Exogenous Application Effects of Ethephon on Olive Oil Quality

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Abstract: This experiment was carried out to evaluate the effects of five different ethephon concentrations (0, 1000, 2000, 3000 and 4000ppm) on olive oil sensory attributes (quality) after one and two weeks of fruits harvesting. Olive fruits Harvested two weeks after 4000 ppm of ethephon application significantly increased free fatty acid and peroxide value of olive oil contents and decreased sensory attributes (fruitiness bitterness, and pungency), indicating a high impact on reducing oil quality. While olive fruits harvested one week after 4000 ppm ethephon application did not affect the olive oil quality traits. In conclusion, 4000ppm ethephon application led to efficient olive fruits harvest without affecting the olive oil quality, when olive fruits harvested after one week.

Keywords: Ethephon, olive, olive oil, quality.

1. INTRODUCTION

Olives (*Olea europaea* L.) is one of the important fruit trees in the Mediterranean region (Wallander and Albert, 2000). It is a drought tolerant crop that adapted to arid and semi-arid environment, which can be grown in low rainfall areas, where supplementary irrigation can be implemented (International Centre for Agricultural Research in the Dry Areas (ICARDA), 2016). One of the difficulties facing olive cultivation in Jordan is the high costs of fruit harvest, which constitutes 50-70% of olive oil production costs) (Ahmad and Ayoub, 2014). Traditional olive harvest by hand needs a large number of workers, but at the same time it ensures a high oil quality if the harvesting process is done directly by hands (Ahmad and Ayoub, 2014).

Jordanians used to start olive fruits harvesting season throughout the first half of November. But in 2021 harvesting season and in Mid-October, it was observed a complete black coloring of the olive fruits, in addition to fruits falling on the ground, which were due to heat waves that olive trees had been subjected to throughout 2021 growing season. According to Ministry of Agriculture (2021), Jordan, was subjected to frequent long heat waves, which has left a clear impact on olive trees in terms of production, flowering stages, fruit formation and maturity.

Ministry of Agriculture (MOA) pointed out that 2021 olive season is one of the abundantly fruitful seasons, and because of long heat waves and scarcity of rains, there was a clear impact on olive's flowers formation, which was reflected on olive yield.

Fruit mass to pedicel's strength is relatively small in olive fruits as compared with other trees with large fruits. Therefore, olive fruits require much more force to remove the fruit from the stalk when mechanical harvesting is

applied (Ben-Tal and Wonder, 1994). Ethephon can facilitate harvesting by forming an abscission layer between olive fruit and pedicle that makes olive fruits picking easier. However, farmers in Jordan are used to harvest olive fruits by hand.

Ethephon (2-chloroethyelphosphonic acid) is a synthetic plant hormone that releases ethelyne when sprayed on the plant and penetrates the plant tissue (Royer et al., 2006), and in consequence promotes pedicel's loosening that makes fruits removal easier during harvesting (Martin et al., 1981; Denney and Martin, 1994; Metzidakis, 1999). In another study, ethephon increased olive fruits yield by 20% when mechanical harvesting is used (Ravetti and McClelland, 2008). Using ethephon as fruits loosening agent increased harvesting efficiency but it did not have and significant effect on leaves drop, however it showed a little effect on olive oil quality parameters such as oil acidity and oil peroxide value. (Lang and Martin, 1989).

There are limited studies on the effects of exogenous application of ethephon and plant growth regulators on oil quality attributes of olives. Generally, high olive oil quality is highly dependent on the olive cultivar, the environmental conditions during the growing season, cultural practices including water availability, temperature and oil extraction method (Nergiz, 2000; Ranalli et al., 2001; Kalua et al., 2005; Baccouri et al., 2008; Dag et al., 2011). Traditional harvest of olive fruits by hand is the common harvest method in Jordan. Therefore, the overall objective of this experiment is to enhance olive fruits harvest using Ethylene Exogenous application on “Nabali Baladi” cultivar grown in Al-Karak area in Jordan. With a specific objective, to study the possible effect of exogenous ethephon application on olive oil quality.

2. MATERIALS AND METHODS

The experiment was carried out on the olive farm, grown at Alsmakiyah village (31° 20 '17.1″N 35°49'56.0 ″E), Northeast of the Karak district, Jordan. The current experiment was composed of the application of five different ethephon levels during 2021 olive harvesting season to investigate the effects of two harvesting time (one week and two weeks) after ethephon application on olive oil quality. The experiment is applied to 10 years old olive trees of Nabali Baladi CV. Thus, ethephon treatments was sprayed in October, 9, 2021. And The first harvest time was one week after ethephon application, on October 15, 2021. While the second harvest time, two weeks after ethephon application, it was on October, 21, 2021.

The experiment was designed using Randomized Completely Block Design (RCBD) with split–split plot arrangement of three replications. The experimental unit was represented by five trees. Two harvest dates (one week and two weeks after ethephon application) were used as main plots, and five levels of ethephon concentrations (0, 1000, 2000, 3000, and 4000 ppm) were used as sub-sub-plots. Ethephon concentrations was prepared according to the following equation mentioned by Uceda and Frias (1975). The dilution formula used the concentration of the starting and ending solution in ppm, using the following formula:

$C1 \times V1 = C2 \times V2$.

Where **C1** is the concentration of the starting solution, $\mathbf{V_1}$ is the volume of the starting solution. **C2** is the concentration of the final solution and $\mathbf{V_2}$ is the volume of the ending solution. Tween 20 (0.05%) was added to ethephon solution as a surfactant. A composite sample of 2.5 to 3 Kg of olive fruits were taken at harvest time from each experimental unit.

Olive oil percentage (Fresh weight basis) was determined by pressing the olive fruits in special olive laboratory machine. According the following equation by (Yousfi et al. 2006) $Oil\%\ on\ fresh\ weight\ basis = \frac{Oil\ weight}{Fresh\ weight} \times 100\%$

Free fatty acid (FFA) was estimated and performed to evaluate the effects of ethephon treatments and the time of olive fruits harvest on oil quality. The olive oil samples (10 g) were weighted into 250 ml flask (conical). And then it was dissolved in 50 ml of the solvent mixture consists from equal volumes (1:1) of 95% ethanol and diethyl ether. The mixture was titrated with 0.1 N potassium hydroxide (KOH) solution and shaked with hand. The FFA was determined when the pink color persists of phenolphthalein indicator for at least 10 sec **(**EC 2568/1991**).** So (FFA) was expressed as (% of oleic acid).

FFA %=(v-b) X N X28. 2/W

V = volume of the titrant.

b = blank volume.

N = normality of NaOH, usually 0.1

W = weight of sample.

Peroxide value was assessed to evaluate the effects of ethephon treatments and the time of harvest after ethephon application on olive oil quality. Peroxide value was measured under diffused daylight. Olive oil sample of 1-2 g was put in 250 ml flask provided with ground neck and stopper (EC 2568/1991). The sample was dissolved in a mixture of 10 ml of chloroform, 15 ml acetic acid and 1 ml of 0.5 N potassium iodide with rapid stirring and shaking for one minute. The mixture was then left for 5 minutes at room temperature (20°C) in the dark. Afterwards, 75 ml of distilled water added, and the mixture was titrated with the sodium Thio-sulphate solution. The flask was continuously shaked and then 10 g starch L^{-1} aqueous used as an indicator. It was determined when the titrated solution turned from a purplish to a yellowish or colorless. The peroxide value (P V) expressed in milli equivalents of active oxygen per kilogram of olive oil (meq O_2 kg^{-1}) and calculated by using the following equation:

$$P\ V = \frac{V \times T}{W} \times 100\%$$

Where:

V = ml of solution of titrant (sodium Thiosulphate).

T = Normality of sodium thiosulphate solution.

W = weight of sample of olive oil in gram.

Oil sensory attributes were determined according to profile sheets for virgin olive oil test. Specialist taster (accredited team) following a standard procedure did the sensory tests. The following parameters were recorded fruitiness, bitterness and pungency. Each attribute was rated using a scale from 1 to 10, where 1 represents the value for the poorest and 10 the best possible oil quality for the sample (Favati et al., 2013).

Analyses of variance (ANOVA) was used to determine the significant differences between means for the main factors (Harvest Time and Ethephon Concentrations) in addition to their interactive effects means. Means were compared using Least Significant Difference (LSD) test at 0.01% level.

3. RESULTS

Biochemical olive oil parameters were assessed one week and two weeks after ethophon application including: fresh fruits oil content (%) and both free fatty acid and peroxide contents. In addition, some olive oil sensory attributes were assessed including bitterness, pungency and fruitiness, the experiment was implemented using “Nabali Baladi” cultivar. Free fatty acid (FFA) and peroxide contents were significantly increased by delaying the Harvest Time. FFA and peroxide contents were increased from 0.5 to 0.98% and from 8.82 to 10.28meq O_2 kg^{-1} after one week and two weeks of harvest of ethephon application, respectively (Table 1). With a similar trend, increasing ethephon application rate significantly increase FFA and peroxide contents. At which FFA increased from 0.45 at 0 ppm ethephon application rate to 0.91and 1.14% at 3000 and 4000 ethephon application rates respectively (Table 1), while ethephon application rates at 1000 and 2000 ppm were not significantly differed as compared to the control. The peroxide value was gradually increased with increasing ethephon application rate. It was increased from 9.20 meqO_2 kg^{-1} at 0 ppm ethephon application rate to 9.28, 9.34, 9.92and 10.11meqO_2 kg^{-1} at 1000, 2000, 3000 and 4000 ppm ethephon application rate, respectively (Table 1).

High rate of ethephon application decreased fruitiness, bitterness and pungency. Fruitiness was decreased at high ethephon concentration as compared with the control. fruitiness at 3000 and 4000 ppm ethephon application (values=2.83 and 2.64 respectively) (Table 1). It was significantly lower than the control treatment (value=3.25). The Bitterness value in the control treatment (0 ppm) was 2.86, which significantly higher as compared with Bitterness at 3000 and 4000 ethephon application rates (values=2.66 and 1.81, respectively). while the Bitterness value at 1000 and 2000 ethephon application rate treatments were (values =2.8 and 2.73, respectively). Pungency was reduced at

the highest ethephon application rate treatment (4000 ppm, value = 2.53) as compared with the control treatment (value=3.14) and other ethephon treatments, where values ranged from 3.09 to 3.05 ppm at 1000 to 3000 ppm ethephon application rate (Table 1).

Table (1): The main effects of harvest time and ethephon concentration and their interactive effect on free fatty acid, peroxide value, fruity, bitterness, and pungency:

Treatment	free fatty acid (% of oleic acid	peroxide value $meqO_2\,Kg^{-1}$	Fruity (0-10)	Bitterness (0-10)	Pungency (0-10)
Harvesting time (HT)					
One week	0.5^{b}	8.82^{b}	3.17^{a}	2.72^{a}	3.11^{a}
Two weeks	0.98^{a}	10.28^{a}	2.78^{b}	2.42^{b}	2.84^{b}
Ethephon concentration (ppm)					
Control (0)	0.45^{e}	9.20^{e}	3.25^{a}	2.86^{a}	3.14^{a}
1000	0.56^{d}	9.28^{d}	3.11^{b}	2.80^{b}	3.09^{b}
2000	0.65^{c}	9.34^{c}	3.07^{c}	2.73^{c}	3.07^{c}
3000	0.91^{b}	9.92^{b}	2.83^{d}	2.66^{d}	3.05^{d}
4000	1.14^{a}	10.11^{a}	2.64^{e}	1.81^{e}	2.53^{e}
Interactive effect **(HT*EC)**	**	**	**	**	**

** Significance at 0.01 probability level, Means followed with the same letter are not significantly different.

The interactive effects of harvest time and ethephon concentrations on some Nabali Baladi olive oil chemical parameters are shown in (Table 2). The highest FFA (0.98%) and peroxide (10.28 $meqO_2\,kg^{-1}$) contents were obtained two weeks after 4000 ppm ethephon application, which meaning that delaying olive oil harvesting two weeks after 4000 ppm ethephon application will produce “virgin olive oil” based on the standards of chemical quality criteria classification. However, harvesting olive fruits one week after 4000 ppm ethephon application gave oil FFA contents of 0.5% and peroxide contents of 8.82 $meqO_2\,kg^{-1}$ (Table 2), which could be classified as “extra virgin olive oil” based on the standards of chemical quality criteria classification.

Table (2): Interactive effects of date of harvest and ethephon concentration on chemical oil quality (free fatty acid and peroxide).

Parameters				
		Date of harvest		
Free fatty acid (%of oleic acid)	Ethephon concentration (ppm)	One week	Two weeks	Mean
	0	0.33^{e}	0.56^{e}	0.45^{e}
	1000	0.43^{d}	0.69^{d}	0.56^{d}
	2000	0.51^{c}	0.80^{c}	0.65^{c}
	3000	0.61^{b}	1.21^{b}	0.91^{b}
	4000	0.64^{a}	1.65^{a}	1.14^{a}
	Mean	0.5	0.98	

Peroxide $MeqO_2\ Kg^{-1}$	0	8.65^{e}	9.76^{e}	9.20^{e}
	1000	8.68^{d}	9.89^{d}	9.28^{d}
	2000	8.71^{c}	9.97^{c}	9.34^{c}
	3000	9.00^{b}	10.84^{b}	9.92^{b}
	4000	9.06^{a}	10.93^{a}	10.11^{a}
	Mean	8.82	10.28	

* LSD to compare two subplot means (averaged over all sub-subplot treatments) at the same levels of the whole plot. **LSD to compare two whole plot means (averaged over all sub-subplot treatments) at the same or different levels of the subplot.

The interactive effects of Harvest Time and Ethephon concentrations on sensory attributes include fruitiness, bitterness and pungency are shown in (Table 3). The fruitiness was decreased from 3.16 in the control to 2.17 in 400 0ppm ethephon treatment Two weeks after ethephon application (Table 3), and based on this indicator the accredited national olive oil taste team classified the olive oil as “virgin olive oil”. However, harvesting olive fruits one week after Ethephon application reduced fruitiness from 3.33 in the control treatment to 3.11 in 4000 ppm treatment (Table 3), where the oil produced can be classified as “extra virgin olive oil” according to the accredited national olive oil taste team evaluators.

The bitterness value was also decreased from 2.78 in the control to 1.49 Two weeks after Ethephon application in the control and in 4000ppm ethephon application rate treatments, respectively (Table 3). The national olive oil accredited team classified the Olive oil based on bitterness in this combined treatment (Two weeks Harvesting and 4000 ppm Ethephon) as “virgin Olive oil”. On the other hand, harvesting olive fruits one week after Ethephon application reduced bitterness from 2.94 in control treatment to 2.12 at 4000 ppm ethephon treatment (Table 3), which considered as “extra virgin oil” according to national olive oil accredited team.

The pungency value, when olive fruits were harvested one week after ethephon treatment decreased from 3.17 in control to 3.04 in 4000 ppm ethephon treatment (Table 3), which considered as “extra virgin Olive oil” according to the accredited national olive oil taste team. The pungency also reduced from 3.11in the control to 2.01 at 4000 ppm ethephon treatment two weeks after ethephon application, where Olive oil is considered as “virgin oil” according to national olive oil taste team standards.

In general, harvesting olive fruits two week after ethephon at 4000 ppm will accelerate olive fruits maturation and consequently led to dramatic reduction in sensory attributes of Olive oil and consequently will produced “virgin oil”.

Table (3): Interactive effects of date of harvest and ethephon concentration on sensory attributes (bitterness, pungency, fruitiness)

Bitterness (0-10)		Date of harvest		
	Ethephon concentration (ppm)	One week	2nd week	Mean
	0	2.94^{a}	2.78^{a}	2.86^{a}
	1000	2.90^{b}	2.69^{b}	2.80^{b}
	2000	2.82^{c}	2.63^{c}	2.73^{c}
	3000	2.81^{d}	2.51^{d}	2.66^{d}
	4000	2.12e	1.49^{e}	1.81^{e}
	Mean	2.72	2.42	

Pungency (0-10)				
	0	3.17[a]	3.11[a]	3.14[a]
	1000	3.12[b]	3.07[b]	3.09[b]
	2000	3.11[c]	3.02[c]	3.07[c]
	3000	3.09[d]	3.00[d]	3.05[d]
	4000	3.04[e]	2.01[e]	2.53[e]
	Mean	3.11	2.84	
Fruitiness (0-10)				
	0	3.33[a]	3.16[a]	3.25[a]
	1000	3.16[b]	3.06[b]	3.11[b]
	2000	3.13[c]	3.02[c]	3.07[c]
	3000	3.12[d]	2.53[d]	2.83[d]
	4000	3.11[e]	2.17[e]	2.64[e]
	Mean	3.17	2.78	

*LSD to compare two subplot means (averaged over all sub-subplot treatments) at the same levels of the whole plot.
**LSD to compare two whole plot means (averaged over all sub-subplot treatments) at the same or different levels of the subplot.

4. DISCUSSION

There is a scarcity of published reports on the effects of ethephon application rate on Olive fruits harvesting in Jordan. The olive quality related traits were recorded including fresh fruits oil content (%) and some olive oil biochemical parameters (oil free fatty acid and peroxide contents) in addition to some olive oil sensory attributes (bitterness, pungency and fruitiness). The experiment was implemented using “Nabali Baladi” cultivar.

In this experiment, the research questions were successfully answered. However, delaying olive harvest two weeks after 4000 ppm ethephon application reduced the oil quality (i.e. gave virgin olive oil), while early olive fruit harvest one week after 4000 ppm ethephon application was efficient in harvesting olive fruits with high quality olive oil (i.e. gave extra virgin olive oil).

Rain fall scarcity could affect fruit ripening stage and ultimately the quality of olive oil (Lavee et al., 1982, 1991; Barone et al., 1994; Inglese et al., 1996 and Barranco et al., 2000). Ethylene accumulation under stresses (high temperature and low rains) could enhance senescence hormones such as ethylene that accelerating olive fruits ripening and early fruits coloring. “Nabali Baladi” is a widely cultivated cultivar in Jordan. It is characterized by a high yield with good oil quality. This cultivar also displays a variation in ripening due to the variation in prevailing environmental conditions that ultimately influences the accumulation of phenolic compounds in olive oil.

Effect of different ethephon doses were tested on harvest efficiency, fruits’ abscission, free fatty acids and peroxide contents and some Olive oil sensory attributes. It is wiser to know the effective concentration of ethephon that facilitate harvesting and keep high olive oil quality by avoiding olive fruits over ripening and excessive leaves loss. From these perspectives, the current study showed that the best combination treatment is using 4000 ppm ethephon and harvesting olive fruits one week after ethephon application to ensure high harvest efficiency and a good olive oil quality.

Harvesting date and its relation with olive fruits and oil quality related parameters two-harvest dates after ethephon application (one week and two weeks) were tested. Delaying olive fruits harvest two weeks after 4000 ppm ethephon application led to accelerated olive fruits ripening and consequently led to a negative impact on olive oil quality. Olive fruit color changes are also reflected on fruit firmness and both chemical and sensory olive oil quality parameters

(Beltran, 2000). The fruit firmness in “Nabali Baladi” cultivar significantly decreased when olive fruits were collected two weeks after ethephon application as compared with fruits harvest one week after ethephon application.

Delaying olive fruits harvest two weeks after 4000 ppm ethephon application led to accelerated olive fruits ripening and consequently led to a negative impact on olive oil quality, table (3). Changes occurred during the ripening are reflected on the changes occurred in fruit firmness and both chemical and sensory olive oil quality parameters (Beltran, 2000). Exogenous application of ethephon one week and two weeks after ethephon application might lead to higher ethylene accumulation in olive fruits. After ethephon spraying, higher levels of endogenous ethylene accumulation is occurred at late stages of olive fruit ripening, 175- 190 days after full bloom and exogenous pre-harvest application of ethephon accelerates ripening of a non-climacteric olive fruit. Changes in chemical and biochemical contents of ripened olive fruits also occurred which are genotype, climate and prevailing growing conditions dependent (Gutierrez et al., 2000). Chemical and biochemical changes in the texture reduced flesh fruits firmness with the progress of fruit maturation (Nanaos et al., 1999). Olive pulp firmness decreases due to losing in olive cell wall (Jimenez et al. 2001).

In general, delaying harvest time of olive fruit until over ripening reduced the olive oil quality by increasing free fatty acid and peroxide contents above the upper limit of extra virgin oil according to international olive oil council. Ripening index (RI) and both free fatty acid and peroxide contents were significantly increased when olive fruits were harvested two weeks after 4000 ppm ethephon application. The RI was 4.96 when olive fruits harvested two weeks after 4000 ppm ethephon application, which is over the upper limit of the standard olive fruit ripening (4- 4.5). The highest free fatty acid values were 0.5 and 0.98 when olive fruits harvested one week and two weeks after ethephon application, where olive oil was classified as “virgin Olive oil” and “extra virgin Olive oil” according to the chemical quality standards, respectively. Increasing fruit ripening by delaying harvesting ultimately influences the phenolic composition of olive oil (Barone et al., 1994; Inglese et al., 1996 and Barranco et al., 2000). So, it is important to mention that olive fruits harvesting should not be delayed until the fruits are over ripened to avoid more accumulation of free fatty acid and peroxide that reduce Olive oil quality.

The peroxide content was increased by delaying harvest time two weeks after 4000 ppm ethephon application. The peroxide values were of 10.28 and 8.82 when olive fruits were harvested two weeks and one week after ethephon application. In this experiment, the olive oil harvested two weeks after 4000 ethephon application was classified as “virgin Olive oil” and that harvested one week after 4000 ppm ethephon application as “extra virgin Olive oil”. These results were in line with (Baccouri et al.2008) who reported a reduction in peroxide content due to lipoxygenase enzyme activity as olive fruits ripened.

The positive sensory attributes of olive oil (i.e. bitterness, and pungency and fruitiness) were recorded in this experiment for different treatment combinations. The sensory attributes are very important for the consumers and olive oil marketing. To obtain high positive fruitiness, harvesting time would play a major role which depends on environment, genotype and harvest and post-harvest process (Taylor and Burt, 2007). When olive fruits were harvested two weeks after 4000 ppm ethephon application, the fruitiness value decreased to 2.78. Yousfi et al. (2006) and Riachy et al. (2012) reported a reduction in phenolic compounds accumulation with the progress of maturity. In addition to fruitiness, the pungency and bitterness values play important role in the classification olives oil quality. The sensory attributes of olive oil must be measured because they affect the evaluation and classification of olive oil quality. With the same trend, bitterness and pungency values were also decreased to 2.42 and 2.84, respectively, when harvesting is delayed two weeks after 4000 ppm ethephon application. Rotondi et al. (2004) reported that Olive oil sensory attributes are influenced by the ripeness stage and genotype. Many authors (Angerosa et al. 2004, Rotondi et al. 2004, Servili et al. 2004, Tripoli et al. 2004, Kalua et al., 2007 and Delgado 2011) showed reductions in sensory attributes by delaying harvest time and increasing ripeness which leading to more bitterness and pungency in olive oil (Bendini et al., 2007).

Olive oil sensory attributes are very important in Olive oil classification and olive oil grading. Even if the chemical properties (free fatty acid and peroxide contents) of olive oil are very excellent and there is any defect in the sensory attributes, the olive oil quality will be reduced. The sensory and chemical attributes should be corresponded to produce “extra virgin oil”. According to the sensory evaluation of the accredited olive oil team at the National Center of Agricultural Research, the sensory and chemical are usually corresponded.

5. CONCLUSIONS

In this experiment, the highest harvesting efficiency was obtained when Olive fruits were harvested one week after 4000 ppm ethephon application without affecting the chemical quality related traits (i.e. free fatty acid and peroxide value) and olive oil sensory attributes (i.e. bitterness, pungency and fruitiness). Olive fruits harvested two weeks after 4000 ppm ethephon application reduced the olive oil quality (i.e. gave virgin olive oil), while early olive fruit harvest one week after 4000 ppm ethephon application was efficient in harvesting olive fruits with high quality Olive oil (i.e. gave extra virgin olive oil).

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