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INSECTICIDAL AND ANTIOXIDANT ACTIVITIES OF AQUEOUS EXTRACTS OF TWO ALGERIAN MEDICINAL PLANTS

BILLAL NIA^{1*}, ADEL LEKBIR² and MOHAMED KAMEL BEN SALAH¹

1 Center for Scientific and Technical Research on Arid Regions (CRSTRA),
Mohamed Khider University Campus, 07000 Biskra, Algeria

2 Food Science Laboratory, Department of Food Technology, Institute of Veterinary and Agricultural Sciences,
Hadj Lakhdar University of Batna, Algeria

*E-mail: nia.billal@crstra.dz / bilalniadz@gmail.com (corresponding author)

Abstract

In this work, we determined the insecticidal activity and antioxidant activity of aqueous extracts of *Nerium oleander* (Apocynaceae) leaves and *Peganum harmala* (Zygophyllaceae) seeds, two well-known Algerian medicinal plants. Four concentrations of each extract were applied in total randomization by spraying directly on the eggs and larvae of *Ectomyelois ceratoniae* (Zeller, 1879) (Lepidoptera: Pyralidae) under laboratory conditions. Our findings revealed that eggs hatching after four days were not affected by the aqueous extract, with a rate of 54% of hatching eggs in both control and treated eggs. Additionally, toxicity by contact on larvae was not evident and the mortality percentage did not exceed 8%, regardless of the extract plant and concentration. Phytochemical screening showed a slight presence of terpenes and saponins and an absence of alkaloids. However, polyphenols were present in medium concentrations in the aqueous extracts of both plants. The antioxidant activities of *N. oleander* and *P. harmala* extracts were found to be 71.80 and 72.94%, respectively. Total phenolic content (TPC) and total flavonoid content (TFC) of the studied extracts were 50.69 mg gallic acid equivalent (GAE)/100 g dry weight (DW) and 3.55 in terms of quercetin equivalent (QE)/100 g DW, respectively, in *N. oleander*. In *P. harmala*, the TPC and TFC were 52.30 mg GAE/100 g DW and 3.49 mg QE/100 g DW, respectively. Our study clearly shows the limited insecticidal effect of the aqueous extracts, although they showed an interesting antioxidant potential, which could help to decrease the incidence of oxidative stress-induced damage.

KEY WORDS: Aqueous extraction, medicinal plants, *Ectomyelois ceratoniae*, insecticidal property, antioxidant activity.

Introduction

Plants are an interesting source of natural substances. They constitute an essential component of Integrated Pest Management (IPM) strategies as alternatives to chemical pesticides. Indeed, multiple adverse effects on

humans and biodiversity as well as the emergence of resistant populations of pests are a result of the successive applications of chemical products (Peres, 2017). Moreover, in recent years, plant-derived antioxidants have fostered considerable interest among food scientists, manufactures and consumers (Lu *et al.*, 2011).

According to MokkaDEM (1999), there are approximately 600 medicinal and aromatic species in Algeria. These plants have been used for centuries, especially in phytotherapy (Reguieg, 2011).

The flowers and leaves of *Nerium oleander* are used in folk medicine for the treatment of a wide variety of diseases including infection, malaria, autoimmunity (Tayoub *et al.*, 2014), and they have shown conspicuous efficiency as an antioxidant (Mohadjerani, 2012) and against some insect pests (Bagari *et al.*, 2013; Nia *et al.*, 2018). In addition, different parts of *Peganum harmala* are used in traditional medicine for the treatment of a variety of human ailments (Siddiqui *et al.*, 1987). There are several reports in the literature indicating a great variety of pharmacological activities in the harmful plant, such as oxidase inhibition (Abolhasani *et al.*, 2015), nematocidal activity (El Allagui *et al.*, 2007) and insecticidal activity (Abbassi *et al.*, 2005; Dehghani & Ahmadi, 2013).

Importantly, an interest in the use of “generally recognized as safe” (GRAS) solvents, such as carbon dioxide, water or ethanol, has grown in recent years (Camel, 2006). Several developments have been made recently to minimize or completely avoid the use of solvents in the extraction step in order to satisfy the requirements of green chemistry (Tobiszewski *et al.*, 2009). In addition, the consumption of energy has been considerably reduced. Water is a “green solvent” and can be used not only for the extraction of polar compounds, but also for extracting slightly nonpolar compounds under the right conditions because of co-solubility issues and because the polarity of water decreases somewhat at high temperatures (Jones & Kinghorn, 2012).

The date moth *Ectomyelois ceratoniae*, also known as the carob moth, is a major pest in all countries producing date palm (Idder *et al.*, 2009). It seriously threatens date exports by reducing the quality of dates and because the presence of larvae inside the date is offensive. Therefore, in this study we aimed to assess the insecticidal activity of the aqueous extracts of two common plants in Algeria, *Nerium oleander* and *Peganum harmala*, against the eggs and larvae (3rd and 4th instar) of this insect pest by using different concentrations in order to find eco-friendly, safe and low-cost biopesticides.

Antioxidant properties were also studied in this work by testing the aqueous extracts of both plants by standard methods of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging (Abolhasani *et al.*, 2015).

Materials and methods

Biological material

Nerium oleander leaves were collected from Ain Touta (Batna), which is situated in the northeast of Algeria at N 35°17'48.29" and E 5°51'53.48" with an elevation of 729 m a.s.l. The leaves were picked in the morning and sent to the laboratory. Then they were dried in an oven at 50°C (Memmert UN110, Germany) for one week. *Peganum harmala* seeds were bought from a herbalist. Both leaves and seeds were transformed into powder using an electric mortar grinder (Retsch RM 200, Germany) and preserved in glass jars.

Individuals of *E. ceratoniae* used in this work were taken from infested dates provided by a farmer. The insects were reared in plastic boxes on an artificial diet based on wheat bran and yeast. The rearing conditions were: 25±1°C, 50±5% relative humidity (RH) and 16:8 light:dark regime.

Aqueous extract test

We took 50 g of the obtained powder to extract it by infusion in 500 ml of boiled distilled water, and after 24 h a double filtration was done with gauze. Four dilutions were made from a stock solution: 25, 50, 75 and 100%. To assess the toxicity of the aqueous extracts, 30 eggs and 10 larvae (3rd and 4th instar) were transferred separately to Petri dishes and sprayed with 100 μ L of the prepared concentrations, with water as a control, with three replications. The experiment was carried out under laboratory conditions (25 \pm 1 $^{\circ}$ C, 50 \pm 5% RH). The hatched eggs were counted after 4 days and the mortality of larvae and adults was determined after 24 h. When no leg or antennal movements were observed, the insects were considered to be dead.

DPPH free radical scavenging assay

The DPPH radical scavenging capacity was determined using the method described by Mansouri *et al.* (2005). From each aqueous extract, we took 25 μ l that added to 975 μ l of DPPH methanolic solution (6 \times 10⁻⁵ M) and vortexed. The mixture was left in the dark for 30 min and the absorbance measured at 515 nm. DPPH radical scavenging capacity was estimated according to the following equation (Lu *et al.*, 2011):

$$\text{DPPH radical scavenging capacity (\%)} = \left[\frac{(A_{DPPH} - A_{Ext})}{A_{DPPH}} \right] \times 100$$

where A_{DPPH} is the absorbance of the control solution (containing only DPPH) and A_{Ext} is absorbance in the presence of the aqueous extracts of each plant.

Phytochemical screening

The major constituents of both aqueous extracts were screened qualitatively as per standard procedures described by Benmehdi *et al.* (2012). Major constituents analyzed were alkaloids, terpenes, saponins and polyphenols.

Test for alkaloids

Five ml of each extract was warmed with 1.5 ml of 2% H₂SO₄ for 2 min. Then a few drops of Dragendorff's reagent (a solution of potassium bismuth iodide) were added. The presence of an orange/red precipitate indicated a positive alkaloid content.

Test for terpenes (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. The forming of a reddish-brown coloration of the interface indicated a positive result for the presence of terpenes.

Test for saponins

Five ml of each extract was shaken with 5 ml of distilled water, which was then heated to boiling point. Frothing (the appearance of a creamy mist of small bubbles) indicated the presence of saponins.

Total phenolic content

The TPC of the aqueous extract of *N. oleander* was measured by the method described by Juntachote *et al.* (2007). To 0.5 ml of the extract we added 5 ml of distilled water, which was vortexed for 1 min using a vortex mixer (Janke & Kunkel IKA, Model: VF2, Germany). One ml of Folin and Ciocalteu's phenolic reagent was added and mixed well. After 5 min, 1 ml of saturated sodium carbonate solution was added and the mixture was vortexed again. The sample was left to develop a blue color for 1 h. The absorbance was measured at 640 nm using a spectrophotometer (UV-120-01; Shimadzu Co., Kyoto, Japan). A standard curve was prepared at the same time with gallic acid (Sigma-Aldrich GmbH, Steinheim, Germany) at concentrations ranging from 0 to 0.2 mg/ml. The TPC of extracts was expressed as mg of GAE/g DW.

Total flavonoid content

The TFC was estimated using the colorimetric assay according to Gursoy *et al.* (2009). One ml of 2% AlCl₃ solution was mixed with 1 ml of aqueous extract. Test tubes were incubated at room temperature for 10 min and the absorbance was determined at 415 nm. A standard curve was prepared with quercetin (Sigma-Aldrich GmbH, Steinheim, Germany) at concentrations ranging from 0 to 30 µg/ml. The TPC of extracts was expressed as mg of QE/g DW.

Statistical analysis

Experimental values are expressed as mean±standard error of mean (SEM). Comparison of the mean values between various treatments was performed by one-way analysis of variance (ANOVA) and means were separated according to Duncan's multiple range test (DMRT) at p<0.05. The Shapiro-Wilk test of normality was used to test the assumption of normal data distribution. The statistical program SPSS statistical software ver. 20 (IBM company, NC) was used for statistical analysis.

Results and Discussion

The Shapiro-Wilk test confirmed the normality of our data. However, results did not show a significant effect of the aqueous extract of either plant on *E. ceratoniae* eggs hatching and larvae survival (Table I). Indeed, over 54% of the eggs hatched and larvae were able to emerge, regardless of the treatment, and the plant extracts showed no differences from the controls. Also, larvae mortality did not differ from the control and the overall mean did not exceed 8%. The phytochemical screening showed the lack of alkaloids and a slight presence of terpenes, two secondary metabolites families known for their insecticidal activity (Table II). Although these two plants are known for their insecticidal effects (Salari *et al.*, 2012; Nia *et al.*, 2018), their aqueous extracts revealed limited effects in this study. Cruz-Estrada *et al.* (2013) applied the aqueous extracts to *Bemisia tabaci* (Hemiptera: Aleyrodidae) nymphs, which were not affected by them. In fact, most secondary metabolites with insecticidal effects are extracted by nonpolar solvents (Djilani *et al.*, 2006; Singh *et al.*, 2014). According to Dhawan & Gupta (2016), various phytochemicals compounds are generally absent in distilled water extraction and this is may be due to the poor solubility of these phytochemicals in water.

Table I. Aqueous extract effects on *E. ceratoniae* eggs and larvae.

Plant species	Development Stage	Concentration (%)	Mean±SEM	Significance
<i>N. oleander</i>	Eggs	Control	54.44±1.11	F=0.93, p=0.48
		25	52.22±1.92	
		50	54.44±1.11	
		75	55.55±1.11	
		100	53.33±1.92	
	Larvae	Control	3.33±3.33	F=0.62, p=0.65
		25	00.00±0.53	
		50	3.33±3.33	
		75	6.66±3.33	
		100	3.33±3.33	
<i>P. harmala</i>	Eggs	Control	55.55±1.11	F=1.5, p=0.27
		25	54.44±1.11	
		50	52.22±1.11	
		75	54.44±1.11	
		100	55.55±1.11	
	Larvae	Control	6.66±3.33	F=0.5, p=0.74
		25	10.00±5.77	
		50	10.00±00.00	
		75	10.00±5.77	
		100	3.33±3.33	

The antioxidant activities of both extracts were high and similar (71.80 and 72.94%, respectively). Oleander possesses effective antioxidant activity, which includes free radical scavenging and reducing power, and it could be used as a natural source of potent antioxidants (Farooqui & Tyagi, 2018). Our results were close to those obtained by Vinayagam & Sudha (2017) who used a methanolic extract of leaves (72.8%), while Lakhmili *et al.* (2014) recorded over 90% of antioxidant activity with aqueous extract. The total level of antioxidant activity was higher in *N. oleander* leaf extract (72.8%) as compared to its flower extract (68%) (Farooqui & Tyagi, 2018).

The aqueous extract of harmal could be used as a natural source of antioxidants (Kaskoos, 2014). Previous research results revealed that it has high antioxidant activity and is a rich source of antioxidant compounds (Abolhasani *et al.*, 2015). Kanwal *et al.* (2016) recorded 56.71% of antioxidant activity, while Kaskoos (2014) recorded 86.37% and Abolhasani *et al.* (2015) 90%.

The antioxidant activity correlated with the amount of TPC present in the plant (Mohadjerani, 2012) and generally samples with a high level of phenolic content also contain flavonoids in great amounts (Shariffar *et al.*, 2009). Indeed, the TPC and TFC found in oleander leaf aqueous extract were 50.69 mg GAE/100 g DW and 3.55 mg QE/100 g DW, respectively (Table II). Many researchers found high levels of both TPC and TFC in *N. oleander* aqueous extract, varying from 0.44 to 46 mg GAE/g and from 4.69 to 33.3 mg QE/g, respectively (Krishnaveni *et al.*, 2013; Dixit *et al.*, 2014; Akhtar *et al.*, 2018).

Table II. Aqueous extracts phytochemical screening and total phenolic and flavonoid content.
 (-): metabolite absent, (+): low concentration, (++): medium concentration, (+++): high concentration

Plant species	Alkaloids	Terpenes	Saponins	Polyphenols	TPC (mg/100 g DW)	TFC (mg/100 g DW)
<i>N. oleander</i>	-	+	+	++	50.69±1.88	3.55±0.07
<i>P. harmala</i>	-	+	+	++	52.3±0.48	3.49±0.00

In harmful seed aqueous extract, the TPC was 52.3 mg GAE/100 g DW and TFC 3.49 mg QE/ 100 g DW. In published researches, the TPC fluctuated from 24.3 to 348.82 GAE mg/g and TFC from 1.14 to 5.5 mg QE/g (Abolhasani *et al.*, 2015; Khadr *et al.*, 2017; Akhtar *et al.*, 2018).

According to Esmaeili *et al.* (2015), a significant correlation was found between the antioxidant activities of extracts and their TPC and TFC, and these metabolites are often extracted in higher amounts in more polar solvents (Abarca-Vargas *et al.*, 2016). Several studies have also shown that infusion is effective for polyphenol extraction (Rababah *et al.*, 2010; Thouri *et al.*, 2017).

Conclusion

Nerium oleander and *Peganum harmala* aqueous extracts could manifest themselves as a good potential natural source of antioxidants, which have a protective role in human health. On the other hand, these extracts revealed no insecticidal activity against *E. ceratorniae*. Nevertheless, further tests including antifeedant activity are recommended in the future.

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ИНСЕКТИЦИДНА И АНТИОКСИДАТИВНА АКТИВНОСТ ВОДЕНИХ ЕКСТРАКТА ДВЕЈУ ЛЕКОВИТИХ БИЉАКА ИЗ АЛЖИРА

БИЛАЛ НИА, АДЕЛ ЛЕКБИР И МОХАМЕД КАМЕЛ БЕН САЛАХ

Извод

У овом раду утврдили смо инсектицидну активност и антиоксидативну активност водених екстраката листова врсте *Nerium oleander* (Аросупасеае) и семенки *Peganum harmala* (Zygophyllaceae), две добро познате алжирске лековите биљке. Четири концентрације сваког екстракта примењене су по принципу случајности прскањем директно на јаја и ларве врсте *Ectomyelois ceratoniae* (Zeller, 1879) (Lepidoptera: Pyralidae) у лабораторијским условима. Наши налази потврдили су да на излегање јаја после четири дана није утицао водени екстракт, са стопом 54% излегања јаја како у контролним тако и у третираним јајима. Поред тога, токсичност контакта са ларвама није била евидентна и проценат смртности није прелазило 8%, без обзира на биљку и концентрацију екстракта. Фитохемијски скрининг показао је незнатно присуство терпена и сапонина и изостанак алкалоида. Међутим, полифеноли су били присутни у средњим концентрацијама у воденим екстрактима обе биљке. Нађено је да антиоксидативна активност екстракта *N. oleander* и *P. harmala* износи 71,80 односно 72,94%. Укупни фенолни садржај (TPC) и укупни садржај флавоноида (TFC) у испитиваним екстрактима били су 50,69 mg еквивалента галне киселине (GAE)/100 g суве тежине (DW) и 3,55 у односу на кверцетин еквивалент (QE)/100 g DW, респективно, за *N. oleander*. За врсту *P. harmala*, TPC и TFC су били 52,30 mg GAE/100 g DW, односно 3,49 mg QE/100 g DW, респективно. Наше истраживање јасно показује ограничено инсектицидно дејство водених екстраката, мада су показали интересантан антиоксидативни потенцијал, који би могао да помогне да се смањи учесталост оштећења изазваних оксидативним стресом.

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