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Insecticidal effect of *Ammi visnaga* L. (Apiaceae: Apial) methanolic extract against a citrus pest, *Toxoptera aurantii* (Aphididae: Homoptera) under controlled conditions

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Abstract
Ammi visnaga L. (*Apiaceae*) is an endemic Algerian aromatic plant, largely used in traditional medicine, and mostly for renal problems with a very few reported studies on its insecticidal activity. Current work is a contribution to the insecticidal effect of methanolic extract of aerial parts of *Ammi visnaga* collected at various stages of growth against *Toxoptera aurantii*. Eight different extract of six different doses were tested which showed a proportional relation between mortality and the applied doses. The maximum of mortality (98.33%) was obtained for the highest dose (30000 ng/aphids) after 24h for steams extract collected before flowering. Cumulative mortality of the treated aphids occurred in the second day with a rate of 100%. The LD_{50} value determined using the linear regression was 310.53 ng/ aphids < [D_5-D_6] for the said dose.

Keywords: *Apiaceae, Ammi visnaga* L., *Toxoptera aurantii*, methanolic extract, controlled conditions

Introduction
Aphids cause extensive damages on reducing the quality and the quantity of Agricultural products [1]. Because of its efficacy and the simplicity of its application, the chemical control (Foliar insecticides) constitute the most rapid and practical way to kill aphids [2]. Abusive use of insecticides causes several problems like pests' resistance, significant damages on human health and on the environment. As a response to this situation, the research of biocide was more than necessary [3]. Sevral species of *Apiaceae* plants are known for their powerful acaricidal and insecticidal effect against desert locust, tick and mosquito [4-6]. *Ammi visnaga*. is an endemic Algerian aromatic plant. It's a biannual herb measured between 0.8-1m high with robust stem all covered with leaves, white umbels 5 to 11.4 cm diameter appears around June. Its ovoid small seeds (2 - 2.5mm) are brownish to greenish brown with violet tinge [7]. This Mediterranean plant is rich in coumarins, flavonoid, essential oil and fixed oil [8]. The major active components of the plant are khellin and visnagin (furanochromones) which have been both reported from the fruits of *A. visnaga* [9-11]. This plant is used in the traditional medicine for the treatment of urinary problems [11-13], cardiovascular problems [8], diabetes [14], vitiligo [15-17], psoriasis [11], painful menstruation [16], menstruation regulation [16], teeth problems [8] and asthma [10-16]. Khella have several properties including: diuretic [16], antispasmodic, antilithiastic [10-12], antioxidant [15], antibacterial [19] and fungicidal [19]. In addition, essential oil and of extract of *A. visnaga* have been reported to be a powerful pesticide against *Mayetiola destructor and Culex quinquefasciatus* [4.20].
The aim of this study was to investigate the insecticidal effect of methanolic extract of aerial parts of *A. visnaga* at different stage of growth collected from Boumerdes (center of Algeria) against the black citrus aphid *Toxoptera aurantii* under laboratory conditions.

Materials and Methods
Biological materials
Pests
Aphides adults (*T. aurantii*, Aphididae: Homoptera) used in this study were deduct from *Citrus sinensis, Thomson* variety from an experimental private station located at Boumerdes (45 Km east of Algiers). The infestation of plants with *T. aurantii* was natural.

Vegetal material

The vegetal material constituted of the aerial parts of the plant *A. visnaga.* (Leaves, stems, flowers and seeds) which were collected at different period of growth stage: before flowering, flowering and fructification period from Bordj-Menaiel (Latitude: 33°44´30´´; Longitude: 3°43´23´´; Altitude: 33 m, in the northern Algeria) respectively in April, June and July 2014 (Table 1). After that, the plant samples were dried in the shade for 20 days at room temperature. A voucher specimen was deposited for identification at the laboratory of agricultural zoology and forestry in the national school of agronomy (INA), Algiers, Algeria (BRAH FAI, Av 15/012).

Table 1: Harvest period, used parts and doses of *A. visnaga.*

Plant	Harvest period	Used parts	**Doses** ng /aphids	Abreviation
A. visnaga.	April 2014 (before flowering)	Leaves, stems	$3.\ 10^4$	D1
			$1.\ 10^4$	D2
	June 2014 (flowering)	Leaves, stems, flowers	5.10^3	D3
			3.10^3	D4
			5.10^2	D5
			250	D6
	July 2014 (after flowering)	Leaves, stems, seeds		

Methanolic extract preparation

The vegetal pulverized powder (1g) representing each part of the plant during the three different stage of growth was macerated in methanol (20 ml) at room temperature for 48 followed by filtration and evaporation of the solvent at 60 °C. The left residue was weighed and dissolved in methanol (3 ml) for further analysis. Eventually, the solutions were stored at -20 °C [21].

Experimental Conditions

The bioassay was realized in the laboratory conditions using random dispositive. The experimental unit constituted of Petri dish which contained twenty adult aphids on fresh leaves of *Citrus sinensis* serving as a support for the pests. Hydrophilic wet cotton was put down under the fresh leaves to keep the leaves fresh during the test period (96h).

Eight methanolic extract of *A. visnaga* were tested against *Toxoptera aurantii.* Five doses of 250, 500, 3000, 5000, 10000, 30000 nanograms /aphids were prepared from each methanolic extract by simple dilution method with methanol.

1µl of each extract was deposed on aphids' thorax using micro-syringe type Hamilton. Control individuals received 1µl of methanol. The Petri dishes were maintained under laboratory conditions (temperature: 28 °C; relative humidity 80%). All experiments were conducted in triplicate.

Determination of mortality rate

To follow the chronological evolution of mortality of aphids submitted to different concentrations of each extract, successive observations were done after treating the aphids with the different extract, after 1, 3, 24, 48, 72 and 96h. The count of the dead individuals was realized with binocular magnifying glass.

The mortality rate was expressed by percentage, taking in consideration the initial population after correction.

The mortality rate was calculated as follows [22]:

$$M(\%) = \left(\frac{P - T}{S} \right) x100$$

M : Corrected Mortality expressed by percentage.
P : Mortality caused by the methanolic extract substance
T : Mortality of control.
S : Number of surviving individual control

Statistical Analysis

The data obtained were processed by analysis of variance (ANOVA) and comparisons were made at a probability level of 5%. The LD_{50} values are determined from the regression lines obtained by transformations in Probit percentage of corrected mortality, and changes in the logarithm of the administered doses and were expressed as nanograms (ng) of methanolic extract per treated aphids. The standard method for evaluating the toxicity of insecticides involves the calculation of toxicity data (LD_{50}) following standard guidelines such as European Council Directive 91/414 on the one hand and that of the Federal Insecticides and fungicides rodenticides in the United States on the other [23.24].

Results and Discussion

The results of toxicity of the eight methanolic extract (Table 1) of *A. visnaga* against *T. aurantii* are represented in figure 1. Each extract was tested at five different doses (ng /aphids): 250, 500, $3x10^3$, $5x10^3$, 10^4 and $3x10^4$. The control mortality was below 10%. We noted that the number of individual death increased with the eight extract administered dose, however, there was a proportional relationship between doses and the observed mortality.

Indeed for the first extract (leaves before flowering period), the highest dose D_1 ($3x10^4$ ng/ aphid) caused 58.33% of mortality after 24 hours and 94.55% after 96h. After 24h, the lowest dose D_6 (250 ng / aphid) caused 11.8% of mortality while the doses between D_2 (10^4 ng/aphid) and D_4 (5.10^3ng / aphid) recorded mortalities that are closer to the order of 46% (Fig.1A).

Concerning the second extract (leaves during the flowering period), the highest dose (30000ng / aphid) has caused 61.67% of mortality, while the lowest dose D_6 (250 ng / aphid) caused 33.33% of mortality after 24 hours (Fig. 1B). 100% mortality is reached after 96h for all the administered doses.

While the third extract (*A. visnaga* leaves collected at the fructification period), a mortality of 88.33% was caused by the highest dose (3.10^4 ng/aphid) after 24h. This mortality reached 100% after 72 hours. While the lowest dose D_6 (250 ng / aphid) caused 43.33% of mortality after 24 hours, the mortality rate continues to increase to 82.27% after 96 h (Fig. 1C).

We note that the highest dose (3.10^4 ng/aphid) of the fourth extract (Flowers) caused a mortality of 88.33% after 24h. This mortality reached 100% after 96h (Fig. 1D). While doses D_2 and D_3 (10^4 and $5.\ 10^3$ng / aphid) caused a mortality of 86.67% and 73.33 respectively after 24 hours. These mortalities increased to reach 100% after 72 and 96 hours.

It was observed for the fifth extract (stems collected before flowering period), that the highest dose ($3x10^4$ng / aphid) caused a mortality of 98.33% after 24h. This mortality reached 100% after only 48 hours. While the dose D_2 (10^4 ng/aphid)

has caused 85% of mortality after 24 hours, the mortality reached 100% after 96h (Fig. 1E). A mortality of 55% was recorded by the lower dose after 24 hours, while 72.73% was recorded after 96h.

Closed mortalities of 86% were recorded for the highest doses D_1 (3.10^4 ng/aphid) and D_2 (10^4 ng /aphid) caused by the sixth extract (stems collected at the flowering period) after 24 hours, the mortality rate reached approximately 98% after 96h (Fig. 1F). In addition, the average doses D_3 (5.10^3 ng/aphid) and D_4 ($3x10^3$ ng / aphid) cause about 80% of mortality after 24h and reached approximately 98% after 96h. The lowest doses D_5 ($5x10^2$ ng / aphid) and D_6 (250 ng / aphid) cause approximately 57% of mortality after 24h, this mortalities increase to reach 78% and 90% of mortality after 96 hours.

For the seventh extract (steams collected at the fructification period), it was noted that the highest dose ($3x10^4$ ng/aphid) generates the highest mortality (88.33%) after 24h (Fig.1G). the second and the third dose (D_2 and D_3) caused 70% of mortality while the doses D_4, D_5 and D_6 generate mortality close to 55%. For doses D_1, D_2, D_3 and D_4 100% of mortality are achieved after only 48h (so, 100% of mortality are noted at 96h). While D_5 and D_6 reached approximately 90% after 96h.

Indeed, the highest dose (3.10^4 ng / aphid) of the eighth extract (Seeds) generated 86.67% of mortality after 24 hours and 100% mortality after 96 hours (Fig. 1H). The lowest dose D_6 (250 ng / aphid) caused only 35% of mortality after 24 hours, and the mortality rate continues to increase to 70.91% after 96h.

The results of the ANOVA (p<0.00001) showed that the treatment effect is highly significant after 24h, 48h, 72h and 96h in terms of sensitivity to all tested extract to *T. aurantii*.

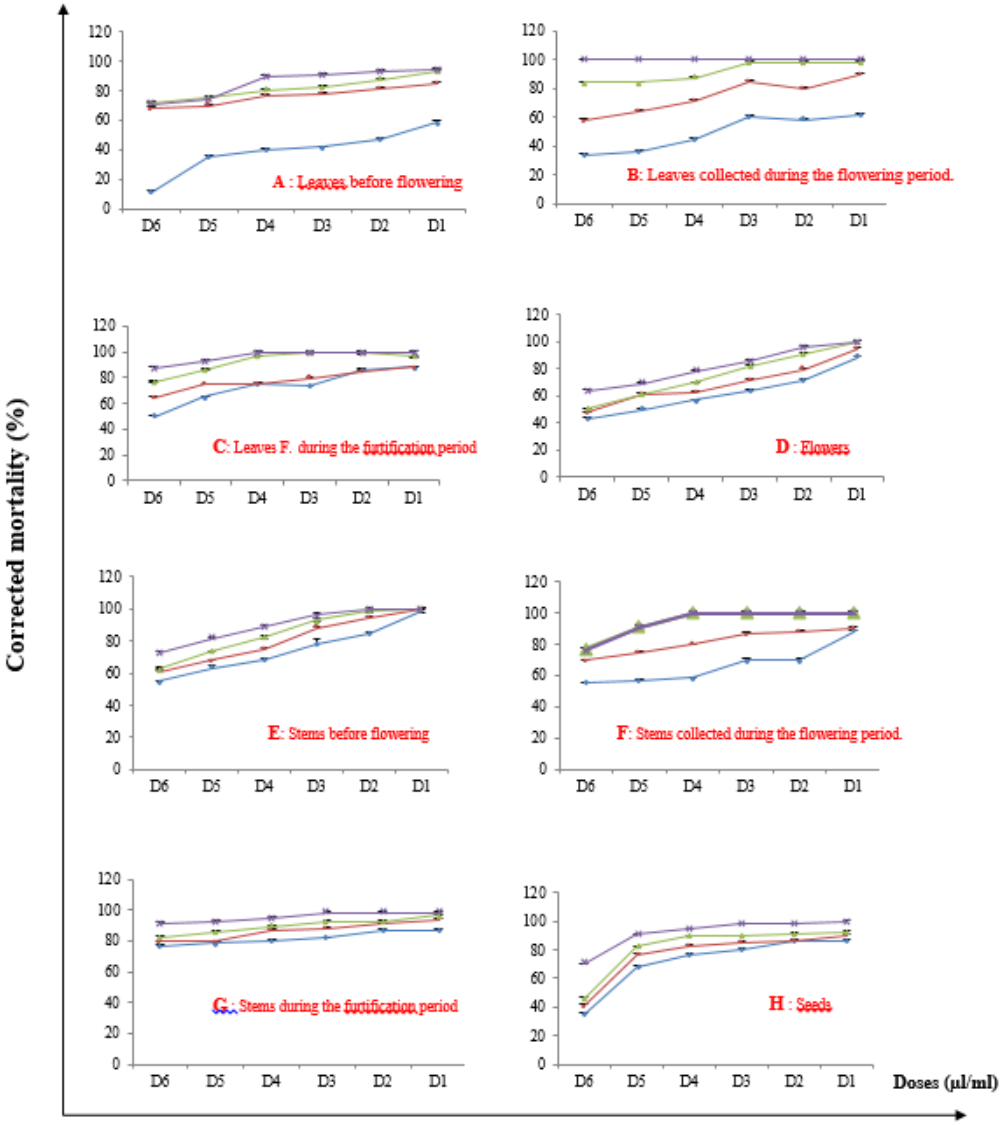

Fig.1 Doses-mortality relation after topic contact with different methanolic extract of *A. visnaga*

Mortality kinetics

The results of the mortality kinetics show that the mortality rate increases with the augmentation of *A. visnaga* methanolic extract doses (figure 2). Aphid's mortality was observed for six doses (ng/aphids): 250, 500, $3x10^3$, $5x10^3$, 10^4 and $3x10^4$.

A proportional relationship was noted between doses and the observed mortality (Figure 2 A.B.C.D.E.F and G). Mortality increases with the administered dose for the eight extract.

Indeed for the first extract (leaves collected before flowering period), the maximum of mortality is reached after 96 h for

doses that are between D_1 and D_4 (Fig. 2 A). Moreover, the second extract (leaves collected during the flowering period) generated 100% of mortality after 96 hours for all administered doses (Fig. 2 B).

While for the third extract (leaves collected during the fructification period), the maximum of mortality (100%) is reached after 96 hours for the doses D_1 and D_4 and after 72 hours for D_2 and D_3 (Fig. 2C). For the lowest doses D_5 and D_6, the maximum of mortality (92.73 and 87.27%) was reached after 96h. Furthermore, 100 % of mortality was achieved after 72 h for dose D_1 for the fourth extract (flowers).Whereas, the maximum of mortality was reached after 96 h for the doses D_2, D_3, D_4, D_5 and D_6 (Fig. 2D).

We record 100% of mortality after 72 hours caused by the sixth extract (steams collected during the flowering period) for doses D_1, D_2, D_3 and D_4 (Fig. 2E). The two lower doses D_5 and D_6 reach their maximum mortalities after 72 hours (91.07% and 76.79% respectively).

For the seventh extract (stems collected during the fructification period), we note that the highest dose D_1 (3.10^4 ng / aphid) reaches 100% of mortality after 96h. Doses D_2, D_3, D_4, D_5 and D_6 reached their maximum of mortality after 96h (Fig. 2F).

Indeed, on the eighth extract (seed), all administered doses recorded the highest mortalities after 96 hours with a percentage equal to 98.18% for D_1, D_2 and D_3 and 94.55%, 92.73%, 90.91% for doses D_4, D_5 and D_6, respectively (Fig. 2G).

Note that the mortality of the aphids increases with the elevation of the dose administered for all the methanol extract tested. Indeed, there is a direct proportional relationship between the dose of administered extract and observed mortality. The maximum of mortality is the most time reached after 96 hours with the exception of the extract of the flowers with dose D_1, the extract of leaves collected during the fructification period with D_2 and D_3 where 100 % of mortality was reached after 72 hours. The highest dose D_1 of stem collected before flowering period extract caused 100 % of mortality after 48 hours.

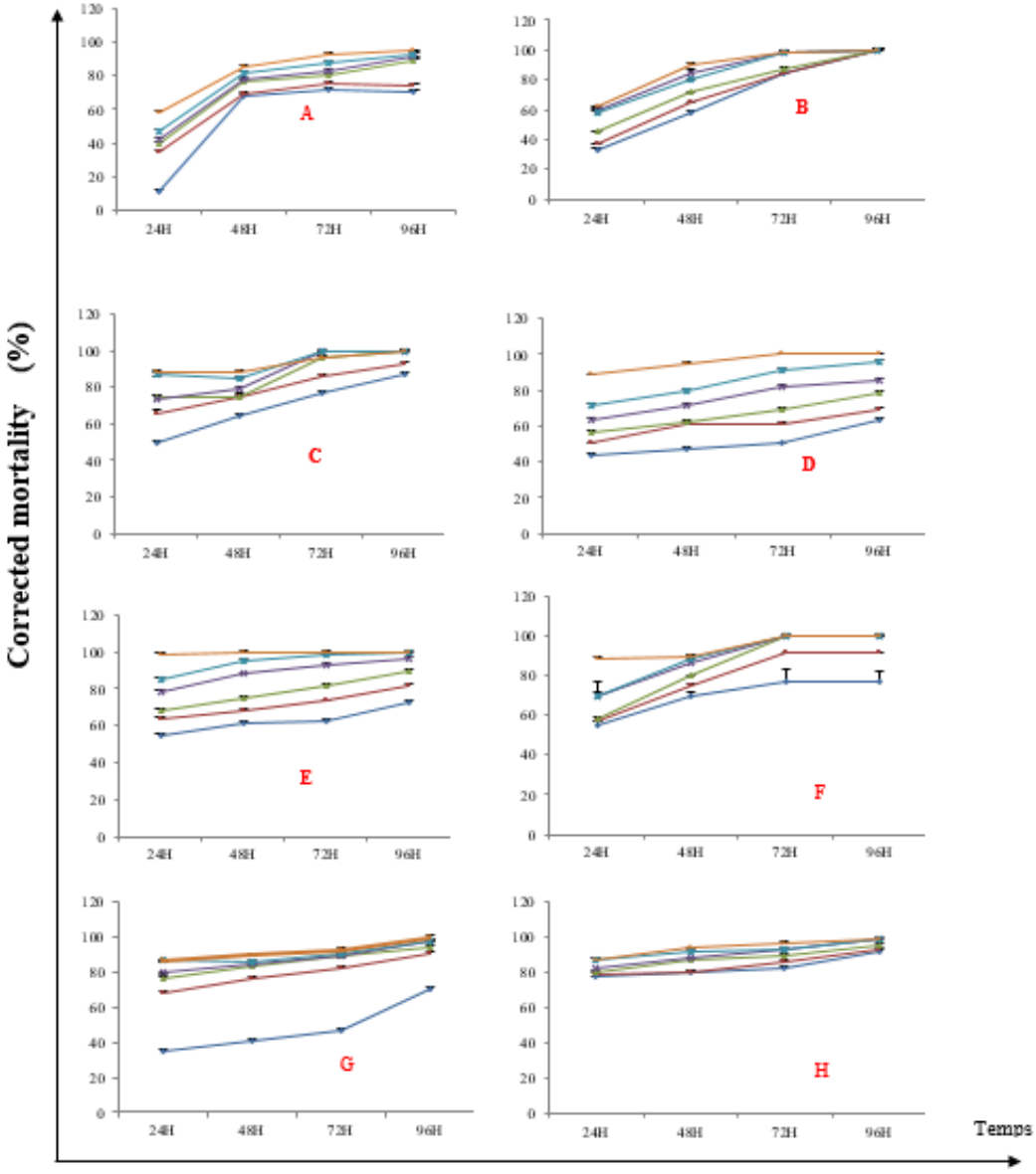

Fig. 2 Mortality Kinetic

— (♦)— D_6 = 250, D_5 = 500 — (■)— , D_4=3000 — (▲) — , D_3=5000 — (×)— ,

—(×)— D_2 = 10000, D_1=30000 — (●) — (Doses en ng/puceron)

With:
A: Leaves collected before flowering, **B** : Leaves collected during the flowering period, **C** : Leaves collected during the fructification period, **D** :Flowers, **E** : Steams collected before flowering, **F** : Steams collected during the flowering period, **G** : Steams collected during the fructification period, **H** :Seeds.

LD_{50} values

After drunkenness intoxication of aphids topically with the eight methanolic extract of *A. visnaga* representing different aerial parts collected during different periods of growth, mortality is monitored for 96 hours. The LD_{50} values for the eight extract tested for aphid are summarized in Table 2.

Table 2: Change in LD_{50} values of *A. visnaga* extract administered to *T. aurantii*.

	DL_{50} (ng /aphids)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
24h	11265.64 [D1 - D2]	3249.92 [D3-D4]	171.56< D_6	706.24 [D_4-D_5]	310.53 [D_5-D_6]	259.53 [D_5-D_6]	281.58 [D_5-D_6]	0.054 < D_6
48h	7.12 <D6	102.86 < D6	16.87 <D_6	383.11 [D_5-D_6]	268.49 [D_5-D_6]	11< D_6	218.91 < D_6	1.345 < D_6
72h	13.97 < D6	11.196 < D6	48.65 < D_6	438.02 [D_5-D_6]	205.82 < D_6	30.77 < D_6	70.17 < D_6	1.258 < D_6
96h	22 < D6	8.09 < D6	13.09 <D_6	238.53 < D_6	113.24 < D_6	30.15 < D_6	54.43 < D_6	0.153 < D_6

LD_{50} values of methanolic extract of the plant *A. visnaga* L. obtained for *T. aurantii* aphids are small. Indeed, after 24 hours the lowest LD_{50} values are respectively equal to approximately 171.65 ng / aphid for the extract of the leaves collected at the fructification period and 0.054 ng / aphid for seed extract topically (Table 2). However, after 48 hours the lowest LD_{50} values are respectively equal to 1.345 ng / aphid for seed extract, 7.12 ng / aphid for leaves collected before flowering period extract, 11 ng / aphid the extract of stem collected during the flowering period and 16.87 ng / aphid the extract of leaves collected during the fructification period topically. Whereas after 72 hours the lowest LD_{50} values are respectively equal to 1.258, 11.19, 13.97, 30.77, 70.17 and 48.65 ng / aphids representing respectively the extract of seeds, leaves of flowering period, leaves before flowering period, stems of flowering period, leaves and stems of fructification period. Thus, at the end of 96h, the LD_{50} values are equal to 0.153 ng/aphid for seed extract, 8 ng/aphid for the extract of leaves of flowering period, 13.09 ng/aphid for the extract of leaves of fructification period, 22 ng/aphid for extract of leaves collected before flowering period, 30.15 ng/aphid for extract from stems of flowering period, and 54.43 ng/aphids for the extract of stems of fructification period topically.

Several species of plant of the family *Apiaceae* are known by their significant acaricidal and insecticidal effect against a large number of insects [5]. It should be noted that few studies are made on the insecticidal effect of the essential oil or various extract of the plant *A. visnaga*. *This* extract have been reported to have an insecticidal activity and inhibitory effects on growth and development of *S. gregaria* [25]. These extract showed disruptive effects on the same locust *S. gregaria* [26]. Pavela [4, 27] mentioned that from 118 methanol extract of Euro-Asian plants tested against *Culex quinquefasciatus*, *A.visnaga* was the only species showing 100% mortality after 24 hours. The LC_{50} value denoted equal to 9 mg /l. The plant extract seems also to provide good protection for stored grain against granary weevil *Sitophilus granarius* [26] and rice weevil *Sitophilus oryzae* [28]. *A. visnaga* essential oils showed the stronger ovicidal effect against *Mayetiola destructor* [20] and a very interesting larvicidal effect against *Culex mosquitoes* [29].

In their whose comparative study, Zekri et al. [30] showed a high insecticidal effect with the *Mentha suaveolens* hydrosol which is more effective against citrus pests than that of *Mentha pulegium* L. So, both hydrosols were toxic toward *T. aurantii*, but *M. suaveolens* hydrosol has the highest insecticidal activity. According to the Zekri *et al.* [30], the degree of insecticidal effect depended significantly on applied doses and the age of tested aphids [30].

Conclusion

The toxicity of methanolic extract of *A. visnaga* was evaluated against *T. aurantii* and presented by the IC_{50} value. The lower values of LD_{50} were obtained after 96h. The most effective extract of *A. visnaga* is the extract of seeds with LD_{50} is equal to 0.153 ng/aphids. So, we concluded the effectiveness of methanolic extract and their LD_{50} values as follows: LD_{50} Seeds (0.054ng/aphids) > LD_{50} leaves of the flowering period (8.09 ng/aphids) > DL_{50} leaves of the fructification period (13.09 ng/aphids) > DL_{50} leaves of before flowering period (22 ng/aphids) > DL_{50} stems of the flowering period (30.15 ng/aphids) > DL_{50} stems of the fructification period (54.43 ng/aphids) > LD_{50} stems of before flowering period (113.24 ng/aphids) > LD_{50} Flowers (238.53 ng/aphids).

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