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# Impact of auxin and rooting media on root formation in semi - hard wood cuttings of *Azadirachta indica* A. Juss (Neem)

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**ABSTRACT:** *Azadirachta indica* is an evergreen woody plant of Meliaceae family, native to Indian subcontinent and distributed in the tropical and subtropical regions of the world. For efficient multiplication and conservation of the genetic resources of neem, the effect auxin, their different concentration in different rooting media on adventitious root formation (ARF) in semi - hard wood cuttings of *Azadirachta indica* was studied. Three different rooting media (sand, vermiculite and soil) were used and the experiment was established using three types of auxin (IBA, IAA and NAA) and 6 concentration (100, 250, 500, 750, 1000 and 1500 mg $L^{-1}$), in a complete randomized block design (CRBD). Significant effects of auxin, their concentration and rooting media on adventitious root formation of neem semi hard wood cuttings were observed. Semi hard wood cuttings were assessed for rooting percentage, number of sprouts, number of roots, root length and number of leaves. Data revealed that there was significant effect ($p < 0.05$) of auxin - vermiculite on rooting percentage, number of sprouts, number of root, length of root and number of leaves. The data showed that a maximum of 65% rooting with 3.00 numbers of sprout, 32.38 number of roots with 5.77 cm root length and 4.92 numbers of leaves was obtained, when these cuttings were treated with 500 ppm IBA. The determination of proper rooting protocols and the use of semi hard wood cuttings were proved important for multiplication of *A. indica*. The rooted plantlets were successfully hardened and acclimatized in poly house and agro shade house. These plants showed a good survival rate of 80% under field conditions.

**Keywords:** semi - hard wood cutting, vegetable hormone, rooting media.

## Influência da auxina e tipos de substratos na formação de raizes em estacas de nim indiano (*Azadirachta indica* A. Juss)

**RESUMO:** *Azadirachta indica* é uma planta lenhosa perene da família Meliaceae, nativa do subcontinente indiano e com distribuição nas regiões tropicais e subtropicais do mundo. Para a multiplicação eficiente e a conservação dos recursos genéticos do nim indiano, avaliou-se os efeitos de diferenes concentrações de auxina e de diferentes meios de enraizamento (substratos) na formação de raízes adventícias (ARF) em estacas de madeira semi-dura de *Azadirachta indica*. Foram avaliados três meios de enraizamento diferentes (areia, vermiculita e solo), três tipos de auxina (IBA, IAA e NAA) e 6 concentrações (100, 250, 500, 750, 1000 e 1500 mg $L^{-1}$), em delineamento completo de blocos causalizados (DBC). Foram observados efeitos significativos do tipo de auxina, da concentração e dos meios de enraizamento na formação de raízes adventícias de estacas semi-duras de nim indiano. As estacas foram avaliadas quanto ao percentual de enraizamento, número de brotos, número de raízes, comprimento da raiz e número de folhas. Os dados revelaram que houve efeito significativo ($p < 0,05$) de auxina-vermiculita em todas as variáveis analisadas. Os dados mostraram que foi obtido um máximo de 65% de enraizamento com médias de 3.00 brotos, 32.38 raízes com comprimento médio de 5.77 cm e 4.92 números de folhas, quando as estacas foram tratadas com 500 ppm de IBA. A determinação de protocolos de enraizamento adequados e o uso de estacas de madeira semi-duras foram comprovados como as melhores opções para a multiplicação de *A. indica*. As plântulas enraizadas foram aclimatadas em estufas de polietileno de baixa densidade e em telados com sombreamento agrícola. Estas plantas mostraram boa taxa de sobrevivência de 80% em condições de campo.

**Palavras-chave:** estacas de madeira semi-dura, hormônios vegetais, substratos, nim indiano.

## 1. INTRODUCTION

The *Azadirachta indica* (Neem) plants is important plant Indian sub-continent. It contain a variety of plant secondary metabolites including limonoids (e.g. azadirachtin, salanin), flavonoids, essential oil, etc. used as biopesticide against insects. The principal bioactive active compound Azadirachtin, is majeroly found in the seed kernel. (SCHMUTTERER,

1990; GAHUKAR, 2000). Neem holds the highly non toxic with effective, and environment friendly means that control or eliminate pests that cause losses in agriculture crop production (GOVINDACHARI et al.,, 1992).

Azadirachtin has received more attention as biopesticide and showed great potential against insects due to low persistence in nature, low toxicity against non-targeted organisms and systemic action (Immaraju 1998; Prakash et al., 2002).Recently, Neem oil can be used as fuel in diesel engines directly or blending with methanol. In recent days, the biodiesel production from *Azadirachta indica* oil on the basis of high yield and quality is very profitable (KARMAKAR et al., 2012). A broad variety of high free fatty acid content of vegetable oils, which is available in neem, that conveys great potential sources for biodiesel production in India (DEEPAK et al., 2013).

Earlier, many research have reported cloning of *Azadirachta indica* using stem cuttings are Sivagnanam et al. (1989), Kijkar (1992), Pal et al. (1992), Pal et al. (1994), Palanisamy; Kumar (1996), Kamaludin; Ali (1996), Gera et al. (1997), Palanisamy; Kumar (2001), Palanisamy et al. (2003), Bhola (2004), Devarnavadagi et al. (2005), Gil et al. (2006), Ehiagbonare (2007), Reddy et al. (2007) and Gehlot et al. (2014 a,b,c), Gehlot et al. (2015).

In above work, limited success to propagate and multiply *Azadirachta indica* using mature stem cuttings was recorded. In the present investigation, making use of earlier work done and other related research work, success has been achieved in standardization of macropropagation protocol through semi hard wood cuttings collected from mature trees. The present study was aimed to develop vegetative propagation protocol of *Azadirachta indica* by the influence of auxin treatment and rooting media under poly house conditions (inter mediate mist).



## 2. MATERIAL AND METHODS

### 2.1. Selection of planting material and Preparation of stem cuttings

Vegetative propagation experiments was carried out at poly house of Arid Forest Research Institute, Jodhpur, Rajasthan (24°40'N, 71°15' E) during July – September (Monsoon season) of 2011. The climate of this region is generally hot and semi-arid but monsoon offers low to medium rainfall with various humid levels.

The selection of Plus Tree or population variants (PT) based on vegetative characters (i.e. general growth, girth of the main stem at breast level, plant height and crown diameter) and reproductive characters (i.e. regeneration ability, initiation of leaf fall, initiation of new leaves, initiation of flowering, number of flower, initiation of fruiting, number of fruits/bunch, fruiting period), seed traits vis. 100 Seed weight (g), oil percentage, seed viability and Azadirachtin percentage). The semi - hard wood cutting was collected from selected Trees, naturally growing at Forest Genetics and Tree Breeding Field, Jodhpur. The semi - hard wood cutting was harvested using sterile pruning scissors in morning time. After harvesting, these were first screened for desired length (30 - 35cm by using scale), then kept in wet cloth (for prevention from damage) for transportation to poly house site. Then, semi - hard wood cutting was treated with aqueous solution of 0.1% Carbendazim (Bavistin 50% WP, Systemic fungicide, BASF India Limited, Bombay) for 10 minute, subsequently washed with distilled water to remove excess fungicide.

The selected cuttings were treated with freshly prepared aqueous solution of root promoting auxins using the Basal Long Soak method. The cuttings dipped in distilled water were considered as (control) or treatments of IBA (indole-3-butyric acid, Duchefa Biochemi, Postbus, Netherland), IAA (indole-3-acetic acid, Duchefa Biochemi, Postbus, Netherland) and NAA (α-naphthalene acetic acid, Duchefa Biochemi, Postbus, Netherland) individually at different concentration of 100, 250, 500, 750, 1000 and 1500 $mgL^{-1}$ (milligram per litre:ppm), respectively.

Twenty cuttings of each treatment were stuck in root trainer (250cc) containing three nursery grade rooting media i.e. sand, vermiculite and soil. To prevent any form of damage to the cambium of cuttings during insertion into rooting medium, holes were made by a glass rod into the root trainer. The cuttings were kept under intermittent mist (misting flow for 60 seconds at 30 minutes interval), maintained at 60 – 80% relative humidity and 25°-30° C/15°-20°C day/night temperature. The cuttings were regularly watered and treated with 0.1% Bavistin to avoid desiccation damage and attack of pathogens respectively, at every 15 day interval.

The rooting experiments were run for 60 days, then rooted cuttings were transferred to polythene bags (16 x 28 cm) containing Soil: FYM (farm yard manure) (5:1) and kept in poly house for 15 to 20 days. The polythene bags were moved daily in order to minimize misting variation. After this, polythene bags containing rooted semi - hard wood cutting were transferred to Agro shade house for hardening. In agro-shade house, plants were manually irrigated by tap water once a day. After 35 - 45 days of hardening, plants were planted in the field and were manually irrigated by tap water once in a week (Figure 1).


Figure 1. Effect of auxin and their concentration on initiation of (A, B, C & D) and rooting (E, F & G) in semi - hard wood cuttings with treatment of 500ppm IBA in sand rooting media during monsoon season during.

Figura 1. Efeito da auxina e sua concentração na brotação inicial (A, B, C e D) e no enraizamento (E, F e G) de estacas de madeira semi-duras de nim indiano, com tratamento de IBA a 500ppm em areia durante a estação chuvosa.

### 2.2. Analysis of rooting in semi - hard wood cuttings

After completion of experiment, all the cuttings from all the treatments were uprooted carefully without harming root and leaves with the help of running water. The number of leaves were recorded just before uprooting of a treatment lot whereas, the rooting success, numbers of primary roots and length (cm) of roots were recorded by observing all semi - hard wood cutting of each replication.

### 2.3. Data collection, experimental design and statistical analyses

The experimental unit is semi - hard wood cutting, which can be assigned at random to a treatment group. In present studies, completely randomized design (CRD) was used for statistical analysis. Resultant data were analyzed through General liner Model (GLM) multi variance factor analysis and one way analysis using Statistical Packages for Social Sciences Software (SPSS 14.0) for all the studied parameters i.e. rooting percentage, number of sprouts, root number, root length and number of leaves. The main effects and their interactions were studied for the test of significance. Means of control, significant factors and their interactions were compared using Duncan Multiple Range Test (DMRT) at 0.05% probability was used to compare the means from main effects. Minimum of 4 replicates with 5 samples (one cutting) were taken per treatment with repetition of experiments thrice. Degree of variations was shown by Mean and standard error. Data given in percentages were subjected to arcsine √X) transformation (Snedecor; Cochran 1967) before statistical analysis.

## 3. RESULTS

The present outcome of the experiment revealed that anxin (IBA, IAA & NAA), their concentration and rooting media (sand, vermiculite and soil) showed significant effect on adventitious root formation in semi – hard wood cuttings of *Azadirachta indica* (Neem). The detail of adventitious root formation in semi – hard wood cuttings of *Azadirachta indica* (Neem) are described below:

### 3.1. Interactive effect of auxin and sand rooting media on rooting

Semi hard wood cutting were used for different auxin treatment in sand rooting media. Data revealed that there were significant effect ($p<0.05$) with auxin concentration on rooting percentage, number of sprouts, number of root, length of root and number of leaves. The outcome showed that, among the different auxin used, IBA showed best results as compared to IAA & NAA. Maximum 60% rooting, 2.83 numbers of sprout, 30.92 number of roots, 5.89 cm root length and 4.92 numbers of

Table 1. Effect of different concentration of auxin (IBA, IAA and NAA) and their concentration on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in semi hard wood cuttings of *A. indica* in sand during monsoon season.

Tabela 1. Efeito de diferentes concentrações de auxina (IBA, IAA e NAA) e sua concentração na porcentagem de enraizamento, número de brotos, número de raízes, comprimento da raiz (cm) e número de folhas em estacas de madeira semi-duras de *A. indica*, em areia durante a estação chuvosa.

| Auxin (ppm) Control | Rooting % 0.00 (1.28)e | Number of sprouts 0.00 ±0.00g | Number of roots 0.00 ±0.00j | Root length (cm) 0.00 ±0.00e | Number of leaves 0.00 ±0.00c |
|---|---|---|---|---|---|
| | | | IBA | | |
| 100 | 25.00 (29.73)bcd | 1.00 ±0.00f | 5.00 ±0.26hij | 2.67±0.49bcd | 3.17±0.40ab |
| 250 | 40.00 (38.95)abc | 1.78±0.22cde | 20.33±2.37cde | 3.94±0.53abcd | 4.78±0.85a |
| 500 | 60.00 (54.21)a | 2.83±0.11a | 30.92±1.26a | 5.89±0.79a | 4.92±0.57a |
| 750 | 45.00 (41.83)abc | 2.17±0.11bcd | 21.67±2.71bcde | 5.83±0.66a | 4.00±0.65ab |
| 1000 | 60.00 (51.05)a | 2.44±0.18ab | 29.70±2.84ab | 5.83±0.74a | 4.80±0.63a |
| 1500 | 35.00 (36.06)abc | 1.29±0.18ef | 22.71±1.55abcde | 5.00±0.93abc | 3.29±1.04ab |
| Mean | 53.00 (41.97) | 1.91 | 21.72 | 4.86 | 4.33 |
| | | | IAA | | |
| 100 | 10.00 (13.92)de | 1.00±0.00f | 2.50±0.29ij | 2.00±0.00de | 2.00±0.00bc |
| 250 | 20.00 (26.56)cd | 1.25±0.25ef | 8.50±0.29fgi | 2.50±0.50cd | 3.00±0.58ab |
| 500 | 40.00 (39.23)abc | 1.50±0.19def | 17.88±2.21def | 4.00±0.71abcd | 3.88±0.81ab |
| 750 | 30.00 (29.74)bcd | 1.50±0.22def | 21.50±0.50bcde | 3.50±0.22abcd | 2.67±0.21ab |
| 1000 | 40.00 (39.23)abc | 1.63±0.18cdef | 23.75±1.26abcde | 5.13±0.67ab | 4.63±0.86ab |
| 1500 | 20.00 (26.56)cd | 1.00±0.00 | 15.00±2.48efg | 4.00±0.58abcd | 2.50±0.29ab |
| Mean | 40.00 (29.22) | 1.31 | 14.86 | 3.44 | 3.11 |
| | | | NAA | | |
| 100 | 10.00 (13.92)de | 1.00±0.00f | 3.00±0.00hij | 2.00±0.00de | 2.50±0.29ab |
| 250 | 20.00 (26.56)cd | 1.00±0.00f | 11.00±2.35fgh | 2.50±0.29cd | 3.00±0.41ab |
| 500 | 55.00 (47.89)ab | 2.00±0.27bcd | 28.09±2.09abc | 4.91±0.53abc | 4.18±0.64ab |
| 750 | 40.00 (38.95)abc | 2.00±0.19bcd | 26.25±2.28abcd | 3.63±0.68abcd | 3.88±0.72ab |
| 1000 | 50.00 (45.00)abc | 2.20±0.20bc | 27.92±3.64abc | 5.20±0.68a | 4.75±0.75ab |
| 1500 | 25.00 (29.73)bcd | 1.20±0.20ef | 16.40±2.66eg | 3.40±0.51abcd | 3.60±0.60ab |
| Mean | 50.00 (40.41) | 1.57 | 18.78 | 3.61 | 3.82 |
| Grand Mean | 32.89 (33.18) | 1.73 | 20.69 | 4.28 | 3.82 |
| | | | AVOVA (Analysis of variance) | | |
| Auxin | | | | | |
| *df* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| *F - value* | 7.90 | 13.93 | 9.40 | 7.03 | 2.68 |
| *p - value* | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 |
| Concentration | | | | | |
| *df* | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| *F - value* | 10.66 | 17.52 | 31.48 | 7.51 | 3.22 |
| *p - value* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Auxin x concentration | | | | | |
| *df* | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| *F - value* | 0.22 | 1.55 | 1.81 | 0.40 | 0.33 |
| *p - value* | 0.99 | 0.12 | 0.06 | 0.94 | 0.97 |

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $p\leq0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.

leaves was observed, when cuttings treated with IBA 500ppm (Table 1, Figure 1).

Analysis of variance (ANOVA) revealed that, the effect of auxin was found significant ($p<0.05$) for rooting percent, number of sprout, number of roots and root length, where as it was not significant for number of leaves. The effect of concentration of auxin showed significant ($p<0.05$) for rooting percent, number of sprout, number of roots, root length and number of leaves when used in sand rooting media. Two factors ANOVA showed that the interaction of auxin and their concentration was not found significant for rooting percent, number of sprout, number of roots, root length and number of leaves.

### 3.2. Interactive effect of auxin and vermiculite rooting media on rooting

In the present investigation, attempts were made for macropropagation from semi hard wood cutting with different auxin treatment used in vermiculite rooting media. Data revealed that there was significant effect ($p<0.05$) of auxin - vermiculite on rooting percentage, number of sprouts, number of root, length of root and number of leaves. The data showed that a maximum of 65% rooting with 3.00 numbers of sprout, 32.38 number of roots with 5.77 cm root length and 4.92 numbers of leaves was obtained, when these cuttings were treated with 500ppm IBA (Table 2, Figure 2).

Analysis of variance (ANOVA) revealed that, the effect of auxin was found significant ($p<0.05$) for rooting percent and root length, whereas not significant for number of sprout, number of roots, number of leaves. Similarly effect of concentration of auxin was significant ($p<0.05$) for rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA showed the interaction of auxin and their concentration was significant ($p<0.05$) for number of sprout, whereas it was not significant for rooting percent, number of roots, root length and number of leaves.

### 3.3. Interactive effect of auxin and soil rooting media on rooting

In the present investigation, semi hard wood cutting were used and auxin treatment were tested in soil rooting media. Data revealed that there was significant effect ($p<0.05$) with auxin concentration on rooting percentage, number of sprouts, number of root, length of root and number of leaves in soil rooting

Table 2. Effect of different concentration of auxin (IBA, IAA and NAA) and their concentration on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in semi hard wood cuttings of *A. indica* in vermiculite during monsoon season.

Tabela 2. Efeito de diferentes concentrações de auxina (IBA, IAA e NAA) e sua concentração na porcentagem de enraizamento, número de brotos, número de raízes, comprimento da raiz (cm) e número de folhas em estacas de madeira semi-duras de *A. indica*, em vermiculita durante a estação chuvosa.

| Auxin (ppm) Control | Rooting % 5.00 (7.60)e | Number of sprouts 1.00±0.00g | Number of roots 2.00±0.00f | Root length (cm) 2.00±0.00ef | Number of leaves 2.00±0.00bcd |
|---|---|---|---|---|---|
| | | | IBA | | |
| 100 | 20.00 (26.56)cd | 1.50±0.29fg | 5.50±0.50def | 2.50±0.29cdef | 3.00±0.00abcd |
| 250 | 35.00 (36.06)abc | 2.29±0.18abcde | 13.71±2.33bcdef | 3.57±0.61abcdef | 2.71±0.36abcd |
| 500 | 65.00 (54.22)a | 3.00±0.00a | 32.38±8.84a | 5.77±0.67a | 4.92±0.81a |
| 750 | 45.00 (42.12)abc | 2.09±0.09abcdef | 25.78±1.47abc | 4.91±0.58abc | 3.64±0.49abc |
| 1000 | 55.00 (48.17)ab | 3.00±0.00a | 28.67±0.70ab | 5.00±0.57ab | 4.75±0.80a |
| 1500 | 30.00 (32.90)bcd | 2.00±0.37cdef | 19.00±2.89abcdef | 3.67±0.84abcdef | 3.83±0.54abc |
| Mean | 41.66 (40.00) | 3.31 | 20.84 | 4.24 | 3.81 |
| | | | IAA | | |
| 100 | 10.00 (13.92)de | 1.60±0.24efg | 3.00±0.00f | 1.60±0.23f | 1.00±0.00d |
| 250 | 25.00 (29.73)bcd | 2.15±0.15bcdef | 7.00±0.84def | 4.00±0.45abcdef | 3.00±0.45abcd |
| 500 | 45.00 (41.83)abc | 2.67±0.17abc | 24.11±2.09abc | 4.33±0.62abcdef | 4.17±0.64ab |
| 750 | 30.00 (32.61)bcd | 2.83±0.31ab | 20.17±1.96abcd | 3.67±0.42abcdef | 3.17±0.60abcd |
| 1000 | 40.00 (38.95)abc | 2.75±0.25abc | 23.63±1.15abc | 4.00±0.66bcdef | 4.11±0.72ab |
| 1500 | 20.00 (26.56)cd | 2.00±0.58cdef | 11.25±2.93cdef | 3.25±1.03 | 3.75±1.11abc |
| Mean | 28.33 (30.06) | 2.46 | 14.86 | 3.48 | 3.03 |
| | | | NAA | | |
| 100 | 10.00 (13.92)de | 1.00±0.00g | 4.50±0.87ef | 2.00±0.00ef | 2.00±0.00bcd |
| 250 | 20.00 (26.56)cd | 1.75±0.48def | 5.00±0.41def | 2.38±0.38def | 2.75±0.48abcd |
| 500 | 55.00 (48.17)ab | 2.50±0.17abcd | 23.80±1.60abc | 4.70±0.56abcd | 4.70±0.47a |
| 750 | 35.00 (32.63)bcd | 2.29±0.18abcde | 14.45±1.81bcdef | 2.57±0.43cdef | 2.71±0.47abcd |
| 1000 | 45.00 (42.12)abc | 2.44±0.18abcd | 23.40±1.76abc | 4.67±0.60abcd | 3.67±0.33abc |
| 1500 | 25.00 (29.73)bcd | 2.00±0.00cdef | 12.25±2.63cdef | 2.50±0.87cdef | 1.50±0.50cd |
| Mean | 31.66 (32.18) | 1.99 | 13.90 | 3.14 | 2.88 |
| Grand Mean | 32.37 (32.86) | 2.27 | 19.11 | 3.95 | 3.57 |
| | | | ANOVA (Analysis of variance) | | |
| Auxin | | | | | |
| *df* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| *F - value* | 6.37 | 1.03 | 1.41 | 2.67 | 2.10 |
| *p - value* | 0.00 | 0.35 | 0.24 | 0.05 | 0.12 |
| Concentration | | | | | |
| *df* | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| *F - value* | 9.99 | 17.06 | 12.19 | 6.89 | 5.56 |
| *p - value* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Auxin x concentration | | | | | |
| *df* | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| *F - value* | 0.29 | 2.34 | 0.79 | 0.93 | 0.92 |
| *p - value* | 0.97 | 0.01 | 0.63 | 0.50 | 0.51 |

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $p\leq0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.


Figure 2. Effect of auxin and their concentration on initiation of sprouting (A, B & C) and rooting (D, E & F) in semi - hard wood cuttings with treatment of 500ppm IBA in vermiculite rooting media during monsoon season.
Figura 2. Efeito da auxina e sua concentração na brotação inicial (A, B e C) e no enraizamento (D, E e F) de estacas de madeira semi-duras de nim indiano, com tratamento de IBA a 500ppm em vermiculita durante a estação chuvosa.

media. Best response was obtained when cuttings treated with IBA 500 ppm on which 55% rooting was obtained with 2.88 number of sprout, 24.50 number of roots, 5.09 cm root length and 4.18 numbers of leaves (Table 3, Figure 3).

Analysis of variance (ANOVA) revealed that, the effect of auxin was significant ($p<0.05$) for rooting percent, root length and number of leaves, whereas it was not significant for number of sprout and number of roots. The effect of concentration of auxin showed significant ($p<0.05$) for rooting percent, number of sprout, number of roots, root length and number of leaves. Two factors ANOVA showed the interaction of auxin and their concentration was not found significant for rooting percent, number of sprout, number of roots, root length and number of leaves.

## 4. DISCUSSION

In the present investigation, semi hard wood cuttings were treated with three auxins (IBA, IAA & NAA) and planted in three rooting media (sand, vermiculite and soil). It was observed that IBA (500 ppm) showed maximum rooting as compared to IAA

Table 3. Effect of different concentration of auxin (IBA, IAA and NAA) and their concentration on rooting percentage, number of sprout, number of root, root length (cm) and number of leaves in semi hard wood cuttings of *A. indica* in soil during monsoon season.
Tabela 3. Efeito de diferentes concentrações de auxina (IBA, IAA e NAA) e sua concentração na porcentagem de enraizamento, número de brotos, número de raízes, comprimento da raiz (cm) e número de folhas em estacas de madeira semi-duras de *A. indica*, em solo durante a estação chuvosa.

| **Auxin (ppm)** | **Rooting %** | **Number of sprouts** | **Number of roots** | **Root length (cm)** | **Number of leaves** |
|---|---|---|---|---|---|
| **Control** | **0.00 (1.28)a** | **1.00±0.00a** | **3.00±0.00f** | **2.00±0.00bcd** | **2.00±0.00ab** |
| | | | IBA | | |
| 100 | 15.00 (20.24)cd | 1.33±0.33gh | 3.00±0.58f | 2.33±0.33bcd | 1.33±0.33b |
| 250 | 25.00 (29.24)abc | 1.60±0.24efgh | 13.80±3.06de | 3.88±0.35abc | 3.20±0.37a |
| 500 | 55.00 (48.17)a | 2.88±0.13a | 24.50±2.63a | 5.09±0.86a | 4.18±0.66ab |
| 750 | 45.00 (41.83)abc | 2.44±0.18abc | 16.67±1.94bcd | 3.56±0.85abc | 3.22±0.62ab |
| 1000 | 55.00 (47.89)a | 2.73±0.14ab | 22.09±1.98ab | 4.73±0.70ab | 4.09±0.79ab |
| 1500 | 30.00 (32.90)abc | 1.67±0.21defg | 12.50±2.53e | 4.00±0.63abc | 3.83±1.49ab |
| Mean | 37.50 (36.71) | 2.11 | 34.18 | 3.93 | 3.31 |
| | | | IAA | | |
| 100 | 10.00 (13.92)de | 1.00±0.00h | 2.50±0.29e | 1.60±0.23d | 3.00±0.00ab |
| 250 | 20.00 (26.56)bcd | 1.00±0.00h | 4.50±1.32e | 3.50±0.87abc | 3.00±0.41ab |
| 500 | 45.00 (41.83)ab | 2.67±0.17abc | 21.89±0.75ab | 4.33±0.37abc | 4.11±0.54ab |
| 750 | 35.00 (32.63)abc | 2.29±0.18abcd | 18.29±2.18abcde | 3.14±0.14abc | 3.14±0.26ab |
| 1000 | 45.00 (42.12)ab | 2.33±0.17abc | 21.55±1.87abc | 3.78±0.40abc | 3.56±0.94ab |
| 1500 | 25.00 (29.73)abc | 2.00±0.00cde | 14.40±3.19cde | 3.00±0.84abc | 2.80±0.58ab |
| Mean | 30.00 (31.13) | 1.88 | 10.81 | 3.23 | 3.27 |
| | | | NAA | | |
| 100 | 15.00 (20.24)cd | 2.00±0.00cde | 4.00±1.00e | 3.00±1.00abc | 2.67±0.33ab |
| 250 | 25.00 (29.73)abc | 1.40±0.24fgh | 5.20±0.80e | 3.90±0.95abc | 2.60±0.40ab |
| 500 | 40.00 (38.95)ab | 2.50±0.22abc | 22.00±0.47ab | 4.40±0.68abc | 3.75±0.65ab |
| 750 | 30.00 (29.46)abc | 2.00±0.27cde | 19.17±2.80abcde | 3.17±0.98abc | 3.17±0.98ab |
| 1000 | 40.00 (38.95)ab | 2.45±0.16abc | 20.13±1.87abcd | 4.38±0.71abc | 3.13±0.30ab |
| 1500 | 30.00 (29.73)abc | 2.17±0.17bcde | 14.50±1.52cde | 4.00±0.77abc | 2.83±0.31ab |
| Mean | 30.00 (31.17) | 2.09 | 14.16 | 3.81 | 3.03 |
| Grand Mean | 30.79 (31.36) | 2.15 | 16.38 | 3.81 | 3.35 |
| | | | ANOVA (Analysis of variance) | | |
| Auxin | | | | | |
| *df* | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| *F - value* | 2.12 | 2.72 | 0.32 | 1.60 | 0.22 |
| *p - value* | 0.12 | 0.07 | 0.72 | 0.20 | 0.80 |
| Concentration | | | | | |
| *df* | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| *F - value* | 10.03 | 22.92 | 33.91 | 2.64 | 1.49 |
| *p - value* | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 |
| Auxin x concentration | | | | | |
| *df* | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| *F - value* | 0.33 | 1.73 | 1.30 | 0.23 | 0.32 |
| *p - value* | 0.97 | 0.08 | 0.23 | 0.99 | 0.97 |

*Arc sine values in parentheses. Values within the column followed by different letters significantly different at $p\leq0.05$ level as determined using Duncan's multiple range test. A value represents mean ± standard error.


Figure 3. Effect of auxin and their concentration on initiation of sprouting (A, B, C & D) and rooting (E, F & G) in semi - hard wood cuttings with treatment of 500ppm IBA in soil rooting media during monsoon season.
Figura 3. Efeito da auxina e sua concentração na brotação inicial (A, B, C e D) e no enraizamento (E, F e G) de estacas de madeira semi-duras de nim indiano, com tratamento de IBA a 500ppm em solo durante a estação chuvosa.

and NAA in all rooting media. In terms of rooting media, semi hard wood cuttings planted in vermiculite showed maximum rooting as compared to sand and soil. The semi hard wood cuttings treated with 500ppm IBA and planted in vermiculite rooting media showed maximum 65% rooting with 3.00 numbers of sprouts, 32.38 number of roots with 5.77 cm root length and 4.92 numbers of leaves. However, Pal et al. (1992) reported none of the auxin treated semi hard wood cuttings rooted. Reddy et al. (2007) reported that rooting response of shoot cuttings of *Azadirachta indica* in different rooting media showed that sand was best medium followed by vermiculite and 1000ppm IAA produced best rooting. Palanisamy; Kumar (1996) reported 2000ppm boric acid treatment gave 86% sprouting in the cuttings as compared to thiamine and auxin in all season. Thiamine 1000ppm and 2000 ppm treatment induced 17% rooting. Singh and Chander (2001) reported semi hard wood cuttings treated with 500 ppm of IBA maximum rooting. Similar observations on role of IBA and vermiculite were reported in semi hardwood cuttings by Tsipouridis et al. (2003), Fabbri et al. (2004), Pio et al. (2005), Yeboah; Amoah (2009) and Purohit et al. (2009).



## 5. CONCLUSIONS

The formation of healthy plants after successfully hardening under outdoor conditions showed that *Azadirachta indica* could be successfully propagated by using semi hard wood cutting. Plants were successfully hardened and planted in the field. For best rooting results, it can be recommended for propagation of other valuable plants also.

## 6. ABBREVIATIONS

IBA: Indole-3-Butyric Acid;
IAA: Indole-3- acetic acid;
NAA: α-naphthalene acetic acid, $mgL^{-1}$: Parts per Milligram;
ANOVA: Analysis of Variance

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