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Application of different concentrations of licorice and willow extracts as rooting stimulator in hardwood cuttings of olive (*Olea europaea L.*)

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Abstract—Rooting in hardwood cuttings of olive was investigated at the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq under application of licorice and willow extracts with concentrations of (0, 3, 6 and 9 g.L⁻¹). The extracts were prepared from licorice root and willow shoots in 25% ethanol heated in a water bath at 40°C for 3 hours, refrigerated for 24 hours and filtered through filter paper. The hardwood cuttings of olive cv. (Sorani) were taken from basal part of one-year-old suckers then soaked in the licorice and willow extract concentrations for 1 hour. The results revealed that the highest (66.66%) rooting was achieved in the cuttings soaked in 6 and 9 g.L⁻¹ licorice extract for 1 hour, they were not different in comparison with control cuttings which gave (49.99%), but rooting percentage was reduced (38.8%) in the cuttings soaked in 6g.L⁻¹ willow extract. The cuttings soaked in 9 g.L⁻¹ willow extract and 6 g.L⁻¹ licorice extract gave the best root number, root length, shoot length, shoot diameter and leaf number. Generally, depending on the obtained results in this study, 6 and 9 g.L⁻¹ licorice extract with 9 g.L⁻¹ willow extract have possibility to use as an alternative to induce root formation and improve root and shoot quality of the cuttings in olive.

Keywords—Licorice extract, willow extract, olive cuttings, root formation.

I. INTRODUCTION

Olive propagation is desirably conducted through cuttings to provide guarantee for genetic uniformity and induce earlier bearing in comparison to seedlings from seeds (Awan *et al.*, 2012). Rooting in olive cuttings is restricted by many internal and external factors which is more evident according to cultivar (Hechmiet *et al.*, 2013). Therefore, to overcome the difficulty in rooting, growth regulators are applied to the cuttings of olive in order to induce rooting, including auxins. It is observed that that 3000 ppm IBA was the best dosage for rooting and enhancing other traits in hardwood cuttings of olive (Rahman *et al.*, 2002). However, using synthetic auxins in propagation are not permitted by European and North American regulations to obtain vegetative propagated materials in organic farming (Centeno and Gómez-del-Campo, 2008). Also, application of synthetic growth regulators is not recommended recently because of they

are not friend of environment and may have toxic effect on plant, human and animals (El-Sherif, 2017); synthetic growth regulators are expensive and are not readily available in local markets as well (El-Shaima *et al.*, 2018). Thereby, extract of some plants and natural products have been used as a substitute for synthetic growth regulators to promote rooting onto the cuttings. The more pronounced are extracts of vermin wash, Coconut water, willow leaf water, honey, humic acid, seaweed extract, Aloe vera, cinnamon powder, licorice and yeast extract (Gad and Ibrahim, 2018; Mohammed *et al.*, 2020; Rajan and Singh, 2021).

Many researches have emphasized on root extract of licorice as a source of natural stimulant compounds which could be used instead of synthetic growth promoters because it contains phenolic compounds, mevalonic acid, amino acids, vitamins, biotin, folic acid, pantothenic acid, and many minerals (El-Dengawyet *et al.*, 2017). Licorice

root extract is also counted as a source of phytohormones which have an important role in root formation onto the cuttings (Rady *et al.*, 2019). Eide *et al.* (2018) reported that licorice extract is among the plant extracts could be considered as an alternative to growth regulators for rooting Picual olive cuttings. On the other hand, willow bark, shoot or leaf extracts contain growth promoting chemicals may effectively enhance rooting onto the cuttings. For example, some root-promoting and diffusate substances were observed in *Salix alba* which synergistically interact with IBA to augment rooting on mung bean cuttings (Kawase, 1970; Al-Amad and Qrunfleh, 2014). In addition, salicylic acid (SA) is a plant hormone prevalently was found in willow extract which stimulates root formation onto the cuttings (Sandoval-Yapiz, 2004; Hayat *et al.*, 2010). Wise *et al.* (2020) summarized that 1.06 $\mu\text{L/L}$ willow bark extract gave the best results with the cuttings of chrysanthemum and lavender. Thus, this study was carried out to determine the best concentration of licorice root extract and willow shoot extract for inducing rooting in hardwood cuttings of olive.

II. MATERIALS AND METHODS

The study was carried out at the College of Agricultural Engineering Sciences, University of Sulaimani, Kurdistan Region-Iraq to determinate the best concentration of willow shoot extract and licorice root extract for inducing root formation onto olive hardwood cuttings cv. (Sorani).

2.1 Preparation of the cuttings

The hardwood cuttings of olive cv. (Sorani) were taken on February 10, 2021 from basal part of the suckers of previous year with 20 cm long and about 4.5-6.5 mm diameter. After preparation, the bases of the cuttings were soaked in (0, 3, 6 and 9 g.L^{-1}) of licorice and willow extracts separately for 1 hour. Following treatment, the cuttings were planted in polyethylene bag with a size of 12×30 cm filled with sand medium. The experiment was laid out in RCBD design with three replications in a lath house, and in each bag six cuttings were planted. Initially, because of low temperature, the cuttings were covered with a polyethylene UV plastic sheet until April 14, 2021 then the plastic cover was removed till the time of taking the results. The average of maximum and minimum temperature inside the plastic cover was between 9-37 °C and inside the lath house after removing the plastic sheet was between 17.2- 41.3 °C.

2.2 Preparation of licorice and willow extracts

The dried root of licorice and dried one- and two-year-old shoots of willow were grinded and weighted in required amount, then the volume was completed with 25% of ethanol and placed in a water bath at 40°C for 3 hours. After that, they were taken out from the water bath and refrigerated for 24 hours. In the next day, the extracts were filtered through filter paper and applied to the cuttings.

2.3 Statistical analysis

After 4 months and 20 days (on June 30, 2021) the cuttings were checked to calculate rooting percentage, root number, root length, shoot length and leaf number. XLSTAT software version 2019.2.2, one-way ANOVA-RCBD and Duncan's multiple-range used for analyzing the data.

III. RESULTS AND DISCUSSION

The data in figure (1) showed that licorice and willow extracts did not significantly induce rooting percentage in hardwood cuttings of olive in comparison with control cuttings. However, rooting percentage was reduced in the cuttings soaked in 6 g.L^{-1} willow extract, and they were statistically different compared to the cuttings soaked in 6 and 9 g.L^{-1} licorice extract. The highest (66.66%) rooting was achieved in the cuttings soaked in 6 and 9 g.L^{-1} licorice extract, but the lowest (38.8%) rooting achieved in the cuttings soaked in 6 g.L^{-1} willow extract, while control cuttings and the cuttings soaked in 3 g.L^{-1} licorice and willow extracts gave (49.99%) rooting. These results could be attributed to that extract of licorice contains many chemicals that may induce root formation in cuttings. Also, licorice extract may synergistically elevate the metabolites in cuttings which have important role in rooting process. El-Shaima *et al.* (2018) found that licorice extracts increased rooting rate and root traits in the cuttings of grape similar to IBA and concomitantly resulted in the highest IAA content of the cuttings which is quite possible one of the factors augments rooting in the cuttings. Besides, Tahoori *et al.* (2019) found quercetin flavonoid in licorice extract, and quercetin was considered to be a substance stimulates root formation onto the cuttings (Tarragó *et al.*, 2005). In that connection, do Prado *et al.* (2015) concluded that quercetin was a compound which enhanced performance of *Eucalyptus* cuttings owing to oxidative stress reduction and the encouragement of cell division. Also, phenols were found in licorice extract (Rao, 1993), and phenols were identified as rooting promoter in cuttings (Wilson and Staden, 1990).

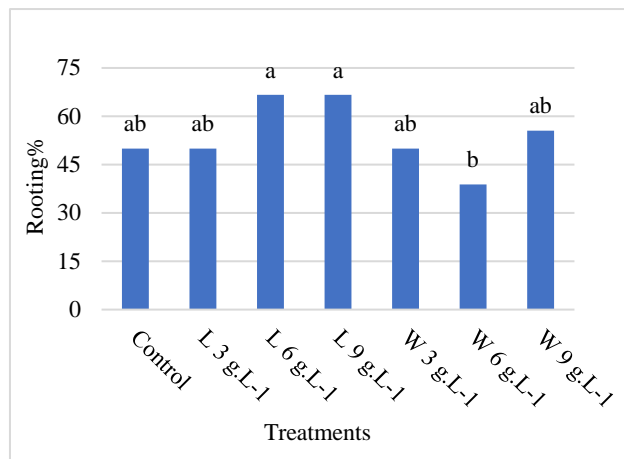


Fig. 1-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on rooting percentage. Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

Despite rooting percentage, extracts of licorice and willow remarkably increased root number (Figure 2). Numerous roots (11.23) found in the cuttings soaked in 9 g.L⁻¹ willow extract followed by (10.5) in the cuttings treated with 6 g.L⁻¹ licorice extract. Control cuttings and the cuttings were treated with 3 g.L⁻¹ willow extract gave the lowest (3.5 and 3.56, respectively) root number. It is noteworthy to mention that root number was increased by increasing concentration of willow shoot extract from 3 g.L⁻¹ to 9 g.L⁻¹. Similarly, the both 6 g.L⁻¹ licorice extract and 9 g.L⁻¹ willow extract significantly improved root length (Figure 3). The longest root (6.2 cm) was obtained in the cuttings soaked in 6 g.L⁻¹ licorice extract along with (5.98 cm) in the cuttings soaked in 9 g.L⁻¹ willow extract. The shortest roots (2.42 and 3.03 cm) were found in cuttings treated with 3 g.L⁻¹ willow extract and control cuttings, respectively. Willow contains phytohormones such as auxin and salicylic acid that may influence rooting and root traits of the cuttings as used in the form of "willow water" (Knapkeet *al.*, 2018). Arena *et al.* (1997) reported greater root number in semi-hardwood cuttings of *Chionanthusretusus* when they were treated with willow diffusates with IBA after 75 days of the treatment. Al-Amad and Qrunfleh(2014) recognized babylon weeping willow extract to be a factor to raise root number in olive cuttings when they were soaked in it for 2 hours. Moreover, licorice extract 100% was found to be the best treatment to increase root number in grape cuttings near to the results of IBA (El-Shaimaet *al.*, 2018). On the other hand, root elongation of the cuttings in the present study might be due to the stimulating agents exist in the two extracts. Rajan and Singh(2021) mentioned that natural decoction of many plants and natural substances have

efficiency to elongate roots in cuttings, this is due to occurrence of IAA and minerals in the extract of those substances. Wise *et al.* (2020) stated that a major component of willow bark extract is salicylic acid which is a phytohormone improved root growth and development. Additionally, rooting promoters accelerate earlier root formation onto cuttings as a result of breakdown and mobilization of carbohydrates and nitrogenous substances at the base of cuttings, particularly at high concentrations, thus roots are elongated excessively due to utilization of more nutrients (Babaieet *al.*, 2014).

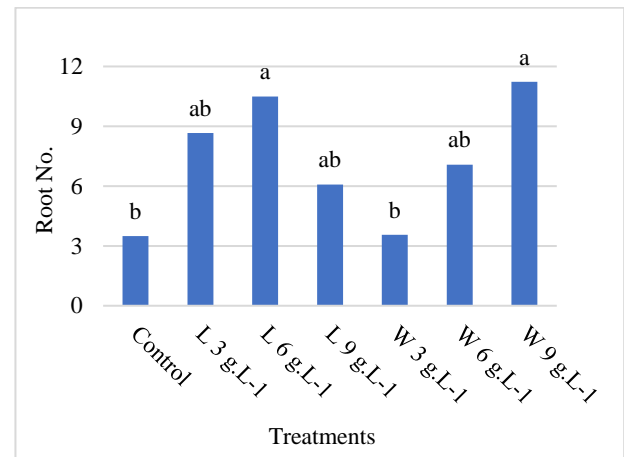


Fig. 2-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on root number.

Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

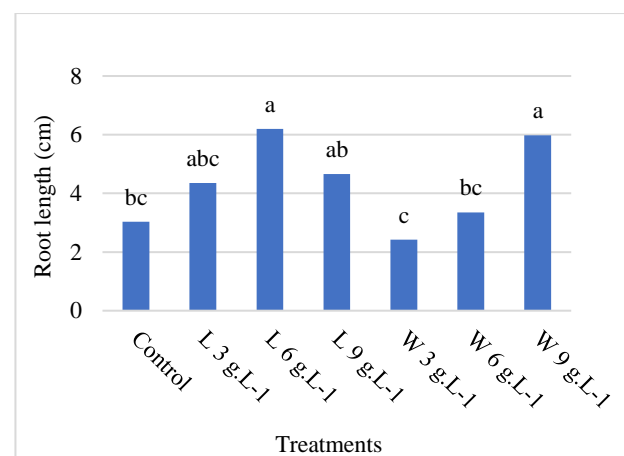


Fig. 3-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on root length (cm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

The results were shown in figure (4) confirmed that licorice and willow extracts gave rise to enhance shoot

length in hardwood cuttings of olive compared to control cuttings. The longest shoot (2.18 cm) was found in cuttings treated with 9 g.L⁻¹ willow extract, but control cuttings gave the shortest shoot (0.74 cm).

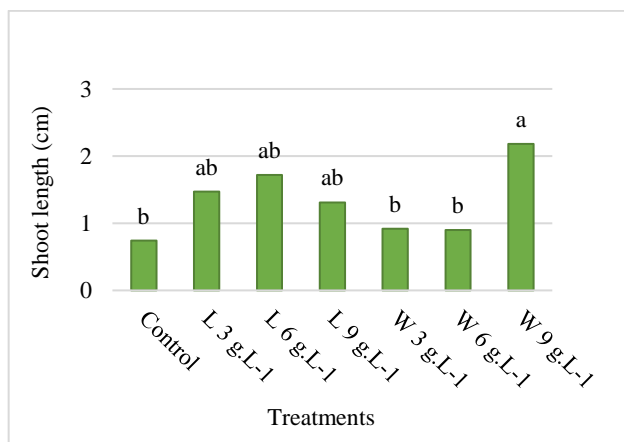


Fig. 4-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on shoot length (cm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

Furthermore, shoot diameter was also significantly different according to concentration of the both extracts (Figure 5); 9 g.L⁻¹ willow and licorice extracts increased shoot diameter significantly and reached in (1.41 and 1.38 mm, respectively), in contrast shoot diameter reduced to the lowest (0.91mm) in control cuttings.

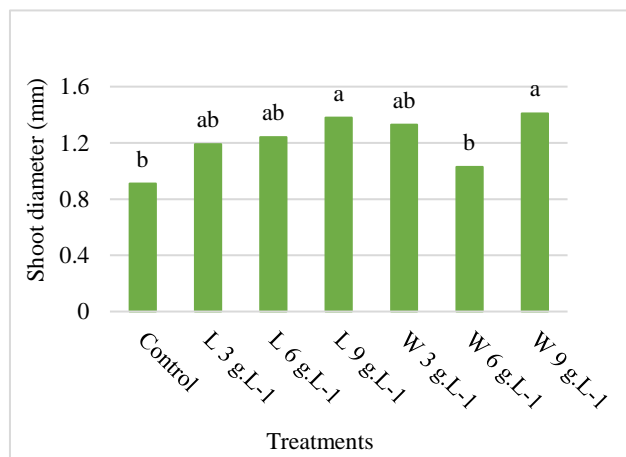


Fig. 5-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on shoot diameter (mm). Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

Additionally, leaf number was notably elevated (17.02) in cuttings soaked in 6 g.L⁻¹ licorice extract (Figure 6),

contrarily leaf number diminished to (7.66) at 3 g.L⁻¹ willow extract and control cuttings (8.13).

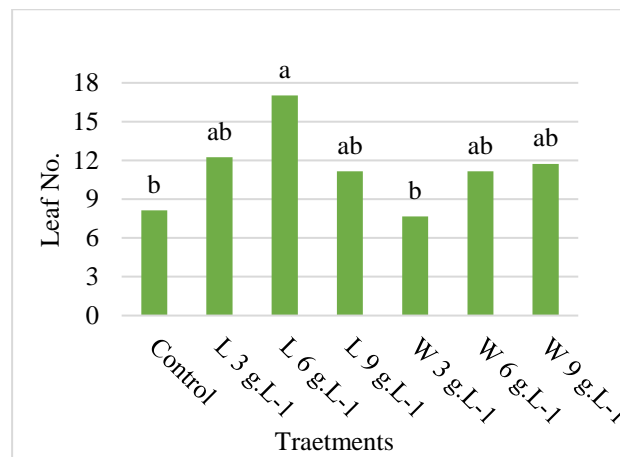


Fig. 6-Effect of different concentrations of licorice root extract (L) and willow shoot extract (W) on leaf number.

Means sharing the same letter are not significantly different according to Duncan's multiple-range Test at $p \leq 0.05$.

The above results revealed that the concentrations of the two extracts which encouraged better root number and length also gave the best shoot traits in terms of shoot length, shoot diameter and leaf number. Therefore, the analysis of correlation of the means in this study confirmed there was a positive association among root number and root length with shoot length and leaf number (Figure 7). According to the correlation analysis, root number and root length positively correlated with shoot length ($r = 0.91$, p -value = 0.005 and 0.004, respectively) and with leaf number ($r = 0.82$, p -value = 0.023 for root number) and ($r = 0.84$, p -value = 0.017 for root length). Perhaps these are because of better root characters lead to absorb more water and nutrients needed to superior shoot growth. Shukla *et al.* (2010) mentioned that growth of shoot in cuttings relies on favorable balance between root and shoot ratio crucial for uptake and translocation of water and nutrients. Branislavet *et al.* (2009) summarized that vigorous shoot growth and development occurred in poplar cuttings subsequent earlier intensive root formation. Besides, it is possible that the extracts directly promoted shoot growth of the cuttings via inducing growth of buds as a result of metabolize reserved food and biostimulant effects on the growth of buds and formed shoots. Chandramouli (2001) showed that high concentration of auxin elongated shoot on the cuttings through elevating better utilization of nutrients such as carbohydrates and nitrogen. Ingle and Venugopal(2009) referred that elongation of shoot caused high node number which in turn means high leaf number. Apart from, a balance of auxin and cytokinin in cuttings is

decisive to the best growth of vegetative parts, and cytokinin and auxin activate the genes involved in growth and regulate cell division in a harmonious manner (Bredmose *et al.*, 2004). Auxin and cytokinin were found in licorice and willow extracts (Fujita *et al.*, 2014; Rehman *et al.*, 2018; Desoky *et al.*, 2019).

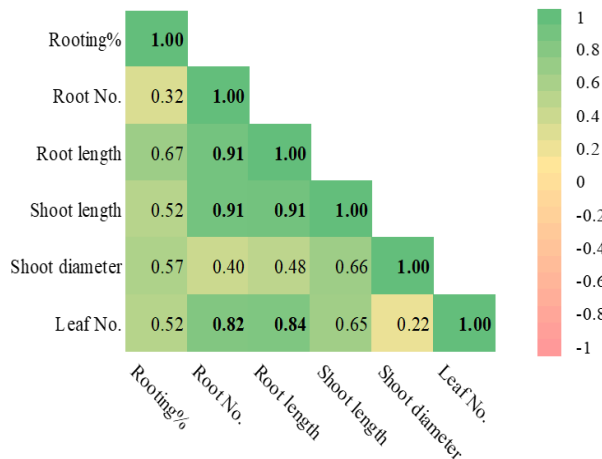


Fig. 7-Pearson correlation analysis of the six studied characteristics.

IV. CONCLUSION

The results of this study indicated that rooting percentage in hardwood cuttings of olive was increased by soaking them in 6 and 9 g.L⁻¹ licorice root extract, while 9 g.L⁻¹ willow shoot extract together with 6 g.L⁻¹ licorice extract improved most other shoot and root characteristics.

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