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# Effect of Hormones (IBA & IAA) on the Propagation of Himalayan Yew in Pakistan: A Conservation Approach

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**Research article**

**Keywords:** Hormone, propagation, endangered, Taxus wallichiana, survival, conservation

**Posted Date:** June 22nd, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-27841/v1

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# Abstract

## Background

Himalayan yew (*Taxus wallichiana*) is one of the endangered medicinal plants species having great importance due to the presence of anticancer drug Taxol. This metabolite is mainly used for the treatment of ovarian, breast, AIDS-related cancers, and other indications. The study being reported here was conducted for the propagation of Himalayan yew by using two different trials of Indole Butyric acid (IBA) and Indole acetic acid (IAA) hormones treatments (2000–7000 ppm) through stem cuttings (140 each). In the same way, 3840 cuttings were treated with IBA and IAA from November 2016 to November 2017 at Lalku valley, Swat, Khyber Pakhtunkhwa (KP), Pakistan.

## Results

The influence of IBA treatment (7000 ppm) showed a survival of 85.22% (average number of roots = 10.4, average length of roots = 15.5 cm, average number of leaves = 92.4 and average number of sprouts = 3.3) while that of IAA treatment (7000 ppm) the survival of 81.11% (average number of roots = 9.1, average length of roots = 14.6 cm, average number of leaves = 84.0 and average number of sprout = 3.0) were more significant followed by 2000–6000 ppm (IBA and IAA). Lowest survival 40–45% (average number of roots = 4.2, average length of roots = 8.0 cm, average number of leaves = 32.2, average number of sprouts = 1.7) was noticed for controlled cuttings.

## Conclusion

The present study enhanced the potential of conservation and propagation of *T. wallichiana*. Hence our study suggests and recommends the application of IBA (7000 ppm) as a better hormone for the conservation and propagation of Himalayan yew.


## Background

*T. wallichiana*is one of the threatened medicinal plants of Himalayan Region (1) commonly known as Himalayan yew and belongs to family Taxaceae (2).Other names given to the species are Banrya in Pushto, Barmi in Hindi and Urdu, Common yew and Himalayan yew in English (3). It is a coniferous, evergreen and a slow-growing tree having needle-like leaves and bright red fruit (Fig. 1) (4) with lengthy seed dormancy period of about 1.5 to 2.0 years and shows growth rate (increase in its circumference) of 0.4 to 1.3 cm per year (5, 6). The species survive for an average life of approximately 600 years (7). It is found in Pakistan, Afghanistan, Bhutan, Indonesia, Nepal, China, Malaysia, Philippines Vietnam, Myanmar, and India (8, 3). In Pakistan, it is present in moist temperate forests of Murree, Galliat, Kaghan, Kurram, Chitral, Kashmir, Swat and Hazara (9). Unlike other common coniferous species, the population of *Taxus* occurs in patches not continuous. Its habitat ismainly characterized by moist, mixed coniferous tree forests or cool broad-leaved forests. Due to shade demanding nature, *Taxus* is usually found in association with large tree species such as *Abiespindrow, Betula utilizes Pinus wallichiana, Acer cesium, Rhododendron arboreuand, Quercus semecarpifolia* (10).

*Taxus* is very important to treat cancer and many other diseases like bronchitis, snake bites, epilepsy, asthma, aphrodisiac, internal injuries, scorpion, diabetes, and for the diseases of lungs (10, 11).

In Pakistan, lack of awareness, slow growth rate, agriculture, construction, habitat loss, forest fires, transformation, grazing, over-harvesting, decoration purposes, medicinal use, accidental mortality, lack of management policies, illicit cutting etc. are major threats to the species (10, 12, 13). Approximately 10 genera of *Taxus* are now declining at the Northern Hemisphere in temperate zones (14). During 2001–2005, Technology, Information, Forecasting and Assessment Council (TIFAC) has reported 45 threatened medicinal plants with specific recommendation for 7 plants including *Taxus* enlisted in Convention for International Trade in Endangered Species (CITES) (Appendix II) in 1995 (15, 16, 17, 18, 19, 20, 21). Recently the existence of Yew is receiving high conservation attention due to the high exploitation rate which has reduced its population by 87% (22, 23, 24, 25). Poor regeneration process, slow growth rate and lengthy seed dormancy period of the species (26) significantly contribute to hurdles in its conservation. Vegetative propagation, therefore, could be one of the practical options to enhance its natural regeneration. The Taxus species has high regeneration potential by adventitious rooting of fresh stem cuttings (27). Unlike other Taxus species, *T. wallichiana* is difficult to root and requires longer time (28). Rooting of Taxus stem cuttings is well documented (29, 30). The present experiment was aimed to enhance the potential of conservation and propagation of *T. wallichiana* using the stem cuttings of mature trees with the application of various doses of IBA and IAA.

# Results

## Test for normality

Results of Kolmogorov-Smirnov and Shapiro-Wilktests are not significant therefore the data fulfill the normally assumption (Table 2)

Table 1
Experimental Design of Hormones applications.

| Trials | No. of Rows | No. of Cuttings in each Row | Total Cuttings | Length of each cutting (inches) | Nos of Replications |
|---|---|---|---|---|---|
| 1st _IBA | 7 | 20 | 140 | 7–8 | 3 |
| 2nd _IAA | 7 | 20 | 140 | 7–8 | 3 |

Table 2
Tests of Normality

| Parameters | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig. | Statistic | df | Sig. |
| **IAA Number of Roots** | .089 | 140 | .008 | .971 | 140 | .004 |
| **IAA Root Length(cm)** | .109 | 140 | .000 | .896 | 140 | .000 |
| **IAA Number of Leaves** | .150 | 140 | .000 | .933 | 140 | .000 |
| **IAA Number of Sprouts** | .209 | 140 | .000 | .898 | 140 | .000 |
| a. Lilliefors Significance Correction | | | | | | |

The Q-Q plots was used to test the assumptions of normality for all the expected and observed values for the four parameters (number of roots, length of root, number of leaves, and number of sprouts). All the values were plotted on the Q-Q plots on a graph which shows that the data is normal (Fig. 4–7).

# Multicollinearity

Table 3 shows that Correlation is significant at the 0.01 level (2-tailed). Correlations were done between the growth parameters (number of roots, root length (cm), number of leaves, and number of sprouts). There is strong to moderate correlation was found among number of roots-root length (0.736), number of roots-number of leaves (0.800), number of roots-number of sprouts (0.519). Significant correlation was also found among root length-number of leaves (0.718), root length-number of sprouts (0.421). Similarly, there is significant correlation among number of leaves-number of sprouts (0.541). Hence data contain no multicollinearity.

Table 3
Pearson Correlations for the Parameters i.e. number of roots, length of roots, number of leaves, and number of sprouts of *T. wallichiana* stems cuttings.

| **Parameters** | | **Number of Roots** | **Root Length(cm)** | **Number of Leaves** | **Number of Sprouts** |
|---|---|---|---|---|---|
| **Number of Roots** | Pearson Correlation | 1 | .736** | .800** | .519** |
| | Sig. (2-tailed) | | .000 | .000 | .000 |
| | N | 140 | 140 | 140 | 140 |
| **Root Length (cm)** | Pearson Correlation | .736** | 1 | .718** | .421** |
| | Sig. (2-tailed) | .000 | | .000 | .000 |
| | N | 140 | 140 | 140 | 140 |
| **Number of Leaves** | Pearson Correlation | .800** | .718** | 1 | .541** |
| | Sig. (2-tailed) | .000 | .000 | | .000 |
| | N | 140 | 140 | 140 | 140 |
| **Number of Sprouts** | Pearson Correlation | .519** | .421** | .541** | 1 |
| | Sig. (2-tailed) | .000 | .000 | .000 | |
| | N | 140 | 140 | 140 | 140 |
| **. Correlation is significant at the 0.01 level (2-tailed). | | | | | |

Since the data fulfil all the assumption to apply MONOVA technique for further analysis.

Since the p value is < 0.05 (Table 4) which means different treatment at different level are significant to each other. Now to check the performance of different combination, which one performs better than the other, for that post hoc test was applied.

Table 4
Multivariate Analysis of Variance

**Multivariate Tests[a]**

| Effect | | Value | F | Hypothesis df | Error df | Sig. |
|---|---|---|---|---|---|---|
| Intercept | Pillai's Trace | .988 | 2574.650[b] | 4.000 | 123.000 | .000 |
| | Wilks' Lambda | .012 | 2574.650[b] | 4.000 | 123.000 | .000 |
| | Hotelling's Trace | 83.728 | 2574.650[b] | 4.000 | 123.000 | .000 |
| | Roy's Largest Root | 83.728 | 2574.650[b] | 4.000 | 123.000 | .000 |
| Harmons | Pillai's Trace | .126 | 4.448[b] | 4.000 | 123.000 | .002 |
| | Wilks' Lambda | .874 | 4.448[b] | 4.000 | 123.000 | .002 |
| | Hotelling's Trace | .145 | 4.448[b] | 4.000 | 123.000 | .002 |
| | Roy's Largest Root | .145 | 4.448[b] | 4.000 | 123.000 | .002 |
| Concentration | Pillai's Trace | 1.082 | 7.785 | 24.000 | 504.000 | .000 |
| | Wilks' Lambda | .109 | 15.905 | 24.000 | 430.306 | .000 |
| | Hotelling's Trace | 6.468 | 32.742 | 24.000 | 486.000 | .000 |
| | Roy's Largest Root | 6.204 | 130.275[c] | 6.000 | 126.000 | .000 |
| Harmons * Concentration | Pillai's Trace | .025 | .130 | 24.000 | 504.000 | 1.000 |
| | Wilks' Lambda | .976 | .128 | 24.000 | 430.306 | 1.000 |
| | Hotelling's Trace | .025 | .126 | 24.000 | 486.000 | 1.000 |
| | Roy's Largest Root | .017 | .353[c] | 6.000 | 126.000 | .907 |
| a. Design: Intercept + Harmons + Concentration + Harmons * Concentration | | | | | | |
| b. Exact statistic | | | | | | |
| c. The statistic is an upper bound on F that yields a lower bound on the significance level. | | | | | | |

For concentration levels (2000 PPM-7000 PPM), Post Hoc test was applied. As by having the same numbers of replications/concentrations of hormones/number of plant cuttings Tukey Test (HSD)

(Table 5) and correlation analysis (Table 4) have been done.

Table 5

Post Hoc Tests (Tukey HSD) applied showed the concentration 7000 ppm is more significant for similar number of cases in each treatment

**Multiple Comparisons**

**Tukey HSD**

| Dependent Variable | (I) Quantity (PPM) | (J) Quantity (PPM) | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| Number of Roots | Control | 2000 | -1.7000* | .44042 | .003 | -3.0197 | − .3803 |
| | | 3000 | -2.3000* | .44042 | .000 | -3.6197 | − .9803 |
| | | 4000 | -3.0500* | .44042 | .000 | -4.3697 | -1.7303 |
| | | 5000 | -3.7000* | .44042 | .000 | -5.0197 | -2.3803 |
| | | 6000 | -4.8000* | .44042 | .000 | -6.1197 | -3.4803 |
| | | 7000 | -6.5000* | .44042 | .000 | -7.8197 | -5.1803 |
| | 2000 | Control | 1.7000* | .44042 | .003 | .3803 | 3.0197 |
| | | 3000 | − .6000 | .44042 | .821 | -1.9197 | .7197 |
| | | 4000 | -1.3500* | .44042 | .041 | -2.6697 | − .0303 |
| | | 5000 | -2.0000* | .44042 | .000 | -3.3197 | − .6803 |
| | | 6000 | -3.1000* | .44042 | .000 | -4.4197 | -1.7803 |
| | | 7000 | -4.8000* | .44042 | .000 | -6.1197 | -3.4803 |
| | 3000 | Control | 2.3000* | .44042 | .000 | .9803 | 3.6197 |
| | | 2000 | .6000 | .44042 | .821 | − .7197 | 1.9197 |
| | | 4000 | − .7500 | .44042 | .615 | -2.0697 | .5697 |
| | | 5000 | -1.4000* | .44042 | .030 | -2.7197 | − .0803 |
| | | 6000 | -2.5000* | .44042 | .000 | -3.8197 | -1.1803 |
| | | 7000 | -4.2000* | .44042 | .000 | -5.5197 | -2.8803 |
| | 4000 | Control | 3.0500* | .44042 | .000 | 1.7303 | 4.3697 |
| | | 2000 | 1.3500* | .44042 | .041 | .0303 | 2.6697 |

| Multiple Comparisons | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3000 | .7500 | .44042 | .615 | −.5697 | 2.0697 |
| | | 5000 | −.6500 | .44042 | .759 | -1.9697 | .6697 |
| | | 6000 | -1.7500* | .44042 | .002 | -3.0697 | −.4303 |
| | | 7000 | -3.4500* | .44042 | .000 | -4.7697 | -2.1303 |
| | 5000 | Control | 3.7000* | .44042 | .000 | 2.3803 | 5.0197 |
| | | 2000 | 2.0000* | .44042 | .000 | .6803 | 3.3197 |
| | | 3000 | 1.4000* | .44042 | .030 | .0803 | 2.7197 |
| | | 4000 | .6500 | .44042 | .759 | −.6697 | 1.9697 |
| | | 6000 | -1.1000 | .44042 | .169 | -2.4197 | .2197 |
| | | 7000 | -2.8000* | .44042 | .000 | -4.1197 | -1.4803 |
| | 6000 | Control | 4.8000* | .44042 | .000 | 3.4803 | 6.1197 |
| | | 2000 | 3.1000* | .44042 | .000 | 1.7803 | 4.4197 |
| | | 3000 | 2.5000* | .44042 | .000 | 1.1803 | 3.8197 |
| | | 4000 | 1.7500* | .44042 | .002 | .4303 | 3.0697 |
| | | 5000 | 1.1000 | .44042 | .169 | −.2197 | 2.4197 |
| | | 7000 | -1.7000* | .44042 | .003 | -3.0197 | −.3803 |
| | 7000 | Control | 6.5000* | .44042 | .000 | 5.1803 | 7.8197 |
| | | 2000 | 4.8000* | .44042 | .000 | 3.4803 | 6.1197 |
| | | 3000 | 4.2000* | .44042 | .000 | 2.8803 | 5.5197 |
| | | 4000 | 3.4500* | .44042 | .000 | 2.1303 | 4.7697 |
| | | 5000 | 2.8000* | .44042 | .000 | 1.4803 | 4.1197 |
| | | 6000 | 1.7000* | .44042 | .003 | .3803 | 3.0197 |
| Root Length (cm) | Control | 2000 | -2.0550* | .56146 | .007 | -3.7374 | −.3726 |
| | | 3000 | -2.4700* | .56146 | .000 | -4.1524 | −.7876 |
| | | 4000 | -2.7450* | .56146 | .000 | -4.4274 | -1.0626 |

**Multiple Comparisons**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5000 | -2.9850* | .56146 | .000 | -4.6674 | -1.3026 |
| | | 6000 | -4.1550* | .56146 | .000 | -5.8374 | -2.4726 |
| | | 7000 | -4.5300* | .56146 | .000 | -6.2124 | -2.8476 |
| | 2000 | Control | 2.0550* | .56146 | .007 | .3726 | 3.7374 |
| | | 3000 | −.4150 | .56146 | .990 | -2.0974 | 1.2674 |
| | | 4000 | −.6900 | .56146 | .882 | -2.3724 | .9924 |
| | | 5000 | −.9300 | .56146 | .646 | -2.6124 | .7524 |
| | | 6000 | -2.1000* | .56146 | .005 | -3.7824 | −.4176 |
| | | 7000 | -2.4750* | .56146 | .000 | -4.1574 | −.7926 |
| | 3000 | Control | 2.4700* | .56146 | .000 | .7876 | 4.1524 |
| | | 2000 | .4150 | .56146 | .990 | -1.2674 | 2.0974 |
| | | 4000 | −.2750 | .56146 | .999 | -1.9574 | 1.4074 |
| | | 5000 | −.5150 | .56146 | .969 | -2.1974 | 1.1674 |
| | | 6000 | -1.6850* | .56146 | .049 | -3.3674 | −.0026 |
| | | 7000 | -2.0600* | .56146 | .006 | -3.7424 | −.3776 |
| | 4000 | Control | 2.7450* | .56146 | .000 | 1.0626 | 4.4274 |
| | | 2000 | .6900 | .56146 | .882 | −.9924 | 2.3724 |
| | | 3000 | .2750 | .56146 | .999 | -1.4074 | 1.9574 |
| | | 5000 | −.2400 | .56146 | 1.000 | -1.9224 | 1.4424 |
| | | 6000 | -1.4100 | .56146 | .164 | -3.0924 | .2724 |
| | | 7000 | -1.7850* | .56146 | .030 | -3.4674 | −.1026 |
| | 5000 | Control | 2.9850* | .56146 | .000 | 1.3026 | 4.6674 |
| | | 2000 | .9300 | .56146 | .646 | −.7524 | 2.6124 |
| | | 3000 | .5150 | .56146 | .969 | -1.1674 | 2.1974 |
| | | 4000 | .2400 | .56146 | 1.000 | -1.4424 | 1.9224 |
| | | 6000 | -1.1700 | .56146 | .369 | -2.8524 | .5124 |

**Multiple Comparisons**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 7000 | -1.5450 | .56146 | .094 | -3.2274 | .1374 |
| | 6000 | Control | 4.1550* | .56146 | .000 | 2.4726 | 5.8374 |
| | | 2000 | 2.1000* | .56146 | .005 | .4176 | 3.7824 |
| | | 3000 | 1.6850* | .56146 | .049 | .0026 | 3.3674 |
| | | 4000 | 1.4100 | .56146 | .164 | −.2724 | 3.0924 |
| | | 5000 | 1.1700 | .56146 | .369 | −.5124 | 2.8524 |
| | | 7000 | −.3750 | .56146 | .994 | -2.0574 | 1.3074 |
| | 7000 | Control | 4.5300* | .56146 | .000 | 2.8476 | 6.2124 |
| | | 2000 | 2.4750* | .56146 | .000 | .7926 | 4.1574 |
| | | 3000 | 2.0600* | .56146 | .006 | .3776 | 3.7424 |
| | | 4000 | 1.7850* | .56146 | .030 | .1026 | 3.4674 |
| | | 5000 | 1.5450 | .56146 | .094 | −.1374 | 3.2274 |
| | | 6000 | .3750 | .56146 | .994 | -1.3074 | 2.0574 |
| Number of Leaves | Control | 2000 | -30.5500* | 2.49479 | .000 | -38.0257 | -23.0743 |
| | | 3000 | -32.3000* | 2.49479 | .000 | -39.7757 | -24.8243 |
| | | 4000 | -33.8000* | 2.49479 | .000 | -41.2757 | -26.3243 |
| | | 5000 | -39.6000* | 2.49479 | .000 | -47.0757 | -32.1243 |
| | | 6000 | -47.8000* | 2.49479 | .000 | -55.2757 | -40.3243 |
| | | 7000 | -59.2500* | 2.49479 | .000 | -66.7257 | -51.7743 |
| | 2000 | Control | 30.5500* | 2.49479 | .000 | 23.0743 | 38.0257 |
| | | 3000 | -1.7500 | 2.49479 | .992 | -9.2257 | 5.7257 |
| | | 4000 | -3.2500 | 2.49479 | .850 | -10.7257 | 4.2257 |
| | | 5000 | -9.0500* | 2.49479 | .007 | -16.5257 | -1.5743 |
| | | 6000 | -17.2500* | 2.49479 | .000 | -24.7257 | -9.7743 |
| | | 7000 | -28.7000* | 2.49479 | .000 | -36.1757 | -21.2243 |

**Multiple Comparisons**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3000 | Control | 32.3000* | 2.49479 | .000 | 24.8243 | 39.7757 |
| | | 2000 | 1.7500 | 2.49479 | .992 | -5.7257 | 9.2257 |
| | | 4000 | -1.5000 | 2.49479 | .997 | -8.9757 | 5.9757 |
| | | 5000 | -7.3000 | 2.49479 | .060 | -14.7757 | .1757 |
| | | 6000 | -15.5000* | 2.49479 | .000 | -22.9757 | -8.0243 |
| | | 7000 | -26.9500* | 2.49479 | .000 | -34.4257 | -19.4743 |
| | 4000 | Control | 33.8000* | 2.49479 | .000 | 26.3243 | 41.2757 |
| | | 2000 | 3.2500 | 2.49479 | .850 | -4.2257 | 10.7257 |
| | | 3000 | 1.5000 | 2.49479 | .997 | -5.9757 | 8.9757 |
| | | 5000 | -5.8000 | 2.49479 | .241 | -13.2757 | 1.6757 |
| | | 6000 | -14.0000* | 2.49479 | .000 | -21.4757 | -6.5243 |
| | | 7000 | -25.4500* | 2.49479 | .000 | -32.9257 | -17.9743 |
| | 5000 | Control | 39.6000* | 2.49479 | .000 | 32.1243 | 47.0757 |
| | | 2000 | 9.0500* | 2.49479 | .007 | 1.5743 | 16.5257 |
| | | 3000 | 7.3000 | 2.49479 | .060 | − .1757 | 14.7757 |
| | | 4000 | 5.8000 | 2.49479 | .241 | -1.6757 | 13.2757 |
| | | 6000 | -8.2000* | 2.49479 | .022 | -15.6757 | − .7243 |
| | | 7000 | -19.6500* | 2.49479 | .000 | -27.1257 | -12.1743 |
| | 6000 | Control | 47.8000* | 2.49479 | .000 | 40.3243 | 55.2757 |
| | | 2000 | 17.2500* | 2.49479 | .000 | 9.7743 | 24.7257 |
| | | 3000 | 15.5000* | 2.49479 | .000 | 8.0243 | 22.9757 |
| | | 4000 | 14.0000* | 2.49479 | .000 | 6.5243 | 21.4757 |
| | | 5000 | 8.2000* | 2.49479 | .022 | .7243 | 15.6757 |
| | | 7000 | -11.4500* | 2.49479 | .000 | -18.9257 | -3.9743 |
| | 7000 | Control | 59.2500* | 2.49479 | .000 | 51.7743 | 66.7257 |

**Multiple Comparisons**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2000 | 28.7000* | 2.49479 | .000 | 21.2243 | 36.1757 |
| | | 3000 | 26.9500* | 2.49479 | .000 | 19.4743 | 34.4257 |
| | | 4000 | 25.4500* | 2.49479 | .000 | 17.9743 | 32.9257 |
| | | 5000 | 19.6500* | 2.49479 | .000 | 12.1743 | 27.1257 |
| | | 6000 | 11.4500* | 2.49479 | .000 | 3.9743 | 18.9257 |
| Number of Sprouts | Control | 2000 | −.3500 | .25820 | .824 | -1.1237 | .4237 |
| | | 3000 | −.5500 | .25820 | .342 | -1.3237 | .2237 |
| | | 4000 | −.8500* | .25820 | .021 | -1.6237 | −.0763 |
| | | 5000 | -1.1000* | .25820 | .001 | -1.8737 | −.3263 |
| | | 6000 | -1.4000* | .25820 | .000 | -2.1737 | −.6263 |
| | | 7000 | -1.5500* | .25820 | .000 | -2.3237 | −.7763 |
| | 2000 | Control | .3500 | .25820 | .824 | −.4237 | 1.1237 |
| | | 3000 | −.2000 | .25820 | .987 | −.9737 | .5737 |
| | | 4000 | −.5000 | .25820 | .460 | -1.2737 | .2737 |
| | | 5000 | −.7500 | .25820 | .064 | -1.5237 | .0237 |
| | | 6000 | -1.0500* | .25820 | .002 | -1.8237 | −.2763 |
| | | 7000 | -1.2000* | .25820 | .000 | -1.9737 | −.4263 |
| | 3000 | Control | .5500 | .25820 | .342 | −.2237 | 1.3237 |
| | | 2000 | .2000 | .25820 | .987 | −.5737 | .9737 |
| | | 4000 | −.3000 | .25820 | .907 | -1.0737 | .4737 |
| | | 5000 | −.5500 | .25820 | .342 | -1.3237 | .2237 |
| | | 6000 | −.8500* | .25820 | .021 | -1.6237 | −.0763 |
| | | 7000 | -1.0000* | .25820 | .003 | -1.7737 | −.2263 |
| | 4000 | Control | .8500* | .25820 | .021 | .0763 | 1.6237 |
| | | 2000 | .5000 | .25820 | .460 | −.2737 | 1.2737 |
| | | 3000 | .3000 | .25820 | .907 | −.4737 | 1.0737 |

| Multiple Comparisons | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5000 | − .2500 | .25820 | .960 | -1.0237 | .5237 |
| | | 6000 | − .5500 | .25820 | .342 | -1.3237 | .2237 |
| | | 7000 | − .7000 | .25820 | .104 | -1.4737 | .0737 |
| | 5000 | Control | 1.1000* | .25820 | .001 | .3263 | 1.8737 |
| | | 2000 | .7500 | .25820 | .064 | − .0237 | 1.5237 |
| | | 3000 | .5500 | .25820 | .342 | − .2237 | 1.3237 |
| | | 4000 | .2500 | .25820 | .960 | − .5237 | 1.0237 |
| | | 6000 | − .3000 | .25820 | .907 | -1.0737 | .4737 |
| | | 7000 | − .4500 | .25820 | .589 | -1.2237 | .3237 |
| | 6000 | Control | 1.4000* | .25820 | .000 | .6263 | 2.1737 |
| | | 2000 | 1.0500* | .25820 | .002 | .2763 | 1.8237 |
| | | 3000 | .8500* | .25820 | .021 | .0763 | 1.6237 |
| | | 4000 | .5500 | .25820 | .342 | − .2237 | 1.3237 |
| | | 5000 | .3000 | .25820 | .907 | − .4737 | 1.0737 |
| | | 7000 | − .1500 | .25820 | .997 | − .9237 | .6237 |
| | 7000 | Control | 1.5500* | .25820 | .000 | .7763 | 2.3237 |
| | | 2000 | 1.2000* | .25820 | .000 | .4263 | 1.9737 |
| | | 3000 | 1.0000* | .25820 | .003 | .2263 | 1.7737 |
| | | 4000 | .7000 | .25820 | .104 | − .0737 | 1.4737 |
| | | 5000 | .4500 | .25820 | .589 | − .3237 | 1.2237 |
| | | 6000 | .1500 | .25820 | .997 | − .6237 | .9237 |
| Based on observed means.<br>The error term is Mean Square (Error) = .667. | | | | | | | |
| *. The mean difference is significant at the .05 level. | | | | | | | |

Since two different hormones IAA and IBA are being used, therefore we interpret the differences by simply looking at their mean values. The results revealed that in both trials for the regeneration of Himalayan yew (*T. wallichiana*) cuttings, by the treatment of hormones (IBA and IAA) with 2000–7000 ppm were applied. Out of all the applied concentrations of hormone (2000–7000 ppm), 7000 ppm shows maximum marginal means for number of roots (10.4, 9.1), roots length (11.82 cm, 10.27 cm), and number of leaves (92.40, 88.30), sprout numbers (3.30, 3.0) and survival percentage (85.22%, 81.11%) throughout the experiment. The next best dozes were 6000 ppm followed by 5000 ppm, 4000 ppm, 3000 ppm, and 2000 ppm. The lowest growth was noticed in control cuttings (Table 6) (Fig. 8 to 12).

Table 6

General Linear Model (Descriptive Statistics), showing the estimated marginal means of the Parameter (number of roots, roots length (cm), number of leaves, and number of sprouts) of *T. wallichiana* cuttings by the application of various concentration of hormones (2000 PPM- 7000 PPM) of IBA, and IAA.

| Parameters | Treatment | Quantity in PPM | Mean ± SD | N |
|---|---|---|---|---|
| Number of Roots | IAA | Control | 2.8000 ± 1.54919 | 10 |
| | | 2000PPM | 4.8000 ± 1.03280 | 10 |
| | | 3000PPM | 5.2000 ± 1.39841 | 10 |
| | | 4000PPM | 5.9000 ± 1.19722 | 10 |
| | | 5000PPM | 6.6000 ± 1.17379 | 10 |
| | | 6000PPM | 7.6000 ± 1.07497 | 10 |
| | | 7000PPM | 9.1000 ± 1.19722 | 10 |
| | | Total | 6.0000 ± 2.23931 | 70 |
| | IBA | Control | 3.7000 ± 1.63639 | 10 |
| | | 2000PPM | 5.1000 ± 1.19722 | 10 |
| | | 3000PPM | 5.9000 ± 1.19722 | 10 |
| | | 4000PPM | 6.7000 ± 1.56702 | 10 |
| | | 5000PPM | 7.3000 ± 1.33749 | 10 |
| | | 6000PPM | 8.5000 ± 1.26930 | 10 |
| | | 7000PPM | 10.4000 ± 2.22111 | 10 |
| | | Total | 6.8000 ± 2.52868 | 70 |
| | Total | Control | 3.2500 ± 1.61815 | 20 |
| | | 2000PPM | 4.9500 ± 1.09904 | 20 |
| | | 3000PPM | 5.5500 ± 1.31689 | 20 |
| | | 4000PPM | 6.3000 ± 1.41793 | 20 |
| | | 5000PPM | 6.9500 ± 1.27630 | 20 |
| | | 6000PPM | 8.0500 ± 1.23438 | 20 |
| | | 7000PPM | 9.7500 ± 1.86025 | 20 |
| | | Total | 6.4000 ± 2.41339 | 140 |
| Root Length (cm) | IAA | Control | 5.8600 ± 3.25958 | 10 |

| Parameters | Treatment | Quantity in PPM | Mean ± SD | N |
|---|---|---|---|---|
| | | 2000PPM | 8.2000 ± 1.04243 | 10 |
| | | 3000PPM | 8.6400 ± 1.24383 | 10 |
| | | 4000PPM | 8.8800 ± 1.40063 | 10 |
| | | 5000PPM | 9.1300 ± 0.97985 | 10 |
| | | 6000PPM | 10.1100 ± 1.19019 | 10 |
| | | 7000PPM | 10.2700 ± 1.51441 | 10 |
| | | Total | 8.7271 ± 2.11571 | 70 |
| | IBA | Control | 7.1700 ± 3.01517 | 10 |
| | | 2000PPM | 8.9400 ± 1.73666 | 10 |
| | | 3000PPM | 9.3300 ± 1.49596 | 10 |
| | | 4000PPM | 9.6400 ± 1.55863 | 10 |
| | | 5000PPM | 9.8700 ± 1.76638 | 10 |
| | | 6000PPM | 11.2300 ± 1.71856 | 10 |
| | | 7000PPM | 11.8200 ± 1.18115 | 10 |
| | | Total | 9.7143 ± 2.27783 | 70 |
| | Total | Control | 6.5150 ± 3.12903 | 20 |
| | | 2000PPM | 8.5700 ± 1.44481 | 20 |
| | | 3000PPM | 8.9850 ± 1.38499 | 20 |
| | | 4000PPM | 9.2600 ± 1.49399 | 20 |
| | | 5000PPM | 9.5000 ± 1.44113 | 20 |
| | | 6000PPM | 10.6700 ± 1.54923 | 20 |
| | | 7000PPM | 11.0450 ± 1.54255 | 20 |
| | | Total | 9.2207 ± 2.24565 | 140 |
| Number of Leaves | IAA | Control | 30.1000 ± 16.25799 | 10 |
| | | 2000PPM | 60.8000 ± 5.11642 | 10 |
| | | 3000PPM | 62.1000 ± 7.48999 | 10 |
| | | 4000PPM | 64.0000 ± 6.97615 | 10 |
| | | 5000PPM | 69.7000 ± 4.71522 | 10 |

| Parameters | Treatment | Quantity in PPM | Mean ± SD | N |
|---|---|---|---|---|
| | | 6000PPM | 77.7000 ± 6.91295 | 10 |
| | | 7000PPM | 88.3000 ± 5.01221 | 10 |
| | | Total | 64.6714 ± 18.69645 | 70 |
| | IBA | Control | 32.1000 ± 11.87387 | 10 |
| | | 2000PPM | 62.5000 ± 6.32895 | 10 |
| | | 3000PPM | 64.7000 ± 7.45431 | 10 |
| | | 4000PPM | 65.8000 ± 7.19259 | 10 |
| | | 5000PPM | 71.7000 ± 3.83116 | 10 |
| | | 6000PPM | 80.1000 ± 6.08185 | 10 |
| | | 7000PPM | 92.4000 ± 6.39792 | 10 |
| | | Total | 67.0429 ± 18.75350 | 70 |
| | Total | Control | 31.1000 ± 13.89396 | 20 |
| | | 2000PPM | 61.6500 ± 5.66870 | 20 |
| | | 3000PPM | 63.4000 ± 7.39417 | 20 |
| | | 4000PPM | 64.9000 ± 6.95777 | 20 |
| | | 5000PPM | 70.7000 ± 4.30544 | 20 |
| | | 6000PPM | 78.9000 ± 6.45552 | 20 |
| | | 7000PPM | 90.3500 ± 5.97605 | 20 |
| | | Total | 65.8571 ± 18.69543 | 140 |
| Number of Sprouts | IAA | Control | 1.5000 ± 1.08012 | 10 |
| | | 2000PPM | 1.7000 ± 0.67495 | 10 |
| | | 3000PPM | 1.9000 ± 0.56765 | 10 |
| | | 4000PPM | 2.3000 ± 0.94868 | 10 |
| | | 5000PPM | 2.6000 ± 0.96609 | 10 |
| | | 6000PPM | 2.9000 ± .73786 | 10 |
| | | 7000PPM | 3.0000 ± .66667 | 10 |
| | | Total | 2.2714 ± .96190 | 70 |
| | IBA | Control | 1.7000 ± .94868 | 10 |

| Parameters | Treatment | Quantity in PPM | Mean ± SD | N |
|---|---|---|---|---|
| | | 2000PPM | 2.2000 ± .63246 | 10 |
| | | 3000PPM | 2.4000 ± .51640 | 10 |
| | | 4000PPM | 2.6000 ± .96609 | 10 |
| | | 5000PPM | 2.8000 ± 1.03280 | 10 |
| | | 6000PPM | 3.1000 ± .87560 | 10 |
| | | 7000PPM | 3.3000 ± .48305 | 10 |
| | | Total | 2.5857 ± .92459 | 70 |
| | Total | Control | 1.6000 ± .99472 | 20 |
| | | 2000PPM | 1.9500 ± .68633 | 20 |
| | | 3000PPM | 2.1500 ± .58714 | 20 |
| | | 4000PPM | 2.4500 ± .94451 | 20 |
| | | 5000PPM | 2.7000 ± .97872 | 20 |
| | | 6000PPM | 3.0000 ± .79472 | 20 |
| | | 7000PPM | 3.1500 ± .58714 | 20 |
| | | Total | 2.4286 ± .95317 | 140 |

The results depict that the cutting responded differently to various levels of concentration of hormones (2000 ppm-7000 ppm) and the modes of application. Table 4.5 shows the multiple comparisons of all the parameters (number of roots, length of roots cm, number of leaves, number of sprouts) marginal mean values by the applied various concentrations of hormones (2000 ppm-7000 ppm) to the cuttings of *T. wallichiana* for the significant. Therefore, Post Hoc test (Tukey HSD) has been applied to check the significance on different levels among the growth parameters (number of roots, length of roots cm, number of leaves, number of sprouts) by applied hormones various concentrations (2000 ppm–7000 ppm). The multiple comparison (Tukey HSD) shows that based on observed means the error term is Mean Square (Error) = 0.667 and (*) the mean difference is significant at the 0.05 level ($p < 0.05$) (Table 5).

# Discussion

The current study indicates that hormone treatment (IBA & IAA) is the favourable and best option for the conservation and vegetative propagation of Himalayan yew. As our study depicts and recommends IBA the best rooting hormones in comparison to the Indole acitic acid ( IAA). In fact, the auxins application is important to produce roots in fresh stem cuttings as they have been experienced for stimulating secondary plant growth that results in the reserve food material mobilization to the root initiation site (31). Auxins application increases rooting and quality of roots in various tree species (32). It is a well-documented that the auxins application is important for the roots formation in the cuttings of plant stem as they have been found in stimulating the cambial activity which results in reserve food mobilization to the root initiation site (31; 33).

Also, a successful rooting in juvenile shoot cuttings of *T. wallichiana* with different auxins has been documented from the other temperate areas as well (34, 35, 36, 38, 39)

Our results are also similar with Nautiyal et al. (1991) (40), who treated hormones on stem cutting of teak for roots induction and concluded that IBA is the favourable and best auxins. Other scientists (41, 44, 37, 42, 45, 46, 43) also documented that the IBA application play a vital role in the propagation of stem cuttings of *Taxus*. Their results clearly mentioned that vegetative propagation (in-situ conservation) can improve the number of juvenile's plants of genus Taxus.

Several primary root formations under the influence of IBA application have been obtained in the present trials (7000 ppm). It must be accepted that a higher root to shoot is satisfactory for achieving a higher survival rate after transplantation of field. It has also been reported that more than optimums concentration of auxins is toxic to the root regeneration, while theoptimum concentration of hormones is favorable (47, 48). In our study we had applied the maximum concentration of 7000 ppm of both the auxins (IBA and IAA) which shows favourable response to the growth of *T. wallichiana* cuttings.

The differential response to changing concentrations of hormones for survivals of *T. wallichiana* were noted in the present study could be like the action of increasing Auxins (2000 ppm – 7000 ppm). The IBA application may have an indirect effect by increasing the translocation speed and the movement of sugar to the cuttings base which in result stimulate rooting (31).

IBA application in the current study significantly increased the survival percentage (number of roots, length of roots, number of leaves, and number of sprouts). Same results were obtained in another study by applying different hormones, for the initiation of a maximum rooting percentage in the juvenile fresh stem cuttings of *T. wallichiana*; IBA is the effective and best hormone (50). It is now decided that IBA is the best rooting auxins for Taxus species and it was confirmed by many workers (36, 29). The time duration of our study was kept 20 weeks after the plantation of the cutting of *T. wallichiana*. Similarly, the auxins effect on growth and induction of adventitious roots of *T. wallichiana* cuttings after 4 and ½ months of application/treatment and plant growing. In general, it has been found that among various auxins IBA was the more active for root germination showed the maximum percentage of survival, rooting percentage, percentage of callusing roots per cutting, and length of root per cutting (37, 41, 42, 43, 45, 46). The researcher studied the application of 1000 ppm and 500 ppm of IBA on the *T. wallichiana* cuttings and found, IBA 500 ppm showed slightly less response than IBA 1000 ppm. IBA 1000 ppm showed the best rooting response on shoot cuttings of *T. wallichiana* in the spring season with a rooting percentage (95%) compared to other treatments in other seasons (51). In our study the best response was given by 7000 ppm in case of both IBA and IAA. By looking into other studies, it clears that by increasing the concentration up to 7000 ppm the growth will be high.

By using IBA, with some other cultivar of yew (49) that showed that treatment of hormone was very important for successful conservation and propagation of *T. wallichiana*. By the treatment of hormones, stem cuttings of some other species were also published (55), their research showed that the best response for rooting due to IBA followed by IAA and NAA in rooting percentage, the cuttings heights, number of roots per cutting in Sage, Rosemary, and Elderberry. Similarly, the treatments of auxins (IBA, IAA, and NAA) on the root development were studied in species like *Melissa officinalis* (52), *Ficus Benjamina* (53), and *Oryza sativa* (54).

Auxins are involved in the formation of root, cambial cells activation, and lateral bud inhibition. They have been found as naturally existing compounds that promotes the formation of root and synthetic auxins also stimulating the emergence of root on cuttings. It is well documented and verified maximum time that auxins applied (naturally or artificially) is needed for adventitious root initiation on stem cuttings. Initial cells division of roots is dependent on either endogenous or applied auxins (56).

Our findings are also in similarity with (57) that showed that root development in stem cuttings of *T. wallichiana* using IBA followed by IAA and NAA and in control where no auxins treatment was given, less root formation was observed in those cuttings, and the lowest survival was found during the observation of cuttings. In this case further the survival becomes very difficult and ultimately the cuttings died. In this respect, our results are similar as obtained by (58).

Thus, our study suggests the utilization of auxins mainly IBA for regeneration of *T. wallichiana* under natural conditions. The results of our research will be beneficial for developing propagation protocol of *T. wallichiana* species especially for the moist temperate climate of the Himalayan Region.

# Conclusion

This study was aimed to conserve and propagate the Himalayan yew (*T. wallichiana*) by finding out the best rooting hormone (IBA and IAA) with suitable concentration applied (2000 ppm-7000 ppm) for the regeneration of the fresh stem cuttings. Out of the two different trial, it has been found that 7000 ppm shows higher survival percentage (IBA 85.22% and IAA 81.11%) irrespective of the mode of application and results were highly significant followed bytheir corresponding. Hormones with concentration 6000 ppm was the next best suitable dose that shows maximum survival (IBA 66.67%, IAA 63.33%) followed by 5000ppm > 4000ppm > 3000ppm > 2000 ppm. Therefore, among all the treatments of auxins (IBA and IAA), IBA shows maximum growth (number of roots, root length (cm), number of leaves, and number of sprouts) and survival (IBA > IAA) **(**Fig. 8–12**).** The lowest survival percentages were noticed by control cuttings in both the trials.

# Methods

# Study Area

Our study was carried out with the aim to enhance the potential of conservation and propagation of the valuable and endangered medicinal plant *T. wallichiana* species in Lalku valley of District Swat, KP, Pakistan. Lalku valley has more Taxus density and offers a rich forest cover of pine and oak trees. It lies at an altitude of 1963 meters above sea level, latitude of 35°.1375 and longitude of 72°.38639. Forest area of Lalku forest range is 8580 ha with a Forest cover of 59.3%. In this region,temperature ranges from − 2ºC to 34ºC. The average annual precipitation ranges from 1000–1200 mm. The study period was during November 2016 to November 2017.

# Collection, Preparation, and Planting of Cuttings

The formal identification of the *T. wallichiana's* plant materials was undertaken by the Directorate of Non-Timber Forest Produce (NTFP), Forest Department, Peshawar; Department of Environmental Sciences, University of Peshawar. Proper permission was granted by the University of Peshawar, Ethical review board. Voucher specimens no. Bot. 20156 (PUP) were deposited in the herbarium of Department of Botany, University of Peshawar.

Cuttings were collected from various mature patches of Himalayan yew. The cuttings were brought to the nursery, raised in Lalku Forest Research Station. The length ofthefinal cuttings were kept 7 to 8 inches and 3 to 4 nodes were retained in each cutting. The needles at the basal portion (about 2 cm) of the stem cuttings were removed and sterilized using 2% benlate (fungicide) before planting. The cuttings were dipped in fungicidal solution for 5 minutesand dried for 20 to 25 minutes in an open environment. The dried cuttings were treated with 50% (Water: Ethanol) concentrated solution of the IBA and IAA (2000–7000 ppm) for 5 minutes and planted in the polythene bags containing soil. The soil was prepared by mixing forest soil, sand and agriculture soil ofthe area in1:1:1 and sieved properly before filling into polythene bags (Fig. 2).

# Experimental Design

The experiment was carried out in a randomized block design with a factorial treatment's arrangement. First trial treatment was carried out with 7 rows and each row containing 20 cuttings. Total of 140 cuttings were taken in the first trial of the experiment (n = 140; x 20 cuttings x 1 type of cuttings x 7 IBA treatments, 3 replications) (Fig. 3). Same was repeated for the second trial of IAA treatments 7 rows with 20 cuttings each, so a total of 140 cuttings were used (n = 140; x 20 cuttings x 1 type of cuttings x 7 IAA treatments, 3 replications) as shown in (Table 1). The trials were evaluated for the number of roots, length of roots, number of leaves, sprouts and survival percentages of cuttings after 20 weeks in 2016-17of planting.

# Statistical Analysis

A Statistical software SPSS version 25.0 was used for the analysis. Mean values of all the parameters (number of roots, length of roots (cm), number of leaves, number of sprouts) were calculated to determine the highest growth of each trial for every applied concentrations of hormones (2000 ppm-7000 ppm).

To compare the average effect of treatment at different level Multivariate Analysis of Variance (MANOVA) technique have been applied. The results of different assumptions which are acquired for applying MANOVA are discussed briefly.

## Abbreviations

IBA: Indole Butyric acid; IAA: Indole acetic acid; KP: Khyber Pakhtunkhwa; TIFAC: Forecasting and Assessment Council; CITES: Convention for International Trade in Endangered Species; MANOVA: Multivariate Analysis of Variance; IAA: Indole acitic acid.

## Declarations

# Ethics approval and consent to participate:

There are no ethical guidelines for research on the conservation of *T. wallichiana*.

### Consent for publication:

Not applicable.

### Availability of data and materials:

The additional data regarding the *T. wallichiana's* in-situ conservation (relationship between the species growth through hormones (IBA, IAA) applied) by looking into each mentioned parameters (Word docs 40 kb).

In current research study the datasets analysed are available from the corresponding author on reasonable request.

# Competing interests:

The authors declare that they have no competing interests.

# Funding:

This project was financially supported by the National Research Project for Universities (NRPU), Higher Education Commission (HEC) Pakistan. The project details are: No: 20-3690/NRPU/R&D/HEC/2014/816. The funding bodies have no role in the design and colllection, analysis, interpretation of the data and in writing of the manuscript.

# Author's contribution:

BK designed the research project plan ; JI did field and laboratory work, also wrote the manuscript; SK review and contributed in the writing; NG presented the concept idea; IAM hepled in field work of this study; NY review the manuscript also helped in the laboratory work; IA provide the field design for In-situ experiment at the forest nursery; SM helped to statistically analyse the results. Final manuscript is read and approved by all of the authors.

# Acknowledgement:

The co-authors are very thankful to all of the organization (Department of Environmental Sciences, University of Peshawar; Pakistan Forest Institute (PFI); Directorate of Non-Timber Forest Produce, Forest Department, Peshawar; and Higher Education Commission Pakistan (HEC)) who contributed positively in the completion of this project.

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# Figures

**Figure 1**

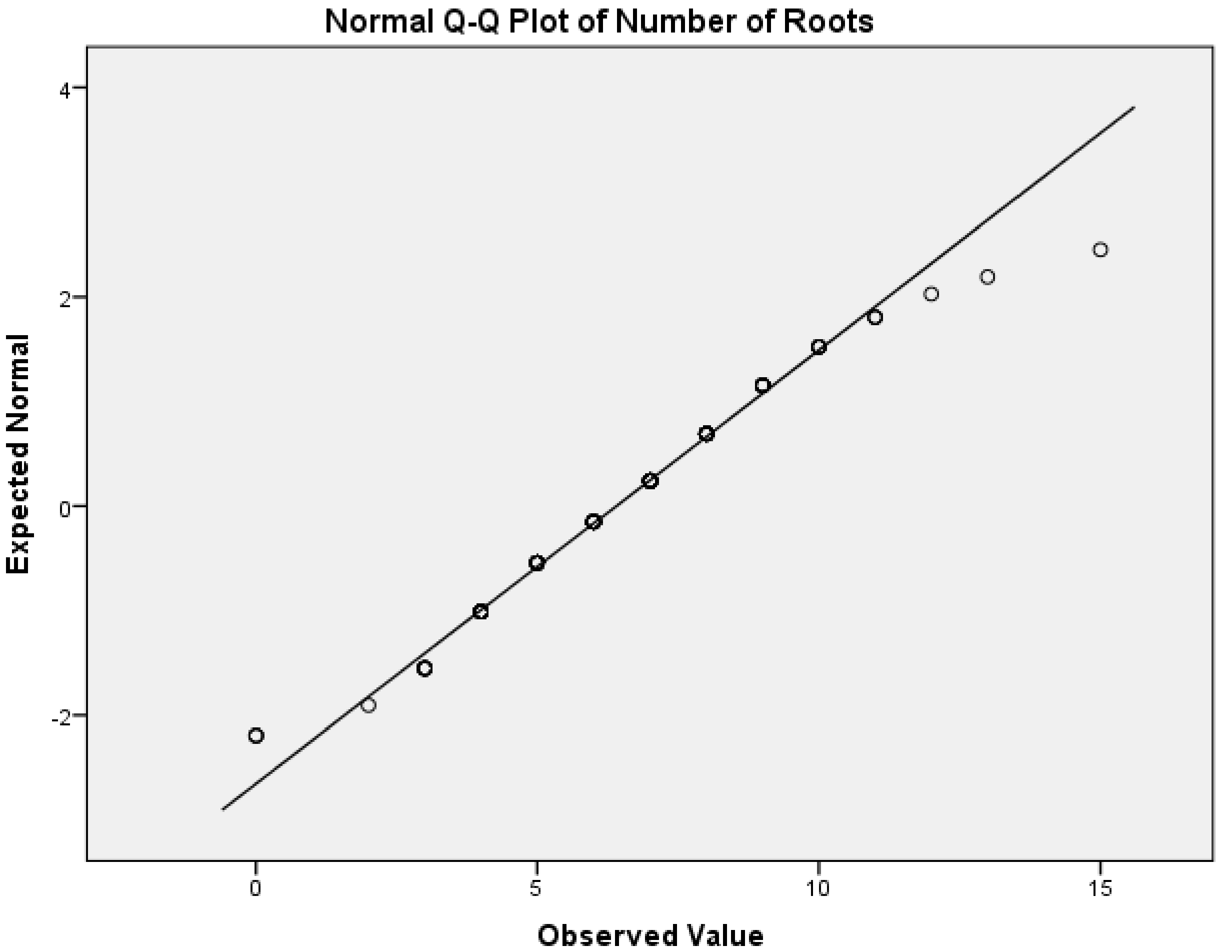
Leaves and fruit (bright red berries) of Himalayan Yew (Taxus wallichiana) from Kalam, Swat (Iqbal et al., 2020).


**Figure 2**

(a) Mixing and sieving of forest, sand and agriculture soil (1:1:1); (b) Filling of Polythene bags; (c) Cuttings treatment with the concentrated hormonal solutions (2000-7000 ppm); (d) T. wallichiana's cuttings preparation with sharp knife; (e) Plant cuttings washing with tap water.

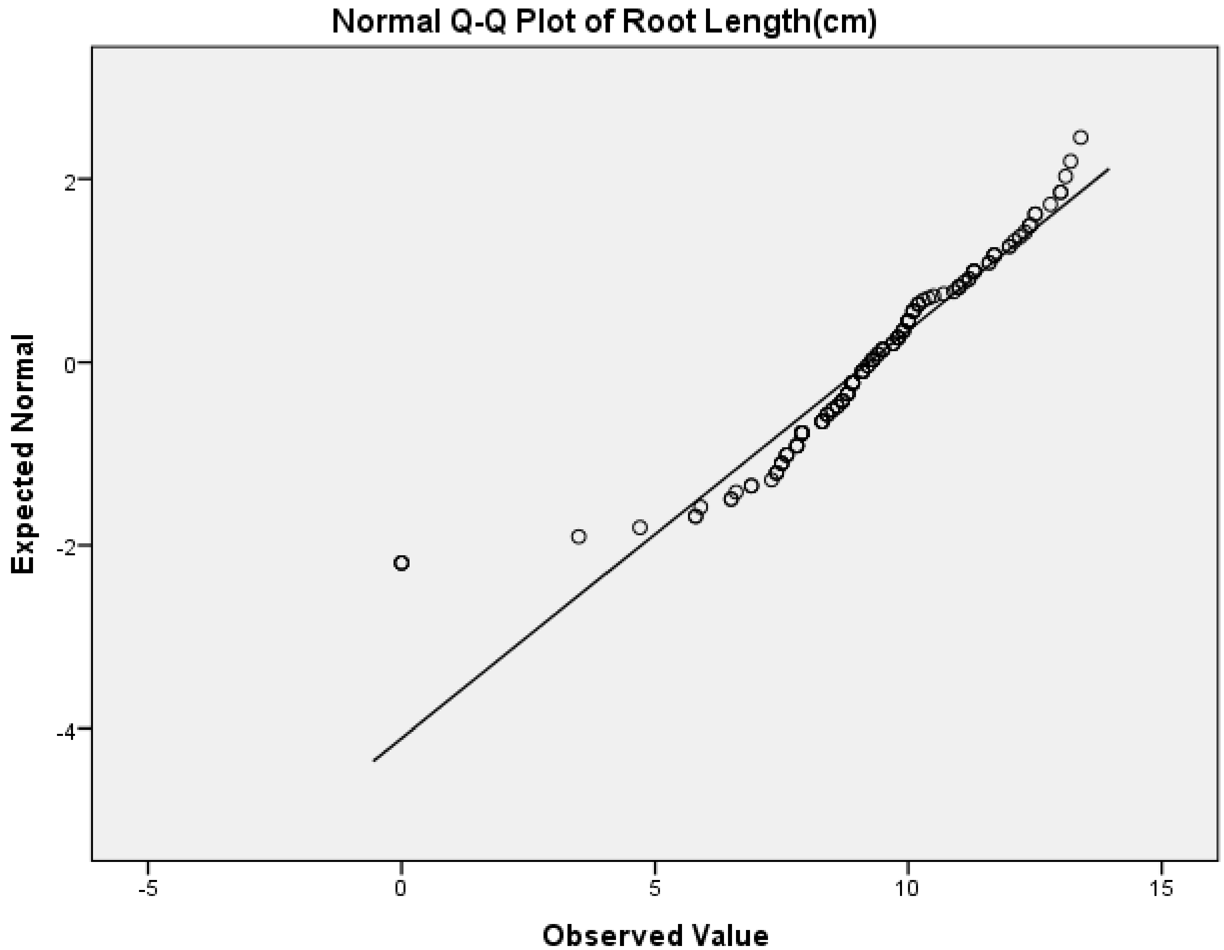

## Figure 3

Himalayan yew (Taxus wallichiana) cuttings grown in the Lalku Valley, Khyber Pakhtunkhwa, Pakistan.



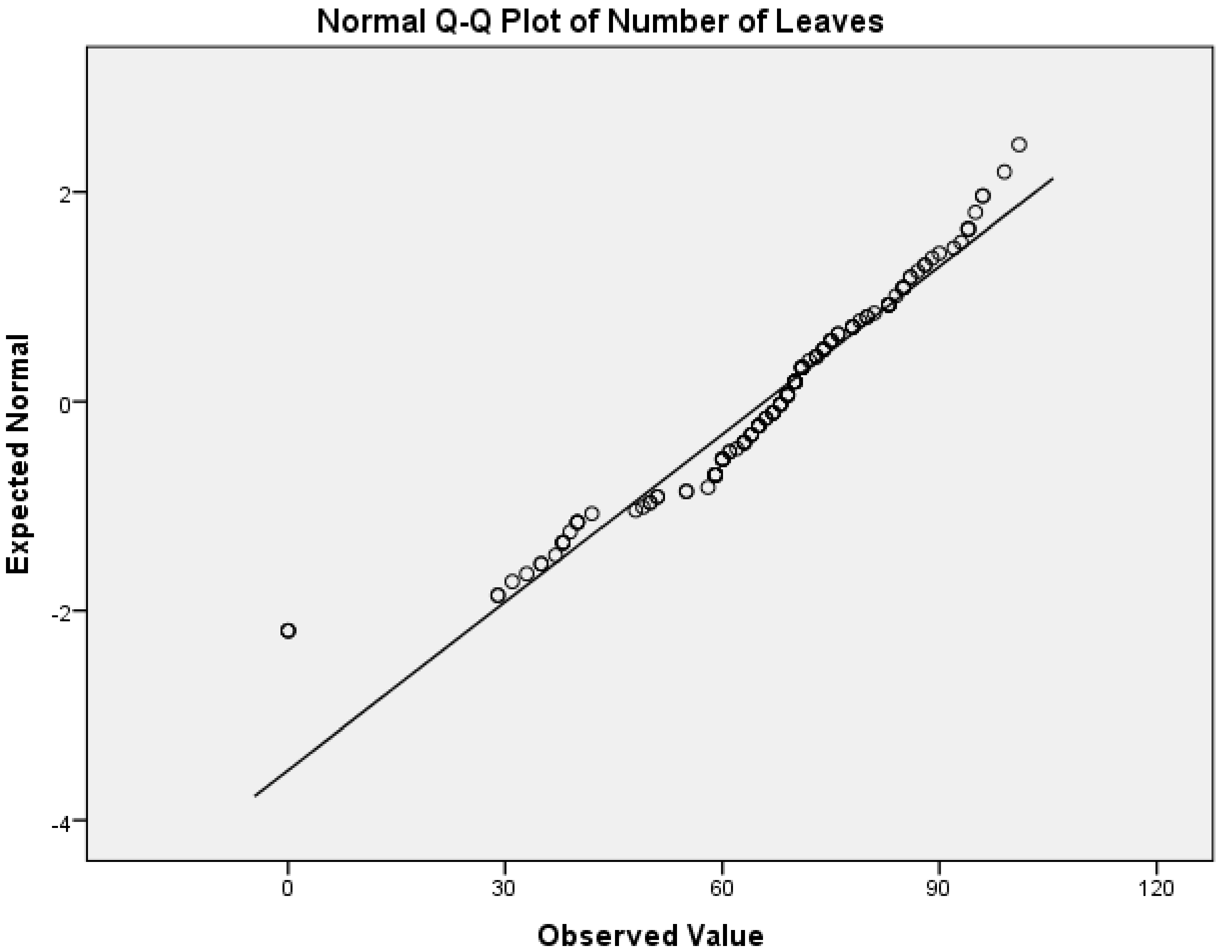

## Figure 4

Normal Q-Q Plot of observed and expected values for number of roots of T. wallichiana Cuttings.




## Figure 5

Normal Q-Q Plot of observed and expected values for length of roots of T. wallichiana’s cuttings.




## Figure 6

Normal Q-Q Plot of observed and expected values for number of leaves of T. wallichiana’s cuttings.

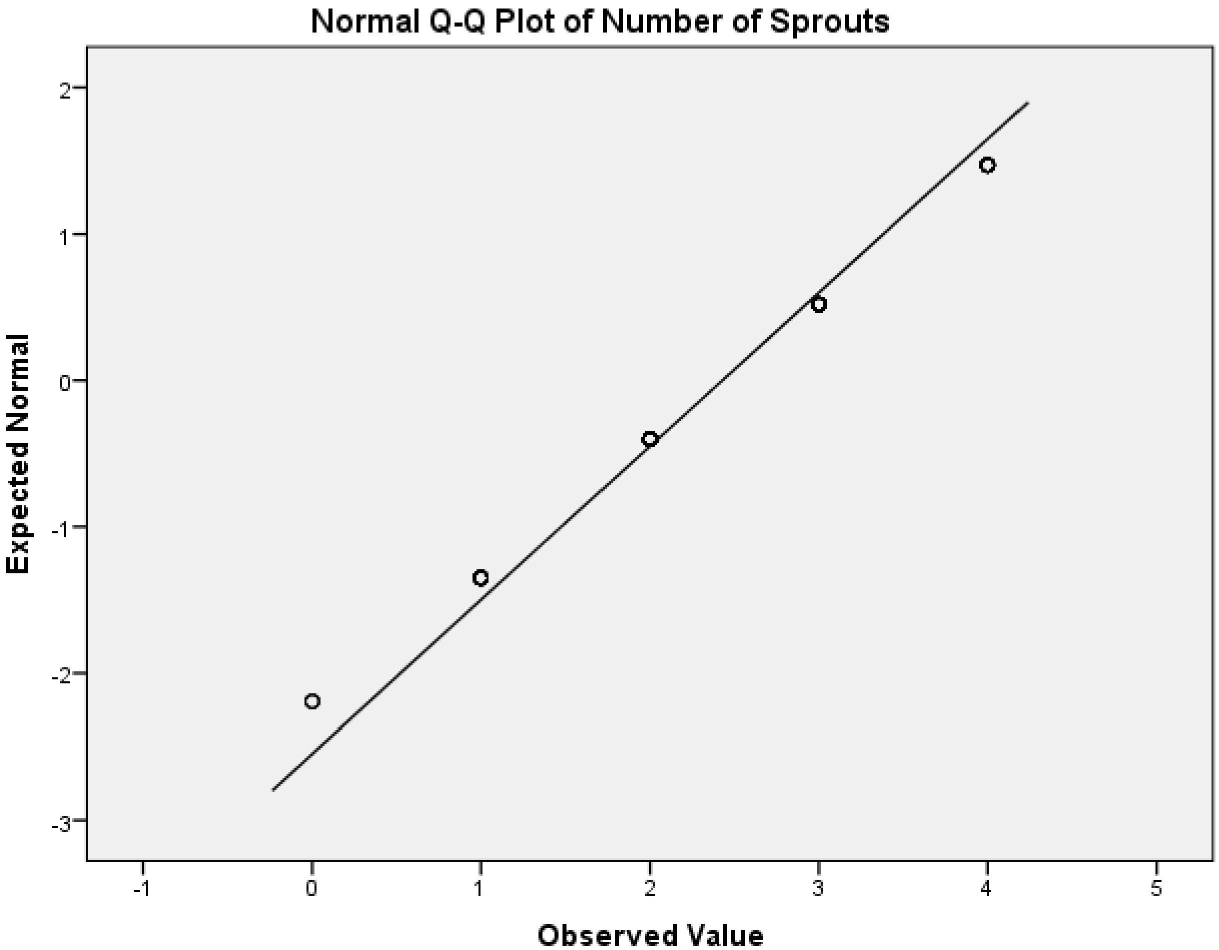
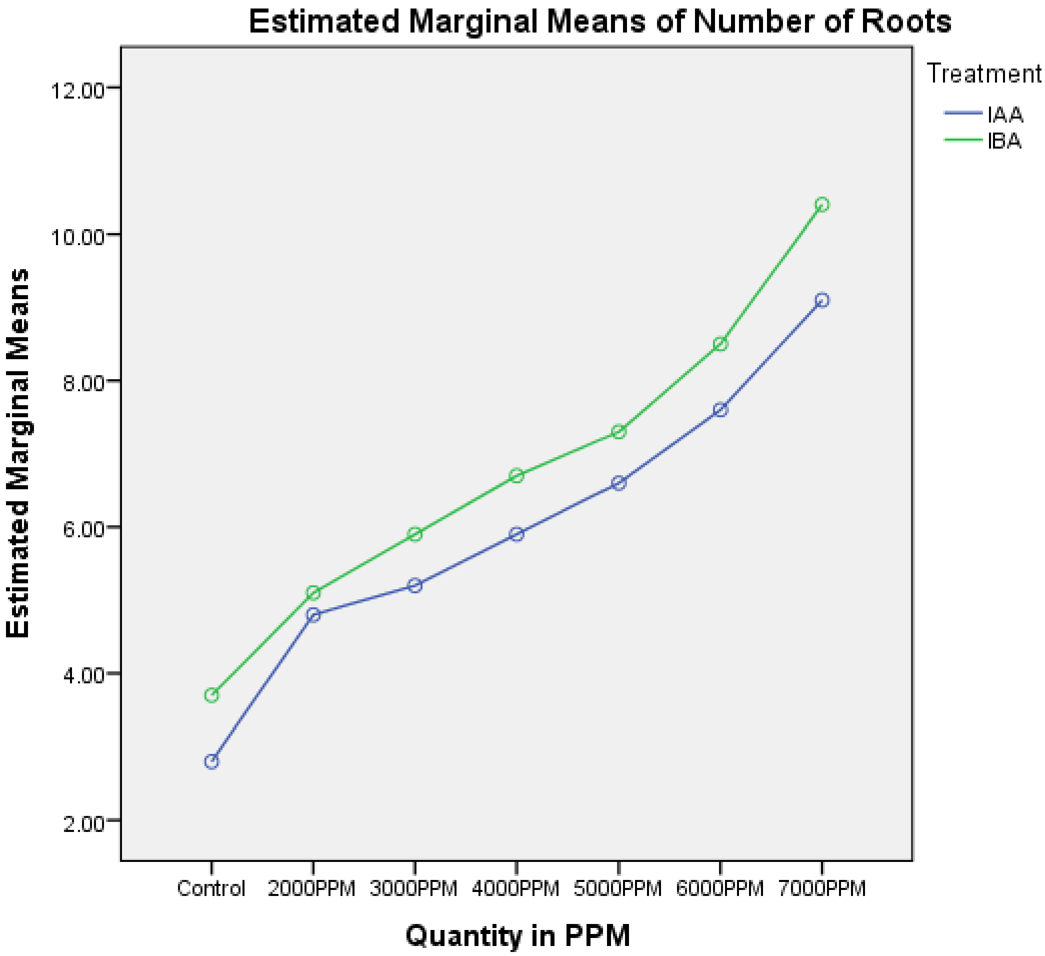


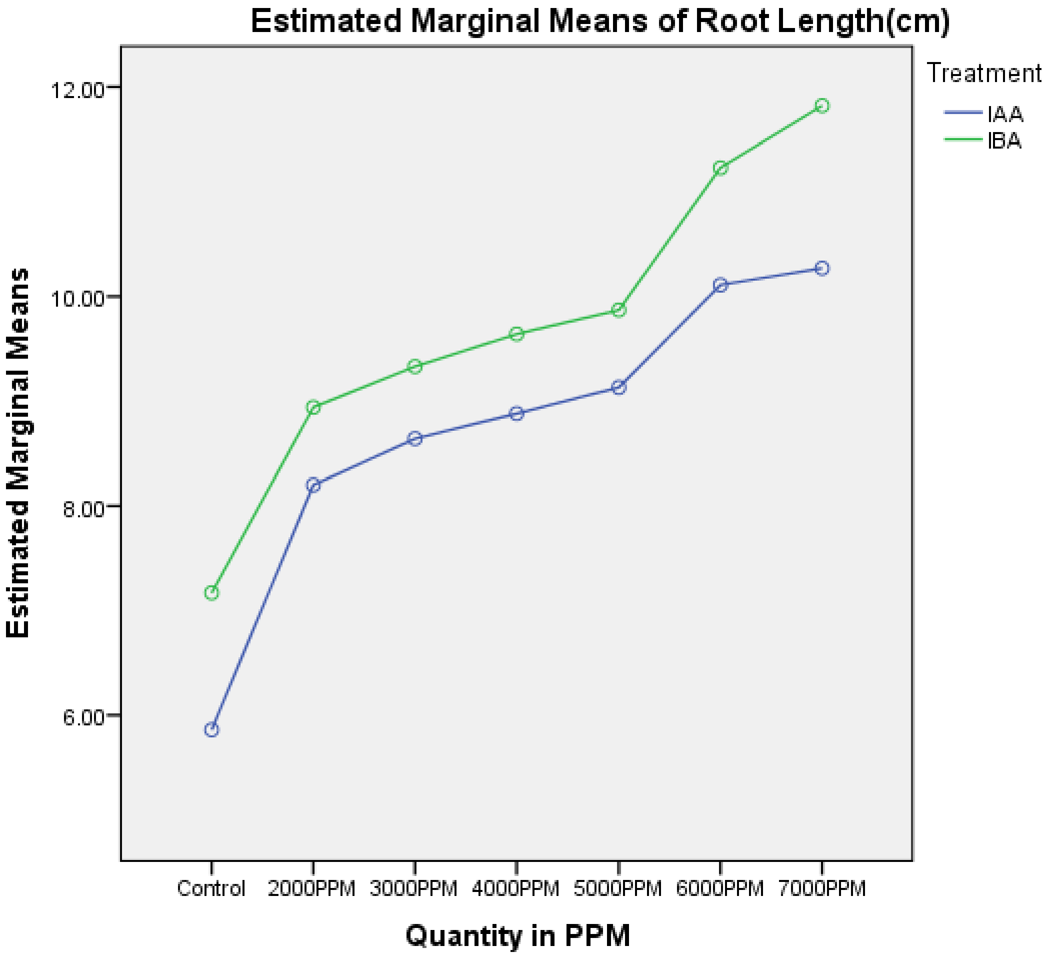

Figure 7

Normal Q-Q Plot of observed and expected values for number of sprouts of T. wallichiana’s cuttings.



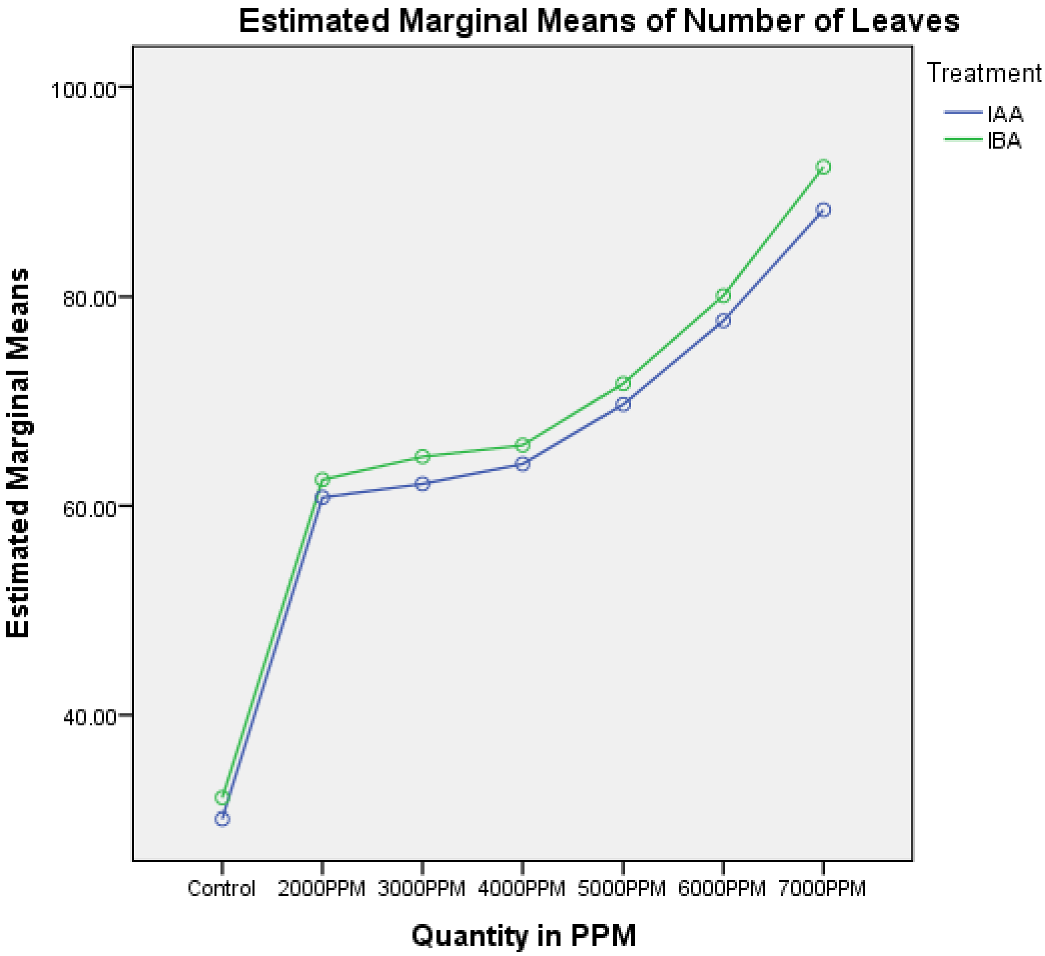

## Figure 8

Comparison of number of roots of T. wallichiana fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).



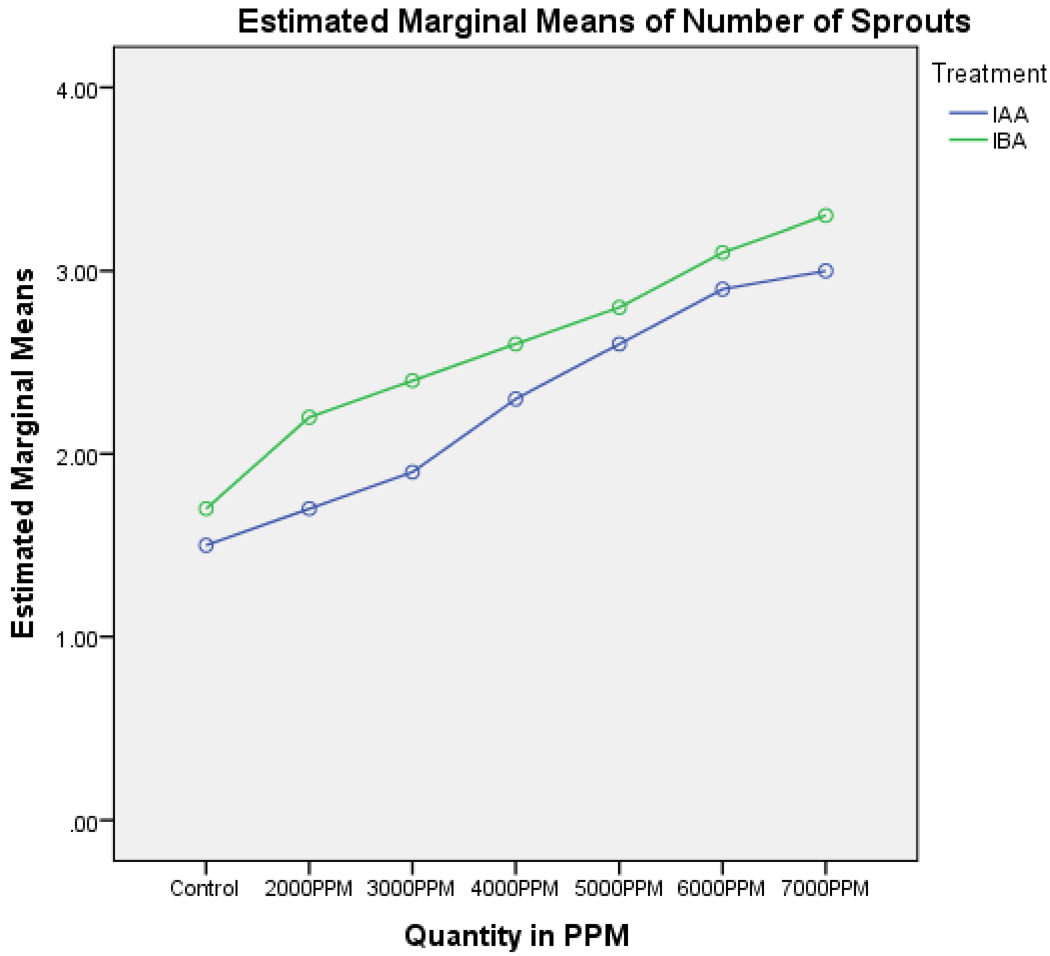

## Figure 9

Comparison of length of roots (cm) of T. wallichiana fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).




### Figure 10

Comparison of number of leaves of T. wallichiana fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).




## Figure 11

Comparison of sprouts of T. wallichiana fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).

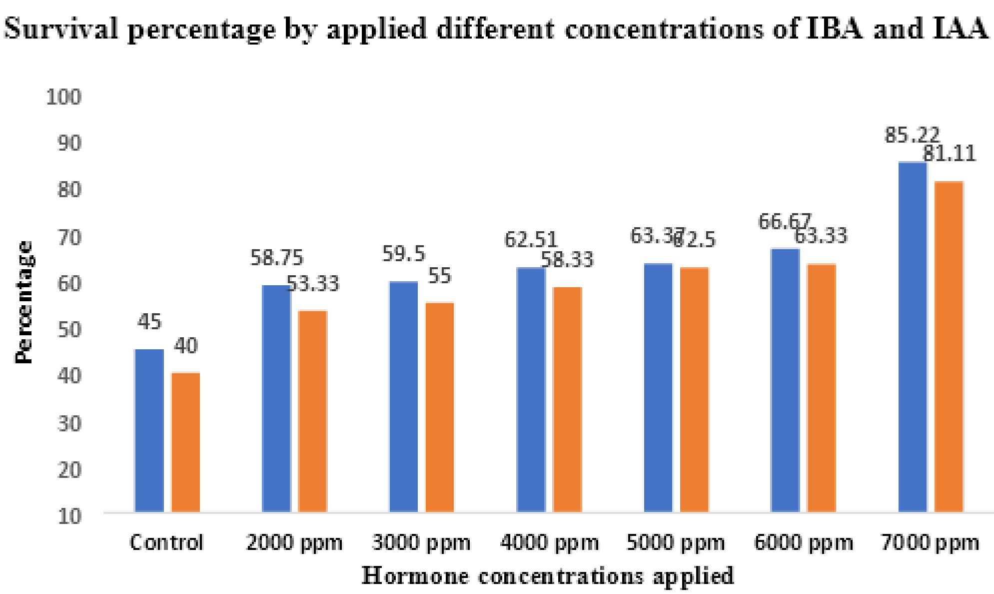


**Figure 12**

Survival percentage of T. wallichiana cuttings by the application of various concentrations of hormones (IBA and IAA).

# Supplementary Files

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