



LC-MS Analysis and Study of the Insecticidal Activity of Phenolic Extracts from the Leaves and Roots of *Urtica dioica* against *Tuta absoluta* M. (Lepidoptera: Gelechiidae) and *Hyalopteruspruni* (Hemiptera: Aphididae)

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ABSTRACT

Background: This study aimed to identify polyphenols in nettle (*Urtica dioica* L.) using Liquid Chromatography coupled with Tandem Mass Spectrometry (LC-MS/MS) and to evaluate the toxicity of leaf and root extracts against larvae of *Tuta absoluta* and adults of the mealy aphid *Hyalopterus pruni*. Extraction yields were determined after maceration and toxicity was assessed through LD₅₀ measurements.

Methods: Fresh leaves of *Urtica dioica* were collected from the experimental farm of the University of Mostaganem, Algeria. The extraction process was carried out through maceration with pure methanol. Phytochemical profiling was conducted using LC-MS/MS analysis on a UHPLC Nexera system coupled with an 8040 triple quadrupole mass spectrometer. The biological activity was assessed using the direct contact method. Four concentrations of leaf extract (10%, 20%, 30% and 40%) and four concentrations of root extract (10%, 20%, 25% and 30%) were tested.

Result: A total of 22 compounds were identified in the roots and 27 in the leaves, mainly phenolic acids including gallic acid, 4-hydroxybenzoic acid, 4-methylguaiacol and ferulic acid. Extraction yields reached 10.9% for leaves and 22.4% for roots. Both extracts showed comparable toxicity, with LD₅₀ values of 17.54% (leaves) and 13.19% (roots) against *T. absoluta* and 8.05% (leaves) and 9.42% (roots) against *H. pruni*.

Key words: Biopesticides, *Hyalopterus pruni*, LC-MS/MS, LD50, *Tuta absoluta*, *Urtica dioica*.

INTRODUCTION

In Africa, the production of vegetables and fruits represents an important field in agriculture (Aworh, 2021), including tomato (*Lycopersicon esculentum* L.) (1768), one of the most popular and commonly grown vegetable crops worldwide. This fruit is a source of potassium, lycopene (antioxidant, anticancer agent), polyphenols and ascorbic acid (Vit C) (Rao *et al.*, 2018; Wang *et al.*, 2023). As well as plum trees with a low-calorie content and a low glycemic index, but a relatively high nutritional value. They contain carbohydrates, primarily sucrose, glucose and fructose, organic acids, *e.g.* (Arslan *et al.*, 2023). citric and maleic acids, fibers (pectins), tannins, aromatic substances, chlorophyll, carotenoids, anthocyanins and enzymes. These substances determine the nutritional value and taste of plums (Ayub *et al.*, 2023). Plum is a fruit rich in phenolic compounds, characterized by a relatively high antioxidant activity, higher than that of orange, apple or strawberries, also rich in minerals and vitamins (C, A, B1, B2) (Maji and Cornelius, 2023; Manzoor *et al.*, 2023).

Tomato and plum are susceptible to insects causing considerable yield reduction. *T. absoluta* is one of the most important tomato pests in open field and greenhouse cropping systems, which invaded Algeria in 2008 (Guenauoui and Ghelamallah, 2008). Considerable economic losses are caused by larvae that mine tomato plants to feed on leaf, stem and fruit mesophyll (Yadav *et al.*, 2022).

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Numerous pests have also been reported on plum trees, which requires well-organized measures to protect fruits, leaves and wood. The most important pests from an economic point of view are plum flies and certain aphids

such as *H. pruni*, previously considered the most dangerous pest (Lozier *et al.*, 2009). *H. pruni* damage shoots almost throughout the growing season which retards their development (Jordan *et al.*, 2021; Vasilev *et al.*, 2019).

Chemical control has always been considered an efficient approach of controlling *T. absoluta* and *H. pruni* populations. However, this method remains expensive and unstable due to the development of insect resistance and the negative impact noted on the environment and human health.

Plants are natural resources that have long been applied to monitor various agricultural pests (Divekar, 2023; Hajjar *et al.*, 2023). Natural substances can be exploited as biopesticides, such as extracts of medicinal plants and antagonists of natural origin (Tarusikirwa *et al.*, 2020). Typically, plant extracts, which are more environmentally friendly, have been widely used as substitute to chemical pesticides (Abdullah and Zahoor, 2023; Ayilara *et al.*, 2023). The insecticidal activity of botanical extracts has been investigated in various reports (da Silva Sá *et al.*, 2023; Lengai *et al.*, 2020).

Stinging nettle, often known as common nettle, burn nettle, stinging nettle or nettle leaf is one of the plant species adopted by man as a bioactive substance in traditional medicine and agriculture (Bhusal *et al.*, 2022). This plant is very rich in secondary metabolites which has several pharmacological properties (Majedi *et al.*, 2021) including antibacterial, antifungal (Rolta *et al.*, 2020; Taheri *et al.*, 2022); anthelmintic (Bhusal *et al.*, 2022); anticancer (Sharifi-Rad *et al.*, 2021), antioxidant (Jaiswal and Lee, 2022) and anti-inflammatory (Scheau *et al.*, 2021) anti-aging (Sifaki *et al.*, 2020) and antiviral activity (Upreti *et al.*, 2023). *U. dioica* is considered an ecological keystone species and very important for biodiversity (Subba and Pradhan, 2022). It is also used as a natural feed additive in poultry nutrition (Mehboob *et al.*, 2025). Among nettle derivatives, manure is the most used by farmers, its use is explained by the results obtained and the simplicity of its manufacture and its use. Nettle manure is used as a fertilizer, as a fungicide against mildew, as an insecticide against aphids and ticks and as a plant growth activator or regulator (Koczkodaj *et al.*, 2023; Opačić *et al.*, 2022).

In this context, we are interested in the study of the insecticidal activity of methanolic extracts of leaves and roots of *U. dioica* L. against the pests of *T. absoluta* and *H. pruni* crops.

MATERIAL AND METHODS

Extraction of polyphenols from leaves and roots

The plant material used in this study was collected in March 2022 from the Mostaganem region, Algeria. The extraction procedure was performed at the Biochemistry Laboratory, University of Mostaganem. The extraction was carried out

according to the previous method (Sujith *et al.*, 2011). A determined quantity of crushed plant material (root, leaf) is mixed with 100 ml of methanol. The mixture was heated at 60°C using water bath for 20 min and then filtered. Three repetitions are carried out for the same plant material to exhaust the active principles. The three filtrates were combined and the solvent was removed using a rotavapor at 40°C.

LC-MS/MS Analysis of *U. dioica* methanol extract

The LC-MS/MS analysis of *U. dioica* (nettle) methanol extract uses a UHPLC Nexera system coupled with an 8040 triple quadrupole mass spectrometer. The extract is filtered and analyzed through a C18 column with a gradient mobile phase containing water, ammonium formate, formic acid and methanol. Mass spectrometry utilizes ESI in both positive and negative modes, with MRM for quantification. The method is validated for linearity, accuracy, precision and detection limits. It successfully profiles up to 27 phytochemicals, demonstrating the plant's medicinal and nutritional value.

Insecticide activity

The bioassay was conducted according to the direct contact method described by (Nasir *et al.*, 2017), in which insects were directly exposed to the tested extracts. The dilutions retained for the leaf extracts are four doses, namely 10%, 20%, 30% and 40% and the same for the roots (10%, 20%, 25% and 30%). The insecticide *in-vitro* tests carried out with regard to the two animal species under temperature conditions of 21±3°C, a relative humidity of 58±9% and a photoperiod of 14:10. For the species *T. absoluta*, healthy tomato leaves were fixed on absorbent paper discs soaked in mineral water in Petri dishes on which were placed three larvae, the latter must be ventilated to avoid asphyxiation larvae tested. The lids of the boxes have been made in such a way as to allow ventilation by the presence of a fine mesh tulle. Using a sprayer, our extract at each dilution is sprayed on the *T. absoluta* larvae. The same procedure was followed for the toxicity test against *H. pruni*, where the healthy tomato leaves were replaced by healthy peach leaves and on which five adults of *H. pruni* were deposited. The leaves are changed every two days to eliminate the nutritional factor in the mortality of the individuals tested. Control tests were applied to consolidate our results, two types were retained, one positive with the use of 10% acetone and the second just with distilled water (negative control). To do this, the same number of *T. absoluta* larvae and *H. pruni* adults were placed in the control.

Statistic study

The statistical analysis was carried out using the MINITAB® 19 statistical software. This software was used to analyze the mortality rates with ANOVA ($\alpha = 0.05$) and the Tukey and Fisher test used for the comparison of the variances, the differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Analysis and determination of phenolic compounds by LC-MS/MS

The analysis of the phenolic profile of *U. dioica*, conducted using LC-MS/MS, identified 22 compounds in the leaves (Fig 1), primarily phenolic acids such as gallic acid, syringic acid and p-coumaric acid, as well as 27 compounds in the roots (Fig 2), including 4-hydroxybenzoic acid. These compounds, particularly gallic acid and phloroglucinol, are recognized for their anti-mosquito activity (Desmarchelier and Fukuto, 1974). 4-Hydroxybenzoic acid exhibits notable insecticidal properties (Safarova, 2022), while resorcinol is used in cosmetic and pharmaceutical applications (Sowmya, 2021). Ferulic acid, which is widely present in plant cell walls, is also known for its insecticidal activity (Zhang *et al.*, 2021). Additionally, p-coumaric acid has demonstrated effectiveness against *Tribolium castaneum* (Abdelkhalek *et al.*, 2020). Extracts of nettle have been evaluated for their potential in biological insect control (González-Macedo *et al.*, 2021).

Insecticidal activity

This research investigated the insecticidal effects of phenolic extracts from *U. dioica* on *T. absoluta* larvae at a temperature of $21 \pm 3^\circ\text{C}$, which is conducive to larval development (Boualem *et al.*, 2012). The leaf extracts demonstrated varying mortality rates on L1 larvae after 24 hours with 44.4% mortality at 10%, 55.5% at 20%, 33.3% at 30% and 77.8% at 40%, while the control showed 0% mortality. For L4 larvae, the 20% dose resulted in 66.7% mortality, while the other doses (10%, 30% and 40%) caused 22.2% mortality (Fig 3). The root extracts exhibited a strong insecticidal effect, achieving up to 100% mortality by the second day at doses of 25% and 30%. For L4 larvae, doses of 10% and 30% showed 65% and 79% mortality on the first day, reaching 100% by the fourth day. The control group showed increasing mortality, reaching 100% by day 8 (Fig 4).

The study evaluated the insecticidal effects of *U. dioica* leaf extract on mealy aphid adults, revealing significantly higher mortality rates compared to the control. At a 10%

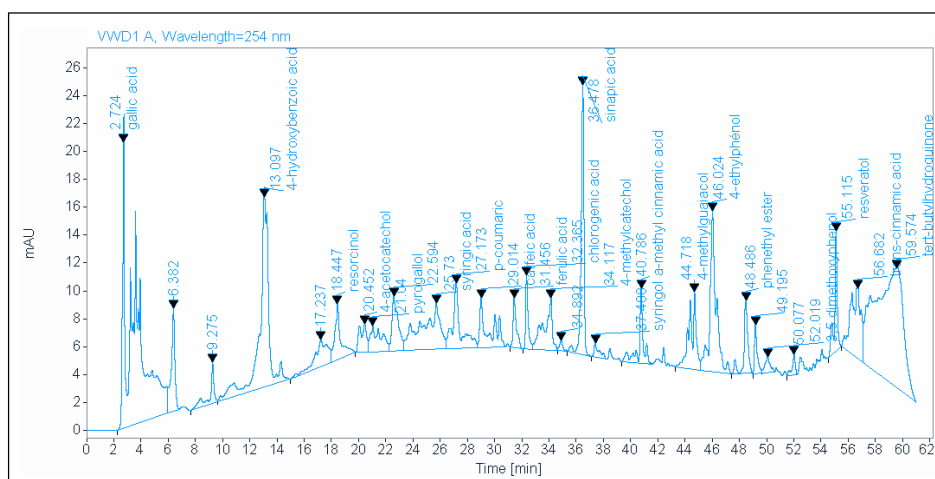


Fig 1: Analysis of the phenolic composition of *U. dioica* roots via LC-MS/MS.

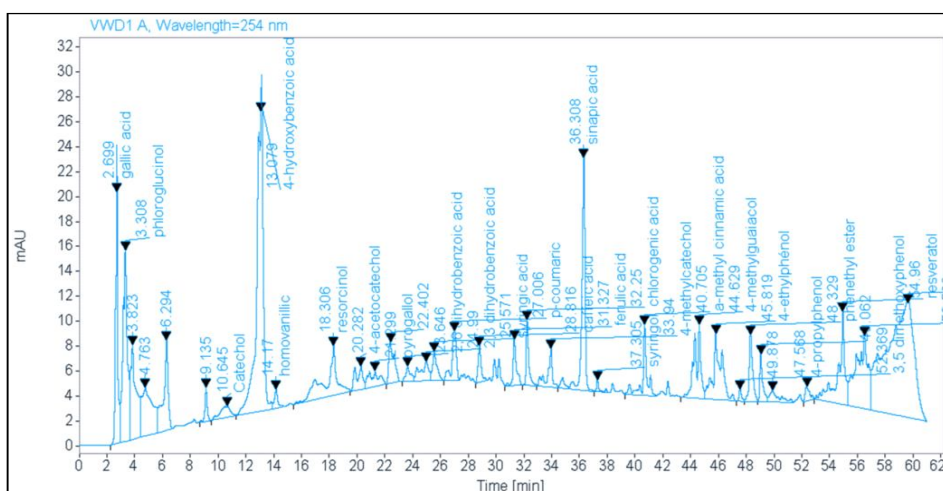


Fig 2: Chromatographic analysis of compounds present in *U. dioica* leaves by LC-MS/MS.

concentration, the mortality rate reached 66.7% after 24 hours and 100% by the third day. Higher doses (20%, 30% and 40%) resulted in mortality rates of 80%, 86.7% and 100%, respectively, while the control group showed only 13.3% mortality after 72 hours. Additionally, the root extract demonstrated a strong insecticidal effect, with 100% mortality in *H. pruni* adults at a 30% dose after 24 hours. No mortality was observed in the positive and negative controls within 48 hours. Overall, mortality rates were directly

related to the concentration of the extract and the duration of exposure (Fig 5).

Estimation of LD₅₀

(Morris-Schaffer and McCoy, 2020) defined the LD₅₀ as the dose of a toxic compound that causes 50% mortality in a population of test animals. According to (Benazzedine, 2010), the effectiveness of a toxic product is measured by its DL50 and DL90 which represent the quantities of toxic

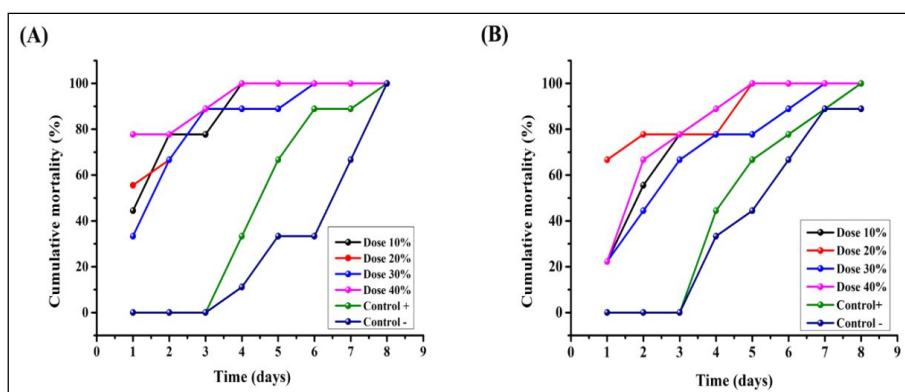


Fig 3: Cumulative mortality of *T. absoluta* larvae exposed to various concentration of extract from *U. dioica* leaf extract (A) larval stage L1 and (B) larval stage L4 at various periods of time.

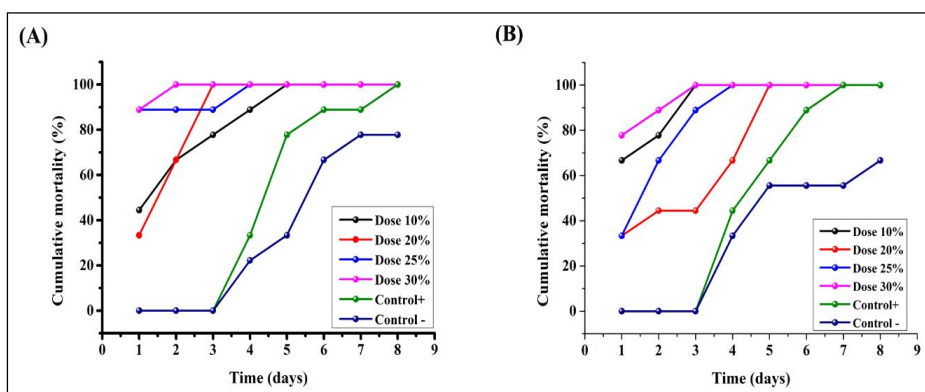


Fig 4: Cumulative mortality of *T. absoluta* larvae exposed to various concentration of extract from the roots of *U. dioica* (A) larval stage L1 and (B) larval stage L4 at various periods of time.

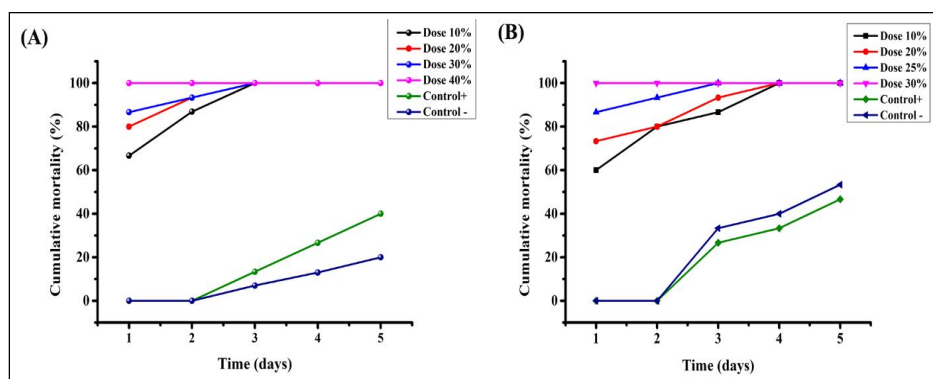


Fig 5: Cumulative percentage mortality of *H. pruni* adults exposed to various concentration of *U. dioica* extract (A) leaf extract and (B) root extract at various periods of time.

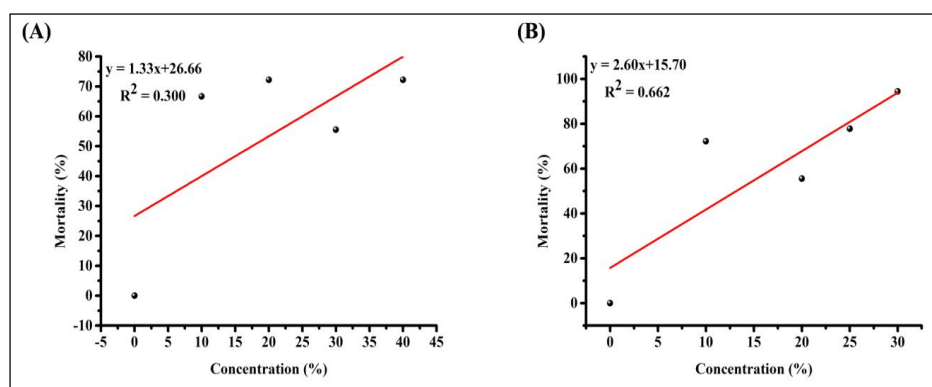


Fig 6: Linear curve of the corrected mortalities of *T. absoluta* larvae treated with the extract of *U. dioica*.

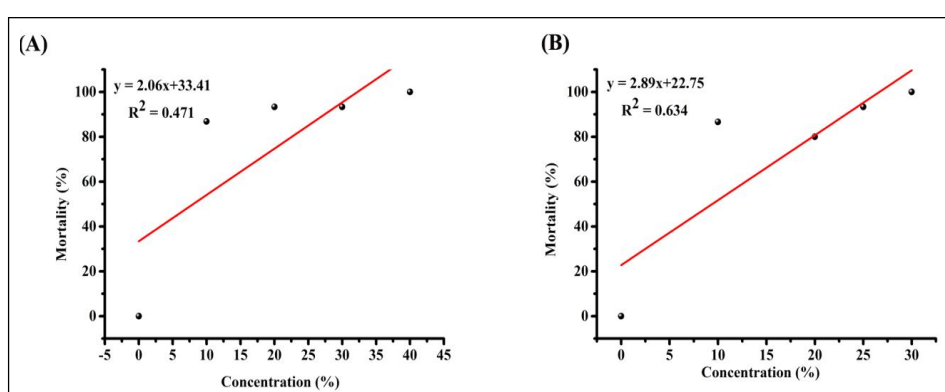


Fig 7: Linear curve of corrected mortalities of adults of *H. pruni* treated with the extract of *U. dioica*.

substance resulting in the death of 50% and 90% of individuals from the same batch respectively which can be deduced from the linear regression equation of the corrected mortality curve (Fig 6 and 7).

The research demonstrated that methanolic extracts of *U. dioica* had a larvicidal effect on *T. absoluta* larvae, with LD₉₀ values of 476240 ppm for leaves and 285769 ppm for roots. Comparatively, extracts from other plants, such as *Annonas quamosa*, showed a mortality rate of 75.1% against *T. absoluta* (Prasanna Kumar *et al.*, 2020). The *U. dioica* extracts exhibited LD₅₀ values of 17.54% for leaves and 13.19% for roots, indicating their potential as biopesticides. While nettle extracts showed no insecticidal effect on certain aphid species, they did exhibit repellent properties and toxicity against others, such as *Callaphisjuglandis*. Nettle leaf extract achieved 100% mortality in tobacco aphids at a concentration of 15 mL/L after 48 hours. Other studies indicated that the ethanol extract of *Artemisia absinthium* had a mortality rate of 75.64% against *H. pruni*, while *Prunus* apricot kernel showed 66.66% mortality. Field trials demonstrated that nettle purine extract has repellent activity against *H. pruni* and *P. juglandi*, suggesting species-dependent effectiveness (Toffolatti *et al.*, 2023). Overall, the findings underscore the potential of *U. dioica* and other plant extracts as biopesticides, contributing to integrated pest

management strategies in agriculture. The research emphasizes the importance of exploring natural alternatives to chemical pesticides for sustainable agricultural practices.

CONCLUSION

Chemical control, although commonly used, leads to numerous negative consequences, which has resulted in a growing interest in natural products. This preliminary study aims to explore bioactive molecules of agronomic and phytosanitary interest by testing the insecticidal activity of the methanolic extract of leaves and roots of *U. dioica* against the larvae of the tomato leaf miner *T. absoluta* and the adult mealybugs of plums *H. pruni*. These insects cause significant damage and can transmit diseases to host plants. *U. dioica* proves to be a promising plant, having shown excellent results against these two pests, with DL₅₀ values of 17.54% and 13.19% against *T. absoluta* and 8.05% and 9.42% against *H. pruni* for the leaves and roots, respectively. *U. dioica* is rich in bioactive compounds that are valuable for agronomy and plant health and could help mitigate associated risks.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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