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Research Article

Effects of wild rose (*Rosa sp.*) crude seed extracts on two legume aphids

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ABSTRACT: Crude hexane seed extracts of four wild species of roses of *Caninae* Section: *Rosa canina* L., *Rosa dumetorum* Thuiller., *Rosa pomifera* Herm. and *Rosa rubiginosa* L., from Kairouan and Beja regions, were screened for their insecticidal activity on two aphid species: black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Scop.) which feed on several cultivated species. The composition of hexane seed extracts permitted the identification of fifteen compounds. *R. dumetorum* was richeest in palmitic acid (7.1%), stearic acid (2.6%), oleic acid (22.2%). *R. ruboginosa* (Kairouan region) was richer in linoleic acid (52.8%) whilst *R. pomifera* was higher in linolenic acid (26.9%).Tests were conducted using *in vitro* spraying of each rose seed extract at four doses: 0, 10, 20 and 40 mg ml^{-1}. Results showed a greater sensitivity of *Aphis fabae* to the rose extracts. Indeed, at the dose of 40 mg ml^{-1}, the mortality varied from 25 to 47% for *Aphis fabae* and from 22 to 34% for *Acyrthosiphon pisum*. The maximum of mortality for the two aphid species was observed with the hexane extracts of *R. dumetorum*. This insecticidal activity may be due to the presence of arachidic acid in the tested extracts.

KEYWORDS: *Acyrthosiphon pisum*, *Aphis fabae*, fatty acids, insecticidal activity, *Rosa canina*, *Rosa dumetorum*, *Rosa pomifera*, *Rosa rubiginosa*

INTRODUCTION

Rose is one of the most important ornamental plants in the world. It is also grown for its fruit (rose hips) which have culinary and medicinal values, because of their high amount of vitamin C, carotenoids, polyphenols and various flavonoids such as anthocyanins.[1] However, the seeds, representing 30% of the rose hip fruit, are considered a low value by product.[2] Rose hip seeds contain 2.7–7.1% of oil (mainly unsaturated fatty acids),[3] 6.9–8.6% protein and 0.22–0.44 mg of ascorbic acid per 100 g of seeds.[2]

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The valuable oil extracted from seeds is used in hair and skin care products because of its high content of linoleic and linolenic acids.[2] Rose hip oil is also recommended for use in sun protection products. It contains natural retinoic acid which is used for the treatment of eye wrinkles, stretch marks and unsightly spots.

Whilst rose oil has long been used for medicinal and cosmetic purposes, it can also be used in the biological control of pests. Indeed, some fatty acids have shown activity on insect pests.[4] Ramswak *et al.*[5] reported insecticidal and an insectistatic activities of linoleic acid on *Anopheles aegyptii* L. (Diptera: Culicidae), *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), *Lymantria dispar* L. (Lepidoptera: Lymantriidae), *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae) and *Orgyia leucostigma* Smith (Lepidoptera: Lymantriidae). Additionally, studies have shown that oleic acid has insecticidal activity on *Anopheles aegyptii* L., *Anopheles stephensi* Liston (Diptera: Culicidae).[6,7] Linolenic acid has activity on *Liposcelis bostrychophila*

Badonnel (Psocoptera: Liposcelididae) and *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae).[8] Studies reported that linoleic, palmitic and stearic acids showed insecticidal activity on *Spodoptera frugiperda* (Lepidptera: Noctuidae).[9,10] Farag *et al*.[11] reported also an insecticidal activity of linoleic and oleic acids on *Spodoptera* littoralis Boisduval.

Aphids are one of the most important groups of insect pests in the world. Actually, about 4,000 aphid species have been described and about 250 species are serious pests to various crops and ornamental plants around the world. Aphids develop at prodigious rates by parthenogenesis and have an efficient dispersal strategy. Their feeding of phloem sap causes stunting, discoloration and deformation of plants, and aphids are major vectors of plant viruses. Although many products belonging to existing insecticide groups are effective on aphids, resistance to insecticides that have a long history of use, such as organophosphates, carbamates and pyrethroids, is a serious problem to farmers and the environment, beneficial insects and natural enemies.[12]

The objectives of this study were to evaluate the insecticidal effects of crude hexane seed extracts of four wild species of roses from Caninae Section on two aphid species: black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Scop.), and to determinate the composition of the crude extracts.

MATERIALS AND METHODS

Seeds oils

Samples of *Rosa* seeds were collected from the North, the Northeast, the Centre and the South of the Tunisian dorsal area, at altitudes between 228–942 m. Four wild species of roses were used: *Rosa canina* L. from Zaghouan region (north), *Rosa dumetorum* Thuill from Seliana region (centre), *Rosa pomifera* L. from Kairouan region (south) and *Rosa rubiginosa* L. from Kairouan and Beja (northeast) regions. All voucher specimens were deposited in the Higher Institute of Agronomy of Chott Meriem - Sousse, Horticultural Laboratory herbarium and were assigned for each one a corresponding number (codes RR 110–RR 114). The seeds were air dried, ground with a coffee grinder and 100 g of each samples was extracted with pure hexane (5 × 200 ml) over 5 days. The extracts were vacuum-filtered and the filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The dried extracts were resuspended in a small volume of hexane and transferred to pre-weighed vials. After evaporation of the hexane, the vials were re-weighted to determinate the yield of the extract.

The fatty acids gas chromatography analysis requires their transformation into methyl esters. 100 µl of the crude extract was evaporated under nitrogen flow, then 2 ml of hexane, 1 ml of heptadecanoic acid methyl ester as an internal standard and 0.5 ml of sodium methylate (3%) were added. After stirring during 1 min and standing for 2 min, the mixture was neutralized by 0.2 ml of H_2SO_4 (1 N), then the methyl esters were washed with 1.5 ml of distilled water. The superior phase was poured off and evaporated under nitrogen flow. Fatty acids methyl esters were analysed by GC–FID in capillary column HP INNOWAX (30 m x 0.25 µm x 250 µm). The identification of the different fatty acids in the extract was achieved by referring to the analytical standard SUPELCO; F.A.M.E. Mix, C8-C22.

Insect culture

Black bean aphid (*Aphis fabae* Scop.) and pea aphid (*Acyrthosiphon pisum* Scop.) were collected from broad bean farm and reared on beans plants in the laboratory under these conditions: temperature 26 ± 2 °C, humidity 70 ± 5% and photoperiod 16 hr.

In vitro toxicity test

Bean leaves infected by aphid were introduced in a Petri dishes covered with moist filter paper. Leaves of rose plants were sprayed with the rose hip seed oils using four doses: 0, 10, 20 and 40 mg ml^{-1}. For each dose, four replications were used. A quantity of tween 20 (1%) was added to provide a good emulsification of the solution. Petri dishes were maintained in the same conditions described before. Mortality was assessed 24 hours after, aphids was examined under stereomicroscope and considered dead when they haven't reaction to mechanic stimulation.

Statistical analysis

The percentage of mortality was subjected to analysis of variance (ANOVA). Differences among treatment means were analysed using Duncan test (SPSS 11) for Windows. Differences between means was considered significant when $P < 0.05$ Correlation between fatty acids and percentage of insect's mortality was calculated using Pearson coefficient.

RESULTS AND DISCUSSIONS

The oil yields in hexane seed extracts of the roses genotypes varied between 10.66% in *R. rubiginosa* from

Kairouan region and 4.76% in *R. canina*. Fourteen compounds identified by GC-FID analysis, representing 99.1% of the extracted materiel of *R. pomifera, R. rubiginosa* (Kairouan region) and *R. dumetorum* and 87.0% of *R. canina* and *R. rubiginosa* (Beja region). The main components were the linoleic acid (52.84%) in *R. rubiginosa* Kairouan, the linolenic acid (26.93%) in *R. pomifera*, the oleic acid (22.24%) and the palmitic acid (7.13%) in *R. dumetorum* (Table 1).

This composition differs from that reported by Barros *et al*.[13] who identified twenty one components in a methanolic seed extract of *Rosa canina*. The main compounds reported were linoleic acid (43.5%), oleic acid (19.0%), linolenic acid (15.8%), palmitic acid (10.1%) and stearic acid (4.6%). Kazaz *et al*.[3] found five components in a petroleum ether seed extracts of *R. canina*. The main compounds were linoleic acid (48.8%), oleic acid (22.1%) and linolenic acid (20.6%).

This composition differs from other species already known for their insecticidal activity. Indeed, Pérez-Gutiérrez *et al*.[10] found nineteen compounds in a seed chloroformic extract of *Carica papaya*. The main components were palmitic acid (24.1%) and oleic acid (45.9%). Adebowale and Adedire[8] found eight compounds in a petroleum ether seed extract of *Jatropha curcas* and the main compounds were linoleic acid (47.3%), stearic acid (17.0%) and oleic acid (12.8%). Ramos-Lopez *et al*.[4] found that the proportion of linolenic acid and linoleic acid in hexane leaf extracts of *Ricinus communis* was 47.7 and 15.8%, respectively.

The toxicity test realized by spraying infected bean leave's with *A. fabae* and *A. pisum*, by seed rose hip oils showed a general increase in insect mortality with increasing doses of seed oil (Figures 1 and 2). The highest insect mortality was observed on leaves treated with *R. dumetorum* seed oil at the dose of 40 mg ml^{-1}: 33.7 and 47% for *Acyrthosiphon pisum* and *Aphis fabae*, respectively. At this dose, the lowest mortality was observed on leaves treated with *R. rubiginosa* Beja region seed oil: 21.9 and 25.1%, for *Acyrthosiphon pisum* and *Aphis fabae*, respectively.

The seed oil of *R. dumetorum* was rich in palmitic, oleic and stearic acids and there was a positive correlation between the percentage of mortality of *A. fabae* and palmitic acid (R^2 = 72%), stearic acid (R^2 = 68%), arachidic acid (R^2 = 72%), behenic acid (R^2 = 76%) and euricic acid (74%) percentages, and also a positive correlation between the percentage of mortality of *A. pisum* and the percentages in stearic acid (R^2 = 69%), arachidic acid (R^2 = 76%) and behenic acid (R^2 = 71%) (Table 2).

In a similar study, the spraying of *Jatropha curcas* oils on broad bean plants infested by *A. fabae* caused a mortality of 56.9% at the concentration of 50 mg ml^{-1} and 88.1% at the concentration of 150 mg ml^{-1}.[14]

Table 1: Composition (%) of four rose species seeds in oil and fatty acids

Oil and fatty acids	Carbon Length	*R. canina* Zaghouan	*R. rubiginosa* Beja	*R. rubiginosa* Kairouan	*R. dumetorum* Seliana	*R. pomifera* Kairouan
Total oil contents	_	4.76	5.71	10.66	6.66	9.11
Caprylic acid	C8:0	0.12	0.19	0.09	0.04	0.05
Capric acid	C10:0	0.02	0.03	0.02	0.01	0.02
Lauric acid	C12:0	0.03	0.03	0.01	0.03	0.25
Myristic acid	C14:0	0.05	0.04	0.04	0.06	0.03
Pentadecanoïc acid	C15:0	0.16	nd	nd	nd	nd
Palmitic acid	C16:0	4.36	5.27	5.87	**7.13**	4.47
Palmitoleic acid	C16:1	0.22	0.23	0.22	**0.32**	0.17
Stearic acid	C18:0	2.50	2.07	2.32	**2.64**	2.42
Oleic acid	C18:1	16.69	16.50	18.40	**22.24**	11.28
Linoleic acid	C18:2	46.70	46.06	**52.84**	45.35	52.75
Linolenic acid	C18:3	15.27	15.70	18.56	19.94	**26.93**
Arachidic acid	C20:0	0.88	0.70	0.83	**1.19**	1.03
Eicosenoic acid	C20:1	0.38	0.30	0.32	0.39	0.28
Behenic acid	C22:0	0.13	0.10	0.12	0.17	0.13
Euricic acid	C22:1	0.20	0.06	0.21	0.33	0.04

nd: not detected.

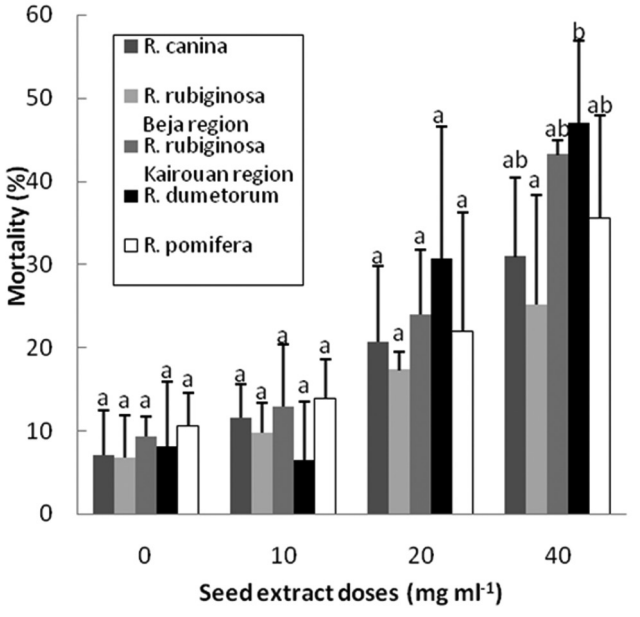

Figure 1. Percentage of mortality of *Aphis fabae* Scop. treated with hexane seed extracts of four wild Tunisian roses. Values with different letters are significantly different at $P < 0.05$. Differences were assessed between oils of different wild species of roses.

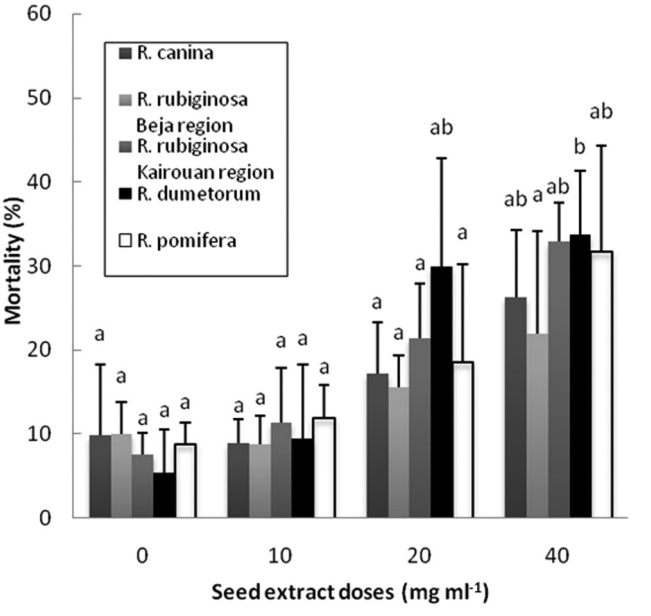

Figure 2. Percentage of mortality of *Acyrthosiphon pisum* Scop. treated with hexane seed extracts of four wild Tunisian roses. Values with different letters are significantly different at $P < 0.05$. Differences were assessed between oils of different wild species of roses.

Table 2: Matrix correlation between fatty acids compounds and percentage of mortality of *Aphis fabae* Scop. and *Acyrthosiphon pisum* Scop

Fatty acids	Carbon length	Mortality of *A. fabae*	Mortality of *A. pisum*
Caprylic acid	C8:0	–0.82	–0.93
Capric acid	C10:0	–0.87	–0.71
Lauric acid	C12:0	–0.08	0.22
Myristic acid	C14:0	0.49	0.25
Palmitic acid	C16:0	***0.72**	0.48
Palmitoleic acid	C16:1	0.49	0.20
Stearic acid	C18:0	**0.68**	**0.69**
Oleic acid	C18:1	**0.54**	0.23
Linoleic acid	C18:2	0.22	0.46
Linolenic acid	C18:3	0.35	**0.60**
Arachidic acid	C20:0	**0.72**	**0.76**
Eicosenoic acid	C20:1	0.33	0.13
Behenic acid	C22:0	**0.76**	**0.71**
Euricic acid	C22:1	0.74	0.52

*Values in bold represent positive correlation between fatty acids and mortality.

CONCLUSIONS

Rose seed oils were found to be rich in unsaturated fatty acids and an insecticidal activity has been demonstrated on *Aphis fabae* and *Acyrthosiphon pisum*. This toxicity increases when the dose of oil increased and the high percentage of mortality was observed with *R. dumetorum* oils. This insecticidal activity could be more important by increasing oil doses and the quantification of dead insects could last until 120 hours.

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