Chapter

Use of Olive Mill Wastewaters as Bio-Insecticides for the Control of *Potosia Opaca* in Date Palm (*Phoenix dactylifera L.*)

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Abstract

The date palm is one of the most economically important perennial plants of the North Africa and in Morocco, where it is extensively cultivated for food and many other commercial purposes. Palm trees are threatened by many pests such as *Potosia opaca* newly identified in Morocco, especially in Marrakesh and Errachidia regions. In addition, olive mill wastewaters (OMW) are an environmental problem in olive oil producing countries such as Morocco. Generally, these effluents are drained into ecosystems without any pre-treatment. To reduce their negative impact and to get benefits in particular from their high phenolic content, OMW were used as bio-insecticides in crude form. The results showed that crude OMW were effective to control this pest causing a weight loss similar to Cordus insecticide (17% *vs.* 15%) and mortality almost similar to Kemaban insecticide. OMW's biocide potential was related principally to their high phenolic content. Based on HPLC analysis, ten phenolic molecules were identified, including two which were revealed as the major monomeric phenolic compounds in OMW, 0.248 g/L of hydroxytyrosol and 0.201 g/L of tyrosol. In this chapter, the potential use of OMW as bio-insecticides for the control of *P. opaca* in date palm is discussed.

Keywords: olive mill wastewaters, *Potosia opaca*, date palm trees, insecticidal activity, biological control

1. Introduction

The date palm trees have many important socio-economic and ecological roles in oases ecosystems [1]. In North Africa and in Morocco, the oases are facing several constraints related to urbanization, drought, salinity, desertification, poor soils in organic matter and nutrients, genetic erosion, aging, diseases like Bayoud palm caused by *Fusarium oxysporum* fsp *albidinis* [2–4] and pests attacks [5, 6]. Palm trees are strongly threatened by the red weevil caused by *Rhynchophorus ferrugineus,* which causes huge economic losses [7]. The red weevil causes economy loss, resulting in millions of dollars each year, related to agricultural production or costs related to pest control [8]. In the Gulf countries and the Middle East, US$ 8 million is spent every year to cut down contaminated trees [8, 9]. In Spain, red palm weevil has appeared since 1999 and damaged almost 20,000 palms of *Phoenix dactylifera* [10]. In the North of Morocco and more precisely in Tangier, the number of *Phoenix canariensis* prospected during 2009–2016 is 244,393 [11]. The number of *P. canariens is* infested with *R. ferrugineus* was 904, which 896 were incinerated [11], whereas no *P. dactylifera* has been infested with *R. ferrugineus*. In Morocco, *Potosia opaca* var. cardui Gyllenhal has been observed for the first time by Meddich and Boumezzough [12]. Indeed, in Marrakesh and Errachidia regions, it attacks *P. dactylifera* L. and *P. canariensis* by consuming their wood, which causes faster degradation. Thus, to remedy the damage caused by *P. opaca*, most farmers were forced to use synthetic pesticides. However, the intensive use of these pesticides are generally effective in protecting crops [13], but they are toxic to wildlife and to organisms from different levels of the ecosystems [14–17]. Over time, the permanent use of insecticides may be accompanied by the development of resistant strains in some treated species. Biocontrol strategies for pests need to be investigated and developed to provide an ecological substitute or alternative approach to the conventional methods. Some sub-products such as olive oil mill waste waters (OMW) are currently used to control pests, which is essential for crop protection [18, 19]. Most of the OMW phenolic compounds derived from olive polyphenols have many other biological properties [20, 21], as well as biocide activities [22] and phytotoxic effects [23]. Due to their particular characteristics, these effluents are a serious problem for the Mediterranean region, which annually produce around 30 million m^3 of OMW with a damaging effect on the environment [23] and accounts for approximately 95% of olive oil production in the world [19]. In addition, different physicochemical methods have been proposed to treat OMW, including natural and forced evaporation [24], electro-coagulation [25], oxidation by ozone and Fenton reagent [26] as well as their agricultural spreading [21], which is an alternative among the suggested solutions. However, the agronomic application of OMW is limited by the doses to be applied and the risk of polyphenols accumulation in the soil after consecutive applications [21–27]. In parallel with researches made on the treatment of OMW, many studies have been carried out aiming the recovery of OMW phenolic compounds. Recent studies tried to take its advantage from the antimicrobial and phytotoxic properties by using it as biopesticide for crops protection [28–30] or as insecticides to control *P. opaca* larvae [31]. This contribution summarized the quality of palm health status, OMW characteristics and its application as insecticides to control *P. opaca* larvae in date palm, especially in *P. dactylifera* L.

2. Materials and methods

2.1 Study area and plant material

This study was conducted during the period of 2014–2016 in the oasis of Marrakesh located in the central region of Morocco and the oasis of Errachidia situated in the southeastern part of the country. The majority of their territory presents arid climate, hot summer and cold winter. Palms (*Phoenix*) constitute one of the important botanical families, and include some of the world's most important economic plants. In North Africa regions, dates production provides jobs for estimated around 50 million people [32]. It plays by now an undeniable role in maintaining human populations in arid regions where natural resources are limited and living conditions are difficult [32, 33]. Like in Zagora, Errachidia, Ouarzazate which are located in the Draa-Tafilalet region. It is built on terraces, crowned by an old Glaoui kasbah of a hill and surrounded by a major oasis of palm grove. This includes in particular the biosphere reserve of the oases of south-eastern Morocco [34], which forms an agglomeration of ksours (small castles). This area is located in an

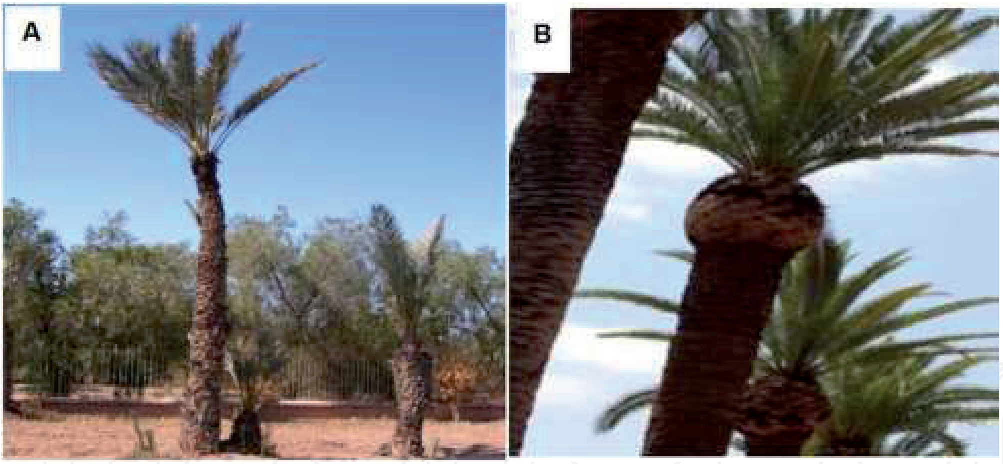

Figure 1.
Pictures of P. dactylifera *(A) and* P. canariensis *(B) in Marrakesh city.*

environment covered by vegetation and many dunes were transformed into regs (vast stone expanses) where the vestiges of khetaras (old local irrigation system) are still visible. As Marrakesh city, the area is under a semi-arid climate regime characterized by relatively cold and humid winters and hot and dry summers with a large diurnal temperature range [35]. This study was performed on *P. dactylifera* and *P. canariensis*. 60-year-old trees with a diameter of 70–90 cm were selected for larvae sampling (**Figure 1**). Analyzed leaves were 4–5 m long with 80–100 segments on each side of the spine. No specific permissions were required for these locations and activities. The field studies did not involve endangered or protected species. The pathogen presence was visually confirmed. No plants were available at very early stage of infection.

2.2 Phytopathological analysis and fungi isolation

The present study used augers to perform localized sampling and perform microbiological isolates. To deepen the diagnosis of *P. canariensis*, we made samplings of rachis, leaflets palms, dry and green leaf bases at the crown. We have carried out cultures and incubation of extracts of rachis (1 cm) and palm leaves puny on selective and non-selective media.

2.3 Sampling techniques and prospecting the crown of palm

During the exploration of the palm crown, using a scaffold (**Figure 2A**), a number of larvae (white grubs) sluggish and arched with strong mandibles were harvested at the base of green and/or dead rachis. Similarly, insect larvae were removed from the base of green and dried palms for laboratory breeding in incubators with controlled conditions of temperature, humidity and photoperiod. Dead rachis (**Figure 2B** and **C**) were brought back to the laboratory to explore them further and also put them in terrariums to ensure the follow-up and development of the larvae trapped inside the hope of having imagos (adult forms). Identification of larvae was performed according to the key proposed by Mico and Galante [36].

2.4 Breeding of larva and nymphs

The collected larvae were immediately placed into breeding boxes; transparent, with holes on the sides and on the top of the boxes to ensure oxygenation and avoid asphyxiation. The holes on the boxes are closed with a fabric scrim (muslin type). Rectangular boxes were used for rearing larvae harvested from *P. canariensis* and

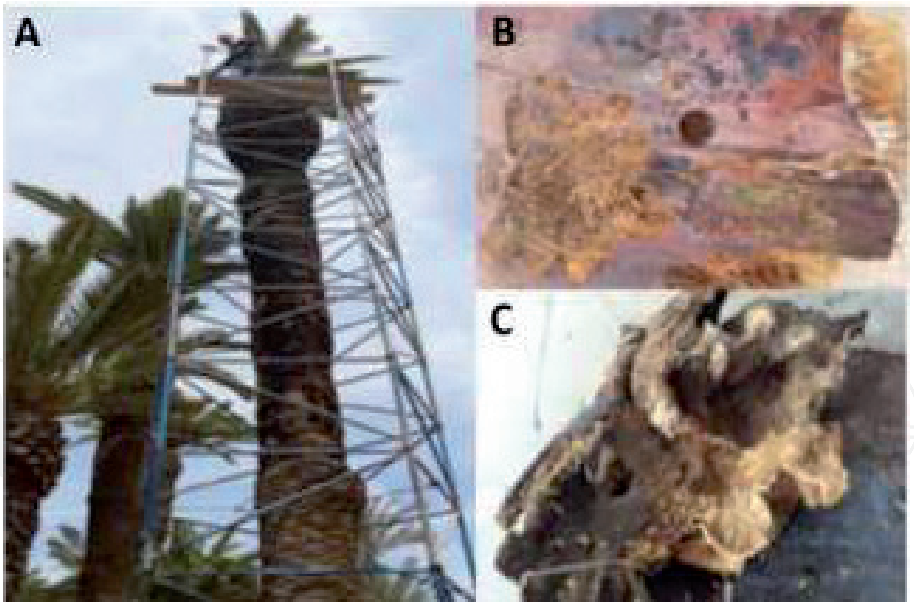

Figure 2.
Exploration of the P. canariensis *palm crown using a scaffold (A); base of leaves (B and C).*

P. dactylifera. The breeding substrate was composed of a mixture of untreated natural soil and debris of dead wood, rotted wood and sawdust. Care was taken not to import diseases on bringing boxes of dry dung in breeding substrate in order to increase its acidity, which disadvantages the development of diseases. The breeding substrate was constantly renewed as soon as the feces of larvae appear in large quantities on the surface and more debris and wood in the rearing environment was observed. This breeding operation continued in incubators refrigerated and illuminated with controlled temperature and humidity. However, larvae are lucifugous (escape behavior of light); the optimum temperature is between 25 and 30°C. The nymphal hulls were placed in boxes with slightly damp peat. The duration of pupation varies according to the temperature supported by the larvae and also the male or female sex. The infected nymphal hulls were removed as soon as possible from the breeding environment to avoid pathological contamination of the rest of the cocoons.

2.5 Chemical properties of palm wastes

Date palm wastes were used as food for *P. opaca* larvae. The sampling of these wastes was carried out in April 2016 in Marrakesh area (Morocco). **Table 1** summarizes the main chemical composition of date palm wastes.

2.6 Main chemical composition of OMW

Sampling of OMW was carried out in a semi-modern three-phase olive mill installed in Marrakesh (Morocco) and the samples were conserved at 4°C. The determination of the volatile matter (VM) was performed by differentiating between the dry matter (DM) obtained by evaporation at 105°C and the ash residue obtained from calcination at 550°C over a two-hour period.

2.7 Phenolic compounds analysis

2.7.1 Phenols extraction from OMW

OMW total phenolic compounds were obtained by liquid–liquid extraction according to the method described by El Abbassi et al. [37]. HCl (2 M) was added to OMW

Parameters	Mean ± SD
Ph	7.03 ± 0.17
TOC (%)	40.80 ± 2.47
NTK (%)	1.06 ± 0.08
C/N	38.60 ± 4.88
Ashes (%)	29.80 ± 1.58
NH_4^+ (mg/g)	738.00 ± 30.1
NO_3^- (mg/g)	0.70 ± 0.08
Available phosphorus (μg/g)	9.00 ± 0.80
NH_4^+/NO_3^- (×1000)	1.05 ± 0.75

TOC, total organic carbon; TKN, total Kjeldahl nitrogen.

Table 1.
Chemical characteristics of date palm waste.

samples (5 mL) to adjust pH to2.0.OMW were defatting using n-hexane and two extractions were performed with ethyl acetate. The aqueous ethyl extracts were dried at 40°C under reduced pressure via a rotary evaporator and then recovered in methanol (5 ml).

2.7.2 Total phenolic content

Estimation of the total phenol content was determined by the Folin–Ciocalteu calorimetric method [38] where gallic acid was used as the standard. Therefore, it was measured as gallic acid equivalent (GAE) and expressed as g of GAE/L of OMW.

2.7.3 OMW phenolic compounds identification

HPLC analysis was conducted at the Center for Analysis and Characterization (Cadi Ayyad University, Marrakesh, Morocco) with C18 column (Eurospher II 100–5, 250 x 4.6 mm) in gradient system (eluting solution A = acetonitrile; eluting solution B = *o*-phosphoric acid/water (pH = 2.6), 5/95 v/v). A volume of 10 μl was injected at a flow rate of 1 mL/min and pressure of 117 bar. The characterization of phenolic compounds was carried out using their UV–Vis diode-array detector at a spectrum of 280 nm and their identification was performed by comparing their retention time (RT) with standards. Then these compounds were quantified through the calibration curve of the corresponding standards. The results obtained were expressed in g/L.

2.8 Larvae cultures

Sampling of larvae of *P. opaca* var. cardui Gyllenhal (**Figure 3**) was conducted according to section sampling techniques. The larvae were reared in round boxes (8 cm x 5 cm: diameter x height) containing a mixture of palm waste (150 g). The larvae culture was maintained in darkness at an optimal temperature between 25 and 30°C inside an incubator. The experiments were conducted in the same conditions as those for the cultures.

2.9 Spray toxicity bioassay

The insecticidal activity of crude OMW and the two commercial insecticides (used as positive controls, Cordus and Kemaban 48 EC) to control *P. opaca* larvae was assessed by a spray toxicity bioassay conducted using 5 g of palm compost in plastic boxes. Cordus and Kemaban are composed of 50 and 48% chlorpyrifos-ethyl, respectively. Chlorpyrifos is an insecticide, acaricide and organophosphate miticide used mainly against insect pests. Concentrations of 0.5 and 1 μL/mL of Cordus and Kemaban were dissolved in distilled water. Many preliminary tests have been performed to select the doses to be used for positive controls. For each solution and crude OMW, a volume of 5 mL was sprayed on the surface of the palm compost every 24 hours for 6 days. Based on dry matter, the dose of OMW was calculated to be 94.86 g/L (≈95 mg/mL) (**Table 2**). The cumulative dosage of OMW polyphenols applied over a six days treatment was calculated. For each treatment, six larvae were placed in each box using ten replicates. Weight loss and mortality of positive controls and OMW were recorded 8 hours per day every 2 hours. Negative control was treated with distilled water. The larva was considered dead, when no movement was recorded when shaking. Observations were made on all treated larvae until their death.

2.10. Statistical analysis

All results were analyzed statistically with the CO-STAT software (Statistical Software, New Style Anova). The study includes an analysis of variance followed by the Newman and Keuls test at the 5% threshold.

Figure 3.
Potosia opaca *larvae isolated from* P. canariensis *and* P. dactylifera *palms.*

Parameters	Mean ± SD
pH	4.70 ± 0.11
EC (mS/cm)	23.50 ± 0.50
TOC (g/L)	26.23 ± 1.40
DM (g/L)	94.86 ± 1.66
TSS (g/L)	21.79 ± 0.50
Ash (g/L)	11.35 ± 0.67
TPC (g GAE/L)	8.38 ± 0.14
Residual Oil (g/L)	2.20 ± 0.30

EC, electrical conductivity; TOC, total organic carbon; DM, dry matter; TSS, total suspended solids; TPC, total phenolic content.

Table 2.
Physicochemical characteristics of crude OMW.

Probit analysis [39] was conducted to estimate lethal times (LT_{50} and LT_{90}) with their 95% confidence interval by SPSS 20.0 Statistical software; LT values were considered significantly different when their respective 95% confidence interval did not overlap.

3. Results

3.1 Microbiological analysis of crown palm

In laboratory, the microbiological analysis showed fungal formations (blackish, whitish and greenish spots) observed on the Petri dishes that contain the extracts [12]. After observation on microscope, it has been found as saprophytic fungi in particular the Dimaties, with blackish spots; those take advantage of the damage already noted and the genus *Fusarium* with a non-virulent strain developed in whitish and pinkish spots with some conidia. The genus *Trichoderma* wide spread, in greenish spots and is often a key fungus in symbiosis and in biological control.

3.2 Sampling techniques and prospecting the crown of palm

During the exploration of the palm crown (*P. canariensis and P. dactylifera*), a number of soft arched larvae (grubs) with strong mandibles were harvested at the base of the green spine and / or dead. The first diagnosis of these larvae showed that they have a powerful mandibles, form "melolonthoides," sub-cylindrical strongly arched, whitish, with head, stigma and brownish legs; the head was always perpendicular to the body axis, with blackish posterior end; related to the Scarabaeidae beetles larvae (3 pairs of legs, antennae with 3 segments). Maximum width of head capsule: 4.6–4.9 mm. These larvae were either rhizophagous (melolonthoids), saprophytophagous or saproxylophagous (Cetoniidae). Initial research has shown that in the forms of phytophagous and saproxylophagous larvae, egg-laying can include about 100 eggs placed directly in the soil or in the wood, sometimes with the aid of an auger [40]. According to field investigations, Meddich and Boumezzough [12] observed the theft of a number of scarabaeidae beetles from the Cetoniidae family, but linking the larvae harvested at the level of the palm crown and the adults captured on the flight seems to be an illusory thing especially since all the larvae of Coleoptera belonging to the family Scarabaeidae were similar and that the systematic identification at the generic and specific level requires a rearing of the larvae under appropriate conditions of temperature and humidity for obtaining the adult. The ultimate stage of development of this Scarabaeidae and whose characteristics were indispensable for the identification of specimens. The evolution of the larvae is carried out at the base of the palms and rachis weakened and attacked by saprophytic fungi, which can lead to the appearance of rot and the dieback of the weakened palm [12]. Their presence at the top of the palm can also be explained by the fact that this beetle found an ideal biotope for the proliferation of larvae. Probably the adult manages to lay his eggs at the base of the weakened rachis and continues its development process while damaging the foliar bases as well as the heart of the palm. The larvae of this insect developing in the crown of the stem can infect the whole leaf mass and cause the crown tilt, which can be fatal to the palm. It should be noted that important attacks are observed at the palms base, at the crown; Attacks marked by the formation of galleries with a rejection of sawdust and dejections of white grubs (**Figure 4**) [12].

3.3 Breeding of larva and nymphs

After one month of larval rearing, the majority of stage III (L3) larvae are transformed into nymphs (the last stage before adult release) [12]. At the end of the last stage of development, the larva becomes more yellowish (accumulation of adipose tissue to the detriment of the stercoral volume of the rectal sac). The premymphal phase lasts a few days during which the elderly larva (L3) no longer feeds and migrates to the bottom of the substratum to build a pupal cocoon, which it generally chooses to make against a support (**Figure 5**).

Pupation occurs in the dorsal position since the larvae move on the back, which distinguishes them from Oryctes larvae (Rhinoceros). In general, its cycle development from the egg to the imago known to be set on one year [40]. However, its metabolic activity is closely related to the ambient temperature, the low temperatures slow down this passage, which may last 2 years [40]. The oases, Moroccan as already said are relatively warm, which could privilege this passage. The nymph of brown-orange color has appendages entirely free and folded down on its ventral surface. During this critical phase of development, the insect does not feed, its mobility is very limited and it is very dependent on the conditions of the environment (temperature, humidity and predation). During this passive stage of development, the nymph gradually acquires a darker color. This pigmentation is perfected in the days before the molt. On the occasion of this final metamorphosis, the insect takes a ventral position, in order to facilitate the deployment of wings and elytra. Its tissues harden progressively in the presence of oxygen from the air. After gaining greater rigidity, the adult perforates its cocoon and migrates to the surface of the substrate in order to begin its phase of aerial life. The adult that has just hatched is sometimes still a little soft and often presents colors less sustained and clearer than its older congeners (**Figure 6**) [12].

Examination of adults under binocular loupe and using a dichotomous key and reference collections from the laboratory led us to the species of Coleoptera Scarabaeidae: *Potosia opaca* var. Cardui Gyllenhal. This species varies greatly in size (from 14 to 24 mm), its morphology with the sides of the pronotum, which can

Figure 4.
*Larvae found in the palm crown (*P. canariensis*) (A) and (B) larvae, (C) leaf base.*

Figure 5.
Observed cocoons (A) and nymphs (B).

Figure 6.
Adult observed in its damaged cocoon (A), (B) and (C) free adults.

be weakly indented before the posterior angles, or not indented. The, the general coloring on the topis black, passes more rarely to the black green (typical form) and even to bronze and green with coppery metallic reflection. On the underside, the color is sometimes black, sometimes bluish, or sometimes greenish or white.

Systematic

Hexapoda (Insecta)

Coleoptera,

Scarabaeidae

Cetoniini

Potosia opaca Fabricius.

3.4 Olive mill wastewater characteristics

3.4.1 Physicochemical characteristics of crude OMW

Table 2 shows the physicochemical characteristics of the OMW according to Boutaj et al. [31]. In addition, these effluents have an acidic pH of 4.7, a high electrical conductivity of 23.5 mS/cm, a residual oil of 2.2 g/L, a high polyphenol content of 8.38 g GAE/L of crude OMW, and an average dry matter content of 94.86 g/L.

3.4.2 Identification and quantification of OMW phenolic compounds

As described by Boutaj et al. [31], OMW present a high phenol content. **Table 3** summarizes the qualification and quantification of these principal phenolic compounds identified by HPLC analysis. Based on comparisons of their retention times and their UV spectra with standards analyzed under the same conditions, 10 free compounds were provisionally identified and quantified in crude OMW (**Table 3**). HPLC analysis revealed that the two main monomeric phenolic compounds in OMW were hydroxytyrosol (0.248 g/L) and tyrosol (0.201 g/L).

3.5 Olive mill wastewater as bio-insecticides to control *P. OPACA*

3.5.1 Weight loss of treated larvae

The effect of OMW spray on the larvae was significantly important compared to the control larvae sprayed with distilled water (**Figure 7**). Over time, larvae treated with OMW showed a significant weight loss from 2.38 to 2.02 g after 216 h. In contrast, the negative control was increased from 2.38 to 2.45 g after 168 h, and then decreased slightly from 2.41 to 2.39 g from 192 to 456 h. In comparison with the crude OMW and the negative control, the two positive controls (Cordus and Kemaban) showed a significant difference (**Figure 7**). Indeed, Cordus is a very effective insecticide resulting from the combination of two active substances, whose weight loss was similar to that of OMW for the two doses applied. The greatest weight loss over the first three days compared to the other treatments was achieved at a dose of 1 μL/mL, which resulted from the slow decrease in weight. Then, a more or less similar weight loss was observed for both doses. Kemaban insecticide is formed by a single active substance and resulted in significant weight loss at both doses compared to control and other treatments (Cordus and OMW) (**Figure 7**). Significant weight loss was seen during the first 4 days at a dose of 1 μL/mL of Cordus compared to 0.5 μL/mL of Kemaban. Thereafter, weight loss was found to be almost similar until 174 h, and then became slight and stable at a dose of 1 μL/mL until 224 h. The stability was the result of larval death.

3.5.2 Mortality rate of treated larvae

Mortality due to crude OMW was 33, 67 and 100% at 216, 224 and 456 h, respectively (**Table 4**). In the same way, Kemaban exhibited 33% mortality at 192 and 174 h with application of doses of 0.5 and 1 μL/mL, respectively. The mortality

Peak	Retention time (min)	Area (mAU)	Concentration (g/L)	Compounds
1	3.950	654.660	0.146	Gallic acid
2	9.967	3191.702	0.248	Hydroxytyrosol
3	13.317	2005.142	0.201	Tyrosol
4	14.450	53.766	0.122	Hydroxybenzoic acid
5	15.167	186.037	0.128	4-dihydroxybenzoic acid
6	16.117	102.685	0.124	Vanillic acid
7	17.317	42.461	0.122	Caffeic acid
8	22.250	53.637	0.122	Coumaric acid
9	30.933	39.469	0.122	Oleuropein
10	44.417	674.747	0.147	Quercetin

Table 3.
OMW phenolic compounds determined by HPLC.

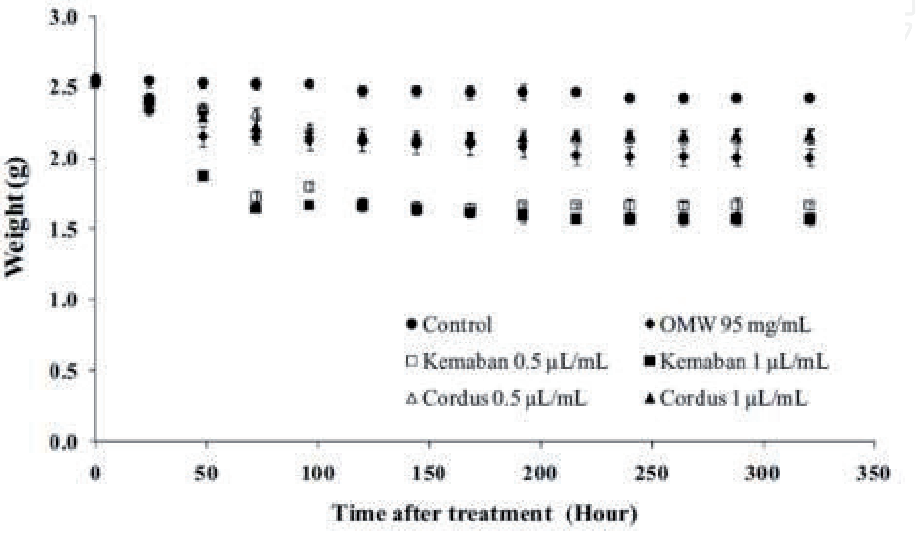

Figure 7.
Weight loss of P. opaca *larvae treated with crude OMW and different doses of commercial insecticides.*

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	Time after treatment (Hour)										
Treatment	**0**	**144**	**146**	**174**	**192**	**198**	**216**	**218**	**224**	**288**	**456**
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Crude OMW											
95 mg/mL	0.00	0.00	0.00	0.00	0.00	0.00	33.33	33.33	66.67	66.67	100
Cordus											
0.5 µL/mL	0.00	0.00	33.33	66.67	66.67	66.67	100	100	100	100	100
1 µL/mL	0.00	33.33	33.33	66.67	66.67	100	100	100	100	100	100
Kemaban											
0.5 µL/mL	0.00	0.00	0.00	0.00	33.33	33.33	33.33	66.67	100	100	100
1 µL/mL	0.00	0.00	0.00	33.33	33.33	66.67	66.67	100	100	100	100

Table 4.
Percentage of dead Potosia opaca larvae for each treatment.

Treatments	LT_{50}(h) (95% CL)[a]	LT_{90} (h) (95% CL)[a]	Slope ± SE[b]	χ^2	Df[c]
Crude OMW					
95 mg/mL	245.39 (224.25–326.42)	323.86 (281.69–568.58)	0.01 ± 0.01	2.17	4
Cordus					
0.5 µL/mL	172.85 (128.56–201.58)	211.00 (188.72–383.17)	0.02 ± 0.01	1.98	4
1 µL/mL	160.02 (66.13–184.92)	199.23 (177.22–430.20)	0.02 ± 0.01	1.30	4
Kemaban					
0.5 µL/mL	208.01 (173.03–249.35)	233.91 (217.58–623.46)	0.04 ± 0.24	2.52	4
1 µL/mL	197.53 (164.23–237.69)	228.65 (209.02–507.80)	0.02 ± 0.02	2.07	4

[a]*95% lower and upper confidence limits are shown in parenthesis.*
[b]*SE, standard error.*
[c]*Df, degree of freedom.*

Table 5.
LT_{50} and LT_{90} values of OMW and two positive controls applied by using spray toxicity bioassay to control Potosia opaca larvae.

rate was 100% when larvae were treated with Kemaban at 218 and 216 h for 0.5 and 1 µL/mL, respectively. Otherwise, mortality of larvae treated with Cordus was significantly higher compared to other treatments. After 144 and 146 h, the effect began with doses of 1 and 0.5 µL/mL, respectively. However, larvae treated with OMW, Kemaban and Cordus started to die for the first time from day 9, 8 and 6 respectively. In contrast, the two commercial insecticides caused 100% mortality within 8 (Cordus) and 9 (Kemaban) days for all doses tested, compared to OMW which showed 33 and 100% mortality after 9 and 19 days, respectively.

3.5.3 Spray toxicity bioassay

Boutaj et al. [31] reported that OMW showed insecticidal activity to control *P. opaca* larvae with LT_{50} and LT_{90} values of 245 h and 324 h, respectively (**Table 5**). Furthermore, a positive correlation was found between treatments and the duration of exposure (Letal time to kill 50% and 90% of the population). The highest efficiency was recorded for Cordus insecticide with LT50 of 160 h and 173 h for 1 and 0.5 µL/mL doses, respectively and LT90 of 199 h and 211 h for 1 and 0.5 µL/mL doses, respectively. Median lethal times (LT_{50} and LT_{90}) generally decrease when insecticide concentrations increase. The least effectiveness was observed in *P. opaca* larvae for crude OMW. However, the results were close to Kemaban at a dose of 0.5 µL/mL.

4. Discussion

The palm represents the symbolism of life in the arid and semi-arid area and being one of the oldest domesticated trees with multifold social and economical status [41, 42]. The *P. dactylifera* is a former species, which constitutes the pivot of the oasis agriculture in the south of Morocco. Out of an overall area estimated at 84500 ha in 1948, the Moroccan palm groves in 1994 covered an area of 44,450 ha occupied by a total of 4.42 million palm trees [9]. This population is currently estimated at 5.12 million palms on an area of 48,000 ha. The importance of the palm by province showed that the provinces of Ouarzazate (1,873,000 palm trees), Errachidia (1,250,000), Tata (Bani) (800,000), Marrakesh (799,000), Tiznit (139,140), Guelmime (138,000) and Figuig (125,500) were the most important and thus constitute the largest phoenicultural areas (quoted by Sedra (2003) and updated by Meddich [43]. Meddich and Boumezzough [12] note that the prospected and infected *P. canariensis* palms were cultivated and located in the North-East palm grove of Marrakesh. This will allow exchanges of attacks between the two palm species studied. For the oasis ecosystem, the problem will cause a lot of damage and a serious socio-economical problem. Moroccan palms suffer from invasions by larvae and adults of *Rhinoceros Borer*; this is the case in Tunisia, the Middle East and Iran [33]. In Morocco, no *R. Borer* larvae or adults were found; also, the colonization of the wounds by the saprophytic fungi can lead to the appearance of rot and the dieback of the weakened palm. According to INRA (Institut National de Recherche Agronomique) reports and their research axis developed on *P. opaca* in oases newly identified in Morocco by Meddich and Boumezzough [12]. Tauzin [40] indicated the presence of this species in Anti-Atlas (Ifni, Tiznit), middle and southern of Morocco. The presence of *P. opaca* in the crown of the *P. canariensis* and *P. dactylifera* palm which can be explained only by the fact that adults have found an ideal biotope for egg laying and larval development. Meddich and Boumezzough [12] showed that *P. opaca* occurred in decaying wood of and *P. dactylifera*, also, where they consumed the wood and promote more rapid decay and laid their eggs in the hollows of branches. The finding of Meddich and Boumezzough [12] was supported by Mico and Galante [36]. Besides, adults of the Cetoniidae fly above the vault of the trees (including the palm tree) and feed on nectar plants and fruit trees and probably the inflorescences of the palm trees. In this way, it can be assumed that this species has undergone mutations by changing biotope and passing from saprophagous larvae (dead organic matter, compost) to saproxylophagous larvae (dead woods, rachis and dead and / or alive palms of the *P. canariensis* and *P. dactylifera*). As finding by Meddich and Boumezzough [12], *P. opaca* larvae was found of all studied sites. The phytophagous species (Scarabaeidae) were active at night. In the broad sense, saproxylophages cause damage by attacking either roots or leaves. Larvae (white worms) were generally the most harmful to palms. However, Meddich and Boumezzough [12] conclude that the degradation of the Moroccan palm grove may be linked to the attack of *P. opaca* larvae. They observed that the frequency of palms prospected and infected with *P. opaca* remains higher in the Marrakesh palm grove than in the south of Morocco. Date palm grove of Marrakesh is more confronted with anthropogenic constraints such as urban extension and air pollution by dust exceeding the tolerance threshold [43, 44]. Suspended particle concentrations in certain areas of Marrakesh are slightly above the limit value for health protection in Morocco [44]. This may induce the creation of biotopes necessary for the development of *P. opaca* larvae in this city.

Many researches carried out in other countries have highlighted the danger of the red palm weevil for both species *P. dactylifera* and *P. canariensis*. In the Arab countries, the efforts deployed to control *R. ferrugineus* were based mainly on modified cultural practices, the application of traditional insecticides and traps that uses pheromones to lure *R. ferrugineus* [38]. Moreover, the control of *P. opaca* insect pests involves spreading chemicals (in the form of toxic pellets) as uniformly as possible in areas where the larvae are causing damage. The elimination of diseased palms (attacked bylarvae) can reduce the spread of the pest and consequently limit the damage.

For preventive treatments of the aerial parts and the crown of the palm:

- Use Imidacloprid (200 g/L) by spraying at the crown (stipe, palm and heart) 2 to 3 times every 3 months. It is a systemic insecticide for the selective control of scarabaeidae beetles.

- Spray acetamiprid 100 g/L at 2–3 times / year.

- Also, use entomo-pathogenic nematodes with a dose of 180 million nematodes per 100 L of water, which act by reducing the size of the larvae and consequently affect the number of female adults capable of laying the eggs.

- Trapping control using pheromones. The use of such specific substances for sexual confusion may be a means.

Efforts now focus on the development of integrated pest management methods based on biological control and pheromone traps rather than on conventional insecticides [45]. Since it is an internal tissue borer, *R. ferrugineus* is difficult to control in the early stage of attack [38–46]. Initial efforts to control red palm weevil in the Kingdom of Saudi Arabia using chemical insecticides were failed [47]. An integrated pest management strategy, developed in India, has successfully suppressed the pest in the date plantations in the Kingdom of Saudi Arabia [38]. The strategy is modeled on the lines of tackling the pest on coconut. The pheromone traps has been used successfully to monitor and mass attract the pest, and it could be considered as the core of in any integrated pest management [48–50].

There is a great danger from chemicals such as insecticides and fungicides in human's food and animal use. Recognizing this real danger, farmers and consumers turned their efforts to environmental and eco-friendly practices by using as well as consuming biological and healthy products. The polyphenols are natural molecules present in OMW from the olive fruit which could be an alternative and an asset for pest control. However, the amount of OMW polyphenols may vary based on multitude factors, such as climatic conditions, olive variety and fruit ripening stage as well as the harvest period [51–53]. The OMW phenolic compounds content, can be considerably affected by the technological processes used for olive oil extraction [54]. In this context, the phenolic compounds content of OMW which, presented potential insecticidal activity has been assessed and investigated by Boutaj et al. [31] in view to develop new valorization strategies. Additionally, an application of a hydroxytyrosol-rich OMW extract by spraying it against olive psyllid (*Euphyllura olivine*), in a drip-irrigated olive orchard for evaluating the insecticidal activity of OMW, was carried out in 2008 and 2009 [29]. The extract from OMW had a strong insecticidal activity control this insect when the applied concentration was 2 g/L. In addition, the authors observed a significant biocide effect depending on OMW phenolic extracts concentration on *E. olivina* larvae as well as adults. Indeed, OMW showed similar toxicity to the Kemaban insecticide at 0.5 μL/mL dose. Nevertheless, it is clear that the obtained results were attributed to the chemical molecules that contain the two commercial insecticides. Cordus presents two active molecules namely chlorpyriphos ethyl and cypermethrin. As for Kemaban contains a single active molecule which, is chlorpyriphos ethyl. These molecules act on the spread of nerve impulses along the axon (cypermethrin action) and inhibit the acetylcholine esterase by blocking the transmission of the nerve flux (chlorpyriphos ethyl action) [55, 56]. The main mechanisms which explain the OMW's biocide effect on invasive species in general including insects are not clarified. It has been suggested that the transmission of the nerve flux may be blocked by the high phenolic compounds content in OMW [57, 58]. A significant inhibition of acetylcholine esterase activity in a marine mollusk (*Mytilusgallo provincialis*) has been reported by Danellakis et al. [57] after exposition to OMW. While, Campani et al. [58] reported that the inhibition of acetylcholine esterase may be attributed to the potential presence in OMW of organophosphates and carbamates, two pesticides which, are strong inhibitors of acetylcholine esterase activity and commonly used to treat the olive fruit fly (*Bactroceraoleae*). However, the authors did not dismiss the inhibition of acetylcholine esterase possible which, could be explained, by the phenolic compounds as well as metals and ammonia contained in OMW. Thus, the used OMW crude showed a toxic effect on *P. opaca* larvae. Danellakis et al. [57] noted that the toxicity is provided by the phenolic compounds and trace metals contained in OMW. Furthermore, Barbera et al. [59] showed that during their growth cycle, no phytotoxic effects were observed when OMW were applied on crops. This is related to plant phenological stage, the application modalities and the applied doses. The absence of harmful residues is the main advantage of OMW application to control plant pests as well as pathogens. Therefore, we can assume that there will be no need for a preharvest interval on crops, after the application of OMW.

5. Conclusions and future directives

In this chapter, we present a new eco-friendly approach to control the spreading of *P. opaca* which started in Morocco. Microbiological analysis show the presence of saprophytic fungi and genus *Fusarium* with a non-virulent strain. On the other hand, the two insecticides used separately and crude OMW are toxic on *P. opaca* var. Cardui Gyllenhal larvae. These results are promising and suggest the possibility of using OMW due to their high content of phenolic compounds as a means of biological control to overcome environmental problems caused by synthetic pesticides. The OMW and their phenolic extract compounds could be used in agricultural systems. Moreover, focused field researches (each plant-pathogen system) could be carried out to understand and evaluate the effects of OMW on specific *in situ* pest problems. Based on the main findings, it is clear that OMW may contribute to improve the date palm protection to control *P. opaca* and could be used as bio-insecticides. Nevertheless, OMW could be used safely as a challenge to control plant pest without affecting negatively the soil and plants. Besides, the use of OMW combined with other pest bio-control practical methods which could be a sustainable approach to minimize the potential risks. In this context and to understand the beneficial effects of OMW, more investigations could be required to assess the feasibility of OMW application in bio-controlled systems at large-scale, and determining the limitations and advantages on the long term. Further, research works are needed to test, besides crude OMW, pretreated OMW (ultra-filtered or heated) and its phenolic extracts as biodegradable pesticides.

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