Effect of plant extract *Ruta graveolens* against green peach aphid, *Myzus persicae* , at Biskra oasis, Algeria

RAHMANI Sameh[1] TARAI Nacer[1] et ZELLOUF Ibtissem[1]

1 Department of Agricultural Sciences, Faculty of Exact Sciences , Natural and Life Sciences , University of Biskra

tarainacer@gmail.com

Introduction

The green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), is one of the most damaging insect pests of greenhouse crops, and has been causing considerable damage in Biskra oasis. To minimize the side effect of chemical use against *M. persicae*, a study was conducted at Oasis of Biskra, by applying plant extract of common rue, *Ruta graveolens* (Sapindales: Rutaceae). Extracts were sprayed on the first, second and sixth day of the week. Different extracts were used; seed extract and dry leaf extract, with three concentrations (10, 30, 50g/l). Extracts were tested on different larval stages and adults in the laboratory and greenhouse, during the autumn and winter period of years 2020-2021. Results showed that the mortality is related to the type and concentration of extracts, especially on larval stages. The highest cumulative larval mortality was found 72 hours after spraying the seed extract, with 83% larval mortality under laboratory conditions and 70% mortality under greenhouse. For adult, 70% mortality under laboratory conditions and 45% mortality under greenhouse.

Fig. 01 Extraction and dilution steps

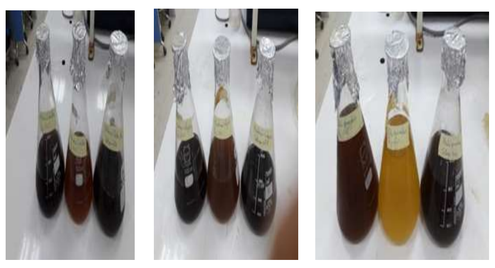


Fig. 02 Aqueous extracts of *Ruta graveolens*

Tab. 01 Retention times of chemical composition standards of *Ruta graveolens* aqueous extract analysed by HPLC

N°	Composites	Retention time (min)	ppm
01	Gardenin B	2.909	5.58
02	Hespéridine	2.992	4.8
03	Tetramethylscutellarein	3.704	-0.59
04	Pyrogallol	11.361	-7.48
05	Catéchol	11.726	-7.12
06	"2,3-Dihydroxy-1- guaiacylpropanone"	12.223	-7.58
07	Acide Gallic	12.24	-10.81
08	Quercétine	12.571	0.39
09	4-Methylcatechol	12.571	-3.89
10	Delphinidin 3-O-rutinoside	12.571	2.79
11	Acide homovanillique	12.604	-2.86
12	2,3-dihydroxy-1-guaiacylpropanone	12.604	5.12
13	Oleoside 11-methylester	12.737	0.63
14	Petunidin 3-O-rutinoside	12.969	-4.46
15	Peonidin 3-O-(6''-acetyl- galactoside)	12.985	-5.91
16	Acide coumarique	13.019	-10.74
17	Acide vanillique	13.4	-2.79
18	acide p-Coumaroyl glycolique	13.499	5.17
19	Piceatannol	13.615	-4.58
20	Pyrogallol	13.648	-7.37
21	Acide férulique	13.88	-3.85
22	5-Acide féruloylquinique	13.88	-5.06
23	Feruloyl glucose	13.715	2.67
24	Biochanin A	13.947	-2.76
25	Acide 3-sinapoyl quinique	14.046	4.49
26	Hespérétine	14.063	-1.5
27	Narirutin 4'-O-glucoside	14.063	2.32
28	Resveratrol	14.394	-2.89
29	Esculetine	14.991	-7.33
30	Anéthole	16.864	15.77

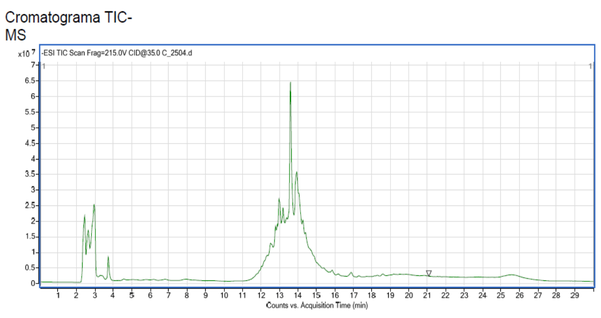


Fig. 04 Chromatography of the aqueous extract of *Ruta graveolens* analysed by HPLC

MATERIALS AND METHODS

1. Aqueous extract preparation

After collection, the plant material was dried at room temperature in the shadow for one month (Koïta et al., 2012). Then, the plant was ground by an electric grinder to obtain a fine powder and conserved at room temperature in a hermetically closed glass bottle until used (N'Guessan et al, 2009). The powdered aerial parts (100 g) were macerated in distilled water (1 L) for 24 h with a constant agitation speed of 200 rpm, at room temperature. The suspension was then filtered on Whatman paper (3 mm) (Aouinty et al., 2006). The filtrate recovered represents an initial stock solution at 100 g/l, i.e. 10%. After preliminary tests on different concentrations, we chose three concentrations: 10 g / l (C1), 30 g / l (C2) and 50 g / l (C3). The prepared extracts are conserved in hermetically closed bottles at a temperature of 4°C until used (Fig. 01, 02).

2. Toxicity tests of aqueous extracts on *Myzus persicae*

2.1. Spraying of the extracts

The treatment was carried out in a greenhouse at Biskra oasis and laboratory where the study of the evolution of the population is effected before the treatment. For each concentration of the three extracts as well as for the control (distilled water), three repetitions were executed. Sampling and calculation of the mortality rate are executed after 24 h.

2.2 Calculation of mortality rates

Larvae and dead adults are counted every 24 hours for a period of 120 hours. The observed mortality is expressed after correction by the formula of Henderson and Tilton (1955):

$$\text{Corrected (\%)} = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}} \times 100\right)$$

n = Nbre of *Myzus persicae* population
T = Treated
Co = Control

2.3 Analysis of the aqueous extract by HPLC

The phenolic composition of the extracts was determined by HPLC analysis. This was realized with an HPLC system coupled to LC-MS Agilent 6520 QTOF masses. The separation was performed on a ZORBAX Eclipse plus 100 mm x 4.6 mm, 3.5 µm column. The mobile phase contained two solvents: A) acidified H_2O solution with 0.1% formic acid and B) acetonitrile MS with 0.1% formic acid. The flow rate was maintained at 0.350 ml/min. The gradient programme was as follows: (95% A / 5% B) (0 min), (95% A / 5% B) (5 min), (10% A / 90% B) (15 min), (10% A / 90% B) (20 min), (95% A / 5% B) (25 min), (95% A / 5% B) (30 min). The analysis was performed in negative mode with a source of 3500V capillary voltage and 180V fragment voltage. Peaks were identified by comparing retention times with those of pure standards available in the enphenol-explorer.eu database

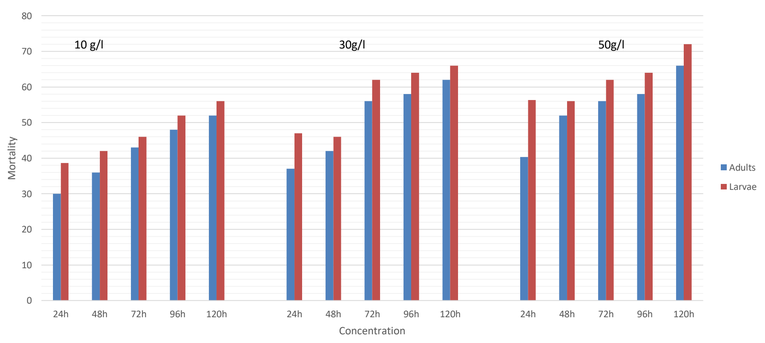
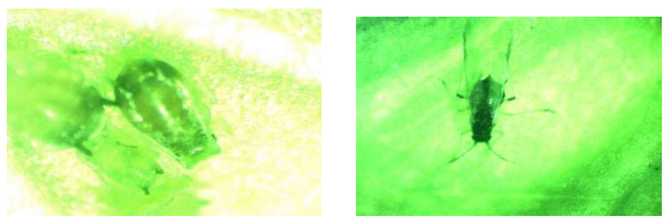


Fig. 05 Mortality rate of *Myzus persicae* treated with the three concentrations of the aqueous extract of *Ruta graveolens* under laboratory conditions



Larva Adult

Fig. 03 *Myzus persicae , larvae and adults* damage on pepper under greenhouse

RESULTS AND DISCUSSION

1. Corrected mortality rates of *Myzus persicae* exposed to *Ruta graveolens* during 24h, 48h, 72h, 120h

1.1 Adults

The results obtained on the efficacy of the aqueous extract of *Ruta graveolens* on the adults, indicate that the concentration 50 g/l represents the highest percentage of mortality compared to the other concentrations 30 g/l and 10 g/l. It is noted that after the first 24 hours of contact with the insecticide, the mortality rate of the adults reached 40.33% with concentration 50 g/l against 30.33% and 37% with concentrations 10 g/l and 30 g/l, respectively. After 48 to 120 hours, the 50 g/l concentration represents the mortality percentage of 50% and 64.33%, respectively (Fig. 05).

1.2 Larvae

The results obtained indicate that the 50 g/l concentration gave the highest mortality rate of *Myzus persicae* larvae during the time of exposure to the aqueous extract of *Ruta graveolens*, followed by the 30 g/l and 10 g/l concentrations, respectively. For example, during the first 24 hours, the mortality rate of the larvae treated with the 50 g/l aqueous extract was 56.33% compared to 45% and 38.67% for the 30 g/l and 10 g/l, respectively. After 48 hours and up to 120 hours of exposure to the biopesticide, the mortality rate of 50 g/l still represents the highest percentage with 66% and 72%, respectively.

HPLC-MS analysis of the aqueous extract of *R. graveolens* identified 30 components. The main components are: anethole (15.77 ppm), gardenin B (5.58 ppm), p-coumaroyl glycolic acid (5.17 ppm), 2,3-dihydroxy-1-guaiacylpropanone (5.12 ppm), hesperidin (4.8 ppm) and 3-sinapoyl quinic acid (4.49 ppm). (Tab. 01).

Aqueous extracts of *Ruta graveolens* is a bioinsecticide against *Myzus persicae* . The same remark is indicated by Flávia et al (2011) against *Tuta absoluta*. Chacko et al (2015), reported that the ether extract of *Ruta graveolens* has a larvicidal activity against Anopheles stephensi and Aedes aegypti with a mortality rate of 100% and 80%, respectively. (Ben Chaaban et al, 2019) reported that Ruta graveolens essential oils were toxic to *Empestait kuehniella* with 88% mortality after 48 h.

(Lee et al, 2004) showed that the high efficacy of *Ruta graveolens* essential oil as a fumigant against *Sitophilus sp*. According to (Perera et al, 2016), *Ruta graveolens* essential oil has a high insecticidal and repellent potential against Sitophilus oryzae, producing the highest mortality rate of rice weevils (100%) after one hour of exposure.

Conclusion

The study of the effect of extract, Ruta graveolens, shows a good insecticidal action against *Myzus persicae*. The plant *R. graveolens* is more efficient against the larvae.

There is a correlation between the concentrations of the aqueous extracts used and the time after treatment.

The results obtained show that aqueous extracts of medicinal plants are effective. The application of biopesticides is of major importance in terms of cost and quality.

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