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Activity of Cellulase and Pectinase Enzymes and ABA Content of Koroneiki Olives as Affected by Spraying Chemical Loosening Agents

[1]E.S. Hegazi, [1]A.A. Hegazi, [2]M.M. Naguib and [2]H.A. El-Attar

[1]Department of Pomology, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Pomology Research, National Research Centre, Dokki, Giza, Egypt

Abstract: Koroneiki olive trees were sprayed with the following loosening agents: ethephon at 300, 600 and 1200 mg/l, monopotassium phosphate (MPK) at 2%, 3% and 4%, phosphoric acid at 75, 125 and 175mM and thiourea at 500, 1000 and 1500 ppm at two times (7 and 15 days before harvesting). Then, trees were harvested with a specific hand vibrator to evaluate the efficiency of these agents in increasing fruit abscission. The obtained results of this study indicated that used loosening agents could facilitate mechanical harvesting and saving costs demanding the minimal trees damage with a fine oil quality. Generally, ethephon spraying was the most effective agent for raising cellulase activity in the three studied seasons. Since 1200, 600 and 300 mg/l of ethephon scored higher cellulase activity, followed by phosphoric acid at 175mM, while the lowest enzyme activity was recorded with the control. Similarly, control treatment scored the lowest values of pectinase activity, while phosphoric acid at 175mM resulted in the highest value followed by spraying MPK at 4% and ethephon at 1200 mg/l. Considering pedicel's ABA content, ethephon sprays at 1200 and 600 mg/l followed by phosphoric acid at 175 mM, were more effective treatments that enhanced ABA content, while the control treatment scored the minimum content of ABA in fruit's pedicels. Moreover, applying the loosening agents 15 days prior harvesting was more effective than those applied 7 days before harvesting.

Key words: Olive · Koroneiki · Loosening agents · Mechanical harvesting · Ethephon · MPK · Phosphoric acid · Thiourea

INTRODUCTION

Olive (*Olea europaea* L.) is one of the oldest cultivated fruits since its fossilized leaves dating to around 37,000 years ago. The world production of olive was 16,555,375 tons [1]. In Egypt, olive cultivation was increased considerably during the last two decades due to the great efforts paid for expanding olive cultivated areas with new cultivars in reclaimed soils. The Egyptian olive production is about 465,000 tons, resulted from acreage 132,000 feddan (one feddan =0.42ha), most of which are processed mainly as table olive and the rest is extracted for olive oil. Unfortunately, it's a fact that the quality of the oil decreases with the increase of mechanization and electric tools because the more gently the olives are treated the better the resulting oil. In spite of this importance, applying mechanical harvesting of olive is quite limited middle east. Olives and their oil nowadays sustain an industry producing billions of dollars annually. It is known for olive's producers that 50–60% of total labor requirement is used for harvesting operations [2], the different harvesting methods are important to understand as harvesting costs can make a big difference in the production cost of the olive oil. In this respect, using loosening agents is spreading among olive growers. The present study deals with the effect of different loosening agents on "Koroneiki" olives specifically with the mechanical harvesting protocol. Loosening agents might be considered as a must since they are effective and highly successful approach for efficient mechanical harvesting. Hegazi *et al.* [3] proved that ethephon applied 2 weeks before harvest, with two olive cultivars showed that, the high concentrations of ethephon (1000 and 1250 mg/l) caused 100% fruit abscission. Moreover, when continuously stem-fed with 75 mM of phosphoric acid, "Manzanillo" olive explants

Corresponding Author: H.A. El-Attar, Department of Pomology Research, National Research Centre, Dokki, Giza, Egypt.

showed significant leaf abscission after 48 hours [4]. As working on "Mission" olives [5], spraying ethephon and thiourea as loosening agents 2 weeks prior to harvest aided in fruit removal and increased the number of fruits harvested by hand vibration. In addition, spraying 3% monopotassium phosphate (MKP) 15 days before mechanical harvest reduced the high fruit retention force (FRF) of the immature fruits and facilitated fruit loosening in "Arbequina" and "Picual" olives. The incorporation of ethephon at 0.05% caused greater FRF reduction and improved mechanical harvest efficiency [6, 7]. In this respect, when two Tabasco (*Capsicum frutescens* L.) lines treated with (1000 $\mu l L^{-1}$) of ethephon, fruit detachment force (FDF) at the fruit- receptacle abscission zone reduction occurred four days earlier when ethephon was applied compared to the control fruits of both lines [8]. Toscano and Casacchia [9] subjected "Leccino" olive to foliar application of 3% monopotassium phosphate solution + ethephon and noticed that the application improved oil quality.

MATERIALS AND METHODS

This work was carried out in a private orchard located at Cairo – Alexandria, desert road, Giza, Egypt during three successive seasons 2010, 2011 and 2012. The present study aimed to facilitate mechanical harvesting of fifteen years old "Koroneiki" olive (*Olea europaea* L.) trees, planted at 5 x 3 m apart, uniform in growth and received the common horticultural practices as scheduled in the orchard program. Trees were sprayed with the chemical loosening agents: ethephon (2- chloroethylphosphonic acid) at the concentrations of 300, 600 and 1200 ml/l, MPK (monopotassium phosphate KH_2PO_4) at 2%, 3% and 4%, phosphoric acid (H_3PO_4) at 75, 125 and 175 mM and thiourea (1,3-di 3-methoxybenzyl) at 500, 1000 and 1500 ppm in two times for each concentration (7 and 15 days before harvesting) then harvested by hand vibrator (Zanon® Oliver electro telescopic Al 200/O) by pass it over the treated branches either with the different loosening agents or with the untreated (Control). Fruit pedicels specimens were collected and then cellulase activity was determined according to the method of Durbin and Lewis [10]. Also, the pectinase activity was determined in a similar fashion to cellulase activity. However, the viscometric assay was performed with 1% pectin solution [11]. Abscisic acid (ABA) content was determined by GLC in concentrated aqueous solution.

Statistical Analysis: The experimental design was randomized completely block design (RCBD) with three replicates and with one tree for each replicate of the studied cultivar for statistical analysis. The obtained data in three seasons were analyzed using MSTAT-C program package for analysis of variance [12]. Means were differentiated using Duncan multiple range test at significance level of 0.05 [13].

RESULTS AND DISCUSSION

Cellulase Activity: Data presented in Table 1 claimed that, spraying ethephon at 1200 mg/l in both dates (7 and 15 days before harvesting) had scored the highest significant cellulase activity in the three studied seasons (21.95, 18.96 and 21.54 Δn/Hr, respectively as means). These results followed by ethephon at 600 mg/l then 300 mg/l that reached high rates of cellulase activity, respectively in the three studied seasons. Eo and Lee [14] noticed that ethylene has a strong role in enhancing abscission. On the other hand, the control sprays in the two dates had scored 8.61, 4.75 and 7.43Δn/Hr as means in the 1st, 2nd and 3rd seasons, respectively. These results are in harmony with those obtained by Wu *et al.* [15], who mentioned that cellulase activity was increased over time and correlated strongly with grapes berry abscission.

Considering the application date, applying the loosening agents at 15 days before harvesting was more effective than that at the other date (7 days before harvesting) in increasing the cellulase activity (16.54, 12.37 and 14.98 Δn/Hr, respectively) in the three studied seasons. Working on orange, Oliva *et al.* [16] found that a relationship between treating fruit abscission zone with jasmonic acid (as a ripening promoter) and cellulase activity increment. Moreover, an increase indicated in the activity of cellulase with ethylene treated explants [17]. This increase was accompanied by a substantial decrease in the force required to separate the petiole from the stem and an increased accumulation of cellulase transcript in the abscission zone. It is concluded that ethylene leads to higher cellulase expression and increased activities of ethylene biosynthesis enzymes in the abscission zone. This may explain ethephon agents' potency in enhancing fruit harvesting due to raising cellulase activity and occurring abscission zone (the weakest FRF).

Pectinase Activity: It is obvious from the data illustrated in Table 2, in the three studied seasons that spraying 175 mM of phosphoric acid had maintained the highest

Table 1: Effect of different loosening agents on cellulase activity (Δn/Hr) of "Koroneiki" olive (2010, 2011 and 2012 seasons)

		2010 season			2011 season			2012 season		
Treatments		7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean
Ethephon (mg/l)	300	20.14^{ab}	21.81^{a}	20.98^{a}	11.80^{d-f}	16.12^{a-c}	13.96^{c}	14.97^{cd}	17.15^{bc}	16.06^{bc}
	600	20.71^{a}	22.67^{a}	21.69^{a}	14.45^{b-d}	18.77^{a}	16.61^{ab}	18.67^{ab}	19.27^{ab}	18.97^{ab}
	1200	21.70^{a}	22.20^{a}	21.95^{a}	18.37^{a}	19.55^{a}	18.96^{a}	20.79^{a}	22.30^{a}	21.54^{a}
MPK (%)	2	9.79^{ef}	12.62^{ef}	11.20^{de}	5.43^{ij}	7.43^{h-j}	6.43^{gh}	9.83^{fg}	11.56^{d-g}	10.70^{ef}
	3	12.63^{c-e}	14.41^{c-e}	13.52^{cd}	7.49^{h-j}	9.49^{e-h}	8.49^{e-g}	13.78^{c-e}	15.38^{b-d}	14.58^{cd}
	4	13.64^{cd}	17.14^{bc}	15.39^{bc}	11.22^{d-g}	12.98^{c-e}	12.10^{cd}	9.44^{fg}	16.14^{bc}	12.79^{de}
Thiourea (ppm)	500	11.93^{d-f}	13.32^{d-f}	12.63^{cd}	8.80^{f-i}	9.69^{e-h}	9.25^{ef}	8.03^{g}	9.92^{fg}	8.97^{fg}
	1000	13.84^{c-e}	15.46^{cd}	14.65^{bc}	8.80^{f-i}	11.16^{d-g}	9.98^{ef}	13.84^{c-e}	13.66^{c-f}	13.75^{cd}
	1500	14.52^{cd}	16.47^{bc}	15.50^{bc}	11.94^{d-f}	14.69^{bc}	13.31^{cd}	14.52^{cd}	14.31^{cd}	14.42^{cd}
H_3PO_4 (mM)	75	12.36^{c-e}	14.08^{c-e}	13.22^{cd}	8.41^{g-i}	8.22^{g-i}	8.31^{fg}	10.01^{e-g}	13.25^{c-f}	11.63^{de}
	125	14.46^{cd}	16.51^{bc}	15.49^{bc}	11.16^{d-g}	11.06^{d-g}	11.11^{de}	12.82^{d-f}	16.06^{bc}	14.44^{cd}
	175	15.37^{cd}	19.60^{ab}	17.49^{b}	11.90^{d-f}	16.77^{ab}	14.33^{bc}	14.46^{cd}	18.42^{ab}	16.44^{bc}
Control		8.54^{f}	8.69^{f}	8.61^{e}	4.65^{j}	4.87^{j}	4.76^{h}	7.56^{g}	7.30^{g}	7.43^{g}
Mean		14.59^{b}	16.54^{a}	--	10.34^{b}	12.37^{a}	--	12.98^{b}	14.98^{a}	--

Means followed by the same letter (s) in each column are not significantly different at 0.05 level of probability

Table 2: Effect of different loosening agents on pectinase activity (Δn/Hr) of "Koroneiki" olive (2010, 2011 and 2012 seasons)

		2010 season			2011 season			2012 season		
Treatments		7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean
Ethephon (mg/l)	300	7.10^{cd}	6.47^{de}	6.78^{c}	9.02^{ef}	11.85^{c-e}	10.44^{cd}	8.95^{cd}	10.86^{bc}	9.90^{cd}
	600	8.79^{b-d}	8.21^{b-d}	8.50^{bc}	12.41^{c-e}	15.25^{b-d}	13.83^{b}	12.13^{bc}	12.64^{a-c}	12.38^{bc}
	1200	11.30^{a-c}	11.09^{a-c}	11.20^{a}	14.57^{b-d}	14.98^{a-c}	14.77^{b}	14.03^{ab}	17.21^{a}	15.62^{ab}
MPK (%)	2	9.48^{b-d}	8.00^{b-d}	8.74b^{c}	13.10^{b-d}	13.95^{b-d}	13.53^{bc}	9.85^{cd}	11.55^{bc}	10.70^{c}
	3	10.88^{a-c}	8.12^{b-d}	9.50^{ab}	13.26^{b-d}	14.84^{b-d}	14.05^{b}	14.73^{ab}	14.87^{ab}	14.80^{ab}
	4	9.93^{a-d}	11.56^{ab}	10.75^{ab}	14.57^{b-d}	16.34^{ab}	15.45^{ab}	15.29^{ab}	15.49^{ab}	15.39^{ab}
Thiourea (ppm)	500	5.95^{de}	6.06^{de}	6.01^{c}	3.13^{g}	4.75^{fg}	3.94^{e}	5.62^{de}	5.95^{de}	5.79^{e}
	1000	6.18^{de}	10.55^{a-c}	8.36^{bc}	5.94f^{g}	7.12^{fg}	6.53^{e}	7.87^{d}	9.17^{cd}	8.52d^{e}
	1500	6.23^{de}	11.05^{a-c}	8.64^{bc}	8.78^{ef}	10.84^{de}	9.81^{d}	9.93^{cd}	11.03^{bc}	10.48^{cd}
H_3PO_4 (mM)	75	7.21^{cd}	12.06^{ab}	9.64^{ab}	12.15^{c-e}	13.24^{b-d}	12.70^{b-d}	11.67^{bc}	14.36^{ab}	13.02^{a-c}
	125	7.64^{cd}	12.27^{a}	9.96^{ab}	14.42^{b-d}	15.46^{a-c}	14.94^{b}	14.10^{ab}	16.81^{a}	15.46^{ab}
	175	10.20^{a-c}	13.90^{a}	12.05^{a}	17.32^{ab}	19.31^{a}	18.32^{a}	15.40^{ab}	16.97^{a}	16.19^{a}
Control		2.71^{e}	2.62^{e}	2.66^{d}	3.63^{g}	4.16^{g}	3.90^{e}	2.48^{e}	2.40^{e}	2.44^{f}
Mean		7.97^{b}	9.38^{a}	--	10.95^{b}	12.47^{a}	--	10.93^{b}	12.26^{a}	--

Means followed by the same letter (s) in each column are not significantly different at 0.05 level of probability

significant pectinase activity rates (12.04, 18.31 and 16.18 Δn/Hr, respectively), followed by ethephon at 1200 mg/l and MPK at 4% in the three studied seasons. On the contrary, spraying water (control) in both dates had showed the lowest significant pectinase activity rates (2.66, 3.90 and 2.44 Δn/Hr). On the other hand, applying the loosening agents 15 days before harvesting date had a better effect than applying them 7 days before harvesting in enhancing the pectinase activity (9.38, 12.47 and 12.26 Δn/Hr, respectively) for the three studied seasons.

ABA Content: It is evident from Table 3, that spraying 1200 mg/l of ethephon, had the best result in increasing ABA content in fruit pedicels significantly to (1.84, 2.30 and 2.39 $\mu g g^{-1}$, successively) in the three studied seasons. Contrarily, spraying water (control) on trees at both dates (7 and 15 days before harvesting) had decreased the fruits pedicels' content of ABA significantly to the minimum values (0.28 and 0.82 and 0.83$\mu g g^{-1}$, successively) in the three studied seasons. Moreover, data revealed that with respect to ABA content, both ethephon at 600 mg/l and phosphoric acid at 175 mM ranked second and third in increasing fruits ABA content in all studied seasons. Zhang and Zhang [18] showed that if ABA ratio is less than 20 $ng g^{-1}$ fresh weight, the abscission zone is not developed to the grade 3 and the berry falling is effectively stopped.

Table 3: Effect of different treatments of loosening agents on ABA content (µgg^{-1}) of "Koroneiki" olive (2010, 2011 and 2012 seasons)

		2010 season			2011 season			2012 season		
Treatments		7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean	7 days before harvesting	15 days before harvesting	Mean
Ethephon (mg/l)	300	1.05^{de}	1.28^{cd}	1.16^{de}	2.00^{ab}	2.20^{a}	2.10^{a}	1.67^{cd}	1.91^{bc}	1.79^{b}
	600	1.43^{bc}	1.49^{bc}	1.46^{bc}	2.18^{a}	2.28^{a}	2.23^{a}	2.07^{b}	2.14^{ab}	2.11^{a}
	1200	1.67^{ab}	2.02^{a}	1.85^{a}	2.28^{a}	2.34^{a}	2.31^{a}	2.31^{a}	2.48^{a}	2.39^{a}
MPK (%)	2	0.66^{fg}	0.70^{ef}	0.68^{f}	1.03^{fg}	1.22^{ef}	1.13^{de}	1.09^{e-g}	1.28^{ef}	1.19^{d}
	3	1.08^{de}	0.93^{d-f}	1.00^{de}	1.33^{de}	1.41^{de}	1.37^{cd}	1.05^{e-g}	1.79^{b-d}	1.42^{cd}
	4	1.17^{cd}	1.30^{cd}	1.23^{cd}	1.44^{de}	1.70^{cd}	1.57^{bc}	1.71^{cd}	1.53^{cd}	1.62^{bc}
Thiourea (ppm)	500	0.94^{d-f}	0.91^{ef}	0.93^{ef}	0.90^{fg}	0.91^{fg}	0.91^{ef}	0.89^{g}	0.86^{g}	0.88^{e}
	1000	0.92^{d-f}	0.93^{d-f}	0.93^{ef}	1.01^{fg}	1.07^{e-g}	1.04^{ef}	0.92^{fg}	0.89^{g}	0.90^{e}
	1500	0.94^{d-f}	0.91^{ef}	0.93^{ef}	1.08^{e-g}	1.11^{e-g}	1.09^{d-f}	0.89^{g}	0.92^{fg}	0.91^{e}
H_3PO_4 (mM)	75	1.37^{bc}	1.69^{ab}	1.53^{b}	1.30^{d-f}	1.38^{de}	1.34^{cd}	1.11^{e-g}	1.47^{d}	1.29^{d}
	125	1.66^{ab}	1.98^{a}	1.82^{a}	1.52^{cd}	1.63^{cd}	1.58^{bc}	1.42^{de}	1.78^{b-d}	1.60^{bc}
	175	1.81^{a}	2.00^{a}	1.90^{a}	1.62^{cd}	1.85^{bc}	1.74^{b}	1.61^{cd}	1.94^{b}	1.77^{b}
Control		0.29^{g}	0.28^{g}	0.29^{g}	0.80^{g}	0.84^{g}	0.82^{f}	0.83^{g}	0.84^{g}	0.84^{e}
Mean		1.15^{b}	1.26^{a}	--	1.42^{b}	1.53^{a}	--	1.35^{b}	1.53^{a}	--

Means followed by the same letter (s) in each column are not significantly different at 0.05 level of probability

ABA enhances the activities of cellulase and polygalacturonase and accelerates the decomposition of cellulose and pectin, which determines the level of development of abscission zone and berry falling. Also, ethephon had enhanced the berry falling. In addition, ABA had enhanced water dropwort (*Oenanthe stolonifera* DC.) fruit abscission without increasing ethylene evolution [13]. However, ABA indicates possible involvement of ethylene in the ABA effect. Considering the application date, spraying the loosening agents 15 days before harvesting was more effective in increasing ABA concentration (1.26, 1.53 and 1.53 µgg^{-1}), successively in 2010, 2011 and 2012 seasons; thus, it was better than spraying them 7 days before harvesting in accumulating ABA in all the studied seasons. In this respect, [14] ABA concentration in Water dropwort (*Oenanthe stolonifera* DC.) was increased gradually after 20 days after anthesis.

CONCLUSION

It could be concluded that the earlier spraying date (15 days before harvesting) contributed in pectinase and cellulase activation and ABA content, more than the later date one, because of time multiply for the enzymatic reaction. Ethephon at 1200 mg/l had led to the highest significant ABA contents while spraying 175 mM of phosphoric acid had maintained the highest significant pectinase activity rates, in spite of that, ethephon sprays at 1200 mg/l in both dates, 7 and 15 days before harvesting, had scored the highest significant cellulase activity, so that the abscission zone occurred within the fruits' pedicels.

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