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RESEARCH ARTICLE

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Insecticidal activity of crude olive pomace oils from Kabylia (Algeria) against the infestation of *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) in stored wheat grains

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The insecticidal effect of four crude olive pomace oils extracted from pomace collected from four localities of Kabylia, refined olive pomace oil and extra virgin olive oil were assessed under laboratory conditions for the control of adults of *Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera: Bostrychidae)) and *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae). The doses used ranged from 0.1 to 0.4 ml/25 g of durum and soft wheat seeds. In addition, the quality indices and the fatty acid composition of these oils were evaluated. The results show that the legal quality indices were well within the legal limits for crude olive-pomace oil (COP), refined pomace oil (ROP) and extra-virgin olive oil (EVO) categories. The effectiveness of these vegetable oils is highly dependent on the insect species, the dose rate, the exposure time and the type of oil tested The main fatty acids from all samples tested, were oleic (61.89–79.25%), palmitic (8.34–15.71%) and linoleic (8.17–16.52%) acids. For both species and substrates tested, mortality is dose and time of exposure dependent. The highest dose (0.4 ml/25 g) causes ≥ 63.75% mortality, after 24 h of exposure. Comparison of LD_{50} (ml/25 g) indicates that olive oil was generally more toxic than crude and refined pomace oils, for both insects and substrates, values varies from 0.005 to 0.189. The most sensitive insect was *S. oryzae* for most vegetable oils tested. Likewise, oils significantly reduced the F1 offspring of both insects and seed weight losses. No progeny were observed in the two pests at the highest dose, and therefore no weight loss was recorded. The results also revealed that the six oils affect the germination capacity of durum and soft wheat seeds when the dosage is increased. The results collected remain encouraging for the recommendation of natural substances as part of integrated pest management programmes against insect pests of stored grains.

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INTRODUCTION

In Algeria, cereals, mainly wheat (*Triticum aestivum* L.), occupy a strategic place in the food supply system and in the national economy (Benbelkacem & Kellou 2000; Lakhdari & Ayad 2009). Touchan et al. (2016) reported that durum wheat (*Triticum turgidum* L.) represents 46% of grain crops in Algeria. Bread wheat (*Triticum aestivum* L.) has a total production of more than 600 million tonnes per year (Bellatreche et al. 2019).

Among the major constraints on effective production and utilisation of cereal crops in Africa are losses resulting from attacks on stored products by insect pests (Phillips & Throne 2010; Midega et al. 2016). For example, insects, mainly coleopterans, are responsible for huge grain losses of up to 57% in Africa (Kumar & Kalita 2017). *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *Sitophilus oryzae (*L.) (Coleoptera: Curculionidae) are among the most common and destructive pests of stored grains worldwide (Gourgouta et al. 2019; Paloukas et al. 2020). They are classified as primary colonisers and are responsible for significant damage if no protective measures are taken (Chintzoglou et al. 2008; Obeng-Ofori 2011; Mason & McDonough 2012).

Currently, the control of these insects is mainly based on the use of synthetic pesticides (Gossen & Mcdonald 20220; Machekano et al. 2019). However, the development of genetic resistance in many species, the demand for food without residues, the growing concern for worker safety and the environmental risks associated with methyl bromide (Boyer et al. 2012) have led researchers to assess the potential use of safer alternative control methods for the protection of stored products (Donahaye 2000; Adak et al. 2019). In this respect, the use of plant-derived products is seen as a promising alternative to traditional pesticides currently used against stored product insects (Guettal et al. 2020 a, b; Kellouche & Soltani 2004; Isman 2006; Saroj et al. 2020). Of these, vegetable oils have been extensively researched as natural insecticides against several insect species of stored products with promising results (Obeng-Ofori & Amiteye 2005; Nikpay 2006; Rahman & Talukder 2006; Demissie et al. 2008; Udo & Harry 2013; Tufail et al. 2015; Wahedi et al. 2015; Wale & Assegie 2015; Jilu et al. 2018). The mode of action of these oils has yet to be confirmed, but most appear to cause the death of the insect egg, larvae or adult by suffocation (Don-Pedro 1989; Aider et al. 2016).

Among the edible vegetable oils, olive oil is an essential ingredient of the Mediterranean diet and it is widely known for its health-promoting proprieties (Foscolou et al. 2018). This oil has also

been traditionally used in the region of Kabylia (Algeria) as a post-harvest grain protectant against insect pests (Kellouche et al. 2004).

Olive pomace (OP) is one of the most important by-products produced by the olive oil industry, it can contain up to 12% of oil and is mainly composed of water, pieces of pit, skin and pulp (Meziane & Kadi 2008). It is estimated that the production of OP may be as much as 2 881 500 t/year worldwide (Yangui et al. 2009; Ravindran & Jaiswal 2016; Nunes et al. 2020).

Appropriate use of this olive pomace production could improve the economic status of olive cultivation, and mitigate environmental problems (Dermeche et al. 2013; Nunes et al. 2019; Ribeiro et al. 2021); it is mainly used for extraction of the residual oil (Clemente 1997; Meziane 2013; Mateos et al. 2020; Ribeiro et al. 2021). The extracted oil is referred to as 'crude olive pomace oil' and must be refined before being consumed (EEC 2001).

Olive oil (*Olea europaea* Linné) has been the subject of several studies by various researchers, who have demonstrated its toxicity to various insects of stored grains, which is attributed to oleic acid (Khalequzzaman et al. 2007; Kellouche et al. 2004; Aider et al. 2016). To our knowledge, there has to date been little research done on the applications of olive by-products as bio-insecticides.

In this context, the aim of this work consisted in assessing the value of a local olive oil by-product, olive pomace, by recovering its residual oil and studying the biological activity of crude oils extracted from olive pomace collected in four localities in Kabylia, refined olive pomace oil and olive oil (reference oil), against two primary insect pests of stored products, *S. oryzae* and *R. dominica*.

MATERIALS and METHODS

Vegetable oils

Samples and oil extraction

Six products were used, four crude olive-pomace oils (COP), one refined pomace oil (ROP) and one extra-virgin olive oil (EVO). The ROP sample was purchased from a commercial refining plant (Mystic Moments, Fordingbridge, U.K.). All samples were stored in dark bottles at 4 °C until use.

Olive-pomace oils

The olive-pomace samples used to extract the oils were obtained from four olive oil mills (with a three-phase centrifugation system) in four localities of Kabylia (Algeria): Tadmaït (altitude 52 m), Maatkas (altitude 620 m), M'Chedellah (altitude 440 m) and Bechloul (altitude 414 m), in December 2016. The olives processed were of the 'Chemlal' variety. The moist olive-pomace samples (initial humidity varied from 40.0 to 51.32%) were dried in an oven at 60 ± 1 °C to reach a residual moisture content of about 8% (6.38 to 7.77%) (Table 1), and were then stored at –5 °C until use. Oil yield of the samples varied from 5.45 to 7.42% (Table 1).

Oil extraction from the olive pomace was effected in a soxhlet apparatus (250 ml), with an organic solvent, hexane (purity 95%), according to regulation EC No. 2568/91 relative to the characteristics of olive oils and olive-pomace oil and the relevant methods of analysis. The weight of cake subjected to the extraction was always 20 g. The miscella was distilled by means of a rotary evaporator. Extracted oil samples were designated by COP1 (Tadmait), COP2 (Maatkas), COP3 (M'Chedellah) and COP4 (Bechloul).

Table 1. Characteristics of the olive pomace tested[1] (Mean ± SD).

Origin of the olive pomace	Initial moisture (%)	Moisture after drying (%)	Oil yield* (%)
Tadmaït	51.32 ± 1.03	6.38 ± 0.56	7.42 ± 0.90[a]
Maatkas	46.63 ± 1.04	6.46 ± 0.39	5.45 ± 0.66[b]
M'Chedellah	43.97 ± 0.49	7.25 ± 0.08	5.89 ± 0.85[b]
Bechloul	40.00 ± 0.78	7.77 ± 0.10	5.53 ± 0.87[b]

[1] Each value is the mean ± SD of three determinations
* Means followed by the same letter are not significantly different (Dunn test at $p = 0.05$)

Olive oil

Olive oil was obtained from olives of the Chemlal cultivar, picked by hand at an optimum stage of ripeness from an orchard located in the Tizi-Ouzou area in the north of Algeria, during the 2016–2017 harvest season. Sampling consists of harvesting 2 kg of olives, from the inside and outside of 10 randomly selected trees (IOC 2011). The olive oil was extracted using a laboratory-scale oil mill in the first 48 h after harvesting. This unit is equipped with a hammer crusher, malaxator and centrifuge. The oil was separated from the waste water by decanting.

Analytical methods

All the pomace oil samples underwent routine analyses for a first characterisation, i.e., determination of free acidity (%), peroxide values (PV), extinction coefficients (*K*232, *K*270) and fatty acid composition, as prescribed by the analytical methods of EC Regulations EEC/2568/91 and subsequent amendments (EEC 2001). Free acidity given as percentage of oleic acid was determined by titration of a solution of oil dissolved in 1:1 ethanol:ether with ethanolic potash. The peroxide value (PV), expressed in milli-equivalents of active oxygen per kilogram of oil (mequiv O2/kg), was determined by reacting oil and 3:2 chloroform:acetic acid with potassium iodide in darkness; the free iodine was then titrated with a sodium thio-sulphate solution. The K232 and K270 extinction coefficients were calculated from absorption at 232 and 270 nm, respectively, collected on a UV mini-1800 instrument (Shimadzu Co., Kyoto, Japan), using 1% oil in cyclohexane and path length of 1 cm. The fatty acid composition was determined after the conversion of triglycerides to methyl esters by vigorous shaking of a solution of oil in hexane (0.1 g in 2 ml) with 0.2 ml of 2 N methanolic potassium hydroxide (ISO 5509: 2000, point 5 IUPAC Method 2.301). A gas chromatograph (GC) (Chrompack CP 9002, Varian Inc, Holland) equipped with split/splitless injector, and flame ionisation detector (FID) was employed. Separations were made on DB23 (50% cyanopropyl) capillary column (30 m × 0.32 mm i.d., 0.25 µm film thickness) with nitrogen as the carrier gas. The injector, detector and oven temperatures were 250, 260 and 220 °C, respectively. The injection volume was 0.5 µl. Three replicates for each determination were analysed per sample; except for fatty acid composition (one replicate was prepared and analysed per sample).

Insects

Adults of *S. oryzae* and *R. dominica* used in the bioassays came from cultures reared in the laboratory, on whole wheat (*T. durum* and *T. aestivum*), in an oven at 30 ± 1 °C, 70 ± 5% RH, and continuous darkness. The initial population was collected from storage facilities and kept in our laboratory for more than 2 years. In the experiments, only unsexed 1- to 7-day-old adults were used.

Substrates

Untreated, clean and infestation- and pesticide-free local seeds of durum wheat and soft wheat were used in the bioassays. The moisture content of the two tested grain commodities, as determined by oven drying, ranged between 11.0 and 11.7%.

Bioassays

The biological tests were carried out in an oven under the conditions described above. The vegetable oils were applied at three doses: 0.1, 0.2 and 0.4 ml/25 g of wheat. For each test (oil-dose-substrate combination), 25 g of grains were treated with the required amount of oil (Kerbel et al. 2021). Each sample was introduced, separately for each combination, into glass Petri dishes (13 cm diameter and 3 cm high), which were shaken for approximately 15 min to achieve equal distribution of the oil throughout the entire grain mass. In addition, a series of additional lots with untreated wheat was used as a control. The Petri dishes were then placed in incubators, under the conditions aforementioned. Subsequently, 20 unsexed adults of *S. oryzae* were introduced into each dish. The same procedure was followed in the case of *R. dominica*. Each test (insect-substrate-oil-dose combination) was repeated four times. Mortality was assessed after 24, 48, 72 and 96 h of exposure for each species (Kerbel et al. 2021) and the percentage of cumulative mortality was corrected using Abbott's formula (Abbott 1925). Insects were presumed dead if they did not move (legs and antennae) when touched with a pin. The dose required to kill 50% of the insects (LD_{50}) was estimated after 72 h of exposure using probit analysis (Finney 1971; Abdelli et al. 2016).

After the last mortality count (96 h), all adults (dead and alive) were removed and the Petri dishes were left under the same conditions for an additional period of 45 days. Then, emerged individuals of *S. oryzae* and *R. dominica* were counted daily in each Petri dish.

After adult emergence, the seeds were weighed to estimate grain weight loss caused by the two pests. The percentage of weight loss was determined as described by Khare & Johari (1984):

$$\% \text{ seed weight loss} = (\text{initial weight} - \text{final weight/initial weight}) \times 100.$$

To determine the effect of vegetable oil treatments on seed germination, we proceeded as follows: 50 grains for each test were randomly sampled from treated and untreated seed lots. The selected seeds were placed on moist cotton in glass Petri dishes (13 cm diameter and 3 cm high) and incubated for 7 days at room temperature (28 to 32 °C). The germination percentage (Nikpay 2006) was calculated as:

$$\% \text{ germination} = \text{number of seeds germinated/total number of seeds} \times 100$$

Data analysis

All analyses were carried out in four replicates and all results were expressed as mean value ± standard deviations (SD). The data were processed using the free R language. The Anderson–Darling normality test applied for the four measured variables (adult mortality, emerged adults, grains weight loss and seeds germination) asserts, by the *p*-values (Table 2), that none of these variables follow a normal distribution. Therefore, the non-parametric Kruskal-Wallis test was used for the four variables with insect mortality as the response variable and type of treatment, insect species, dose rate, exposure time and type of substrate as the main factors. The same procedure was carried out for the progeny production, the weight loss and germination rate of the grains with the type of treatment, insect species, dose rate and type of substrate as the main factors. Dunn's multiple comparison test with Bonferroni adjustment was used to assess differences between different groups. Differences were considered statistically significant at $p < 0.05$ (Dagnelie 2012).

RESULTS

Characterisation of the six oils

The results obtained show that for the ROP and EVO samples, the free acidity, peroxide value (PV) and specific absorptions at

Table 2. Anderson–Darling normality test

Variable	*p*-value
Adult mortality	$<2.2 \times 10^{-16}$
Emerged adults	$<2.2 \times 10^{-16}$
Grains weight loss	$<2.2 \times 10^{-16}$
Seeds germination	1.287×10^{-6}

Table 3. Analytical characteristics of the six oils (

Parameters	COP1	COP2	COP3	COP4	ROP	EVO	Legal limits EEC/2568/91		
							COP	ROP	EVO
Acidity (% oleic acid)	13.98 ± 0.71^{c}	34.13 ± 0.04^{a}	33.28 ± 0.06^{b}	11.48 ± 0.35^{d}	2.19 ± 0.04^{e}	0.43 ± 0.22^{f}	–	≤0.3	≤0.8
Peroxide value (meq O_2/kg)	42.02 ± 0.03^{b}	50.65 ± 0.19^{a}	23.46 ± 0.001^{d}	35.05 ± 0.00^{c}	3.39 ± 0.04^{f}	10.83 ± 0.76^{e}	–	≤5.0	≤20.0
K232	3.13 ± 0.21^{c}	3.14 ± 0.08^{c}	3.05 ± 0.01^{c}	4.05 ± 0.01^{a}	0.82 ± 0.02^{b}	0.98 ± 0.05^{b}	–	–	≤2.50
K270	1.06 ± 0.32^{a}	0.55 ± 0.02^{c}	0.72 ± 0.01^{c}	0.77 ± 0.01^{c}	0.26 ± 0.01^{b}	0.21 ± 0.008^{b}	–	≤2.0	≤0.22
Fatty acid composition (%)									
C16:0	11.46	15.71	15.54	14.88	8.34	15. 06	–	–	7.5–20.0
C16:1	1.83	1.97	2.01	2.11	0.18	1.94	–	–	0.3–3.5
C18:0	2.97	2.77	2.17	2.46	2.86	2.67	–	–	0.5–5.0
C18:1	64.09	65.10	63.95	61.89	79.25	66.44	–	–	55–83
C18:2	13.63	12.56	14.83	16.52	8.17	11.97	–	–	3.5–21.0
C18:3	0.56	0.58	0.57	0.53	0.32	0.46	–	–	≤1.0
C20:0	0.66	0.51	0.54	0.71	0.08	0.71	–	–	≤0.6
C20:1	0.55	0.46	0.34	0.42	0.27	0.40	–	–	≤0.4
C22:0	0.25	0.26	trace	0.22	0.48	0.12	–	–	≤0.2
MUFA/PUFA	4.68	5.13	4.30	3.78	9.39	5.53	–	–	–
Oleic acid/ linoleic acid	4.70	5.18	4.31	3.74	9.70	5.55	–	–	–
UFA/SFA	5.26	4.19	4.48	4.46	7.50	4.38	–	–	–

Notes: COP= crude olive-pomace oil; ROP = refined pomace oil; EVO = extra-virgin olive oil
C16:0 = palmitic acid; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:1 = oleic acid; C18:2 = linoleic acid; C18:3 = alpha linolenic acid; C20:0 = arachidic acid; C20:1 = eicosenoic acid; C22:0 = =behenic acid; MUFA/PUFA = monounsaturated fatty acids/polyunsaturated fatty acids; UFA/SFA = unsaturated fatty acids/saturated fatty acids
Means followed by the same letter are not significantly different (Dunn test at $p = 0.05$)

232 and 270 nm, falls generally within the ranges established by EC regulation 2568/91 (1991) for these categories (Table 3). For COP oils, the values of free acidity vary from 11.48% (COP4) to 34.13% (COP2). This increase in free acidity is due to triglyceride hydrolysis influenced by the drying and extraction temperature of the pomace, the relatively long extraction time and the nature of the solvent (Kmieciak et al. 1991; Yanik 2017). Moreover, PV and K232 were used as indicators of olive oil primary oxidation, while the K270 values are indicative of secondary oxidation. The mean levels of PV range from 23.46 to 50.65, K232 from 3.05 to 4.05, and K270 from 0.55 to 1.06. The chemical characteristics of the crude olive-pomace oils are similar to those reported in previous papers for these oils (Kmieciak et al. 1991; Gomes & Caponio 1997, 1998; Amarni & Kadi 2010; Yanik 2017). The fatty acid (FA) composition, from all samples tested, corresponded to the normal range indicated for EVO (EEC 2001). The main fatty acids were oleic, palmitic and linoleic acids. Stearic, palmitoleic, linolenic, arachidic, eicosenoic and behenic acids were also detected in small amounts (Table 3).

Effect of oils on adult mortality

All factors (dose: $p < 0.001$; exposure time: $p < 0.001$; insect species: $p < 0.001$ and type of treatment: $p < 0.001$) significantly influence the mortality rate, except the substrate ($p = 0.36$) (Table 2).

Mortality of *R. dominica*

According to the results obtained (Figure 1), after 24 h of exposure, all the oils tested (COP, RPO and EVO) at the highest dose (0.4 ml/25 g) caused total mortality (100%) in adults of *R dominica*, in the presence of durum wheat seeds, with the exception of treatment with olive oil. For this substance, the same effect was only reached after 72 h of exposure. In the case of soft wheat, the six treatments showed similar trends of mortality to that observed in the presence of durum wheat seeds; however, no product caused total adult mortality of *R. dominica* even after 96 h of exposure, with the exception of RPO, tested at the highest dose (0.4 ml/25 g) (Figure 2). For the two substrates tested, the viability of adults of *R. dominica*, treated with COP, ROP and EVO at the lowest dose (0.1 ml/25 g), remains high even after 96 h of exposure (Figures 1 and 2).

Mortality of *S. oryzae*

The results obtained (Figure 1) show that after 24 h of exposure, all adults of *S. oryzae* died in the presence of durum wheat treated with RPO and EVO at the highest dose (0.4 ml/25 g). For COP, mortality increased with exposure time and generally reaches 100% after 72 h. Similar mortality rates were also recorded for soft wheat, but after 96 h of exposure (Figure 2). In contrast to lesser grain borer, the tested oils ($P < 0.001$), at the lowest dose (0.1 ml/25 g) on both substrates, caused mortality higher than 47% after 96 h, except COP4, causing low weevil mortality (<19%) (Figures 1 and 2).

These data show that *S. oryzae* was more sensitive than *R. dominica* to the different treatments (Figures 1 and 2, Table 4). The toxicity of the six oils, based on the LD50 (ml/25 g) values for 72 h mortality data, is shown in Table 4. For example, in the case of *S. oryzae* infesting durum wheat, the order of toxicity was as follows: EVO (0.025), COP3 (0.038), ROP (0.059), COP1

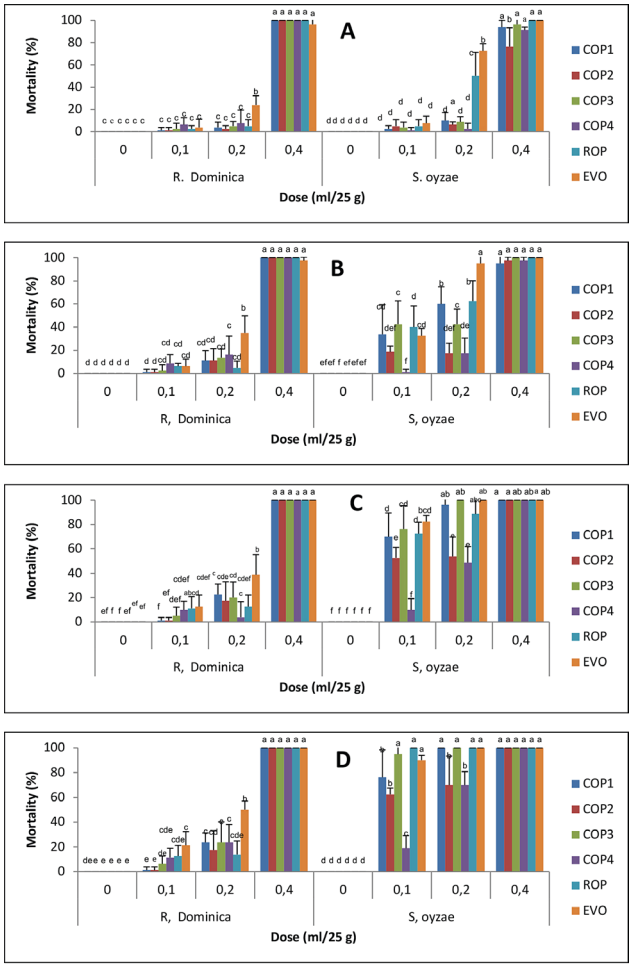

Figure 1. Percentage cumulative mortality (% ± SE) of *R. dominica* and *S. oyzae* adults exposed for 24 h (**A**), 48 h (**B**), 72 h (**C**) and 96 h (**D**) on durum wheat grains treated with the six oils, at three doses (bars with the same letter are not significantly different; Dunn test at $p = 0.05$).

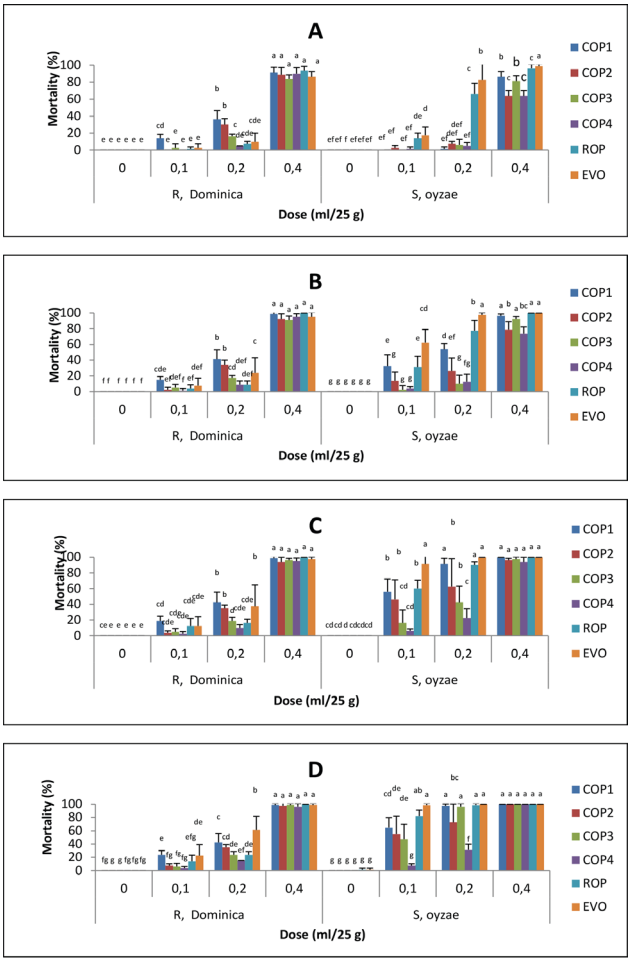

Figure 2. Percentage cumulative mortality (% ± SE) of *R. dominica* and *S. oyzae* adults exposed for 24 h (**A**), 48 h (**B**), 72 h (**C**) and 96 h (**D**) on soft wheat grains treated with the six oils, at three doses (bars with the same letter are not significantly different; Dunn test at $p = 0.05$)

Table 4. Contact toxicity (LD_{50} median) of the six oils against adults of *S. oryzae* and *R. dominica* infesting durum and soft wheat seeds after 72 h of exposure

Insect species	Substrate	COP1	COP2	COP3	COP4	ROP	EVO
		LD_{50} median (ml/25 g)					
S. oryzae	Durum wheat	0.076	0.120	0.038	0.180	0.059	0.025
	Soft wheat	0.084	0.122	0.178	0.224	0.079	0.005
R. dominica	Durum wheat	0.220	0.227	0.211	0.198	0.210	0.183
	Soft wheat	0.170	0.217	0.222	0.247	0.203	0.189

Note: COP= crude olive-pomace oil; ROP = refined pomace oil; EVO = extra-virgin olive oil

(0.076), COP2 (0.120), COP4 (0.180). However, for *R. dominica*, the order of toxicity was: EVO (0.183), COP4 (0.198), ROP (0.210), COP3 (0.211), COP1 (0.223), COP2 (0.227) (Table 4).

Effect of oils on progeny production

Progeny production was significantly affected by the insect species ($P < 0.001$), the dose rate ($P < 0.001$) and type of treatment ($P = 0.036$). However, substrate type had no impact ($P = 0.78$) (Table 2).

For the two tested substrates, the number of progeny in untreated lots (control) ranged from 40.25 to 105.0 for *R. dominica* and from 20.25 to 27.00 for *S. oryzae* (Figure 3).

Results (Figure 3) show a significant reduction in the offspring numbers of both insect pests in all the treated lots, compared with the controls. Thus, for *R. dominica* infesting both substrates, adult viability gradually decreased with increasing doses; progeny production was completely suppressed in treatments with ROP, at doses ≥ 0.2 ml/25 g and at a dose of 0.4 ml/25 g for treatments with COP and EVO. For *S. oryzae,* no progeny were observed on seeds of durum wheat and soft wheat treated with COP, ROP and EVO at doses ≥ 0.1 ml/25 g (Figure 3).

Effect of oils on grain weight loss

All factors (dose rate: $p < 0.001$; insect species: $p < 0.001$ and substrate: $p = 0.027$) significantly influence the grain weight loss, except the type of treatment ($p = 0.20$) (Table 2). The box-plots for grain weight loss versus treatment type, dose rate, substrate and insect species are shown in Figure 1c.

For the two substrates tested, the results (Figure 4) show the highest weight losses in the untreated lots, ranging from 4.30 to 8.68%, and from 1.71 to 2.75%, for *R. dominica* and *S. oryzae*, respectively. The COP, ROP and EVO caused a considerable reduction in percentage weight loss, compared with the controls. For all oils tested, the treatment with the highest dose (0.4 ml/25 g) completely protected wheat seeds from attacks by *R. dominica*. In the case of *S. oryzae,* no weight loss was recorded in lots treated with doses ≥0.1 ml/25 g in all treatments (Figure 4).

Effect of oils on seed germination

All factors significantly affected the germination: dose rate ($p < 0.001$), the substrate ($p < 0.001$); the type of treatment ($p < 0.001$) and the insect species ($p < 0.001$) (Table 2). For both insect pests, results (Figure 6) show that the germination rates of the control lots (untreated and non-infested grains) ranged from 91 (durum wheat) to 96.5% (soft wheat). Seed germination was significantly adversely affected as oils concentration increased; however, seed germination was more drastically reduced at the highest dose (0.4 ml/25 g), ranging from 20.00 to 50.0% (*R. dominica*), and from 8.00 to 47.00% (*S. oryzae*) (Figure 5).

DISCUSSION

The results of this study reveal that COP, ROP and EVO have shown significant insecticidal activity against *S. oryzae* and *R. dominica* infesting durum and soft wheat seeds. The oils

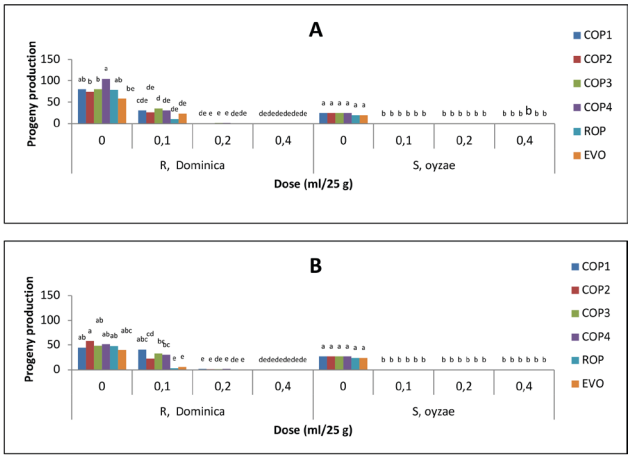

Figure 3. Mean progeny production (number of individuals/dish ± SE) on durum (**A**) and soft wheat (**B**) treated with the six oils at three doses, for each species (bars with the same letter are not significantly different; Dunn test at $p = 0.05$)

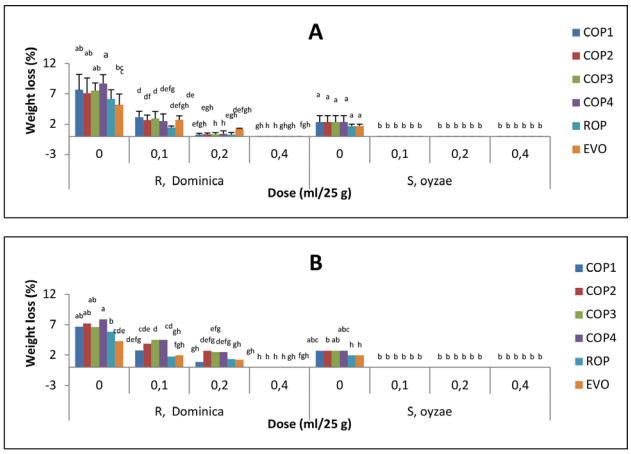

Figure 4. Percentage weight loss (% ± SD) on durum (**A**) and soft (**B**) wheat seeds treated with the six oils at three doses, for each species (bars with the same letter are not significantly different; Dunn test at $p = 0.05$)

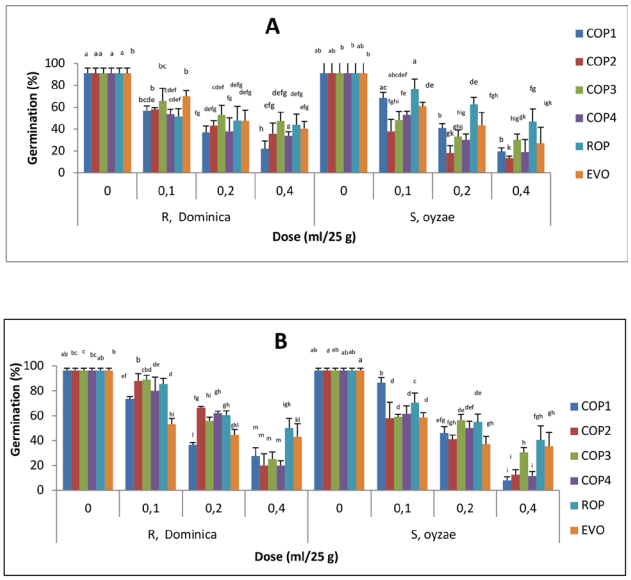

Figure 5. Percentage germination (% ± SD) of durum (**A**) and soft wheat (**B**) seeds, treated with the six oils at different rates and infested by the two insect pests (bars with the same letter are not significantly different; Dunn test at $p = 0.05$)

showed contact toxicity against adults of these pests, a significant reduction of its offspring and consequently a reduction in weight

loss caused to grains. The effectiveness of these vegetable oils is highly dependent on the insect species, the dose rate, the exposure time and the type of oil tested.

Under our experimental conditions, the mortality of the two beetle species tested varied with the dose rate and the exposure time. These oils showed contact toxicity against adults of *S. oryzae* and *R. dominica* infesting durum and soft wheat seeds; this corroborates previous work assessing the effectiveness of different vegetable oils on the same pests. We can cite, in particular, those of Ivbijaro et al. (1985) on coconut, groundnut and palm oils (5–10 ml/kg of maize grains), Tembo & Murfitt (1995) on groundnut, rape seed and sunflower oils (10 ml/kg of wheat grain). Similar results were obtained by Obeng (1995) with cottonseed, soybean, corn, groundnut and palm oils (10 ml/kg of maize and sorghum), Nikpay (2006) on chamomile, sweet almond and coconut oils (10 ml/kg of wheat). In addition, Kellouche et al. (2004) and Aider et al. (2016) have shown the effectiveness of treatments with different vegetable oils, including olive oil, and the major fatty acids contained in olive oil (oleic, linoleic, stearic and palmitic) against *Callosobruchus maculatus* (Fabricius,1775) (Coleoptera: Chrysomelidae) (0.8 ml/50 g of cowpea).

The characteristics of vegetable oils can influence their insecticidal activity (Kellouche et al. 2004). Despite the fact that all oils tested revealed strong contact toxicity against both insect species, olive oil showed the highest biological activity in the presence of the two infested substrates. Pacheco et al. (1995) noted that crude castor oil was more effective than refined soybean oil against *C. maculatus* and *Callosobruchus phaseoli* (Gyllenhal, 1833) (Coleoptera: Chrysomelidae) in stored chickpeas. The toxic effects of crude oils could indeed be attributed to possible synergistic activity between their constituents (Kher 2006; Aider et al. 2016).

In our study, *S. oryzae* was shown to be more susceptible to the six treatments than *R. dominica*. Differences in insect pest responses to different vegetable oils have already been reported (Shaaya et al. 1997), and could be attributed to morphological and behavioural differences between the insects. For example, Hill (1990) reported that *S. oryzae* is considered as particularly agile, so the possibility of contact with the toxic substance increases. Sighamony (1986) also reported that clove, cedarwood and karanja oils, applied at 25–100 ppm, were considerably more effective against *S. oryzae* than *R. dominica*.

For both species studied, the difference in the effectiveness of oils with respect to the type of grain used (durum wheat or soft wheat) is not significant. However, it is well known that the type of diet directly affects the development and reproductive rates of *S. oryzae* and *R. dominica* (McGaughey et al. 1990). In addition, grain type is a critical factor affecting the insecticidal efficacy of vegetable oils against pests of stored products (Khalique et al. 1988). Dey & Sarup (1993) tested the effectiveness of eight vegetable oils on adults of *S. oryzae* on three varieties of stored maize, and observed different levels of efficacy between these varieties.

Regarding the effects of the six vegetable oils on the progeny of *S. oryzae* and *R. dominica,* all the products significantly reduced the emergence of adults of the first generation. This decrease may be due to increased adult mortality and the ovicidal and/or larvicidal properties of vegetable oils, as illustrated in the findings of Kumar & Okonronkwo (1991) and Obeng & Amiteye (2005) with vegetable oils such as palm oil, soybean oil, groundnut oil and coconut oil. These authors suggested that these oils significantly reduce the emergence of adult progeny of *R. dominica*, *S. zeamais* and *S. oryzae*, respectively. Other authors such as Obeng & Reichmut (1999) have reported that treatments with vegetable oils (coconut, sunflower, sesame and mustard) inhibited progeny production in *Sitophilus granarius* (Linnaeus, 1758) (Coleoptera: Curculionidae), *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae), *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) and *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrychidae). In addition, similar results have been obtained with other vegetable oils against *C. maculatus* on cowpea (Ramzan 1994; Shaaya et al. 1997; Rahman & Talukder 2006).

On the other hand, all oils tested significantly reduced the feeding activity of *R. dominica* and *S. oryzae,* thus reducing the weight loss of infested wheat grains, compared to untreated control lots. No weight loss was observed in the grains treated with 0.4 ml/25 g for the six oils. These results are consistent with other studies that show a positive correlation between the density of emerged adults and the percentage of grain weight loss (Ivbijaro 1985; Braga-Caneppele et al. 2003; Law-Ogbomo & Egharevba 2006).

The mode of action of vegetable oils is not clearly defined; it has been suggested that mortality caused by oils is due to anoxia (Don-Pedro 1989) or to the disturbance of normal respiration causing suffocation. The oils could also act as anti-feedants or alter the storage micro-environment, thereby discouraging insect penetration and feeding (Obeng 1995; Weaver & Subramanyam 2000). Other authors attribute insecticidal activity to triglyceride fractions and oleic acid content (Hill & Schoonhoven 1981; Lienard et al. 1993).

Finally, the results obtained also revealed that the six oils tested affect seed germination. This phytotoxicity increases with vegetable oil concentration. Data provided by Tembo & Murfitt (1995) indicate that the application of high doses of groundnut, rape seed and sunflower at doses of 10 ml/kg can significantly reduce seed germination. Yuya et al. (2009) and Wale & Assegie (2015) also reported severe reductions in germination in maize grains treated with high levels of Niger seed oil *(Guizotia abyssinica* Cass.) and castor bean oil (*Ricinus communis* L.), respectively.

CONCLUSION

The experiments carried out have shown the effectiveness of treating wheat grains with natural substances such as olive oil and olive-pomace oil to reduce the damage caused by the two main insect pests of stored grains. Olive oil is used in large quantities in local cuisine and can therefore be safely used to process wheat grains. It does not pose any danger to humans or animals, even when the grains are used for consumption. The results obtained are encouraging for integrated crop protection systems that can lead to a reduction in the use of conventional pesticides that are known to have an impact on human health and on the environment. The study also opens the way towards an alternative way to make use of the olive by-products responsible for severe environmental pollution in our region. We recommend that further studies be conducted to determine the efficacy, technical and economic feasibility of the application of these oils at pilot scale, and to assess the effect of these substance on the organoleptic and nutritional quality of grains. In addition, the toxicity of crude inedible olive-pomace oils must also be investigated. It would also be interesting to determine the biological activity of other constituents of the tested oils, as phenolic compounds.

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CONFLICT OF INTEREST

We declare that we have seen, read, and understood our guidelines on copyright. We also declare that the authors and co-authors of this work have no conflict of interest with any company or institution.

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