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Larvicidal effect of *Ruta graveolens* L. plant extract against *Myzus persicae* (Sulzer) (Homoptera: Aphididae) in *Capsicum annum*

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Abstract

Capsicum annum, one of the most important subsistence greenhouse vegetable crops in the Biskra region, has been destroyed by the more damaging pest *M. persicae*. Biological control using aqueous extracts of medicinal plants could be a viable alternative for decreasing the use of chemical products. The current study aims to assess the larvicidal activity of the aerial component of *Ruta graveolens* L. on green peach aphid larvae *M. persicae via* contact. Three concentrations with four replications were applied to *M. persicae* larvae, and corrected mortality was measured after 24 hours, 48 hours, and 72 hours. The results showed that an aqueous extract of *R. graveolens* was effective on *M. persicae* Sulzer at 5, 10 and 20% concentrations with a 97% mortality rate after 72 hours with the 20% dose. The value of the current work is that an aqueous extract of *R. graveolens* was poisonous to the green peach aphid; these findings can help to produce biopesticide.

Key words: *Capsicum annum*, *Ruta graveolens* L, larvae, *Myzus persicae* (Sulzer) L, aqueous extract, biopesticide

Introduction

Capsicums are members of the Solanaceae family and are widely grown vegetables that play an important role in human nutrition (Orobiyi *et al.*, 2013). Capsicums are native to Central and South America but have spread rapidly to subtropical regions and thrive in tropical climates due to their preference for hot, humid conditions (Al-snafi, 2015). As one of the original food spices, capsicum ranks among the top 40 vegetable species globally, with *C. annuum* L., *C. frutescens* L., and *C. chinense* Jacq being the most widely cultivated and recognized species.It has undergone significant growth, with production reaching 174234.1 tonnes (MADR, 2020). Biskra is one of the country's leading producers of greenhouse vegetable crops, with *Capsicum annum* playing an important role in Algerians' daily diets (Bettiche *et al.*, 2017). However, this crop is susceptible to a variety of Orthropod pests and disease attacks, which can cause a wide range of damage. *M. persicae* is the most common insect pest of capsicums in greenhouses (Erdoğan and Yıldırım, 2016).

M. persicae is a cosmopolitan and polyphagous insect (Yadav and Patel, 2017). The primary host can be a peach or a plum. This insect directly targets *capsicum* crops, causing damage to all stages of plant development, including leaves, blossoms, and fruits (Akesse *et al.*, 2015). It causes much damage to host plants, sucks sap and produces necrotic spots in tissues, wilting and death and (Harmel *et al.*,2010; Yadav and Patel, 2017). Aphids are major pests of crops due to their biological and behavioural characteristics, such as their biotic potential and their ability to adapt to the maximum exploitation of their environment through their polymorphism; the green peach aphid demonstrates an amazing ability to adapt to pesticides and acquire resistance quickly (Benoufella-kitous *et al.*, 2019) . decreased agricultural yields, wilting, and death. Indirect damages include the transmission of viral diseases, which can transmit over 100 plant viruses (Dewhirst *et al.*, 2012). The excretion of honeydew, which serves as a substrate for the growth of sooty moulds, also interferes with photosynthesis (Yadav and Patel, 2017). Currently, aphid populations are resistant to synthetic insecticides, making control challenging (Erdoğan and Yıldırım, 2016).

Although chemical pesticides are the most commonly used method of controlling this insect, excessive use can have serious consequences (Nia *et al.*, 2015). This method is a quick and simple way to stop the pest's spread. However, the widespread use of these chemical products has negative consequences such as environmental pollution, biodiversity loss, and potential health risks (Lingakari and Bandi, 2023; Ait Tadaouit *et al.*, 2012). As a result, applying bio-pesticides could be a potential solution to reduce the impact of this insect (Abhishek *et al.*, 2021). Derived from plants, they are often less effective than their chemical counterparts in the short term when used alone (Ait Tadaouit *et al.*, 2012).

In this context, we chose *R. graveolens* (Rutaceae). The *Ruta* genus has approximately 700 spontaneous species in warm temperate regions, including *R. angustifolia, R. chalpensis,* and *R. montana* (Hamiche and Azzouz, 2013). Originating from the Latin words gravis, meaning strong, and olère, meaning smell, a strong and unpleasant odour (Oliva *et al.*, 2003). It is a dicot herb with both aromatic and medicinal properties (Chacko *et al.*, 2015). *R. graveolens* is a perennial semi-shrub that grows in rocky and secluded areas, reaching a height of 65 to 75 cm (Jayakody *et al.*, 2011). Furthermore, it has been used to treat diabetes, thrombogenesis, inflammation, spasms, and hypertension. It works as a vermifuge, antihistamine, and rubefyant. Its beneficial eupeptic properties make it suitable for stomach problems. (Naguib *et al.*, 2007).

The current study aimed to contribute to the phytosanitary protection of vegetable crops in greenhouses as part of our strategy to find alternatives to synthetic insecticides and promote the cultivation of crops. Specifically, to confirm the efficacy of aqueous extracts derived from *R. graveolens* Linn against a common pest (*M. persicae*) affecting *C. annuum* cultivation.

Materials and methods

Plant material: The plant tested in this study was *R. graveolens* family (Rutaceae), collected from the region of Biskra, located in the southeast of Algéria in 2018, where it grown naturally. The aerial part was carefully dried in shade, then finely crushed to obtain a fine powder using an electric grinder. The resulting powder was added to distilled water to create a solution, which was stirred for 30 minutes before being filtered through a Wathman filter paper (Dehliz *et al.*, 2017). The process involved using 100g of the aerial plant material in 1000 mL of distilled water. The obtained filtrate was used as the mother solution (Aouinty *et al.*, 2006).

Animal material: *M. persicae* larvae were taken from healthy leaves of *C. annum* plants cultivated in the greenhouse of the Agronomy Department in Biskra. These leaves were collected in April 2019 and subsequently placed in petri dishes (9 cm diameter) for the purpose of conducting treatments.

Biological test: The method involved placing larvae individually on the lower surface of the leaf discs, which have been designed using a cookie cutter with a diameter of 2 cm. Once transferred, the discs are placed in Petri dishes containing well-hydrophilic cotton and soaked with water to prevent leakage of individuals and maintain adequate relative humidity (Elkertati *et al.*, 2013). From the initial extract of 100g/L, 3 concentrations were prepared, C1=5%, C2=10%, and C3=20%. The toxicity test was done by direct spraying on the *M. persicae* larvae. Mortality was assessed at 24, 48, and 72 hours. A fine paintbrush bristle was used to gently push the aphids, and those unable to move were considered dead. Each leaf disk contained 20 individuals, with two disks placed per box. For each concentration, three boxes were used, with larvae of any stage combined. The experiment was repeated four times for each dose. To account for natural mortality, corrections were applied using the Abbott formula (1925), based on the percentage of mortality observed in control boxes.

MC% = (MO-MT)/ (100-MT)100.

MC: the percentage of corrected mortality.

MT: the mortality rate in the control lots.

MO: the proportion of treated batch deaths.

Statistical analysis: *In vitro* assays were conducted using an aqueous extract to evaluate its larvicidal effect against *M. persicae* (green peach aphid). Statistical analysis was performed using ANOVA, and means were compared using Duncan's Multiple Range Test, with significance determined at α = 0.05. SPSS version 25 was used for data analysis.

Results

Larvicidal activity of the aqueous extract: The results presented in (Fig. 1) indicated that the mortality rate varied depending on different concentrations and exposure times. According to the results obtained, a high mortality rate of *M. persicae* larvae was attained, reaching 97% for the 20% dose after 72 hours. However, the low dose of 5% resulted in a mortality rate of 52% after 24 hours, which gradually rose to 81% after 48 hours and to 88% after 72 hours.

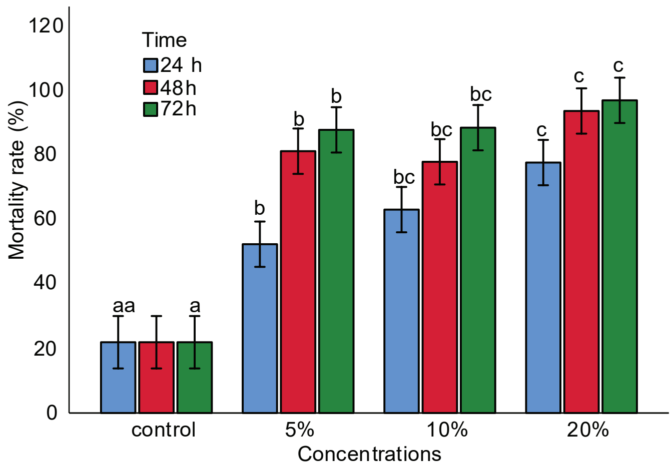

Fig. 1. Efficiency of aqueous extract of *R. graveolens* applied on larvae of *M .persicae.* Means followed by the same letter are not significantly different according to Duncan's multiple range test at *P*<0.05.

***R. graveolens* extract concentrations and *M. persicae* mortality:** the study showed that the efficacy of the *R. graveolens* aqueous extract and its impact on the mortality rate revealed a proportional correlation between the different doses and the mortality rate of *M. persicae* larvae, demonstrating a positive correlation, as the concentrations increased with a coefficient r = 0.72. (Fig. 2).

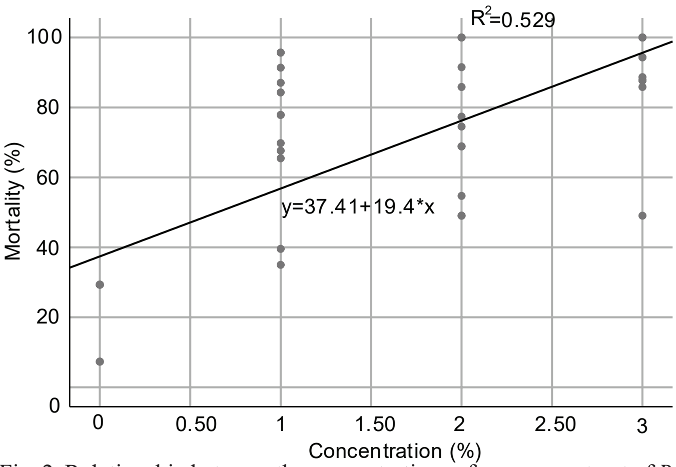

Fig. 2. Relationship between the concentrations of aqueous extract of *R. graveolens* and the mortality rate of *M. persicae* larvae.

Toxicity of *R. graveolens* extract on *M. persicae* larvae: The various concentrations of the *R. graveolens* aqueous extract were tested on *M. persicae* larvae, the mortality rate results for both control and treated groups with *R. graveolens* aqueous extract are summarized in (Table 1). According to the results of the treatment, we noted a mortality rate that exceeded 50% for the highest concentration 20%, reaching 77.83±19.22 after 24 hours and 97.17±3.27 after 72 hours of treatment. Conversely, the lowest concentration (5%) exhibited the least toxicity with mortality rates of 52.46±17.71 after 24 hours and 88±7.62 after 72 hours of treatment. The results suggest a relation between the toxic impacts of the *R. graveolens* aqueous extract and the elevation of both concentration and exposure time.

Table 1. Larvicidal activity of different concentrations of *R. graveolens* aqueous extract on *M. persicae* larvae as a function of exposure time

Exposure time (h)	Larva mortality			
	Control	5%	10%	20%
24h	22±12.70^{a}	52.46±17.71^{b}	63.21±24.68^{bc}	77.83±9.22^{c}
48h	22±12.70^{a}	81.37±11.75^{b}	78.07±14.86^{bc}	93.87±7.08^{c}
72h	22±12.70^{a}	88±7.62^{b}	88.68±9.53^{bc}	97.17±3.27^{c}

Value is represented as mean±SD. The means value followed by the same letter is not significantly different according to Duncan's multiple comparison at the P>0,05.

The impact of dose and exposure times on mortality rates: The analysis of variance (ANOVA) results presented in Table 2 indicate that both dose and time had significant effects on the mortality of *M. persicae* larvae. The associated *P*-value of 0.001 demonstrates a highly significant effect of dose on larval mortality. Similarly, the corresponding *P*-value of 0.001 indicates a significant influence of time on mortality.

Overall, the results suggest that while both factors significantly affected mortality rates, the effect of dose was more pronounced, as reflected by the higher F-value compared to that of time.

Table 2. Temporal variation in the mortality of *M. persicae* larvae in function to dose and time

Factors	Some of squares	Ddl	Meduim of squares	F	P
Doses	25967.631	3	8655.877	43.614	0.000
Times	3217.155	2	1608.578	8.105	0.001

Discussion

Plants are known to contain bioactive compounds with insecticidal properties such as repellency, antifeedant, and antioviposition effects. Botanical insecticides are gaining attention as alternatives to synthetic chemicals (Erdoğan and Yıldırım, 2016). Our results show that aqueous extract of *R. graveolens* has a significant larvicidal effect on *M. persicae* larvae, with mortality rates increasing from 52% at 5% concentration after 24 hours to 97% after 72 hours.

R. graveolens contains pharmaceutically active compounds such as alkaloids, coumarins, volatile oils, and flavonoids, which have demonstrated antimicrobial properties. It inhibits the growth of microorganisms like *Staphylococcus aureus*, *Salmonella typhimurium*, and *Bacillus subtilis* (Reddy and Alrajab, 2016). Saeed *et al.* (2023) found that *R. graveolens* leaves possess antibacterial and antioxidant activities. Similarly, Ncibi *et al.* (2023) reported that the methanol extracts of *Artemisia judaica*, *R. graveolens*, and *Suaeda monoica* contain metabolites with significant antioxidant, antibacterial, and cytotoxic effects. In another study, Benchaaban *et al.* (2019) evaluated hydroethanolic extracts from *Sambucus australis*, *Melia azedarach*, and *R. graveolens* against *Pediculus*. At a concentration of 54.54 µL/L air, mortality rates for *Ephestia kuehniella* after 48 hours reached 88%, 100%, and 100% for *R. graveolens*, *Ocimum basilicum*, and *Mentha pulegium* essential oils, respectively.

The corresponding dose also resulted in mortality rates of 62.88% and 100% for *Ectomylois ceratoniae*. Chacko *et al.* (2015) found that the crude petroleum ether extract from *R. graveolens* had strong inhibitory effects against larval species, achieving 100% inhibition at 250 µg and a minimum of 48.98% at 31.25 µg against *Anopheles stephensi*. In parallel studies, Mouas *et al.* (2017) reported a low mortality rate of 25% for aphid adults after 24 hours, which increased to 100% after 96 hours. Lawel *et al.* (2015) observed high effectiveness of neem extract against *M. persicae*, while Acheuk *et al.* (2017) showed that *Artemisia judaica* ethanolic extract caused complete mortality in *Aphis fabae* adults within two hours at a dose of 12 mg/mL. Behi *et al.* (2017) reviewed several studies and confirmed the insecticidal properties of essential oils for aphid control. Déla *et al.* (2014) demonstrated that neem leaves reduced survival and reproduction in *M. persicae* nymphs through ingestion. In a study by Pavela (2009), *Pongamia glabra*, *Azadirachta indica*, and *Chrysanthemum* extracts showed 100% mortality against *Spodoptera littoralis*, *M. persicae*, and *Tetranychus urticae*. Lai and Mimsheng (2010) reported that *Allium sativum* extract displayed high toxicity against *M. persicae* in both lab and field conditions. Nia *et al.* (2015) demonstrated that etheric extracts from *Eucalyptus camaldulensis*, *Rosmarinus officinalis*, and *Artemisia herba-alba* caused 100%, 53%, and 60% mortality, respectively, at the highest concentration. Yadav *et al.* (2017) found that *Cassia angustifolia* seeds and leaves were effective against *M. persicae*, resulting in up to 96.67% mortality after 72 hours at a 10% concentration.

Conclusively, the aqueous extract of *R. graveolens* exhibited strong insecticidal activity, achieving 97% mortality in *M. persicae* larvae at a 20% dose after 72 hours. This suggests the potential for plant-based biopesticides, which are environmentally friendly and less toxic to human health than chemical alternatives, to be further explored in real-world pest control.

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