Chapter

Valorization of Olive Mill Wastewater in the Control of *Aphis pomi* De Geer 1773 (Hemiptera, Aphididae) Infesting Apple Plants in Nurseries

Nahid Haouache, Soukaina El Asri, Adil Asfers, Abdelhadi Ait Houssa, Bouchra Tazi and Ahmed Boughdad

Abstract

Olive mill wastewater (OMW), are the liquid residues generated during the extraction of oil by traditional and modern three-phase type crushing units. These effluents are characterized by an acidic pH and composition rich in water, organic matter, minerals and polyphenols. In general, they are directly discharged into natural ecosystems. Their danger is linked to the enormous quantities produced in a short period between October and March. To mitigate the effects of vegetable waters on the environment, their valorization in different areas is discussed. As biopesticides, crude OMW have been shown to be very toxic to *Aphis pomi*; the LC50 and LC95 varied respectively from 27.17 to 45.59 and from 77.19 to 134.57 mg of OMW/L of water; they vary according to the stage of the aphid considered. The young stages of *A. pomi* were more sensitive than the elderly are. Therefore, the OMW can be used as a means of controlling aphids. However, before operating on a large scale, it is necessary to repeat the trials in field and assess their impact on non-target organisms and treated crops.

Keywords: Olive Mill wastewater, Biopesticides, *Aphis pomi*, Apple plants, Nursery

1. Introduction

In Morocco, with an area of 49731 ha and an annual production of 809762 t [1], apple cultivation is exposed to the pressure of various harmful biological agents; approximately 182 synthetic pesticides are registered against these organisms [2]. Aphids are small, soft-bodied insects with long, slender mouthparts used to pierce stems, leaves and other tender parts of plants and, suck up sap from the host plant. They are among the most dangerous pests of crops; they directly weaken plants by sucking their sap; these results in curling and deformation of the leaves of young shoots, which affects the photosynthetic function of the attacked plant. Among the indirect damage, aphids are vectors of many phytopathogenic viruses and the secretion of honeydew favoring the development of sooty mold on leaves and fruits [3, 4]. The green apple aphid, *Aphis pomi* De Geer (Hemiptera, Aphididae) is 1.3–2.3 mm long and light green or yellowish green in color, with short antennae and black or dark brown siphunculi; asexual development goes through 4 Nymphs and an adult (**Figure 1**). It is a monoecious holocyclic species, i.e., the aphid has one sexual generation and several asexual (parthenogenetic) wingless and /or winged generations; they grow on the same plant species or on plants of related species. The aphid is widely distributed in the northern hemisphere [5]. This species is very harmful to pome fruit (Rosaceae), especially apple trees; its infestations are rife regularly. The species is particularly harmful in nurseries and young orchards. To control aphids, apple growers only use synthetic insecticides; thus, 82 pesticides are registered against aphids [2]; these pesticides are broad spectrum and effective against many pests other than aphids; they mainly belong to the groups of organophosphates, carbamates, pyrethroids and neonicotinoids. However, the intensive use of these products raises health, environmental and ecotoxicological problems (e.g., [6–8]). The use of these pesticides also generates resistance phenomena in pests [9–11]. In addition, they can cause the resurgence of secondary pests [12]. This latter phenomenon is characterized by a reversal of the biological response such as the shortening of the duration of the development, the increase in fecundity with fertility and longevity due to the application of the sublethal doses of the pesticides used [13]. Besides the unwise use of pesticides increases the mortality of natural enemies that contribute to pest control [14, 15]; which increases the cost of production and affects the efficiency of the techniques applied and the environmental sustainability of the agroecosystem [16].

To mitigate the ecotoxicological, environmental and social consequences of synthetic pesticides; the research for effective, economical, safe and ecological alternative methods compatible with sustainable development is therefore imperative. In other words, adopt the concept of integrated pest management (IPM) [17, 18]. Among the products likely to replace synthetic pesticides and, at the same time reduce pollution of natural ecosystems; valorization of OMW in plant protection responds well to this dilemma.

Around the world, there are more than 800 million productive olive trees, occupying an area of 10 million hectares; olives are used either as table olives or for the production of olive oil. Global table olive production was 2900000 tons,

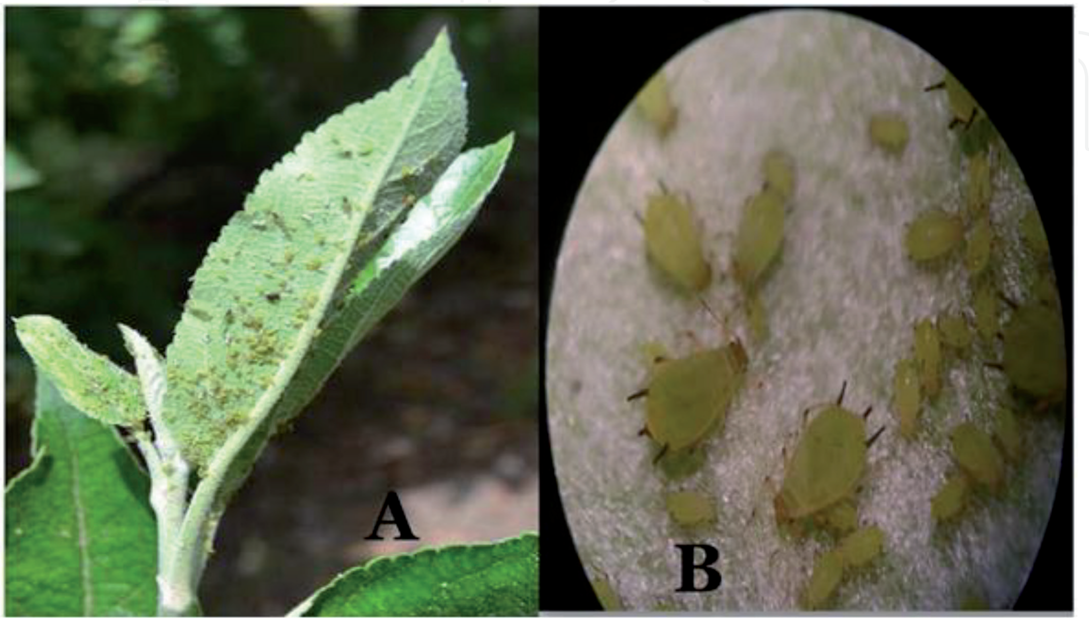

Figure 1.
Colony of Aphis pomi on an apple plant (A) and magnified 35 times under a stereomicroscope (B).

while olive oil production exceeded 3300000 tons [19]. The Mediterranean region alone provides 97% of the total world olive oil production, being close to 3 million tons [20]. The oil extraction is carried out by three processes, namely the traditional process (press process), the two-phase process and the three-phase process [21]. However, olive oil production is also accompanied by the generation of huge amounts of by-products and waste that leave a cluttered environmental footprint [22]. Indeed, during the extraction of oil by press or three-phase systems, enormous quantities of vegetable water are produced annually, the reported volumes are estimated at around 440 L of OMW for 100 kg of processed olives [23], i.e., from 10 to 30 million m^3 per year [24]. These effluents are acidic and very rich in organic compounds including polyphenols [24–26]. The management of OMW is generally unregulated; they can be discharged directly into terrestrial and aquatic ecosystems without prior treatment. This results in disastrous environmental consequences due to their high polluting capacity (e.g., [27–30]). Thus, non-target organisms in these natural compartments are negatively affected (e.g., [31–35]). In addition, the presence of organic and inorganic pollutants makes this waste flow toxic for bacteria and other microorganisms used in biological treatments [32]. In Morocco, where olive tree occupies an area of 1073493 ha and produces 1912238 t of olives [1], oil extraction is carried out mainly by traditional press and, in few cases by the three-phase or two-phase system; thus, it generates enormous quantities of OMW. Like other countries, the dumping of vegetable waters is also disposed mainly on soils and in rivers. Indeed, as indicated in **Figure 2**, during the extraction of oils from the olives, the OMW are evacuated in a channel (**Figure 2A**) discharging them either in a watercourse (**Figure 2B** and **D**) either in a terrestrial environment (**Figure 2C**).

Figure 2.
Olive mill wastewater (OMW) disposed into ecosystems ((A) discharge channel, (C) soil receptacle, (B and D) Oued receptacle).

With regard to the harmful effects of OMW and to avoid or mitigate the issues associated with them, the scientific community has felt the need to promote them in several areas. This is because vegetable waters contain valuable components, such as water, organic compounds and a wide range of nutrients that could be recycled. Then, OMW could be recycled and used as a feedstock to generate cost effective compounds such as antioxidants, enzymes, biogas, soil conditioners, feeds, foods, fertilizer, etc. (e.g., [36–42]).

In crop pest management, OMW have been tested against weeds, fungi and nematodes [43], olive psyllid [44], green apple aphid [45], date palm white worm [46], tomato broomrape [47], plant pathogens [48], Mediterranean fruit fly fruits [49]. For full details, bibliographic reviews provide a synthetic overview on the use of OMW in plant protection [50, 51]. For our part, after having evaluated the toxicity of OMW with respect to *Gammarus gauthieri* (Amphipoda, Crustaceae) [31] and earthworms [33], we tried to valorize crude OMW in the control of pests by testing them against *A. pomi*. This work aimed at replacing synthetic pesticides used in aphid control and, at the same time reducing pollution of natural ecosystems owing to OMW. To this end, the valorization of OMW in plant protection responds well to this dilemma. In this chapter, the results relating to the efficacy of crude olive mill wastewater with respect to the green apple aphid are presented, as well as their physicochemical composition.

2. Materials and methods

2.1 Location of the study nursery

The experiment was carried out in summer in a 2-year-old apple tree nursery (*Malus communis va*r. Golden Delicious, Rosaceae) located near the National School of Agriculture in Meknes (GPS coordinates: 33° 50′ 37″ N, 5° 28′ 39″ W) in plastic tunnel under ambient conditions. It is installed inside an orchard of the same culture.

2.2 Origin and physicochemical composition of olive mill wastewaters

The vegetable water samples were collected from a traditional olive oil extraction unit located in the Sefrou-Morocco region (GPS coordinates: 33° 49′ 50″ N, 4° 50′ 15″ W). Sampling was carried out immediately after olive oil extraction and OMW production; the effluents were taken, on three times with an interval of 15 min between two successive sampling. The samples were brought back to the Laboratory of the National School of Agriculture in Meknes in airtight boxes and stored at 4°C to avoid any alteration of their chemical composition.

OMW analysis (pH, electrical conductivity or EC, crude protein level, potassium, phosphorus, magnesium, calcium, copper, manganese, iron, zinc, sodium, cadmium and nickel) was carried out at the Official Laboratory of Chemical Analysis and Research, Casablanca Morocco (www.loarc.ma) according to standard methods. The content of suspended solids, the chemical oxygen demand (COD), the biological oxygen demand (BOD), concentrations of nitrites, ammonium, sulphate, nitrate, orthophosphat and chlorine were determined at the Laboratory of the Department of Basic Sciences of the National School of Agriculture in Meknes as methods described in Rodier et al. [52]. The total polyphenol content was determined according to the method of Folin Ciocalteu [53, 54]; to this end, 200 μL of OMWW were mixed with 1 ml of a freshly prepared Folin–Ciocalteu reagent (10 times diluted) and 0.8 ml of 7.5% sodium carbonate (Na_2CO_3). The whole mixture was incubated for 30 minutes at room temperature (20° C). The reading was carried out using a UV–visible spectrophotometer (Shimadzu 1601, Europe) at a wavelength of 765 nm. The concentration of polyphenols is expressed in milligrams of gallic acid equivalent per gram of OMW).

2.3 Strain of *Aphis pomi* and biotest

The different stages of the green apple aphid (L1, L2, L3, L4, Wingless adult) (**Figure 1**) were sampled with apple leaves infested in the nursery where the experiment occurred; the nursery has not undergone any insecticide treatment before. The leaves with the insects were then sorted by stage and placed in Petri dishes 9 cm in diameter and 1.5 cm in height ventilated and closed to prevent the aphids from escaping. Each box contained 10 individuals belonging to the same stage. Leaves bearing aphids were kept turgid by wrapping their peduncle with cotton wool soaked in water. The experiment was carried out in the nursery growing under a plastic tunnel in ambient conditions.

To determine the quantity of the crude OMW to be recommended to the farmer, i.e., the dose necessary to control aphids, we sought to determine the content of crude vegetable water to be diluted in water, i.e., the concentration of these effluents to be dissolved in the water required to kill a percentage of aphids. To this end, the concentrations were determined beforehand according to the formula of [55], i.e., concentrations capable of causing the mortality of 5% and 95% of the insects used. Then, the concentrations used are 5, 10, 20, 40 and 80 mg of OMW/liter of distilled water. The solutions of the vegetable waters were prepared using a magnetic stirrer for 10 min. At the same time, two control treatments were carried out, the negative one consisting of distilled water; while the positive one using Imidacloprid (Confidor (Active matter content 200 g/L) according to the recommended dose against aphids on apple trees (50 cc/hL) [2]. For each concentration or controls, 5 replicates were performed with 10 individuals each according to a randomized complete block design. The application of OMW to the leaves bearing the aphids was performed using 1-liter hand sprayer maintained at 0.3om from the targets. The count of dead insects was carried out 48 hours after spraying; each individual shaken by a paintbrush and not moving was considered dead. Aphid mortality was assessed under a stereomicroscope.

2.4 Statistical analysis of data

The lethal concentrations (LC50 and LC95 i.e., concentrations capable of killing 50 and 95% of the population treated) of the products tested against *A. pomi* were determined by Probit analysis [55] using SPSS version 21 (IBM Corp. Released 2015. Armonk, NY: IBM Corp.). The values were considered significantly different, when their 95% confidence intervals did not overlap. To identify the toxicity caused exclusively by OMW, the mortalities recorded with each concentration were corrected with Abbott formula [56]. The comparison of the lethal effects of OMW and the pesticide on aphid stages was performed by two-factor variance analysis (Stages* products) followed by the Tukey test (HSD) at 5% as post-hoc for the multiple comparison of means. The homogeneity and normality of the variance of the dependent variables were verified by Levene and Shapiro–Wilk tests, respectively; the dataset was transformed into arsin $\sqrt{}$ (percentage) before analysis of variance according to Sokal and Rohlf [57]. Linear models relating stage mortality to OMW concentration have been established; their choice was made based on the low values of the Akaike Information Criterion (AIC) and standard errors as well as on the high values of R^2 and their significance by the analysis of variance (F). To classify the different stages of the aphid according to their responses to OMW, a hierarchical classification was carried out on the LC50 and LC95 using Statistica version 7 software (Statsoft Inc. USA). The data are summarized as graphs or tables.

3. Results

3.1 Physicochemical properties of OMW

The physicochemical characteristics of OMW obtained from an extraction unit by the traditional press process are presented in **Table 1**. The effluents are reddish-black in color and darken during storage (**Figure 2**); they have a cloudy appearance and a strong odor of olive oil. It is an acidic liquid (pH = 4.90) with an electrical conductivity of 13.5mS/cm which is characteristic of wastewater. The contents of solid matter, chemical oxygen demand (COD) and biological oxygen demand (BOD5) weigh 40, 160 and 90 g/L of OMW, respectively, they are high. Crude proteins represent approximately 1% of the composition of vegetable waters; while

OMW properties	Units	Values
pH		4.90
Electrical conductivity	mS/cm	13.5
COD	g/L	160
BOD5		90
Solid matter		40
Crude protein	%	1.4
Potassium		1.0
Calcium		0.1
Sodium		0.06
Magnesium		0.045
Phosphorus	mg/kg	641.8
Iron		40.0
Manganese		4.5
Zinc		10.0
Copper		2.2
Nickel		˂ 0.75
Cadmium		˂ 0.015
Sulfate	mg/L	1591.84
Ammonium		1342.53
Nitrate		743.39
Nitrite		260.57
Chlorine		184.6
Orthophosphate		12.43
Polyphenols	mg of GAE/g	66,19

Table 1.
Physicochemical properties of OMW collected from a traditional processing unit in Sefrou region (GAE: gallic acid equivalent).

potassium, calcium, sodium and magnesium represent from 1 to 0.05% of the total (**Table 1**). The samples of vegetable water analyzed are relatively rich in inorganic ions (P, Mn, Cu, Fe, Zn, Ni, Cd), their contents vary from approximately 642 to 0.015 mg/Kg. Sulfates, ammonium, nitrates, nitrites, chlorine and orthophosphate

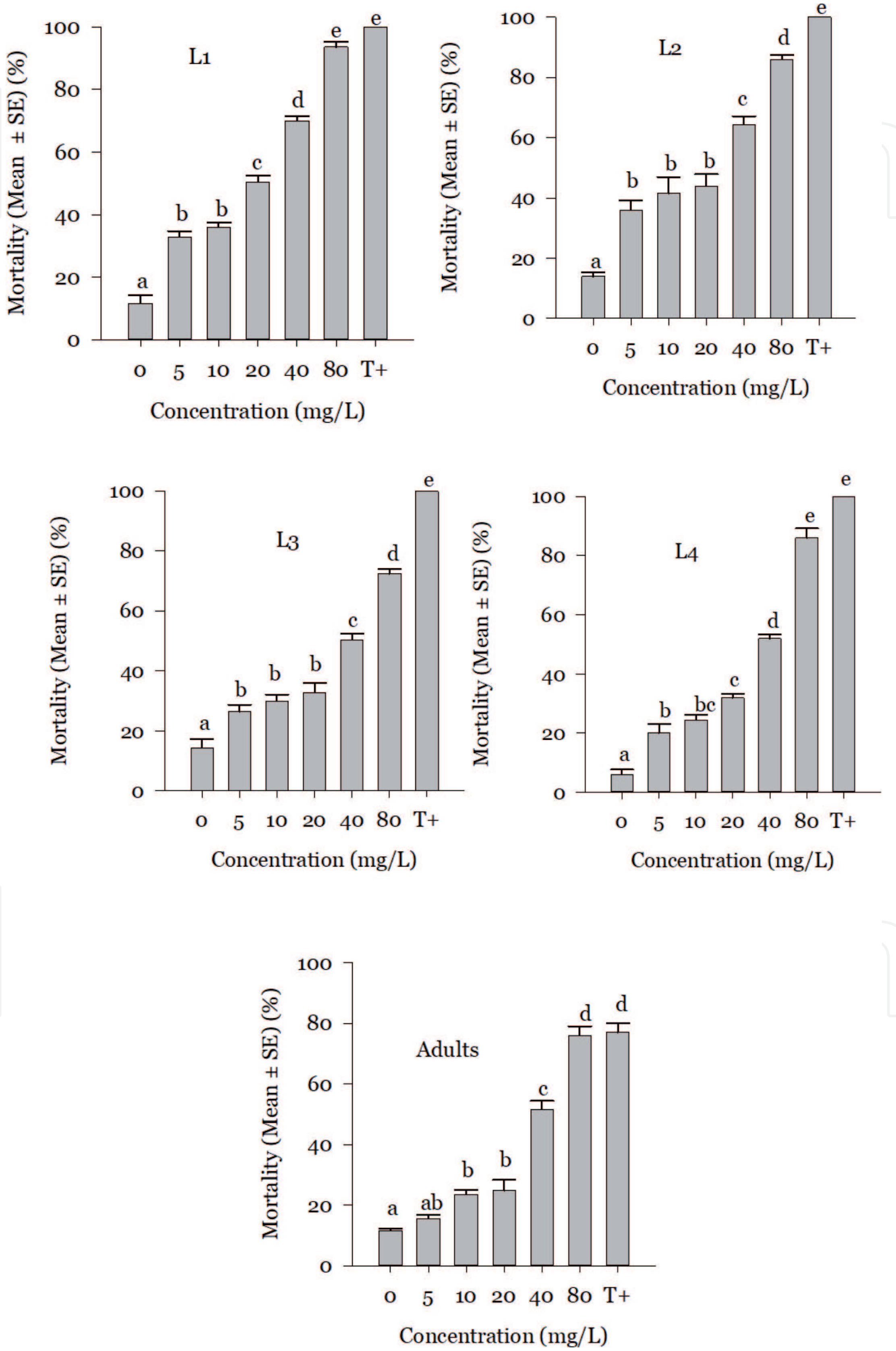

Figure 3.
Mortality of the different stages of Aphis pomi *treated with crude OMW (SE: standard error; T +: imidacloprid; the bars with the same letter do not differ statistically from each other, ANOVA2F followed by the Tukey HSD test at 5%).*

are also dissolved in vegetable waters, their concentrations ranging between approximately 1592 and 12 mg/L; while the total polyphenol content is around 66 mg /L of OMW (**Table 1**).

3.2 Aphid toxicity

Crude vegetable water from olives has been shown to be very toxic to *A. pomi*, their effects are amplified with concentration (**Figure 3**). The response of the treated individuals varies significantly between stages ($F = 59.93$, $df = 4, 141$, $P < 0.001$) and according to the concentration ($F = 871.75$, $df = 6, 141$, $P < 0.001$). Compared to the negative control, crude OMW significantly affect the survival of the different stages of the green apple aphid, the percentages of mortalities are approximately 4 to 38 times higher than the control. Whereas all stages have a comparable mortality rate in untreated lots ($P > 0.05$). On the other hand, compared to the positive control (Imidacloprid), only the high concentration (80 mg/L) which makes it possible to induce statistically comparable mortalities, the other concentrations cause mortalities markedly lower than this,

Stages	Models	R^2	$Df^{Residual}$	F	P
L1	y = 0.93x + 24.98	0.92	4	47.19	0.0024
L2	y = 0.78x + 27.42	0.91	4	38.11	0.0035
L3	y = 0.67x + 20.32	0.97	4	126.11	0.0004
L4	y = 0.94x + 12.45	0.98	4	252.45	<0.0001
Adults	y = 0.82x 12.70	0.98	4	171.21	0.0002

Table 2.
Relationship between concentration (x in mg of OMW/L of distilled water) and mortality (y in %) of Aphis pomi treated with crude olive mill wastewater during 48 h.

Stages	Number	Slope[b] ± SE	Constante ± SE	LC50 (CI[c]) mg/L	LC95 (CI) mg/L	χ^2	*Df*[d]	P[e]
L[a]1	300	0.03 ± 0.004	−0.75 ± 0.11	24.17 (19.08, 29.78)	77.19 (65.18, 96.25)	4.92	4	0.30
L2	300	0.02 ± 0.003	−0.62 ± 0.11	27.33 (20.61, 34.75)	100.00 (82.42, 130.04)	6.44	4	0.17
L3	300	0.02 ± 0.003	−0.82 ± 0.11	44.67 35.93, 57.10)	134.57 (108.30, 183.10)	2.26	4	0.69
L4	300	0.03 ± 0.003	−1.10 ± 0.12	39.08 (33.10, 46.50)	97.56 (83.28, 119.65)	3.40	4	0.49
Adults	300	0.02 ± 0.003	−1.06 ± 0.12	45.59 (38.27, 55.35)	116.37 (97.48, 147.32)	2.30	4	0.68

[a]*L = nymph.*
[b]*Probit model = slope – constante, SE: standard error.*
[c]*95% lower and upper confidence limits are shown in parenthesis expressed in mg of OMW/L of distilled water.*
[d]*Df: degree of freedom.*
[e]*Probaility.*

Table 3.
Toxicity parameters calculated for Aphis pomi exposed during 48 hours to crude OMW.

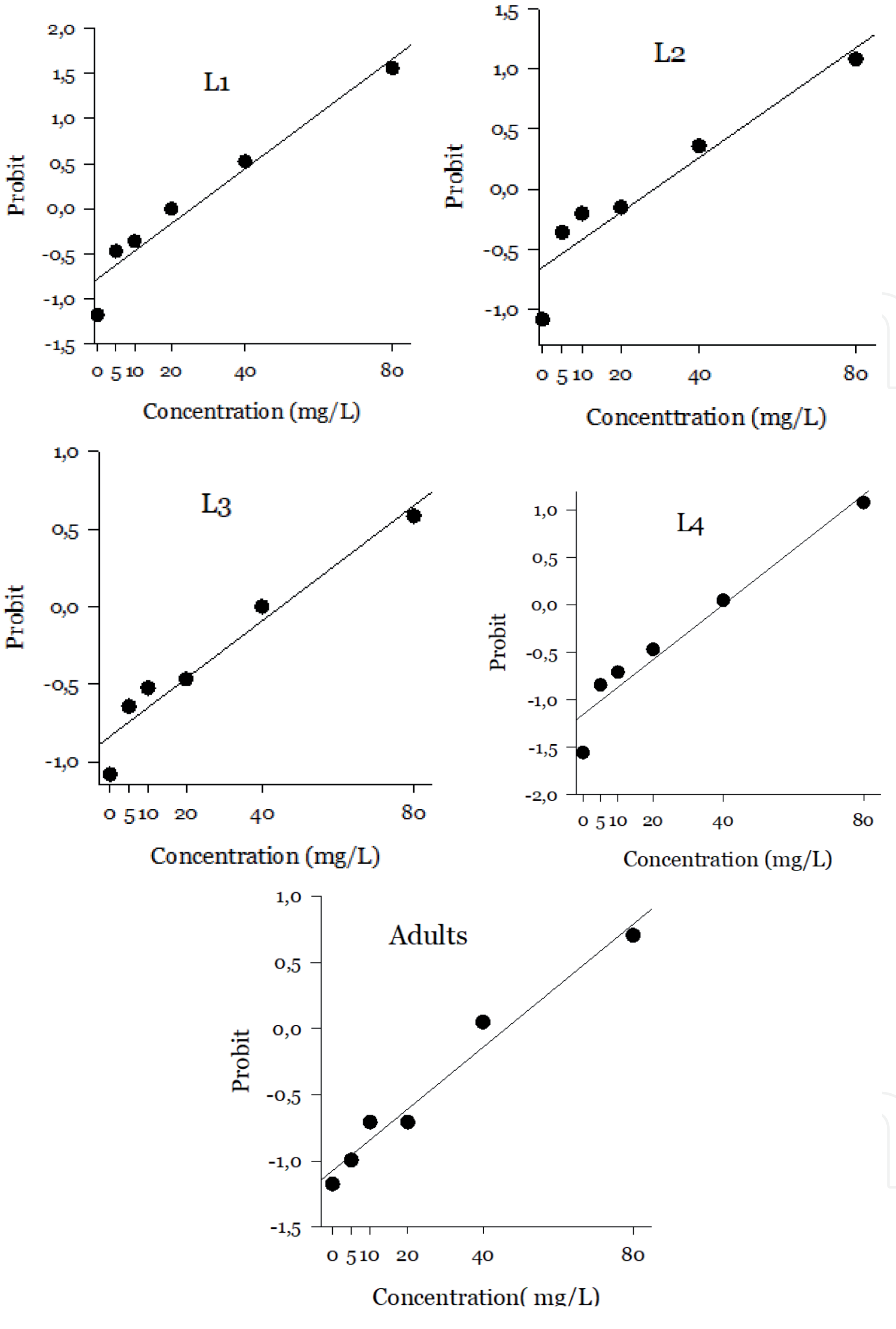

Figure 4.
Toxic activity of crude OMW against the different stages of Aphis pomi after 48 hours of treatment (on the x-axis are presented the concentrations instead of their logarithms).

P < 0.05 (**Figure 3**). With regard to the same concentration of OMW, the aphid's response varies depending on the stage. With 5 mg /L, the L1 and L2 are affected by the same mortality rate (P > 0.05); the same is true for L3 and L4 or L4 and adults compared in pairs. The same pattern is obtained with 10 mg/L. Treated with 20, 40 or 80 mg/L, the L1 and L2 were shown to be much more vulnerable than the other stages, P < 0.05.

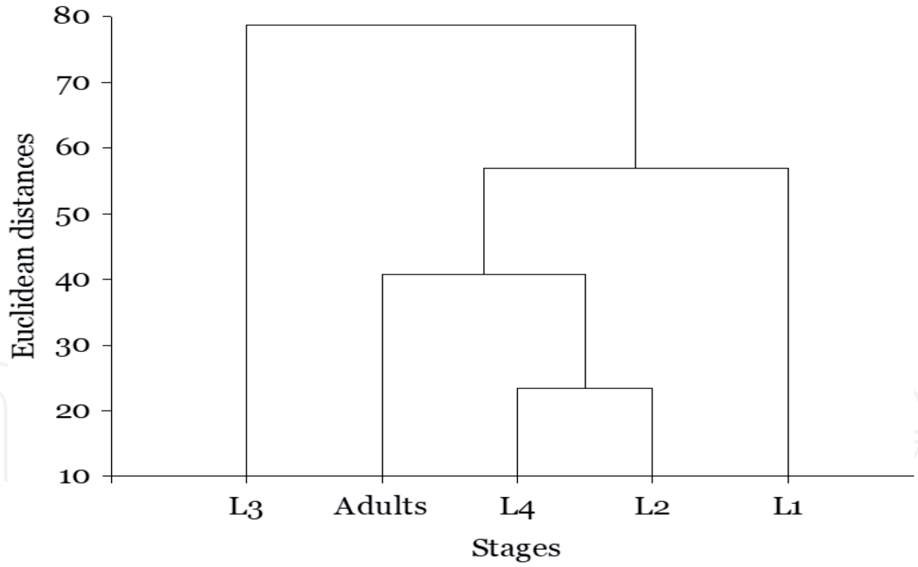

Figure 5.
Classification of the different stages according to their sensitivity to OMW (unweighted averages of lethal concentrations).

Moreover, the relationship linking the mortality of the stages of the aphid to the concentrations of OMW makes it possible to note that for each stage of the green apple aphid, the mortality therefore depends linearly and positively on the concentration of OMW (**Table 2**). In terms of corrected mortality, i.e., due exclusively to crude OMW, the percentages of dead insects vary from around 2 to 98% depending on the concentration and the stage considered with strong variations (Coefficients of variation = 4–70%).

Furthermore, the observed data fit well to the log-probit model and no statistically significant deviation from the regression equation was detected, $P > 0.05$ (**Table 3** and **Figure 4**). Lethal concentrations vary depending on the stage of the aphid considered; in fact, the extreme LC50 and LC95 vary from approximately 19 to 55 and 65 to 147 mg of OMW/L of distilled water, respectively. With regard to the values of the LC50, the tolerance of *A. pomi* increases with age, the sensitivity of the young stages is greater than that of the older ones. At high concentration (LC95), values increase with stage; while for L4 and adults, lethality is variable (**Table 3** and **Figure 4**).

With regard to lethal concentrations, the hierarchical classification of the stages of *A. pomi* according to their sensitivity to crude OMW allows them to be classified in decreasing order L1 ≤ L2 ≤ L4 < Adults <L3. The nymphs L1, L2 and L4 were therefore more vulnerable to crude OMW than adults and L3 (**Figure 5**). In terms of efficiency, the application of 135 mg of crude OMW/L of water allows to reduce the level of aphid populations below the threshold adopted by producers (15–20% of infested shoots).

4. Discussion

4.1 Physicochemical characteristics of OMW

Olive oil extraction by traditional and three-phase processes generates huge amounts of vegetable waters rich in various organic and inorganic compounds during a short period of the year (October–March). OMW have a high biological and chemical oxygen demand values, as well as high contents of organic matter, suspended matter, inhibitor substances (phenolic compounds) and minerals, especially potassium, phosphorus, magnesium and calcium [58]. The values of the physicochemical parameters presented in this work are similar to those reported in Morocco (eg, [26, 59–62]). However, the chemical composition of OMW depends on the olive variety with the stage of maturity of the olives, the harvest period and the extraction techniques [63, 64]. Their physicochemical characteristics confer them the status of polluting substances posing serious environmental problems. Indeed, certain elements present in OMW are responsible for their harmful effects. Thus, the high concentration of phenolic compounds in vegetable waters generates phytotoxic effects [63]. Moreover, according to [65], heavy metals (Cu, Ni, Pb, Cr, Zn) affect the performance of anaerobic digestion by inhibiting microorganisms; indeed, during the production of biogas by the anaerobic digestion process from of the olive mill waste, the methanogenic bacteria are inhibited. The recovery, recycling, and reuse of these by-products are considered the best options for a sustainable water management program. Thus, their use as bio-pesticides is one of the valorization modalities that we have tried to apply in this work.

4.2 Valorization of OMW in aphid control

The damage caused by *A. pomi*, especially in nurseries and young plantations, force producers to treat frequently the pest with synthetic insecticides. The misuse of synthetic pesticides, however, raises safety and environmental issues [6–8]. At the same time, olive oil extraction by traditional and three-phase systems generates enormous volumes of vegetable waters, which are generally discharged into natural ecosystems [24–26]. To mitigate the undesirable effects of synthetic pesticides and at the same time those of OMW, it has proved to be imperative to replace synthetic aphicides by valorizing vegetable water in the management of *A. pomi*; this will contribute to crop protection while solving the environmental and health problems raised by the two categories of products. The treatment of the aphid with crude OMW has made it possible to prove their effectiveness. Thus, the crude vegetable waters tested against *A. pomi* were toxic to aphids. Indeed, the applied concentrations cause a variable mortality according to the stage and dependent on the concentration. The LC50 and LC95 vary from 27.17 to 45.59 and from 77.19 to 134.57 mg of crude OMW /L of water, respectively; the young stages were more vulnerable to vegetable waters than the older ones and adults. By high concentration, crude OMW equal the efficacy of the reference product, imidacloprid, used at the recommended dose in the field. In terms of efficiency, to reduce the level of populations below the threshold adopted by producers, ie, 15–20% of infested shoots, an average dose of 135 mg of crude OMW/L of water can satisfy the farmer to control aphids.

Various studies have evaluated the effectiveness of the OMW or their polyphenols in the management of insect pests on different rops. Thus, for example, the treatment of *Euphyllura olivina* Costa (Hemiptera, Psyllidae), olive psyllid, with polyphenols from OMW at a rate of 2 g of hydroxytyrosol/L of water in an olive grove, allowed to control 41.1% of larvae and 72% of adults. In contrast, with regard to eggs, the products was ineffective [44]. Against *A. pomi*, crude OMW or their polyphenols cause significantly higher mortalities than the controls used; their toxicity depends both on the stages of the insect and on the concentration of the products tested; the average LC50s are around 25.10 ml of crude OMW and 42.8 mg of polyphenols/L of distilled water. The toxicity caused by crude OMW depends in 67% to approximately 100% of cases on that of the polyphenols contained in them [45]. Spraying the larvae of *Potosia opaca* (Coleoptera, Scarabeidae), a date palm pest in Morocco, with crude OMW, allowed to obtain the same efficacy as with the reference insecticides (chlorpyriphos-ethyl or chlorpyriphos-ethy + cypermethrin) 19 days after treatment; vegetable waters are both toxic and affect the weight of survivors; their toxicity depends directly on their polyphenol content [46]. Tested on the Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann) (Diptera, Tephriditae), the polyphenolic fractions of OMW inhibit egg hatching and female fecundity without affecting larval development [49]. Overall, from all the studies cited in this paragraph, it emerges that the toxicity caused by OMW depends mainly on their polyphenol content (*op. Cit.*). In addition, although, the biochemical modes of action of OMW have not yet been elucidated in insects, the high levels of phenols present in vegetable water could block the transmission of nerve impulses [66, 67]. However, in this case, it is not excluded that the vegetable waters contained insecticides, in this case organophosphates and/or carbamates, used against the olive fly and which inhibit acetylcholinesterase (eg, [68]).

5. Conclusions and perspectives

Rejected agricultural by-products offer multiple opportunities for recovery and have significant potential not only in the agricultural and agrifood sectors but also in plant protection. In fact, in this work, crude OMW tested against *A. pomi* were effective in reducing the level of their populations to economically tolerable levels. However, the effect of products tested in nursery pest management must be compatible with integrated pest management (IPM) concept. Since, some plant producers also carry out augmentative releases of natural enemies (Unpublished data). Therefore, like conventional pesticides, risk assessment of side effects of OMW is still necessary [17, 18]; the evaluation of the effects of OMW on non-target organisms must include both lethal and sublethal effects (e.g., [14, 15]). In the event that the natural enemies bred massively and purchased by plant producers, their releases must be carried out outside the treatment periods. It is also possible to spray against pests with OMW outside the activity of natural enemies; preferably during vegetative rest against overwintering forms.

Moreover, knowing that OMW can also show phytotoxicity [69], an evaluation in this direction is planned. Our work can help to enhance the use of MOW to control the green apple aphid among other pests while integrating the ecological services provided by beneficial organisms in agroecosystems, and at the same time avoid the harmfulness of OMW. At the industrial level, the large-scale direct extraction of polyphenols for the production of biopesticides would result in high added-value. The identification and quantification of the constituents of polyphenols with their biochemical modes of action in treated pests should precede the economic estimation of pest control based on OMW and their polyphenols.

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References

[1] FAOSTAT, 2019. Crop statistics, Food and Agriculture Organization of the United Nations. Available from: http://www.fao.org/faostat/fr/#data/QC (Acceced: 2019-07-19)

[2] ONSSA, 2019. http://eservice.onssa.gov.ma/IndPesticide.aspx (Acceced June 2021)

[3] Beers E. H., D. M. Suckling, R. J. Prokopy and J. Avilla. Ecology and Management of Apple Arthropod Pests. In Ferree D.C. and I.J. Warrington (eds), Apples: Botany, Production and Uses, CAB International 2003, pp: 489-519.

[4] Alford D.V. A Colour Handbook Pests of Fruit Crops, Second Edition. CRC Press Taylor & Francis Group; 2014. 434 p.

[5] Van Emden H. F. and Harrington R. (eds),. Aphids as Crop Pests. CABI, Second Edition. www.cabi.org; 2017. 686 p.

[6] Guedes R. N. C., Smagghe G., Stark J. D., & Desneux N. Pesticide-Induced Stress in Arthropod Pests for Optimized Integrated Pest Management Programs. Annual Review of Entomology. 2016; 61(1), 43-62. https://doi:10.1146/annurev-ento-010715-023646

[7] Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. Science of the Total Environment. 2017. 575:525-235. https://DOI:10.1016/j.scitotenv.2016.09.009

[8] Carvalho F. P. Pesticides, environment, and food safety. Pesticides, Food and Energy Security. 2017; 6(2): 48-60. https://doi:0.1002/fes3.108

[9] Tamaš N., Dojnov B., Margetić A., Vujčić M., Špirović B., Miletić N., Stević M. and Vujčić Z. Resistance to common organophosphate and carbamate insecticides in Aphis pomi (Hemiptera: Aphididae). Fruits. 2015; 70(3): 135-142. https://doi.org/10.1051/fruits/2015005

[10] Guedes R. N. C, Walse S. S. and Throne J. E. Sublethal exposure, insecticide resistance, and community stress. Current Opinion in Insect Science. 2017; 21:47-53. http://dx.doi.org/10.1016/j.cois.2017.04.010

[11] Paula, D. P., Menger, J., Andow, D. A., & Koch, R. L. Diverse patterns of constitutive and inducible overexpression of detoxifying enzyme genes among resistant Aphis glycines populations. Pesticide Biochemistry and Physiology. 2020; 164:100-114. https://doi.org/10.1016/j.pestbp.2019.12.012

[12] Cutler C., G., Ramanaidu, K., Astatkie, T., & Isman, M. B. Green peach aphid, *Myzus persicae* (Hemiptera: Aphididae), reproduction during exposure to sublethal concentrations of imidacloprid and azadirachtin. Pest Management Science. 2009; 65(2):205-209. https://doi.org/10.1002/ps.1669

[13] Guedes, R. N. C., & Cutler, G. C. Insecticide-induced hormesis and arthropod pest management. Pest Management Science. 2014; 70(5): 690-697. https://doi.org/10.1002/ps.3669

[14] Stark JD, Banks JE. Population-level effects of pesticides and other toxicants on arthropods. Annual Review of Entomology. 2003; 48: 505-519. https://doi.org/10.1146/annurev.ento.48.091801.112621

[15] Desneux, N., Decourtye, A., Delpuech, J.M. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 2007; 52:81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

[16] Ribeiro, L.P., Zanardi, O.Z., Vendramim, J.D., Yamamoto, P.T. Comparative toxicity of an acetogenin-based extract and commercial pesticides against citrus red mite. Exp. Appl. Acarol. 2014; 64, 87-98.

[17] Radcliffe, E. B., Hutchison, W. D., Cancelado, R. E. (Eds.), Integrated pest management: concepts, tactics, strategies and case studies. Cambridge University Press. 2009.

[18] Abrol D.P. Integrated pest management: current concepts and ecological perspective. Academic Press, San Diego, Calif; 2014. 576p.

[19] IOOC, 2018. International Olive Oil Council Webpage, World Olive Oil Figures. Available from: http://www.internationaloliveoil.org. (Accessed: 2021-08-05)

[20] Vossen, P. Growing olives for oil. In: R. Aparicio & J. Harwood (Eds.). Handbook of olive oil: Analysis and properties. Springer; 2013. (pp. 19-56). https://doi:10.1007/978-1-4614-7777-8_2

[21] Azbar, N., Bayram, A., Filibeli, A., Muezzinoglu, A., Sengul, F., Ozer, A. A review of waste management options in olive oil production. Critical Reviews in Environmental Science and Technology. 2004; 34 (3): 209-247. https://doi.org/10.1080/10643380490279932

[22] Galanakis C. M. (ed.). Olive Mill Waste Recent Advances for Sustainable Management. Academic Press; 2017. 284p.

[23] Alu'datt, M.H., Alli, I., Ereifej, K., Alhamad, M., Al-Tawaha, A.R., Rababah, T. Optimisation, characterisation and quantification of phenolic compounds in olive cake. Food Chemistry. 2010; 123(1):117-122. https://doi.10.1016/j.foodchem.2010.04.011

[24] El-Abbassi, A., Kiai, H., Hafidi, A. Phenolic profile and antioxidant activities of olive mill wastewater. Food Chem. 2012; 132(1), 406-412

[25] Jail A., Boukhoubza F., Nejmeddine A., Sayadic S., Hassani L. Co-treatment of olive-mill and urban wastewaters by experimental stabilization ponds. Journal of Hazardous Materials. 2010; 176: 893-900. https://doi:10.1016/j.jhazmat.2009.11.120

[26] El Yamani, M. E., Sakar, E. H., Boussakouran, A., Ghabbour, N., & Rharrabti, Y. Physicochemical and microbiological characterization of olive mill wastewater (OMW) from different regions of northern Morocco. Environmental Technology. 2020; 41(23): 3081-3093. https://doi.org/10.1080/09593330.2019.1597926

[27] Salomone, R., Ioppolo, G. Environmental impacts of olive oil production: a Life Cycle Assessment case study in the province of Messina (Sicily). Journal of Cleaner Production. 2012; 28:88-100. https://doi.org/10.1016/j.jclepro.2011.10.004

[28] Uyar, B., Kars, G., Eroğlu, İ., Yücel, M., Gündüz, U. Hydrogen production via photofermentation, in: Azbar, N., Levin, D.B. (Eds.), State of the art and progress in production of biohydrogen. Bentham Science Publishers, United Arab Emirates; 2012. pp. 54-77

[29] Dermeche S., M. Nadoura, C. Larroche, F. Moulti-Matia, P. Michaud. Olive mill wastes: Biochemical characterizations and valorization strategies Process Biochemistry. , 2013; 48(10): 1532-1552. https://doi.org/10.1016/j.procbio.2013.07.010

[30] Haddad, K., Jeguirim, M., Jellali, S., Thevenin, N., Ruidavets, L., & Limousy, L. Biochar production from Cypress sawdust and olive mill wastewater: Agronomic approach. Science of The

Total Environment. 2020; 141713. https://doi:10.1016/j.scitotenv.2020.141713

[31] Haouache N. A. Bouchaleta. Olive mill wastewater: Effect on the survival and reproduction of the ecological indicator Gammarus gauthieri. Journal of Materials and Environmental Science. 2016; 7 (7): 2288-2294

[32] Souilem, S., El-Abbassi, A., Kiai, H., Hafidi, A., Sayadi, S., Galanakis, C.M. Olive oil production sector: environmental effects and sustainability challenges. In: Galanakis C.M., .ed. Olive Mill Waste: Recent Advances for Sustainable Management. Academic Press. 2017. Pp: 1-28. https://doi.org/10.1016/B978-0-12-805314-0.00001-7

[33] Haouache N. Bouchelta A., Boughdad A., Bouabid R., Hannour K. Effect and Chemical composition of olive mill wastewater on the survival and the fecundity of *Eisenia foetida* (Haplotaxida, Lumbricidae, Savigny, 1826). International Journal of Academic Studies. 2018; 4(02): 36-49

[34] Mekersi N., Kadi K., Casini S., Addad ., Bazri K., Marref S., Lekmine S., Amari A. Effects of single and combined olive mill wastewater and olive mill pomace on the growth, reproduction, and survival of two earthworm species (*Aporrectodea trapezoides, Eisenia fetida*). Applied Soil Ecology. 2021; 168, 10412. https://doi.org/10.1016/j.apsoil.2021.104123

[35] Patsios S. I., Kontogiannopoulos K. N. and Banias G. F. Environmental impact assessment in agri-production: a comparative study of olive oil production in two European countries In: Bochtis D, Achillas C., Banias G. & M. Lampridi (eds). Bio-Economy and Agri-production Concepts and Evidence. Academic Press; 2021. Pp:83-116. https://doi.org/10.1016/B978-0-12-819774-5.00005-9

[36] Muktadirul Bari Chowdhury, A. K. M., Akratos, C. S., Vayenas, D. V., & Pavlou, S. Olive mill waste composting: A review. International Biodeterioration & Biodegradation. 2013; 85, 108-119. https://doi:10.1016/j.ibiod.2013.06.019

[37] Kourmentza, C., Koutra, E., Venetsaneas, N., Kornaros, M. Integrated biorefinery approach for the valorization of olive mill waste streams towards sustainable biofuels and bio-based products, in: Kalia, V., Kumar, P. (Eds.), Microbial Applications Vol. 1. Springer; 2017. pp. 211-238.

[38] Gullón, B., Gullón, P., Eibes, G., Cara, C., De Torres, A., López-Linares, J.C., Ruiz, E., Castro, E. Valorisation of olive agro-industrial by-products as a source of bioactive compounds. Sci. Total Environ. 2018; 645: 533-542

[39] Sarris, D., Rapti, A., Papafotis, N., Koutinas, A. A., & Papanikolaou, S. Production of added-value chemical compounds through bioconversions of olive-mill wastewaters blended with crude glycerol by a Yarrowia lipolytica strain. Molecules. 2019 ; 24(2), 222. https://doi.org/10.3390/molecules24020222

[40] Ahmed P.M., Fernández P.M., Figueroa L.I.C., Pajot H.F. Exploitation alternatives of olive mill wastewater: production of value added compounds useful for industry and agriculture. Biofuel Research Journal. 2019; 22:980-994. https://doi:10.18331/BRJ2019.6.2.4

[41] Zema, D.A.; Esteban Lucas-Borja, M.; Andiloro, S.; Tamburino, V.; Zimbone, S.M. Short-Term Effects of Olive MillWastewater Application on the Hydrological and Physico-Chemical Properties of a Loamy Soil. Agric. Water Manag. 2019; 221: 312-321.

[42] Ahmad, T., Belwal, T., Li, L., Ramola, S., Aadil, R. M., Abdullah, Zisheng, L. Utilization of wastewater from edible oil industry, turning waste

into valuable products: A review. Trends in Food Science & Technology. 2020; 99:21-33. https://doi.org/10.1016/j.tifs.2020.02.017

[43] Cayuela, M. L., Millner, P. D., Meyer, S. L. F., & Roig, A. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. Science of The Total Environment. , 2008 ; 399(1-3): 11-18. https://doi:10.1016/j.scitotenv.2008.03.031

[44] Debo, A., Yangui, T., Dhouib, A., Ksantini,M., Sayadi, S. Efficacy of a hydroxytyrosolrich preparation from olive mill wastewater for control of olive psyllid, Euphyllura olivina, infestations. Crop. Protection. 2011; 30(12): 1529-1534. http://dx.doi.org/10.1016/j.cropro

[45] Haouache N, Boughdad A. Utilisation des margines contre Aphis pomi (De Geer, 1773) (Homoptera, Aphididae). Neuvième Congrès de l'Association Marocaine de Protection des Plantes –Rabat Maroc. 2014. 15p.

[46] Boutaj, H., Boutasknit, A., Anli, M., Ait Ahmed, M., El Abbassi, A., & Meddich, A. Insecticidal Effect of Olive Mill Wastewaters on Potosia opaca (Coleoptera: Scarabeidae) Larva. Waste and Biomass Valorization. 2020 ; 11 (7): 3397-3405. https://doi:10.1007/s12649-019-00682-1

[47] Qasem, J. R. Control of branched broomrape (*Orobanche ramosa* L.) in tomato (*Lycopersicon esculentum* mill.) by olive cake and olive mill wastewater. Crop Protection. 2020; 129:105021. https://doi.org/10.1016/j.cropro.2019.105021

[48] Sciubba F, Chronopoulou L, Pizzichini D, Lionetti V, Fontana C, Aromolo R, Socciarelli S, Gambelli L, Bartolacci B, Finotti E, Benedetti A, Miccheli A, Neri U, Palocci C, Bellincampi D. Olive Mill Wastes: A Source of Bioactive Molecules for Plant Growth and Protection against Pathogens. Biology. 2020 ; 9(12):450. https://doi.org/10.3390/biology9120450

[49] Di Ilio1 V., & Cristofaro M. Polyphenolic extracts from the olive mill wastewater as a source of biopesticides and their effects on the life cycle of the Mediterranean fruit fly *Ceratitis capitata* (Diptera, Tephriditae). International Journal of Tropical Insect Science. 2021; 41:359-366. https://doi.org/10.1007/s42690-020-00224-6

[50] El-Abbassi A, Saadaoui N, Kiai H, Raiti J, Hafidi A. Potential applications of olive mill wastewater as biopesticide for crops protection. Science of the Total Environment. 2017 ; 576 (15): 10-21. https://doi.org/10.1016/j.scitotenv.2016.10.032

[51] Bailón-Salas, A. M., Ordaz-Diaz, L. A., López-Serrano, P. M., Flores-Villegas, M. Y., and Dominguez-Calleros, P. A. Wastewater as a resource for pest control: An overview. BioResources. 2021 ; 16(3): 6401-6425. https://doi:10.15376/biores.16.3.Bailon-Salas

[52] Rodier J., Legube B., Merlet N. Analyse de l'eau. Dunod, Paris. 2009. 1526 p

[53] Blainski, A., Lopes, G., & de Mello, J. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules. 2013 ; 18 (6): 6852-6865. https://doi:10.3390/molecules18066852

[54] Rover, M. R., & Brown, R. C. Quantification of total phenols in bio-oil using the Folin–Ciocalteu method. Journal of Analytical and Applied Pyrolysis. 2013; 104: 366-371. https://doi:10.1016/j.jaap.2013.06.011

[55] Finney, D.J. Probit Analysis. 3rd ed. Cambridge University Press. Cambridge. 1971. 333p.

[56] Abbott, W.S. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 1925; 18, 265-267.

[57] Sokal R. R., F. J. Rohlf, Biometry: The Principles and Practices of Statistics in Biological Research. Third edition. Publisher: W. H. Freeman. 1995. 899p

[58] Patsios, S.I., Kontogiannopoulos, K.N., Mitrouli, S.T, Plakas, K.V., Karabelas, A.J. Characterisation of agricultural waste co- and by-products, Report, AgroCycle project Deliverable 1.2. 2016. Available online at: http://www.agrocycle.eu/files/2017/10/D1.2_AgroCycle.pdf (Accessed: 2021-7-20)

[59] Ben Sassi, A., Boularbah, A., Jaouad, A., Walker, G., & Boussaid, A. A comparison of Olive oil Mill Wastewaters (OMW) from three different processes in Morocco. Process Biochemistry. 2006; 41(1): 74-78. https://doi:10.1016/j.procbio.2005.03.074

[60] Zbakh, H., El Abbassi, A. Potential use of olive mill wastewater in the preparation of functional beverages: a review. Journal of Functional Foods. , 2012; 4(1):53-65. https://doi:10.1016/j.jff.2012.01.002

[61] Belaqziz, M., El-Abbassi, A., Lakhal, E. K., Agrafioti, E., & Galanakis, C. M. Agronomic application of olive mill wastewater: Effects on maize production and soil properties. Journal of Environmental Management. 2016; 171: 158-165. https://doi:10.1016/j.jenvman.2016.02.006

[62] Elabdouni, A., Haboubi, K., Merimi, I., & El Youbi, M. S. Olive mill wastewater (OMW) production in the province of Al-Hoceima (Morocco) and their physico-chemical characterization by mill types. Materials Today: Proceedings. 2020; 27(4):3145-3150. https://doi.org/10.1016/j.matpr.2020.03.806

[63] Magdich, S., Jarboui, R., Rouina, B.B., Boukhris,M. and Ammar, E. A yearly spraying of olive mill wastewater on agricultural soil over six successive years: Impact of different application rates on olive production, phenolic compounds, phytotoxicity and microbial counts. Science of the Total Environment. 2012; 430(15): 209-216. https://doi.org/10.1016/j.scitotenv.2012.05.004

[64] Bene C. D., Pellegrino E., Debolini M., Silvestri N., Bonari E. Short- and long-term effects of olive mill wastewater land spreading on soil chemical and biological properties. Soil Biology & Biochemistry. 2013; 56: 21 – 30

[65] Alcrudeashdeh, K.A.b.; Gul, E.; Yang, Q.; Yang, H.; Bartocci, P.; Fantozzi, F. Effect of Heavy Metals in the Performance of Anaerobic Digestion of Olive Mill Waste. Processes. 2020; 8, 1146. https://doi.org/10.3390/pr8091146

[66] Danellakis, D., Ntaikou, I., Kornaros, M., Dailianis, S. Olive oil mill wastewater toxicity in the marine environment: alterations of stress indices in tissues of mussel *Mytilus galloprovincialis*. Aquatic Toxicology. 2011; 101(2): 358-366. https://doi:10.1016/j.aquatox.2010.11.015

[67] Campani, T., Caliani, I., Pozzuoli, C., Romi, M., Fossi, M.C., Casini, S. Assessment of toxicological effects of crude and bioremediated olive mill waste in the earthworm Eisenia fetida: a biomarker approach for sustainable agriculture. Applied Soil Ecology. 2017; 119:18-25. https://doi:10.1016/j.apsoil.2017.05.016

[68] Casida, J. E., & Durkin, K. A. Neuroactive Insecticides: Targets, Selectivity, Resistance, and Secondary Effects. Annual Review of Entomology.

2013; 58(1): 99-117. https://doi:10.1146/annurev-ento-120811-153645

[69] Enaime, G., Baçaoui, A., Yaacoubi, A. et al. Phytotoxicity assessment of olive mill wastewater treated by different technologies: effect on seed germination of maize and tomato. Enviro nmental Science and Pollution Research. 2020; 27: 8034-8045. https://doi.org/10.1007/s11356-019-06672-z