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INSECTICIDAL ACTIVITY OF ALGERIAN CASTOR EXTRACTS *RICINUS COMMUNIS* AGAINST THE GREEN ROSE APHID *MACROSIPHUM ROSAE*

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Abstract

Description of the subject: Presently, biopesticides are gaining more and more attention from researchers as an alternative to persistent chemical pesticides.
Objective: The insecticidal activity of leaves extracts from Castor (*Ricinus communis* L.) was evaluated against green rose aphids *Macrosiphum rosae* L. affecting the propagation of ornamental plants.
Methods : The biological extracts of the Castor, namely the aqueous extract, the ethanolic extract, the methanolic extract, and the acetonic extract were obtained by extraction with an appropriate solvent. The functional groups of the biomolecules have been determined by spectroscopy. The polyphenols are assayed by spectrophotometry according to the Folin-Ciocalteu method. The toxicity tests against aphid larvae were carried out under laboratory conditions.
Results : The highest yield was obtained from the aqueous extract (31±0.69%). Analysis by spectrophotometry indicates that the acetonic extract is the richest in polyphenols (151.66±0.85 mg EGA / g). The bioinsecticidal effect shows that the acetonic extract is the most effective against *Macrosiphum rosae* L. with a mortality rate of 93.33 ± 6.69% after 48 h of exposure and of 100% after 96 h of contact. The LC_{50} and LT_{50} values are 0.62 mg/ml and 19.19h, respectively.
Conclusion : The present study shows the effectiveness of plant extracts, in particular *Ricinus communis* L. in controlling the green rose aphid *Macrosiphum rosae* L. This will allow farmers to control pests with minimal negative impact on the environment.
Keywords : Insecticidal activity ; Biopesticides ; Green aphid ; Acetonic extract ; *Ricinus communis* L.

ACTIVITÉ INSECTICIDE DES EXTRAITS DE RICIN D'ALGÉRIE *RICINUS COMMUNIS* CONTRE LE PUCERON VERT DU ROSIER *MACROSIPHUM ROSAE* (HEMIPTERA : APHIDIDAE)

Résumé

Description du sujet : A l'heure actuelle, les biopesticides attirent de plus en plus l'attention des chercheurs comme une alternatif de lutte aux pesticides chimique persistants.
Objectifs : L'activité insecticide d'extraits de feuilles du Ricin (*Ricinus communis* L.) est évaluée contre le puceron vert du rosier *Macrosiphum rosae* L. affectant la propagation des plantes ornementales.
Méthodes : Les extraits biologiques du Ricin à savoir l'extrait aqueux, l'extrait éthanolique, l'extrait méthanolique, et l'extrait acétonique ont été obtenus par extraction avec un solvant approprié. Les groupements fonctionnels des biomolécules ont été déterminés par spectroscopie infrarouge. Le dosage des polyphénols est effectué par spectrophotométrie en utilisant la méthode de Folin – Ciocalteu. Les tests de toxicités vis-à-vis les larves de puceron ont été réalisé dans des conditions du laboratoire.
Résultats : Le rendement le plus élevé a été obtenu à partir de l'extrait aqueux (31±0,69%). L'analyse par spectrophotométrie indique que l'extrait acétonique est le plus riche en polyphénols (151,66±0,85 mg EGA/g). L'effet bioinsecticide montre que, l'extrait acétonique est le plus efficace contre *Macrosiphum rosae* L. avec un taux de mortalité de 93,33±6,69 % après 48 h d'exposition et de 100 % après 96 h de contact. Les valeurs de CL_{50} et de TL_{50} sont respectivement 0,62mg/ml et 19,19h.
Conclusion : La présente étude montre l'efficacité des extraits de plantes, en particulier *Ricinus communis* L. dans la lutte contre le puceron vert du rosier *Macrosiphum rosae* L. Cela permettra aux agriculteurs de lutter contre les ravageurs avec un minimum d'impact négatif sur l'environnement.
Mots clés : Activité insecticide ; Biopesticides ; Puceron vert ; Extrait acétonique ; *Ricinus communis* L.

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INTRODUCTION

Roses are one of the most beautiful ornamental flowers in the world [1], which are always refered to "the Queen of flowers". Besides to their use in decoration, in the perfume and cosmetics industry, mainly to produce rose oil, rose water and essential oils. Roses flowers are used also for medicinal purposes [2, 3]. However, numerous insect pests infest rose among them, the rose aphid *Macrosiphum rosae* L. which is considered to be the most important pest of roses due, not only, to the significant economic damage it causes [4], but also the lowering of the ornamental value of shrubs and urban landscapes [5]. *Macrosiphum rosae* L. attacks many of fruit trees and shrub among them photinia and pyracantha, which are closely related to the same family as roses (Rosaseae) [6]. In case of severe infection, it causes deformation of the petals which reduces the beauty of the flowers as well as the transfer of phytopathogenic viruses and their diseases [1, 5, 7]. In addition, this aphid secretes honeydew, which causes the development of saprophytic fungi on plants [1, 8]. The high abundance and diversity of aphids in the different regions of Algeria is serious and requires emergency intervention [9]. Although the use of chemical insecticides seems to be effective, several studies indicate that aphid populations often cause resistance and environmental problems [10], in addition to health problems and harmful effects on non-target organisms [3]. This situation requires the installation of a new alternative with low risk and without side effects to control insect pests such as the use of plant-based biopesticides which has proven its effectiveness, according to recent researches [11, 12]. Castor (*Ricinus communis L.*.) is an Euphorbiaceae plant widely recognized for its insecticidal power [13]. However, no study has been undertaken on the effect of its biological extracts on *Macrosiphum rosae* L. The main objective of which is to find an alternative to chemical control by using organic extracts of *Ricinus communis L.*in order to remedy the damage caused by these harmful insects.

MATERIEL AND METHODES

1. Plant material

The leaves of *R. communis* L. were harvested during the month of February 2021 in the region of Ouled Haddadj, wilaya of Boumerdes (36 ° 462 003 north, 3 ° 282003 east) situated at 45 km east of Algiers, Algeria. The choice of Castor is based on its availability and its use as a bioinsecticidal purposes.

2. Animal material

The insects *Macrosiphum rosae* L. were collected from an untreated site in the garden of the Faculty of Science of the University of Boumerdes, Algeria. The climate of the Boumerdes area is Mediterranean. This last belonged to the sub-humid bioclimatic stage with a mild winter and a hot and dry summer, despite the disturbances of recent years due to problems related to the phenomenon of climate change. Aphids were collected from infested plants using brushes and transported to the insectarium in Petri dishes. The specimens were sorted, identified and then distributed over different groups for the evaluation of the biotests.

3. Extracts preparation

The leaves of *R. comminus* L. were washed in running water and dried at room temperature (18 to 24° C) in a ventilated place away from light for Three weeks. Then, they were powdered in an electric mixer (Moulinex, France). The extracts were prepared either by infusion for the aqueous extract [14, 15], or by maceration in the various organic solvents (ethanolic, methanolic, and acetonic extracts) by mixing 20g of the plant powder with an appropriate volume of the solvent for 24, 48 and 72h. The whole is put under magnetic stirring. After each time interval, the extract is filtered and the residue obtained and taken up for a second extraction with a renewal of solvent. At the end of the extractions, the collected filtrate is subjected to vacuum evaporation at 40 °C to remove the solvent using a rotavapor (Stuart RE300DB, UK) [16].

4. Dosage of polyphenols

The Folin-Ciocalteu method was used for the determination of polyphenols [17]. For this purpose, 0.5 ml of each extract was diluted in 5 ml of distilled water. Then 0.5 ml of Folin-Ciocalteu reagent was added. After 3 min of reaction, 0.5 ml of 20% sodium carbonate (Na_2CO_3) was also added.

The mixture was then incubated for 1 h at room temperature and protected from light. The absorbance of the extract was measured at 760 nm by a spectrophotometer (Optizen 2120 UV/Vis, Korea). The concentration of polyphenols was deduced from the calibration curve established by gallic acid at different concentrations under the same conditions as the sample. The polyphenol content is expressed in micrograms equivalent gallic acid per gram of dry matter (mg EGA / g).

5. Infrared spectroscopy

The spectroscopic characterization was carried out using a spectrophotometer of the Thermo Scientific Nicolet IS 10 type, equipped with the Smart ITR ATR Thermo Scientific module. The analysis was carried out with 40 scans and 2 cm^{-1} resolution, in the range of 4000 to 600 cm^{-1}. The main component of the ATR module (SMART Itr) is a highly sensitive Zn SE crystal. In ATR mode, it does not support liquid samples for this purpose; it is recommended that liquid samples be prepared in KBr pellets for processing in transmission mode.

6. Insecticidal toxicity test

The insecticidal activity of the Castor leaves was carried out on *Macrosiphum rosae* L. larvae aged from 3 to 4 days. For each extract, three repetitions were carried out. The control group was treated only with distilled water. The solutions to be tested were prepared the same day as the treatment. For each extract, five concentrations were prepared (D1, D2, D3, D4 and D5) which correspond to 0.625; 1.25; 2.5; 5 and 10 mg/ml respectively. For the aphicidal test, groups of 20 aphid larvae were placed in Petri dishes containing a filter paper with 9cm of diameter (Whatman No. 1, 63.62 cm² of surface) previously soaked with the extracts to be tested. The kinetics of mortality were followed and the mortality rate was determined after 24h, 48h, 72h and 96h. Insects were considered dead, when no leg or antennal movements were observed [18]. The mortality rate was calculated and corrected according to Abbott's formula [19]:

$$Mortality\ rate\% = \frac{Death\ number}{Total\ number\ of\ individuals} x100$$

$$Corrected\ mortality\ (\%) = [Mortality\ in\ treated\ group\ -\ Mortality\ in\ the\ control\ group\ /100 - Mortality\ in\ the\ control\ group]\ x100$$

7. Statistical analysis

Results of aphicidal activity were expressed as mean±SEM. The statistical comparison between the groups was performed with one-way ANOVA analysis followed by the Tukey test using the statistical presentation system Origin 2018. A value of $p<0.05$ was considered significant. In order to determine the values of LC_{50} and LT_{50}, regression lines Probits=f (log concentration) and Probits=f (log time) were respectively plotted.

RESULTATS

1. Phytochemical study of R. communis

The extract obtained has a caramelized appearance of dark green color. The values relating to the yields of the different extracts and to the dosage of polyphenols are represented in the following table (Table 1). The highest yield is obtained by the aqueous extract (31±0.69 %), followed by the ethanolic extract (17.7±0.34%), then the methanolic extract (12.3±0.34%). The lowest rate is obtained for the acetonic extract with 5.39±0.36 %. In addition, for the polyphenol concentrations, our results revealed a high polyphenol richness of the acetonic extract with a value of 151.66±0.85 mg EGA / g, followed by the aqueous extract (60.71±0.82 EGA / g) and methanolic extract (44.32±0.21 EGA / g MS). However, the lowest levels are attributed to the ethanolic extract with 34.98±0.61 mg EGA / g.

Tableau 1: Extraction yield and concentration of polyphenols in *R. communis* extracts

Extracts	Yield (%)	Dosage (mg GA/g)
AcEx	5.39±0.36	151.66±0.85
AqEx	31±0.69	60.71±0.82
MeEx	12.3±0.34	44.32±0.21
EtEx	17.7±0.34	34.98±0.61

AcEx: acetonic extract, AqEx: aqueous extract, MeEx: methanolic extract, EtEx: ethanolic extract, mg EGA/g: milligram equivalent gallic acid per gram

2. Infrared spectral analysis

The infrared spectrum representing the absorbance values (A%) as a function of the wave number (cm^{-1}) of the leaves of *R. communis* is represented in the following figure (Fig. 1). The infrared spectrum of the *R. communis* L. leaves shows the presence of different functional groups in the Castor biomolecules. These bonds correspond mainly to the Phenol (O-H), Methylene (C-H) and Primary amines (N-H) functions, with respective wave numbers of 3291.29, 2925.84 and 1610.05 cm^{-1}.

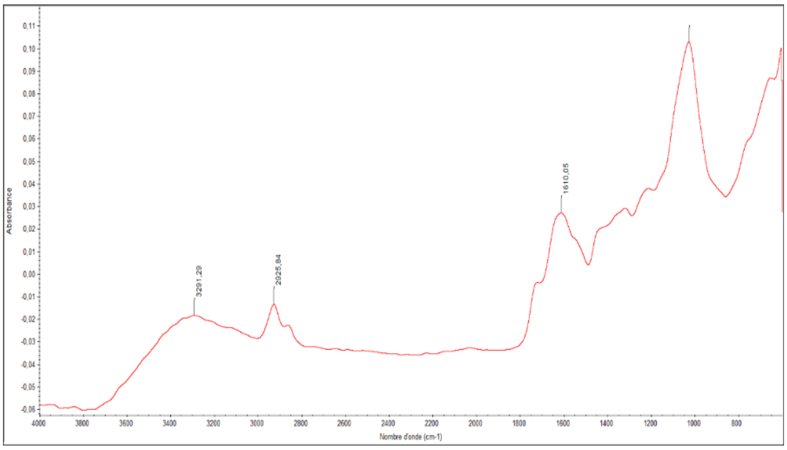

Figure 1 : Infrared analysis spectrum of leaves of *R. communis*

3. Bio-aphicide tests

For the aphicidal effect, the mortality rate of *M. rosae* larvae vary proportionally with the concentration used and the duration of exposure for each of the extracts of *R. communis*. In fact, the best aphicidal activity was obtained with the acetonic extract. This extract at the concentration of 5 mg/ml caused a mortality of 93.33±6.69% after 48 h and a mortality of 100% after 96 h of contact. At the lowest concentration (0.25 mg/ml), the extract reduced the *M. rosea* population by more than half (63.33±6.69 %). The highest concentration of aqueous extract (10 mg/ml) resulted in mortality of 76.67±4.42 % after 48 h and 95.00±2.09 % after 96 h of contact. Similarly, methanolic extract caused mortality of 68.33±6.03 % after 48 h and 100 % after 96 h at the same concentration. The lowest aphicidal effect is attributed to the ethanol extract. This extract at a higher concentration (10 mg/ml) caused only 53.33±6.69 % after 48 h of exposure. It should be noted that no significant difference with the control batch was observed for this extract at the low and medium concentrations (0.25 and 0.5 mg/ml) causing mortality of 13.33±7.29 and 16.67±4.42 respectively after 48 h of contact (Fig. 2).

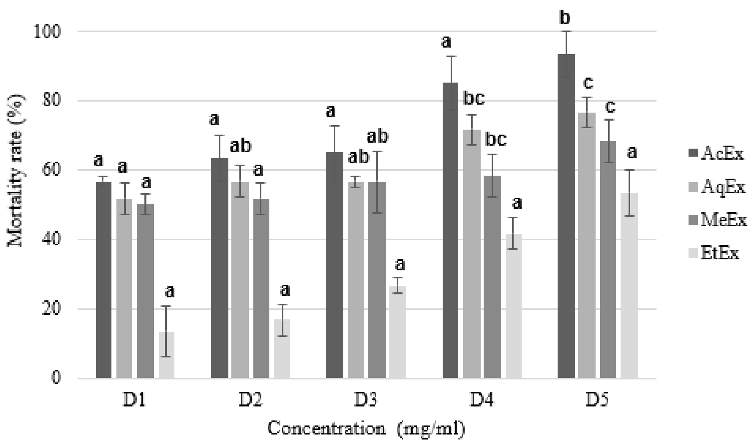

Figure 2: Mortality rate of *M. rosae* larvae treated with extracts of *R. communis*. after 48 h of contact. Values with the same superscript letters within the same extract at different concentrations do not show statistically significant differences by Tukey-Kramer test (p> 0.05). The results represent the mean of three assays

The efficacy of Castor acetonic extract against *M. rosae* larvae was demonstrated by recording the shortest LT_{50} (19.19 h) and the lowest LC_{50} (0.62 mg/ml). This concentration is comparable to the lowest concentration used for this study. In addition, the aqueous extract and the methanolic extract also had very low LC_{50} values (0.71 and 0.86 mg/ml respectively), but were characterized by a more time-spread mortality kinetics (22.67 and 28.65h respectively). Finally, the ethanolic extract was the least effective with an LC_{50} of 9.72 mg/ml and LT_{50} of 36.56 h (Table 2).

Table 2: Toxicity parameters of *R. communis* L. extracts *on M. rosae* L. larvae

Ex	Regression equation/ R^2	LC_{50} mg/ml	P	MS	F	DF	Regression equation/ R^2	LT_{50} (h)
AcEx	y=1.1195x+5.2325 R^2 = 0.9034	0.62	<0.001	3228.89	30.997	5	y=1.0381x+3.668 R^2=0.9637	19.19
AqEx	y=0.5877x+5.0864 R^2=0.8964	0.71	<0.001	2245.56	55.752	5	y=1.1558x+3.4333 R^2=0.9277	22.67
MeEx	y=0.3714x+5.0237 R^2=0.8881	0.86	<0.001	1749.17	19.678	5	y=3.9566x–0.7654 R^2=0.9198	28.65
EtEx	y=1.0073x+4.0051 R^2=0.9804	9.72	<0.001	1148.06	12.155	5	y=4.7666x–2.4504 R^2=0.9258	36.56

DISCUSSION

Plants are a rich source of bioactive compounds with a wide variety of pharmacological properties [20]. The use of these natural products in pest control has been the subject of research in many countries, mainly due to the increased resistance of these organisms to synthetic pesticides. This resistance is due to the indiscriminate use of chemicals, that can cause enormous damage to humans and the environment by pervasive chemical waste [21]. According to Ait Taadaouit et *al.* [22], a yield of 18 % was obtained from 20 g of the methanolic extract of the *R. communis* L. grains harvested in southern Morocco. This value is comparable to that obtained in this study from the ethanol extract (17.7 %). A higher extraction yield (36.5 %) was obtained from 20 g of castor powder was revealed using the Soxhlet method [23]. This variation can be explained not only by the extraction method used, but also by abiotic ecological factors influencing the richness and quantity of secondary metabolites in plants. In this study, the acetonic extract was the richest in polyphenols (151.66±0.85 mg EGA/g) compared to other extracts of the same plant. Thus, higher values were obtained from the methanolic extract of Castor (623.7 mg of EGA/g for leaves and 502.28 mg of EGA/g for roots) [24]. The extraction method adopted is based on the differential solubility in water and organic solvents. Indeed, geographical, climatic and genetic factors, but also the degree of maturation of the plant and the shelf life have a strong influence on the polyphenol content [25]. Infrared spectral analysis of *R. communis* leaves indicate the presence of several molecules with various functions, including phenol (O-H), methylene (C-H), and primary amines (N-H). Indeed, the FTIR spectra of silver nanoparticles prepared from the extract of *R. communis* leaf showed transmittance peaks at 1263.2, 978.6, 849.1, 710.5, 662.8, 502.7 and 435.6 cm-1. the carbonyl group formed amino acid residues that capped the silver nanoparticles indicated by these peaks [26]. Moreover, the presence of peaks at 1400 and 1410 nm which correspond to the chemical information of the first harmonics (O-H) were observed in Castor seeds [27]. This information is attributed to ricinoleic acid, which contains a $C_{12}OH$ group, and these seeds exhibited major spectral absorptions in the range of 1107-1205 nm, 1210-1270 nm, 1340-1483 nm, and 1630-1701 nm with distinct peaks at 1155, 1185, 1223, 1379, 144 00 and 1662 nm, respectively. These spectral regions, outside the region with the peak at 1400 nm, correspond to the stretch (C-H) and are linked to the chemical functional groups of the fatty acids. Recently, several studies have evaluated the aphicidal efficacy of plant extracts, however, little work has been done on *M. rosae*. this study attempts to evaluate for the first time in Algeria the aphicidal activity of the extracts of the Castor against the green rose aphid *M. rosae.*

In this study, extracts from *R. communis* showed significant activity against *M. rosae*. The acetonic extract was the most effective with an LC_{50} of 0.62 mg/ml.

In addition, the aqueous extract and the methanolic extract also exhibited significant activity with very low LC_{50} values (0.71 and 0.86 mg/ml respectively), with a slower action than the acetonic extract. Ethanol extract was the least effective (LC_{50}=9.72 mg/ml). This variation in the action efficiency is probably due to the fact that the bioactive molecules of the plants do not have the same solubility in the different organic solvents. These results are consistent with those found by Sadok and Bentounes [23], these authors showed that the entire population of citrus aphids *Aphis spiraecola* was sensitive to the polyphenolic extract after 72h (LC_{50}=9.9 %). In addition, similar results were reported, showing considerable differences in insect mortality relative to the acetonic extract of *Otostegia persica* (Labiatae) in three species of aphids, *Aphis fabae*, *Aphis gossypii*, and *Myzus persicae* [28]. Similarly, a mean mortality of 100 %, 53 % and 60 % was obtained, by testing the effect of ether extract from three plants (*Artemisia herbalba* Asso, *Eucalyptus camaldulensis* Dehnh and *Rosmarinus officinalis* L.) on the green peach aphid, *Myzus persicae* [16]. Moreover, Arab et *al.* [29], while working on the evaluation of the effect of methanolic extract from black poplar leaves (*Polpulus nigra* L.) on citrus black aphid (*Toxoptera aurantii*), found a very highly significant decrease (p<0.001) in the toxicity of the extract recorded as a function of the different dilutions of the stock solution. Interesting results have also been obtained by Bouchekouk et *al.* [30], by evaluating the bio-aphicidal potential of the essential oil of *Pteridium aquilinum* on the black bean aphid (*Aphis fabae*). These authors noted that the application of 80µl/ml, 40µl/ml and 20µl/ml of the essential oil caused respectively 96.49 %, 84.21 % and 82.46 % of mortality, after 48h, with a LD_{50} value of 4.26 µl/ml. Furthermore, previous studies have confirmed that extracts from *R. communis* leaves contain several bioactive compounds, that can act as natural insecticides [31].

CONCLUSION

The botanical extracts of Castor show toxicity against the green aphid of roses *M. rosae*. The best insecticidal activity is attributed to the acetonic extract, suggesting the future use of extracts *of R. communis* as an alternative for pest control, with minimal negative impact on health and the environment.

However, it will be interesting to carry out a more detailed phytochemical analysis, in order to identify and purify the compound(s) responsible for the insecticidal effect.

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