Evaluation of the Insecticidal Activity of Bioactive Compounds Obtained from Azolla pinnata and Azolla Microphylla

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Abstract

Plant extracts offer an alternative approach to safeguarding stored food products. Our research is focused on assessing the insecticidal properties of aqueous and ethanolic extracts containing bioactive compounds obtained from two species of Azolla; *A. pinnata* and *A. Microphylla* against pests that affect stored food items. Two drying processes were conducted in order to compare the yield of bioactive compounds (freeze-drying and oven-drying). We employed two extraction methods (maceration and decoction), using ethanol and water as solvents. Phytochemical screening of both extracts was carried out by CG-MS analysis. The insecticidal properties of the obtained extracts were assessed using the spraying method on larvae and adults of *Tribolium castaneum*. The results indicated that the freeze-dried samples had the highest yield, with the ethanolic extract by decoction recording the highest value at 26.07%. GC-MS analysis for both species confirmed the existence of primarily fatty acid, terpenoid, steroid, coumarin, and flavonoid derivatives compounds Overall, the various extracts exhibited high toxicity against both larvae and adults. The mortality rate was increased according to the increasing concentration of extracts. The lowest LC_{50} of *A. pinnata* and *A. microphylla* extracts were found to be 872.42 µg/mL and 894,65 µg/mL, respectively. The aqueous extract caused higher toxicity, reaching 96%. The results of this study may indicate that the dry matter from *A. pinnata* and *A. Microphylla* demonstrated effective toxicity against individuals of *T. castaneum*. The potency of this effect is evidenced by the mortality of both the larvae and adults.

1. Introduction

Cereals occupy an important place in both human and animal nutrition. They constitute an essential source of basic food for a large part of the world's population and are also widely used in animal feed (Ngamo and Hance 2007). Cereal storage is of great importance to ensure food availability; allowing surplus crops to be preserved for periods when production is reduced or when there is increased demand and also for seeds for future agricultural seasons.

On the other hand, cereals are generally attacked by insects, fungi and rodents. Stored wheat insects represent a very important part of stored grain pests, such as *Sitophilus granarius, Rhyzoperta dominica* and *Tribolium castaneum*. (Kučerová et al. 2003; Rahman et al. 2007). The damage caused by these insects is the most important (Ndomo et al. 2009). They can cause significant losses by reducing the quality and quantity of stored products. These losses are of the order of 10–40% in countries where modern storage technologies have not been introduced (Lee et al. 2003).

Grain protection methods can be used to prevent insect and pest attacks. This may include the use of insecticides, fumigation or appropriate chemical treatments (de Sousa et al. 2023). However, these chemical insecticides can induce chronic poisoning of consumers, resistance in pests. Furthermore, the ongoing use of synthetic chemical controls and their frequent application has led to the development of resistant mosquitoes and environmental pollution (Ravi et al. 2018). The focus on replacing chemical

treatments is growing. According to the European Commission, the number of low-risk or non-chemical pesticides approved for pest control has doubled since 2009 (de Sousa et al. 2023).

To achieve effective protection of foodstuffs during storage, it is necessary to find an alternative that does not cause health problems or any harm to consumers and the environment (Ngamo and Hance 2007).

Biological control is the most favoured method as an alternative in research programs given its economic and agro-environmental interests which allow the maintenance of a bioecological balance (Amari et al. 2014). Much research has been done to find a suitable biopesticide than chemical pesticide. Natural substances which present a broad spectrum of action in pharmacology, such as bactericides, fungicides, acaricides, etc., can also be used as replacement insecticides.

Plant-derived pesticides were the first recorded pest control agents used by humans. Sulfur, elemental compounds, toxic inorganic salts, and mineral oils were the first chemical pesticides used (Dalavayi Haritha et al. 2021). These crude pesticides were very hazardous to humans. The sulfur preparations, despite effective fungicides, were injurious to humans and still are considered moderately toxic. The other materials were highly toxic to humans. The use of these materials was an attempt to control pests with less harmful material. This concept led to the use of inorganic and highly toxic organic compounds and is the driving force today for the use of botanicals and organic compounds (Souto et al. 2021).

Among the natural springs available, we have an aquatic fern called the Azolla. There are at least eight species of azolla well known (Malek et al. 2008). All species fall into two subgenera: the Euazolla subgenus, encompassing *Azolla fliculoides*, *Azolla mexicana, Azolla caroliniana, Azolla microphylla*, and *Azolla rubra*, and the Rhizosperma subgenus, comprising *Azolla pinnata, A. nilotica*, and *A. imbricate* (Yang et al. 2022).

Among them, *Azolla pinnata* and *Azolla microphylla* which are small aquatic fern used in traditional medicine. It also a good quality supplement for animal feeds, especially for poultry and pigs. Numerous studies showed that pigs and poultry fed Azolla meal grow faster and have better feed conversion rates than those fed standard diets (Swain et al. 2022; Alagawany et al. 2024).

In previous studies, it was concluded that biological extracts obtained from Azolla have several effects such as; Antioxidant, antibacterial activity (Chander and Kumar 2017). There is therefore a need to produce purified bioactive substances for use in a wide field of application.

Thus, the valorization of molecules obtained from this aquatic fern as therapeutic agents has significant economic potential and an alternative to the use of synthetic products and conventional agents. In fact, this research work is part of this context with the purpose of evaluating the insecticidal properties of aqueous and ethanolic extracts from two Azolla species (*A. pinnata* and *A. microphylla*) obtained by maceration and decoction, against the red flour beetle (*Tribolium castaneum*).

2. Materials and Methods

2.1. Biological materials

2.1.1. Plant material

The collection of Azolla samples was carried out from the region named Honaine –Tlemcen province. Samples are collected manually from the culture basins as shown in Fig1. Samples should be transported in a cooler. Their storage must be done at 4°C and in the dark until preparation for analysis.

2.1.2. Insect material

The insect material used for our study is wheat larvae and insects. The collection of insects (larvae, adults) was carried out at the cereal cooperative where the wheat is stored (Tiaret province). There are several species of insect pests, in particular the species of *Tribolium castaneum, Sitophilus granarius* and *Trogoderma granarium*. We were interested in working with *Tribolium castaneum* because it is the most abundant in wheat silos. *T. castaneum* commonly called the Red Flour Tribolium or small flour worm (Atta et al. 2020). It is a pest of stored foodstuffs, best known in tropical and subtropical regions. The adult measures 3 to 4 mm, uniformly reddish brown in color (Fig2).

The mass breeding of insects was carried out in a glass jar containing damaged wheat grains, and placed in an oven set at a temperature of 37 °C and a relative humidity of 70%. To separate the insects (larvae and adults) from the wheat grains, we have used a 0.5 mm diameter sieve.

2.2. Experimental methods

2.2.1. Sample preparation

Both of azolla samples (*A. pinnata* and *A. microphylla*) were washed thoroughly with tap water followed by rinsing with distilled water and drying. Most literature available concentrated on evaluating the effect of extraction protocol in plant extracts. However, the drying method before extraction is also important. In order to evaluate the effect of the sample preparation method on the extraction yield; two drying methods were used, freeze drying and by oven dry.

Lyophilization was carried out by a Christ Martin ALPHA 1-2 LD plus type lyophilizer. The frozen samples of azolla approximately 100 g of each species was introduced directly into the vacuum freeze dryer for 48 hours where the temperature was reduced to -51 °C and the pressure to 0.011 mbar.

The fine powder was obtained from the dried material using a mixer grinder for the oven-dried material and a mortar for the freeze-dried material. The plant powder was stored in a desiccator in order to use it to obtain the extracts.

2.2.2. Extraction of bioactive compounds from azolla dry matter

In our study we started with the extraction of the bioactive compounds present in samples dried by two methods (lyophilization and oven). The extraction was carried out by maceration and decoction processes using water and ethanol as solvent.

Maceration involves exposing powdered plant material to a solvent for extended periods of time to extract the active ingredients. It is extracted at room temperature, which has the advantage of preserving heat-sensitive substances (Bouchouka 2016). Decoction is a method of extracting the active compounds from general herbal preparations by boiling them in water. It is generally applied to the hardest parts of the plant: roots, seeds, bark, wood (Stéfane et al. 2022).

1g of each sample is mixed with 60 ml of (water; ethanol), the whole being incubated with a layer of aluminum foil in the dark with magnetic stirring (at 25°C for 24 hours) for maceration and (at 60°C for 3 hours) for decoction. The mixtures are filtered on Wathman filter paper (n°: 1), then evaporated in an oven at 44°C to obtain a dry residue.

2.2.3. Determination of extraction yield

The extraction yield is expressed as a mass percentage relative to the quantity of dry matter according to the formula:

$$R(\%) = \left[\frac{M_1}{M_0}\right] \times 100$$

From where: R (%): Yield of extracts; M1: Mass of the dry extract obtained (g); M_0: Initial mass of dry matter (g).

2.2.4. Analysis of extracts by gas chromatography coupled to mass spectrometry (CG-MS).

Only the ethanolic extract of each species was used for the analysis of possible bioactive compounds. GC-MS analysis was conducted using a Perkin Elmer Clarus 680 gas chromatograph, paired with a Perkin Elmer Clarus SQ8 mass spectrometer, both operated by Turbo Mass v. 6.1 software and featuring a 2011 NIST MS Search 2.0 database. According to the method reported by Boussoum et al. (2022); the samples are derived first to facilitate the detection of all compounds present. For this, 2 mg of each azolla extract were mixed with 50 µL of N,O-Bis (trimethylsilyl) trifluoroacetamide (bstfa) + TMSCl 1% as silylation agent in a 2 mL pillbox. In order to allow the reaction of the derivation agent on extracts; the preparation was placed in an oven at 70 °C for 120 min. Then, the pillbox was then opened to allow the evaporation of bstfa. After that, 1 mL of ethyl acetate was added to dissolve the silylated extracts. Finally, by the splitless method, 1 µL of the solution was injected into the GC via injector port (250 °C). The DB-5ms was used as stationary phase (dimethyl-/ diphenyl-polysiloxane 95%/5%; length: 30 m; internal diameter: 0.25 mm; film thickness: 0.25 µm) through a 40 min furnace temperature program (80°C to 300°C at the rate of 6°C/minutes). Helium was used as a carrier gas with a flow rate of 1

ml/minute. At the end of the chromatographic separation, the compounds are sent to the mass spectrometer via a transfer line thermostated at 250 °C before to be ionized at 70 ev.

2.2.5. Evaluation of insecticidal activity

a. Preparing the extracts

For comparison purpose, each of the dry extracts of both species obtained by the two extraction methods (maceration, decoction), from the lyophilized sample only, are dissolved in DMSO with a volume of 5mL and five different concentrations of ethanolic and aqueous extracts (500, 1000, 1500, 2000, 2500 µg/mL) in order to conclude the lethal concentration (LC_{50}). The prepared solution were poured into glass spray bottles, and then stored in the refrigerator at 4 °C until further use.

b. Insecticidal tests

This test consists of studying the effect of bioactive compounds from *A. pinnata* and *A. microphylla* on the mortality of adults and larvae of *T. castaneum*. Techniques for measuring the insecticidal activity of natural extracts have a major impact on the result. The application technique consists of spraying the insects with the prepared solutions. The larvae and adults are placed separately in petri dishes. Each box contains 10 individuals with some infested wheat grain for their food; three repetitions were carried out for each solvent plus a control. The mortality rate was calculated after 8, 16 and 24 hours respectively.

c. Calculation of mortality rate

The percentage of larvae and adults mortality of *T. castaneum* is processed and calculated by the following formula:

$$\textbf{Observed mortality} = \left[\frac{\textbf{Number of dead individuals}}{\textbf{Number of dead individuals}}\right] \times 100$$

d. Mortality rate correction:

The number of dead individuals in a population treated with the prepared solutions does not represent the real number of individuals influenced by the bioactive compounds containing in solutions. The ABBOT formula (1925) makes it possible to correct this mortality rate:

$$M(\%) = \frac{M_O - M_C}{100 - M_C} \times 100$$

M (%): percentage of corrected mortality; M_O: percentage of mortality observed in the treated population; M_C: percentage of mortality observed in the control population

2.3. Statistical analysis

The manipulations were carried out in triplets and the statistical analysis is carried out using the computerized statistical software ANOVA. Values were expressed as means ± standard deviation.

3. Results and discussions

3.1. Extraction of bioactive compounds

The extraction of bioactive compounds is necessarily a complex and delicate operation. Its aim, in fact, is to break down the cell walls to extract the bioactive compounds locked in the cells. For comparison purpose, two different drying methods, different extraction processes and two solvents were used to investigate their extraction and compare their respective yields.

3.1.1. Extraction yields

Extraction yields vary considerably depending on the drying method used and the extraction method adopted. The results of the extraction of azolla samples by the different drying methods and in the different solvents used are represented in tables 1.

Table 1: Extraction yield of azolla samples by the different methods used.

		Extraction yield (%)			
		Azolla pinnata		*Azolla microphylla*	
		Ethanol	*Water*	*Ethanol*	*Water*
Lyophilization	***Maceration***	24.8 ± 1.02	*10.2 ± 0.74*	21.8 ± 1.22	*11.6 ± 1.04*
	Decoction	**26.07 ± 0.44**	***11.6 ± 0.68***	**22.03 ± 1.23**	***13.03 ± 1.62***
Oven dry	***Maceration***	19.5 ± 1.24	*9.7 ± 1.45*	19.16 ± 1.43	*10,12 ± 0.88*
	Decoction	21.2 ± 0.57	*11.02 ± 1.13*	19.8 ± 1.17	*12.02 ± 0.92*
***Moyen* ± SD**		16.75 ± 0.9		16.19 ± 1.18	

We have found that, for the decoction method, ethanol gives the best extraction yield for both species and for the freeze-dried and oven-dried samples, respectively (26.07% and 22.03%), followed by aqueous extracts. (11.6% and 13.03%),

Lyophilization, also known as freeze-drying, and oven drying are two common methods used for drying and preserving biological materials, including plant extracts. Each method has its advantages and disadvantages, and their impact on extraction yield can vary depending on the specific characteristics of the material being processed. The results of our study have shown that freeze-dried samples in both solvent have lead to higher extraction yields compared to oven drying for both of *A. pinnata* and *A.*

microphylla. It can be explicated by the fact that samples are containing delicate compounds that may degrade or evaporate at higher temperatures. By preserving the integrity of the sample, lyophilization can facilitate the release of bioactive compounds during subsequent extraction processes (ElNaker et al. 2020). Whereas, oven drying may be more suitable for materials that are not sensitive to high temperatures and require rapid drying (Bennour et al. 2020). Mphahlele et al. (2016), demonstrated that the best drying method to preserve total pomegranate phenols is freeze-drying compared to oven drying. Also, according to Galaz et al. (2017), high drying temperatures had reduced the polyphenol content.

In the other hand, samples that are decocted record high values compared to those that are macerated. These results are very close to those obtained by Mahmoudi et al. (2013), who found that the best extraction yields of total polyphenols, flavonoids and condensed tannins from artichoke by four solvents (water, methanol, ethanol and acetone), are recorded by the decoction, an average of 17.34% versus 15.64% for maceration.

3.1.2. CG-MS analysis

The results of previous studies about the qualitative analysis indicated the presence of phenols, flavonoids, alkaloids, steroids, tannins, cardio glycosides, and saponins in the ethanolic extract of azolla (Sreenath et al. 2016). Hence, in the present work, this quantitative analysis was used for the further studies.

The GC-MS analysis of the ethanolic extracts obtained from *A. pinnata* and *A. microphylla* conducted to the identification of more than 20 different compounds. The results are presented in table 2.

Table 2: GC-MS analysis of the ethanolic extracts obtained from *A. pinnata* and *A. microphylla*

Compounds		*A. Pinnata* (Area %)	*A. Microphylla* (Area %)	
			Our results	*(Sreenath et al., 2016)*
1,3-di-iso-propylnaphthalene	$C_{16}H_{20}$	0.5	NA	0.3
3-ethyldibenzothiophene	$C_{14}H_{12}S$	0.84	0.41	0.52
Tetradecanoic acid, 12-methyl ester	$C_{16}H_{32}O_2$	1.03	0.33	0.51
Neophytadiene	$C_{20}H_{38}$	0.83	1.02	1.64
2-[(Z)-9-octadecenyloxy] ethanol	$C_{20}H_{40}O_2$	0.72	NA	0.23
9-hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	2.11	1.25	1.04
Hexadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	8.04	5.6	4.8
3-cyano-12-isopropoxy-6,11-methanocyclodeca[g] imidazo[5,1-c] (1,2,4) triazine	$C_{18}H_{15}N_5O$	2.04	1.38	1.15
cis-13-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.28	0.76	1.05
Hexadecanoic acid, 2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	0.91	NA	0.2
9-Octadecenoic acid	$C_{18}H_{34}O_2$	22.16	18.12	15.04
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	1.81	1.03	0.5
(1R)-2-(1S)-1-[2-(Methoxymethoxy) phenyl] ethyl} amino) oxy]-1-phenylethanol	$C_{18}H_{23}NO_4$	0.34	0.4	0.69
3-[(tert-Butyldimethylsilyl) oxy]-1,4,4a, 9a-tetrahydro-1-phenyl-9H-xanthen-9-one	$C_{25}H_{30}O_3Si$	1.22	0.52	0.76
Cholest-2-en-1-ol	$C_{27}H_{46}O$	NA	0.73	0.39
Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	1.27	0.26	0.34
(2R/S,4S/R,6R/S)-4-Hydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadeca-9,12-dien-11-one	$C_{26}H_{42}O_4$	1.53	4.21	2.04
3á-(Peroxymethyl)-5-vinyl-A,B-bisnor-5á-cholestane	$C_{28}H_{48}O_2$	0.74	0.57	0.34
2,3,4,5,2',6'-Hexamethoxy-4',5'-methylenedioxychalcone	$C_{22}H_{24}O_9$	38.32	71.04	55.47

Cucurbitacin B, dihydro-	$C_{32}H_{48}O_8$	NA	NA	0.15
Chol-8-en-24-al 3-hydroxy-4,4,14-trimethyl-	$C_{27}H_{44}O_2$	0.93	NA	0.17

NA: *Not available*

For *A. pinnata* extracts, the highest area percentage value was noted for the compounds "$C_{22}H_{24}O_9$" with 38.32%, while almost the double of area percentage value was observed for the same compound in *A. microphylla* extract (71.04%). Sreenath et al. (2016) has reported the same major compound in term of area percentage with (55.47%). The findings for both species confirmed the existence of primarily fatty acid, terpenoid, steroid, coumarin, and flavonoid derivatives compounds.

These compounds have been noted to exhibit a range of biological and pharmacological effects (Sakthivel and Guruvayoorappan 2013).

3.2. Insecticidal test

In order to test the insecticidal effect of the bioactive compounds of Azolla, we have only used the extracts obtained from the freeze-dried dry matter. Therefore, we were interested in studying the effect of the two extraction methods (maceration and decoction) and the polarity of the solvents used. The results obtained made it possible to determine the insecticidal effect of the bioactive molecules present in *A. pinnata* and *A. microphylla* evaluated through the mortality recorded in adults and larvae of *T. castaneum* by a contact test at different periods after treatment. The percentage mortality of larvae and adults was increased according to the increasing concentration of extracts. The results of LC_{50} for both species are presented in table 3.

Table 3: Mean LC_{50} efficacy of *A. pinnata* and *A. microphylla* extracts after 24 h of exposure on *T. castaneum*.

		Extracts	LD_{50} (µg/mL)	Regression equation
A. pinnata	Larvae	*Ma-ethanolic*	1103,44	y = 14,08x + 19,02
		Ma-aqueous	-	y = 5,5x + 15,5
		Dec-ethanolic	-	y = 5,8x + 16,2
		Dec-aqueous	987,31	y = 15,1x + 23,7
	Adults	*Ma-ethanolic*	1000	y = 10,71x + 25,78
		Ma-aqueous	1589,25	y = 11,5x + 4,5
		Dec-ethanolic	-	y = 3,2x + 14,4
		Dec-aqueous	872,42	y = 12,7x + 38,9
A. microphylla	Larvae	*Ma-ethanolic*	1165,23	y = 11,4x + 23,4
		Ma-aqueous	2500	y = 7,5x + 11,5
		Dec-ethanolic	2080,58	y = 7,7x + 15,3
		Dec-aqueous	968,12	y = 11,6x + 29,2
	Adults	*Ma-ethanolic*	1500	y = 6x + 31,2
		Ma-aqueous	2087,46	y = 7,6x + 16
		Dec-ethanolic	-	y = 4,8x + 11,2
		Dec-aqueous	894,65	y = 7,9x + 33,5

As it is shown in Fig3, the *T. castaneum* larvae bioassay demonstrated a significant rise in mortality as the concentration of *A. pinnata* extract increased. The LC_{50} of ethanolic and aqueous extracts were found to be 1103.44 µg/mL and 987.31 µg/mL, respectively. Bhattacharjee et al. (2022) have reported the same larvicidal activity exhibited by the *A. pinnata* extracts. The aqueous extracts by decoction had significant insecticidal toxicity against the adults at 500 µg/mL and above. At 872.42 µg/mL, 50% mortality of the adults was achieved, and at 2500 µg/mL, almost a complete mortality was observed (Fig4). Comparatively, the *A. microphylla* extracts were less toxic towards the *T. castaneum* larvae and adults with LC_{50} of 1165.23 µg/mL and 968.12 µg/mL, respectively (Fig5 and 6).

3.2.1. Impact of the extraction method on the insecticidal capacity of the extracts

We have conducted two extraction techniques in our study to determine the effectiveness of the extracted compounds. It should be noted that mortality was observed in batches of up to 3/10 individuals. Therefore, the toxicity of crude phytopreparations was estimated by evaluating the corrected mortality. The results are presented in table 4 below.

Table 4: Mortality rate of larvae and adults of *T. Castaneum* treated with both species extracts (2500 µg/mL) after 24 hours.

		Mortality (%)			
		Larvae		***Adults***	
		Ethanol	***Water***	***Ethanol***	***Water***
A. pinnata	***Maceration***	84,44 ± 1.35	40 *± 3.74*	75,55 ± 1.22	60 *± 3.54*
	Decoction	43 ± 1.3	*96 ± 2.68*	30 ± 3.23	*96 ± 6.62*
A. microphilla	***Maceration***	76 ± 1.24	*50 ± 1.45*	60 ± 2.43	*55 ±2.88*
	Decoction	55 ± 1.57	*84 ± 2.13*	38 ± 1.17	*70 ± 1.92*

The observation of the petri dishes of the larvae treated with the different extracts (2500 µg/mL) obtained from *A. pinnata* by maceration revealed that the ethanolic extract gave a very high mortality of 84.44%, comparing with the aqueous extract (40%). Identical observation was noted in adult's petri dishes (75.55% and 60%, respectively) While, for extracts obtained by decoction we were able to see that the aqueous extracts caused significantly higher mortality, reaching 96% against both of larvae and adults.

The same principles were followed for the *A. microphylla* extracts, but they exhibited lower mortality rates (76% ethanolic extract and 84% for aqueous extract) compared to *A. pinnata* extracts. These results are in concordance with the previous study, mentioned that azolla plant has potential as a bioinsecticide to address single chemical compound resistance issues (Ravi et al. 2018).

The insecticidal power of this fern is due to the presence of phenolic compounds, tannins and saponins (Ekanayake et al. 2007). The insecticidal effect of these constituents has been mentioned by several authors. Phenolic compounds have both pesticidal and fungicidal properties. Tannins have insecticidal, larvicidal and repellent properties (Wardell 1987).

Previous studies have shown that these natural compounds can cause symptoms indicative of neurotoxic activity, such as hyperactivity, convulsions and tremors followed by paralysis and death of the insect which are very similar to the effects produced by pyrethroid insecticides (Richardson et al. 2019).

The comparative analysis of these results shows that there is a positive correlation between polarity of the extract tested and the stage of development of the killed insect. According to Hinwood (1997), who states that a polar solvent dissolves a polar solute better than a non-polar solvent. One might think that this difference in biological activity linked to the polarity of our various biocidal products could only be due to a quantitative and/or qualitative difference in the active compounds present there.

3.2.2. Impact of exposure time on the insecticidal activity of extracts

We tried to study the effect of the contact time of the extracts to larval and adult individuals during this work. Therefore, the mortality reading was made after every 8 hours up to 24 hours. The results are presented in the Figure 5. It was found that the average mortality of adults and larvae of *T. Castaneum* increases depending on the duration of exposure to the extracts used by contact, since an increase in mortality was recorded as we move forward in the exposure time.

The effectiveness of the extracts began from the first reading (after 8 hours) for all the extracts with the exception of the ethanolic extract by decoction which only gave effect after 10 hours. On the other hand, the aqueous extract by decoction was very effective with a mortality rate of 60% from the first reading. In fact, the mortality of individuals increased mainly after 16 hours of exposure.

The findings of the present study may suggest that the dry matter of *A. pinnata* and *A. Microphylla* gave a good result for its toxicity on individuals of *T. castaneum*. This effectiveness is confirmed by the death of larvae and adults of this pest. The results for both species confirmed the existence of primarily fatty acid, terpenoid, steroid, coumarin, and flavonoid derivatives compounds. Activity was always positively associated with an increase in concentration of the bioactive compound. These biologically active molecules have been noted to exhibit a range of biological and pharmacological effects. Ongoing research is being carried out to clarify the elements accountable for the insecticidal effects, along with any potential pharmacological or toxicological properties of such extracts.

Declarations

Acknowledgement/Disclaimers/Conflict of interest

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The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this article.

Contributions

AA Conceptualization, Methodology, Investigation, Formal Analysis, Data Curation, Validation, Writing—Original Draft, Writing—Review and Editing. WD Resources, Methodology, Writing—Review and Editing. MOB Conceptualization, Methodology, Investigation, Writing—Review and Editing, Validation, Resources, Funding Acquisition, Supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Informed consent

All authors have reviewed the manuscript and approved its submission to the Journal of Plant Disease and Protection.

Human or animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Figures

Figure 1

Azolla sampling site in Honaine - Tlemcen province

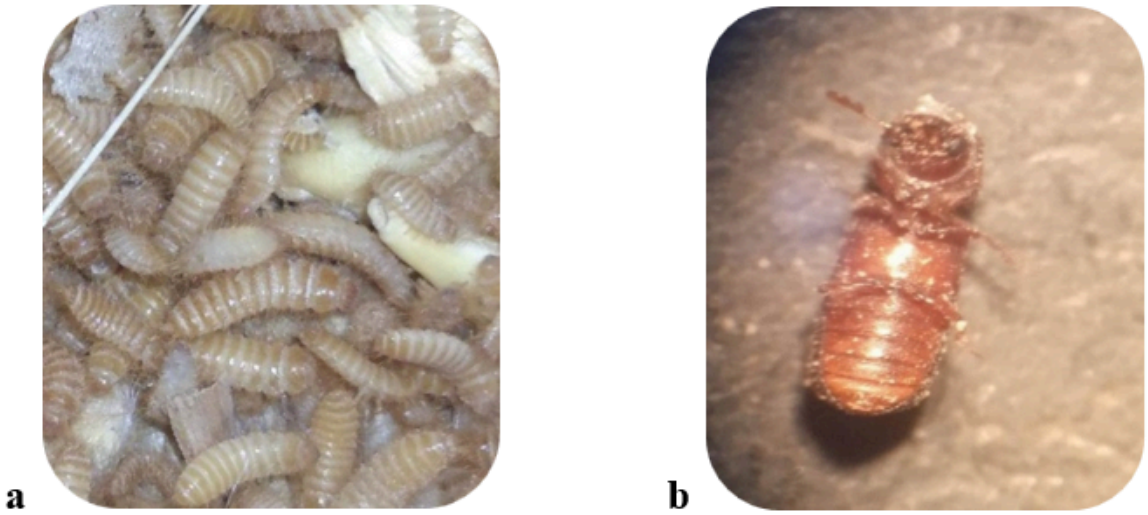

Figure 2

Tribolium castaneum (**a:** larvae **b**: adults)

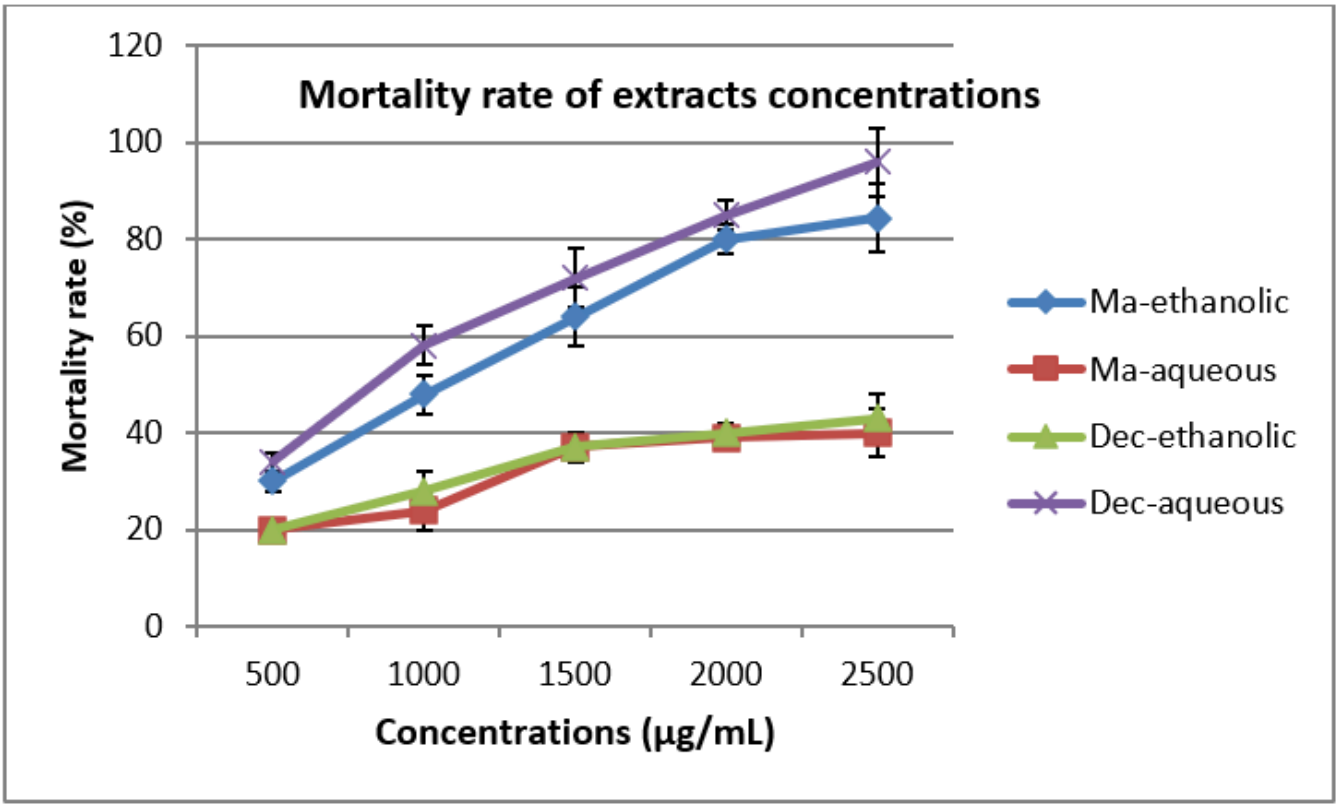

Figure 3

Mortality rate of *T. Castaneum* larvae depending on concentrations of different extracts obtained from *A. pinnata*

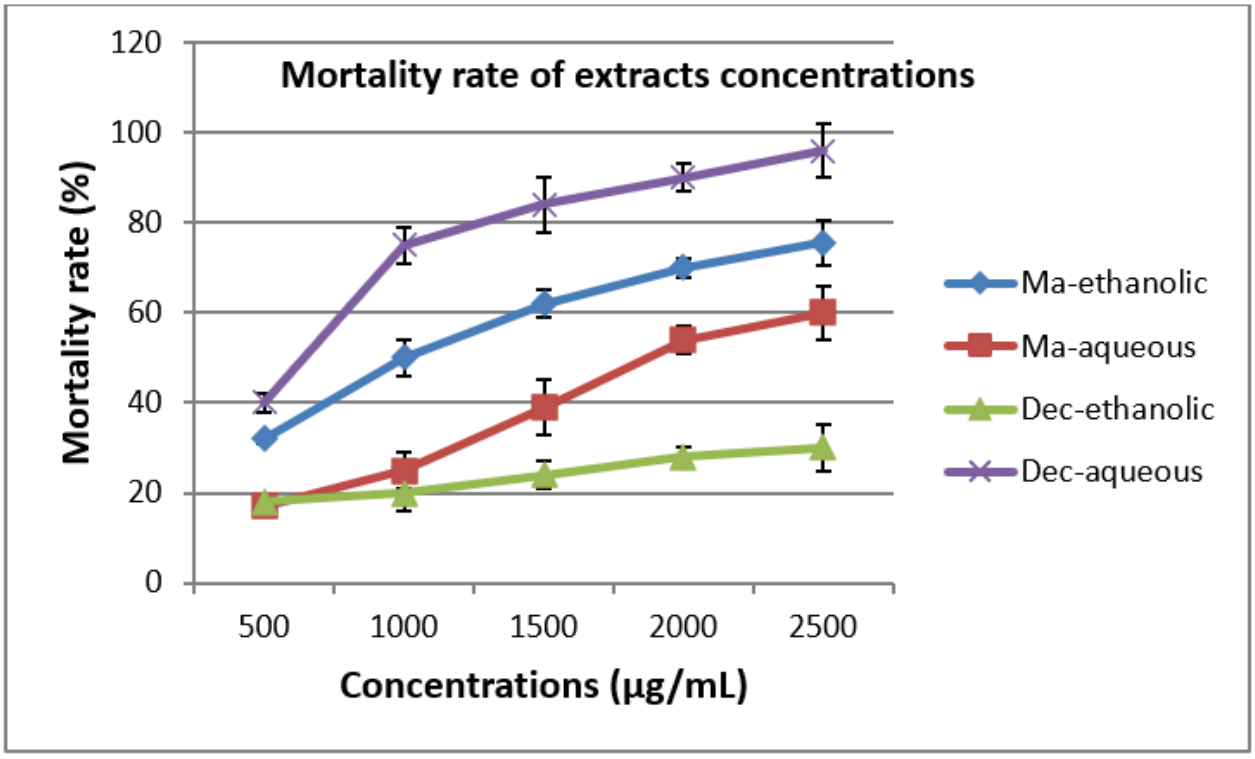

Figure 4

Mortality rate of *T. Castaneum* adults depending on concentrations of different extracts obtained from *A. pinnata*

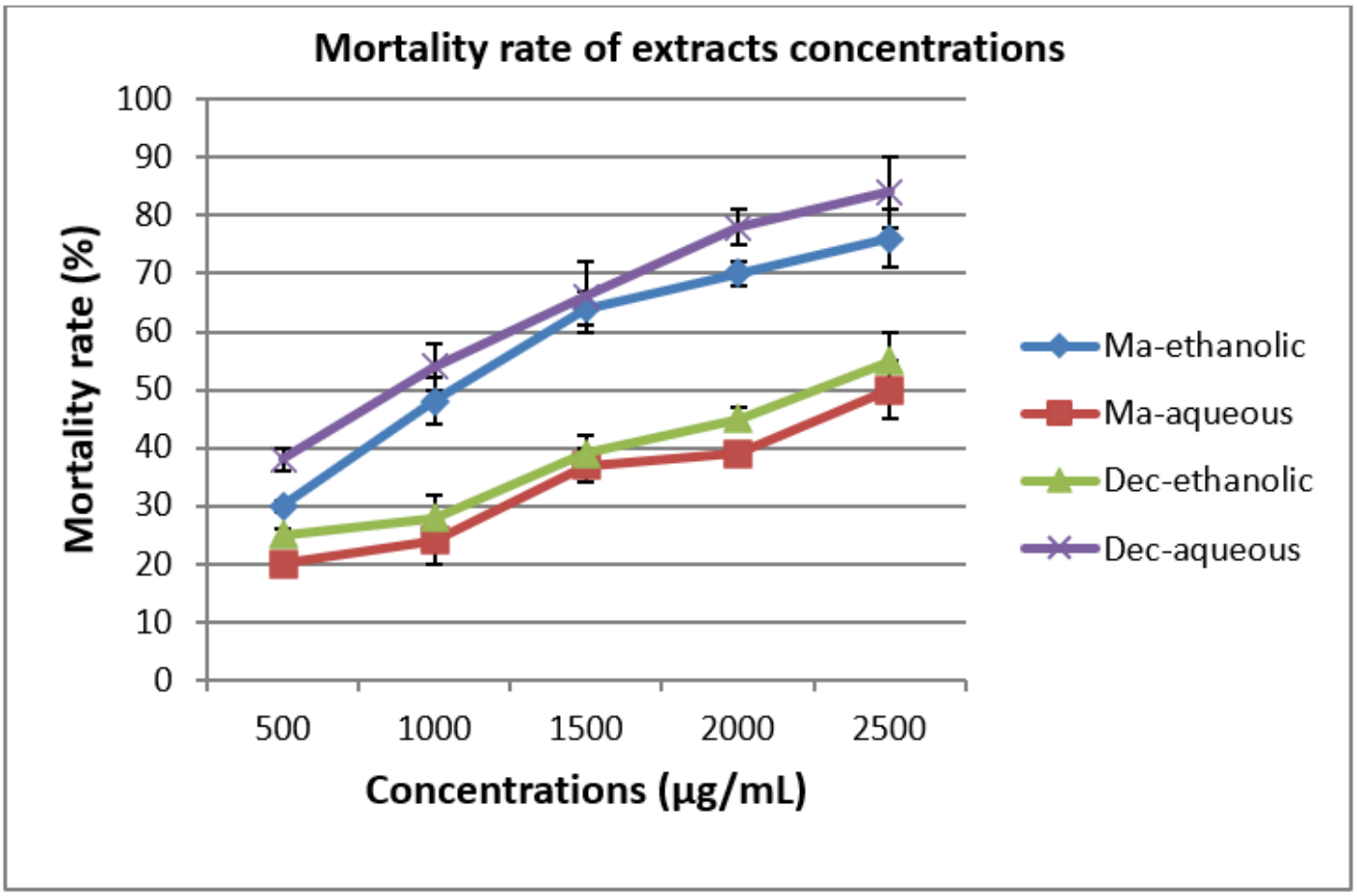

Figure 5

Mortality rate of *T. Castaneum* larvae depending on concentrations of different extracts obtained from *A. microphylla*

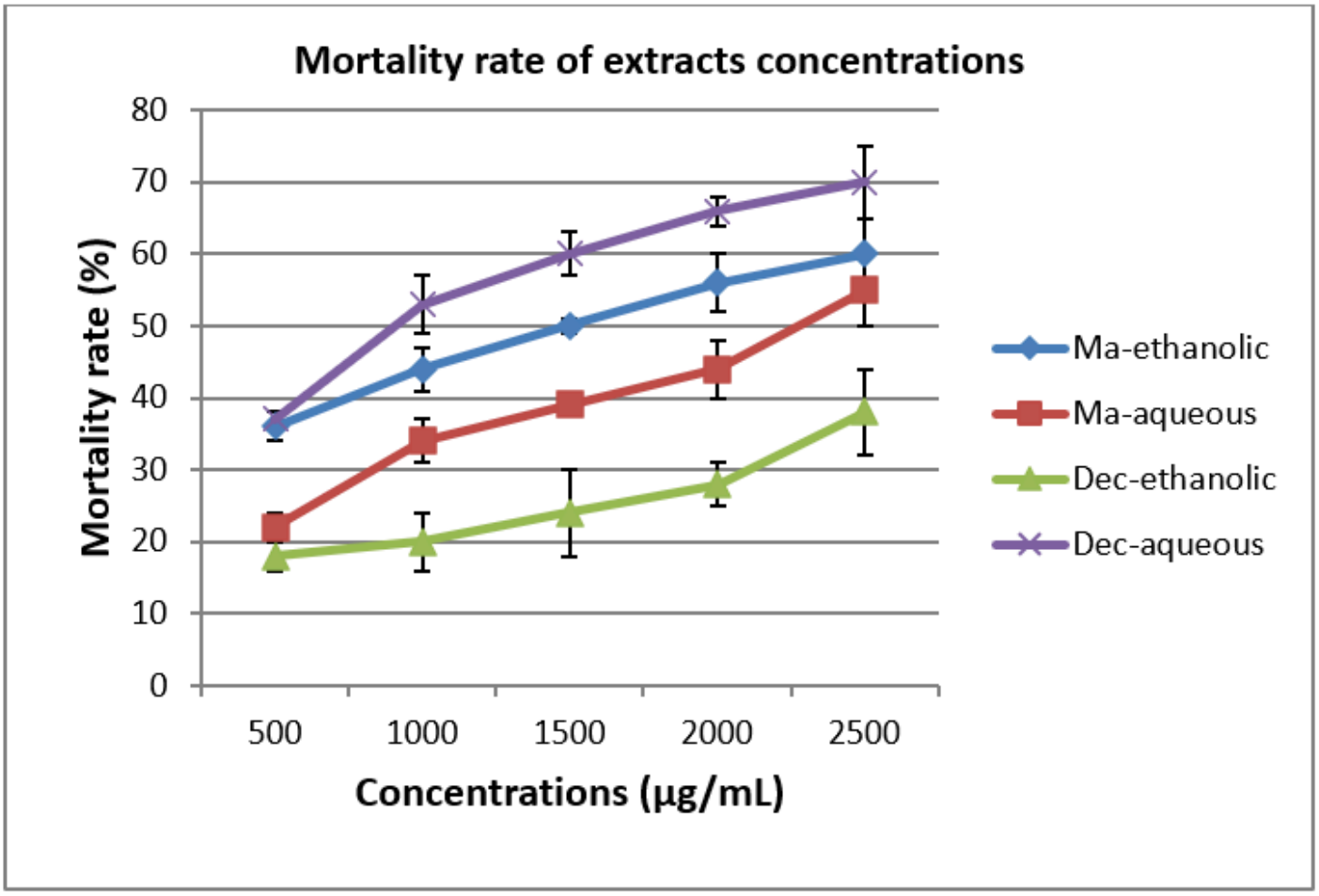

Figure 6

Mortality rate of *T. Castaneum* adults depending on concentrations of different extracts obtained from *A. microphylla*

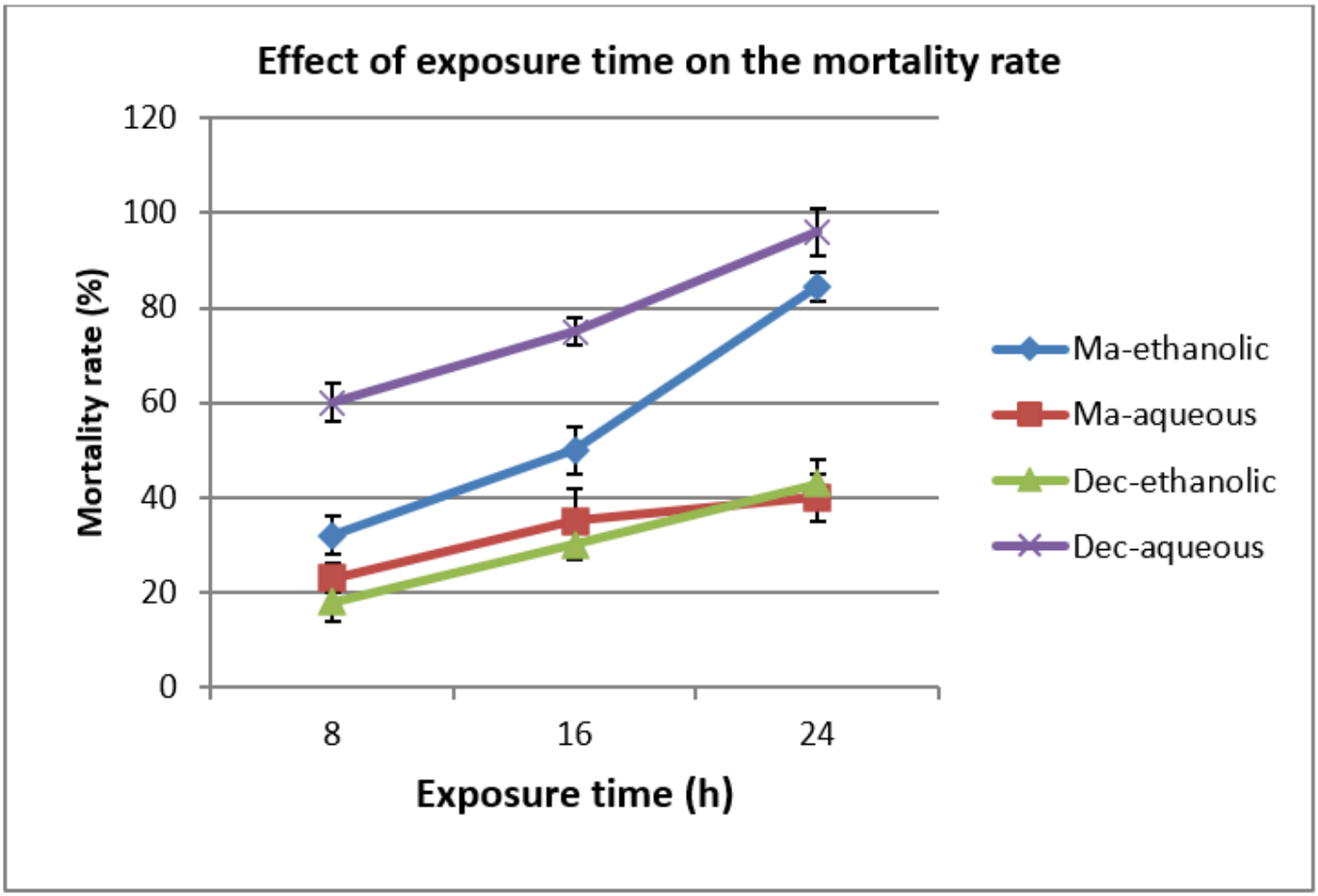

Figure 7

Mortality rate of *T. Castaneum* larvae depending on exposure time of different extracts obtained from *A. pinnata*