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Facilitation of olive harvest by microbial indole acetic acid and an enzyme mixture

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Abstract

In this study, the effect of indole acetic acid (IAA) and/or a commercial enzyme mixture on the olive harvest was analyzed. IAA was produced by *Gibberella fujikuroi*. IAA (1500 and 3000 ppm) and/or the commercial enzyme preparation (1%) was mixed and applied to the olive trees before (3 weeks and 1 week) the harvest. The changes in protein, pectin and cellulose contents of fruits were determined monthly. While the cellulose and protein contents showed a decrease in 2 periods of 3 months (July-September and October-December), pectin contents increased in the same period. IAA and/or enzyme applications did not lead to any significant changes in the cellulose and protein contents of the fruit (p>0,001). However, the pectin amount in the trees where the enzyme application was performed before the harvest, showed a tendency to fall. The most convenient application with regard to the fruit and leaf fall was 3000 ppm IAA+1% enzyme application 3 weeks before harvest. While the fruit fall amount increased more when compared to the control and other groups with this application, the leaf fall decreased significantly.

Keywords: *Gibberella fujikuroi*, Pectinolytic Enzymes, Protein, Pectin

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Introduction

The harvest is one of the most significant processes of the olive cultivation because the choosing of the form and time of the harvest affects the quantity and quality and manufacturing cost of the annual yield, and the yield of the next years.

The olive harvest both constitutes more than 40% of the manufacturing cost (Castillo-Ruiz et al., 2015) and affects particularly the physiology of flowering of a tree along with the yield and quality. The harvest can be done with mechanical and traditional methods. However, in Turkey, the utilization of the harvest machines used in the countries such as Italy and Spain, where the modern cultivation is made, is rather difficult. This is because of the fact that the condition of the some gardens and trees is not suitable for the use of these machines (Guler and Cesur, 2011).

The traditional harvesting methods are hand-picking, whisking, shaking, dropping with a rod. Especially as a result of the harvest done with rods, both the fruit and the annual shoots carrying the buds that will yield next year's products are damaged. Therefore, in the harvest done with the rod, a contamination from the cancerous areas to the healthy ones occurs with the branch and offshoot breaking. This situation both reduces the quality of the fruit and increases the severity of the periodicity (Bulbul, 2008). From the point of the yield quality, the best form of the harvest is hand-picking (Bulbul, 2008; Morales-Sillero and Garcia, 2015).

The joint usage of the hormones for the plant development and some enzymes (cellulolytic and pectinolytic) can be an alternative in order to prevent the damage in the harvest, either with the machine or traditional methods, and the loss of leaf.

The hormones can be used for the purpose of thinning the blossoms and fruit, rooting the slips, germination, fruit setting and parthenocarpy, affecting the dormancy mechanism, gender formation, blossoming, increasing the fruit quality, diminishing the pre-harvest falls, slowing down the aging process, preservation, fighting the diseases and weed (Barut, 1995). Auxins rank among the first-discovered plant hormones. Auxin is a general term used for the compounds having the characteristic of initiating the growth of the shoot cells. The most significant representative of this class is indole-3-acetic acid (IAA). The IAA is responsible for the regulation of each stage of the plant development (Palavan-Unsal, 1993). It was determined that not only the plants (Palavan-Unsal, 1993) but also fungi (Cakmakci, 1981; Ergun, 1997; Unyayar, 2000), lichen, algae (Ergun, 1997) and bacteria (Cakmakci, 1981; Martinez-Toledo et al., 1988; Davies, 2010) form IAA.

In this study, in order to create an alternative to the existing harvesting practices, the IAA hormone produced naturally by *Gibberella fujikuroi* was extracted and its facilitative effects on the olive harvest were observed by using it with a commercial enzyme preparation.

Materials and Methods

Materials

In this study, *Gibberella fujikuroi* was used in the production of IAA. This fungus was obtained from Hacettepe University, Faculty of Science, Department of Biology Prof.

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The experiment was carried out in the olive garden belonging to Murat Paşa Foundation in Antalya. Five olive trees of Gemlik cultivar were chosen for the experiment. A commercial enzyme preparation that contains a small amount of cellulase and hemicellulase enzymes as well as pectintranseliminase, polygalacturonase, and pectinesterase enzymes (Pectinex Ultra SP-L, Novo Nordisk Ferment Ltd., Switzerland) was used for facilitation of harvest.

Methods

IAA Production

The enriched synthetic Czapek-Dox broth was used for the IAA production (Mahadevan & Sridhar, 1982). The medium was inoculated (1%) with the suspension prepared from *Giberella fujikuroi* spores. Then the culture was incubated for 8 days at 30 ^{0}C and 150 rpm (Yalcinkaya, 1993). During the incubation, the IAA levels were determined in the samples taken on the 2nd, 5th, and 8th days (Cakmakci, 1981). Concentrated HCl (0.05 mL) was added to the 5 mL sample for the IAA extraction. After the samples had been thoroughly mixed, they were extracted two times with 5 mL ether. Then ether was evaporated under the nitrogen gas. The residue was dissolved in water and the IAA level was spectrophotometrically determined. The IAA extract (3 mL) was added into 1.5 mL 0.05 M $FeCl_3.6H_2O$ + 35% perchloric acid mixture. It was waited for 20 min until the characteristic red color to form. Afterwards, the absorbances were measured at 525 nm on a spectrophotometer (UV-VIS 1601 Shimadzu) against to the blind. IAA amount was calculated by comparison to the standard curve.

The IAA and/or enzyme application to the olive trees

The applications performed with the IAA produced by *Gibberella fujikuroi* and/or commercial enzyme preparation are shown in Table 1.

Table 1. The amount of IAA and/or enzyme

Trees	*3 weeks before the harvest*	*1 week before the harvest*
1	1500 ppm IAA	1500 ppm IAA
2	1500 ppm IAA + 1% enzyme	1500 ppm IAA + 1% enzyme
3	3000 ppm IAA	3000 ppm IAA
4	3000 ppm IAA + 1% enzyme	3000 ppm IAA + 1% enzyme
5	Control	Control

IAA and enzyme mixture were applied to the trees by a back pulverizer. The experiment was properly conducted according to the randomized parcel design. The picking of the olive samples began in July, at the fruit set stage, and it was sustained at intervals of one month until the harvest stage. The sample picking process ended on 30th December. The olive samples were maintained at -20ºC in 250-gram portions until they were analyzed.

The analyses of protein, pectin, and cellulose contents

Olive seeds were removed and the flesh was homogenized before the analysis. The protein, pectin, and raw cellulose amounts were determined in the samples. The protein content was determined by the Kjeldahl method (AOAC, 1990). The crude cellulose analysis was made according to ASTM (1980) with slight modifications and the pectin ratio according to Cemeroglu (1976).

Statistical Analyses

The analysis results of the olive samples were subjected to the variance analysis in accordance with the randomized parcel design. The SAS package program was used in the statistical evaluations and the results were analyzed by using the univariate analysis (ANOVA) test (SAS Institute Inc., 2011).

Results and Discussion

Gibberella fujikuroi produced the maximum IAA with 5-day incubation. It was observed that the amount of IAA increased gradually within the period of 5-day incubation and on the 5th day, the maximum production was reached. Following the 5th day, the production of IAA declined rapidly and was determined to be close to the level of the initial values. The production of IAA reached 38850 ppm (unpublished data) in consequence of triplicate performed. In the other studies, it was observed that the production rates of various fungi were lower and their incubation periods were longer (Palavan-Unsal, 1993; Yalcinkaya, 1993; Khan et al., 2012; Jaroszuk-Ściseł et al., 2014). The increasing of the production level and the shortness of the incubation period are significant in terms of reducing the production cost.

Cellulose, protein, and pectin contents of the olive fruit samples

The cellulose, pectin, and protein amount of the olive within the period from the fruit set to the harvest (July-December) is shown in Table 2.

It is possible to analyze the changes which occurred during the olive ripening in two periods. A rapid change occurred in the cellulose amount in the July-September period. Fewer changes were observed in the October-December period. In various studies, in which the changes in the polysaccharide compounds on the cell wall were analyzed, it was notified that the cellulose amount of the cell wall of the olive fruit decreases at the unripe, green, turning to green and turning to purple stages (Vierhuis et al., 2000; Mafra et al., 2001).

The differences in the protein amount in the ripening period compared to the beginning were also considered to be statistically significant ($p<0.001$). It was determined that the changes in the protein contents, as the ones in the cellulose amount, could also be analyzed in 2 periods. While the decline in the protein amount in the July-September period was considered to be statistically insignificant ($p<0.001$), the differences between this period and the October-December period were considered to be significant ($p<0.001$). Especially, the protein contents of the samples of October showed a certain decline compared to the samples of September. Similar findings were encountered in the studies where the domestic and foreign olive cultivars were analyzed. Lazovic & Miranovic (1999) examined the protein content and amino acid composition of the olive fruit and determined that the protein content in the fruit was 1.50%-2.61%. They used Picholine, Itriana, and Zutica cultivars and notified that the average protein content in the September-November period declined at the rates of 12% in Picholine type, 23% in Itriana and 38% in Zutica. The highest protein content was detected in the shoots, then in the leaf and the lowest in the fruit. In the study conducted by Ozay & Borcaklı (1995), it was found that the protein content of Gemlik olive changed between 1.76-1.95 g/100 g after it had been processed as the table olive. The pectin amount showed a regular increase. This result resembles the findings of Vierhuis et al. (2000) which notify that the pectin amount

Table 2. Changes in the cellulose, pectin, and protein amount in the ripening period

	Months					
	July	August	September	October	November	December
Cellulose (%)	7.34±0.125a*	6.92±0.300b	6.18±0.076c	5.98±0.031cd	5.74±0.014d	5.66±0.029d
Protein (%)	1.97±0.113a	1.88±0.118a	1.82±0.091a	1.43±0.046b	1.37±0.016b	1.30±0.016b
Pectin (%)	1.70±0.043c	1.79±0.051cb	1.89±0.043b	2.13±0.078a	2.29±0.094a	2.26±0,024a

***Means in the same row with different superscript letters are significantly different (P<0.001).**

As a result of the studies conducted to determine the effects of the IAA and enzyme applications on the cellulose, protein, and pectin contents, it was concluded that the applications performed 3 weeks and 1 week before the harvest did not cause any changes in the cellulose and protein amount (p>0.001). However, the pectin amount showed the tendency to decline in the trees on which the enzyme applications are performed (p<0.001). However it was determined that the IAA application did not significantly affect the pectin content (p>0.001).

Harvest Results

The trees, on which the enzyme and/or hormone application were made 1 or 3 weeks before the harvest, were harvested on the same day. Harvesting was performed with a rod. The main branches of the trees, on which the application was performed, were hit once in a way that their external skin was not damaged and the olives that fell onto the tarp that was stretched under the tree were picked. In the control trees, this practice, because of not being effective in the fruit fall, was realized with the traditional method by hitting the areas where the fruit was present with the stick. The amount of the leaf and fruit that fell due to natural causes and practices are shown in Table 3.

Table 3. The amount of fruit and leaf that fell with the effect of the application

Trees	Fruit and leaf	1st Application (4th December)			2nd Application (18th December)		
		Before Harvest	Harvest	Total	Before Harvest	Harvest	Total
1*	Fruit (g)	1980	750	2730	1000	400	1400
	Leaf (pcs)	1966	99	2065	59	103	162
2	Fruit (g)	2211	1100	3311	1000	1250	2250
	Leaf (pcs)	650	203	853	76	160	236
3	Fruit (g)	2040	900	2940	750	570	1320
	Leaf (pcs)	1382	113	1495	48	79	127
4	Fruit (g)	4550	600	5150	850	600	1450
	Leaf (pcs)	741	153	894	232	253	485
5	Fruit (g)	850	3500	4350	200	4350	4550
	Leaf (pcs)	908	1250	2158	524	1250	1774

***1; 1500 ppm IAA, 2; 1500 ppm+%1 enzyme, 3; 3000 ppm IAA, 4; 3000 ppm+%1 enzyme, 5; Control**

As can be seen in Table 3, as a result of hormone and/or enzyme, a significant amount of the fruit fell before the harvest procedure. The amounts that fell before the harvest were rather close to each other in the groups on which 1500 ppm IAA and/or enzyme was applied 3 weeks before the harvest. While the fruit amount that falls due to natural causes before the application shows close values with each other, the differences occurring after the application can be clearly observed. The amount of the fruit that fell from the trees, on which 1500 ppm IAA and/or enzyme was applied, during the harvest showed rather close values with each other. In consequence of the comparison of the same experimental groups in terms of the application periods, it was determined that the applications performed 3 weeks before the harvest were more efficient.

In the groups in which the IAA concentration was increased to 3000 ppm, it was observed that a significant amount of the fruit fell before the harvest. It was observed that the use of the enzyme in addition to 3000 ppm IAA expedites the fruit fall. Since a significant amount of the fruit fell before the harvest, the fruit amount obtained after the harvest was less that the one before the harvest. Upon considering the total amount of the fruit, it can be said that 3000 ppm IAA+1% enzyme with 5150 g yield provided the best result. However, the similar effect could not be provided with the applications performed 1 week before the harvest. Therefore, in order for the harvest to be expedited, 3000 ppm IAA+1% enzyme application, 3 weeks before the harvest can be recommended. With the studies conducted before, it was aimed to expedite the harvest by using various chemicals. In the study conducted by Cavusoglu (1973), the Ethrel® (2-chloroethylphosphonic acid) was used in various concentrations in order to expedite the harvest. The Ethrel® caused the offshoot break and the high level of leaf loss. In another study, Ethrel® was considered to be ineffective in pre-harvest fall and insufficient in fruit fall during the harvest (Cavusoglu, 1977). The chemical applications could not find a common usage area because they leave residues as well as cause leaf loss.

According to the results, it was determined that an effective harvest performed with the rod, a total of 4550 g fruit was obtained and 1250 leaves fell during the harvest as a result of hitting the tree multiple times with the rod. Along with 1432 leaves that fall due to natural causes before the harvest, the total loss is 2652 leaves. However, with the effect of 3000 ppm IAA+1% enzyme application, a total of 894 leaves fell.

The most leaf fall was caused by 1500 ppm IAA application performed 3 weeks before the harvest. Although the data obtained in 1500 ppm IAA application did not show any significant differences compared to the control, upon taking all the applications into account, the decline of the leaf fall compared to the control, along with expediting the fruit harvest, is significant for the efficiency of the application. From the data obtained, it can be said that while the IAA+enzyme application creates a synergistic action in fruit fall, it can offer an advantage in terms of declining the leaf fall. The harvest method practiced in the control group causes both the aggravation of the periodicity and diseases, as well as the high level of leaf loss, because of the damages it creates in the branches and the cells. However, upon considering the leaf loss occurring during the harvest, it was observed that the most leaf fall occurs in the tree on which 3000 ppm IAA+1% enzyme was applied 1 week before the harvest and on the other hand, the leaf loss was more in the control tree. Percentage ratios of the leaf loss caused by the applications were calculated by accepting the leaf number of the branches on which the application was made to be 5000 pieces (Table 4).

The leaf loss at the rate of 10-57.6% was determined with the effect of the application performed 3 weeks before the harvest. In the control group, the loss was determined as 57.9%. The loss in the applications performed 1 week before the harvest was much less compared to the control tree (96.15%). The total loss caused by 3000 ppm IAA+1% enzyme application 3 weeks before the harvest, recommended in this study, was limited to 2%.

The different concentrations (2000, 4000 and 8000 ppm) of the Ethrel® were applied to the Manzanialla olive 1 month before the harvest. These trees were harvested with a mechanical shaker. One of the 2 control trees was harvested by hand and the other was harvested with a pneumatic shaker. The amount of the fruit that fell as a result of the harvest was found to be 68.7% at 2000 ppm; 95.1% at 4000 ppm; 99.1% at 8000 ppm, and 100% in the control. In consequence of this application, it was determined that the Ethrel® causes a lot of leaf fall. The leaf fall at the rate of 0.2% at 2000 ppm; 18% at 4000 ppm; 22% at 8000 ppm, and 32% in the control was notified. The highest pre-harvest fall was determined to be 59.93% at 8000 ppm. This rate is 7.23% in the control tree (Ozguven et al., 1998). There are some resemblances between the two studies in terms of the leaf fall in the control. However, the loss caused by the IAA and enzyme applications is much less. In another study, it is notified that usage of glycerol and NaH_2PO_4 increased the leaf loss from 9% to 18% in addition to increasing the fruit fall from 50% to 80% (Martin, 1994).

The pectolytic enzyme preparation used in this study will affect the quality and amount of the oil in addition to the positive effects of the fruit and leaf fall because the technical enzyme preparations are used for this purpose in the olive oil production. More products of much higher quality are obtained by means of changing the structure of pectic polysaccharides on the cell wall of the olive fruit with the pectolytic enzyme. It was determined that with the effect of the enzyme, the methyl esterification decreased, the molecular weight profile changed, the galactan chains that are bound with the (1-4) bond broke (Vierhuis et al., 2003). The pectolytic and cellulolytic enzymes are the enzymes bound to the cell wall of the olive fruit and of which amount increases with the ethylene production during the ripening. This enzyme group, formed with seven glycosidases and Cx-cellulose, tears down the cell wall and ensures that the fruit softens and ripens (Fernandez-Bolanos et al., 1995). Depending on the increase of the ethylene production, the enzyme synthesis or activation in especially the black olives is promoted (Fernández-Bolaños et al., 1997; Morales-Sillero and García, 2015). Therefore, the studies aimed at using the chemicals promoting the ethylene production were conducted in order to expedite the harvest (Cavusoglu, 1973; 1977; Ben and Wodner, 1993; Martin, 1994; Ozguven et al., 1998). It will be possible to benefit mutually from the pre-harvest application with the determination of the effects of the enzyme applications, performed in order to expedite the harvest, on the yield and quality of the olive oil. Thereby, an advantage in terms of the cost can also be created.

Conclusion

The olive harvest is an efficient and important factor on the quality and amount of the yield. The procedures carried out during the harvest are significant in developing these features. The findings of this study, conducted in order to diminish the yield loss in Turkey, which is an important olive

Table 4. Amount of the leaves that fell as a result of the applications

Trees	Applications*	Leaves that fell before the harvest (%)	Leaves that fell by the wind, etc. before the harvest (%)	Leaves that fell before the harvest with the effect of the application (%)	Leaves that fell during the harvest (%)	Leaves that fell as a result of the application (%)
1	1	56.78	51.56	5.22	43.21	48.43
	2	32.20	21.18	11.01	67.79	78.81
2	1	95.20	43.97	51.23	6.39	57.62
	2	36.41	30.86	5.55	0	5.55
3	1	91.00	89.90	1.68	8.41	10.09
	2	73.31	16.07	57.23	26.68	83.92
4	1	93.00	90.80	2.20	7.80	10.00
	2	47.83	10.30	37.52	52.16	89.69
5	1	42.07	42.07	-	57.92	57.92
	2	3.84	3.84	-	96.15	96.15

*1; 3 weeks before harvest, 2; 1 week before harvest producer, and meet the product quality that the world markets demand, have shown that the IAA and enzyme applications can give the successful outcome. It is thought that the hormones and enzymes used in the experiment do not pose any danger to the health as they are already present naturally in the plant tissues. With the studies to be conducted from now on, the effect of the IAA and enzyme application on the periodicity, amount, and quality of the olive oil will be determined.

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