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Screening for insecticidal potential and acetylcholinesterase activity inhibition of *Urginea maritima* bulbs extract for the control of *Sitophilus oryzae* (L.)

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ABSTRACT

Interest in botanical insecticides derived from plants has increased as a result of environmental concerns and insect resistance to chemical insecticides. In this study, we explored the insecticidal activity of *Urginea maritima* bulbs extract and its inhibitory effect on acetylcholinesterase enzyme system in the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Phytochemical screening and HPLC-ESI/TOF-MS analysis revealed the presence of bioactive molecules with high insecticidal potential. Content of polyphenols (130.88 ± 0.44 mg GAE/g FW), flavonoids (50.81 ± 0.25 mg RE/g FW) as well as alkaloids (12.09 ± 0.16 mg AE/g FW) were high. HPLC-ESI/TOF-MS analysis results demonstrated that ferulic acid (124.19 μg/g FW), vanillic acid (75.84 μg/g FW) and 4-hydroxybenzoic acid (59.73 μg/g FW) were the major phenolic compounds of *U. maritima* bulbs extract. The toxic potential against the rice weevil using contact and repellent bioassays showed the highest insecticidal potential of *U. maritima* bulbs extract. The LD_{50}, LC_{50} and RC_{50} values were 19.03 μg/insect, 2.35 μg/cm^2 and 0.009 μg/cm^2 for contact bioassay by topical application, contact bioassay by treated filter paper method and repellent bioassay, respectively. Furthermore, *U. maritima* bulbs extract inhibited acetylcholinesterase (AChE) activity and median inhibition concentration IC_{50} was evaluated to 66.08 μg/ml. The findings of the present investigation confirmed that *Urginea maritima* may be recommended as an eco-friendly alternative to synthetic insecticides.

Introduction

Grains produced for daily consumption of human come from cereals like wheat, rice and maize and represent the stable foods in the developing countries of the world such as Tunisia (Jarraya, 2003). Storage of grains is a vital component of food security. During storage, grains are destroyed by insects causing severe damage to the cereal products (Rajendran and Sriranjini, 2008) especially in northern Africa countries due to favorable climatic conditions (Bekele et al., 1997). Insect infestation may occur from time of harvest to consumption and it is the principal cause of grain losses (Shazali, 1987).

In many agricultural systems, chemicals are used for pest management. However, synthetic insecticides have been reported to have dangerous effects to human health due to the persistent toxicity on grains and environment. Insects' resistance, non-biodegradable toxic residues and increasing costs of synthetic insecticide stimulated researches through the insecticidal potential of plants to control pests (Dubey et al., 2008).

Plants are a rich source of bioactive molecules (Isman, 2006) and their insecticidal activities against insects have been demonstrated (Isman, 2000). In fact, plant preparations such as powders, plant extracts and essential oils induced toxicity, mortality, growth inhibition, suppression of reproductive behavior and reduction of fertility (Kohli et al., 1998; Zettler and Arthur, 2000; Lee et al., 2001; Choi et al., 2006). Furthermore, developing acetylcholinesterase (AChE) inhibitors, primary targets of organophosphates and carbamates insecticides, from plants emerged as an important strategy with promising results for insect control (Houghton et al., 2006; Rajendran and Sriranjini, 2008).

U. maritima, a medicinal plant native from the Mediterranean area,

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belongs to *Liliaceae*. This plant grows naturally in the wild, rather than being specifically cultivated (Deb and Dasgupta, 1987). The medicinal parts are the bulbs harvested, sliced transversely and grounded to be ready for use (Gentry et al., 1987). *Urginea* genus has been used in traditional medicine due to its cardiotonic, antiepileptic, antiasthmatic, dermatological and diuretic properties (Gentry et al., 1987).

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is one of the most widespread and destructive insects of stored grains (Athanassiou et al., 2008). It causes quantitative damage to grain by weight loss due to insect feeding and qualitative damage by loss of nutritional value due to grains alterations leading to the increase in levels of rejects in the grain mass (Padín et al., 2002). Control of rice weevil relies on the use of synthetic insecticides such as organophosphates, pyrethroids or gaseous insecticides (Shaaya et al., 1997). The excessive use of chemical insecticides promoted emergence of resistant *S. oryzae* (Ben Halima et al., 2004) and supported the development of new and safe bioinsecticides to control the rice weevil.

To the best of our knowledge, few references are available regarding the insecticidal activity of *U. maritima*. In earlier reports, Hassid et al. (1976) studied effect of *Urginea* leaves on the growth of *Spodoptera littoralis* and isolated azetidine carboxylic acid as the active insecticidal compound. In addition, Pascual-Villalobos and Fernández (1999) reported growth inhibition of *Tribolium castaneum* larvae when *U. maritima* bulbs extract was mixed at 10% in the diet.

Thus, the objectives of this work were (1) identification of chemical composition of *U. maritima* bulbs extract; (2) determination of *U. maritima* bulbs extract insecticidal potential throughout contact and repellent bioassays toward *S. oryzae* adults and (3) evaluation of its effect on rice weevil acetylcholinesterase enzyme.

Materials and methods

Chemicals

Rutin ≥ 94%, catechin ≥ 99%, sodium nitrite ($NaNO_2$) ≥ 97%, aluminum chloride ($AlCl_3$) 98%, sodium hydroxide (NaOH) ≥ 97.0%, gallic acid 97%, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3) 99.99%, trichloroacetic acid (TCA) ≥ 99.0%, vanillin 99%, hydrochloric acid (HCl) 37%, methanol ≥ 99.8%, dimethyl sulfoxide (DMSO) ≥ 99.5%, Triton x100, sodium chloride (NaCl) ≥ 99%, chloroform ≥ 99.8%, atropine ≥ 99%, bromocresol green sodium salt (BCG), acetylthiocholine iodide ≥ 99% and 5,5-dithiobis-2-nitrobenzoic acid (DTNB) 99%, galanthamine hydrobromide from *Lycoris* sp. ≥ 94%; All chemicals used were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant material

U. maritima (L.) bulbs were collected from the region of Ras Jbel in the North East of Tunisia (37° 12′ 54″ North 10° 07′ 26″ East). The fresh bulbs were cleaned with water to remove all residues (debris and damaged portions) and grounded into small pieces. The plant material was then stored at 4 °C until further uses.

Preparation of U. maritima bulbs extract

Five grams of powdered fresh bulbs were macerated with 50 ml of methanol (1/10: w/v). After shaking for 24 h, the mixture was centrifuged at 3500 rpm for 20 min. The supernatant was then concentrated under reduced pressure at 40 °C. The pellet was re-suspended in the appropriate solvent for the evaluation (1 ml of methanol or DMSO). The extracts were stored at 4 °C for further uses.

Insects

Sitophilus oryzae adults were obtained from cultures in durum wheat (*Triticum durum* L.) maintained at Laboratory of Biotechnology Applied to Agriculture at The National Agricultural Research Institute of Tunisia (INRAT). Insects were reared in plastic containers in a growth chamber at a temperature of 25 ± 1 °C, a relative humidity (R.H.) 65 ± 5% and 16/8 (L/D) photoperiod without exposure to any insecticide. Adult insects, 7 days old, were used for all bioassays. All bioassays were carried out under the same environmental conditions as the cultures.

Acetylcholinesterase solution

Enzyme solution was prepared according to the protocol reported by Kim et al. (2013). Enzyme solution was extracted from rice weevils. The rice weevils were soaked in Tris–HCl buffer (0.1 M, pH 7.8) containing 20 mm NaCl, 0.5% Triton X-100 under ice-cold conditions. The extract was centrifuged at 15.000 rpm for 15 min at 4 °C, and the supernatant was purified from the insect tissue debris (pellet). The supernatant was used for acetylcholinesterase activity inhibition test.

Determination of bioactive compounds content

Determination of total polyphenols and flavonoids content

Total polyphenols content was assessed using modified colorimetric Folin–Ciocalteu method (Singleton and Rossi, 1965). Fifty microliters of the methanolic *Urginea* bulbs extract were mixed with 125 µl of Folin–Ciocalteu reagent. After 5 min of incubation at room temperature in the dark, 125 µl of 16% Na_2CO_3 were added. The volume of the mixture was made up to 1 ml with distilled water. After 60 min in the dark at room temperature, the absorbance of the mixture was measured at 760 nm. This assay was performed in triplicate and the results were expressed as mg of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

Total flavonoids content was estimated according to the method of Dewanto et al. (2002). Thirty microliters of the methanolic *Urginea* bulbs extract were mixed with 30 µl of 16% $NaNO_2$. After 60 min of incubation at room temperature, 200 µl of 1 M NaOH solution, 60 µl of 10% $AlCl_3$ and 700 µl of H_2O were added to the solution. Absorbance of the mixture was measured at 510 nm. The analysis was performed in triplicate and the results were expressed as mg of rutin equivalent per gram of fresh weight (mg RE/g FW).

Determination of total condensed tannins

Total condensed tannins were determined using the method of Sun et al. (1998). Ten microliters of the methanolic *Urginea* bulbs extract were mixed with 0.6 ml of vanillin (4%) and 0.3 ml of concentrated hydrochloric acid. After stirring, the mixture was incubated at room temperature for 15 min. Absorbance of the mixture was measured at 500 nm. The analysis was performed in triplicate and the results were expressed as mg of catechin equivalent per gram of fresh weight (mg CE/g FW).

Determination of total alkaloids

Total alkaloid content was determined according to Shamsa et al. (2008) protocol. Forty microliters of the methanolic *Urginea* bulbs extract were mixed with 1 ml of 2 N HCl and then filtered. One milliliter of this solution was transferred to a separating funnel and washed with 10 ml chloroform for three times. NaOH 0.1 N was added to the solution to adjust pH to neutral. Five milliliters of BCG solution and 5 ml of phosphate buffer (2 M, pH 4.7) were added. The mixture was shaken and extracted with 1, 2, 3 and 4 ml chloroform successively. Chloroform phases were collected and the volume was made up to 10 ml with chloroform. Absorbance of the mixture was measured at 470 nm. This assay was performed in triplicate and the results were expressed as mg of atropine equivalent per gram of fresh weight (mg AE/g FW).