



## Insecticidal effect of polysaccharides extracted from *Prunus amygdalus amara* seeds

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### Abstract

Searching natural molecules with insecticidal effects and less harmful to environment is one of scientific and agricultural concern. In this work, the polysaccharidic fraction from *Prunus amygdalus amara* was tested for its insecticidal potential against *Tribolium confusum*. Water-soluble polysaccharides of *P. amygdalus* were extracted at 80°C and filtrated using sevag milieue. Their chemical composition was estimated using FTIR analysis. Contact toxicity and repellent assays were performed by exposing *T. confusum* to different concentrations of the extract, in petri dishes. Levels of malondialdehyde were measured in individuals surviving after 7 days of exposition to 4 mg/ml of PAP. Our results show the richness of PAP in many aromatic and sulfonated compounds that are probably complexed to proteins. The contact toxicity revealed that insects' mortality was dose and time-dependant. For example, when applied at 8 mg/ml for 15 days, PAPs induced 80 % demise of adult. The repellent effect varied between 66 % and 100 % for the studied doses, after 6 hours of exposition. The analysis of malondialdehyde proved that exposition to PAPs induced oxidative stress at the organismic level, thus explaining in part their toxic effect on *T. confusum*. It is concluded that polysaccharides extracted from *P. amygdalus amara* exert insecticidal effect against coleopteran and might be considered for the development of new "eco-friendly" insecticides.

### 1. INTRODUCTION

Agriculture has been facing several ravaging insects that significantly reduce the quantities and qualities of the production values, particularly in arid and semi-arid regions that exhibit extreme conditions. Among ravaging insects, several species of coleoptera like the *Tribolium confusum*, *Tribolium castaneum* (Tenebrionidae family) and *Rhynchophorus ferrugineus* (Dryophthoridae family) did respectively destroy fruits and the plant trunk and induce severe degradation of date palms' productivity (Ahmed et al, 2022, El-Shafie et al, 2018, Hassan et al, 2013). Finding an eco-friendly way to counteract pests remains a big challenge facing scientists and farmers. In such issue, numerous scientific investigations focused on herbal extracts as reasonable insecticides in safe and sustained developmental agriculture-approaches (Taha and Gadalla, 2017). In fact,

using plant extracts has garnered multiple natural bioactive molecules with great interest in food industry, cosmetology, pharmacy, as well as insecticides. They might exert wide range of biological activities such as antimicrobial, antitumor, antiviral, and antioxidant (Luiz et al., 2017; Nazeam et al., 2017; Xie et al., 2016; Chen et al., 2016). The present study is dedicated to highlight the importance of *Prunus amygdalus amara* seeds' extract as biological insecticide. This tree is a member of the Rosaceae family and was proved to be a valuable source of phytochemicals including fatty acids, proteins, vitamins, minerals, as well as phenolic compounds. It was investigated for its benefits in many domains like cosmetics, skin healing, and anti-inflammation. This work evaluates the phytochemical composition and insecticidal effects of *Prunus amygdalus amara*'s water-soluble polysaccharides.

## 2. MATERIAL AND METHODS

### 2.1. Plant material and extraction of polysaccharides

*Prunus amygdalus amara* (bitter almond) from the Rosaceae family were grown at the region of Sidi Aich, Tunisia (34.683N, 8.8E, Altitude 522 m). Their seeds were collected at the maturation stage and left to dry at ambient temperature before being finely grounded. Water-soluble carbohydrates were extracted by dissolving 50 g of the powder in 1L of distilled water. The mixture was placed for 4 h at 80 °C and then filtered. The obtained filtrate was centrifuged at 12,000 g for 15 min at 4 °C and the supernatant was mixed in Sevag reagent (butanol: chloroform; 1/4) for an overnight at 4 °C, in order to eliminate proteins. The remaining solvents were thereafter evaporated at room temperature (18 to 22°C). the final obtained solution was, then, stored in dark container until further use. The yield of crude polysaccharides (PAP) was calculated according to Chouana (2017), by the following formula:

$$R\% = (\text{Weight of crude extract of water-soluble polysaccharides}) / (\text{Weight of dry plant material}) \times 100$$

### 2.2. Determination of total sugars' content

The amount of total carbohydrates contained in the crude polysaccharide extracted from the seeds of *P. amygdalus amara* (PAP) was determined by the phenol-sulfuric acid method described by Dubois et al. (1956). To do so, 1 mL of the sample was introduced into test tubes containing 1 mL of phenol (5%). The tubes were shaken and stored for 20 min before the addition of 5 mL of concentrated sulfuric acid. The mixture was incubated at 100 °C for 20 minutes. The absorbance of the pink-salmon colored solution was read at 490 nm. The amount of carbohydrate was determined by referring to a calibration curve using D-glucose as a standard.

### 2.3. FTIR analysis

The Fourier transforms infrared (FTIR) (Shimadzu, FTIR-8400S, CE) equipped with an IR 1.10 Shimadzu solution analysis software) was used to predict the different chemical classes contained in both the water-soluble polysaccharides fraction and the aqueous extract. The spectra covered the infrared region 4000-500 cm<sup>-1</sup>.

### 2.4. Insecticidal activity

Insecticidal activity of PAP was carried out on adult *Tribolium confusum* (Du val) (Coleoptera: Tenebrionidae) which are commonly known as grain borer and the flour beetle, using methods of contact toxicity and repellent effect. Each assay was made in triplicate. The used adult insects were aged between 7 and 10 days that were reared on flour obtained from insecticides-free wheat.

#### 2.4.1. Contact toxicity assay

To evaluate the toxicity of PAP, three different concentrations were prepared (0 mg/ml, 2 mg/ml, 4 mg/ml and 8 mg/ml) by diluting the crude PAP into distilled water. A fifth group of insects was exposed to a world-wide used systemic chloro-neonicotinyl insecticide, the Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidazolidin-2-ylideneamine) at a dose of 200 mg /L as a reference. The contact toxicity assay was made using 20 wheat grains that were previously immersed into one of the PAP solutions and left to dry at ambient temperature. Grains were thereafter placed into petri dishes and used to feed 10 *T. confusum* individuals, for 21 days at 28 ± 2°C and 55 % air humidity in dark room. (Arifuzzaman et al., 2014). Mortality was defined when insects lose their articulate appendices (antenna and or legs) and mobility. It was recorded at 1, 2, 7, 15 and 21 days after treatment. The percentage of mortality was calculated using the following equation

$$M_c = \frac{(M_a - M_b)}{(100 - M_b)} \times 100$$

Ma: Percentage of mortality of treated insect

Mb: Corrected mortality percentage of control

MC%: Corrected mortality percentage

#### 2.4.2. Repellent activity

The repellency test was carried out according to (Talukdar and Howse 1994). Briefly, bottom of petri dishes was covered by Whatman - filter papers that were divided into two halves. The first half was submersed by 1 ml of one of the prepared solutions of PAP (2mg/ml, 4 mg/ml or 8 mg/ml), where the second half was similarly treated using distilled water. Before *T. confusum* introduction, petri dishes were left to dry. Ten insects were released at the center of each petri dish. Six hours later, the percent of insects present at each part of dishes was counted and the percentage of repulsion (%PR) was calculated using the following formula:

$$\% PR = (NC - 50) \times 2$$

Where, % PR = percentage of repulsion, NC = percentage of insects present in the control half. Only positive values of PR designated a repellent effect that was categorized according to the following scale Class 0 (PR less than 0.1 %), class I (0.1 % < PR < 20 %), class II (20 % < PR < 40 %), class III (40 % < PR < 60 %), class IV (60 % < PR < 80 %), and class V (PR over 80 %) (McDonald et al. 1970).

### 2.4.3. Evaluation of the oxidative stress

To evaluate the level of the oxidative stress, surviving individuals on the 7th day of contact toxicity assays, were used. Briefly, 1 g of insects were shredded, immersed in 2mL ice-cold Tris-buffered saline (TBS, pH = 7.4), and centrifuged (5000 x g, 30 min, 4°C). Supernatants were collected and used in order to determine the malondialdehyde concentration according to the Thiobarbituric acid reactive substances (TBARS) method described in (Yagi, 1976).

### 2.5. Statistics

All experiments were performed in triplicates. Comparison of results were carried out using Mann-Whitney non parametric test, and significant differences were retained at 5 % (SPSS for windows 20 program, IBM corporation).

## 3. RESULTS AND DISCUSSION

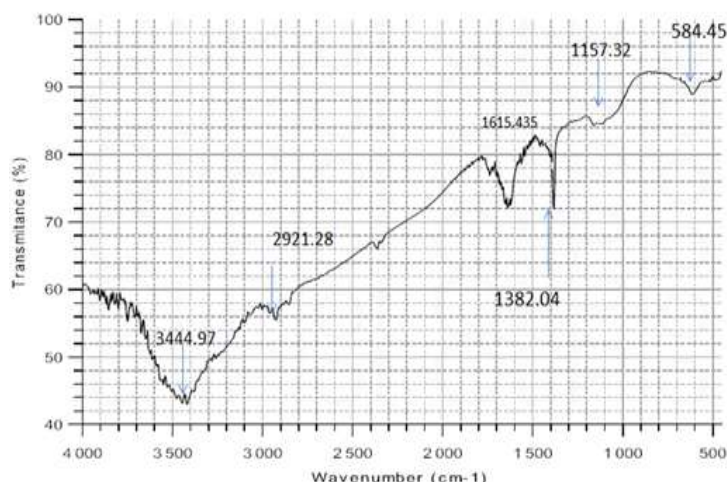
While chemical pesticides are of great efficacy in controlling several ravaging insects that quantitatively and qualitatively degrade vegetables and crops' production, there is growing consensus of their severe outside effects on human health and environment. Thus, many scientists were focusing on searching natural and eco-friendly alternatives to manage ravaging insects (Stamopoulos et al, 2007).

In this report, the insecticidal potential of polysaccharides extracted from *P. amygdalus amara* seeds (PAP) against the flour beetle (*T. confusum*) was investigated. This specie is one of the most known coleopteran that are involved in decreasing the quality of palm dates (*Phoenix dactylifera*), particularly in arid and semi-arid regions (Bibars et al, 2018). Our results, revealed the richness of PAP (which represent 23.41% of the seed dry matter) in reducing sugars (5.16 %) and molecules within several chemical classes including aromatic compounds, sulfonates, imino and hydroxyl groups (Table 1), as found in FTIR spectra (Fig. 1).

This goes along with previous studies that proved the presence of many bioactive molecules like veratic acid, rosmarinic acid, protocatechuic acid, 3-O-caffeoylquinic acid, dihydroquercetin- hexoside, coumaroyl-quinic

**Table 1.** Chemical functions found by FTIR in Polysaccharides extracted from *P. amygdalus amara*.

Wave length cm <sup>-1</sup>	Chemical group
3444.97	Hydroxy -OH stretch // Heterocyclic N-H stretch
2921.28	Methylene C-H stretch
1615	Aromatic compound // Aromatic ring stretch // NH bend // Quinone or conjugated ketone // Open chain Azo or imino (C=N or N=N)
1157.32	Aromatic C-H // Dialkyl/aryl sulfones / sulfonates // Tertiary alcohol // Secondary amine (CN stretch)
584.45	Aliphatic iodo-coumpounds // Disulfides



**Fig. 1.** FT-IR spectrum of crude polysaccharides extracted from *Prunus amygdalus amara*.

acid, vanillic acid and many other compounds, in almond (Moqbel et al, 2018, Moussa et al, 2019). According to El- Hawary and his colleagues (2014) it is also enriched in amino acids that might be complexed to carbohydrates (Capek and Kostalova, 2022). In particular, Bitter almond contains high levels of cyanogenic diglucoside amygdalin that when hydrolyzed liberates toxic cyanides (Lee et al, 2013). The application of the water -soluble carbohydrates extracted from *P. amygdalus* amara resulted in a dose and time dependent lethality of *T. confusum* that reached 100 % within 21 days for all the studied doses. The highest insecticidal effect was observed using 8 mg per milliliter of PAP that caused an augmenting mortality (16± 8 % to 100 ± 00 % respectively for the 1<sup>st</sup> and 21<sup>st</sup> day of the experiment) (Fig. 2). PAP did also induce a dose -dependent repellent effect ranging from 40 % to 100 %, respectively for 2 mg/ml and 8 mg/ml, within one hours after its spread.

The PAP toxicity was weaker than imidacloprid that resulted in 100 % mortality of adult *T. confusum* within the first 24 hours, at a dose of 200 µg/ml. Polysaccharides from *P. amygdalus* amara did also show good repellent effect ranging from 40% to 80 % at 1hour and 66 % to 89 % at 6 hours (Table 2). Similar insecticidal effects against this coleopteran have been obtained using different plant extracts (Abdulhay, 2012, Mirzaeva et al, 2022, Patil et al, 2020, Salim et al, 2019). As shown in Fig. 3, the exposure to PAP induced a dose -dependent increase of the oxidative stress (from 4,7 ± 0,6 to 10,2 ± 1,2 mmol/mg of proteins, respectively for 2 and 8 mg/ml of PAP). This reflects the toxicity mechanisms of PAP.

It is suggested that cyanogenic diglucoside contained in *P. amygdalus* amara seeds might bio-accumulate in insects' body and lead to increase in oxidative stress resulting in their demise.

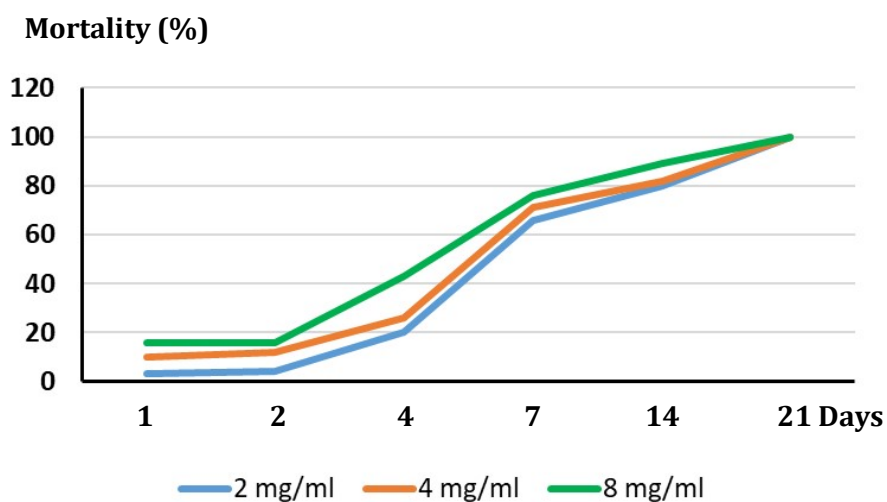


Fig. 2. Lethal effect of polysaccharide of *Prunus amygdalus* amara on *Tribolium confusum* at different exposure time and different concentrations.

Table 2. Repellent effect of polysaccharides extracted from *Prunus amygdalus* amara against *Tribolium confusum*

Dose (mg/mL)	Repellency rate (%)				
	1h	3h	6h	Average	Class
2	40±20	73±23	80±34	64±21	IV
4	80±30	66±30	66±11	66±09	III
8	80±00	86±30	100±03	89±10	V

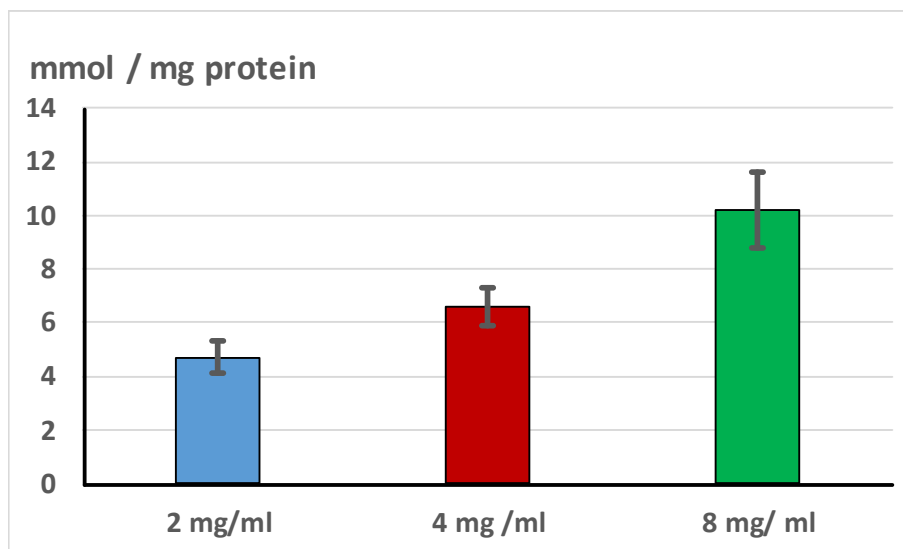


Fig. 3. Levels of malondialdehyde in *T. confusum* exposed to different concentrations of polysaccharides extracted from *P. amygdalus amara*.

#### 4. CONCLUSION

*P. amygdalus amara* seeds contain several polysaccharides complexed with peptic fractions, like cyanogenic diglucoside amygdalin. Water soluble PAP might release cyanides that exhibit insecticidal and repellent effects against coleopteran. The toxicity of PAP against *T. confusum* might involve oxidative stress as mechanistic scheme of action. The water-solubility and insecticidal activities of polysaccharides obtained from *P. amygdalus amara* seeds' are two desirable properties for the development of an ecofriendly agent to manage crops and vegetables' pests. Further investigations are envisaged to better explore these properties and to ascertain PAP's health safety for Humans and animals.

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