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RESEARCH ARTICLE

In Vitro Evaluation of Nematicidal and Insecticidal Properties of *Urginea Maritima* Aqueous Extract

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ABSTRACT

This study evaluates the nematicidal and insecticidal potential of aqueous extracts from the leaves and bulbs of *Urginea maritima* against the root-knot nematode (*Meloidogyne* spp.) and the grain weevil (*Tribolium castaneum*). Both parts exhibited significant bioactivity, with leaves demonstrating higher efficacy. At the highest tested doses, the leaf extract achieved a lethal dose 50 (LD50) of 0.788 mg/mL for nematodes and 10.320 mg/mL for insects. Similarly, it reduced lethal time 50 (LT50) to 39.06 hours and 1.0899 days, respectively. These results suggest that the secondary metabolites in *Urginea maritima* extracts could serve as effective agents for biological pest control, offering a sustainable alternative to chemical pesticides.

Keywords: *Meloidogyne* spp., *Tribolium castaneum*, *Urginea maritima*, nematicidal, insecticidal, *in vitro*.

INTRODUCTION

Since the launch of the National Plan for Agricultural and Rural Development (PNDAR) in Algeria in the year 2000, agricultural production has shown steady growth, particularly in certain sectors such as cereals, horticulture, arboriculture, and viticulture. The Total Agricultural Area (TAA) is 42.4 million hectares, representing 18% of the country's total land area, while the Useful Agricultural Area (UAA) is 8.458 million hectares, representing 20% of the TAA. Agriculture accounts for about 12% of the Gross Domestic Product (GDP). The sector directly and indirectly supports 21% of the national population (Bayer Algeria, 2021).

In Algeria, the cereal and vegetable crop sectors are two of the main agricultural production sectors. In the 2021/2022 agricultural season, the cereal sector produced 41 million quintals (Algeria Presse Service,

November 8, 2012). In 2017, the vegetable crop sector produced 130.2 million quintals (Algeria Presse Service, June 25, 2018).

However, this growth is threatened by numerous abiotic and biotic constraints that severely impact crop productivity and quality. Among biotic factors, pests, including root-knot nematodes (*Meloidogyne* spp.) and arthropods such as the grain weevil (*Tribolium castaneum*), cause significant economic losses. *Meloidogyne* spp., particularly prevalent in Mediterranean regions, account for average global agricultural losses of 14–25% (Whitehead, 1998; Agrios, 2005). Similarly, stored cereals suffer approximately 30% annual losses due to arthropod infestations (Haubruge *et al.*, 2000).

Chemical control methods are widely used to combat these pests, but they are associated with numerous

drawbacks, including environmental contamination, health risks, reduced biodiversity, and the development of resistance among target organisms (Silva *et al.*, 2016; Usman *et al.*, 2023; Ahmad *et al.*, 2024; Ali *et al.*, 2024). Consequently, there is an urgent need for effective, environmentally friendly pest control strategies. Biological control, including the use of plant-based biopesticides, has gained attention as a sustainable alternative (Zaid *et al.*, 2019).

Currently, many research efforts are focusing on the use of alternative methods, such as the use of plant-based biopesticides, which are considered as a potential source of less toxic, naturally occurring bioactive molecules (Jovana *et al.*, 2013). In the literature, many wild and cultivated plants are known for their nematocidal and insecticidal activity. These plants are being studied for their potential use as alternatives to conventional insecticides (Kemassi *et al.*, 2010) and nematocides (Erdoğan, 2022).

Urginea maritima (commonly known as squill) has emerged as a promising candidate for biopesticide development. This plant is rich in bioactive secondary metabolites, including bufadienolides, which exhibit various biological activities, including antimicrobial, nematocidal, and insecticidal effects (Zhang *et al.*, 2022). Studies have demonstrated the potential of *U. maritima* extracts against different pests, highlighting its efficacy as a natural pesticide (Kemassi *et al.*, 2010). However, further research is needed to fully explore and validate its applications in pest management, particularly against *Meloidogyne* spp. and *T. castaneum*.

This study aims to investigate the nematocidal and insecticidal properties of aqueous extracts from the leaves and bulbs of *U. maritima*. The *in vitro* evaluations allowed the determination of the lethal dose (LD50) and lethal time (LT50) against *Meloidogyne* spp. and *T. castaneum*, and the analysis of the bioactive compounds responsible for these effects. It was demonstrated by this study that *U. maritima* extracts provide a sustainable and effective alternative to synthetic pesticides, offering a natural solution for biological pest control.

MATERIALS AND METHOD

Plant Biological Model: The plant material used for the preparation of pyto-preparations was limited to a wild plant that is very common in the Mediterranean region, *Urginea maritima* (L.) Baker (*Asparagale / Asparagaceae*). The aerial parts (leaves) and the root part (bulb) of the

plant were collected in May 2020 in the Blida province (Soumaa region, Algeria). After separating the leaves and the bulb, these were sorted, washed, and dried with a microfiber cloth. The dried material was then ground using an immersion blender. The fresh powder was stored for the preparation of the extract that was used for testing in our study.

Animal biological model: *Meloidogyne* spp.: Samples of tomato roots infested with root-knot nematodes, *Meloidogyne* spp., were collected at the end of the growing season in the Tipaza Province (Douaoueda Region, Algeria) and transported to the laboratory. The roots were washed with tap water and then with distilled water and placed in a glass Pandri dish to extract the egg masses. This operation was carried out under a binocular loupe at magnification (x10) or (x25), using forceps and two entomological needles. The egg masses isolated from the females of *Meloidogyne* (15 to 30 masses) were placed in small plastic sieves 2 to 4 cm in diameter. These were placed in Pandri dishes containing distilled water and then placed in an incubator at 25°C for mass hatching. After hatching, the larvae (L2) that were released gradually into the water were collected and counted daily. For our trials, we counted and distributed the *Meloidogyne* larvae into batches of 20 larvae (L2) in saltshakers containing 0.5 ml of water. A total of approximately 600 larvae were counted.

Sitophilus granaries: The biological material used to evaluate the effectiveness of the pyto-preparations applied is limited to individuals of *Sitophilus granarius* (*Coleoptera: Curculionidae*) from the stocks of the I.T.G.C. (Field Crops Technical Institute) in El Harrach (Algeria). Insect rearing was carried out in the laboratory of the department of life and nature sciences at the University Dr. Yahia Fares in Medea; it took place in a ventilated incubator at a temperature of 25°C and a relative humidity of 65 %. Individuals of *Sitophilus granarius* were placed in a glass jar, containing wheat covered with a fine-meshed and held by a ribbon that allows the insects to breathe while preventing the escape of individuals.

Study methods

Procedure for extracting aqueous extracts: The extraction method used in our experiment is aqueous maceration, which involves keeping the plant material in contact with water at room temperature for an extend period. This process aims to release and extract all the active components present in the studied plant.

The method for preparing the aqueous extract is described by (Aghofack-Nguemezi and *al.*, 2015). Specifically, 250 g of the fresh plant material (including ground leaves and bulbs of *Urginea maritima*) was added to 1 L of distilled water, placed in sterile and airtight flasks, and subjected to agitation for 3 days at room temperature. After filtration on a muslin cloth, the filtrates were centrifuged for 10 minutes at 14,000 rpm at room temperature. Then each sample was filtered on filter paper N°1 and stored at 4 °C until use.

Solution Range Preparation: To estimate the effectiveness of the extracts, four doses of extract (D1=100%, D2=50%, D3=25% and D4=12.5%) were prepared. The tested dilutions were prepared according to the protocol of sensitivity tests with multiplied concentrations (r^2 ratio progression) standardized by the World Health Organization (WHO), for biocides used in control campaigns (O.M.S., 1963; O.M.S., 2017 and Tchaker F.Z. and *al.*, 2020).

Experimental sandup of the bioassays: Bioassays of the nematocidal activity of the extract: The tests were carried out in the wells of cell culture microplates, each well containing 0.5 ml of distilled water added to 20 second-stage larvae previously counted. The extracts and their dilutions were then added to the larval suspension at a rate of 1 ml each. To compare the effectiveness of the treatments, a control was prepared with sterile distilled water. The toxic effect of the different treatments was evaluated after an immersion time of 24, 48, and 72 hours. Each treatment was repeated five times.

Bioassays of the insecticidal activity of the extract: The bioassays were carried out in the laboratory in boxes, at an average temperature of $24 \pm 1^\circ\text{C}$, a relative humidity of $85 \pm 5\%$. The modes of action were performed by two applications, respectively one by direct contact and a second by ingestion. A lot of 20 adult insects taken from the breeding medium were introduced into the Pandri dishes, lined with filter paper, where the extract of *Urginea maritima* at various doses were sprayed directly. Each batch of wheat, weighing approximately 5 grams, was sprayed with extract of *Urginea maritima* at various doses, after which the targand population *Sitophilus granarius* was introduced directly onto the treated wheat for ingestion. The boxes, containing 20 adult individuals, were closed, and placed in the breeding room. For the control batch, spraying with tap water was applied. Five renditions were performed for each dose and the counting of dead adult

insects was carried out every 24 hours for 7 days.

Calculating mortality rates: The mortality rate of *Meloidogyne* larvae and the wheat weevil treated with extracts of *Urginea maritima* at various doses was calculated using the following formula (Marmonnier *et al.*, 2006):

$$\text{mortality rate\%} = \frac{\text{Number of dead individuals}}{\text{total number of individuals}} \times 100$$

The observed mortality was expressed as corrected mortality using Abbott's formula $p=(P(\text{obs})-m)/(1-m)$ with (Abbott W.S., 1925) with p is the probability resulting from a logistic regression model corresponding solely to the effect of the dose, and if m is the natural mortality, considering the natural mortality observed in the control groups.

Estimating the LD50 and LT50: The median landhal time (TL50) was calculated from the regression line of probits corresponding to corrected mortality percentages as a function of the logarithms of treatment times (Ould El Hadj *et al.*, 2003; Kemassi *et al.*, 2010). Finney's formula (Finney D.J., 1971) and the probit table were used for this purpose. To calculate the median landhal dose (LD50), the doses were transformed into decimal logarithms and the corrected mortality values were converted to probits using the probit table (Ndomo *et al.*, 2009).

STATISTICAL ANALYSIS

Results were reported as mean \pm standard error of three replicates based on a coefficient of variation (CV) of less than 15%. Comparisons of mortality means were performed using one-way analysis of variance (ANOVA). The variables that contributed most frequently to the total variance and were significant at $p < 0.05$ were selected. The ANOVA was performed using XLSTAT v. 16 software (SPSS, Inc., 2016). The data were also analyzed using Systat (SPSS v.12) software. GLM-type analysis of variance was performed using the F-test for normally distributed variables, and Tukey's test was used to post-hoc test for multiple comparisons. The landhal doses (LD50) were dander mined using the probit method according to Finney (1971). Mortality was corrected using Abbott's formula (1925). Median landhal times (TL50), required for the death of 50% of the individuals exposed to different doses of the mandhanolic and aqueous extracts, were estimated.

RESULTS AND DISCUSSION

Evaluation of the nematocidal effect of aqueous extracts: Temporal fluctuations in *meloidogyne* larval

populations under the influence of pyto-preparations:

The results represented in figure 1 indicated that the mortality rate of free *Meloidogyne spp.* Larvae increased with the concentration of pyto-preparations based on *Urginea maritima* leaves (Figure 1). Doses D1, D2, and D3 (100%, 50%, and 25%) were highly effective and caused 100% mortality within 24 hours of exposure. Dose D4 (12.5%) is less effective, but still caused a mortality rate of

over 75% after 72 hours.

Pyto-preparations based on *Urginea maritima* bulb exhibited lower efficacy compared to those based on leaves. Doses D1 and D2 (100% and 50%) resulted in 100% mortality within 24 hours of exposure, whereas dose D3 (25%) reached this rate after 72 hours. Dose D4 (12.5%) only induced a mortality rate of 36.25% after 72 hours (Figure 1).

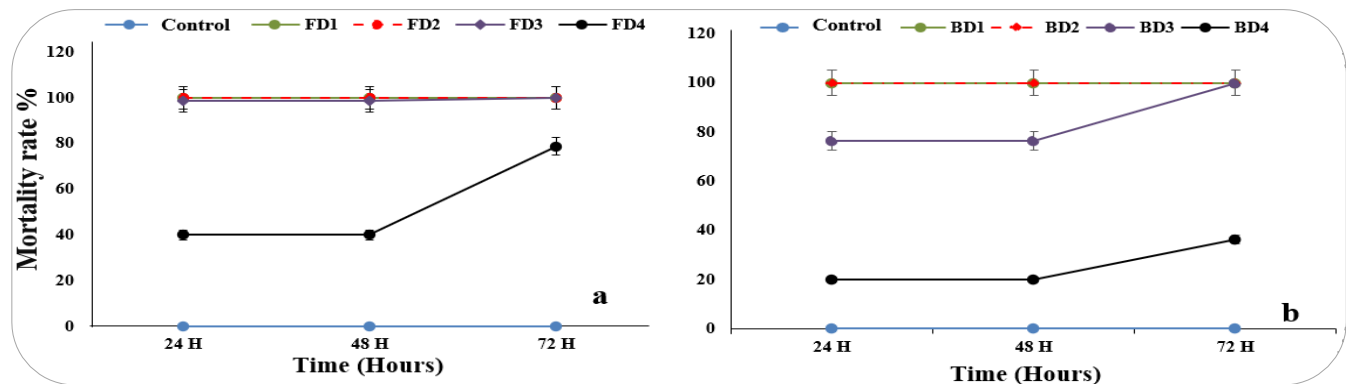


Figure 1. Time course of mortality rate of *Meloidogyne spp.* under the effect of applied aqueous extracts (a: Leaves; b: Bulb)

To visualize the toxic effects of pyto-preparations derived from the leaves and bulb of *Urginea maritima* on *Meloidogyne spp.*

larvae, a graphical representation based on the Generalized Linear Model (G.L.M.), was utilized, as depicted in Figure 2.

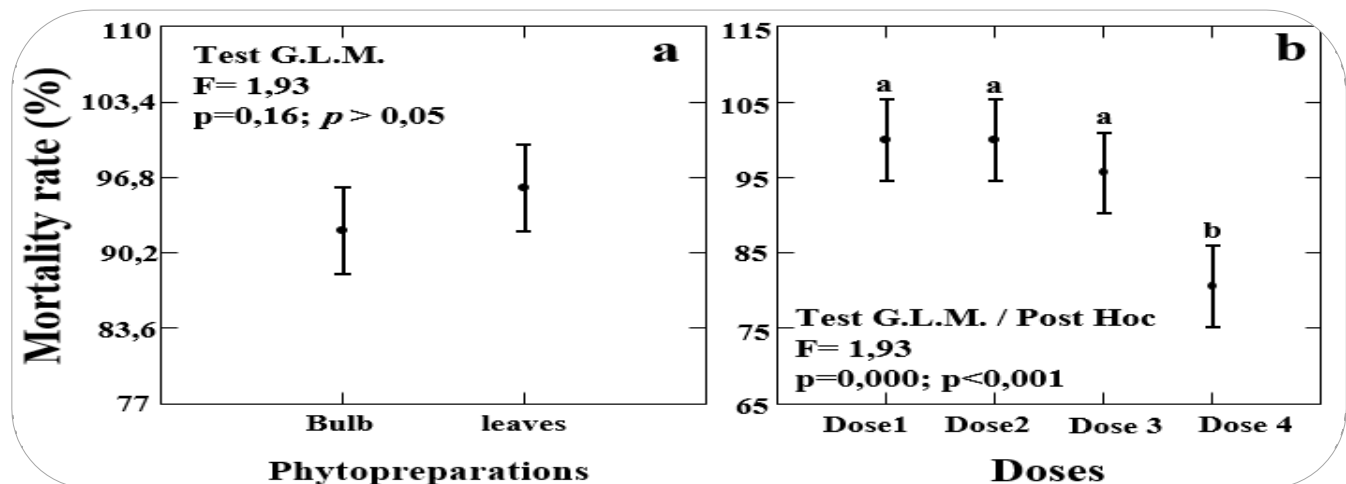


Figure 2. Comparative study of the effect of aqueous extracts on the mortality rate of L2 larvae of *Meloidogyne spp.*

The graphical presentation demonstrated a slight toxicity of the leaves compared to the bulbs. However, the probabilities from the G.L.M. test did not reveal any significant difference between the two compartments ($p = 0.16; p > 0.05$).

In contrast, highly significant differences were observed based on the applied concentrations ($p = 0.000; p < 0.01$). The biocidal effect of the pure pyto-preparations

(D1: 100%), dose 2 (D2: 50%), and dose 3 (D3: 25%) was higher than that of the diluted dose at 12.5% (D4).

Determination of the lethal dose 50 (LD50) and lethal time 50 (LT50) of the pyto-preparations: To assess the LD50 of pyto-preparations derived from the leaves and bulbs of *Urginea maritima* applied to *Meloidogyne* gall nematode larvae, a probit regression was conducted (Figure 3).

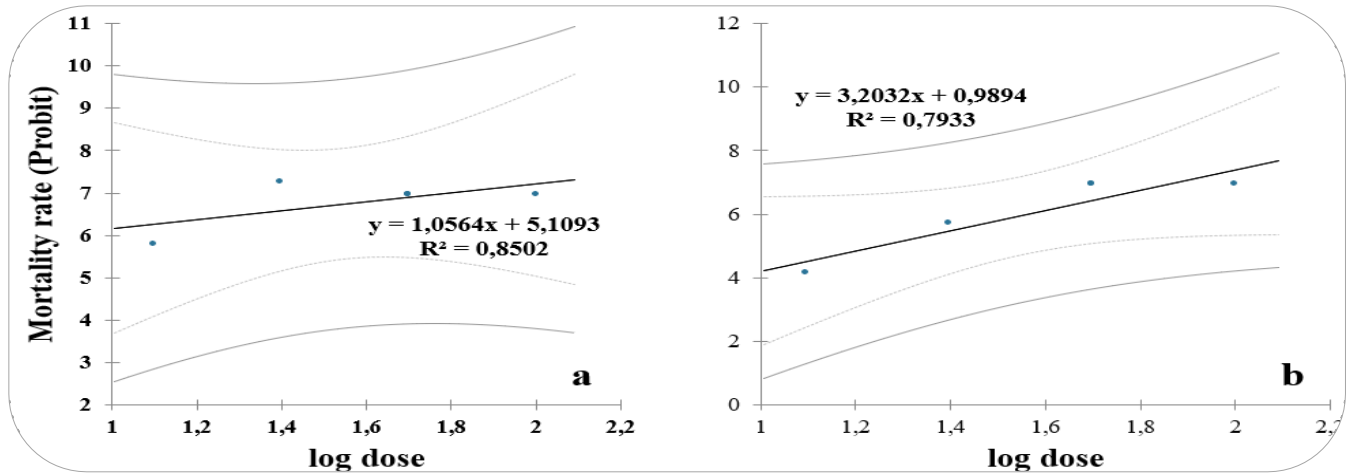


Figure 3. Larval mortality rate of L2 larvae as a function of the logarithm of doses of aqueous extracts from *Urginea maritima*. (a: Leaves; b: Bulbs)

By analyzing the equations obtained from the regression curves, it is noteworthy that the lowest LD50 was observed after the application of leaf extracts, with a value of 0.788 mg/ml, while the highest LD50 was obtained with the aqueous extract of *U. maritima* bulbs, reaching 17.87 mg/ml.

The evaluation of lethal time 50 (LT50) of the aqueous extract from the leaves and bulbs of *Urginea maritima* on

second-stage larvae of *Meloidogyne* gall nematodes was conducted.

Using the equation derived from the regression line (Figure 4), the shortest lethal time 50 (LT50) was recorded following the direct application of aqueous extracts from leaves, at 39.06 hours, whereas the longest LT50 was recorded under the effect of aqueous extracts from bulbs, at 241.43 hours.

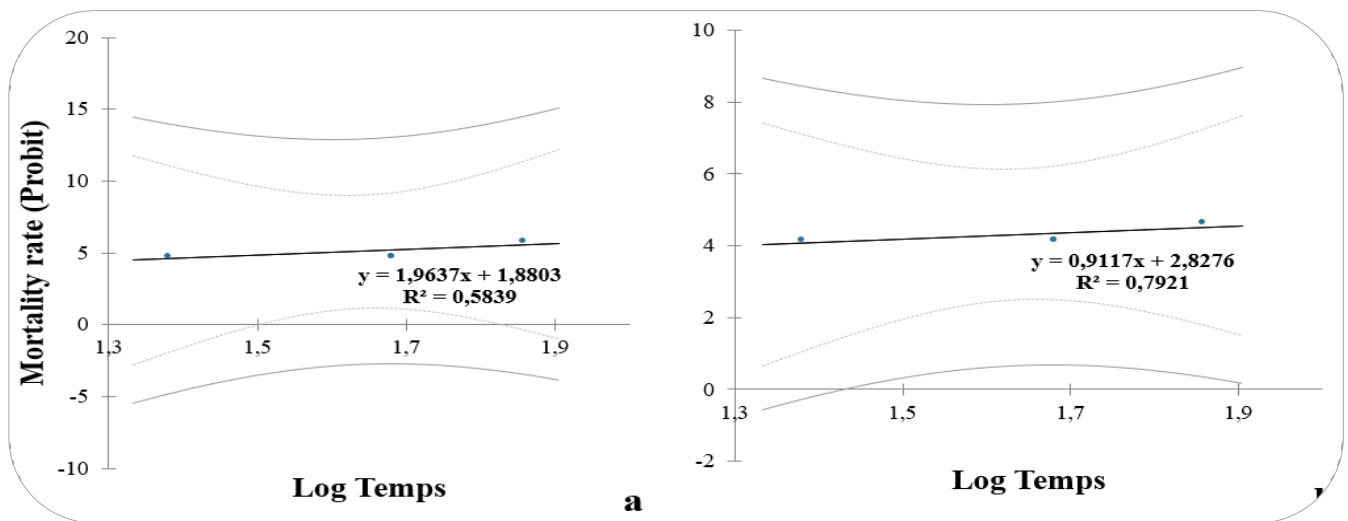


Figure 4. Larval mortality rate of L2 larvae as a function of the logarithm of time for aqueous extracts of *Urginea maritima*. (a: Leaves; b: Bulbs)

Insecticidal activity: Variation in the mortality rate of *Sitophilus oryzae* under the effect of aqueous extract of *Urginea maritima*: The results in figure 5 (a and b) indicated that the prepared aqueous extracts of *U. maritima* exhibit significant toxicity in terms of increasing the mortality rate of *Sitophilus oryzae*

individuals compared to the control (Figure 5). From the second day of application (J2), a significant increase in the mortality rate of the treated populations was observed. This upward trend in mortality persisted throughout the monitoring period. The mortality rate was influenced by the doses applied, demonstrating a

gradual increase as the concentration decreased from the highest dose (D1 = 1000 mg/ml) to the lowest (D4 = 125 mg/ml) (D4 < D3 < D2 < D1). Additionally, the

results indicated that leaf-based extracts of *Urginea maritima* exhibited greater efficacy against the weevils compared to bulb-based extracts (Figure 5. a and b).

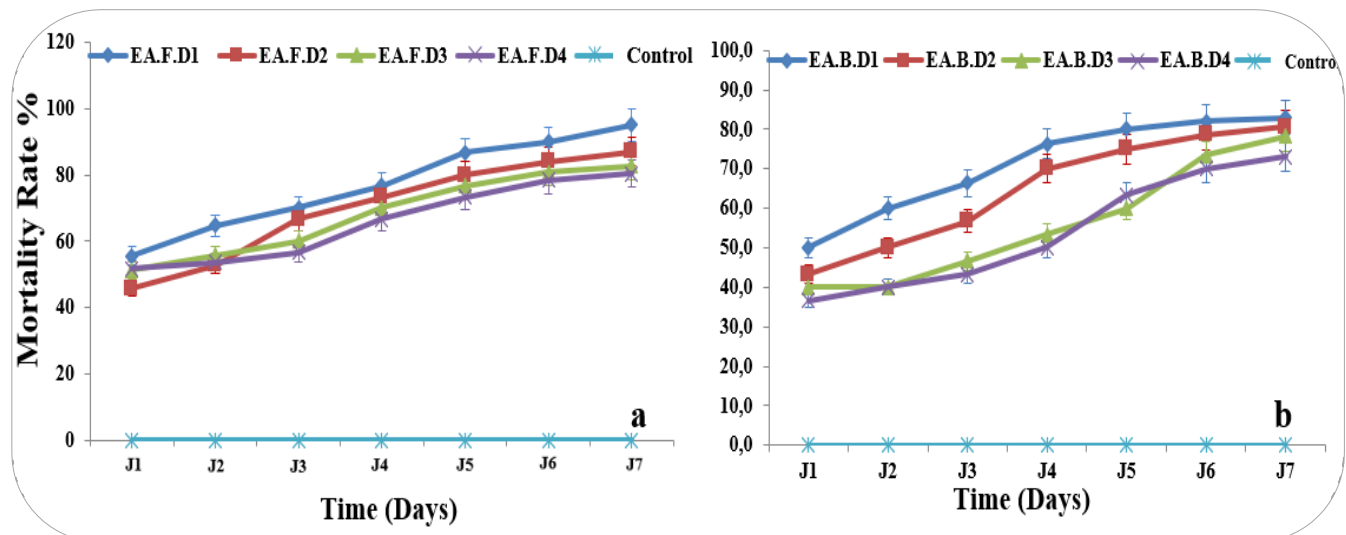


Figure 5. Temporal fluctuation of the mortality rate of *Sitophilus oryzae* under the effect of applied aqueous extracts. (a: Leaves; b: Bulbs)

Based on the results of the G.L.M. test, it was observed that the aqueous extracts from different compartments have a toxic effect on the population of *S. oryzae*, with a highly significant difference ($p < 0.001$) (Figure 6.a). The results clearly demonstrate the powerful effect of the aqueous extract derived from the leaves of *Urginea maritima* (L.) (Homogeneous group a), as compared to the extract from the bulb of the same plant (Homogeneous group b) (Figure 6.a).

The analysis of variance revealed that the dose factor has a significant impact on the increase in mortality rate in *S. oryzae* ($p < 0.001$) (Figure 6.b). Post-Hoc comparison analysis reveals the presence of three homogeneous groups related to the biocidal efficacy levels of the extracts. The first level is observed in the population exposed to the first dose, which has the highest mortality rate and is affiliated with homogeneous group (a), while the third level, corresponding to the populations exposed to the third and fourth doses, has the lowest mortality rate and is associated with homogeneous group (c) (Figure 6.b).

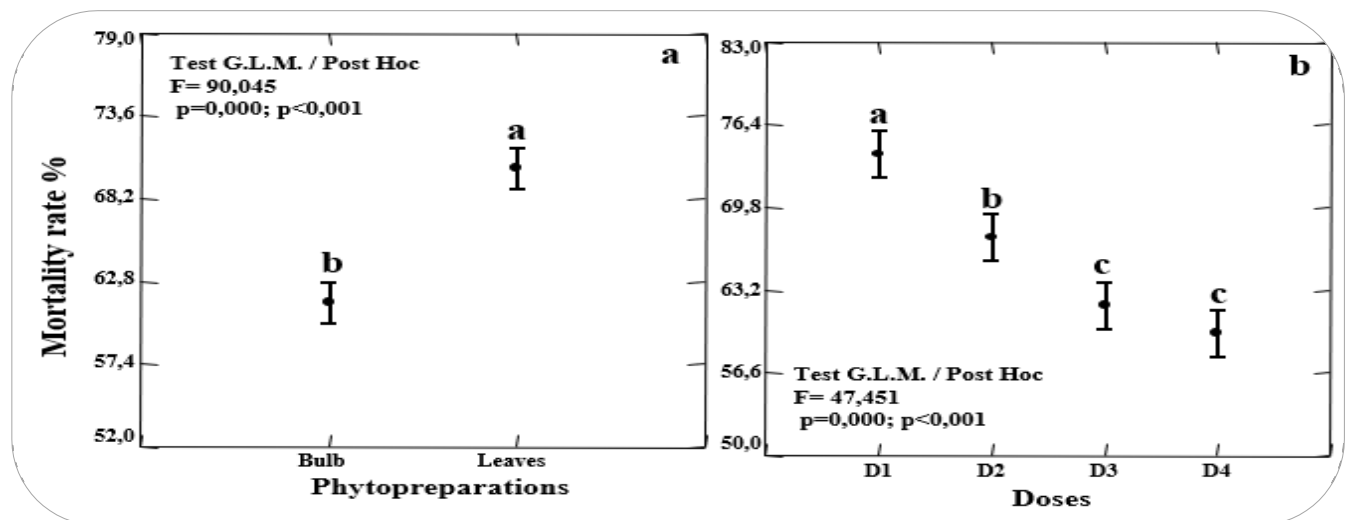


Figure 6. Comparative study of the effect of aqueous extracts on the mortality rate of *Sitophilus oryzae*

Determination of the lethal dose 50 (LD50) and lethal time 50 (LT50) of the pyto-preparations: To determine the LD50 of the aqueous extracts of *U. maritima* leaves and bulbs tested on adult *S. oryzae*, regression curves were plotted by correlating probits corresponding to the mortality percentages with the logarithms of extract doses

(Figure 7). By analyzing the equations derived from the regression curves, It was noteworthy that the lowest LD50 value of 10.320 mg/ml was observed following the application of leaf extracts, whereas the highest LD50 value of 53.689 mg/ml was obtained with the aqueous extract of *Urginea maritima* bulbs.

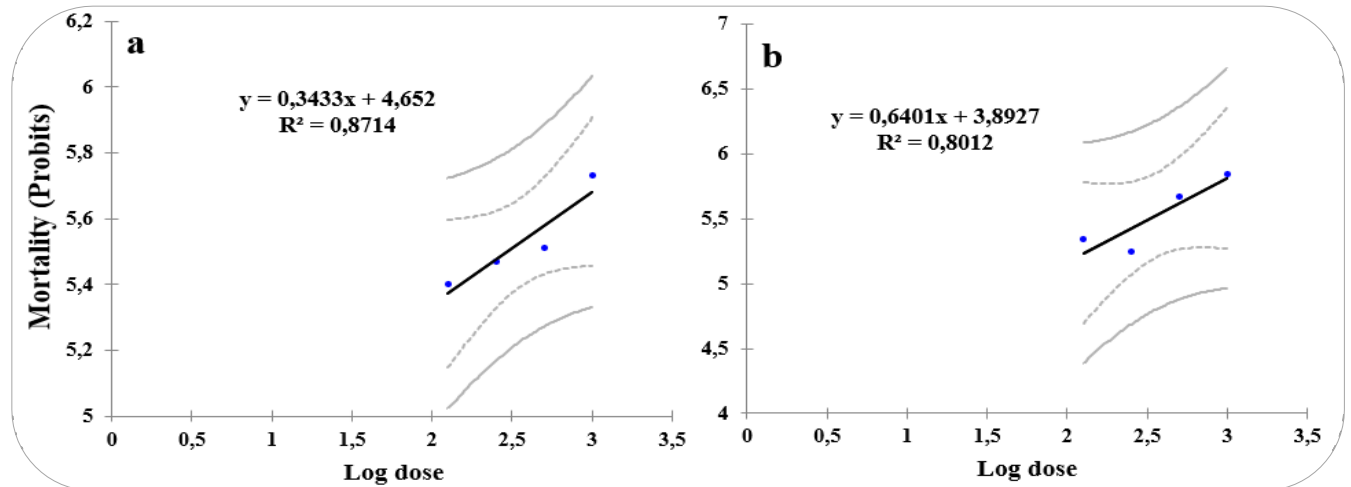


Figure 7. Mortality rate of *Sitophilus oryzae* as a function of the logarithm of doses of aqueous extracts from *Urginea maritima*. (a: Leaves; b: Bulbs)

Based on the probit analysis of cumulative mortalities and the logarithms of time for *Urginea maritima* leaf- and bulb-based aqueous extracts, regression lines were plotted to

determine the 50% lethal time (LT50) for the *Sitophilus oryzae* population (Figure 8).

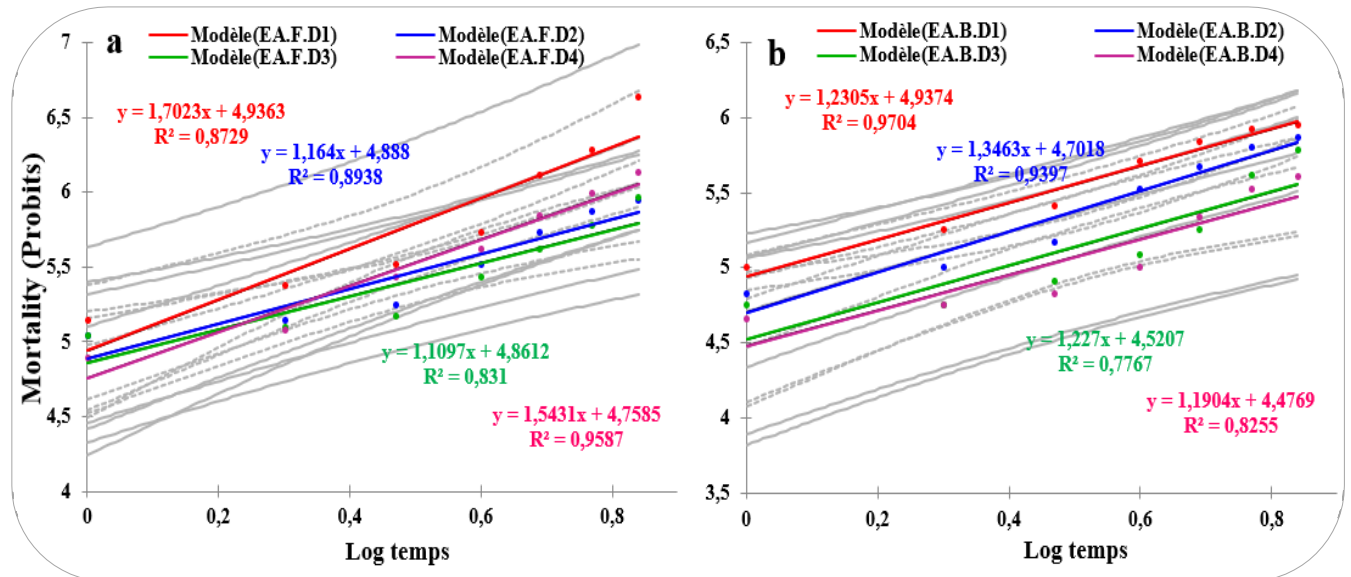


Figure 8. Mortality rate of *Sitophilus oryzae* as a function of the logarithm of treatment times applied. (a: Leaves; b: Bulbs)

The LT50 values, calculated from the equations derived from these regression lines, indicated that the shortest LT50 (1.0899 days) was obtained with high-dose leaf-

based aqueous extracts. In contrast, the longest LT50 (2.7506 days) was recorded with low-dose bulb-based aqueous extracts (Figure 8; Table 1).

Table 1. Regression line equations and LT50 values

Aqueous extracts of <i>Urginea maritima</i>				
Leaves			Bulbs	
	Equation	LT 50(Days)	Equation	LT 50(Days)
D1	$y = 1,7023x + 4,9363$	1,0899	$y = 1,2305x + 4,9374$	1,1242
D2	$y = 1,164x + 4,888$	1,2480	$y = 1,3463x + 4,7018$	1,6653
D3	$y = 1,1097x + 4,8612$	1,33376	$y = 1,227x + 4,5207$	2,4582
D4	$y = 1,5431x + 4,7585$	1,4338	$y = 1,1904x + 4,4769$	2,7506

DISCUSSION

Plants produce active substances with insecticidal, aseptic, or plant and insect growth-regulating properties. Most often, these active substances are secondary metabolites that originally protect plants (Deravel *et al.*, 2014). Plant extracts, which concentrate these molecules, are increasingly being studied for their potential to replace conventional chemical treatments, which are often harmful to the environment and human health (Yakhlef, 2010).

These results confirm the findings and conclusions of previous research that examined the effects of plant extracts on *Meloidogyne* spp. larvae. Our results are consistent with the work of Erdoğan (2022) and Nebih *et al.* (2019), all of whom demonstrated the nematocidal effect of different plant extracts on *Meloidogyne* larvae. This previous work has established a link between the nematocidal effect and the ability of plants to produce specific secondary metabolites.

Some subclasses of molecules express a bio-regulatory capacity against nematodes. Thus, in the alkaloid's family, non-protein amino acids, and pyrrolizidine alkaloids, they are recognized for their toxicity. In the phenolic compounds, we find flavonoids, specifically glycosylated flavones and flavonols. Among terpenoids, glycosylated cardenolides, and saponins possess these toxic characteristics (Andang, 2012).

The secondary metabolites identified in *Urginea maritima* extracts, such as alkaloids, non-protein amino acids, pyrrolizidine alkaloids, flavonoids, glycosylated flavonols, glycosylated cardenolides, saponins, catechic tannins, cardiotonic heterosides, mucilages, flavonones, anthocyanins, coumarins, and leuco-anthocyanins, demonstrate significant biochemical diversity. These compounds are well known for their varied biological properties, some of which may influence nematocidal activity (Andang, 2012).

Numerous studies have identified the presence of bioactive secondary metabolites in extracts of *Urginea*

maritima, supporting their potential use in pest management. Kord *et al.* (2020) conducted thin-layer chromatography (TLC) screening of *U. maritima* extracts, identifying 15 distinct compounds, including catechic tannins, cardiotonic heterosides, mucilages, flavonones, anthocyanins, and coumarins. These metabolites are known for their biological activity, including nematocidal and insecticidal effects. Bouhadjeb *et al.* (2018) further demonstrated that aqueous extracts of *U. maritima* are particularly rich in phenolic compounds, likely due to their higher solubility in water compared to ethanol extracts.

The phytochemical composition of *U. maritima* aligns with the observed efficacy of its aqueous extracts in this study. The lower LT50 and LD50 values recorded with the leaf-based aqueous extracts can be attributed to the higher concentration of water-soluble secondary metabolites, such as phenolics and flavonoids, which are known to disrupt the physiological processes of pests. These findings underscore the role of secondary metabolites in enhancing the nematocidal and insecticidal activities of *U. maritima* extracts, supporting their potential as a natural pest control agent.

Our results are consistent with previous studies highlighting the role of plant-derived substances in the development of new therapeutic substances as well as alternative biological control methods to manage pests (Soltani *et al.*, 2022).

According to (Pascual-Villalobos M.J., and Fernández M., 1999), *U. maritima* is a widely recognized and commonly used plant in phytotherapy. Several authors have noted that this plant exhibits biological activity, attributed to its chemical composition mainly comprising cardiac glycosides, anthocyanins, flavonoids, fatty acids, polysaccharides, calcium oxalate, tannins, reducing compounds, combined anthraquinones, mucilage, triterpenes, and steroids (Cogne *et al.*, 2001; Mami *et al.*, 2017; Belhaddad *et al.*, 2018). According to Mami A. and *al.* (2017), the insecticidal activity of *Urginea maritima*

extracts demonstrated good toxicity against *S. oryzae*. Furthermore, the toxic effect of aqueous extracts from both leaves and bulbs of *U. maritima* has been confirmed against the larvae and adults of *Tribolium castaneum* according to (Benhamouda *et al.*, 2016).

The aqueous extract of *U. maritima* leaves is more toxic against rice weevil than that of the bulbs. This difference is explained by the variation of secondary metabolites in the different parts of the plant, as confirmed by other studies. The composition of these molecules varies both quantitatively and qualitatively depending on the part of the plant used, the age of the plant and the period of the vegetative cycle (Chaouati *et al.*, 2023).

Furthermore, the findings of our study highlight that the biocidal capabilities of the treatments increase proportionally with the applied dose. These findings are consistent with those of (Rahuman *et al.*, 2009), who observed a correlation between the toxicity level of the extracts and the dose used.

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CONCLUSION

In this study, the aqueous extracts of *Urginea maritima* demonstrated significant nematocidal and insecticidal activity against *Meloidogyne* spp. and *Tribolium castaneum*. These results highlighted the potential of *U. maritima* extracts as a targeted biological control agent for managing these specific agricultural pests. The high efficacy observed, particularly with the leaf-based extracts, underscores the importance of the plant's rich secondary metabolite composition in pest control.

While the findings are promising, their application is currently limited to the pests investigated in this study. Further research is necessary to explore their effectiveness against a broader range of pests, optimize extraction methods, and assess their environmental safety and compatibility with existing agricultural practices. By advancing this research, *U. maritima* extracts could represent a sustainable alternative to synthetic pesticides, contributing to environmentally friendly pest management strategies.

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Safiddine fadhila	:	Performed the nematode experiments, collected and analyzed the nematode data, w
Tchaker F. Zohra	:	Conducted the entomological experiments, analyzed the data,
Hantabli rym	:	linguistic correction (scientific English)