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Antioxidant and insecticidal properties of *Euphorbia helioscopia* L. Aqueous extracts

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Abstract
The phytochemical composition, the antioxidant and the insecticidal activities of Tunsian *Euphorbia helioscopia* L. (Euphorbiaceae) leaves and flowers aqueous extracts were revealed. A variation in phenolic and flavonoid contents was observed between samples. Leaves aqueous extract revealed the best phenolic contents (25.2 mg GAE/g DW). However, flowers extracts were characterized by the highest flavonoid contents (8.53 mg RE/g DW). The level of antioxidant capacity estimated by free radical scavenging activity (DPPH) varied significantly among organs. Leaves aqueous extract revealed the best results (IC_{50}=35.11 µg/ml). *E. helioscopia* aqueous extracts were also evaluated for their insecticidal effect against *Tribolium castaneum,* with three concentrations 10 (T1), 5 (T2), and 2.5% (T3). Probit analysis revealed that flowers aqueous extracts of *E.helioscopia* exhibited the lowest LC50 with 2.9 mg/mL.

Keywords: *Euphorbia helioscopia,* aqueous extracts, antioxidant, insecticidal effect

Introduction
There is an increasing interest in medicinal plants to screen and to use in the fields of agriculture, agroalimentary and pharmacology, due to their capacity as a source of natural biologically active compounds (Jaouadi *et al*., 2022) [1]. The use of antioxidants to prevent oxidative degradation of foods by free radicals have been widely recognized. To inhibit the oxidative chain reaction, adequate antioxidants are supplied as natural or synthetic ones (Ben El Hadj Ali *et al*., 2015) [2]. However, synthetic antioxidants have many side effects. Hence, plant based natural antioxidants acts as a good source to produce a wide range of natural antioxidants. Therefore, the development and the use of natural antioxidants obtained from plants, are desired (Asha *et al*., 2016) [3].

Being natural products and less persistent in nature, they are also eco-friendly to surrounding flora and fauna (Sanna *et al*., 2004; Tapondjou *et al*., 2005; Saroukolai *et al*., 2010; Regnault-Roger *et al*., 2012) [4, 5, 6, 7]. Many researchers have evaluated plant extracts for the management of different insect pests (Elimem *et al*., 2019) [8]. In this perspective, there is an increasing interest to explore plant compounds or their derivatives as botanical insecticides for protecting crops (Selin-Rani *et al*. 2016) [9].

The genus *Euphorbia*, with more than 2000 species belongs to “Euphorbiaceae” family. *Euphorbia helioscopia* L. also known as sun spurge, (=Sun *Euphorbia),* is an annual plant (10 to 50 cm high) with milky latex, rising with erected reddish stem, oval alternate leaves and small yellow green flowers. It has a wide distribution in Eurasia and North Africa (Su *et al*., 2019) [10]. *E. helioscopia* gained a great interest due to the biological and medical properties of their chemical compounds. Many bioactive compounds (i.e. polyphenols, steroids, lipids, and volatile oils) have been isolated and identified. Among them, diterpenoids and flavonoids are the most prominent and abundant ones (Yang *et al*., 2021) [11]. It was reported to be widely used in folk medicine and known for its antitumor, antiviral, antibacterial, nematicidal, an antifungal and antioxidant properties (Devi and Gupta, 2000; Ramezani *et al*., 2008; Al younes and Abdullah, 2009; Uzair *et al*., 2009) [12, 13, 14, 15].

Few studies were reported on *E. helioscopia*. The aim of this work was to evaluate the total phenolic and flavonoid contents from leaves and flowers of *E. helioscopia* and to assess their antioxidant and insecticidal activities.

Material and methods
Plant material
Euphorbia helioscopia was collected from Bir Mcherga (Latitude: 36°26'54.32''N and Longitude: 10° 04'09.32''E, Altitude 750m) at the flowering period. Before analyses, plant organs (leaves and flowers) were separated and air dried at room temperature for two weeks, then ground to powder before analysis.

Preparation of the plant extracts
20 grams of each organ were ground and mixed with 200 mL of distilled water. After filtration, each extract was stored at 4 ◦C prior to further analysis.

Determination of Total Phenolic and Flavonoid Contents
Total phenolic content
The total phenolic content was determined using the method of Chetoui *et al*. (2013) [16]. 0.5 mL of diluted sample was added to 2 mL of Folin-Ciocalteu reagent. A volume of 2.5 mL of Na_2CO_3 (7.5%) was added, after incubation for 5 min. The absorbance at 760 nm was read, after incubation for 90 min. Total phenols were expressed as gallic acid equivalents/ g DW (mg GAE/g DW)

Total flavonoid content
The total flavonoid content was determined using the method of Chetoui *et al*. (2013) [16]. 1mL of diluted extract was mixed with 1 mL of 2% $AlCl_3$. The absorbance was measured at 430 nm, after incubation for 15min. The percentage content of flavonoids was expressed as mg rutin equivalent/g DW (mg ER/g DW).

Antioxidant activity
The antioxidant activity was carried out using free radical scavenging activity DPPH (2, 2-diphenyl-1- picrylhydrazyl), as reported by Zaouali *et al*. (2010) [17]. 3 ml of DPPH ($4*10^{-5}$ M) was added to 1 ml of diluted extracts. The absorbance was measured at 517 nm after incubation for 30min. Trolox was used as positive control.

Assessment of insecticidal activity
The insecticidal efficiency of *E. helioscopia* leaves and flowers extracts was evaluated against *T. castaneum*. The pest specie was extracted from the infested wheat kept at the Laboratory of Entomology at the High School of Agriculture of Mograne.
Three concentrations (10 (T1), 5 (T2), and 2.5% (T3)) of *E. helioscopia* aqueous extracts were used. Filter paper disc was placed in a Petri dish and 10 adults of *T. castaneum* were placed in each Petri dish. After 24 hours the number of dead insects was recorded after 24 hours. Water was used as a negative control. All Petri dishes were stored in a climate room at 25±1°C, 60-70% Relative Humidity, and a photoperiod of 16:8 (L:D) h.
Mortality rates of different treatments were estimated and corrected using the Abbott's formula (Abbott, 1925) [18].

Statistical analysis
All analyses were performed in triplicate and the results were reported as means ± standard deviation of three measurements. For each analysis, the results were compared by ANOVA followed by Duncan's multiple range test using SPSS software version 26.0 for Windows.
For the insecticidal activity, results were obtained using the Probit analysis.

Results and discussion
Total phenolic and flavonoid contents
In the present study, total phenolic and flavonoid contents varied significantly among plant organs (Table 1). The leaves aqueous extract exhibited the best contents of polyphenols (25.2 mg GAE/g DW). However, the highest total flavonoid content was revealed in flowers aqueous extract (8.53 mg ER/g DW).

Table 1: Total phenolic and flavonoid contents of leaves and flowers aqueous extracts

Assays	Leaves extract	Flowers extract
Total phenolic and flavonoid contents		
Polyphenols (mg GAE/g DW)	25.2^b±0.0	17.23^a±0.3
Flavonoids (mg RE/g DW)	5.03^a±0.11	8.53^b±0.2
Antioxidant activity		
DPPH (IC_{50} µg/ml)	35.11^a±0.0	50.12^b±0.3

Numbers in lines followed by the same letter are not significant at p>0.05 (Duncan's multiple range test).

As compared to previous literature data, Maoulainine *et al*. (2012) [19] reported that *E. helioscopia* flowers extracts showed the best total phenolic and flavonoid contents compared to leaves and stems extracts. This discrepancy could mainly be linked to genetic factors (species, organ, phenological stage, and environmental factors) (Maoulainine *et al*., 2012) [19]. Moreover, the accumulation of phenolic compounds depends on processes of transport involved in the distribution of these polyphenols at the plant level and the phonological organ growth (Fico *et al*., 2020) [20].

Antioxidant activity
As other biological effects, phenolic compounds were reported to display antioxidant capacity. The antioxidant capacity of *E. helioscopia* samples was evaluated by the DPPH scavenging assay (Table1).
Our data showed that leaves aqueous extracts revealed the best antiradical capacity (IC_{50}= 35.11 µg/mL), which might be due to the abundance of total phenolics in this plant organ compared to flowers. Phenolic compounds have been widely known for their significant antioxidant capacities (Swallah *et al*., 2020) [21]. Lower activity was revealed by Maoulainine *et al*. (2012) [19] for flowers and leaves methanolic *E. helioscopia* extracts (IC_{50}=26.66-65.25 µg/mL), from Tunisia, and even for *E. hirta* methanolic extracts (IC_{50}=0.2 mg/mL) (Sharma *et al*., 2014) [22].

Effect of *E. helioscopia* on *Tribolium castaneum*
In order to evolve environmental safe methods for insect control, natural bioactive compounds can be used (Jbilou *et al*., 2006) [23]. They generate toxicity, mortality, growth inhibition and suppression of reproductive behavior of insects (Zettler and Arthur, 2000; Lee *et al*., 2001; Choi *et al*., 2006) [24, 25, 26].
E. helioscopia aqueous extracts were evaluated for their insecticide effect against *T. castanaeum*. Results revealed that percent of mortality in control petri dishes were very low during the six first days of observation and they ranged between 0 and 20 % (Figure1). Mortalities of *T. castanaeum* were observed at various exposure time and concentrations of both leaves and flowers aqueous extracts. The mortality rate ranged between 15%-100% and 10-100%, for leaves and flowers extracts, respectively.

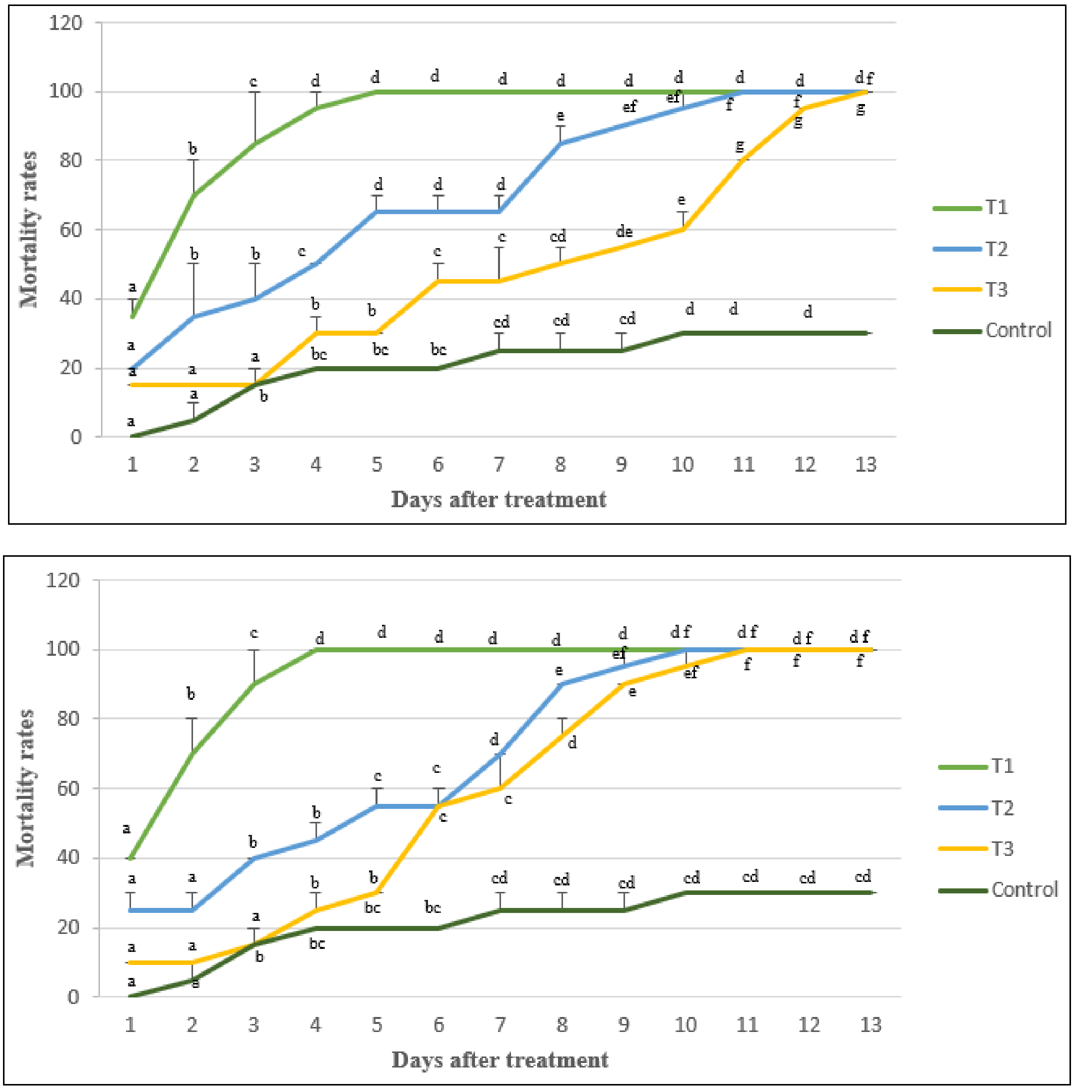

Fig 1: Effect of leaves (A) and flowers (B) aqueous extracts on *T. castaneum* (Values followed by the same letter are not significant at $p>0.05$ (Duncan's multiple range test).

T. castaneum mortality was affected by the applied concentration of extracts, as well as the exposure time. In fact, for leaves and flowers extracts, mortality rates observed in T1 reached more than 60% during second day after treatment. However, the highest mortality rate for T2, revealed 35 and 25% for leaves and flowers, respectively, during the second day. Toxicity of aqueous extracts could be related to the expanded contact between insects and *E. helioscopia* bioactive compounds and their increased passage through insects during period of exposure. In line with that, Maazoun *et al.* (2017) [27] reported that plant polyphenols are toxic to insects and cause rapid death.

Probit analysis revealed that flowers aqueous extracts of *E. helioscopia* exhibited the lowest LC50 with 2.90 mg/mL (equation of the regression line: $Y = -2.964 + 0.911 * X$ and LC90 with 4.31 mg/mL (Table 2).

Table 2: LC_{50} (mg/ml) and LC90 (mg/ml) values of *E. helioscopia* aqueous extracts against *T. castaneum.*

	LC_{50}	LC_{90}	Equation of the regression line
Leaves	3.97	6.05	Y= -2.442+0.616*X
Flowers	2.90	4.31	Y = -2.964+ 0.911*X

This activity can be associated to flavonoids accumulated in flowers extracts. Alonso *et al*. (2002) [28] reported that flavonoids have effects on growth reduce, pupal mass, fecundity, and increasing mortality of insects. In fact, a number of flavones have been explored as feeding deterrents against many insect pests (War *et al*., 2012) [29]. In line with that, Selin-Rani *et al.* (2016) [9] revealed that the Quercetin (flavonol), isolated from *E. hirta*, produced 90% mortality at 50 ppm.

Conclusion

Our study on the chemical composition and biological activity of Tunisian *E. helioscopia* aqueous extracts varied significantly. The leaves aqueous extracts exhibited the highest phenolic contents and the best antiradical activity. These results may highlight the use of this specie in diverse industrial fields. In addition, *E. helioscopia* showed significant insecticidal activity against *T. castaneum*. Therefore, it is a good source for insect control. It can present a substitute to damaginig chemical insecticides. However, further research on the characterization of bioactive compounds in *E. helioscopia*, should be carried out.

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