Original Research Article

Effect of indole butyric acid and zinc sulphate in different media on rooting of olive cuttings

Abstract

Olive (*Olea europaea* L.) is an oil fruit crop with the physiological potential to survive and produce in rain-fed areas. In Pakistan, it is grown in upper Punjab and Baluchistan due to their climate suitability for better production. Due to the increasing trend of olive oil, peoples show more interest in planting disease-free plants for better oil production. The successful nursery of olives mainly depends on growth substrates with rooting hormones like Indole butyric acid. A recent study was conducted to observe the influence of growing substrate and combination of IBA with zinc sulphate during the vegetative stage of two cultivars of olive. Semi-hardwood olive cuttings were treated with indole butyric acid (IBA) at a level of 3000 ppm with zinc sulphate at 2000 ppm and 3000 ppm and cultured on various types of soil in pots under plastic tunnel conditions under completely randomized design with three factors and each treatment replicated thrice. Both varieties with all treatments significantly affect all traits. The maximum number of shoots were recorded in the media that contain sugarcane press mud with 3000ppm IBA and 3000ppm zinc sulphate that showed the maximum number of shoots (5. 66), branches (6. 66), shoot length (17. 01), number of roots (5. 33), root diameter (0. 23), plant survival percentage (73. 66) and root length (16. 86). In contrast, the minimum number of shoots (1. 0), branches (1. 00), shoot length (3. 77), number of roots (1. 00) root diameter (0. 01), plant survival percentage (44. 33), root length (4. 92) were observed in control media in cultivar Ottobrattica with the treatment of IBA at 3000 ppm+ zinc sulphate 2000 ppm. Results from all treatments revealed a favorable correlation between medium and IBA when combined with zinc sulphate for all measured growth parameters. The 50% sugarcane press mud, 50% silt soil with IBA 3000 ppm+ zinc sulphate 2000 ppm proved to be the best approach for better germination of olive cuttings in pots. The resulting outcomes play a significant role in the better germination percentage under dry climatic conditions. Analysis of variance was used to examine the data obtained (Statistix 8.1). The least significant test (LSD) was used to compare significant means at a 5% probability level.

Keywords: olive, IBA, Zinc sulphate, Physical parameter, cuttings

Introduction

Olive (*Olea Europaea*) is a broad-leaved subtropical plant that belongs to the family Oleaceae. it has edible fruit and is enriched with oil contents. It is commonly known as "zaitoon" (Asadollahi *et al.*, 2019). Olive is well known for its nutritional value as edible fruit and oil which plays a key role in its popularity (Ribeiro *et al.*, 2017). It originated from the islands of Greece; its cultivation has been started in 3000 BC. After Greece, it was widespread in Europe and around the globe in almost every country since about 6000 BC. Olive bears fruit for the first time at the age of 4-8 years and proper yield potential is achieved after 15 years of plantation. Mature fruits are processed for oil extraction while immature fruits are processed for food processing and product development. Olive cultivated varieties are divided into two categories that include table verities and oil-producing varieties. Table verities are used for medicinal purposes as well as raw fruits while another category is solemnly used for oil extraction (Anguita-Maeso *et al.*, 2021).

Olive plants can grow in low irrigation facilities or in arid zones which have proven to be its natural habitat. Wild olive plants have been found in various regions of all provinces that have which indicates that Pakistan could be self-sufficient in olive production (Cabezas *et al.*, 2020). Millions of acres are found to provide favorable climatic zones for its production and Pakistan's Pothwar valley is entitled as "Olive valley" to achieve self-sufficiency in oil production Pakistan's agro-climatic conditions are not exactly like Mediterranean countries but favorable climate results in the exponential growth of verities reported to be the best performing in Baluchistan include, Arbequina, Arbosana Frantoio, Leccino, Coratina, Kalamata, Gemlik, Oliana, Manzanilla, Hojiblanca, Picual, Chakwal-I, and Chakwal- II (Chaudhry, 2018). According to earlier research, olive oil quality attributes are influenced by genetic and environmental factors, including the olive variety (Kosma et al., 2016), geographic origin, climate, and soil quality, growing conditions, including harvest timing and irrigation strategies (Borges *et al.*, 2017).

Pakistan's environment is ideal for growing olives, but there isn't much of it being cultivated. Since a number of decades, olive orchards have been planted in Pakistan (Toensmeier, 2016). Commercial olive cultivation is limited to latitudes between 30 and 450. It needs a chilling temperature of 8°Cto 15.6 °C with day and night temperatures for 85 to 115 days in order to flower (Greer, 2018). As a rain-fed crop, olives typically require 100 to 200 cm of annual precipitation. It thrives in a variety of soil types with good water retention and aeration. The ideal soil has a pH range of 6.5-8. However, muddy ground and standing water are not appropriate (Ferreira *et al.*, 2018). Olive can be successfully reproduced both asexually and sexually by stem cuttings, root cuttings, ovules, suckers, and tissue culture methods. Because it uses the fewest resources and is the simplest methodology, cutting-based propagation is the best strategy for underdeveloped countries (Ak *et al.*, 2021). The transporting of tree cuttings is simple and doesn't require much area. However, stem cutting, which is used extensively in commercial nursery production, is the most simple and affordable way. Success of cuttings depends on their capacity to generate adventitious roots, and different cultivars of olive respond differently to rooting (Barrett *et al.*, 2016). Consequently, there is a significant need for numerous nursery plants in appropriate varieties for these places. The health of the roots is important since it affects how nursery plants function. An effective propagation strategy can enhance the health of roots (Jan *et al.*, 2015). The easiest and least expensive way is cutting-based propagation, which is best for developing nations like Pakistan. The physical properties of the propagation substrate significantly influenced the success of cuttings, and the physiological state of the cuttings (Hoogenboom, 2022). A recent study was conducted to evaluate the germination percentage and other physical parameters of olive cuttings of two varieties using IBA and zinc sulphate with various concentrations of different media in pots.

Objectives

- Determine the impact of IBA and various zinc sulphate concentrations on olive cuttings
- To observe the interaction between zinc and auxin.
- To evaluate the performance of two different olive types side by side.

Materials and Methods

Study site

The Bahauddin Zakariya University Multan Department of Horticulture's nursery was the site of the study. The Barani Agriculture Research Institute (BARI) in Chakwal provided the propagation material. The 10-year-old tree from which the one-year-old branches originated was harvested. The cuttings were transported in jute bags to the testing location. To prevent drying of the branches and maintain the freshness of the cuttings in the resources at hand, the bags were maintained moist. The experiment was carried out in pots with CRD. Cultivars, medium, and IBA + zinc sulphate concentrations were the three variables. BARI-1 and Ottobrattica were concentrated with IBA + zinc sulphate, evaluated with five rooting substrates including control media. To maintain the moisture level, bags were repeatedly wetted.

Treatments

There were three variables: cultivars, medium, and IBA+Zinc sulphate concentrations. Bari-1 and Ottobrattica, two concentrations of IBA+Zinc Sulphate, five rooting substrates, and a control media were all utilized.

Cuttings preparation

Fresh cuttings from branches with scature were made in the experimental area. Branch cuttings were made that were 6-7 inches long and had a pencil-thickness. Before using it in the autoclave, was sterilized. To prevent contamination, the scatures were also dipped while being worked on. In order to provide more surface area for hormone and substrate contact, the upper side has a round cut while the lower side has a slanty cut.

Solution preparation

Auxin (IBA) solution was freshly made in the laboratory. 3 grammes in order to make 3000 ppm solutions, IBA was first dissolved in a 1% NAOH solution and then the volume was increased to 1 litter. A half-hour was spent stirring the solution to homogeneity. The 1000 ml beaker with the fluid inside was brought to the testing location. In order to make 2000 and 3000 ppm of zinc sulphate heptahydrate, separately 2 and 3 grammes of zinc sulphate were dissolved in IBA solution 1000ml (Table 1).

Table 1. The concentration of IBA and Zinc sulphate in two varieties of olive

Variety	**Chemical**	**Concentration (ppm)**
Ottobrattica	Indole butyric acid	2000
		3000
BARI-1	Zinc sulphate	2000
		3000

Media preparation

The cuttings were planted on peat moss, sugarcane, farm yard manure, sawdust, and five other media. Each medium was made by mixing an equal amount of each with 50% media and 50% soil by volume. The media was placed inside clear polythene bags. The bags measured 7 inches in length and 5 inches in width (Table 2).

Table 2. The various types soil were used in the experiment

Media	**Soil type**	**Percentage**
M_0	Silt soil	100%
M_1	Silt soil, Sugarcane press mud, Saw dust,	25% each
M_2	Saw dust Silt soil	50% 50%
M_3	Farm yard manure Silt soil	50% 50%
M_4	Peat mass Silt soil	50% 50%

M_5	Sugarcane press mud Silt soil	50% 50%

Shifting of cuttings

Cutting was shifted in each bag containing all media after being dipped in the IBA+Zinc solution. Transparent polythene covering was then placed over the test area. The data were recorded after 5 months of experiment. Following physical parameters were recorded.

Physical parameters

- No. of shoots
- No. of branches
- No. of roots
- Shoot length (cm)
- Root length(cm)
- Root diameter(mm)
- Plant survival percentage

Statistical analysis

The recorded data of physical parameters and survival percentage were analyzed using analysis of variance (ANOVA). Means and standard error were recorded using statistics 8.1 software and least significance test (LSD) at a 5% probability level.

Results and Discussion

The analysis of variance of study of media in combination with IBA 3000 ppm and Zinc sulphate at a concentration of 2000 ppm and 3000 ppm revealed that results are highly significant for media, auxin, and varieties. Significant variations in rooting length, shoot length, and the number of shoots was founded regarding rooting substrate, cultivars, and their interactions. Analysis of variance was significant regarding root diameter and numbers of roots relating to auxin, media, and cultivars and their interactions (Fernández-Paz et al., 2021) (Table 3).

Table 3. Mean square values of the number of roots, branches, shoots, root diameter, length and plant survival percentage

SoV	DF	NB	NR	RD	RL	NS	PS%
Media×Treatment×Variety	5	0.32*	0.18**	0.00**	0.67**	0.08	0.70
Error	48	0.05	0.01	0.00	0.13	0.06	0.55

SoV= Source of variation, DF= degree of freedom. *= significant, **=highly significant, DS= days to sprout, NB=number of branches, NL= number of leaves, NR= number of roots, RD= root diameter, RL= root length, NS=number of shoots, PS%=plant survival percentage

Number of Roots

In sugarcane press mud BARI-1 variety concentrated with IBA at 3000 ppm + Zinc Sulphate at 3000 ppm as opposed to Ottobrattica and in M0 (Control) media with both of IBA + Zinc Sulphate, the most information regarding the effects of various media, treatments, and varieties on various roots was recorded. Auxin may encourage the development of adventitious roots and root growth by stimulating the production of ethylene, according to some research. The BARI-1 performs better in terms of root ability than Ottobrattica because to some genetic variance. Many authors have established the crucial function auxin plays in the development of adventitious roots. The amount of auxin-type Phytohormones used to treat cuttings has a significant impact on root initiation, which also increases the rooting percentage. Additionally, IBA concentration-related rooting induction research was conducted by (Cirillo *et al.*, 2017). The cambial activity, which aids in the induction of roots and is sped up by the use of external IBA application, may be the cause of the large number of rooting (Jan *et al.*, 2015). Due to the synergistic effects of zinc, cuttings with higher zinc concentrations and IBA grow more roots. Because of the silt media's small particle size and high water retention capacity, which suffocates cuttings at their base, it is not suited for rooted cuttings. A smaller number of roots were recorded as a result. In the experiment, the sugarcane press mud media produced more roots than the other types of media because it was soft and nutrient-rich. However, having a medium with sufficient drainage is the key to success for commercial production (Ganesh *et al.*, 2015). It was discovered that treatments with 3000 ppm zinc sulphate in addition to 2000 ppm IBA showed the greatest numbers of roots. The start and growth of cuttings are essential to their success. Success in rooting is influenced by the variety, cutting kinds, indole butyric acid concentrations, and physical characteristics of the rooting medium, especially for cultivars that were challenging to root (EL Sabagh, 2021) (Table 4).

Number of Branches

In both IBA + Zinc treatments, the cultivar Ottobrattica developed the fewest branches in control rooting media and M1 (mix media), whereas BARI-1 produced the fewest branches in control media. The greatest number of branches were visible in V2 (BARI-1) after treatment with IBA + Zinc Sulphate and rooting media containing IBA 3000ppm sugarcane press media (Table 4).

Root Diameter

The results of comparing the media, treatments, and varieties in terms of root diameter revealed that the maximum root diameter was (0.23mm) in M5 (sugarcane press mud) in V2 (BARI-1) with T2 (IBA at 3000 ppm + Zinc Sulphate at 3000 ppm), and the minimum root diameter was observed in V1 (Ottobrattica) in M0 (Control) media with T1 (IBA at 3000 ppm) (0.01mm). Root diameter serves as an indicator of root integrity. The root diameter is a product of the environmental conditions in the plant's root zone. The mud from the sugarcane press has greater physical and chemical properties, such as the nutrients needed for root growth (Table 4).

Root Length

In contrast to media, treatment, and variety, it was discovered that the root length varied greatly. According to estimates regarding root length, the greatest root length was observed in M5 (sugarcane press mud) in V2 (BARI-1) with T2 (IBA at 3000 ppm + Zinc Sulphate at 3000 ppm), and the smallest root length was observed in V1 (Ottobrattica) in M0 (Control) media with T1 (IBA at 3000 ppm + Zinc Sulphate at 2000 ppm). The characteristics of sugarcane press mud that were crucial for root growth led to the longest possible root length. The physical and chemical features of the material promote root development. With better nutritional conditions, farmyard manure may contain some harmful compounds that slow root growth. Likewise, sawdust is lingo-cellulosic and does not degrade readily during biodegradation. Cuttings' capacity to develop adventitious roots determines how well they take, and different cultivars of olive exhibited varying rates of rooting. Because there are more roots and healthier shoots, which produced more auxin, IBA has a direct impact on root length. Conversely, Auxin lengthened the root (Al-Hamdani *et al.,* 2017) (Table 4).

Numbers of shoots

The results revealed that M5 (sugarcane press mud) in V2 (BARI-1) with T2 (IBA at 3000 ppm + Zinc Sulphate at 3000 ppm) had the highest number of shoots (5.66), whereas V1 (Ottobrattica) and V2 (BARI-1) had the lowest number of shoots (1) in M0 (Control) and M1 (mix media) for both IBA + Zinc. The ability of a cultivar to take root differently may lead to distinct shoots in that cultivar. The health of the plant's root zone's nutrients has a direct impact on the growth of a shoot. Roots carry nutrients to shoots from the root zone. The healthy substrate gave rise to healthy roots, and the state of the roots and nutrients promotes shoot growth through an increase in sprouting points. All of the aforementioned parameters for cuttings are satisfied by using sugarcane press mud as a substrate for roots. A combination of media (Peat + Sand) was discovered to be superior to just peat (Suman, 2017) (Table 4).

Shoot Length

The length of the shoots and branches on sprouted cuttings is a direct indicator of their health. Root quantity and growth have an impact on shoot length (Jan *et al.,* 2015). Exogenous application of the IBA has been shown by numerous investigations to improve rooting substrate. A superior shoot system is produced as a result of the synergistic interaction between auxin and zinc during rooting. When combined with zinc sulphate, the sugarcane press mud, Barri-1, and IBA have a synergistic effect on one another. Press mud is nutrient-rich, providing more of the nutrients needed for growth and development. Zinc was used to increase IBA's effectiveness. The ability of olive cuttings to root and the length of the shoots vary from variety to variety. As documented in mango cuttings, the combination of zinc and auxin promotes the rooting of cuttings because zinc is involved in the synthesis of IAA (Ashiono *et al.*, 2017) (Table 4).

Plant survival percentage

The comparison of the media, the amount of nutrients present, and the type of plant with regard to plant survival rate revealed statistically different results, according to the analysis of variance. The highest plant survival rate was noted in M5 (sugarcane press mud) in V2 (BARI-1) with T2 (IBA at 3000 ppm + Zinc Sulphate at 3000 ppm), and the lowest plant survival rate was noted in V1 (Ottobrattica) in M0 (control) media, according to the study. The nutritional quality of a plant and the health of its roots are both important for its survival. Cutting survival after IBA treatment is correlated with Phytohormones' capacity to promote adventitious roots. Auxin and zinc work together to promote the development of more robust roots, which improves plant survival. In terms of root length mean values, topsoil performed the best. In terms of the plant's survivability, sugarcane press mud has a greater chance than peat moss, FYM, sawdust, and mix. The sugarcane press mud outperforms all other rooting substrates in terms of all physical and chemical characteristics (Das *et al.*, 2020) (Table 4).

Table 4. Means of interaction (Growing media × IBA + Zinc concentrations × varieties) for no. of branches, roots, root diameter, length, plant survival % age, shoot length and no. of shoots

soil media × IBA + Zinc conc. × varieties	No. of branches	No. of Roots	Root Diameter	Root Length	Plant survival % age	Shoot Length	No. of shoots
M0 T1V1	1.00 g	1.00 f	0.01 r	4.92 r	44.33 o	3.77 u	1.0 g
M0 T1V2	1.00 g	1.00 f	0.03 pq	6.69 p	50.66 m	5.93 s	1.0 g
M0 T2V1	1.00 g	1.00 f	0.02 q	5.88 q	47.00n	5.16 t	1.0 g
M0 T2V2	1.00 g	1.00 f	0.03 p	7.20 op	53.33 l	6.40 r	1.0 g
M1 T1V1	1.33 g	1.00 f	0.05 o	7.46 o	54.00 l	7.00 q	1.00 g
M1 T1V2	2.00 f	1.00 f	0.07 n	8.45 mn	56.00 jk	8.70 n	1.00 g
M1 T2V1	2.00 f	1.00 f	0.07 n	8.17n	55.33 k	7.41 p	1.00 g
M1 T2V2	2.00 f	2.00 e	0.09 m	9.04 lm	56.00 jk	8.29 o	1.00 g
M2 T1V1	2.00 f	2.00 e	0.09 m	8.60 mn	57.00 j	9.09 n	1.66f
M2 T1V2	3.00 e	2.00 e	0.10 kl	9.64 jk	60.33 h	10.69 l	2.00 ef
M2 T2V1	2.33 f	2.00 e	0.09 lm	9.33 kl	58.66 i	9.62 m	2.00 ef
M2 T2V2	3.00 e	2.00 e	0.10 jk	10.04 ij	62.00 g	11.46 k	2.00 ef
M3 T1V1	3.00 e	2.00 e	0.11 ij	10.23 hij	62.66 g	11.89 j	2.33 e
M3 T1V2	3.00 e	2.00 e	0.12 gh	10.69 gh	64.33 f	12.42 hi	3.00 d
M3 T2V1	3.00 e	2.00 e	0.12 hi	10.35 hi	63.00 g	12.32 i	3.00 d

M3 T2V2	3.66 d	3.00 d	0.13 fg	11.01 g	65.00 f	13.16 g	3.0 d
M4 T1V1	4.00 d	3.00 d	0.13 ef	11.26 fg	65.00 f	12.73 h	3.00 d
M4 T1V2	4.00 d	4.00 c	0.14 e	12.04 de	67.66 d	13.78 ef	3.66 c
M4 T2V1	4.00 d	3.00 d	0.14 e	11.77 ef	66.33 e	13.50 fg	3.00 d
M4 T2V2	5.00 c	4.00 c	0.15 d	12.48 cd	68.00 cd	14.15 de	4.00 c
M5 T1V1	5.00 c	4.00 c	0.16 c	12.96 c	69.00 c	14.49 e	4.00 c
M5 T1V2	6.00 b	5.00 b	0.19 b	14.11 b	73.00 ab	16.19 b	5.00 b
M5 T2V1	5.00 c	4.00 c	0.17 c	13.67 b	71.83 b	15.46 c	4.66 b
M5 T2V2	6.66 a	5.33 a	0.23 a	16.86 a	73.66 a	17.01 a	5.66 a
LSD	0.38	0.19	7.97	0.59	1.21	0.40	0.43

M = type of substrate, T = concentration of IBA and Zinc sulfate and V = variety in the text, but If the Authors could add below the table the legend of different initials (M0, M1, M3, M4 and M5; T1 and T2; V1 and V2) for facility of the lecture.

Conclusion:

The experiment's findings indicated that cultivar Ottobrattica in all-medium with IBA at 3000ppm+Zinc Sulphate at 2000ppm excelled. Bari-1 on rooting substrates containing 50% sugarcane press mud and 50% silt soil (v/v). According to the results of the aforementioned experiment, 50% sugarcane press mud, 50% silt soil, IBA at a rate of 3000 ppm, and a dose of 3000 ppm zinc sulphate are the best alternatives for the asexual propagation of olive cuttings.

Novelty Statement

Indole butyric acid with zinc sulphate work best under sugarcane press mud soil media for better germination and survival percentage of olives cuttings.

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