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Standardization technique of vegetative propagation in *Melia Dubia* cav.

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Abstract
Melia dubia, commonly known as Malabar neem, is a fast-growing indigenous species. It is considered a major wood in plywood and paper industries, and the plant is put into various medicinal uses, preferred for use in packing cases, cigar planks etc. There is a dearth in the availability of good planting material. The main difficulty in establishing forest plantation of this species is the poor germination capacity of seeds. An experiment was done to standardize the vegetative techniques in *Melia dubia* using three different hormones at different concentrations. The results of different hormones IBA, IAA and NAA on the rooting and sprouting percentage of the cuttings obtained from the two-month-old seedlings showed that the hormone IBA gave the best results. It was observed that the sprouting and rooting percentages of cuttings were 56 and 48.6 per cent, respectively. The values for these observations were lower when cuttings were treated with the hormones IAA and NAA. Effect of growth regulators and their concentrations on the percentage of root initiation and sprouting of the Melia hardwood cuttings showed that the hormone IBA at 6000 mg l^{-1} concentration was the best combination as far as the number of days to sprout, and the percentage of root initiation was considered. For standardization of clonal progenies, cuttings from the seedlings, semi-hardwood cuttings from mature trees and root suckers by damaging the roots were taken to develop a method for mass multiplication of the *Melia dubia*. Three different auxins, IBA, NAA, and IAA, at different concentrations were administered. The result showed that the best treatment for the cuttings and root suckers was IBA at 1000 ppm. For the semi-hardwood cuttings, monoclonal technology was used, and the best treatment observed was IBA at 6000 ppm.

Keywords: *Melia dubia*, Clonal propagation, Root sucker, Hormones

Introduction
Till recently, exotic species dominated the farmlands. There are many controversies regarding the cultivation of exotic trees, so preference is given to the fast-growing indigenous species (Sharma *et al.*, 2019) [38]. *Melia dubia* is one of the suitable alternate species. It is an indigenous fast-growing tree species and is considered as a major wood in plywood and paper industries. The plant is also put into various medicinal uses, preferred for use in packing cases, cigar planks etc. It is highly suitable for agroforestry in India, with adaptability to a wide range of climatic conditions (Kumar *et al.*, 2017) [17]. In southern parts of India, there are instances where some are planting Melia as a plantation species replacing rubber. This created an increased demand for nursery-grown seedlings largely among farmers, which resulted in an urgent need to produce large stock of healthy and vigorous seedlings in a short period of time. However, the main hurdle in establishing forest plantation of this species is the poor germination percentage of seeds. For any large-scale afforestation programme to be successful, the most important prerequisite is uniform germination of seeds with good vigour. An attempt has been made in different states of India to improve this species. Some of the southern states have gone further and have released varieties of *Melia dubia*, whereas, in Kerala, such an attempt has not been reported. As already mentioned, the main difficulty in establishing forest plantation of this species is the poor germination capacity of seeds, and also it shows poor recruitment in the wild. With this background, a study was done with the objective to develop commercially viable clonal propagation protocol for the species.

Clonal propagation
Macro clonal propagation studies
Clonal propagation in tree breeding programmes is emerging as a strong attraction to the traditional seed orchard breeding system. It has been widely used for the preservation of genotypes in clonal banks and for clonal seed orchard establishment.

(Zobel and Talbert, 1984; Surendran *et al.*, 2000) [49, 44]. It helps in the mass production of desired individuals for planting purposes, developing and maintaining a genetic base population for advanced generation. Clonal propagation is widely used for the multiplication of elite plants obtained in breeding programmes or that are selected from natural populations (Hartman *et al.*, 1990) [12]. This helps to produce plants which are identical in genotype with the source plant (ORTET). Large genetic advances can be made in a single step by selecting unique superior trees from a population of seed-producing trees and reproducing by vegetative propagation. Vegetative propagation also helps in the removal of biological constraints associated with seed collection, viability, storage, germination and pest. To increase productivity, planting disease-resistant parent stock or selecting parent stock showing a wide range of adaptability can be used (Saini, 2001) [34]. In many forest tree species, techniques have been standardized, which has helped for large-scale propagation with cost less than that for the seedling production. Clonal techniques are also used for exploiting the considerable amount of genetic variability already existing in the natural population of forest tree species that have been suitable for vegetative propagation. It is extensively used for the domestication and improvement of tropical tree species. This has helped in the yield improvement. Several approaches, such as stem cutting, hardwood cutting, and sucker, can be used. The causes of variation of the genetic component may be attributed to the lack of endogenous auxins or any of rooting co-factors, lack of enzymes for synthesis of auxin-phenol complexes, presence of inhibitors. Within clone variation, among plants is considered to be mainly due to the physiological condition of the stock plant, which in turn may be affected by the environment and season, position of the harvested shoots on the plants, age and size of the tree and the incidence of pathogens (Leakey, 1987) [18].

The ability of plants to reproduce vegetatively depends on the extraordinary capacity of plant cells to dedifferentiate and redifferentiate, forming new roots or shoots (Pacurar *et al.*, 2014) [22]. Many studies have been conducted to understand the entire process of root formation. The studies show that adventitious root formation is a heritable quantitative genetic trait controlled by many internal and external factors. The most important among them are auxin, light, temperature and mineral nutrients (da Costa *et al.*, 2013) [6]. Two significant types of AR formation have been identified, which vary with the species a) in some species performed, AR's initials are already present, and under favourable conditions, they become active, e.g. Salix, Populus and Jasminum B) in other species, no preformed cells are present. The cells undergo dedifferentiation during an induction phase first to acquire competence for cell division and organ regeneration (Pijut *et al.*, 2011) [27]. The physiology of auxin action indicated that auxin was involved in plant growth activities such as stem growth, adventitious root formation (Haissig and Davis, 1994) [11]. It has been confirmed that auxin is required for adventitious root initiation on stems. Further, it has been shown that divisions of the first root initial cells are dependent upon either applied or endogenous auxins (Gasper and Hofinger, 1988) [7]. External application of auxins has been shown to induce roots. The results have been found to be predictable and consistent, irrespective of the plant species. Among the auxin, IBA is generally used as a rooting hormone because of its higher root-inducing capacity compared with other auxins. One of the reasons is its greater stability to light than IAA (Kurepin *et al.*, 2011) [18]. The use of different rooting hormones for the development of stem/branch cuttings of forest tree species is widely studied (Baul *et al.*, 2010) [50]. Different studies show that the rooting hormone applied for cuttings, especially IBA, had a significant result in rooting of various tropical forest tree species (Tchoundjeu *et al.*, 2002; Tchoundjeu *et al.*, 2006; Husen and Pal, 2007) [51, 52, 13]. However, it is generally observed that tree species and even clones respond differently to the individual and mixed applications of auxin at differing concentrations, even when many other factors are constant; hence, studies are usually done based on this.

The growth of soft, semi-hard and hardwood branch cuttings of 8 to 10-year-old *Azadirachta indica* trees treated with IAA, IBA and 2, 4-D at 500, 1000 and 1000 mg l^{-1} were observed for rooting. Nearly all auxin treatments increased growth over control. The maximum increase in main branch length and number of leaves of hardwood cuttings were in the 500 mg l^{-1} IBA and 1000 mg l^{-1} IAA treatments. Palanisamy and Pramod Kumar (1996) [24] studied the variation in rooting of branch cuttings of neem collected from superior 25-year-old trees from proximal, middle and distal portions of the branch of different lengths (5, 15 and 25 cm) treated with 1000 mg l^{-1} IBA, cuttings of 25 cm long and 0.5 cm diameter from the distal end gave 100 per cent rooting. Percentage rooting decreased significantly when the length of the cutting was reduced, and no rooting was observed in 5 cm long cuttings, irrespective of the position. The two-year-old sapling cuttings of *A. catechu* gave good rooting performance (63.2%) in 100 mg l^{-1} IBA (Verma *et al.* 1996) [47]. Cladode cuttings of *Casuarina equisetifolia* rooted significantly high with 2000 mg l^{-1} of IBA hormonal treatment compared to its control (Gurumurthi and Bandari, 1988) [10]. In *Eucalyptus tereticornis*, micropropagation was successful when the branch cuttings were treated with IBA at the concentration of 2000 mg l^{-1} (Satish Chandra *et al.*, 1992). Several reports are available for clonal multiplication of *Eucalyptus tereticornis*. Successful clonal propagation techniques were developed using leaf cuttings on treatments with 5000 mg l^{-1} IBA as a powder from formulation. In soft stem cuttings of *Prosopis alba*, the rooting was observed in the IBA 3000 mg l^{-1} + Kinetin 1300 mg l^{-1} treatment (43%) (Karoshi *et al.*, 2000) [15].

Taymour Rostami Shahraji *et al.* (2007) reported that the highest rooting percentages were 21.1% for hardwood and 71% for semi-hard cuttings when they were treated with IBA 8000 mg l^{-1} with mixed media. The combination of IBA with mixed media is highly effective in increasing rooting capacity when compared to the perlite. The medium-sized cuttings of *Pterocarpus dalbergioides* treated with IBA 100 mg l^{-1} exhibited maximum rooting per cent (62%), root length (28.0 cm) and shoot length (38.3 cm) (Venkatesh and Pandey, 2006) [46]. Reddy *et al.* (1992) [32] reported that terminal cuttings, when treated with IBA at 1000 mg l^{-1}, were promising in *Prosopis juliflora*. A successful vegetative propagation method has been developed for *P. juliflora* softwood terminal cuttings on treatment with 1000 mg l^{-1} IBA. In *P. juliflora*, stem cuttings treated with IBA at 2000 mg l^{-1} showed a good rooting response (Goel *et al.*, 1997) [56]. Parthiban *et al.* (1999) [26] reported that double nodal stem cuttings of superior genotypes of *Ceiba pentandra* treated

with IBA at different concentrations (1000, 2000, 3000 and 4000 mg l^{-1}) on a quick dip basis showed high percentage of rooting in IBA at 2000 mg l^{-1} and 3000 mg l^{-1}. Singh and Bhatt (2009) [40] reported that the branch cutting of *Dalbergia sissoo* was collected from higher elevation populations and exhibited a higher per cent of rooting as well as other growth parameters. On average, there was 25 per cent rooting, with a maximum (45.0%) rooting recorded. The response of branch cuttings of *D. sissoo* proved to be much more effective for root induction at 400 and 800 mg l^{-1}.

Manmohan Jagatram (2003) [14] reported that the maximum rooting per cent was found in IBA 2000 mg l^{-1} for hardwood cuttings (21.67%), while softwood cuttings, 1000 mg l^{-1} IBA exhibited maximum rooting (21.67%) in *Madhuca latifolia* in nursery mixture medium. Prasad and Kulkurani (1988) [29] observed maximum rooting percentage (55.8%) in the coppiced shoot of Eucalyptus hybrid after treatment with 2000 mg l^{-1} IBA. Meena Bakshi (1998) [5] reported that the best treatment for maximum vegetative propagule production through coppice shoots of nodal cuttings is IBA 4000 mg l^{-1} powder. Valluri *et al.* (1995) [43] reported that shoot terminal cuttings of *A. mangium* rooted well in 500 mg l^{-1} IBA. IBA was the most effective auxin, giving 40% (100, 200 or 500 mg l^{-1}) rooting in branch cuttings of *Acacia nilotica* made in November (Gurumurthi *et al.*, 1994) [53]. Surendran *et al.* (1996) [43] reported that rooting of branch cuttings of *Casuarina junghuahniana* (two-year-old tree) was good in 100 mg l^{-1} of IBA. Bhupendra Singh *et al.* (2011) [41] reported that the application of IAA and IBA induced a greater number of cuttings collected from a ten-year-old mother tree to root compared to control in *Dalbergia sissoo*. Gera *et al.* (1998) [8] studied the variation in rooting response in ten provenances of *A. indica* and reported that treatment of cuttings with 1000 mg l^{-1} IBA increased rooting by 85.5 per cent, number of roots per cutting by 24.8 per cent, maximum root length by 68 per cent and root dry weight by 218.7 per cent over control.

Shamet and Kumar, 1988 [37] reported that the most effective treatments were IAA at 5000 and 10,000 mg l^{-1}, IBA at 5000 mg l^{-1} and NAA 200 mg l^{-1} in *D. sissoo* cuttings and all the increased rooting to 40% compared with 5% for the controls. The positive response of rooting in treatment with 2000 mg l^{-1} IBA in *Acacia albida* has been reported (Ahmed, 1988) [1]. Puri (1990) [30] concluded that IBA was the most effective growth hormone than IAA and NAA for cutting the propagation of *Casuarina equisetifolia*. Verma *et al.* (1996) [47] studied the rooting of neem cuttings with 100, 500 and 1000 mg l^{-1} of NAA and IBA and reported that all IBA and NAA treatments stimulated rootings and sprouting of neem cuttings, while control cuttings did not root or sprout. In *Juniperus procera*, a threatened plant, the studies for the response of branch cutting showed that among the three hormones *viz*., IAA, IBA and NAA tried, the response for IBA was better when compared to other hormones (Berhe and Negash, 1998) [54]. Mohd Aslam *et al.* (2007) [3] observed that among different auxins, IBA at 500 mg l^{-1} performed best of all the treatments of stem cutting regarding rooting behaviour *viz;* callusing percentage (11.3%), rooting percentage (76.66%), number of roots (12.33) and length of roots (12.50 cm) per cutting. In *Swietenia macrophylla*, it was observed that the percentage of rooting and sprouting was significantly higher in cuttings treated with 4000 mg l^{-1} IBA (Azad and Matin, 2015) [57]. Kulkarni and Jakawale (1999) [16] observed the highest rooting (61.66%) in combination of 2500 mg l^{-1} IBA + 1250 mg l^{-1} kinetin, compared with a value of 28.33% in the control in *D. sissoo*. Karoshi *et al.* (2000) [15] reported that rooting was promoted in *Casuarina cunninghamiana* best using 3000 mg l^{-1} IBA + 1300 mg l^{-1} kinetin in mistless. Branch cuttings of *Melia azadirachta* treated with IAA and IBA triggered rooting and the best treatment was 50 mg l^{-1} IBA collected during February and treated with 50 mg l^{-1} IAA for the cuttings collected in May (Gupta *et al.*1989) [9]. Badji *et al.* (1991) [4] concluded that IBA (8%) promoted better rooting than 2% IBA, 0.2% NAA or 1% IAA in gum arabic (*Acacia Senegal*) cuttings during the rainy season. Palanisamy *et al.* (1996) [24] reported that the stem cuttings collected in February from neem trees showing complete leaf fall or at the bud break stage developed significant adventitious roots, but the rooting percentage was poor in cuttings collected during the vegetative season and very poor in those collected in flowering and fruiting seasons. IBA at 1000 mg l^{-1} was the best hormonal treatment, including 80 per cent rooting in February cuttings compared with 23 per cent rooting in untreated cuttings. Palanisamy *et al.* (1998) [25] recorded increased rooting over that observed in controls, with IBA the best treatment in *Pongamia pinnata* cuttings. Rooting was best in March cuttings, followed by September cuttings, and there was no rooting in July cuttings. IBA induced 100 per cent rooting and the greatest number of roots in the March cutting phase. Palaniswamy and Promod Kumar (1997) [55] observed in *Pongamia pinnata* that 800 mg l^{-1} IBA induced 100 per cent rooting and more shoots during the month of March.

Palanisamy and Bisen (2001) [58] reported that IAA 200 mg l^{-1} gave maximum rooting and root length in *Dendrocalamus* as compared to other treatments of IBA and boric acid. The cuttings responded with good rooting only in particular seasons, i.e. March (53%), January (50%), February and April (35%), and in the remaining months, the rooting was less than 20%.

Palanisamy and Pramod Kumar (2001) [58] reported that IBA 1000 mg l^{-1} induced 80% rooting and luxuriant root system in branch cuttings of mature neem tree (*Azadirachta indica*) only in leaf fall season (February).

In some of the studies, it was also observed that the inter-species variations were observed when they were subjected to the same treatment. Pal *et al.* (1994) [23] observed 62 per cent rooting in the segments treated with 5000 mg l^{-1} IBA – talc in *Casuarina equisetifolia* while in *C. glauca* treatment with 5000 mg l^{-1} IBA-talc caused rooting in about 35% segments with 72% survivability. In *Dalbergia sissoo* and *D. latifolia*, among different concentrations of IBA imposed, 5000 mg l^{-1} IBA has shown the best effect on all parameters of rooting behaviour. It was found that *D. sissoo* showed comparatively more response than *D. latifolia* (Sharma and Pandey, 1999) [39].

The literature indicates that clonal propagation using stem cutting has been successfully used in *Melia dubia*, where the response when dipped in 4000 mg l^{-1} and 5000 mg l^{-1} of IBA was better for rooting percentage (50%) as reported by Nair *et al.*, 2005. In another study, it was also reported that the cuttings from one-year-old branches from four-year-old trees responded best for the treatment IBA at 4000 mg l^{-1}. The rooting percentage was reported as 90 per cent (Ram *et al.*, 2014). In a study done in six-month-old Melia seedlings, stem cuttings obtained from the seedlings were rooted without any hormone treatment, and it was found that the percentage of

established seedlings was 76. It was demonstrated that a small number of plants produced by seed germination can be multiplied about sevenfold (Tilakaratna, 1996) [59] through clonal propagation.

Mini clonal technology

Rooting of stem cuttings was not suitable for a majority of economically important forest tree species. The popular method of rooted stem-cutting has major limitations, *viz.* quick loss of rooting competence due to ontogenetic ageing, intra-clonal variation resulting from apophysis and poor-quality root system that negatively affect the genetic expression of several clones. For many clones, such deformations prevented their full genetic expression, consequently reducing the ratio between the selected trees and the number of cuttings effectively available for plantation use (Tahir Mushtaq *et al.*, 2017) [20]. Because of such limitations of rooting stem cuttings, alternative methods were developed for commercial cloning of tree species; one such method is mini-clonal technology. Mini clonal technology exhibits a great potential of substituting rooted stem-cuttings owing to its technical and economic advantages as well as the evidenced success of rooting in the auxiliary sprouts. The success of this method is considerably dependent upon the rooting medium and optimal nutrient concentration.

Compared to stem cuttings, mini-cuttings improve rooting potential, rooting speed, and root system quality, besides reducing costs. Additionally, this system also offers the opportunity for physiological homogenization of propagules and drastically reduces apophysis effects. Development of this super–intensive cloning system has emerged as a novel and effective method for mass clonal propagation in Eucalyptus and other tree species. In India, propagation by mini-cutting represents the most modern concept of commercial cloning in Eucalyptus, Casuarina, *Melia dubia*, *Dalbergia sissoo* and *Neolamarckia caramba.*

It is reported that plants have endogenous auxins. The removal of the shoot apex causes a reduction in the production of these auxins, thus causing a reduction in the number of adventitious roots. Hence the presence of hormones at lower concentrations may be required to compensate for the reduction in the endogenous auxins due to the removal of the shoot apex (Kurupin *et al.*, 2011). At higher concentrations, the hormones might have a deleterious effect on the growth of the roots. Studies have shown that the high auxin application produces toxicity, and NAA is more toxic than IBA (Zeng and Lu, 1988) [48].

In a study done to standardise the rooting hormone in monoclonal technology in teak where different hormones (IBA, IAA and NAA) at various concentrations *viz*., 1000, 2000, 3000, 4000, 5000 and 6000 mg l^{-1} were applied to the mini cuttings. The result showed that the maximum percentage of rooting and sprouting was for the treatment IBA at 6000 mg l^{-1}. (Packialakshmi and Sudhagar, 2019) [21]. Superiority of IBA over other hormones has been reported earlier by many investigators in *Acacia albida* (Ahmed, 1988) [1], *Woodfordia floribunda* (Shah *et al.*, 1994) [36], in *Parkia biglandulosa* (Reeves *et al.*, 1996) [33], *Azadirachta indica*, *Casuarina equisetifolia*, *Ceiba pentandra*, *Gmelina arborea and Thespesia populnea* (Parthiban *et al.*, 1999) [26], *Ceiba pentandra* (Rajendran *et al.*, 2002) [31] and *Pterocarpus dalbergioides* (Venkatesh and Pandey, 2006) [46] and *Lannea coromendalica* (Prabhakaran, 2013) [28].

The review shows that even though vegetative propagation has been standardized in other states of India, techniques suitable to our climatic conditions are lacking.

To standardize the clonal propagation

Stem cuttings from seedlings

The seedlings in the nursery and the polybags were used for the standardization of clonal propagation. Stem cuttings were obtained from two-month-old seedlings when they reached a height of 25-30 cm. The shoot was cut from the base of the seedlings leaving a height of 5-8 cm from the base. This was left for the sprouting of new shoots. The excised shoots were then cut into 5 cm long (2-3) nodes, and the base was treated with the three auxins (Table 1) to stimulate root formation. For this, the base of the shoots was dipped in the solution containing auxins. Two to three cuttings were obtained from each seedling. The cuttings were planted in trays filled with sand. The cuttings started to sprout within one week. After taking the observations of the sprouting and rooting percentage of the rooted cuttings, they were transferred to polybags of size 10 x 15. The potting media used for this purpose was a mixture of soil, sand and FYM in the ratio of 2:1:1. The experiment was done in Completely Randomized Block design with three replications. Ten cuttings were planted per replication. Altogether 480 plants were maintained. The observations were taken after 30 days of transplanting. Following this, the rooted seedlings in the nursery were treated with nutrient solutions to enhance epicormic shoot production. After the emergence of the new shoots, again, the shoots were excised and rooted, with the best concentrations of IBA, IAA and NAA, which were standardized during the experiment was done.

Collection of data on vegetative propagation

Different growth parameters on which observations were recorded at the end of the study were as follows.

Sprouting per cent = Number of sprouted cuttings / Number of cuttings planted x 100

Rooting per cent = Number of rooted cuttings / Number of cuttings planted x 100

Collar diameter (mm): The diameter of cuttings at the collar region was measured using a digital calliper and expressed in millimetres.

Sprout length (cm): The length of the sprout was measured from the base of the sprout to the tip using a scale and expressed in centimetres.

Stem length (cm): Total stem length was measured from the collar region to the tip of the sprout using a scale and expressed in centimetres.

Root length (cm): The length of the longest root was measured from the collar region to the root tip by running a thread along with the root and then measuring thread length using a scale. Root length is expressed in centimetres.

Number of leaves: The number of total leaves in the cutting was counted.

Number of roots: The number of roots in the cutting was totalled.

Stem cuttings from matured trees using mini-clonal propagation techniques

In order to standardize the stem cutting from matured trees, shoots were severed from the mother plants, wrapped with wet gunny bag material, and transported to the laboratory. The shoots were then treated with a fungicide (2% Bavistin solution). Cuttings with 5-12 cm diameter and 1m length were used for planting. These were treated with 2% Bavistin (Carbendazim solution 50% WP) for 10 minutes and were subsequently washed with distilled water. The cuttings were planted in polybags containing the potting media soil, sand and FYM in the ratio of 2:1:1. These polybags were placed in the mist chamber. After 20-30 days, the new shoots started emerging, and at two leaves stage, they were served from the mother plants, and the base of the shoots was treated with different concentrations of indole acetic acid (IAA), indole 3-butyric acid (IBA) and naphthaleneacetic acid (NAA). Concentrations of auxins ranged from 1000 to 9000 mg l^{-1}. In total, there were 28 treatments, including control (Table 2).

Preparation of the growth regulators

The growth regulator was prepared as a liquid formulation. The different rooting hormones *viz*., IBA, IAA and NAA were used at 1000 mg l^{-1}, 2000 mg l^{-1}, 3000 mg l^{-1}, 4000 mg l^{-1}, 5000 mg l^{-1}, 6000 mg l^{-1},, 7000 mg l^{-1}, 8000 mg l^{-1} and 9000 mg l^{-1} concentrations. The stock solutions of these hormones were prepared by dissolving the weighed quantity of each hormone (i.e., 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9 g/l respectively) first in sodium hydroxide solution and making into known volume with distilled water. The experiments were laid out in CRD with four replications, with each replication having five cuttings each.

Collection of data on vegetative propagation

Sprouting per cent = Number of sprouted cuttings / Number of cuttings planted x 100

Rooting per cent = Number of rooted cuttings / Number of cuttings planted x 100

Experiment on the clonal progeny evaluation

Collection of propagules

Propagules were collected from all the twenty-five plus trees located in the natural forests at selected locations. Propagules used for the experiment were of two types *viz*. a) Semi-hardwood cuttings with 5-12 cm diameter with 1 m height. b) Root suckers from the injured roots. Semi-hardwood cuttings were collected from the plus trees from the leading branches of the tree. For initiation of the root suckers, the roots thinner than 10 cm diameter were severed during the month of January. Suckers started arising from the injured part of the roots after one month of the root injury. These root suckers were used for the study. The coppice shoots were collected with the help of secateurs which were transported in iceboxes to the laboratory to avoid the desiccation.

Preparation of cuttings

The plants were produced from the semi-hardwood cuttings as mentioned above. The best auxin and the concentration to produce the plants from the cuttings standardised during the experiment were IBA at 6000 mg l^{-1}, which was used for this experiment.

The root suckers brought to the laboratory were cut into 10-15 cm length with a minimum of three nodes. Prepared cuttings were treated with two per cent Bavistin solution (Carbendazim 50% WP, Systemic fungicide) for 5 minutes and were subsequently washed with distilled water. Then the cuttings were treated with the growth regulators IBA (1000 mg l^{-1}), as standardized earlier before the start of this experiment. The suckers were immediately planted in the polybags of size 15 cm x 25 cm. The potting media used for this purpose was a combination of soil, sand and FYM mixed in the ratio of 2:1:1.

Standardization of clonal propagation

Effect of different hormones and concentrations on the cuttings from the seedlings of the *Melia dubia*

Sprouting percentage

The results of different hormones at different concentrations on the sprouting percentage are shown in Table 3. It was observed that the hormones at different concentrations were significant. The sprouting percentage of the cuttings treated with IBA was the highest (56%), followed by IAA (45.77%) and NAA (41.55%). The concentration had a negative effect on the sprouting percentage, irrespective of the hormone. The highest sprouting percentage (68%) was observed when the concentration of the hormone was 1000 mg l^{-1}, and the lowest for the concentration was 5000 mg l^{-1}. The interaction between hormones and concentrations was significant (Fig. 1). The hormone and concentration interaction showed that the IBA at 1000 mg l^{-1} was found to be the best combination as the sprouting was 82 per cent and NAA at 5000 was the lowest performing combination (15%).

Rooting percentage

It was observed that the hormones at different concentrations were significant (Table 4). The rooting percentage of the cuttings treated with IBA was the highest (48.6%), followed by NAA (43.93%) and IAA (39.8%). The different concentration levels had a negative effect on the sprouting percentage, irrespective of the hormone. The highest rooting percentage (67.89) was observed for the concentration 1000 mg l^{-1}, and the least rooting percentage (12.61) was observed for the concentration 5000 mg l^{-1}. The interaction between hormones and concentrations was significant (Fig. 2). The hormone and concentration interaction showed that the IBA at 1000 mg l^{-1} was found to be the best combination (75.33%) and 1AA at 5000 was the lowest performing combination (6.67%).

Root length

The effect of hormones at different concentrations on the root length is shown in Table 5. It was observed that the hormones at different concentrations were significant. The root length for IBA (10.22 cm) and IAA (9.36 cm) were at par but were significantly higher when compared to NAA (7.61 cm) hormone treatment. The highest root length (14.39 cm) was observed for all the hormones at concentration 1000 mg l^{-1}, and for concentrations 2000 mg l^{-1} and 3000 mg l^{-1}, they were at par. Similarly, root length for the concentrations 4000 mg l^{-1} and 5000 mg l^{-1} were at par. The highest root length was observed for the treatment IBA at 1000 mg l^{-1} (17.33 cm),

while the lowest value (6.0 cm) was observed for the treatment IBA at 5000 mg l^{-1}. The interaction between hormones and concentrations was significant (Fig. 1). It was observed that the highest root length (17.33 cm) was observed for the cuttings subjected to the treatment T1 (IBA 1000 mg l^{-1}); this was followed by the treatment T6 (IAA 1000 mg l^{-1}), with root length (15.33 cm). The control performed better than some of the treatments, particularly when the concentration of the hormone was higher.

Shoot length

The results of different hormones at different concentrations and their interactions are shown in Table 6. It was observed that the hormones at different concentrations were significant. The interaction between hormones and concentrations was insignificant. The shoot length of the cuttings treated with IBA was highest (6.97 cm), followed by IAA (5.93 cm) and NAA (5.2 cm). The concentration had a negative effect on the shoot length, irrespective of the hormone. The highest shoot length (8.29 cm) was observed when the concentration of the hormone was 1000 mg l^{-1} and the lowest (4.5 cm) when the concentration was 5000 mg l^{-1}. The shoot length did not differ significantly for the concentrations 2000 mg l^{-1} and 3000 mg l^{-1}.

Collar diameter

The effect of hormones at different concentrations on the collar diameter (mm) is shown in Table 7. Collar diameter differed significantly with different hormones. There was no significant difference in the concentration and for the hormone and concentration interactions. The collar length for IBA (3.12 mm) was the highest. The collar diameter was at par for the cuttings treated with the hormones IAA and NAA.

Number of leaves

The effect of hormones at different concentrations on the number of leaves is shown in Table 8. Collar diameter differed significantly with different hormones and for different concentrations. The interactions between hormones and concentrations were insignificant. The number of leaves for IBA and IAA were on par. The highest number of leaves was observed for the concentration of 1000 mg l^{-1}

Effect of different hormones and concentrations on the cuttings of *Melia dubia*

The effect of hormones at different concentrations and the interactions between hormones and concentration on the cuttings collected from different plus trees of *Melia dubia* are shown in Table 9. The result showed that there was a significant difference in the percentage of root initiation of the epicormic shoots from the cuttings. Root initiation was observed only for the concentrations 5000 mg l^{-1}, 6000 mg l^{-1} and 7000 mg l^{-1}. For all the other concentrations, the cuttings failed to root, irrespective of the hormones. The result showed that of the three hormones experimented with, the effect of IBA was significant, and the observed percentage of root initiation was 49.48. This was followed by IAA (32.49%) and NAA (29.67%). The maximum root initiation (57.23%) was observed for the concentration of 6000 mg l^{-1} for all the hormones. This was followed by 31. 28 and 23.13 for the concentrations 5000 mg l^{-1} 7000 mg l^{-1}. The best treatment combination was observed for T6 (IBA at 6000 mg l^{-1}) and the least performing combination was for the treatment T25 (NAA at 7000 mg l^{-1}). The values obtained were 78.1% and 19.6% respectively.

The result showed that different hormones at different concentrations had a significant variation with respect to the observed value of the number of days taken to sprout. However, the variations for the interaction were non-significant (Table 10). The value for the number of days taken to sprout (37.76%) was less for the treatment where IBA was used, which was followed by the treatment with IAA (48.5%) and for NAA (50.92%). For the different concentration levels, the best treatment was observed the concentration 6000 mg l^{-1}, where the number of days taken to sprout was least (38.17%), followed by 5000 mg l^{-1} concentration (46%) and 7000 mg l^{-1} (52.92%). It can be concluded that the hormone IBA at 6000 mg l^{-1} concentration was the best combination as far as the number of days to sprout and percentage of root initiation was considered.

Standardization of clonal propagation

Effect of different hormones on the cuttings from the melia seedlings

The results of different hormones IBA, IAA and NAA on the rooting and sprouting percentage of the cuttings from the seedlings showed that the hormone IBA gave the best results (Table 22-27). It was observed that the sprouting and rooting percentages of cuttings were 56 and 48.6 per cent, respectively. The values for these observations were lower when cuttings were treated with the hormones IAA and NAA. It is a fact that a number of internal and external factors influence the process of adventitious root formation in the cuttings. The application of root-promoting growth regulatory substances (auxins) is probably the single most effective external factor that enhances rooting in stem cuttings and thus helps to achieve successful propagation (Sevik and Guney, 2013) [35]. IBA is found to be more stable to light when compared to other auxins, and this may be one of the reasons for using IBA, commonly as rooting hormone. However, the differential roles of IBA, IAA and NAA in promoting root formation are little understood (Pacurar *et al.*, 2014) [22]. It was observed that the increase in the concentration of the hormone level, irrespective of the hormones used, had a negative effect on the root initiation and rooting percentage of the cuttings (Table 20 and Table 21). It is reported that plants have endogenous auxins. The removal of the shoot apex causes a reduction in the production of these auxins, thus causing a reduction in the number of adventitious roots. So, the presence of hormones at lower concentrations may be required to compensate for the reduction in the endogenous auxins due to the removal of shoot apex (Kurupin *et al.*, 2011), but at higher concentrations, the hormones might have a deleterious effect on the growth of the roots. Studies have shown that the high auxin application produces toxicity, and NAA is more toxic than IBA (Zeng and Lu, 1988) [48]. It was observed that the treatment control (T 28) performed better than most of all combinations of the hormones where the concentrations of the hormones were higher. In some species of *Salix*, *Populus* and *Jasminum* presence of preformed adventitious roots initials, which are lying dormant, is reported. These adventitious root initials start growing when the stem is cut and placed in a condition favourable for the growth of roots (Geiss *et al.*, 2009; Pijut *et al.*, 2011) [27]. However, studies are meagre in these aspects, and to confirm the result of our study, further study in this subject, especially

in Melia cuttings, is required. The effect of the hormones on the root length of the cuttings from the seedlings showed that the treatments T 1 (IBA + 1000 mg l^{-1}) and T 10 (NAA + 1000 mg l^{-1}) were on par. The values for root length values were 17.33 and 15.33 cm, respectively. The shoot length, collar diameter and number of leaves were highest for the treatment T 1.

Effect of growth regulators and their concentrations on the percentage of root initiation and sprouting of the melia hardwood cuttings

It was observed that the hormone IBA at 6000 mg l^{-1} concentration was the best combination as far as the number of days to sprout and the percentage of root initiation was considered. The physiology of auxin action indicated that auxin was involved in plant growth activities such as stem growth, adventitious root formation (Haissig and Davis, 1994) [11]. It has been confirmed that auxin is required for adventitious root initiation on stems. Further, it has been shown that divisions of the first root initial cells are dependent upon either applied or endogenous auxins (Stromquist and Hasen, 1980; Gasper and Hofinger, 1988) [42, 7].

The use of different rooting hormones for the development of stem/branch cuttings of forest tree species is widely studied (Leakey *et al.*, 1990; Husen and Pal, 2006; Baul *et al.*, 2010) [50]. Different studies show that the rooting hormone applied for cuttings, especially IBA, had a significant result in rooting of various tropical forest tree species (Tchoundjeu *et al.*, 2002; Tchoundjeu *et al.*, 2006; Husen and Pal, 2007) [51, 52, 13]. In *Juniperus procera*, a threatened plant, the studies for the response of branch cutting showed that among the three hormones *viz*., IAA, IBA and NAA tried, the response for IBA was better when compared to other hormones (Berhe and Negash, 1998) [54]. In *Swietenia macrophylla*, it was observed that the percentage of rooting and sprouting was significantly higher in cuttings treated with 4000 mg l^{-1} IBA (Azad and Matin, 2015) [57].

In a study done to standardise the rooting hormone in monoclonal technology in teak where different hormones (IBA, IAA and NAA) at various concentrations *viz*., 1000, 2000, 3000, 4000, 5000 and 6000 mg l^{-1} were applied to the mini cuttings. The result showed that the maximum percentage of rooting and sprouting was observed for the hormone IBA at 6000 mg l^{-1}. (Pakialakshmi and Sudhagar, 2019) [21]. The result corroborates our findings. Superiority of IBA over other hormones has been reported earlier by many investigators in *Acacia albida* (Ahmed, 1988) [1], *Woodfordia floribunda* (Shah *et al.*, 1994) [36], in *Parkia biglandulosa* (Reeves *et al.*, 1996) [33], *Azadirachta indica*, *Casuarina equisetifolia*, *Ceiba pentandra*, *Gmelina arborea and Thespesia populnea* (Parthiban *et al.*, 1999) [26], *Ceiba pentandra* (Rajendran *et al.*, 2002) [31] and *Pterocarpus dalbergioides* (Venkatesh and Pandey, 2006) [46] and *Lannea coromendalica* (Prabhakaran, 2010) [28].

Table 1: Treatment combinations for the rooting of stem cuttings from seedlings.

Treatment no.	Growth regulator	Concentration (mg l^{-1})
1.	IBA	1000
2.	IBA	2000
3.	IBA	3000
4.	IBA	4000
5.	IBA	5000
6.	IAA	1000
7.	IAA	2000
8.	IAA	3000
9.	IAA	4000
10.	IAA	5000
11.	NAA	1000
12.	NAA	2000
13.	NAA	3000
14.	NAA	4000
15.	NAA	5000
16.	Control	0

Table 2: Treatment combinations for the rooting of stem cuttings from mature trees are given as below.

Treatment no.	Growth regulator	Concentration (mg l^{-1})
1.	IBA	1000
2.	IBA	2000
3.	IBA	3000
4.	IBA	4000
5.	IBA	5000
6.	IBA	6000
7.	IBA	7000
8.	IBA	8000
9.	IBA	9000
10.	IAA	1000
11.	IAA	2000
12.	IAA	3000
13.	IAA	4000
14.	IAA	5000
15.	IAA	6000

16.	IAA	7000
17.	IAA	8000
18.	IAA	9000
19.	NAA	1000
20.	NAA	2000
21.	NAA	3000
22.	NAA	4000
23.	NAA	5000
24.	NAA	6000
25.	NAA	7000
26.	NAA	8000
27.	NAA	9000
28.	Control	0

Table 3: Effect of different hormones for various concentrations on the sprouting percentage of stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	82.00(1.13)^{a}	70.67 (1.00)^{b}	61.67 (0.90)^{c}	42.00 (0.70)^{d}	23.67 (0.51)^{e}	56.00 (0.85)^{a}
IAA	59.67 (0.88)^{a}	60.00 (0.89)^{a}	53.00 (0.82)^{b}	32.33 (0.60)^{c}	21.33 (0.48)^{d}	45.27 (0.73)^{b}
NAA	62.50 (0.91)^{a}	51.57 (0.80)^{b}	48.33 (0.77)^{b}	30.67 (0.59)^{c}	14.67 (0.39)^{d}	41.55 (0.69)^{c}
Mean	68.06 (0.98)^{a}	60.74 (0.90)^{b}	54.33 (0.83)^{c}	35.00 (0.63)^{d}	19.89 (0.46)^{e}	

Values in the parenthesis show the logarithmic (Log x) values.

Table 4: Effect of different hormones and concentrations on the rooting percentage of stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	75.33 (1.05)^{a}	62.33 (0.91)^{b}	55.00 (0.84)^{b}	36.00 (0.64)^{c}	14.33 (0.39)^{d}	48.60 (0.77)^{a}
IAA	65.00 (0.94)^{a}	55.67 (0.84)^{b}	48.33 (0.77)^{b}	23.33 (0.50)^{c}	6.67 (0.26)^{d}	39.80 (0.66)^{c}
NAA	63.33 (0.92)^{a}	49.67 (0.78)^{b}	47.17 (0.76)^{b}	42.67 (0.71)^{b}	16.83 (0.42)^{c}	43.93 (0.72)^{b}
Mean	67.89 (0.97)^{a}	55.89 (0.84)^{b}	50.17 (0.79)^{c}	34.00 (0.62)^{d}	12.61 (0.36)^{e}	

Values in the parenthesis show the logarithmic (Log x) values.

Table 5: Effect of different hormones and concentrations on the root length (cm) of the stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	17.33 (1.24)^{a}	11.17 (1.05)^{b}	9.17 (0.96)^{c}	7.43 (0.87)^{d}	6.00 (0.76)^{e}	10.22 (0.98)^{a}
IAA	15.33 (1.18)^{a}	8.67 (0.94)^{b}	8.50 (0.93)^{b}	6.93 (0.84)^{c}	7.37 (0.87)^{bc}	9.36 (0.95)^{a}
NAA	10.50 (1.02)^{a}	7.33 (0.86)^{bc}	7.50 (0.87)^{b}	6.07 (0.78)^{c}	6.67 (0.82)^{bc}	7.61 (0.87)^{b}
Mean	14.39 (1.15)^{a}	9.06 (0.95)^{b}	8.39 (0.92)^{b}	6.81 (0.83)^{c}	6.68 (0.82)^{c}	

Values in the parenthesis show the logarithmic (Log x) values.

Table 6: Effect of different hormones and concentrations on the shoot length (cm) of the stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	10.17 (1.01)	6.97 (0.84)	6.80 (0.83)	5.73 (0.76)	5.17 (0.71)	6.97 (0.83)^{a}
IAA	7.30 (0.86)	5.67 (0.75)	6.33 (0.80)	5.67 (0.75)	4.67 (0.66)	5.93 (0.76)^{b}
NAA	7.40 (0.87)	5.43 (0.73)	5.50 (0.74)	4.00 (0.59)	3.67 (0.56)	5.20 (0.70)^{c}
Mean	8.29 (0.91)^{a}	6.02 (0.78)^{b}	6.21 (0.79)^{b}	5.13 (0.70)^{c}	4.50 (0.64)^{d}	

Values in the parenthesis show the logarithmic (Log x) values.

Table 7: Effect of different hormones and concentrations on the collar diameter (cm) of the stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	3.08	2.90	3.40	3.05	3.16	3.12^{a}
IAA	2.83	2.47	3.03	3.18	2.75	2.85^{b}
NAA	2.75	2.93	2.70	2.80	2.59	2.76^{b}
Mean	2.89^{a}	2.77^{a}	3.05^{a}	3.01^{a}	2.83^{a}	

Values in the parenthesis show the logarithmic (Log x) values.

Table 8: Effect of different hormones and concentrations on the no. of leaves of the stem cuttings from seedlings of *Melia dubia*

	Concentration (mg l^{-1})					
	1000	**2000**	**3000**	**4000**	**5000**	**Mean**
IBA	10.00 (0.99)	6.67 (0.82)	7.00 (0.84)	6.00 (0.77)	5.33 (0.73)	7.00 (0.83)^{a}
IAA	11.00 (1.04)	7.00 (0.84)	6.00 (0.77)	5.33 (0.73)	5.00 (0.69)	6.87 (0.81)^{a}
NAA	8.00 (0.90)	6.00 (0.77)	4.33 (0.63)	4.33 (0.63)	4.00 (0.59)	5.33 (0.73)^{b}
Mean	9.67 (0.98)^{a}	6.56 (0.81)^{b}	5.78 (0.75)^{bc}	5.22 (0.71)^{cd}	4.78 (0.67)^{d}	

Values in the parenthesis show the logarithmic (Log x) value

Table 9: Mean table showing the effect of different hormones and concentrations on the sprouting percentage of root initiation of cuttings from *Melia dubia.*

	Concentration (mg l^{-1})									Mean
	1000	**2000**	**3000**	**4000**	**5000**	**6000**	**7000**	**8000**	**9000**	
IBA	0	0	0	0	41.55 (0.70)^{b}	78.1 (1.09)^{a}	28.8 (0.57)^{c}	0	0	49.48 (0.79)^{a}
IAA	0	0	0	0	27.55 (0.55)^{b}	49.03 (0.78)^{a}	20.9 (0.48)^{c}	0	0	32.49 (0.60)^{b}
NAA	0	0	0	0	24.75 (0.53)^{b}	44.58 (0.74)^{a}	19.68 (0.46)^{c}	0	0	29.67 (0.57)^{c}
Mean	0	0	0	0	31.28 (0.59)^{b}	57.23 (0.87)^{a}	23.13 (0.50)^{c}	0	0	

Table 10: Mean table showing the effect of different hormones and concentrations on the percentage of root initiation of cuttings from *Melia dubia*.

	Concentration (mg l^{-1})									Mean
	1000	**2000**	**3000**	**4000**	**5000**	**6000**	**7000**	**8000**	**9000**	
IBA	0	0	0	0	39.25	28.25	45.50	0	0	37.67^{c}
IAA	0	0	0	0	48.75	42.25	54.50	0	0	48.50^{b}
NAA	0	0	0	0	50.00	44.00	58.75	0	0	50.92^{a}
Mean	0	0	0	0	46.00^{b}	38.17^{c}	52.92^{a}	0	0	48.50^{b}

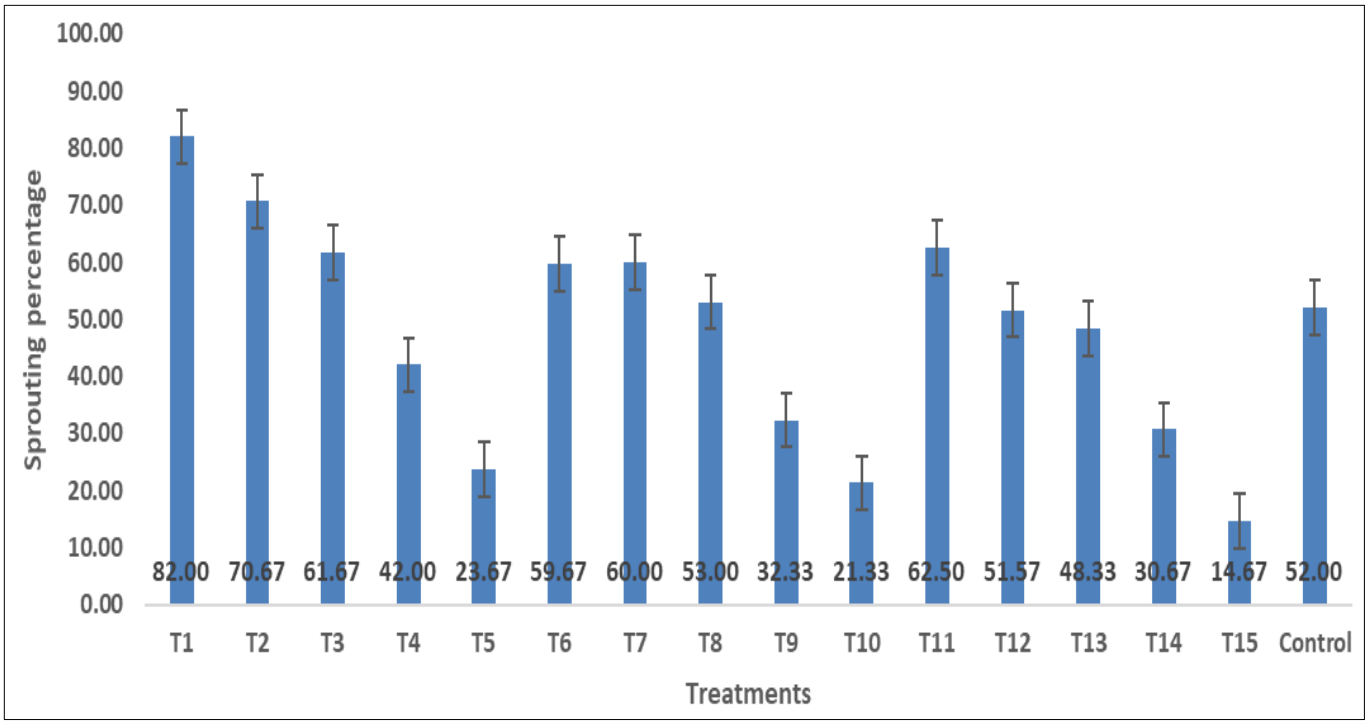

Fig 1: Effect of different treatments on the sprouting percentage of the cuttings

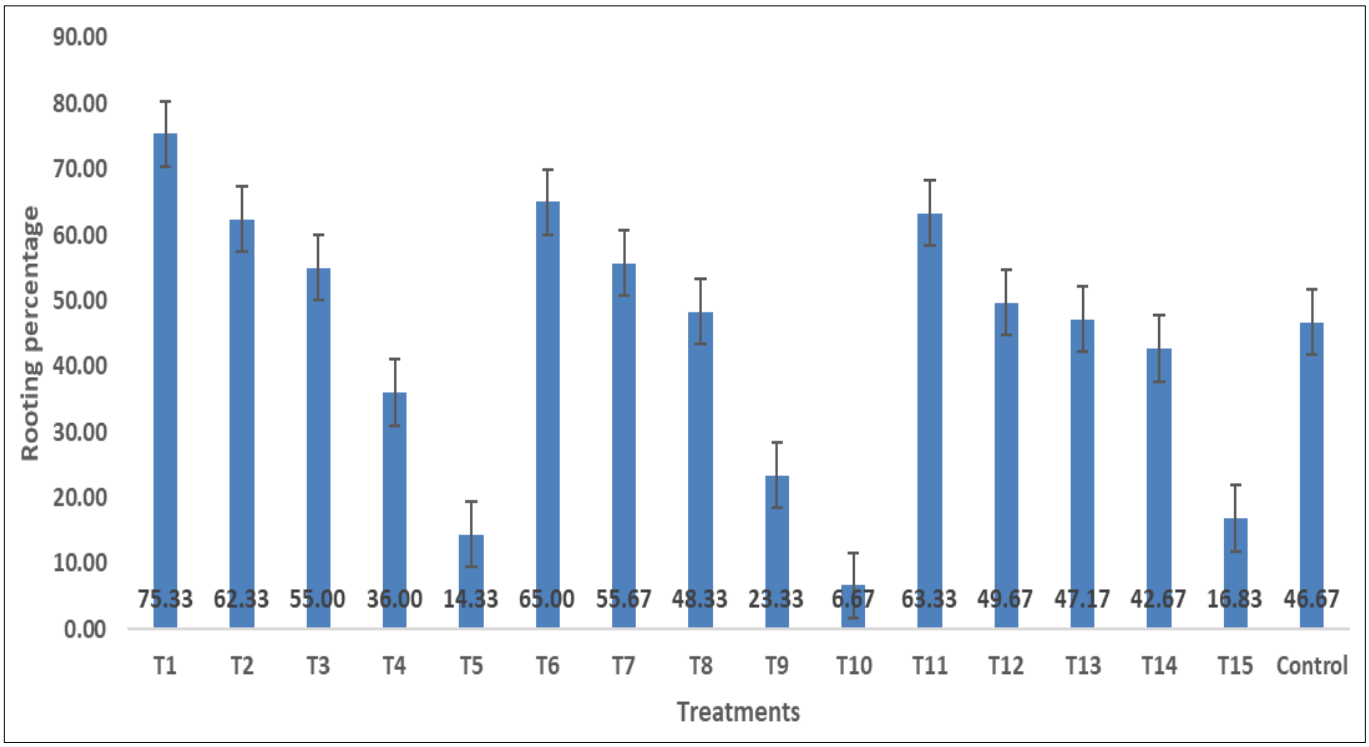

Fig 2: Effect of different treatments on the rooting percentage of the cuttings

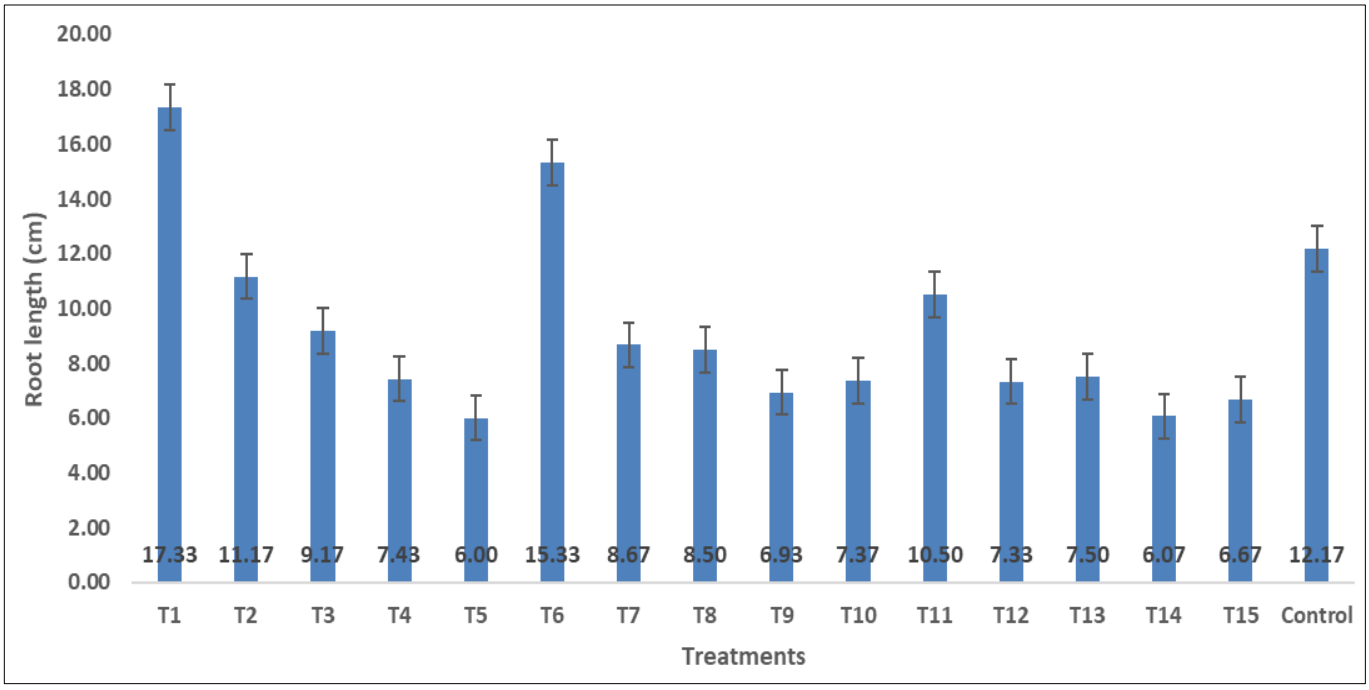

Fig 3: Effect of different treatments on the root length (cm) of the cuttings

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