Research Article

Evaluation of Different Chemicals Response on Rooting Behavior of Olive (*Olea europaea* L.) Semi-Hardwood Cuttings

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Abstract | Olive (*Olea europaea* L.) is an important emerging oil crop in Pakistan. To ensure the provision of true to type healthy saplings, there is a dire need to optimize the protocols for nursery production. In order to attain success in this sector, investigation was carried out to improve the rooting ability of a widely grown olive cv. "Arbequina". This research aimed to enhance the rooting ability of semi-hardwood cuttings through application of different separate and combined concentrations of indole butyric acid (IBA), urea phosphate (UP) and paclobutrazol (PB). These chemicals were evaluated under seven treatments in controlled greenhouse environment and tested for different rooting parameters. The results obtained showed significant effect on rooting ability of the cuttings. The appealing results obtained at T_6 (IBA+UP+PB) where maximum rooting 56.8% with average number of roots per cutting 4.4 and root length 4.3 cm was observed. This treatment also showed higher callusing 80.5%, sprouting 83.3% and survival rate 51.1% as compared to all other treatments. Among other treatments T_5 (IBA+PB) and T_4 (IBA+UP) are ranked second and third respectively in term of higher rooting ability. The minimum rooting was observed in T_7 (UP+PB). The present study demonstrated the effect of combined treatment of IBA+UP+PB on stimulation of rooting and survival of cuttings compared with a treatment of IBA (T_1) being conventionally used.

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Introduction

The cultivated olive (*Olea europaea* L.) is a member of Oleaceae family and is a perennial, evergreen tree among the ancient cultivated fruit plants (Hussein *et al.*, 2017). The olive cultivation originated from Palestine, Lebanon, North West Syria and Cyprus and latterly its cultivation spread to other parts of the Mediterranean region especially Italy, Spain and North Africa (Jan *et al.*, 2014; Jan *et al.*, 2017). More than 95% of the world olive groves are located in the Mediterranean countries, which produces around 95% of the olive oil and 75% of the table olives which are consumed for various purposes (Gad and Ibrahim, 2018).

Olive is a recent introduction in sub-continent where it has an enormous potential especially in Pakistan. Millions of wild olives (*Olea cuspidatae*) trees are growing in various parts of the country. Which indicates the favorable environment for cultivated specie (*Olea europaea* L.) in these regions particularly Pothwar (Punjab), Khyber Pakhtunkhwa, Baluchistan, Islamabad Capital Territory, Azad Jammu and Kashmir and Gilgit Baltistan. Presently, it gained momentum in its cultivation due to wider adoptability in western part of Indus River and Pothwar. Hundreds of its varieties are cultivated in world but in Pakistan most of its cultivars are under trials and few are widely and successfully cultivated. Therefore, federal and provincial governments showed keen interest during recent past for mass scale cultivation of olives in Pakistan under various development projects. Resultantly, a massive cultivation of 3.654 million olive trees over an area of 33, 947 acres has been completed in these areas during last eight years (Anonymous, 2021). Being a new crop, country required a lot of attentions in different sectors of olive culture. To ensure the sustainability of this massive plantation and establishment of new olive orchards in the country, the demand for true to type healthy olive saplings is increased.

Olive propagation through seeds is not practiced in the world as the seedling plants remained in juvenility for longer period of time and they produce inferior quality fruit unfit for oil extraction and product development. The cultivated olives are propagated through various vegetative means but commercially semi-hardwood cuttings are used commonly to produce olive nursery plants because this method is least expensive and the easiest one among conventional methods of olive propagation (Dvin *et al*., 2011). Moreover, the nursery plants produced by cuttings possess similar traits as their mother plants have and they bear fruits of desirable characteristics and start fruiting much earlier than those plants which produced from seeds. Many scientists in the past documented that the main constraint in olive propagation is the poor ability of regeneration that ultimately leads to the poor rooting success (Fabbri *et al*., 2004; Hechmi *et al*., 2013).

Plant growth regulators are the synthetic chemicals which facilitate the various growth and development activities within the plants. Their function is to control and modify the different physiological processes of plants. Indole butyric acid (IBA) is the most commonly used plant growth regulator for successful rooting of olive stem cuttings (Isfendiyaroglu and Ozeker, 2008). It is also documented for commercial rooting of wide range of woody plant species (Moshtaghi and Shahsavar, 2011). Adventitious root formation can be stimulated by IBA in olive stem cuttings but in some cultivars, it remained ineffective to stimulate rooting or stimulate it only slightly. Hartmann *et al*. (2011) documented that rooting capacity and the survival of stem cuttings of many woody plant species can be improved by using some plant growth retardants along with IBA application. Salari *et al*. (2017) reported that triazole group of plant growth retardants can affect some physiological processes during rooting phenomenon and it is also reported that a critical balance between endogenous stimulatory and exogenously applied inhibitory factors controls the rooting success of stem cuttings (Ali *et al*., 2017).

Paclobutrazol is a plant growth retardant which belongs to the Triazol group. It is used to inhibit the synthesis of gibberellins inside plant parts which resulted in the low level of gibberellins inside the cuttings used for propagation purpose because gibberellins are considered as anti-rooting hormones in plant species. The other chemical selected for this study was Urea phosphate (UP) which is a common nutrient element that supplies nitrogen and phosphorous to the plants. UP has been reported to improve the absorption and trigger the activity of plant hormones like gibberellins and auxins. Wiesman *et al*. (1989a); reported that UP improves the efficacy of IBA on rooting of peach stem cuttings (Abdi and Raburi, 2009).

Therefore, the present investigation was designed to evaluate the individual as well as combine effects of IBA, a root promoting substance with PBZ, a growth retardant and urea-phosphate; a nutrient supplement on the rooting response of olive cv. Arbequina stem cuttings.

Materials and Methods

The present study was carried out at olive nursery unit located in the Centre of Excellence for Olive Research and Training (CEFORT) at Barani Agricultural Research Institute (BARI) Chakwal having latitude 32.917° and longitude 72.750° in Punjab Pakistan during 2019-20. The study was conducted under semi-controlled greenhouse conditions. The internal greenhouse environment was maintained by controlling temperature at 10-25°C, relative humidity at 85-90% with adequate light intensity. The rooting media was prepared by mixing equal proportion of perlite and peat moss (1:1) and filled in black polythene bags (4 x 8 inches) for the plantation of cuttings.

Olive cuttings were collected during mid of October 2019 from selected 6-years old mother plants of said variety grown at the olive germplasm unit of CEFORT at BARI Chakwal. The uniform semi-hardwood cuttings were prepared from vigorous one-year shoots about 6-7 mm diameter and 22.5 cm length. All the lower leaves were removed except the upper most pair of leaves to keep the photosynthetic activities alive. The prepared cuttings were treated collectively with fungicide to reduce the effect of other biotic factors. The basal end (1-2 cm) of cuttings was dipped in 3000 ppm for each treatment (Table 1) of IBA for 10 seconds, UP (3000 ppm) and PB (3000 ppm) for 10 minutes each before planting. For the combined treatments, cuttings were first dipped in IBA for 10 seconds, then in aqueous solution of PB for 10 minutes followed by dipping in UP also for 10 minutes as developed by Wiesman and Lavee (1995). The cuttings were irrigated with mist system to maintain microclimate conditions suitable for rooting and their survival. The growing media, environmental conditions and all agronomic practices were kept constant for all the treatments. To evaluate the rooting ability of the treated cuttings, a systematic scheme was followed according to the attainment of that particular parameter stage (Rafique *et al*., 2021). First of all, the sprouting and callusing percentage was recorded after one month of planting. Then rooting and survival percentage was recorded after 120-130 days of planting along with number of roots and root length.

Statistical analysis

The experimental design was completely randomized (CRD) with three replications and each replication consisted of 300 cuttings. Statistical analysis was carried out using Statistix 8.1. Analysis of variance was performed with least significant test at $p<0.05$ and the graphs were drawn by using MS-Excel software.

Results and Discussion

Survival percentage

The survival percentage of cuttings was recorded after one year of treatments application. The response of IBA, UP and PB in combination and individual as well had a significant effect on survival percentage of olive cuttings. T_6 documented the maximum survival percentage (51.1%), compared with T_5 (44.8%) and T_4 (42%). All other treatment showed lower survival percentage as compared to treatments described above.

Table 1: *Plant growth regulators and their combinations evaluated to improve the rooting ability of semi-hardwood cuttings of olive cv. Arbequina.*

Treatment	Chemical	Concentration (%)
T_1	Indole-3-butyric acid (IBA)	0.3
T_2	Urea-phosphate (UP)	0.3
T_3	Paclobutrazol (PB)	0.3
T_4	IBA+UP	0.3+0.3
T_5	IBA+PB	0.3+0.3
T_6	IBA+UP+PB	0.3+0.3+0.3
T_7	UP+PB	0.3+0.3

Sprouting percentage

After the one month of planting cutting, the sprouting percentage was measured which showed appealing results as maximum sprouting was found in T_6 which was 83.3%. All other treatments showed good results that ranged from 69 to 76.9%. Among these treatments no significant difference observed except the treatment T_6 whereas T_5 and T_4 recorded 76.9% and 73.1% cuttings sprouting respectively. Minimum sprouting (69%) was recorded in T_3, containing only paclobutrazol application, followed by T_2 (69.2%) in which urea phosphate was applied to the olive semi-hardwood cuttings.

Rooting percentage

Significant differences were observed in the percentage of rooting in T_6 (IBA+UP+PB) with maximum rooting (56.8%) as compared to all other treatments followed by T_5 (IBA+PB) and T_4 (IBA+UP) with 49.8% and 46.6% rooting, respectively (Table 2). The most likely reason for maximum rooting percentage is the mutual effect of all the three plant growth regulators. All the other treatments showed lower rooting percentage ranged from 35% to 40.3%. The treatments which are applied in combinations with indole butyric acid showed better results as compared to the others having no or alone IBA application. Among the best three treatments, the T_6 (IBA+UP+PB) also has significant difference as compared to other two treatments i.e. T_5 (IBA+PB) and T_4 (IBA+UP). So, the combination

Table 2: *Effect of different plant growth regulator (PGRs) treatments (0.3% concentration) on rooting parameters of olive cv. arbequina semi-hardwood cuttings.*

Treatment	Rooting %	No. of roots	Root length (cm)	Sprouting %	Callus %	Survival %
T_1 (IBA)	38.8 cd	1.8d	3.4abc	71.5 bc	48.1 d	34.9 cd
T_2 (UP)	37.1±1.40 d	2.1±0.96d	2.7±1.06c	69.2±2.96 c	55.1±2.58 cd	33.4±1.35 d
T_3 (PBZ)	40.3±1.78 cd	2.5±0.98cd	3.1±1.10bc	69.0±2.90 c	61.5±2.63bc	36.3±1.40 cd
T_4 (IBA + UP)	46.6±1.96bc	3.3±1.02bc	3.9±1.18abc	73.1±2.98bc	70.4±2.94 ab	42.0±1.60bc
T_5 (IBA + PB)	49.8±2.04 ab	4.1±1.10ab	4.3±1.26ab	76.9±2.98 ab	72.9±2.96a	44.8±1.64 ab
T_6 (IBA +UP+ PB)	56.8±2.57a	4.4±1.05a	4.3±1.20a	83.3±3.02a	80.5±3.02a	51.1±1.95a
T_7 (IBA + PB)	35.0±1.48 d	0.3±1.15e	0.4±0.02d	71.3±2.94bc	56.2±2.49 cd	31.5±1.30 d

*IBA: Indole butyric acid; UP: Urea phosphate; PB: Paclobutrazole. *Significance at P < 0.05. Means for factors. Different letters indicate significant differences between factors (LSD test).*

of indole butyric acid with paclobutrazol and urea-phosphate is proved better for the enhancement of rooting ability of semi-hardwood cuttings of olive cv. Arbequina.

Number of roots

The results regarding average number of roots per cutting showed significant effects of different treatments. Among the all treatments, the T_6 showed higher number of roots i.e. 4.4 roots per cuttings followed by T_5 and T_4 with 4.1 and 3.3 roots per cuttings. The other treatments showed lower number of roots per cuttings ranged from 0.3 to 2.5 which are not important with respect to the combinations used for treatments 6, 5 and 4. The results regarding the number of roots again showed the better output in combination with IBA as compared to use alone.

Root length (cm)

The different treatments had a significant effect on average root length per olive cv. Arbequina semi-hardwood cuttings and the same trend was found as for rooting percentage and mean number of roots. Both the treatments T_6 and T_5 produced maximum root length i.e. 4.3cm, compared with T_4 (3.9cm), T_1 (3.4cm), T_3 (3.1cm), and T_2 (2.7cm). T_7 combination of urea phosphate and paclobutrazol developed a significantly smaller root length i.e. 0.4 cm than all other treatments.

Callus percentage

Callus percentage at one-month age of olive cuttings were statistically different (p<0.05) between different treatments. Callus percentage under T_6 (80.5%) and T_5 (72.9%) were significantly higher than those recorded under T_4, T_3, T_2 and T_7 (Table 2). The indole butyric acid alone (T_1) yielded significantly lower callus percentage (48.1 cm) than all other treatments (Table 2).

After 4 months of olive semi-hardwood cutting transplanting, different rooting parameters were recorded as shown in Table 2. According to the results, T_6 (IBA+UP+PB) at 0.3% each chemical showed good rooting success, sprouting and callus formation of olive cv. Arbequina semi-hardwood cuttings after four months of root development as compared with other treatments. While T_7 (UP+PB) at 0.3% each chemical produced poor rooting response of olive semi-hardwood cuttings of the same variety. However, poor callus percentage was recorded in T_1 (IBA) at 0.3% concentration which is being used conventionally for vegetative propagation of olive semi-hardwood cuttings in Pakistan. The rooted cuttings under all treatments were kept until 1-year-old, when they were measured for survival percentage as shown in Table 2. The data presented (Table 2) here showed a very good rooting response, sprouting, callus and survival percentages were obtained as a result of IBA combination with UP and PB. On the other hand, IBA (T_1), UP (T_2) and PB (T_3) alone did not produced desirable results with respect to rooting, sprouting, callus and survival of olive semi-hardwood cuttings of cv. Arbequina. Hence, these individual treatments are not favorable for rooting of arbequina semi-hardwood cuttings. These results are in agreement with Awan *et al.* (2003), who signaled the lowest rooting of olive cuttings recorded in control treatment (without IBA) due to minimum exposure of the cambium to develop root primordial.

Among plant hormones; auxin is known for stimulation of root induction in the cuttings of woody plant species. Therefore, adventitious root formation in olive stem cuttings can be triggered by auxins, particularly by IBA. However, in some olive cultivars; the auxin remains insufficient to promote rooting (Moshtaghi and Shahsavar, 2011). Hence, two root promoting chemicals were evaluated to improve rooting of olive cv. Arbequina under this study. The maximum rooting percentage, average number of roots per cutting, mean root length, sprouting percentage, callus percentage and survival percentage of olive semi-hardwood cuttings was observed under T_6 containing combination of IBA (0.3%), UP (0.3%) and PB (0.3%). This combination proved as the best root promoting substance in semi-hardwood cuttings of olive cv. arbequina as compared with all other treatments, while the same growth parameters were found to be lowest with T_7 containing UP (0.3%) and PB (0.3%), (Figures 1 to 6). The higher rooting percentages at T_6 might be the favorable composition of the indole butyric acid, urea phosphate and paclobutrazol for better root development of olive cuttings. As it is generally accepted that auxin promotes rooting, while gibberellins inhibit it. PB, which is a plant growth retardant and inhibits gibberellins biosynthesis and UP reported to improve hormone uptake and its activity resulted in improving rooting and survival of cuttings (Abdi and Raburi, 2009). The minimum rooting percentage was recorded for T_7, containing urea phosphate and paclobutrazole, followed by T_2 where only urea phosphate was applied. From these results, it might be concluded that exogenous application of auxin is essential for optimum rooting success in semi-hardwood cuttings of olive cv. Arbequina. Many publications in past have summarized the usefulness of auxin as rooting hormone for cutting propagation and indicated that IBA is considered to be a well rooting auxin in olive cuttings (Hechmi *et al*., 2013). The application of PB as growth retardant together with IBA has been used to improve the rooting capacity of cuttings in some plant species (Henrique *et al*., 2006). The synergies effect of IBA plus UP and PB treatment might be due to increased endogenous auxin level. As Salari *et al*. (2017) reported that IBA together with PB treated cuttings rooted at a higher frequency than those treated with IBA only.

Successful rooting is determined not only by rooting percentage but also by the number and the length of roots formed (Hechmi *et al*., 2013). The results of our findings are in accordance with Salari *et al*. (2017) who reported that treating olive stem cuttings with PB plus IBA produced longer roots through increasing number of newly formed roots than in cuttings treated with IBA alone. The combination of urea phosphate and paclobutrazol (T_7) yielded significantly lesser number of roots i.e. 0.3 only than rest of the treatments. These results showed that a combination of nutrient element (urea phosphate) and growth retardant (paclobutrazol) are incapable of producing sufficient roots in the absence of a suitable auxin i.e. IBA in olive semi-hardwood cuttings. Similarly, the highest survival under T_6 also observed which could be due to a greater number of roots produced under this treatment. T_7 (UP+PB), gave a significantly poor survival than all other treatments which might be occurred due to poor root development under this (T_7) treatment. The enhancement in the survival of the cuttings could be partly explained by the initiation and successful rooting of IBA with urea-phosphate and paclobutrazol treated cuttings. It has also been reported to improve the effect of IBA on rooting of softwood peach cuttings (Wiesman *et al*., 1989) and kiwifruit stem cuttings (Ali *et al*., 2017).

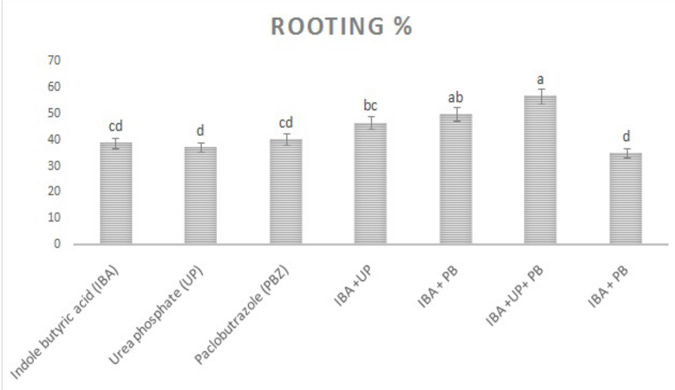

Figure 1: *Rooting (%) of cv. Arbequina semi-hardwood cutting against different chemicals.*

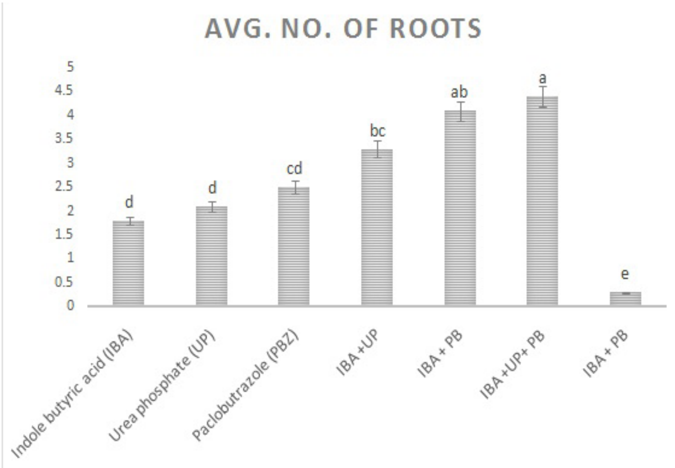

Figure 2: *No. of Roots of cv. Arbequina semi-hardwood cutting against different chemicals.*

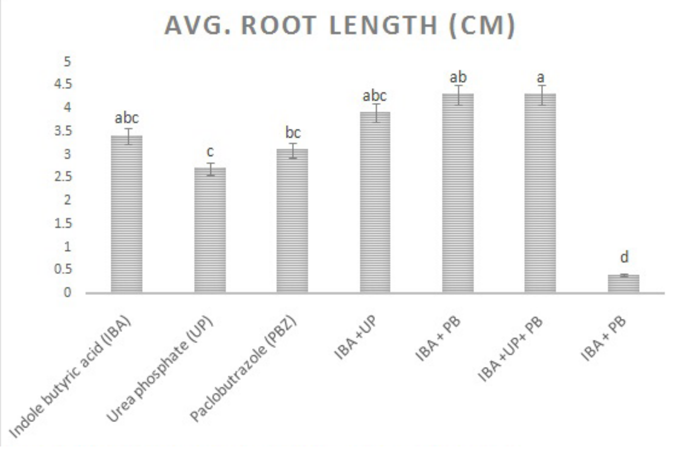

Figure 3: *Root length (cm) of cv. Arbequina semi-hardwood cutting against different chemicals.*

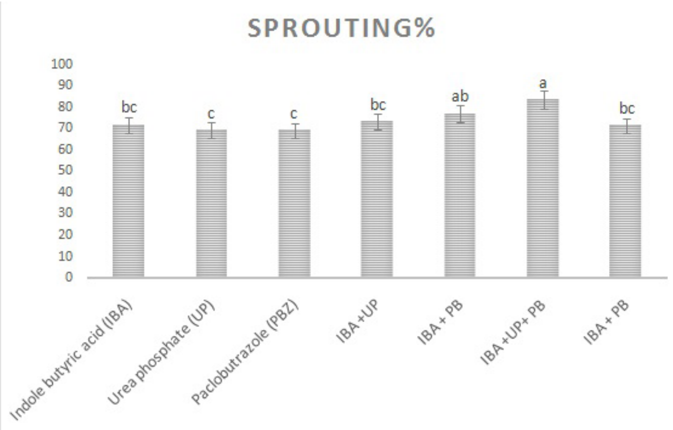

Figure 4: *Sprouting (%) of cv. Arbequina semi-hardwood cutting against different chemicals.*

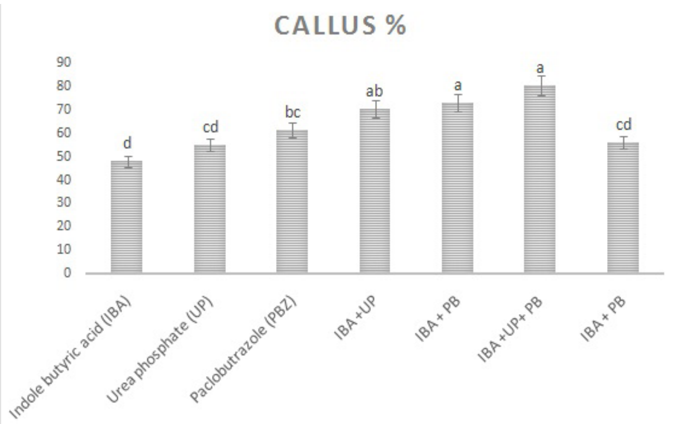

Figure 5: *Callus (%) of cv. Arbequina semi-hardwood cutting against different chemicals.*

This study was conducted systematically as the sprouting percentage was recorded just after one month of planting because the sprouting is primarily dependent on the environmental conditions and food reserves in the cuttings (Hussein *et al*., 2017). The well sprouted cuttings help the endogenous production of root promoting hormones. Similarly, the callus induction at this stage is critically important for successful rooting and the treatments of IBA with other combinations showed good rooting as compared to other combinations without IBA. So, the data regarding callusing was recorded just after measuring the sprouting. The cuttings have well developed callus at sprouting stage showed good rooting percentage whereas the cuttings have not or poor callus induction died gradually which showed sprouting promote the rooting ability only in the presence of callus which was inducted by exogenous rooting hormones. The overall survival was recorded after sixth months of successful rooting and the maximum survival of olive semi-hardwood cuttings in treatments having IBA along with other combinations prove that it helps the callusing. The other chemicals in combination of IBA like the paclobutrazol has also been reported to increase the rooting of cuttings of various fruit trees such as peach (Wiesman *et al*., 1989) and sweet cherry (Lauri, 1993) in which cuttings responded to PB by improved callus formation and prolonged viability (Wiesman and Lavee, 1994). UP is a common fertilizer which enhances the hormone uptake and activity. Wieseman and Lavee (1995) reported that PB and UP interacts with IBA to improve the rooting and survival of mung bean and peach cuttings. In conclusion, combination of IBA, UP and PB (T_6) can be recommended as an appropriate treatment to achieve the good rooting response for olive semi-hardwood cuttings as compared with conventionally used sole application of IBA.

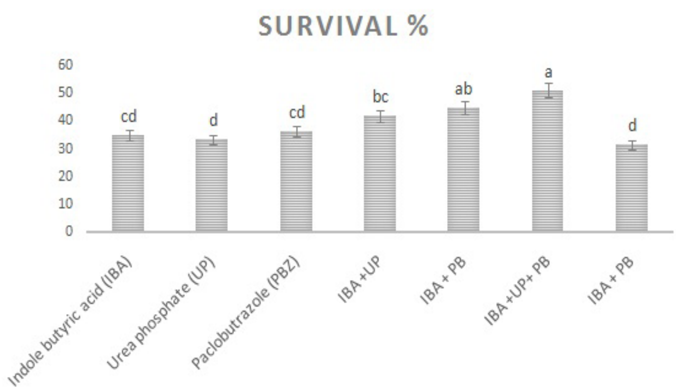

Figure 6: *Survival (%) of cv. Arbequina semi-hardwood cutting against different chemicals.*

Conclusions and Recommendations

The rooting success of olive semi-hardwood cuttings can be increased by using a combination of Indole Butyric Acid (IBA), Urea Phosphate (UP) and Paclobutrazol (PB) in cv. Arbequina; one of the leading variety being cultivated in Pakistan.

Novelty Statement

A combination of plant growth regulators is essential to enhance the rooting capacity of hard to root olive stem cuttings.

Author's Contribution

Inam Ul Haq: Conceived idea and wrote abstract, introduction and methodology.
Humara Umar: Provided technical input and wrote results/ discussion and conclusion.
Farah Umar: Ensured overall management of the manuscript.
Naeem Akhtar: Analyzed data/ review of literature.
Muhammad Jan: Collected data and references.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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