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Determining the Most Suitable Time to Harvest Olive Fruits Infected with Olive Fruit Fly '*Bacterocera oleae*' Larvae Based on the Quality and Quantity of Fruit Oil

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ABSTRACT

This research aimed to determine the most suitable time to harvest olive fruits (*Olea euopeae* cv. Zard) infected with olive fly larvae based on the quality and quantity of the extracted oil. The experiment factors included the fruit type at two levels (1: infected fruits, 2: healthy fruits) and the harvest time at six levels (0: the time of olive fly larvae exit, 1: one week after the fly larvae exit, 2: two weeks after the olive fly larvae exit, 3: three weeks after the olive fly larvae exit, 4: four weeks after the olive fly larvae exit and 5: five weeks after the fly larvae exit). The fresh oil of fruit was extracted and analyzed for measuring qualitative and quantitative characteristics. The results showed that the effect of fruit type was significant on the extinction coefficient at 232 and 270nm (K232 and K270). The effect of harvest times was significant on K320, oil percent ($p \leq 0.05$) and acidity ($p \leq 0.05$), but had no significant effect on K270 and peroxide ($p \leq 0.05$). The interaction effect of fruit type and harvest time was significant only on acidity ($p \leq 0.05$) and was not significant on other parameters. Based on the results, the healthy fruits had lower acidity and peroxide number and higher oil percentage than infected fruits and therefore had better quality. It can be concluded that the best time to harvest the fruit was two weeks after the larva leaves the fruit because, after that, the negative effects of olive fly larvae on the quantity and quality of fruit oil are high, while before that, quality indicators of the fruit are not affected by olive fly larvae.

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1. Introduction

Olive (*Olea europaea* L.) is one of the most important products of the Mediterranean region. A major part of the product is used for its oil and a significant part is processed for various uses (Marsilio *et al*., 2001). The olive fruit fly (*Bactrocera oleae*) is one of the most important and major olive pests in the world. It became widespread in olive-growing regions of Iran in 2004 and caused economic damage to the olive orchards of these regions. This pest needs olive fruit, both wild and cultivated types, for laying eggs, nutrition and growing its larvae (Katsoyanos, 1992; Tzanakis, 1989).

B. oleae larvae infest olive fruits and cause premature fruit drop (Collier and Van Steenwyk, 2003). In addition, it causes bacterial and fungal contamination of fruit (Zalom *et al.*, 2009). The symbiosis between the olive fruit fly and Candidatus *Erwinia dacicola* has been demonstrated as essential for the fly's larval development and adult physiology (Sacchetti *et al*., 2019). In addition to fruit drop, olive fly larvae degrade the quality and quantity of their oil (Pucci *et al*., 1982), due to the entry of pathogenic fungi emitting from the larvae that increase the acidity of the oil (Kapatos, 1989). This study aimed to determine the most suitable time to harvest olive fruits (*O. euopeae*) infected with olive fly larvae in Tarom-Sofla city, Qazvin province, based on the quality and quantity of oil.

2. Materials and methods

In the summer of 2016, a factorial experiment was conducted in the form of a randomized complete block design with 6 treatments and 3 replications on olive fruits (*O. euopeae* cv. Zard). The experiment factors

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included the type of fruit at two levels (a1: infected fruits, a2: healthy fruits) and the harvest time at six levels (b1: the time of olive fly larvae exit, b: one week after the fly larvae exit, b3: two weeks after the olive fly larvae exit, b4: three weeks after the olive fly larvae exit, b5: four weeks after the olive fly larvae exit and b6: five weeks after the fly larvae exit). Each repetition included 1500 grams of fruit.

2.1. Oil percentage

To measure the percentage of oil, after separating the fruit pulp from the stone, the fruit pulp was placed in an oven at 80 °C for 48 hours to dry completely. Five grams of dry matter were weighed in cellulose thimbles and inserted into the Soxhlet apparatus. The oil extraction was carried out at 70 ^{0}C using 125 mL of petroleum ether as solvent. The total extraction process was completed within one hour. The oil was separated from the solvent by rotary evaporator apparatus and the oil percentage per dry matter was determined (Avidan *et al*., 1997).

2.2. Qualitative properties of oil

Firstly, the olive fruits along with the stones, were crushed by a mill and slowly mixed for 30 minutes at room temperature to obtain a uniform paste. Then the prepared paste was centrifuged at a speed of 5000 rpm for 15 minutes to separate the oil (Khaleghi *et al*., 2015). The extracted oil was stored in a refrigerator at 0 degrees Celsius for quality tests.

2.2.1. UV absorption

To determine the quenching coefficient, 250 mg of the extracted oil was diluted with 25 ml of cyclohexane (spectrophotometry degree). Then it was homogenized using a vortex for 30 seconds and absorbance was recorded at 232 and 270 nm using a spectrophotometer (model 2100-UV, made by Unico, USA). Cyclohexane was considered as blank (Commission Regulation., 1991).

2.2.2. Free acidity

Free acidity (% oleic acid per 100 g oil) was determined according to the European Community EEC Reg. 2568/91 (Commission Regulation, 1991). 50 ml of ethanol: chloroform solvent (50:50) was added to 10g of the oil. Then in the presence of phenolphthalein reagent, it was titrated with 0.1 N potassium hydroxide ethanol solution. Finally, free acidity in terms of oleic acid was expressed in 100 g of olive oil (Commission Regulation, 1991).

$$\text{Free acidity}(\%) = \frac{\text{ml of titrant} \times \text{normality of titrant} \times \text{molecular weight of oleic acid}}{\text{sample weight} \times 10} \quad (1)$$

2.2.3. Peroxide value

30 ml of solvent (mixture of acetic acid and chloroform) was added to 5 g of oil. Then about 0.5 ml of potassium iodide was added and the mixture was allowed to remain for one minute. Then 30 ml of distilled water and a few drops of the starch solution were added to the solution and titrated with 0.02 N thiosulfate solution. The value of peroxide was obtained through the following equation. To determine the amount of product due to oxidation, the peroxide number was determined in milliequivalents of oxygen per kilogram of oil (Horwts, 1975).

$$\text{Peroxide value}(\text{meq } O2 \text{ /kg}) = \frac{\text{ml of titrant} \times \text{normality of titrant} \times 1000}{\text{ml of sample}} \quad (2)$$

2.3. Statistics

The obtained data were analyzed using MSTATC statistical software and Duncan's multi-domain method was used for the comparison of the means.

3. Results and discussion

Variance analysis of studied traits (Table 1) showed that the effect of factor A (fruit type) was significant on traits K232 (extinction coefficient at 232 nm), K270 (extinction coefficient at 270 nm), acidity and peroxide value. The analysis of variance for the fruit harvesting time factor also showed that the effect of this parameter was significant on K320, oil percent and acidity. The interaction effect of fruit type and harvest time was significant only on acidity ($P \leq 0.05$) (Table 1).

3.1. Oil percentage

The results showed that only the simple effects were significant on oil content and the interaction effect was insignificant. The oil percentage in dry flesh of healthy fruits (75.61%) was statistically ($P \leq 0.01$) higher than infected fruits (59.65%). As can be seen in Fig. 1, the amount of oil in fruits infected with larvae was lower. Also, more oil was observed at time 4 (three weeks after the larvae exited the fruit).

3.2. UV absorption

The results showed that the effect of fruit type on K270 and K232 was significant and these parameters were lower in healthy fruits than in infected fruits (Fig. 2). The effect of harvest time was significant only on K232 and was not significant on K270. The interaction effect of fruit type and harvest time was insignificant (P≤0.05). The trend of changes in extinction coefficient in K270 and K232 in infected and healthy fruits as well as in different harvest dates, are shown in Figs. 2 and 3, respectively. As can be seen, these parameters are higher in infected fruits and increase overtime.

Table 1. Analysis of variance of qualitative and quantitative traits of oil in olive fruits.

Source of Variations	df	Oil Content (%)	K232	K270	Oil acidity	Peroxide
Replication	2	7.256	0.009	0.002	0.109	2.965
Fruit type(A)	1	39.957**	0.195*	0.004*	0.342**	40.768**
Harvest time (B)	5	3.477*	0.191*	0.001 ns	0.368**	1.0 ns
A*B	5	1.423ns	0.08 ns	0.001ns	0.126*	2.276 ns
Error	22	1.321	0.052	0.001	0.034	1.829
C.V. (%)		1.89	12.3	19.26	24.29	23.06

ns, * and ** are respectively non-significant, significant, at the probability of 5% and 1%.

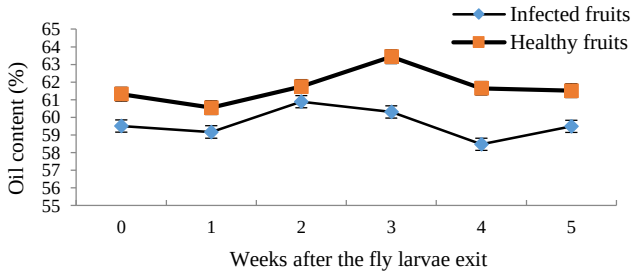

Figure 1. Changes of oil percentage in the dry matter at different harvest dates in two pest-infected and healthy samples.

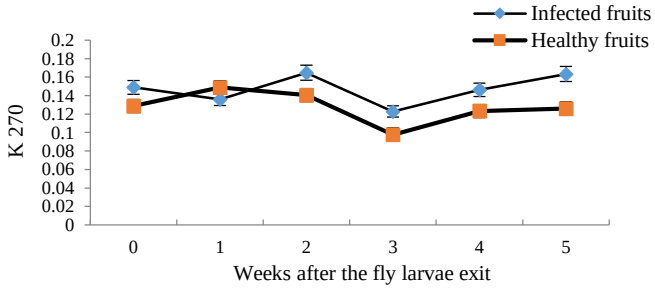

Figure 2. Changes of oil K270 at different harvest dates in two pest-infected and healthy samples.

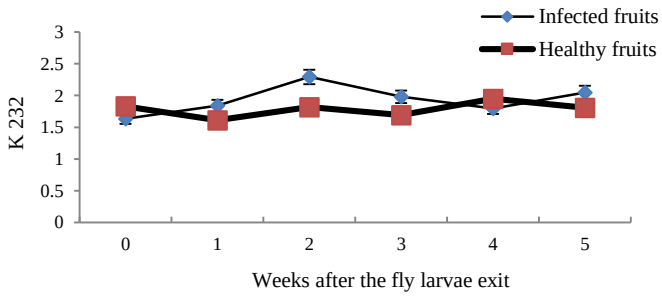

Figure 3. Changes of oil K232 at different harvest dates in two pest-infected and healthy samples.

3.3. Oil acidity

In terms of oil acidity, the simple effect of fruit type, harvesting time and their interaction was significant. A comparison of the mean interaction effect showed that healthy fruits had less acidity than infected fruits and therefore, their quality was better. The oil acidity also increased with the delay of harvesting and as a result, the quality of the fruit decreased (Fig. 4).

3.4. Oil peroxide

Regarding the peroxide parameter, the results showed that only the simple effect of fruit type was significant ($P \leq 0.01$) and the simple effect of harvest time and the interaction effect of fruit type and harvest time were insignificant ($P \leq 0.05$). The peroxide number in healthy fruits (4.799 milliequivalents of gram oxygen per kilogram) was lower than that of infected fruits (6.928 milliequivalents of gram oxygen per kilogram) and this difference was statistically significant (Fig. 5).

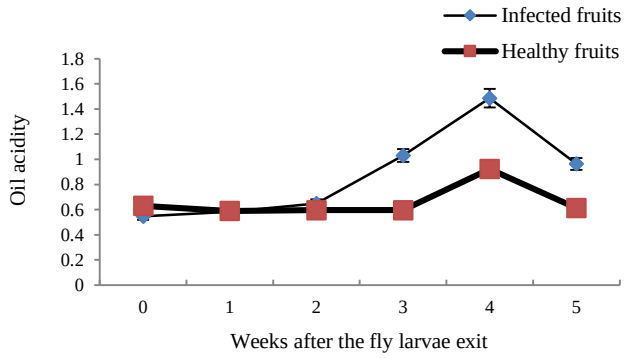

Figure 4. Changes of oil acidity at different harvest dates in two pest-infected and healthy samples.

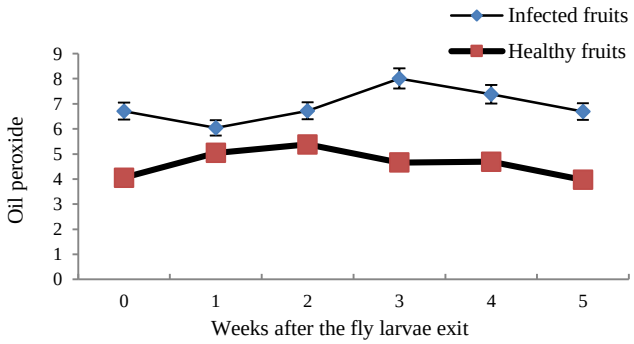

Figure 5. Changes of oil acidity peroxide in two pest-infected and healthy samples.

Topuz and Durmusoglu (2008) investigated the effect of early harvesting of olive fruits infected with the olive fly on the quality and quantity of obtained oil. The lowest population of adult insects was related to July and August and the highest population of adult insects was related to October. The hole number of the fruit increased significantly after the middle of November. Therefore, earlier harvesting will prevent a large infestation of the pest. Bento et al. (1999) showed that early harvesting in the second half of September has a significant reduction in the amount of olive fruit fly damage. Gomez-Caravaca et al. (2008) investigated the effect of olive fruit flies on olive oil properties such as acidity, peroxide, fatty acid composition, moisture content, phenolic compounds and stability against oxidation, etc. The results showed that there was a direct relationship between the degree of pest infestation with acidity and peroxide, but there was an inverse relationship between the amounts of phenolic compounds and stability against oxidation with the

degree of pest infestation. Sousa et al. (2007) researched the effect of olive fruit fly and olive anthracnose on the quality of olive oil. In their research, three groups of olives infected with olive fruit fly, olives infected with anthracnose and healthy olives were investigated based on the characteristics of the oil extracted from them. It was found that the amount of acidity in olives infected with anthracnose was twice that of healthy olives and had the worst oil quality. The amount of acidity in olives infected with olive fruit flies was reported to be 50% higher than in healthy olives. There was no difference between the composition of fatty acids in olives infected with olive fruit flies and healthy olives, but the amount of unsaturated fatty acids was lower than in healthy olives. Mustafa and Al-Zaghal (1987) investigated the effect of olive fruit fly damage on the acidity of oil obtained from three local cultivars, Shami, Kheli, and Rasel, in Jordan and showed that in all three cultivars, the acidity of olive oil was higher than healthy olives. Pucci et al. (1979) showed that there is a direct relationship between the acidity of oil and the level of contamination, and on the other hand, the percentage of oil quantity decreases with the increase of pest infestation. Neuenschwander and Michelakis (1978) investigated the effect of harvesting time and storage life in the fruits of two olive cultivars (Tsounati and Koroneiki) infected with olive fruit fly on the quantity and quality of olive oil extracted in Greece. Olive fruit fly caused a 3% reduction in the oil content in the Tsounati variety and 20% in the Koroneiki variety. Pest infestation by olive fruit fly caused an increase in acidity and a decrease in the quality of olive oil in both cultivars. The amount of acidity is directly proportional to the number of larvae holes.

4. Conclusion

The oil percentage and quality of infected fruits were lower (higher absorption in the ultraviolet spectrum, higher acidity and peroxide) than healthy fruits. Due to the negative effect of olive fly larvae on the quantity and quality of fruit oil, it is recommended to harvest the fruits two weeks after the larva leaves the fruit when the amount of oil is high and the quality indicators of the fruit are not affected. Therefore, in the Tarom-Sofla area, if there is the damage of olive fly pest, it is suggested to harvest the fruits within two weeks after observing the opening of the larval exit hole, which coincides with December 7, so as not to lead to a significant decrease in the quality of the extracted oil.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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