257–264, 2018

DOI: 10.24425/122942

Received: May 7, 2018
Accepted: August 13, 2018

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Abstract

Aphids are one of the most important economic pests and vectors of viral diseases in crops. *Brevicoryne brassicae* L., one of the most serious aphid pests in Brassicaceae, if not controlled, often reaches very high densities. The present study compared the systemic effects of ethanolic, methanolic and aqueous *Melia azedarach* L., *Peganum harmala* L., *Calendula officinalis* L. and *Otostegia persica* Boissier extracts with two systemic pesticides, acetamiprid and pirimicarb (at the maximum label-recommended rate). Population growth percentages of *B. brassicae* through leaf spraying under greenhouse conditions were assessed. The chemicals were sprayed on one of the leaves in greenhouse condition. The results indicated that all the plant extracts have systemic effects at different levels. Among different extracts, *O. persica* ethanolic extract, *P. harmala* methanolic extract and *M. azedarach* aqueous extract resulted in a reduction of the *B. brassicae* population.

Keywords: acetamiprid, cabbage aphid, pirimicarb, plant-derived chemicals, systemic toxicity

Introduction

The cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae) is one of the most important cosmopolitan pests in the cultivation of canola. This pest attacks the leaves, stems and flowers. They may reduce plant growth as well as the content of canola seed by feeding on plant sap and covering the plants with white wax (Gabrys 2008). Also, they impose damage by secreting honeydew, which leads to the growth of sooty mold fungi which affects physiological plant processes (Gratwick 1992). Another damage caused by this aphid is transmission of plant viruses (Bouchery *et al.* 1990).

Nowadays, using systemic insecticides is common in controlling this aphid, but overusing artificial pesticides may cause many problems such as environmental pollution, increased resistance to pesticides in pests and side effects on non-target insects and natural enemies. Considering these problems it is necessary to find a safe, effective and natural way for pest control. Plants provide an alternative to currently used pesticides for pest control, as they are a rich source of bioactive chemicals (Kim *et al.* 2005). Botanical products are compatible with ecosystems, have lower toxicity to mammals and non-target organisms and lower persistence in the environment than synthetic insecticides (Liu *et al.* 2006).

Various studies have been carried out on the insecticidal activity of plant extracts of *Melia azedarach* L., *Peganum harmala* L., *Calendula officinalis* L. and *Otostegia persica* Boissier which are important medicinal plants in Asia against other pests.

Carpinella *et al.* (2003) reported antifeedant and insecticidal properties of a limonoid in *M. azedarach* fruit extract. The effects of *M. azedarach* aqueous extract on the leaf miner *Liriomyza huidobrensis*

Blanchard in the laboratory and field have been studied (Banchio *et al*. 2003). The efficacy of extract from callus leaves and fruits of *M. azedarach* against adults of the *Bemisia tabaci* Gennadius, has been evaluated (Abou-Fakhr Hammad *et al*. 2001). Active compounds isolated from the extract of *M. azedarach* include nimbin, nimbolide, gedunin and azadirachtin (Biswas *et al.* 2002). Moreover, the systemic effects of aqueous extract of *M. azedarach* seeds against *B. brassicae* and its predator *Coccinella septempunctata* L. have been determined (Kibrom *et al*. 2012). In their study, powdered *M. azedarach* seeds were used to prepare a 5% concentration of aqueous solution and sprayed. The results revealed that the aphid population decreased from the 1st week of treatment to the 6th week and the reduction in the predator population was not statistically significant. This study clearly indicates that *Melia* extract was not harmful to aphid predator coccinellids. Also, Hu *et al*. (1998) found systemic insecticidal action of methanolic extract of *M. azedarach* through the root of the host plant.

Peganum harmala a member of the Zygophyllaceae family, is known for its exceptional wealth of harman, harmine, harmaline, harmalol, tetrahydroharmine and peganine (Pulpati *et al.* 2008). Insecticidal and repellent activities of *P. harmala* have been reported against *Tribolium castaneum* Herbst and *Rhyzopertha dominica* Fabricius (Nenaah 2011), *Aphis fabae* Scopoli, *A. gossypii* Glover, *A. nerii* Boyer de Fonscolombe, *Myzus persicae* Sulzer and *T. castaneum* (Salari *et al*. 2012). In another study, Dehgani et al. (2011) investigated the effect of methanolic extract of *P. harmala* on the greenhouse whitefly *Trialeurodes vaporariorum* Westwood.

Pot marigold, *C. officinalis* is a member of the Asteraceae family. The flowers of *C. officinalis* contain flavonol glycosides, triterpene oligoglycosides, oleanane-type triterpene glycosides, saponins (Sharrif Moghaddasi and Kashani 2012). Jankowska and Wilk 2009 studied the impact of aqueous extract of *C. officinalis* on the occurrence of *B. brassicae* and its parasitoid *Diaeretiella rapae* M'lntosh. The effect of *C. officinalis* extracts was reported on consumption-utilization indices of *Spodoptera litura* Fabricius larvae (Medhini *et al.* 2010). The effect of these extracts on nutrient components of different tissues of tobacco cutworm (Medhini *et al.* 2012) was also determined.

Otostegia persica, "Goldar" in Persian, is a member of the Lamiaceae family. It has a range of phytochemical compounds, of which only a few molecules have been characterized including flavonoids, steroids, tannins and terpenoiedes (Tofighi *et al*. 2009). Therefore, complementary investigations are needed to identify new compounds in this species (Sadeghi *et al*. 2014), Salari *et al.* (2010) studied the effects of acetonic extract of *O. persica* on *A. fabae*, *A. gossypii*, *M. persicae* and adults of *T. castaneum*.

Acetamiprid is a neonicotinoid and systemic insecticide intended to control sucking insects on crops such as leafy vegetables, pome fruits, cotton, canola and ornamental plants (Nauen *et al*. 1998). Acetamiprid is translaminar, meaning it protects both sides of a leaf surface, and works by antagonizing the nicotine acetylcholine receptors in the neural pathways (Simon-Delso *et al.* 2015).

Pirimicarb is a selective aphicide which belongs to the group of chemicals called carbamates that is used extensively on a broad range of crops, including vegetable, cereal and orchard crops, but does not affect useful predators such as ladybirds that eat them. The mode of action of pirimicarb is by inhibition of acetylcholinesterase activity (Roberts 1999).

The main objective of this study was to explore the systemic effects of plant extracts for the management of *B. brassicae* on canola plants by ethanolic, methanolic and aqueous extracts from four medicinal plants through leaf spraying, and compare them with two conventional insecticides.

Materials and Methods

Insect rearing

Aphids were collected from ornamental cabbage plants found in the Shahid Bahonar University Park in Kerman, Iran. They were reared on canola (*Brassica napus* L., cultivars, Colvert) in 90 × 80 × 80 cm fine cloth cages in the experimental teaching greenhouse of Shahid Bahonar University of Kerman, Kerman, Iran.

Extraction of plant material

Melia azedarach fruits, *C. officinalis* seeds, *O. persica* leaves and *P. harmala* seeds were collected from natural areas, Mahan [30°3'29.93" N, 57°17'39.91" E, 1908 m (altitude)], Kerman [30°18'4.6" N, 57°1'57.6" E, 1761 m (altitude)], Orzooiyeh [28°27'0" N, 56°21'0" E, 1044 m (altitude)] and Ekhtiyarabad [30°19'47.62" N, 56°55'41.06" E, 1748 m (altitude)] region in Kerman province, Iran, respectively. The plant materials were dried at room temperature under shade conditions for 1 week. Approximately 200 g of dried plant material were individually powdered with an electric stainless steel blender for 5 min. Considering the type of experiment, for the extraction of the active ingredients, the *M. azedarach* fruit, *C. officinalis* seed, *O. persica* leaf and *P. harmala* seed powders were individually mixed with 200 ml ethanol (96%), methanol (90%) and distilled water in an Erlenmeyer flask that was covered with aluminum foil as a dark place. The soaked samples were kept for 24 h at 4°C in a refrigerator. Then, they were filtered with Whatman filter paper (9 cm

diameter). The extracts were placed in a sample vial at 18°C to be used for the assay.

Greenhouse bioassay

All bioassays were performed at 25 ± 5°C and a relative humidity of 70 ± 10% and 16 : 8 h (L : D) photoperiod in the modern experimental greenhouse of Shahid Bahonar University of Kerman, Kerman, Iran. Each experimental unit consisted of a canola plant (with 5–6 leaves) in plastic pots. Four female aphids were placed on each leaf. Then the pots were placed in a thick clear plastic cylindrical container (60 cm high and 27 cm diameter), which were joined on ceramic tiles (40 × 40 cm) by silicone aquarium sealant. These containers were covered with a suitable mesh. The pots were kept inside these containers to the end of the experiment. After 3 days, the number of adult cabbage aphids and pre-adult stages on each leaf were recorded. Then all leaves except one were covered with cellophane. The free leaf was treated with different commercial insecticides at their maximum label-recommended rates [acetamiprid (20 mg · l^{-1} active ingredient) and pirimicarb (50 mg · l^{-1} active ingredient)] as well as plant extracts (at 60 mg · ml^{-1} concentration) separately. The cellophane was then removed and the treated leaf was covered with new cellophane. After 24 h, the treated leaf was removed. Control plants were treated with distilled water, ethanol (96%) and methanol (90%) (due to each experiment). The systemic effects of commercial insecticides and plant extracts were determined by using a spraying test bioassay under the same conditions described above with the same volume (2 ml for each pot). In this study, the number of adult, live cabbage aphids and pre-adult stages on each canola plant were counted 48, 96 and 120 h after spraying.

Statistical analysis

The population growth percentages were calculated from the formula:

$$\text{Population growth percentage} = \frac{n_i - n_{i-1}}{n_{i-1}} \times 100,$$

where: $i = 2, 3, 4\ldots$; n_i = population mean on day ith.

In order to affirm the basic assumptions of the data to be analyzed, they were first tested for the normal distribution and the homogeneity of variance using the Bartlett test (Köhler *et al.* 2002). The data which did not conform to the assumptions of normal distribution were transformed to conform to the assumptions using the Box-Cox formula:

$$Y = \ln X \text{ if } \lambda = 0,$$

$$Y = \frac{X^2 - 1}{\lambda}, \text{ if } \lambda \neq 0,$$

where: Y – the transformed value, X – the untransformed value, and $0 < \lambda < 1$ (Anonymous 1996).

For statistical comparison of the mean data, the data were subjected to a one-way analysis of variance followed by the Fisher LSD method ($p \leq 0.05$). Statistical analyses were run in Statplus version 4.9, 2007.

Results

The mean population increase percentage of *B. brassicae* treated with acetamiprid and pirimicarb in three repetitions is demonstrated in Figure 1. At all recordings, there were significant differences between control (water) and commercial insecticides ($p \leq 0.0001$). Acetamiprid showed the least population increase rate and there were significant differences with pirimicarb as well ($p \leq 0.03$).

The results of ethanolic extracts on population growth of *B. brassicae* are shown in Figure 2. At the first recording (48 h after spraying), there were significant differences between ethanol (as control) and *M. azedarach*, *C. officinalis*, *P. harmala* and *O. persica* treatments ($p \leq 0.02$). *Otostegia persica* showed the least population increase percentage as well as significant differences with *C. officinalis* ($p \leq 0.004$) and *M. azedarach* ($p \leq 0.00001$). At the second recording (after 96 h), the mean population growth rate in the control decreased compared to the first recording. *Otostegia persica* was significantly different from the control ($p \leq 0.007$) and other extracts ($p \leq 0.00001$). At the third recording (after 120 h), the mean population growth rate in *O. persica* decreased more and had the lowest population increase. Also, at the third recording, *O. persica* significantly differed from the other extracts ($p \leq 0.00001$) and the control ($p \leq 0.02$). Overall, *O. persica* extracts were the most effective treatment for the three repetitions of the experiment followed by *P. harmala* and *C. officinalis* treatments. In addition, there were significant differences between the three recordings of *O. persica* ($p \leq 0.00001$).

The mean population increase rate of the aphids treated with methanolic extracts recorded at three different times is demonstrated in Figure 3. At the first recording, there was no significant difference between methanol (as control) and *C. officinalis*, *P. harmala* as well as *O. persica*. In *M. azedarach* treatment, the mean population growth percentage was more than the control. But at the second recording, the results were completely different. The mean population growth rate increased in the control, and it showed significant differences with the extracts ($p \leq 0.00001$). At this recording, *O. persica* showed the least population increase percentage and there were significant differences with

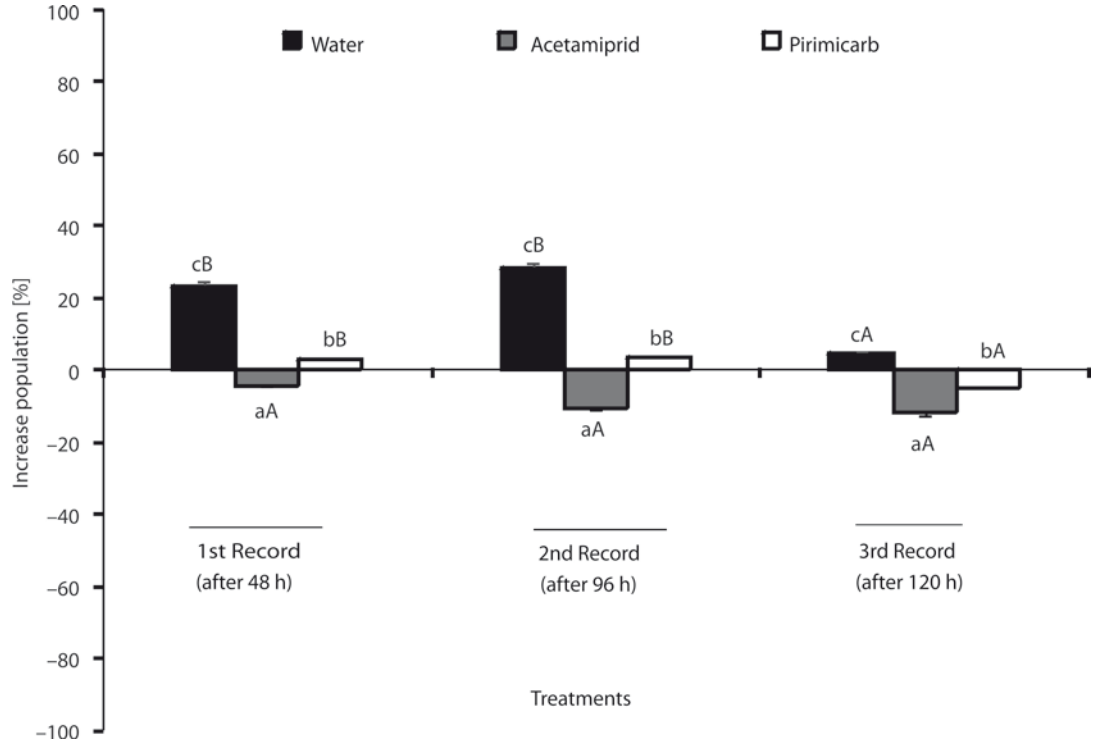

Fig. 1. The mean population growth rate of *Brevicoryne brassicae* L. (adults and pre-adult stages) treated with two commercial insecticides. (Bars with different small letters indicate significant differences between different chemicals at the same time record. Bars with different capital letters indicate significant differences between different time records with the same chemical)

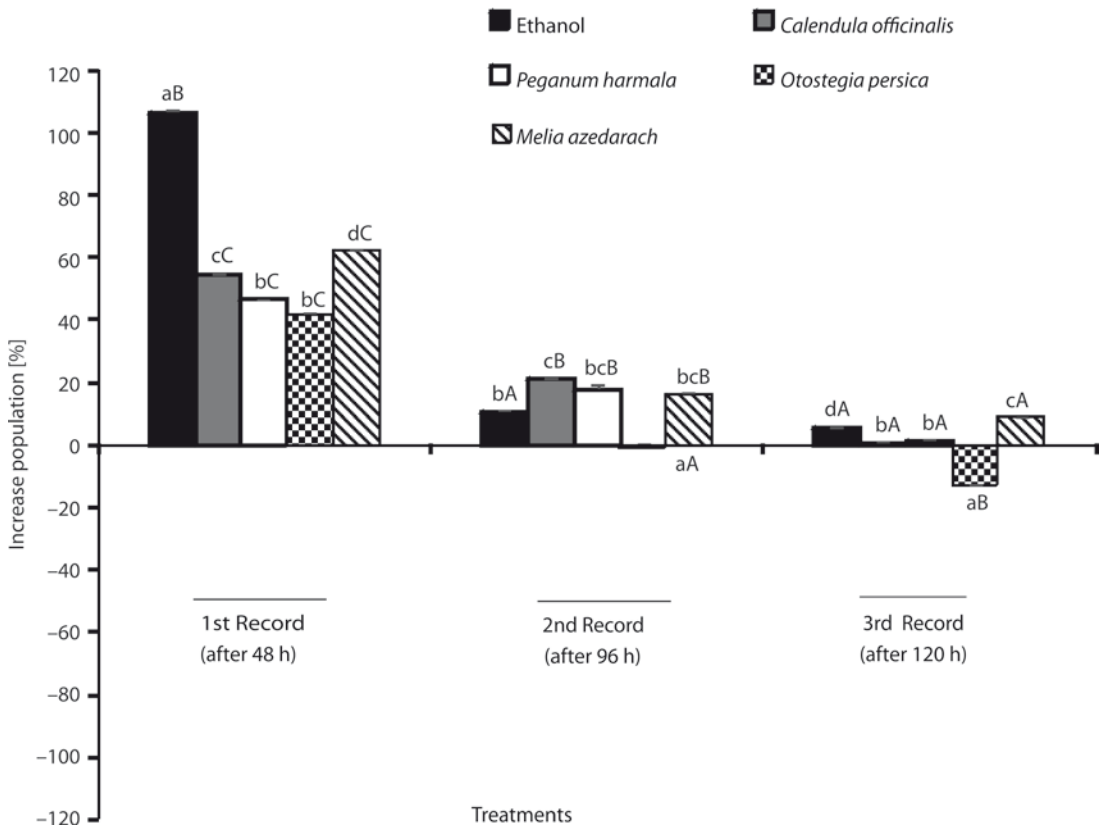

Fig. 2. The mean population growth rate of *Brevicoryne brassicae* L. (adults and pre-adult stages) treated with four ethanolic plant extracts. (Bars with different small letters indicate significant differences between different chemicals at the same time record. Bars with different capital letters indicate significant differences between different time records with the same chemical)

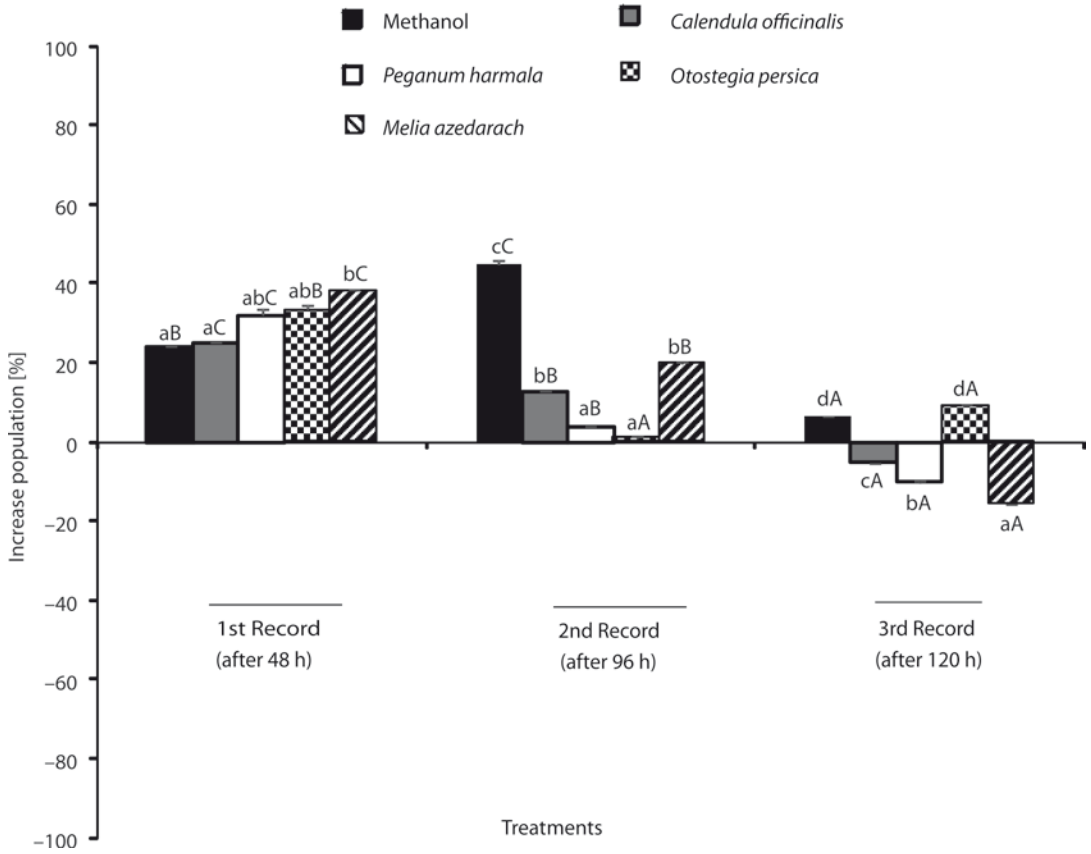

Fig. 3. The mean population growth rate of *Brevicoryne brassicae* L. (adults and pre-adult stages) treated with four methanolic plant extracts. (Bars with different small letters indicate significant differences between different chemicals at the same time record. Bars with different capital letters indicate significant differences between different time records with the same chemical)

C. officinalis ($p \leq 0.01$) and *M. azedarach* ($p \leq 0.00001$) as well. At the third recording, the mean population growth rate in the control decreased compared to the second recording, but it showed significant differences with *C. officinalis*, *P. harmala* and *M. azedarach* ($p \leq 0.00001$). *Melia azedarach* showed the least population increase rate and it showed significant differences with the second recording ($p \leq 0.00001$), while *O. persica* showed the most population increase at this recording.

According to the results of aqueous plant-derived chemicals experiments at the first recording (Figure 4), there were significant differences between water (as control) and *C. officinalis* and *P. harmala* ($p \leq 0.01$), while *C. officinalis* and *M. azedarach* showed the least and the most population increase percentages, respectively. At the second recording, there were significant differences between control and plant extracts with the exception of *O. persica* ($p \leq 0.003$). In addition, the results showed significant differences between the first and second recordings in *C. officinalis* ($p \leq 0.003$) and *M. azedarach* ($p \leq 0.2$). At the third recording, the mean population growth rate in the control decreased more. It showed significant differences with *C. officinalis* and *M. azedarach* ($p \leq 0.02$). *Melia azedarach* had the greatest population decrease, followed by *C. officinalis* and *P. harmala*. There were significant differences between *M. azedarach* and other extracts ($p \leq 0.00001$). Also, *M. azedarach* showed significant differences with the second recording ($p \leq 0.02$). In this experiment, *O. persica* had the lowest impact on population decrease.

Discussion

The present study aimed to evaluate the systemic efficiency of different plant extracts against *B. brassicae*. The results indicated that all the plant extracts have systemic effects at different levels. It is obvious that recording the aphid population at three different times is important for evaluating the time of aphid population reduction and emergence of systemic effects of plant extracts, the speed of the extracts' movement in plant sap and the extracts' stability.

With the use of commercial insecticides, with pirimicarb treatment there were no significant differences between the first and second recordings while in the third recording a negative population growth was

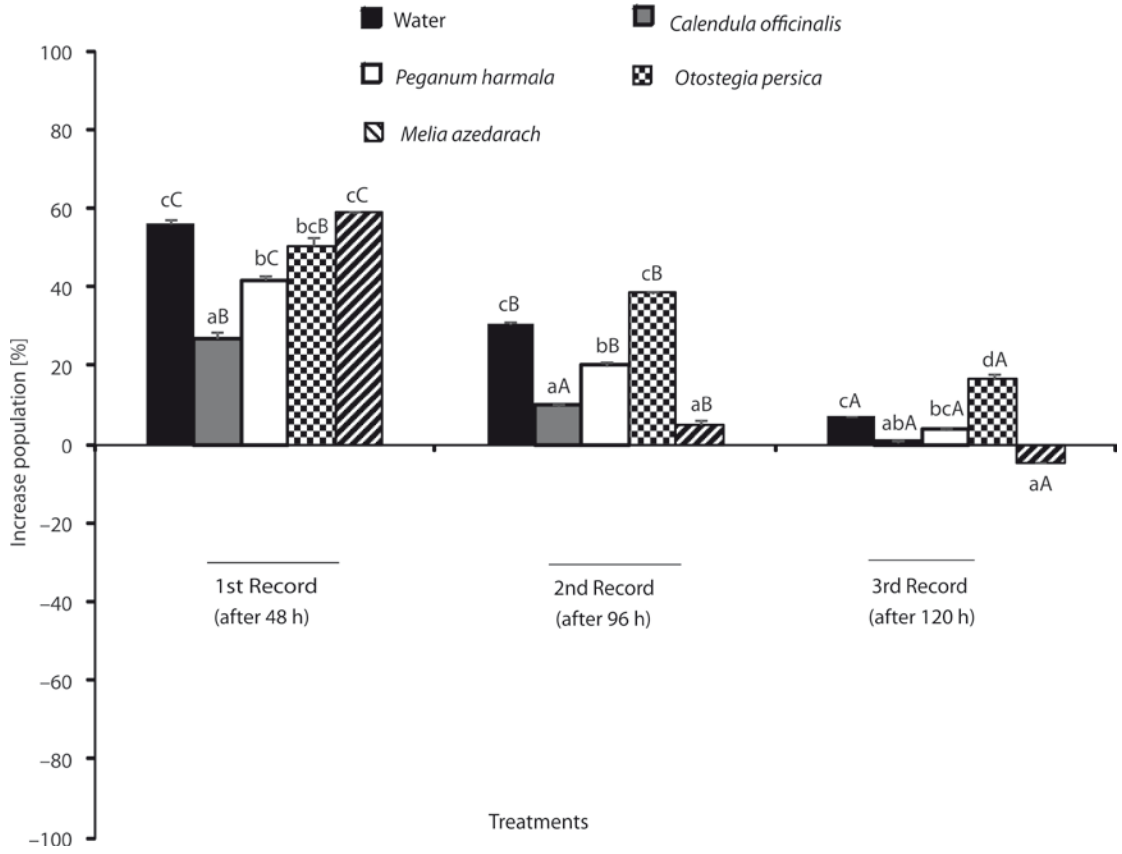

Fig. 4. The mean population growth rate of *Brevicoryne brassicae* L. (adults and pre-adult stages) treated with four water plant extracts. (Bars with different small letters indicate significant differences between different chemicals at the same time record. Bars with different capital letters indicate significant differences between different time records with the same chemical)

observed. This means that through leaf application of pirimicarb it takes 120 h for systemic effects to emerge. This finding is in agreement with the report of McLeod (1991) about the systemic effects of five insecticides on mortality speed of *M. persicae* through the upper surface of spinach leaves. It was reported that the systemic activity of pirimicarb was low. With acetamiprid treatment at the first recording, the population growth rate was negative and systemic effects appeared fast. In addition, the population growth became more and more negative each time. This can be related to the extract's stability in plant sap until the last recording. According to studies performed by Chen *et al.* (2007) foliar sprays of acetamiprid against *Contarinia nasturtii* Keiffer (Diptera: Cecidomyiidae) on cauliflower transplants, acetamiprid can effectively control *C. nasturtii* on cauliflower seedlings, especially in the early stage of insect occurrence.

Among the ethanolic extracts, *O. persica* strongly affected cabbage aphid population growth. There are two remarkable points about *O. persica* ethanolic extract worth mentioning. The first point is the existence of a significant difference between *O. persica* extract and the control at the first recording (48 h after spraying) which can be indicative of the quick systemic spread of this extract, so that a negative growth population was observed at the second recording. The second point implies a significant difference between all-time recordings of *O. persica* which shows the slow effect of this extract on the population growth rate.

According to the results of methanolic extracts, all plant-derived compounds showed a significant decrease in the mean population growth rate at the second recording, while at the third recording the mean population growth of *O. persica* was greater than at the second recording which can be related to the extract's stability. However, the mean of population growth in *M. azedarach*, *C. officinalis* and *P. harmala* was negative. Also, there was no reduction in the cabbage aphid population at the first recording and systemic effects appeared after the second recording. Moreover, there were significant differences between all the recorded times in *P. harmala* that shows the slow spread of this extract into plant sap. The effect of different *C. officinalis* extracts containing methanolic and aqueous extracts on consumption-utilization indices of *S. litura* larvae were studied by Medhini *et al.* (2010). The results indicated that *C. officinalis* extracts exert various effects on the growth and physiology of

S. litura. At higher concentrations tested, larval growth decline was associated with reduced food consumption and utilization.

By using aqueous extracts, *M. azedarach* and *C. officinalis* extracts could strongly affect the cabbage aphid population. With *C. officinalis* treatment, systemic effects appeared faster than with *M. azedarach* because of significant differences between *C. officinalis* and control at the first recording. Moreover, *M. azedarach* has slower impact speed than *C. officinalis*, and showed greater decrease in the mean population growth than *C. officinalis*. These findings are in agreement with the report of Kibrom *et al.* (2012) in which they evaluated the effect of water extract from yellow fruit of *M. azedarach* against *B. brassicae* and its predator *C. septempunctata* through leaf spraying. According to the results of their study, *Melia* seed extract is effective against *B. brassicae* and safer to the predator *C. septempunctata*. The effectiveness of *M. azedarach* was associated with starvation. As a consequence, their strong antifeedant activity could play a significant role in the high mortality values observed on treated *B. brassicae*. The remarkable thing is that unlike *O. persica* ethanolic extract which showed the greatest decrease in the mean population growth rate among ethanolic extracts, *O. persica* aqueous extract showed the least population decrease compared to the control and other aqueous extracts. This means that the derived fractions extracted by ethanol and water are different.

Ghanim and Abdel Ghani (2014) did a study related to the systemic effects of plant extracts. They investigated the effects of different aqueous plant extracts against *A. gossypii* through spraying plants under greenhouse conditions with basil, geranium, chinaberry, onion and garlic aqueous extracts. Geranium showed the highest reduction percentages of aphid populations.

Experiments on the mean cabbage aphid population growth rates and the resulting negative population growth of treated aphids, found that plant extracts can compete with commercial insecticides in terms of reducing cabbage aphid populations. Among different extracts, *O. persica* ethanolic extract due to negative population growth at the second and third recordings, and therefore the appearance of systemic effects, has the closest similarity to acetamiprid. According to the population growth decrease at the second recording, even systemic effects of some plant extracts appeared faster than pirimicarb. Overall, the current study showed that these plant extracts have a considerable capacity to control cabbage aphids in a systemic way. However, there is a need to organize natural sources, carry out more investigations and larger-scale implementation, develop quality control and modify regulatory mechanisms.

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