RESEARCH PAPER

Cultivation of Ginkgo biloba L. - A medicinally valued plant with selected plant hormones

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Abstract
Its excellent medical potential and widespread demand for its herbal products have led to indiscriminate exploitation and a severe risk of extinction. A durable and effective method has been created for both seed and semi-hard stem cutting propagation. Certain growth hormones have been applied at varying concentrations. At 10 mg/l (96.67%), catchin acid had the highest sprouting percentage, whereas tanic acid had the lowest at 2.5 mg/l (24.0%).In the second year, IBA 250 mg/l (85.33%) demonstrated statistically significant rooting (%) and root length/plant. IBA 250 mg/l, 13.67 cm, IBA + Phloroglucinol 250+10 mg/l, and Salicyclic acid 25 mg/l, 7.33. 250 mg/l (14.67 cm) were used to assess the average root length/plant in NAA. Seed germination rates obtained with NAA 500 mg/l (84.73%) and IBA 250 mg/l (85.74%) were statistically significant and showed similar levels of effectiveness. From 2005 to 2013, monthly measurements of the FYM doses—15, 30, 45, and 60 t/ha with control—were made and statistically examined annually.The purpose of the FYM x Spacing project was to look at the leaf producing industry's economic feasibility. From 2009 to 2013, FYM 30 t/ha yielded the most leaves. This study focused on improving propagation techniques through semi-hard stem cuttings and hormone-guided seed germination.IBA showed superior performance in both rooting of cuttings and seed germination.Interestingly, a sizable percentage of sprouting and rooting was also induced by catchin and galic acid.

Keywords : *Ginkgo biloba* L., IBA, IAA, Phenolic substances, FYM.
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Introduction

Ginkgo biloba was recognized as a living Jurassic fossil in the world scenario, highlighting its relic status [1]. Ginkgo biloba L., a member of the Ginkgoaceae family, is indigenous to China. Its "fruit," which may be eaten raw or fried, was first mentioned in Chinese herbals in the fourteenth century A.D. Although its survival as a wild state was questioned, it might still persist in some of China's more isolated mountain valleys, including those in Zhejiang province in the country's east. Because of the leaves' bilobed structure, the plant is known by its name, biloba.The form and veining of the leaves are similar to those of the maidenhair fern. It is revered and grown in temple gardens in China and Japan. Around 1730, it was introduced to Europe, the tree is now commonly grown as an ornamental species in Western parks and streets, and as a medicinal plant in countries including China, France, Germany, and the United States.Because female trees yield a lot of foul-smelling seeds, male trees are preferable for planting. Ginkgo is a long-lived, aesthetically pleasing tree that is extremely resilient to air pollution, germs, viruses, and insects. According to aresearch by a management consultant located in London, G. biloba is a species that urgently needs sustainable conservation due to its widespread use in herbal treatments [2].

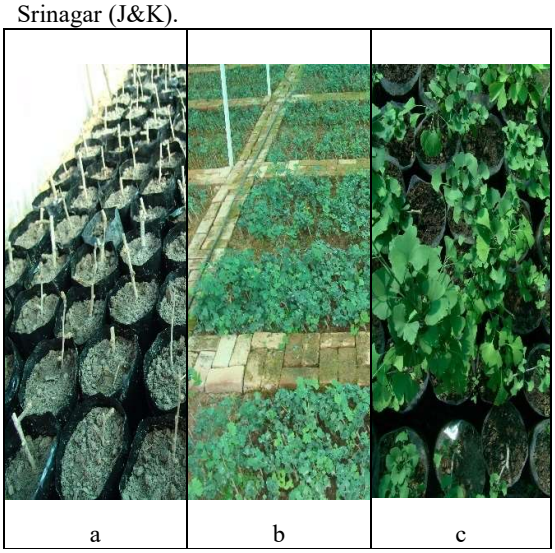
According to Li-Shih Chen's 1596 publication "Pen Ts'ao Kang MLL" (Great Herbal), G. biloba seeds have therapeutic qualities. According to a review, G. biloba has been used to treat a variety of conditions, including multiple sclerosis, brain damage, circulatory diseases, eye disorders, diabetes, headaches, asthma, tinnitus, impotence, allergies, Alzheimer's disease, and as a free radical scavenger [3]. G. biloba leaf extract has been applied to myocardial ischemia, reperfusion damage, and cardioprotective mechanisms [4]. Its effectiveness against environmental pollution has been documented by some workers [5,6].According to Huang et al. (1990) [7], 51 species of broad-leafed deciduous trees, evergreen coniferous trees, deciduous shrubs, etc. in Beijing have been found to accumulate sulfur (s) and be tolerant to SO2. Due to extensive exploitation by pharmaceutical businesses worldwide, the plant is on the verge of going extinct. According to Purohit et al. (2009) [8], G. biloba Linn is a Living Fossil that is endangered because of illicit exploitation, human pressure, and ignorance of sustainable harvesting practices. Through the use of seeds and semi-hard stem cuttings for vegetative growth, we have attempted to establish it throughout India. The best plants were chosen, multiplied in the nursery, and then moved to the test area.Over 50% of the G. biloba population was discovered to be close to the

extinction stage, according to surveys conducted in India (54 numbers in five years). Due to its rarity and great demand worldwide, the demand for its product and extract is rising by roughly 26 to 32 percent per year. In order to prevent the extinction of this species, sustainable agricultural methods are used to develop the vegetative propagation and multiplication technique. Large-scale nurseries and commercial plantations have been created in India through the actual application ofthe standardized propagation strategy.

Importance of Conservation of Ginko biloba- According to a report by a management consultancy located in London, G. biloba is a species that urgently needs to be conserved due to its uses in herbal and various other treatments. Every year, global demand for G. biloba rises from 26% to 32%. Therefore, it is imperative that G. biloba be cultivated on a wide scale [2]. The G. biloba said to be oldest tree species still living. It was noted following 54 surveys that were carried out throughout India during a five-year period, from 2004 to 2008. Of the approximately 30 G. biloba trees known to exist in India, roughly 60% are younger than 30 to 35 years old, and the remaining 40% are semi-dried, meaning they are on the verge of dying as a result of illicit commercial extraction. The majority of the plant's branches and bark have been cut off. The present state of G. biloba in India has already been documented by the author (Gopichand et al., 2009). G. biloba is what Charles Darwin referred to as a "living fossil." According to reports, ginkgo reached its peak of diversity on several continents during the Jurassic period, and it began to quickly decline at the end of the early Cretaceous period [9]. Mesozoic remains of this species have been discovered in Eastern China, where it is native. G. biloba was the only species to survive the First and Second Planet Wars, whereas all of its related species disappeared from other regions of the world. Japan brought it to Europe in 1730, and the USA adopted it as a decorative tree in 1784 (http://w.w.w.worldagroforestrycentre.org). First stated in Lan Mao's Dian Nan Ben Cao (Southern Yunnan's Pharmaceutical Natural History), ginkgo leaves are known as bai-guo-ye in Chinese medicine. The commercial work was registered in 1505 [10], and it was published in 1436 during the Ming dynasty. More than 300 scientific investigations into the pharmacology, chemistryand therapeutic effects of its leaf were carried out. The treatment of bacterial and fungal infections, heart disease,brain damage, eye conditions,dementia, chronic cerebral insufficiency, senility, and poor circulation, vertigo, tinnitus, and a wide range of clinical conditions have all entered a new era in human society based on experimental studies. Ginkgo leaf extracts are finding new applications in blood circulation, memory, erectile dysfunction, and circulatory issues. More advantageous characteristics of the Egb 761 include leaf bio-flavanoids (ginkgolides A–C and J, along with bilobalide). Platelet-activating factor (PAF) is used to treat respiratory, cardiovascular, and inflammatory conditions, among others. It is used to combat pollution, particularly in urban forestryand vesicular arbiscular mycorrhiza formation in ginkgo biloba roots colonized by Glomus epigaeum. It is critically necessary to preserve and establish as many of the aforementioned positive traits as feasible.

2. Methodology

The Biodiversity farm of Institute of Himalayan Bioresource Technology, Palampur (1320m, msl, 320 06'05"N 76034'110"E) in the Indian state of Himachal Pradesh's mid-hill region, was the site of the experimental work from 2005 to 2013. The cutting of the semi-hard stem was taken from the field. Additional parameters recorded included GPS coordinates, plant height and diameter, soil and leaf samples, as well as stem cuttings collected for vegetative multiplication.Nevertheless, some plants are extremely young, some are semi-dried, and some lack stem cuttings. The semi-hard stem cuttings, which had five to six nodes and an average length of 15-20 cm with a diameter of 6.0 to 9.0 mm, were chosen for vegetative propagation.The final week of November through the middle of December was when the stem cuttings were gathered. Certain phenolic acids and growth hormones were chosen for rooting. For comparison in terms of quality characteristics, Stem cutting material was additionally collected fromRanikhet,Mussoori, Dehradun, Nanital (Uttrakhand), Manali, Shimla, Kalpa (H.P.), and Srinagar (J&K).



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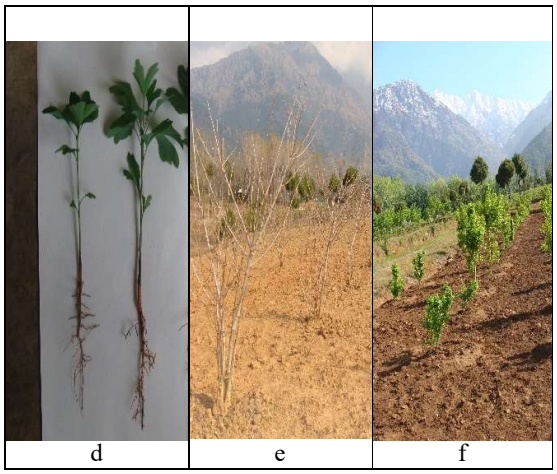

Fig. 1(a). Sprouted semi hard cuttings of G. biloba after one year. Fig. 1(b). Sprouting of semi hard cuttings after 2 years. Fig. 1(c). Seedlings transferred in the poly sleeves by seeds raised. Fig. 1(d). Rooted seedlings raised by seeds. Fig. 1(e). A field trial (FYM x Spacing) in dormant period. Fig. 1(f). A field trial in fully growth season (July-August).

The roots experiments were independently constructed using different hormone concentrations (each hormone applied separately at 250, 500, and 1000 mg/l) to evaluate the combined effects of the different treatments.IBA + Phloroglucinol (250 mg/l+10 mg/l), (250 mg/l+20 mg/l), (500 mg/l+10 mg/l), (500 mg/l+20 mg/l), (1000 mg/l+10 mg/l), (1000 mg/l+20 mg/l), NAA 250 mg/l, 500 mg/l, and 1000 mg/l, IAA 250 mg/l, 500 mg/l, and 1000 mg/l, Ascorbic acid 25 mg/l, ascorbic acid 2.5 mg/l, Tanic acid 5 mg/l, and 10 mg/l 2.5 mg/l, 5 mg/l, and 10 mg/l of catchin acid and 2.5 mg/l, 5 mg/l, and 10 mg/l of galic acid were utilized. Untreated cuttings were immersed in distilled water after all hormones had been dissolved in aqueous ethanol (v/v). All semi-hard cuttings underwent a two-hour immersion at 20°C in either phenol or individual hormone solutions. For this experiment, 16 treatment combinations (16 × 3) were formulated. Each treatment included 150 cuttings (Fig. 2) arranged in three replications of 50 cuttings each. Mean values for each treatment were derived from the recorded observations.

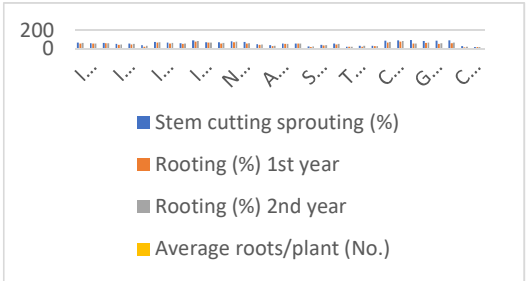

Fig- 2: The effect of different hormones and phenolics of the sprouting of semi hard stem cuttings of G. biloba.

In October 2007, Wuhan All Green Seed, m/s Yu Wangsheng, 27-2-3-301, Changqing Garden, China, provided the ginkgo biloba seeds. For a month, the seeds were kept at 50 degrees Celsius in an incubator. After soaking in regular water for five to six minutes, the seeds were wiped with a fresh cloth and water droplets wereextracted from seed coat's outer layer. Once more, the seeds were maintained at 5.0C in an incubator. This procedure was used after a ten-day break. The previous bed soil was removed up to 8" before planting. The deep surface of the beds was covered with a 2" layer of fine sand.A 4" layer was spread over the fine sand layer in the middle layer after a 1:1:1 ratio (one-part garden soil, one part FYM, and one-part sand) was completely mixed. Once more, a 2" layer of fine sand was dispersed at the top. The seeds were planted in the sand layer at a depth of 2". On December 27, 2007, the seed germination trials were set up **Fig. 1(b) and Fig. 1(c)**. IAA, IBA, NAA, and Salicyclic acid concentrations of 250 mg/l, 500 mg/l, and 1000 mg/l were measured, and the seeds were immersed for an hour. For every concentration, 90 seeds were used **(Fig 3)**. Ninety seeds were used as the control group, and they were submerged into water forabout 60 minutes. First set was being sown inside polyhouse and the second was being sown on an open bed that was only three feet high and covered with 50% agro-net for a partial shed. When necessary, the irrigation was carried out. The same procedure was employed as in the prior instance, but we simply used a 1:1:1 ratio of soil texture outside in the open bed (part garden soil,FYM andfine sand). The seeds were only planted two inches deep. On March 17, 2008, G. biloba seeds began to germinate, and this process continued until April 30, 2008, when between 32.27 and 85.74 percent of the seedsgerminated.

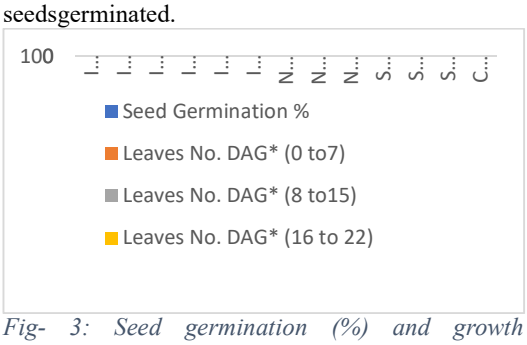

Fig- 3: Seed germination (%) and growth performance of seeds raised saplings after germination under the treatment of different hormones.

After 128 days, germination began and lasted for 192 days. From November to February, Palampur experiences exceptionally chilly agroclimatic conditions. Following germination, the number of leaves and germination percentage were noted. Plant height, root count, and root length were also noted at the time of transplanting **Fig. 1(d)**. From

June 2008 to January 2011, the growth rate and performance various hormone treatments were also documented.

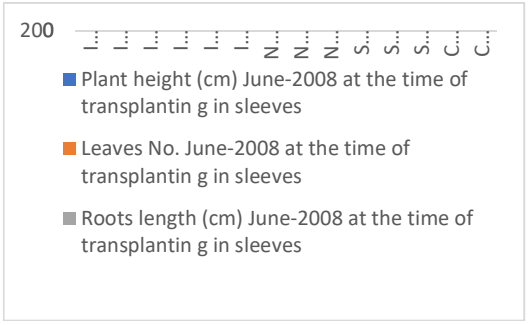

Fig- 4: Vertical growths of seeds raised saplings under the treatments of different hormones.

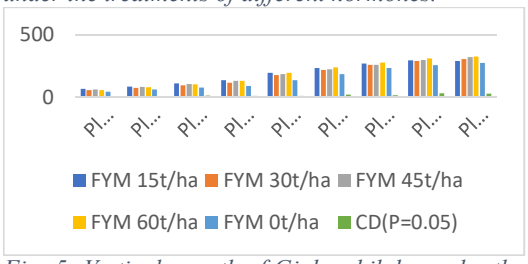

Fig- 5: Vertical growth of Ginkgo biloba under the treatment of different doses of F.Y.M. in different year's (in six year).

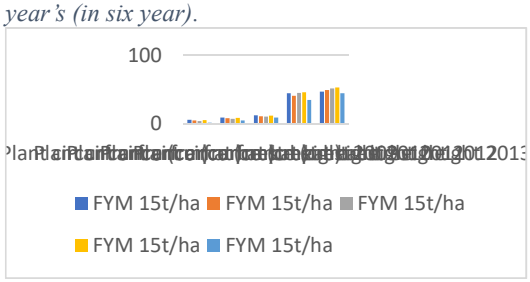

Fig-6: Plant circumference at the Brest height (BDH) of Ginkgo biloba at different treatment of F.Y.M. and their fresh leaves weight.

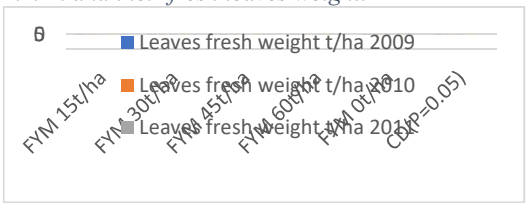

Fig-7: Fresh weight of leaves of Ginkgo biloba t/ha in consequent five years in different FYM application.

Fig-8: Dry weight of leaves of Ginkgo biloba t/ha in consequent five years in different FYM application.

Four distinct doses of FYM were also used in a field study; 15 t/ha, 30 t/ha, 45 t/ha, and 60 t/ha were taken, and FYM was administered based on calculations made for each pit. The FYM doses were administered annually in December and prior to planting; the hole measured 60 x 60 x 60 cm. The trail of 315 plants were planted in five different treatments, each consisting of 63 plants, in the experimental plantation, which was completed in January 2005. Plants were spaced 3 m × 3 m from one another and from row to row in each treatment. Top soil and subsoil samples were collected at planting time and their physico-chemical characteristics examined. The soil had a silty clay loam texture, a high percentage of organic carbon, and a normal to slightly acidic soil reactivity. High and low N, P&K are available. Every month, measurements of plant height and their diameter was taken at ground level. Beginning in April 2005, the observations were made every year until 2013. Additionally, the FYM doses were administered annually in December. The breast height diameter was also measured during the course of the previous five years, from 2009 to 2013. In the past five years, the leaves have also been gathered for commercial use. The STATISTICA-7 statistical tool was used to statistically examine each parameter.

3. Study on seni hard Stem cutting, seed germination and (FYM X Spacing) in Field Experiment

Regarding the sprouting of semi-hard G. biloba cuttings and the rooting response under different concentrations of IBA combined with phloroglucinol, the treatment IBA (500 mg/l + phloroglucinol 10 mg/l) produced the highest and statistically significant rooting percentage (63.3%) in the first year. This was followed by the treatment IBA (250 mg/l + phloroglucinol 10 mg/l) (Fig. 1a, 1b). Regarding average roots/plant in the first and second years as well as rooting (%), the findings were statistically significant **(Fig 2)**. However, in the first and second years, a statistically significant percentage of sprouting and roots were produced by the growth hormones given separately at different doses of IAA, IBA, and NAA (250, 500, and 1000 mg/l) **(Fig 2)**. When compared to IAA, the maximum sprouting percentage (91.33%) was obtained with NAA and IBA 250 mg/l.

Interestingly, both galic acid and catchin acid simultaneously showed statistically significant root and sprouting percentages in the first and second years when compared with control **(Fig 2)**. In our independent experiment, we also attempted to evaluate the percentage of soft stem cuttings that sprouted against hard cuttings. Compared to hard cuttings, very few soft cuttings develop. Cuts from side branches that were three years old and thicker than pencils, however, had the finest results.

The purpose of the seed germination experiment was to investigate how hormones affect seed germination. The dormant season in Palampur's climate lasts from November to mid-March **Fig.1(e)**. From the time of seed sowing until the beginning ofgermination of seed, which occurred after 128 and up to 192 days, the germination parameter was recorded **(Fig-3)**. IBA treatment at 250 mg/l had highest seed germination rate (85.74%). The germination rate was 84.73% in NAA 500 mg/l, however the values were statistically significant and comparable to IBA 250 mg/l. In comparison to 250 mg/l, the germination percentage was statistically significant at higher IAA concentrations of 500 and 1000 mg/l; nevertheless, higher concentrations are equivalent.All of the hormonal treatments were statistically significant when compared to the control. Weekly measurements of the plant's height and leaves were also made for 192 days. According to **Fig-3**, only plant height was significant. Following the transfer of one-year-old plants in poly sleeves for hardening, the growth metrics continued until January 2011 (Fig-3). At the time of transfer, measurements were made of the height of the plants, the number of leaves, the length of the roots, and the number of roots per plant. These measurements were made until January 2011 **(Fig-4)**. In the final week of March marks the beginning of the new sprouting. It has been noted that G. biloba grows only in a cyclical pattern every year between April and May **Fig.1(f)**. It occurs at the end of the inactive phase. When the saplings were moved into the sleeves, which was during the final week of June 2008, the root length and number of roots per plant were simultaneously measured **(Fig-4)**. Furthermore, the number of roots per plant varied between the hormone treatments and the control group in a way that was statistically significant. When compared to all other treatments, the control group's root length and number per plant were much lower. **Fig. 1(d)**.

The FYM dosages were applied at 15, 30, 45, and 60 t/ha in the field trial. When compared to the control, plant height and relative growth rate per year wasalso significant. Plant height has improved with lower dosages of FYM 15t/ha compared to larger doses **(Fig 5)**. At lower doses of FYM 15 t/ha, the findings are likewise statistically significant in terms of plant circumference **(Fig-6)**. Plant height at breast measurement and circumference was also assessed for past five years, from 2009 to 2013 **(Fig-6)**. The results show that the FYM application produced statistically significant results when compared to the control. Fresh leaves were taken in November of each year from 2009 to 2013 as part of the field experiment (FYM x spacing) **(Fig-7)**. That being said, in May of 2009, a hailstorm damaged 10 to 15 percent of the leaves. In the past two years, 2012 and 2013, there have been severe hailstorms in April, May, and September that have severely damaged between 40 and 50 percent of the leaves and caused numerous tears. FYM dose 30t/ha produced batter performance (fresh weight of leaves) in 2009 and 2010, whereas 45t/ha provided statistically significant fresh weight in 2011 **(Fig-7)**. Both the fresh and dry weight of leaves declined at the same time in 2012 and 2013.

4. Discussion

The final objective was to Standardizing the agro technological and propagation methods for batter leaf growth of this economically significant plant species through seed germination and semi-hard stem cuttings, in conjunction with established culture field procedures, was the ultimate goal. All growth hormone and salicylic acid concentrations were shown to have beneficial and statistically significant impacts on cutting sprouting/bud break and seed germination when compared to the control. Additionally, it was noted that the plants' height and diameter considerably increased in comparison to the control as the FYM doses rose. The best growth regulators for vegetative and seed propagation are IAA and IBA. These results validated our findings. Stratification of seeds for 30 to 60 days at 5 0C before sowing has been proposed [11-13]. When semi-hard stem cuttings are pre-treated with growth hormones and phenolic acids, auxins—which promote rooting—have become more abundant and endogenous in the surface area of cut ends [13]. Auxins have also been observed to encourage adventitious root formation in stem cuttings of various species, either alone or in conjunction with phenolic compounds [14,15].

Plant height, cell division and its elongation, leaf count and chlorophyll synthesis were all enhanced by IBA and IAA. (MuKaila et al. 1996-97).These chemicalscooperate to activate enzymes and mobilize food sources once the dormancy barrier has been overcome, facilitating efficient embryo growth and, eventually, germination in viable seeds. Low doses of growth regulators have also been shown to increase plant height, leaf number, root length, and their quantity in a variety of vegetable and tree species [16]. The relationship between endogenous hormones and ginkgo seed growth and decline was investigated [17]. Auxin moves between plant cells by a combination of

membrane diffusion and carrier-mediated transport, claim Kramer and Bennett (2006) [18]. Research has also been done on how applying nitrogen and phosphate affects the growth of G. biloba. N fertilizer allowed G. biloba trees to retain normal foliar N-dynamics even during a lengthy drought when foliar P dynamics were significantly altered [19]. The effect of fertilizer on development and leaf yield was also examined in plantations that produce ginkgo leaves [20]. Del-Tredici (1992) [21] has studied the ginkgo plant's natural regeneration from cotyledonary. The preservation and use of this medicinal plant have been the subject of much investigation in China [22]. It may be grown up to 2000 meters above sea level, and its leaves can be used to create commercial therapeutic goods, according to Zhang and Zhang (1994) [23]. The relationship between seasonal variations and the amounts of ginkgolides and bilobalides in leaves has been demonstrated [24-25]

5. Conclusion

Standardizing propagation techniques using semi-hard stem cuttings and seed germination affected by certain growth hormones and phenolic compounds was the main objective of the current study. In the situations of seed germination and semi-hard stem cuttings, the IBA produced the most notable results. Surprisingly, catchin and galic acid also caused a significant portion of sprouting and roots. An experiment was also conducted in the field (FYM x spacing) to examine the growth characteristics and, ultimately, the leaf production in each treatment for commercial importance. A lower FYM dose (15 t/ha) had significant results in terms of growth characteristics in the field experiment (FYM x spacing). Whether there were significant impacts on leaf production from the FYM dosage (30 t/ha).

Conflict of Interest

The authors declare no conflict of interest.

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