INTRODUCTION

Olive (*Oleaeuropaea* 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 (Heywood, 1978). 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.

Olives are now known to contain significant amounts of vitamin E, essential fatty acids, antioxidants and other nutrients.

The world production of olives was 20,344,342 tons. The major producer countries are Spain, Italy, Greece, Turkey, Morocco, Syria, Tunisia, Egypt, Algeria, Portugal and others, (FAO, 2013).

In Egypt, olive cultivation increased considerably during the last two decades due to the great efforts paid for expanding olive cultivated areas with new cultivars in reclaimed areas. El-Nobaria, Marsa Matruh, El-Fayoum, North Sinai, New Valley and South Sinai are the most important areas of olive production. The Egyptian olive production cultivation was about 563,070 tons produced from acreage 202,743 feddan (1 Feddan = 0.42 Hectare), most of which are processed mainly as table olive and the rest is extracted to olive oil, (Ministry of Agriculture and Land Reclamation, 2013).

Fifty to sixty percent of total production costs is used for harvesting operations (Özarslan et al., 2001) with higher than $300 per ton (Rosa et al. 2009). Also in Egypt harvesting operation costs about 0.5–0.75 EGP/Kg. The higher cost and slow rate of hand harvesting make mechanical harvesting highly demanded.

Olives and olive oil played an important role in ancient Mediterranean economies. Today, olives contribute billions of dollars to the global economy which gives a strong motive to develop and facilitate harvest techniques. There are over 800
million olive trees across the globe. Olives and their oil now sustain an industry producing about $10 billion annually. This is why it is extremely important for all growers to try maximizing this efficiency and to lower harvesting costs.

In spite of this value, in many regions of the Mediterranean and most parts of the world, olives are still picked by hand, using wooden tools, or beaten from the trees with poles and caught in canvases or nets placed under the tree to collect the fallen fruits. This type of harvesting is time consuming and involves intensive labor. In addition, it results in high level of fruit damage.

It is difficult to integrate all the factors that affect final processed olive quality; cultivar, orchard spacing, production practices, particularly irrigation and canopy shape as a result of pruning, fruit maturity, the machine itself, how, when and by whom it is operated, and the final use of the olive (Kauraba et al., 2004). The last factor complicates the mechanical harvesting experimentation further as the physiologically immature olives required for some table processing cures do not have a fully developed abscission zone and therefore require more force to detach the olive.

In Greece, the “Koroneiki” is considered ‘the queen of olives’ and is recognized globally as one of the preferred varietals for oil production. Also in Egypt, “Koroneiki” is considered one of the most important varieties and most widely cultivated in order to produce olive oil.

Therefore, the present work was established to investigate the following:

1. Reduce total costs by applying mechanical harvesting.
2. Increasing fruit removal efficiency without bruising the fruits by using some loosening agents.
3. Study the effect of used loosening agents on oil quality.
4. Anatomical study of fruit pedicels, abscission zone and its locations.
5. Study the economics of mechanical harvesting.
REVIEW OF LITERATURE

In an agricultural context, abscission may become a major limiting factor for crop productivity. Domestication of major crops included the selection of plants that did not naturally shed ripe fruits or seeds. The understanding of abscission is of great importance to control fruit production and to improve harvesting practices (Estornellet al., 2013).

The previous work dealing with the effect of different loosening agents aid in mechanical harvesting, plant and fruit vegetative characteristics, oil chemical analyses, enzymes and hormones contents finally the histological structure of different plants will be discussed as follows:

1. Loosening agents

In early trials, (Cartechini and Standardi, 1971; Casilliet al., 1973; Trona and Cantagalli, 1974; Natali and Vitagliano, 1975; Sanchez-Raya et al., 1975 and Vitagliano, 1975) tried ethephon as a loosening agent and revealed that it had facilitated mechanical harvesting of 80% of olives.

Moreover, (Lavee, 1976; Piccarolo and Paschino, 1976; Klein et al., 1978; Rosati and Zocca, 1978; Troncoso et al., 1978 and Antognozziet al., 1980) reviewed that the ethylene-releasing compounds resulted in olive within an hour, and that between 65 and 90% of the fruits were recovered.

Weis et al. (1988) concluded that abscission responses of detached reproductive olive shoots differed depending on sources of ethylene (applied ethylene or ethylene evolved from ethephon breakdown), organ type, developmental stage of the inflorescence, and site of the potential abscission zone. Disparities in response resulted from differences in sensitivity between 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.

Working on “Gordal” olive, Atawia and Abd El-Rahman (1998) treated trees with NAA and thiourea 15 days before harvesting. They showed that thiourea at 1000 ppm had increased fruit abscission percentage and all the tested fruit abscission agents had no positive or negative effects on fruit physical and chemical properties.
Barrancoet al. (2002) applied 3% monopotassium phosphate (MKP) as mechanical harvest aid agent to “Picual” and “Arbequina” olives and defined three fruit separation zones as a result of the treatment.

Working on banana (*Musa acuminate*), Anaya et al. (2011) used phosphoric acid (H$_3$PO$_4$) at 80 and 85% concentrations as acid hydrolysis of starch-rich banana fruit. They apprehended good results when hydrolysis is activated with the acid.

2. Effect of loosening agents on

a. Leaves drop percentage

Casilli et al. (1973) found that applying 200 g/hectoliter of ethrel (ethephon) 19 days before harvest caused the natural fall of 60% of the fruit compared with 17% from untreated controls, and it increased by 14% the yield of fruit harvested by a mechanical shaker. It caused no serious leaf fall.

Moreover, Cavusoglu (1973) applied ethrel at 1000, 2000 or 3000 ppm on 3 dates and pointed out that the leaf drop was highest after the third application of 3000 ppm.

Moreover, Antognozzi (1981a) sprayed "Frantoio" and "Moraiolo" olive trees in two dates with 2000 ppm CEPA (ethephon), 2000 ppm Alsol, 2000 ppm Hol, and other ERCs compounds. They had recorded undesirable side effects such as leaf drop; but Alsol gave the most promising results and no side effects.

In this respect, Hegazi et al. (1987) applied ethephon and CGA-13586 (Alsol) two weeks before harvest to "Hamed" and "Chmlali" olives. Results reveled that ethephon at 750, 1000 and 1250 mg/l caused a marked leaf abscission in both cultivars whereas Alsol had no such effect.

Regarding leaf abscission, Atawia and Abd El-Rahman (1998) treated "Gordal" olive trees with Alsol, NAA and thiourea 15 days before harvesting and reported that NAA increased leaf abscission percentage, followed by Alsol and thiourea at 1000 or 500 ppm.
a. Leaves burns percentage

Working on "Hamed" and "Chelmali" olives, Hegazi et al. (1987) pointed out that ethephon treatments at 750, 1000 and 1250 mg/l resulted in leaves color reduction whereas Alsol treatments did not affect leaves.

In Italy, (Baratta et al., 1990) and (Baratta et al., 1992) sprayed 2, 4 or 6 % urea on "Nocellara del Belice" olive trees at full bloom (FB) or 10 or 20 d later FB and they noticed that applying 6% urea caused some scorch on leaves.

b. Fruit burns percentage

In this respect, Lavee and Haskal (1975) treated three olive cvs. with ethephon to loosen the fruit for harvesting and noticed that for all cultivars, concentrations exceeded 1500 ppm had decreased flower bud differentiation considerably.

c. Fruit removal force (FRF)

The efficiency of fruit removal by shaking is inversely related to fruit detachment force (FDF). To reduce the detachment force and facilitate harvesting operation, abscission chemicals have been used (Martin 1994; Al-Jalilet al., 1999; Metzidakis 1999; Whitney et al., 2000; Peterson et al., 2003 and Sessiz and Özcan 2006). Some abscission chemicals have been identified for enhancing mechanical harvesting efficiency by loosening pedicles strength of fruit.

Tombesi (1970) applied ethrel at 5000 ppm to the fruits and/or the leaves of "Dolce Agogia" and "Frantoio" olives. The force needed to detach the fruits was reduced when the fruits themselves were treated but not when only the leaves were treated. High quantities of ethylene were produced under the influence of ethrel in treated organs but fruit adjacent to treated leaves and leaves near treated fruit produced no more ethylene than usual.

Moreover, Lavee and Haskal (1975) treated three olive cultivars namely; "Manzanillo", "Souri" and "Nabali" with ethephon to loosen the fruit for conventional or mechanical harvesting. They mentioned that for all cultivars, concentrations in the order 1500 ppm were satisfactory and increased the efficiency of both mechanical and manual picking. Morning sprays were more effective than those given at noon.
Working on "Leccino" olive, Toscano and Casacchia (2006) used foliar application of a 3% solution of monopotassium phosphate (NPK 0-45-35) + ethephon at 9.6 g/kg. They recorded FRF (fruit retention force) reduction with treatments.

Moreover, Atawia and Abd El-Rahman (1998) noticed that treating "Gordal" olives with thiourea at 1000 ppm produced the second highest significant reduction of fruit detachment force after Alsol at 1000 ppm.

Working on "Picual" olive, El-Deeb (2000) subjected trees to harvest aid treatments by pre-harvest sprays of sodium monophosphate+10% glycerol and ascorbic acid. Results showed that control trees (water sprayed) recorded the highest FRF and the highest percentage of handpicked fruits. Sodium monophosphate 2000 ppm+10% glycerol increased the percentage of pre-harvest dropped and vibration harvested fruits.

Whereas, Barranco et al. (2002 and 2004) reported that spraying 3% monopotassium phosphate (MKP) 15 days before mechanical harvest reduced the high 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.

Working on "Leccino" olive, Toscano and Casacchia (2006) used foliar application of a 3% solution of monopotassium phosphate (NPK 0-45-35) + ethephon at 9.6 g/kg. They recorded FRF (fruit retention force) reduction with treatments.

d. Oil chemical analyses

Antognozzi and Standardi (1980) noticed that pre-harvest sprays of olive trees in November with 2000 ppm ethephon or 2000 ppm Alsol resulted in earlier drop, however, led to a lower oil yield.

Rotundo and Pugliano (1981) sprayed thirty-year-old “Rotondella” olives, with ethrel on 16th of September after pit hardening. They proved that ethrel at 100 and 500 ppm increased oil content by 18% and 22%. Total oil analyses showed that a 30% greater oil yield would be obtained if the olives were harvested in late December and early January rather than at the conventional time 30-40 days later.
In this respect, Hassan (2002) sprayed chemical loosening agents on Mission olives 2 weeks prior to harvest: Alsol at 1500 or 2000 ppm; ethephon at 1500 or 2000 ppm and thiourea at 500 or 1000 ppm. He reported that all compounds enhanced oil composition.

Moreover, Barranco et al. (2004) pointed out that 3% MKP application 15 days before harvest resulted in quality oil production of both “Arbequina” and “Picual” olive trees.

ea. Enzymes and hormones

Working on “Hojiblanca” olive, Heredia et al. (1990) had harvested the fruits at different degrees of ripeness (green, changing color and black). They recorded the cellulase activity increased from zero in unripe fruits to 2500 relative units’ g\(^{-1}\)h\(^{-1}\) in over-ripe fruits. The initial increase in cellulase activity coincided with the increase in ethylene production which confirmed that ethylene was the initiating agent of cellulase activity.

Zhang and Zhang (2009) treated grape cells in the abscission zone with growth regulators and mentioned that ABA enhances the cellulase activity, and accelerates the decomposition of cellulose and pectin, which determines the level of development of abscission zone and berry falling. 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. The results indicated that ethephon and exogenous ABA enhanced the berry falling.

f. Histological characteristics

Precisely identifying separation location is critical for testing the effectiveness of harvest procedures, including the mode of action of different fruit loosening chemicals.

Polito and Lavee (1980) serially sectioned olive to characterize ethylene-induced abscission zone. They recorded the absence of perivascular fibers and shortened vascular elements occurred at the petiole base of mature leaves. Separation, preceded by swelling of cell walls and the dissolution of the middle lamella between adjacent separation layer cells, began in the abaxial cortical parenchyma in the region
of the vascular tissue. Neither cell divisions, tylose formation, nor lignifications occurred in abscission zone cells up to the time of leaf drop. The mechanism of leaf abscission is compared with that for fruit abscission, and found to be structurally similar.

Estornellet al. (2013) summarized that abscission consists in the detachment of entire organs due to cell separation processes occurring at the abscission zones (AZs) at specific 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.

Barrancoet al. (2002) 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.
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 uniform “Koroneiki” olive trees planted at 5 x 8 m apart in a sandy soil. The trees received the common horticultural practices such as fertilization, irrigation, pruning, and pest control.

Trees were sprayed with the following loosening agents: ethephon (2-chloroethylphosphonic acid) at the concentrations of 300, 600 and 1200ml/ L, Mono potassium phosphate MPK (KH₂PO₄) at 2%, 3% and 4%, Phosphoric acid (H₃PO₄) 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 which was sprayed with water. Trees were sprayed at two times (7 and 15 days before harvesting). Hand vibrator harvester (Zanon® Oliver electro telescopic Al 200/O) was used by pass it over the treated branches with the different treatments at the harvesting date, that was about 150 ± 20 days after full bloom (AFB) in each season, when the fruit color-turning to purple for more than half of skin, but not complete (pulp is still white).

1. Plant vegetative measurements
   a. Leaf drop percentage

   Number of abscised leaves per branch were counted after harvesting and divided on their number before treatments and multiplied to 100:

   \[ \text{Leaf drop}\% = \frac{A}{B} \times 100 \]

   Where:
   
   A= Number of abscised leaves per branch after harvesting
   B= Total number of leaves per branch before treatments

   b. Leaf burns percentage

   Number of burned leaves per branch were counted after harvesting and divided on the total number of leaves before treatments and multiplied to 100:

   \[ \text{Leaf burn}\% = \frac{A}{B} \times 100 \]

   Where:
   
   A= Number of burned leaves per branch after harvesting
B= Total number of leaves per branch before treatments

c. Fruit burns percentage

Number of burned fruits per branch were counted after harvesting and divided on the total number of fruits before treatments and multiplied to 100:

\[
\text{Fruit burns\%} = \frac{A}{B} \times 100
\]

Where:
A= Number of burned fruits per branch after harvesting
B= Total number of fruits per branch before treatments

d. Fruit removal force (FRF)

At the harvesting day, branches of the sprayed trees by water (control) and the different loosening agents in each concentrations were selected randomly from the four sides of each tree to measure fruit removal force using a digital force gauge (Lutron® FG-5000), by hanging it over the fruit pedicels’ then pulling it down.

2. Chemical properties of oil

a. Oil content

Fruit oil content was determined by means of the Soxhlett fat extraction apparatus using Hexan of 60-80°C boiling point as described by (A.O.A.C. 1975).

Oil was extracted from the samples using chloroform: methanol mixture (2:1, v/v) according to the method described by Kates (1972). The extracts were centrifuged to remove insoluble material and evaporated to dryness on rotary evaporator. The extracted oil was kept in brown glass bottles at 4°C until analysis.

b. Peroxide value

The peroxide value was determined according to Jacobs (1959) by dissolving 5 gm of the oil in a mixture consisting of 60% glacial acetic acid + 40% chloroform. The solution was treated with approximately 0.5 ml of saturated solution of potassium iodide in glass stoppered flask. The flask was shacked in rotary for exactly two minutes, after which 30 ml of distilled water added, and the liberated iodine was titrated with 0.01 N sodium thiosulphate using 1% starch solution as external indicator.
The results were calculated in milli moles per kilogram oil according to the following equation.

\[
\text{Peroxide value} = \frac{0.5 \times N \times V \times 100}{\text{Weight of sample}}
\]

Where:
- \(N\) = Normality of sodium thiosulphate solution
- \(V\) = Volume in ml. of sod. thiosulphate needed for titration

**c. Iodine no.**

The degree of unsaturation of oil was determined by measuring the amount of halogen absorbed by the oil as stated in (A.O.A.C., 1975). (Ca 0.1 – 0.5) of oil was dissolved in 10 ml of chloroform and 25 ml of Hanus iodine solution were added. After 30 min; 10 ml of 15 % potassium iodine solution and 100 ml of freshly boiled cooled distilled water were added. The liberated iodine was titrated with 0.1 N sodium thiosulphate using starch indicator.

**d. Acid value**

Five grams of oil were weighed in 250 ml dry conical flask with 100 ml of neutralized (50 % ethanol + 50 % petroleum ether) to dissolve the oily sample. Acid value was determined by titration with 0.1 N potassium hydroxide solution in the presence of phenolphthalein as indicator (A.O.C.S., 1964). The acid value was calculated by using the following equation.

\[
\text{Acid percentage} = \frac{V \times N \times 5.61}{\text{Weight of sample}} \times 100
\]

Where:
- \(V\) = Volume of KOH solution
- \(N\) = Normality of potassium hydroxide solution

**3. Enzymes activity and hormones analysis**

**a. Cellulase activity**

Cellulase activity was determined according to (Durbin and Lewis, 1988) method with some modifications. The crude protein extraction buffer consisted of
10mM Na$_2$HPO$_4$ / NaH$_2$PO$_4$, 10mM mercaptoethanol, 1mM Na$_2$-EDTA, 0.1% triton X-100 and 0.15M NaCl. One gram of the frozen fruit pedicels was homogenized with 2ml of the extraction buffer in an ice cooled mortar. The mixture was then centrifuged at 10000g for 10 minutes at 4 °C. After centrifugation, 0.8ml of 0.3% sodium carboxymethyl cellulose (CMC) was added to 0.4ml of the enzyme extract (supernatant after centrifugation) and mixed well. The mixture was then incubated at 30 °C for 1 hour. For the control, the enzyme extract was substituted with 0.4ml of distilled water. To determine viscosity, the time needed for 200µl of the reaction mixture to pass through a micro-viscometer was determined using a stopwatch. Viscosity data were converted to intrinsic viscosity and relative units of activity; providing a linear relationship between viscosity and enzyme activity. The linearized cellulase activity unit was calculated also as follows (Durbin and Lewis, 1988).

$$B = \{ (\eta^\alpha - \eta^0) Cs \}/t$$

Where:

- **B**: Linearized cellulase activity Unit
- **η**: Intrinsic viscosity after incubation, $= 8 \ (T/T_0 - 1)^{1/8} /Cs$
- **η$^0$**: Intrinsic viscosity before incubation, $= 8 \ (T/T_0 - 1)^{1/8} /Cs$
- **Cs**: Substrate concentration, g/l;
- **t**: Incubation time, s;
- **T**: Time needed for reaction mix to pass through viscometer, s;
- **T$_0$**: Time needed for control mix to pass through viscometer, s;
- **A**: Substrate constant, for CMC γHP, $\alpha=3.66$

b. Pectinase activity

The pectinase activity was determined in a similar fashion to cellulase activity. However, the viscometric assay was performed with 1% pectin solution (contained 79.5% galacturonic acid and 8.1% methoxy). Data was expressed as relative activity, defined as percent reduction in viscosity of pectin solutions by pectinase extract compared with controls without enzyme extracts(Abeles and Takeda, 1990). All assays were run in duplicate and the results presented are the average of two assays.
c. ABA content

The concentrated aqueous phase was acidified to pH 2.8 with 1% HCL then shacked well with ethyl acetate 1:1. After that, ethyl acetate phase used for ABA determination by GLC. The retention time (RT) of peaks of authentic samples were used in identification of peaks of the samples under investigation compared with relative retention time (RRT) of ABA standard peaks, (Wasfy et al., 1975).

4. Anatomical study

It was intended to carry out a comparative microscopic examination on treated fruits pedicel which showed the most prominent response with both ethephon and H$_3$PO$_4$ 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 the method of (Sass, 1940). Then, Pedicels were embedded in paraffin wax at 60 ºC. 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 the method of (El-Agamy et al., 2002) then dehydrated, cleared with xylene. After that, the slides mounted in Canada balsam (Johansen, 1940), then covered with a fine glass covers.

Measurements (µ) and counts were applied using micrometric lens on light microscopy with magnification power (6*10 = 60x)

5. Economic study

A partial budget consists of two columns (Ronald et al., 2004), which are shown in (Table 4), a subtotal for each column and a grand total. The right hand column has the items that increase income while the left hand column notes those that reduce income for a farm business, taking into consideration that all the factors that were not mentioned in this budget is one of the constants in both methods of harvesting. So, the budget can be divided into four parts:
The net impact of the above effects will be the positive financial changes minus the negative financial changes. A positive net indicates that orchard income will increase due to the change. While, a negative net indicates the change will reduce orchard income, so, it is not recommended to apply this change in this case.

6. Experimental design and statistical analysis

The experiment was laid out as a factorial experiment in randomized complete block design (RCBD), where the experiment involved twenty six treatments with three replicates, each one of them represented by one tree of the studied cultivar. Thus, seventy eight trees were allotted for each season. The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using MSTAT software (1998). Means were differentiated using Duncan multiple range test at significance level of 0.05 (Duncan, 1955).