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# Anatomical Study on the Junction and Location of Abscission Zone of Olive Fruit Pedicels as Affected by Chemical Loosening Agents

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**Abstract:** The present study was conducted during three successive seasons 2010, 2011 and 2012 in a private orchard located at Cairo – Alexandria desert road (far about 50 Km from Cairo). Fifteen - year - old of uniform "Koroneiki" olive trees planted at 5 x 8 m apart in a sandy soil under drip irrigation system. The present work was established to investigate the effect of loosening agents on the anatomical of fruit pedicels, abscission zone and its locations in olive fruits of Koroneiki cv. Loosening agents showed predominant effects on the anatomical features of the pedicel structure. Ethephon at 1200mg/l and MPK at 175 mM of applied in both dates., 7 and 15 days before harvest, dramatically produce comparatively similar anatomical effects, as the symptoms of abscission was found in all tissues shared in pedicel internal structure. Fruit pedicel showed different diameter and length. The most common abscission sites were; fruit- pedicel and pedicel – rachis junctions or in both sites. It is obvious that a well distinguish abscission layer developed in both marginal direction and extended toward the pedicel core. The cells of formed abscission layer generally, maintained heavy suprenized cell walls, also showed a dark color and turn to the protective layer after fruit drop. The fruit vascular trace within the pedicel, that connects the fruit with the rachis, was completely free of abscission symptoms within the vascular trace. The other studied separation site was the pedicel- rachis junction. The pedicels at this area showed constant thick abscission zone that compressed several (more than 11 row) of superinzed cells. Also, the abscission zone occurred just below the point of pedicel attachment with rachis. The vascular tissue showed a complete separation where no real attachment was realized between the vascular trace of the rachis and the pedicel. These symptoms were realized clearly with ethephon and Phosphoric acid ($H_3PO_4$) more than the other loosening agents or their respective control.

**Key words:** Olive · Koroneki · Chemical loosening agents · Anatomy · Fruit pedicels · Abscission zone

## INTRODUCTION

Olive (*Olea europaea* L.) is one of the oldest cultivated fruits since its fossilized leaves dating to around 37, 000 years ago have been discovered on the Aegean island of Santorini. The olive belongs to the Oleaceae family, which comprises of 29 genera and the genera *Olea* is one of them with 35 species [1]. The domesticated olives belong to the genus *Olea*, species *europaea*, subspecies sativa and the number of the cultivated species is estimated more than 2500 cultivars.

The olive species include many cultivars which are used for oil extraction such as "Koroneiki" and "Coratina", or table olives as "Kalamata" and "Teffahi" and double purpose such as "Picual" and "Manzanillo". Growers prefer olive cultivation because of its resistance to drought and salinity conditions in addition to low fertilization needs comparatively with other fruit trees.

Identifying the abscission location of olive fruit pedicels is critical for testing the effectiveness of harvest procedures, including the mode of action of different fruit loosening chemicals.

Hegazi *et al.* [2] cleared that after applying ethephon to the olive cutivar "Chemlali" olive, abscission zone formation occurred at two sites; one at the distal end of the pedicel and the other at the junction between the pedicel and peduncle.

In another study, Weis *et al.* [3] pointed out that abscission response of detached reproductive olive shoots differed from differences in sensitivity between

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leaves and inflorescences and among the same organs of differing maturities and possibly from quantitative differences in amount of ethylene between abscission zones at similar positions. In addition to peduncular abscission, abscission occurred at numerous sites within the inflorescence with ethylene treatment but not with ethephon.

Bartolini *et al.* [4] worked on the activation of fruit abscission zones during ripening of olive cultivars "Frantoio" and "Leccino" using 2000 ppm of ethephon. The early-ripening fruits "Leccino" olive was treated 20 days earlier than "Frantoio". They revealed that, ethephon treatment caused a detachment recorded more frequently at peduncle-pedicel and pedicel-fruit junctions in both cultivars. In "Frantoio", abscission of fully black-ripe fruits occurred more often close to the fruit, affecting only pedicel tissue. Moreover, according to Barranco *et al.* [5] applied 3% monopotassium phosphate (MKP) as mechanical harvest aid agent to "Picual" and "Arbequina" olive cultivars and defined three fruit separation zones: (1) peduncle - branch, (2) Pedicel - rachis and (3) Fruit - pedicel. Results indicated that fruit separation was considerable in all three zones, but predominated in zone 1.

In addition, Castillo-Llanque and Rapoport [6] pointed out that olive fruit abscission can occur at different positions, mainly fruit-pedicel, pedicel rachis and peduncle-branch, apparently varying according to cultivar, fruit weight and fruit maturation.

Working on longkong crop, Taesakul *et al.* [7] reported that ethylene induced fruit drop at the junction area between the main peduncle and the calyx, at a clear abscission zone. There may or may not be an abscission zone at this site.

Estornell *et al.* [8] summarized that the abscission restricted in the detachment of the entire organs may be due to cell separation processes occurring at the abscission zones (AZs) at speci?c positions of the plant body. From an evolutionary point of view, abscission is a highly advantageous process resulting into fruit and seed dispersal as well as the shedding of no longer useful organs. Therefore, the present investigation was established to investigate anatomical of fruit pedicels, abscission zone and its locations.

## MATERIALS AND METHODS

The present study was conducted during three successive seasons 2010, 2011 and 2012 in a private orchard located at Cairo – Alexandria desert road (far about 50 Km from Cairo). Fifteen - year - old of uniform "Koroneiki" olive trees planted at 5 x 8 m apart in a sandy soil under drip irrigation system. The trees received the common horticultural practices such as fertilization, irrigation, pruning and pest control.

Olive trees cv. Koroneiki were sprayed with different agents: ethephon (2- chloroethyl phosphonic acid) at the concentrations of 300, 600 and 1200 ml/ L, mono potassium phosphate MPK ($KH_2PO_4$) 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 addition to the control treatment (sprayed with water). Trees were sprayed at two times 7 and 15 days before the expected harvest time which is that was about 150 ± 20 days after full bloom (AFB) in each season and when the fruit color-turning to purple for more than half of skin.

It was intended to carry out a comparative microscopic examination on the treated fruits pedicel which showed the most prominent response with both ethephon and $H_3Po_4$ as compared with their control. Pedicel specimens 1 cm in length was fixed in F.A.A. solution (formalin, acetic acid and alcohol (95%) as 5:5:90, respectively). Specimens were transferred from F.A.A. and were dehydrated in a graded series of alcohol (tertiary butyle alcohol TBA and ethanol) according to Sass [9]. Then, Pedicels were embedded in paraffin wax at 60°C. Series paraffin blocks were cut into longitudinal sections of 10 µ in thickness then prepared using a hand rotary microtome. Sections were stained with (Safranin O Dye content =85 %) according to El-Agamy *et al*. [10] then dehydrated, cleared with xylene. The slides were mounted in Canada balsam according to Johansen [11], then covered with a fine glass covers. Measurements (in microm) and counts were recorded using micrometric lens on light microscopy with different magnification power (6*10 = 60x)

## RESULTS AND DESCISSION

**Abscission Zone Proliferation in Olive Fruit Pedicel:** Microscopic measurements and counts of certain histological characteristics as shown in the longitudinal sections through fruit pedicel junction of olive fruit are presented in Table (1) and Figures (1, 2 and 3). According to the classification of abscission zones suggested by Bartolini*et al.* [4] the results indicated that, fruit pedicel showed different diameter and length. The recorded results proved that pedicel diameter ranged between 475.2 to 512.6 mµ. However, the pedicel length varied between 3352.6 mµ to 6897.4 mµ. The most common abscission sites were; fruit- pedicel abscission and pedicel – rachis

Table 1: Longitudinal sections in "Koroneiki" olive fruit pedicels as affected by some fruits loosening agents

| | Control | | Ethephon 1200 mg/l | | $H_3PO_4$ 175 mM | |
|---|---|---|---|---|---|---|
| | Days after treatments | | | | | |
| Measurements (mμ) | 7 | 15 | 7 | 15 | 7 | 15 |
| Average pedicel thickness | 1899 | 1851 | 2003 | 1970 | 1885 | 1785 |
| Average cortexthickness | 344 | 318 | 421 | 335 | 312 | 230 |
| Average cortical cell length | 73 | 69 | 87 | 81 | 76 | 74 |
| Average vascular tissue thickness | 121 | 105 | 169 | 154 | 159 | 151 |
| Average pith diameter | 970 | 999 | 722 | 689 | 1060 | 925 |
| Average pith cell diameter | 97 | 112 | 75 | 72 | 88 | 81 |
| Average cell wall thickness | 1.21 | - | 0.65 | - | 0.81 | - |

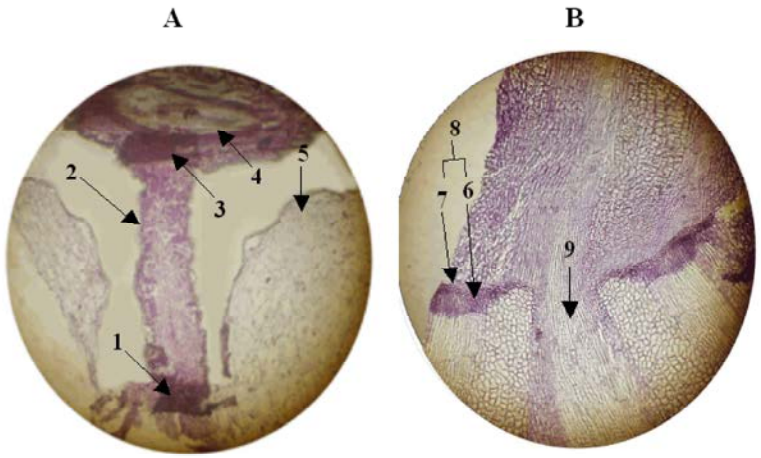


Fig. 1: Longtidunal sections in olive fruit pedicel showing abscission zone

A) Abscission sites (X 30) B) Magnified abscission zone (X150)
1) Fruit- pedicel junction 2) Pedicel 3) Pedicel – rachis junction
4) Rachis 5) Fruit 6) Protoctive layer 7) Abscission layer
8) Abscission zone 9) Vascular trace

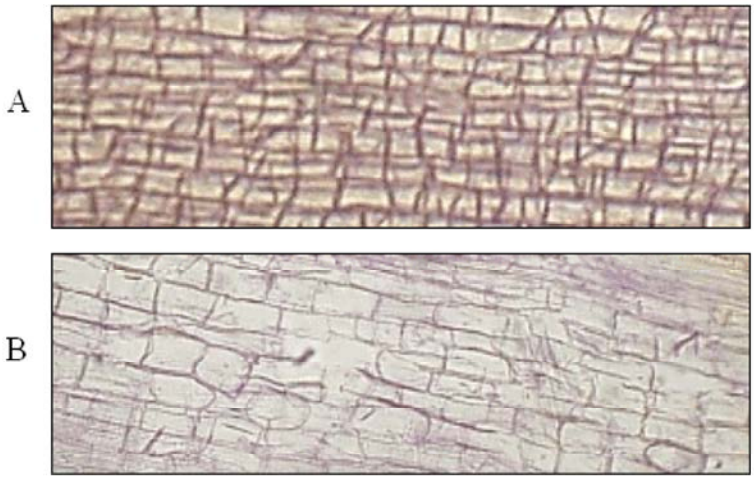


Fig. 2: Longtidunal sections in "Koroneiki" olive fruit pedicel showing differences in thickness and degradation of parenchyma cell wall affected by the loosening agents. A) Before treatments (X 200) B) After treatments (X200)

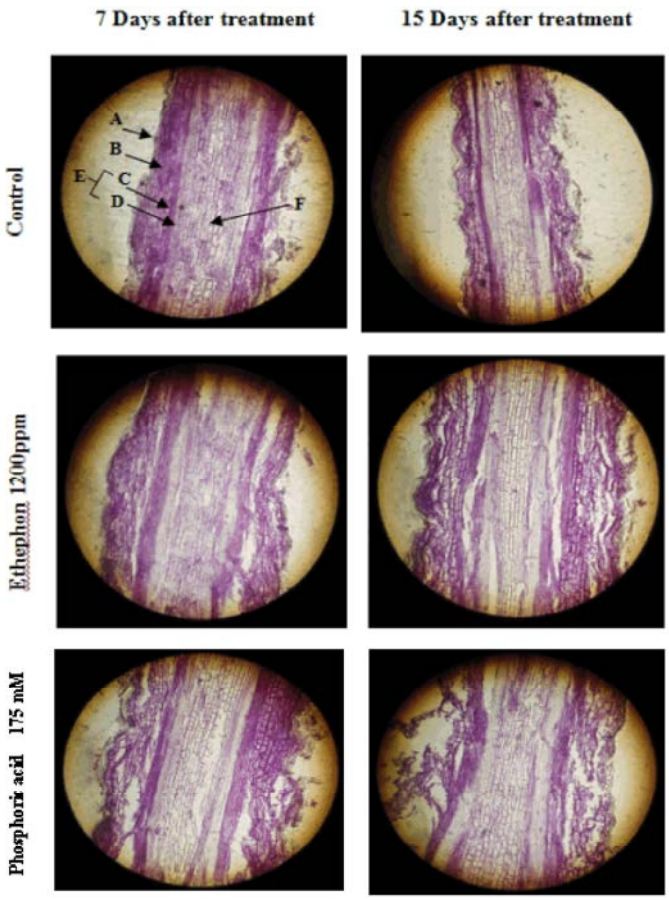


Fig. 3: Longtidunal sections in "Koroneiki"olive fruit pedicel showingpedicel internal structure affected by the loosening agents. (X 75)
A) Prederm B) Cortex C) Phloem D) Xylem E) Vascular bundle F) Pith

junction or in both sites [6]. It is obvious that a well developing abscission layer took place in both marginal direction and extended toward the pedicel core, Fig. (1). The cells of this abscission layer generally, maintained heavy suprenized cell walls. Moreover, these cells showed irregular thickness, as they are thicker in outer pedicel layer (146.3 mμ) as compared with the inner area (46.8 mμ). Histological study proved that this suprenized layer showed dark color and turn to the protective layer after fruit drop [8]. The fruit vascular trace within the pedicel was completely free of abscission symptoms. Where, no separation area found within the vascular trace that connect the fruit with the rachis.

The other studied separation site was the pedicel- rachis junction, Fig. (1). The pedicel at this area showed constant thick abscission zone that compressed several (more than 11 rows) of suprenized cells. These cells extend transversely and contradict the normal pedicel tissue. It was noticed that the abscission zone occurred just below the point of pedicel attachment with rachis. The recorded data indicated that separation zone averaged 308 mμ in thickness and 568.8 mμ in length, Fig. (1). The vascular tissue showed complete separation where no real attachment was realized between the vascular trace of the rachis and the pedicel. Therefore, the separation zone occurred within the vascular gape (the area in between vascular connections).

The separation position is variety linked. For example, Reed and Hartmann [12] observed fruit- pedicel abscission in "Manzanillo". Barranco *et al.* [13] suggested that abscission predominated at the peduncle-shoot union in "Arbequina", while in "Frantoio" and "Leccino" separation occurred at both pedicel-rachis and fruit –pedicel [4]. It is previously reported that many researchers noticed olive fruit separation at the pedicel [14-16]. The other position at the pedicel - rachis junctions [12, 4]. The third position the peduncle - shoot union [5].

**Effect of Loosening Agents' Treatments on the Pedicel Anatomical Structure:** Measurements in microns and counts of the studied longitudinal sections in "Koroneiki" olive fruit pedicel are presented in Table (1) and Fig. (3). The recorded data showed that the average pedicel thickness as well as the cortex thick did not show any appreciable differences due to the date of application (7 or 15 days before harvesting date). The average thickness of the vascular bundle showed comparatively the same measurements (73 and 69 mμ). Moreover average pith diameter did not show extraordinary differences due to the time of harvest. The most remarkable differences were recorded in the average pith cell diameter as the obtained results emphasized that the average pith cell diameter was greater (112 mμ) at 15 days as compared with those of 7 days that scored (97 mμ) pith cell diameter. This is in harmony with the previous report by Young and Biale [17] who concluded that, cell permeability was maintained by application of ethrel that may reflect in average final cell size.

Moreover, we could ensure that applying any of these agents produces comparatively similar anatomical mode of effects. For the adopted treatments it is obvious that the formation of identical abscission zone was not realized in the studied pedicel longitudinal sections. As the symptoms of degradation, after loosening agents' treatments, were found in all tissues shared in pedicel internal structure, Fig. (3). These symptoms were realized with ethephon and $H_3PO_4$ treatments as compared with their respective control. Since, the cortex cells were greatly affected by remarkable destruction and corrosion without notable changes in number of cell rows. It is also, realized that the cortex of both ethephon and phosphoric acid treated was thicker 421 and 312 mμ for ethephon and $H_3PO_4$ treatments, respectively. The average length and diameter of cortical cells were enlarged due to these treatments. The recorded data proved that, relative to the control the average cortical cell length in case of ethephon application was greater by 19.18%. While, the corresponded enlarge percentage in case of $H_3PO_4$ treatments was 4.11 %. The other major feature was the changes which occurred in the average thickness of the vascular bundle, Table (1). Also, both the phloem and xylem diameters as seen in transverse section were greatly affected by loosening agent treatments compared with the control. The counted percentages of these increments were 42.9% and 37.5% in the average thickness of the vascular bundle for ethephon and $H_3PO_4$ treatments, respectively. It is worthy to mention that the realized enlargement in vascular bundle dimension was not due to any increase in either number or size of the vascular elements. The xylem vessels were more loosely and did not show the regular constantly that characterized xylem tissue, Fig. (3). According to the previously reported results with regard to estimated fruit removal force, it was reported that, all ethephon treatments showed the lowest removal force as compared with the control and the other adopted treatments. Moreover, regardless to the time of application, the obtained anatomical differences were confirmed by the estimated contents of pectinase and cellulase in both ethephon 1200 mg/l and $H_3Po_4$ 175 mM treatments. The high pectinase and cellulase activity due to these treatments were manifested on the anatomical features of the pedicel cortex layers and internal pith parenchyma.

The other major distinct difference was the constant decrease in the thickness of parenchyma cell wall as a result of the loosening agent's treatments, Fig. (2). These decrements were more apprehended in case of ethephon 1200 mg/l application (0.65mμ) while, $H_3Po_4$ 175 mM application resulted in (0.81 mμ), Table (1) and Fig. (3). The estimated contents of cellulase and pectinase enzymes activity in pedicel tissues was confirmed by the obtained anatomical study. Also, the average parenchyma cell wall thickness was thinned due to high pectinase and cellulase enzyme activities that linked with both ethephon 1200 mg/l and $H_3Po_4$ 175 mM treatments. This result was previously reported as the applied loosening agents affected cellulase and pectinase enzymes activity that serve in either cell wall degradation, Fig. (2). So, the applied loosening agents showed predominant effects on the anatomical features of the pedicel structure and this was confirmed by either the pectinase and cellulase enzyme activities or the estimated fruit removal force.

The alteration occurred in anatomical features of the pedicel due to loosening agents' treatments as shown in longitudinal section Fig. (3) was also confirmed by the obtained results of abscisic acid (ABA) estimates in pedicel tissues. The obtained readings and counts proved that both ethephon and $H_3Po_4$ treatments caused high loosening effects and correlated with high ABA percentages in pedicel tissues. These results were confirmed previously by many workers such as Devlin [18] who reported that, abscisic acid (ABA) had the effect powerfully inhibition of cell elongation during process of differentiation and it usually causes slow cell division [19].

Generally, the obtained anatomical outcomes were previously confirmed by Neals and McLeod [20], they reported that, auxin has been shown to occur in the xylem

sap in several plant species this may be due to the active ethrel compound which can be imported or generated into xylem vessels as its sight of auxin synthesis and affects the cohesion of xylem components; vessels, fibers and parenchyma. The anatomical features of cortex and pith of the studied treated plants showed an appreciable shrink in average parenchymatous cell length. These stunting cells were found throughout the pith tissue of all the studied treatments Relative to the control, the average decrease percentages in pith cells length were -29.8% with ethephon treatments. While, the corresponded decrease percentage was -13.8% with $H_3PO_4$ treatments.

Finally it could be concluded the results as follows: The used loosening agents showed predominant effects on the anatomical features of the pedicel structure. Fruit pedicel showed different diameter and length. The most common abscission sites were; fruit- pedicel and pedicel – rachis junctions or in both sites. Well distinguish abscission layer developed in both marginal direction and extended toward the pedicel core. Histological study proved that the cells of this abscission layer generally, maintained heavy suprenized cell walls, also showed a dark color and turn to the protective layer after fruit drop. The fruit vascular trace within the pedicel, that connects the fruit with the rachis, was completely free of abscission symptoms within the vascular trace. At pedicel- rachis junction, the pedicels at this area showed constant thick abscission zone that compressed several (more than 11 rows) of suprenized cells. Also, the abscission zone occurred just below the point of pedicel attachment with rachis. The vascular tissue showed a complete separation where no real attachment was realized between the vascular trace of the rachis and the pedicel. Applying any of the used agents could be produced comparatively similar anatomical effects, as the symptoms of abscission was found in all tissues shared in pedicel internal structure after loosening agents' treatments. These symptoms were realized clearly with ethephon and $H_3PO_4$ treatments as compared either with the other loosening agents or the respective control.

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