

Effect of indole-3-acetic acid, indole-3-butyric acid and 1-naphthalene acetic acid on the stem cutting and vegetative growth of *Lawsonia inermis* L.

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ABSTRACT

Conventional methods for the propagation of *Lawsonia inermis* L. encounter challenges such as limited seed viability, susceptibility to diseases and pests, and inconsistent seed propagation due to the conditions of their natural habitat, which hinder large-scale production. In vitro micropropagation also shows limitations for mass production, primarily due to low hardening rates and extended time requirements for generating the desired leaf biomass. Consequently, vegetative propagation through stem cuttings emerges as a practical approach to enhance productivity in leaf biomass by multiplying superior, healthy plants. The application of auxins significantly influences the rooting and shooting processes of cuttings. An experiment was conducted at the Botanic Garden, University of Chittagong, located in southeastern Bangladesh, to assess the effects of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and 1-naphthalene acetic acid (NAA) on the vegetative growth potential of *L. inermis* stem cuttings using the quick dip method and a completely randomized design. The results indicated notable variability in root and shoot development, which directly impacts the leaf biomass production of *L. inermis* cuttings. All three auxins, IAA, IBA, and NAA, demonstrated significant effects on the development of shoots and roots, except for control cuttings, which managed to develop sufficient roots without the application of hormones. The findings suggest that IBA-treated cuttings are more effective in producing higher leaf biomass compared to those treated with IAA and NAA, highlighting their potential importance in the herbal medicine sector and in economically developing nations such as Bangladesh. It is recommended to further explore the combined effects of IAA, IBA, and NAA, along with other plant growth regulators, on the rooting and shooting capabilities of *L. inermis* to maximize leaf biomass production.

Keywords: *Lawsonia inermis*, leaf biomass, IAA, IBA, NAA, vegetative growth potential.

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INTRODUCTION

Lawsonia inermis L, commonly known as “Henna” (Global) and “Mehedi” (Bangladesh), is a member of the family Lythraceae and the sole species in the genus *Lawsonia*, mostly spread in tropical regions (Gupta, 2003; Jones and Luchainger, 1979; Sastri, 1962). In general, this glabrous, much-branched shrub or small tree is supposed to be native to Africa, Asia, and the Middle East. The leaves of this greyish-brown bark plant are opposite, sub-sessile, elliptic, or broadly lanceolate, entire, acute, or obtuse, 2-3 cm long, and 1-2 cm wide (Muhammad and Muhammad, 2005).

The plant possesses antifungal, antibacterial, virucidal, antiparasitic, anti-inflammatory, analgesic, and anticancer properties, as well as hepatoprotective, immunomodulatory, anthelmintic, and anti-oxidant activities (Ahmadian et al., 2009; Badoni Semwal et al., 2014; Singh and Luqman, 2014). Besides pharmacological properties, it also bears tremendous economic importance. According to the Faridabad Henna Manufacturing Association (2012), the sale of henna from Faridabad, India, is worth an estimated US\$ 31-38 million (₹250-₹300 crore) annually. The principal coloring

matter of henna is lawsone; besides lawsone, other constituents present are gallic acid, glucose, mannitol, fats, resin (2%), mucilage, and traces of an alkaloid (Badoni Semwal et al., 2014). Leaf powder derived from this small tree, which contains an active dye (red-orange pigment), lawsone (2-hydroxy-1,4 naphthoquinone), has been widely used in cosmetics and textile industries to decorate skin, hair, fingernails, leather, silk, and wool, and in medicinal industries since time immemorial (Anand et al., 1992; Hema et al., 2010; Nadkarni and Nadkarni, 1982; Singh and Luqman, 2014). Lawsone accumulates in the aerial part of the plant, with the highest amount of 1.0–1.4% in young leaf petioles. Physical conditions influence the dye properties and percentage of lawsone in henna (Bakkali et al., 1997).

Natural medicines and health supplements are increasingly gaining popularity and are preferred over synthetic ones (Forney et al., 2002). About 80% of the world's population still depends solely on traditional or herbal medicine to treat diseases, mostly in Africa and other developing nations (Okoye et al., 2014). Day by day, in developing and even developed countries, the demand for traditional herbal medicine is increasing because of its fewer side effects and affordable costs. The cultivation of medicinal and economically important plants can serve as a good source of income for rural people. Habitat loss and overexploitation are the two main threats to the medicinal plants of Bangladesh, and immediate attention is needed for the conservation of the medicinal plants of Bangladesh (Bakkali et al., 1997).

Auxins in liquid or powder (talc) form are commonly used in commercial cutting propagation as shoot- and root-promoting chemicals. Commercial formulations commonly contain IAA (indole-3-acetic acid), IBA (indole-3-butyric acid) and NAA (1-naphthalene acetic acid), or a combination thereof.

Research on the propagation of *L. inermis* in Bangladesh is limited, and this is the first experiment conducted through plant hormones, i.e., auxins (IAA, IBA, and NAA).

Conventional methods of propagation of *L. inermis*, sexual as well, are beset with many problems that restrict their multiplication on a large scale. Propagation through seed is unreliable because of disease and pest problems, short viability, and heavy rains during the seeding season in the natural habitat (Rout et al., 2001). According to Davis and Haissig (1990), the rooting of cuttings was strongly influenced by plant hormones.

On the other hand, vegetative propagation is recognized as a conservation approach for those species that are economically important and difficult to grow through seeds and other means (Kavita et al., 2015). Vegetative propagation is the most popular method used for large-scale propagation of plants. Vegetative propagation through stem cutting is a simple, feasible, inexpensive, and less time-consuming method for the

conservation of threatened and medicinally important plants (Deepak et al., 2016). Several reports are available where vegetative propagation through stem cutting was used for conserving and mass cultivating threatened, economically, and medicinally important plants such as *Salacia oblonga* (Deepak et al., 2016), *Paris polyphylla* (Kavita et al., 2015), *Saraca asoca* (Smitha, 2013), *Celastrus paniculata* (Yashaswini et al., 2010), *Berberis aristata* (Ali et al., 2008), *Aconitum heterophyllum* (Beigh et al., 2006), *Picrorhiza kurrooa* (Chandra et al., 2006), and *Enantia chlorantha* (Gbadamosi and Oni, 2005). The above plants are either endangered or critically endangered and have several medicinal values.

In this situation, the present study aims to assess the effect of IAA, IBA, and NAA on the vegetative growth potential of the traditional medicinal and natural dye-yielding plant *L. inermis*.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Botanic Garden, University of Chittagong, Chattagram (4331), in the southeastern part of Bangladesh. The study area lies within 22.46 North latitudes and 91.78 East longitudes, at an altitude of 29 m above the sea level of Bangladesh. Completely Randomized Design (CRD) was followed in this whole experiment. *L. inermis* was used as an experimental plant, and auxins (IAA, IBA, and NAA) were used as chemical treatments. In the present study, firstly, cuttings of 8–10 cm each with 2-3 nodes with 3 replications were treated with 250, 500, 750, 1000, 1250, 1500, and 1750 ppm solutions of IAA, IBA, and NAA, respectively (a total of 22 treatments, including the control that is without hormone), following the quick dip method (Bhagya and Sreeramu, 2013) and planted in the sand-filled polybag. Parameters measured included the number of shoots, length of shoots, number of roots, length of roots, number of leaves, fresh weight (FW) of leaf biomass, and dry weight (DW) of leaf biomass per cutting. Vegetative growth data were collected at 30, 60, and 90 DAP (days after planting). All the data were subjected to analysis of variance (ANOVA) and statistical analysis was done using MS Excel 2021.

RESULTS AND DISCUSSION

Generally, there were gradual increments in the shooting and rooting as the experiment progressed with 60 DAP and 90 DAP. T₁₇ (NAA-500 ppm) and T₁₂ (IBA-1000 ppm) treated cuttings recorded the highest number of shoots (mean value 4.33) and longer shoots (mean value 40.10 cm) at 90 DAP. And T₁₃ (IBA-1250 ppm) and T₁₂ (IBA-1000 ppm) treated cuttings recorded the highest number

of roots (mean value 8.00) and longer roots (mean value 5.73 cm) at 90 DAP, while T₁ (control) cuttings recorded a smaller number of shoots and roots (Table 1).

This may imply that the auxin-treated cuttings develop more shoots and roots than the untreated cuttings,

probably because of their greater growth and higher nutrition uptake and absorption capability. The hormone-treated stem cuttings seem to be more physiologically active than the untreated cuttings, as they seem to respond better to IAA, IBA, and NAA.

Table 1. Changes in the growth behavior of *L. inermis* influenced by IAA, IBA, and NAA at 30, 60, and 90 DAP.

Treatment	No. of shoot / cuttings			Length of shoot (cm)			No. of root / cuttings			Length of root (cm)		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T ₁	1.00	1.33	1.67	1.37	2.24	2.73	1.00	2.00	3.00	0.57	1.37	1.73
T ₂	1.33	2.33	2.67	12.37	23.97	25.67	1.33	3.33	4.00	0.62	2.50	2.70
T ₃	2.33	3.00	3.33	10.77	11.17	17.13	1.67	2.33	4.00	1.26	1.48	3.47
T ₄	2.00	3.33	3.67	10.50	24.17	24.73	1.33	3.67	4.33	0.65	2.37	2.93
T ₅	2.67	2.67	3.33	6.47	7.50	34.83	1.33	4.67	5.00	0.68	2.16	4.90
T ₆	2.00	2.33	2.33	12.70	19.23	21.17	3.67	4.00	4.00	2.60	2.56	4.53
T ₇	2.00	3.33	4.00	7.00	11.00	19.87	2.67	3.00	6.67	2.06	2.60	2.60
T ₈	1.33	1.33	2.67	1.45	2.50	14.07	1.00	2.00	3.00	0.65	1.63	3.27
T ₉	1.33	1.33	1.67	1.41	15.07	16.23	1.00	7.00	7.67	0.69	2.78	3.70
T ₁₀	1.33	1.33	1.67	3.00	16.77	18.57	2.67	2.67	3.67	0.91	2.48	3.67
T ₁₁	1.67	2.33	2.67	2.53	20.83	21.17	3.00	4.33	4.67	1.30	2.27	5.61
T ₁₂	1.67	3.00	3.33	2.60	7.27	40.10	2.67	3.00	5.00	0.87	3.38	5.73
T ₁₃	2.00	2.00	2.33	7.43	25.33	28.23	3.33	7.00	8.00	1.03	3.68	4.63
T ₁₄	2.33	3.00	3.33	12.50	15.20	15.40	4.67	5.00	7.67	3.71	3.80	4.90
T ₁₅	1.33	1.33	2.33	1.46	10.20	15.40	1.00	2.33	3.33	0.71	1.82	1.83
T ₁₆	1.33	1.33	1.67	6.50	17.00	20.63	2.33	3.00	3.33	0.98	3.22	2.47
T ₁₇	1.67	1.67	4.33	16.33	17.47	24.87	3.00	3.67	4.33	2.23	2.51	2.55
T ₁₈	1.67	2.00	2.67	7.50	16.82	19.90	3.33	5.00	5.33	2.52	2.55	4.53
T ₁₉	2.67	3.00	3.33	7.13	16.68	17.87	3.67	3.67	5.00	1.50	3.26	3.70
T ₂₀	2.33	2.33	3.00	3.80	11.03	19.97	2.33	4.00	7.33	0.76	2.18	3.17
T ₂₁	2.00	2.67	2.67	2.37	7.55	12.73	1.33	4.67	5.00	0.64	1.86	2.47
T ₂₂	1.33	1.33	2.00	1.48	15.93	16.60	1.00	2.67	3.67	0.67	2.39	3.27

Note: T₁: Control; T₂: IAA-250ppm; T₃: IAA-500 ppm; T₄: 750ppm; T₅: 1000ppm; T₆: 1250 ppm; T₇: IAA-1500ppm; T₈: IAA-1750ppm; T₉: IBA-250ppm; T₁₀: IBA 500ppm; T₁₁: IBA 750ppm; T₁₂: IBA-1000ppm; T₁₃: IBA-1250ppm; T₁₄: IBA-1500ppm; T₁₅: IBA-1750ppm; T₁₆: NAA-250ppm; T₁₇: NAA-500ppm; T₁₈: NAA-750ppm; T₁₉: NAA-1000ppm; T₂₀: NAA-1250ppm; T₂₁: NAA-1500ppm; T₂₂:1750ppm; **DAP**: Days after planting.

Shoot number and length

T₁₇ (NAA-500 ppm) treated cuttings were recorded as the highest number of shoots with a mean value of 4.33 at 90 DAP; T₇ (IAA-1500 ppm) and T₄ (IAA-750) were recorded as the second and third highest number of shoots with a mean value of 4.00 and 3.67, respectively, at 90 DAP. T₁ (control) exhibited the smallest number of shoots (mean value 1.00) at 30 DAP (Table 1, Figure 1).

In terms of length of the shoot, the longest length of the shoot (40.10 cm) was recorded in T₁₂ (IBA-1000 ppm) at 90 DAP, whereas T₁ (control) showed the smallest length of the shoot (1.37 cm) at 30 DAP. T₅ (IAA-1000 ppm) and T₁₃ (IBA-1250 ppm) recorded the second and third longest shoots with a mean value of 34.83 cm and 28.23 cm, respectively, at 90 DAP (Table 1, Figure 2).

However, cuttings treated with IAA, IBA, and NAA produced a greater number of shoots and longer shoots than the cuttings without IAA, IBA, and NAA treatment.

This confirms that *L. inermis* cuttings are affected by NAA (Quainoo et al., 2014).

Root number and length

T₁₃ (IBA-1250 ppm) treated cuttings were recorded as the highest number of roots with a mean value of 8.00 at 90 DAP; T₁₄ (IBA-1500 ppm) and T₂₀ (NAA-1250 ppm) were recorded as the second and third highest number of roots with the mean values of 7.67 and 7.30, respectively, at 90 DAP. T₁ (control) exhibited the smallest number of roots (mean value 1.00) at 30 DAP (Table 1, Figure 3).

This confirms that *L. inermis* cuttings respond to auxins that are required for adventitious root initiation of stem cuttings (Gaspar and Holfingers, 1987). This outcome may be due to the translocation of carbohydrates from the leaves, which plays an important role in root development (De Carvalho and Zaidan, 1995).

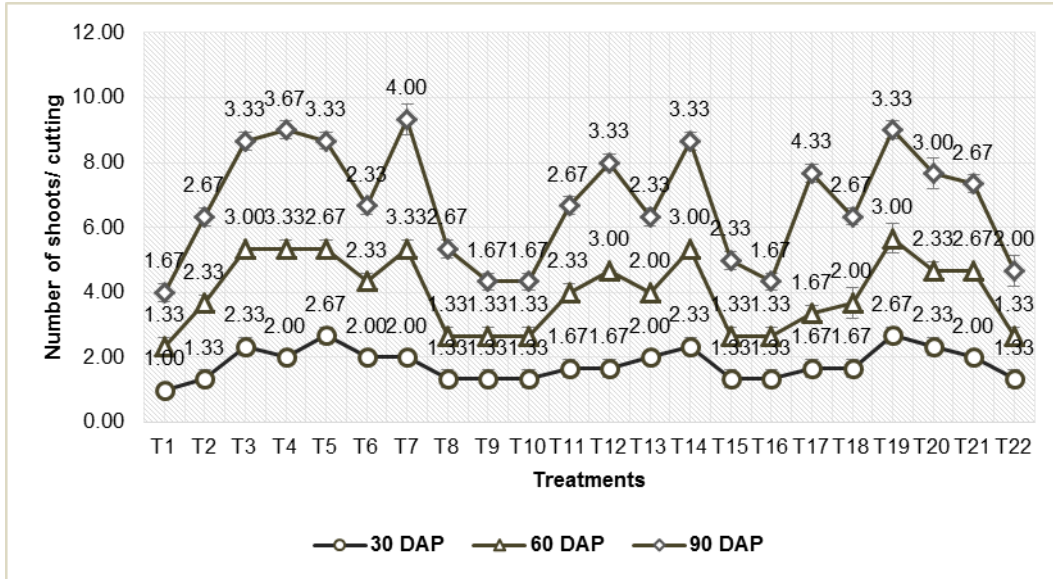


Figure 1. Comparison of changes in the number of shoots of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

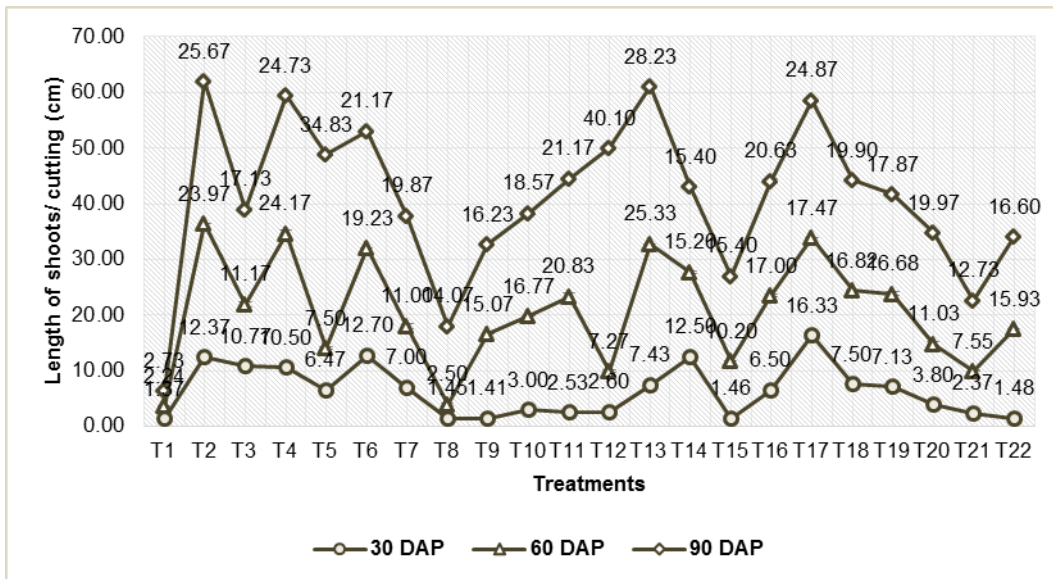


Figure 2. Comparison of changes in the length of shoots of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

In terms of the length of the root, the longest length of the root (5.73 cm) was recorded in T₁₂ (IBA-1000 ppm) at 90 DAP, whereas T₁ (control) showed the smallest length of the root (0.57 cm) at 30 DAP. T₁₁ (IBA-750 ppm) and T₅ (IAA-1000 ppm) recorded the second and third longest roots with a mean value of 5.61 cm and 4.90 cm, respectively, at 90 DAP (Table 1, Figure 4).

This increase in root length may be due to the early initiation of roots affected by auxins and more utilization of food materials due to the early formation of roots.

Number of leaves

T₁₂ (IBA-1000 ppm) treated cuttings were recorded as the highest number of leaves with a mean value of 45.33 at 90 DAP; T₅ (IAA-1000 ppm) and T₆ (IAA-1250 ppm) were recorded as the second and third highest number of roots with the mean values of 44.33 and 39.33, respectively, at 90 DAP. T₁ (control) exhibited the smallest number of roots (mean value 2.67) at 30 DAP (Table 2, Figure 5).

This confirms that *L. inermis* cutting's physiological

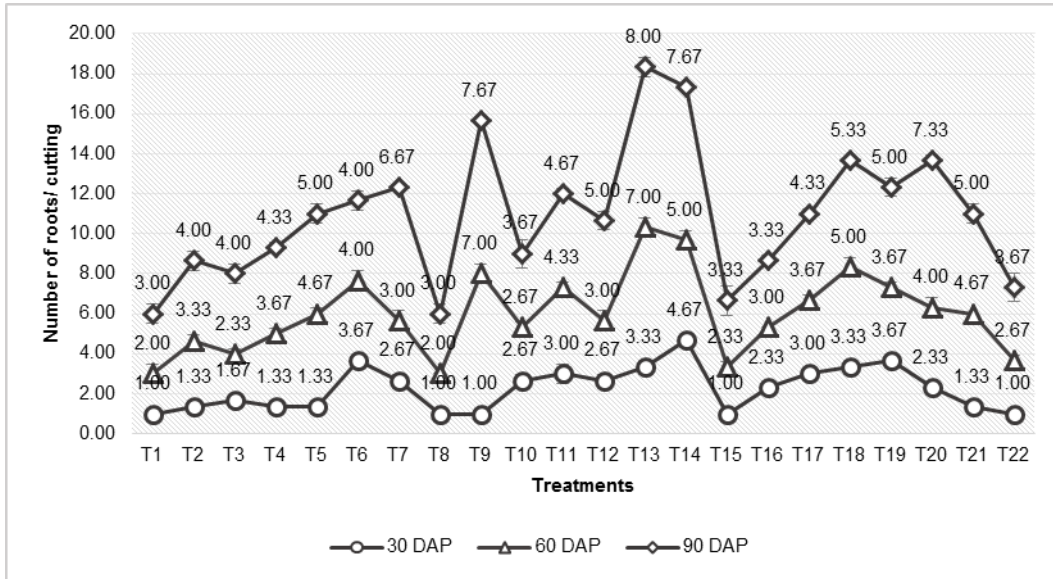


Figure 3. Comparison of changes in the number of roots of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

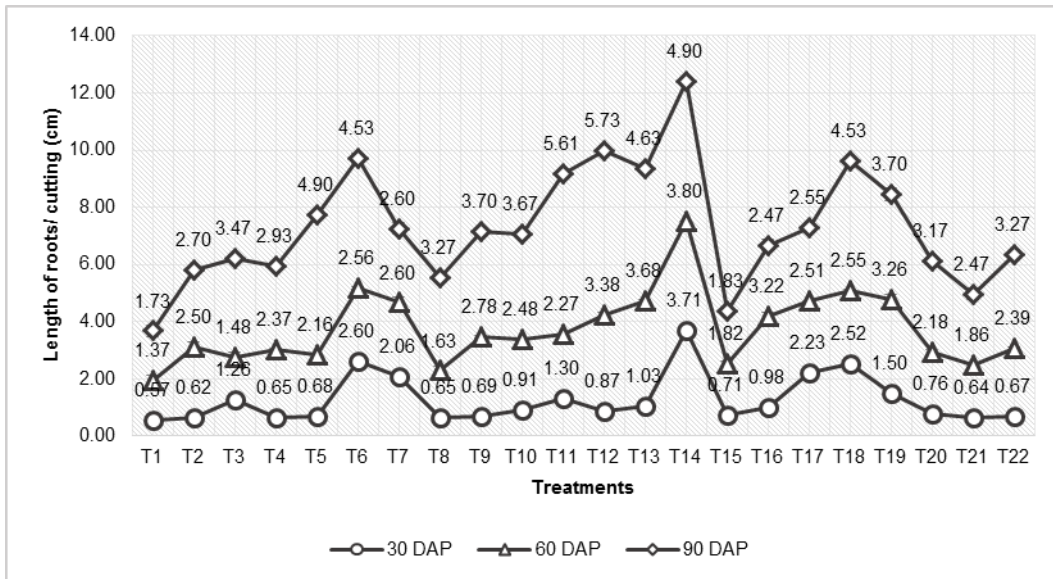


Figure 4. Comparison of changes in the length of roots of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

growth in leaf formation is affected due to auxins having the longest root and comparatively more root number in T₁₂ (IBA-1000 ppm) treatment.

FW of leaf biomass and DW of leaf biomass

The fresh weights of leaf biomass increased gradually over time from 30 to 90 DAP. T₁₂ (IBA-1000 ppm) treated cuttings were recorded as the highest FW of leaf biomass

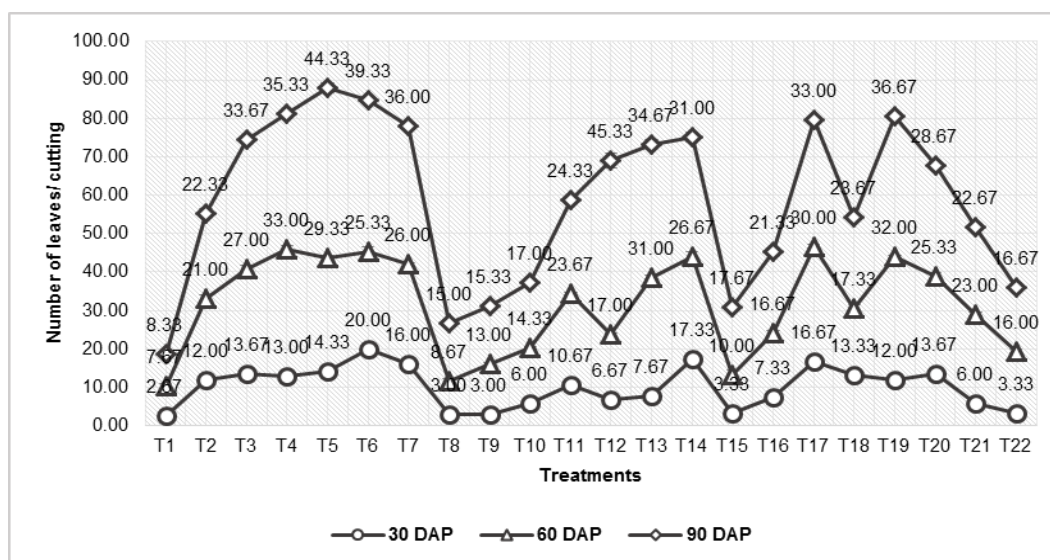
with a mean value of 4.39 gm at 90 DAP; T₅ (IAA-1000 ppm) and T₄ (IAA-750 ppm) were recorded as the second and third highest FW of leaf biomass with the mean values of 3.99 gm and 3.33 gm, respectively, at 90 DAP. T₁ (control) exhibited the lowest FW of leaf biomass (mean value 0.35 gm) at 30 DAP (Table 2, Figure 6).

In terms of the dry weight of the leaf biomass, the highest DW of leaf biomass (3.41 gm) was recorded in T₁₂ (IBA-1000 ppm) at 90 DAP, whereas T₁ (control) exhibited the lowest DW of leaf biomass (0.25 gm) at 30

Table 2. Changes in the growth behavior of *L. inermis* influenced by IAA, IBA, and NAA at 30, 60, and 90 DAP.

Treatment	No. of leaves/ cuttings			FW of leaf biomass/ cuttings (g)			DW of leaf biomass/ cuttings (g)		
	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP	30 DAP	60 DAP	90 DAP
T ₁	2.67	7.67	8.33	0.35	0.70	0.77	0.25	0.62	0.67
T ₂	12.00	21.00	22.33	1.13	1.98	2.09	0.76	1.67	1.78
T ₃	13.67	27.00	33.67	1.28	2.72	3.09	0.87	2.10	2.55
T ₄	13.00	33.00	35.33	1.29	3.20	3.33	0.85	2.74	2.66
T ₅	14.33	29.33	44.33	1.41	2.71	3.99	0.96	2.07	3.02
T ₆	20.00	25.33	39.33	2.06	2.67	3.25	1.53	1.93	2.63
T ₇	16.00	26.00	36.00	1.61	2.55	3.14	1.13	2.00	2.21
T ₈	3.00	8.67	15.00	0.37	0.77	1.20	0.26	0.57	0.90
T ₉	3.00	13.00	15.33	0.37	1.17	1.25	0.28	0.99	0.87
T ₁₀	6.00	14.33	17.00	0.64	1.40	1.50	0.34	1.06	0.91
T ₁₁	10.67	23.67	24.33	1.11	1.51	2.38	0.79	1.99	1.02
T ₁₂	6.67	17.00	45.33	0.68	1.70	4.39	0.38	1.19	3.41
T ₁₃	7.67	31.00	34.67	0.76	2.82	3.01	0.43	2.33	2.30
T ₁₄	17.33	26.67	31.00	1.35	2.48	2.74	0.87	2.09	1.95
T ₁₅	3.33	10.00	17.67	0.43	1.04	1.08	0.26	0.84	0.82
T ₁₆	7.33	16.67	21.33	0.74	1.60	1.62	0.35	1.13	1.07
T ₁₇	16.67	30.00	33.00	1.68	2.81	2.52	1.27	2.11	1.79
T ₁₈	13.33	17.33	23.67	1.42	1.68	1.87	1.12	1.15	1.10
T ₁₉	12.00	32.00	36.67	1.26	3.03	3.11	0.95	2.53	2.44
T ₂₀	13.67	25.33	28.67	1.63	1.93	2.13	1.22	1.45	1.36
T ₂₁	6.00	23.00	22.67	0.64	1.37	2.14	0.33	1.82	0.93
T ₂₂	3.33	16.00	16.67	0.43	1.05	1.54	0.33	0.78	0.81

Note: T₁: Control; T₂: IAA-250ppm; T₃: IAA-500 ppm; T₄: 750ppm; T₅: 1000ppm; T₆: 1250 ppm; T₇: IAA-1500ppm; T₈: IAA-1750ppm; T₉: IBA-250ppm; T₁₀: IBA 500ppm; T₁₁: IBA 750ppm; T₁₂: IBA-1000ppm; T₁₃: IBA-1250ppm; T₁₄: IBA-1500ppm; T₁₅: IBA-1750ppm; T₁₆: NAA-250ppm; T₁₇: NAA-500ppm; T₁₈: NAA-750ppm; T₁₉: NAA-1000ppm; T₂₀: NAA-1250ppm; T₂₁: NAA-1500ppm; T₂₂:1750ppm; **FW**: Fresh weight; **DW**: Dry weight; **DAP**: Days after planting.

**Figure 5.** Comparison of changes in the number of leaves of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

DAP (Table 1). T₅ (IAA-1000 ppm) and T₄ (IAA-750 ppm) recorded the second and third highest DW of leaf biomass with mean values of 3.02 gm and 2.66 gm, respectively, at 90 DAP (Table 2, Figure 7).

T₁₂ (IBA-1000 ppm) treated cuttings recorded the highest fresh (4.39 gm) and dry weights (3.41 gm) of leaves, followed by T₅ (IAA-1000 ppm) and T₄ (IAA-750 ppm) treated cuttings, respectively. Generally, cuttings

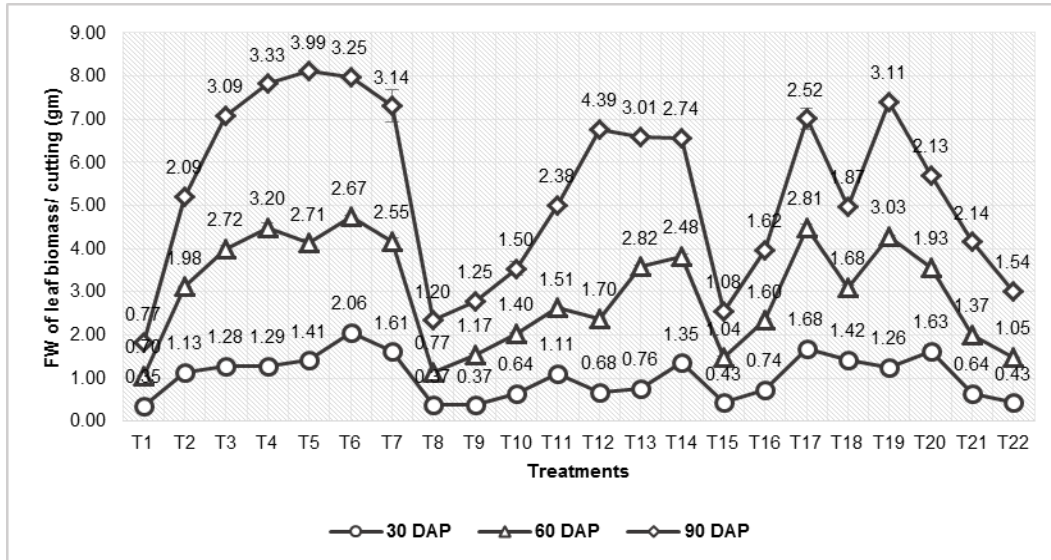


Figure 6. Comparison of changes in the FW of leaf biomass of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

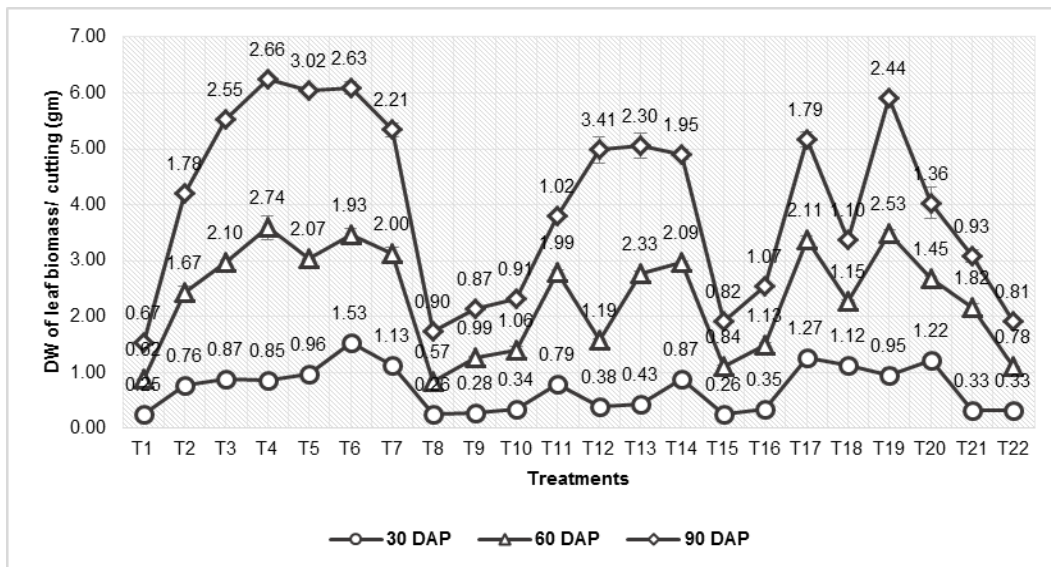


Figure 7. Comparison of changes in the DW of leaf biomass of *L. inermis* influenced by different treatments at 30, 60 and 90 DAP.

treated with IBA had higher FW and DW of leaf biomass than cuttings treated with IAA and NAA treatment.

This result may imply that the IBA application may have induced sufficient roots that facilitated the absorption of water and nutrients from the soil medium. This agrees with the findings of Uniyal et al. (1993) who reported that the higher the number of roots developed by the cuttings, the more they can absorb sufficient water and nutrients to cause an increase in the growth of the aerial portion of the plant.

CONCLUSION

The study showed significant variability in root and shoot development, which influences leaf biomass production in *L. inermis* cuttings. Treatments with IAA, IBA, and NAA had significant effects on shoot and root development, except in control cuttings, where adequate root formation occurred without hormone application. The study concludes that cuttings treated with IBA (T₁₂ IBA-1000 ppm) can be considered highly effective for producing

greater leaf biomass, making them ideal propagation material for *L. inermis*, which may play a vital role in the herbal medicine industry and benefit economically developing countries like Bangladesh. Further investigation is recommended to understand the combined effects of IAA, IBA, NAA, and other plant growth regulators on *L. inermis* root and shoot development for optimal leaf biomass production.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Mohammed Sala Uddin made substantial contributions to the conception and design of the study and thoroughly supervised the whole research work; Md. Abu Shale Musa completed the experiment, drafted the manuscript, and contributed to writing the manuscript. All authors approved the draft of the manuscript.

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