Effect of growth hormone and growth media on the rooting and shooting of *Zanthoxylum armatum* stem cuttings

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The common method of propagation is through seeds but seed germination in *Zanthoxylum armatum* is very low due to the presence of hard seed coat, which might be a great hurdle for large scale production of plantlets. So an attempt was made in this study to see the effect of different growth hormones, their concentrations and different rooting media on the rooting and sprouting of *Z. armatum*. The stem cuttings of *Z. armatum* were treated with two types of auxins namely Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) at different concentrations (2000 ppm, 3000 ppm and 5000 ppm), while the untreated cuttings were used as control. The cuttings were planted in three different rooting media: sand, neopeat and mix (containing a mixture of sand, soil and vermin-compost). The completely randomized design was used for the experiment. The total number of stem cuttings of *Z. armatum* used in the experiment was 1080 for 18 treatments in three replicates (20 cuttings per treatment x 18 treatments x 3 replicates). The experiment was set up in controlled green-house conditions at Dabur Nepal Private Limited Nursery, Banepa, Kavre District. The parameters evaluated were root length, shoot length and number of roots per cutting. The collected data were analyzed statistically using R-program with Agricola. Least significant difference (LSD) and Duncan multiple Range Test (DMRT), as mean separation technique was applied to identify the most efficient treatment in the rooting and shooting behavior of *Z. armatum* (Gomez and Gomez, 1984). Hormone concentration and growth media significantly affected the rooting and shooting ability of *Z. armatum* stem cuttings. IBA was found to be more effective than NAA. Neopeat medium was better than sand and mix media. The highest number of roots (6.5) and root length (11.6 cm) were recorded under IBA 5000 ppm in neopeat medium.

Key words: growth hormones, growth media, sprouting, stem cuttings, rooting, *Zanthoxylum armatum*,

Zanthoxylum armatum DC. (Eng. winged prickly ash; Nep. Timur) belonging to family Rutaceae, is a popular Nepalese spice plant (Manandhar, 2002). The plant is an erect shrub or a small tree up to 6 m in height with dense glabrous foliage and straight prickles on stem, commonly occurring in hot valleys of subtropical to temperate Himalayas (Kashmir to Bhutan), north-east India and Pakistan, Laos, Myanmar, Thailand, China, Bangladesh, Bhutan, Japan, North and South Korea, North Vietnam, Taiwan, Lesser Sunda Islands, Philippines, Malaya peninsula and Sumatra (Grierson and Long, 1991; Nair and Nayar, 1997). In Nepal, it is distributed from west to east at an elevation range of 1000 to 2500 m in open places or in forest undergrowth (DPR, 2007). It is an important medicinal plant with a high trade value having diverse uses in Ayurveda, pharmacy and industry. It has been used in several traditional medicinal practices to cure several diseases such as abdominal pain, carminative, antispasmodic, rheumatism, skin diseases, cholera, diabetes and asthma (Singh *et. al*, 2016). Among the eight species of *Zanthoxylum* found in Nepal, *Z. armatum* is the most common and one of the

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30 medicinal plants and it is prioritized by the government for cultivation and agro-technology development (DPR, 2006).

The plant grows well in open pastures, degraded slopes, shrub lands, natural forests and wastelands with adequate rainfall, deep soils exposed to sun. Clay or loam soil with high organic matter is suitable for the cultivation of this species. The flowering starts in five year old plants in April-May and fruiting in August-October and can be harvested from October to January (Anonymous, 2008). The plants are ready to harvest after three years of plantation and the average annual yield of five years old plant is about 3.5 kg (ANSAB, 2011). *Z. armatum* is generally free from disease, insect or nematode attack; however seven insect pests mostly causing defoliation were reported by Tara *et al.* (2011).

The growing demand of *Z. armatum* in both domestic and international markets, unsustainable harvesting from the wild and lack of proper conservation strategies have led to a sharp decline in the natural population of this valuable plant (Phuyal *et al.,* 2018). The common method of propagation is through seeds but seed germination in *Z. armatum* is very low due to the presence of hard seed coat (Chadha, 1976) which might be a great hurdle for large scale production of plantlets. Furthermore the solitary seeds in the fruit also limit the quantities of seed in *Z. armatum* (Singh and Rawat, 2017). Hence vegetative propagation through stem cuttings could be a viable option for mass scale nursery production of quality planting materials of required genotypes. The increased genetic gains through mass propagation have been obtained in several horticultural plants (Leakey *et al*., 1994; Poupard *et al.,* 1994; Swamy *et al.,* 2002). However, root initiation in cuttings is affected by various factors like plant growth regulators, age of the plant, growth media, size of the cuttings (Hartmann *et al*., 2002; Husen and Pal, 2006).

The information on the propagation techniques of *Z. armatum* is still meager. Therefore a low cost and reliable technology for the propagation for *Z. armatum* has to be developed to integrate its manifold applications into agroforestry systems for the overall benefit of the rural communities as well as the *ex-situ* and *in-situ* conservation of this important plant. Commercial farming by developing suitable agro-technology could be very crucial for enhancement of the marginalized and disadvantaged rural communities. Hence an attempt was made in this study to see the effect of different growth hormones, their concentrations and different rooting media on the rooting and sprouting of *Z. armatum*.

Materials and methods

The study was conducted at the green house of Dabur Nepal Nursery Private Limited, Banepa during February 2017.

Collection of plant materials

Fresh branches were collected from 4–5 years old healthy plants of *Z. armatum* grown at the Nursery premises. The semi-hard wood branches were cut into 15 cm long segment with 2–3 nodes, and all the leaves were removed.

Surface sterilization

All the selected cuttings were surface sterilized by soaking in freshly prepared 1 % Bordeaux mixture (Calcium Hydroxide and Copper Sulphate) for 10-15 minutes.

Hormone concentration

Different concentrations *i.e.* 2000, 3000 and 5000 ppm of two plant growth hormones IBA and NAA were prepared according to the procedure described by Hartmann *et al*. (2002). The surface sterilized cuttings (cut ends only) were then dipped in a bucket containing the respective hormones solutions for 24 hours so as to enhance the absorption of hormones.

Growth media

Three different growth media (rooting media) viz. sand, neopeat (coconut fiber) and mix (containing a mixture of sand, soil and vermi-compost, in the proportion of 2:1:1) were used for studying the rooting behavior of *Z. armatum*. After dipping in hormones, the cuttings were planted directly into the rooting media. Plastic trays (No.21) having 20 cells/cavities and holes at the bottom were used for planting the cuttings. The length and breadth of the tray are 54 cm and 28 cm, respectively. The length of each cell is 6.8 cm whereas the top diameter is 6 cm and the bottom diameter is 2.7cm. The cavities in the trays were filled with the respective rooting media. A single cutting per cavity was inserted obliquely up to a depth of 3 cm.

Experimental design

The completely randomized design was used for the experiment. The total number of stem cuttings of *Z. armatum* used in the experiment was 1080 for 18 treatments in three replicates (20 cuttings x 18 treatments x 3 replicates). Hundred cuttings treated with Bordeaux mixture and washed with distilled water were used as control.

Growth conditions

After planting the cuttings, all the planting trays were labeled clearly and they were transferred to the green house and placed in the controlled environment. The temperature and relative humidity were maintained at 21.9ºC and 75%, respectively throughout the research/study period. Inside the greenhouse, agro-meteorological parameters were recorded through the sensor system run by ARGUS Control and the data were recorded in the computer. Relative humidity was maintained through misting.

Data collection

Numbers of roots, root length and shoot length of individual cutting was recorded after 90 days of planting. A cutting was considered to be rooted if it had at least one primary root of about 1 mm long. For measurement, the cutting was uprooted gradually and then it was cleared off the rooting media carefully so that the roots do not get damaged. The number of primary roots was counted and root and shoot length were measured with a ruler.

Statistical analysis

The collected data were analyzed statistically using R-program with Agricola. Least significant difference (LSD) and Duncan multiple Range Test (DMRT), as mean separation technique was applied to identify the most efficient treatment in the rooting and shooting behavior of *Z. armatum* (Gomez and Gomez, 1984).

Results and discussion

Both growth hormones, IBA and NAA at different concentrations and different growth media *viz.* sand, mix and neopeat had a significant effect ($p< 0.05$) on the number of roots, length of roots and shoots of stem cuttings of *Z. armatum.* The values obtained for IBA and NAA were close to each other (Table 1), however, IBA was found to be more effective than NAA and neopeat was the best growth medium as compared to sand and mix media (Table 3). Furthermore, the measured values (root length, shoot length and number of roots) showed steady increment with the increase in concentration of the growth hormones from 2000 ppm to 5000 ppm. But for NAA, the values increased from 2000 ppm to 3000 ppm concentration and decreased in 5000 ppm concentration except for number of roots, which was the highest in concentration 5000 ppm of NAA. Similarly, the shoot length for concentration 3000 of IBA was lower than concentration 2000 ppm (Table 2). IBA produced more number of roots per cutting as compared to NAA as well as the root and shoot lengths were also longer in IBA than NAA. The values obtained in the treated groups were relatively higher than those of the untreated groups (control).

The number of roots per cutting was affected by the type and concentration of growth hormones and the different growth media but not by the interaction between hormone concentration and growth media. The number of roots produced by IBA and NAA are not significantly different. The value is 4.6 for IBA and 4.4 for NAA (Table 1). The concentration 5000 ppm of IBA had the highest mean number of roots *i.e.* 5.9,

Table 1: Effect of different hormones on the rooting and shooting of *Z. armatum* stem cuttings

Growth hormones	Root length (cm)	Shoot length (cm)	Number of roots
IBA	9.0a	28.0a	4.6a*
NAA	8.1a	25.3b	4.4a
Control	5.4b	22.0c	2.4b*

Means with the same letter in the same column are not significantly different ($P \geq 0.05$).

Table 2: Effects of hormone concentrations on *Z. armatum* stem cuttings

Hormone concentration	Root length (cm)	Shoot length (cm)	Number of roots
IBA2000	7.6b	28.6a	3.4c
IBA3000	8.3b	26.8b	4.5b
IBA5000	11.3a	28.7a	5.9a
NAA2000	7.6b	25.9b	3.2b
NAA3000	9.8a	26.1b	4.3c
NAA5000	6.9b	23.9c	5.2a
Control	5.4c	22.0c	2.4c

Means with the same letter in the same column are not significantly different ($P \geq 0.05$).

while control had the least mean number of roots *i. e.* 2.4 (Table 2). Likewise, the maximum average number of roots was 5 in the growth medium neopeat, while it was 4.2 in sand (Table 3). On the other hand, the highest number of roots was observed in the interaction of concentration 5000 ppm of IBA with neopeat medium with an average of 6.5 and the lowest value was 2 for the interaction between control and sand (Table 4).

Hormone concentration had significant effect on the root length of *Z. armatum* stem cuttings. The concentration 5000 ppm of IBA had the longest root length (11.3 cm) and control had root length of 5.4 cm (Table 2). There was no significant effect of growth media and interaction between hormone concentration and growth media. The interaction between hormone concentration and growth media showed that the concentration 5000 ppm of IBA with neopeat and mix media had the highest mean length of root of 11.6 cm each, while the least value was 5cm for control in sand (Table 4). Among the growth media, neopeat had the longest mean root length of 9.2 cm, while the least value was 8.3cm for sand (Table 3).

The length of shoots was not significantly affected by different growth media and the interaction between different hormone types and concentration. The highest mean shoot length (27cm) was in neopeat, while it was 26.4 cm in both sand and mix media (Table 3). Similarly the interaction between growth media and hormone concentration had the highest shoot length (29.7cm) in the combination of IBA 2000 ppm and neopeat and the lowest mean shoot length of 21.2cm in control with sand (Table 4). On the other hand, the different hormone types and concentration had significant effect on the shoot length. IBA had the highest mean shoot length (28 cm) (Table 1) and IBA 5000 ppm had the best effect on the shoot length with a mean value of 28.7cm (Table 2).

Table 3: Effects of rooting media on the performance of *Z. armatum* stem cuttings

Growth media	Root length (cm)	Shoot length (cm)	Number of roots
Sand	8.3	26.4	4.2b
Neopeat	9.2	27.0	5.0a
Mix	8.8	26.4	4.4ab

Means with the same letter in the same column are not significantly different ($P \geq 0.05$)(ns: not significant)

Table 4: Interaction of hormone concentration and growth media on *Z. armatum* stem cuttings

	Growth media								
	Root length (cm)			Shoot length (cm)			Number of roots		
	Sand	Neopeat	Mix	Sand	Neopeat	Mix	Sand	Neopeat	Mix
Hormone Concentration									
IBA2000	6.9	8.0	8.0	28.8	29.7	27.8	2.7	3.3	3.8
IBA3000	8.5	9.1	7.4	27.5	27.2	25.8	3.8	5.1	4.8
IBA5000	10.7	11.6	11.6	28.2	28.5	29.0	5.5	6.5	5.6
NAA2000	7.4	7.1	8.3	26.0	25.4	26.3	4.5	5.8	5.0
NAA3000	7.0	11.3	11.3	26.3	27.3	24.8	4.4	5.9	3.7
NAA5000	7.0	8.2	5.4	22.1	25.0	24.4	3.9	3.4	3.2
Control	5.0	5.8	5.5	21.2	22.6	22.4	2.0	2.3	2.9

There were significant differences in effect of different concentrations of IBA and NAA and different growth media (sand, neopeat and mix) on the rooting and shooting of stem cuttings of *Z. armatum*.. The exogenous application of growth hormones to induce rooting on stem cuttings has been widely established by several researches (Leakey *et al*., 1994; Poupard *et al*., 1994; Hartman *et al*., 2002; Tchoundjeu *et al*., 2004). The widely used sources of growth hormones for rooting stem cuttings are the different types of auxins: IAA, IBA and NAA, which are known to increase the rate of rooting as well as number of roots per cutting (Gehlot *et al*., 2014; Ibrahim *et al*., 2015).

Auxins are responsible for the overall development in plants from cell division to cell expansion (Taiz and Zeiger, 1998). The initial cell division during root formation in the cuttings depends on the level of auxins, be it exogenous or endogenous (Ludwig, 2000; Kochhar *et al*., 2005). In this experiment also, IBA and NAA had a significant effect on the number of roots per cutting as well as the length of the roots and shoots as compared to the untreated groups. This might be due to the accumulation of metabolites at the auxins application site, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins, and cell division (Strydem and Hartman, 1960). IBA was found to be more effective than NAA in enhancing root formation. The exogenous application of adequate IBA might have caused the vascular differentiation of cells and production of more number of roots. Increase in length of the roots and shoots at higher concentrations might be due to the early formation of roots and more utilization of the nutrients (Banjara, 2017).

The effectiveness of IBA in enhancing root proliferation as well as root numbers have been well documented by several earlier studies in different species. The cuttings of *Jatropha curcas* treated with IBA had the highest mean number of roots than cuttings treated with NAA (Adekola and Akpan, 2012). The highest rooting rate was obtained in *Aesculus indica* cuttings treated with IBA (Majeed *et al*., 2009). *Stereospermum suaveolens* cuttings produced the longest root with IBA (Baul *et al*., 2008). Similar results were obtained for the cuttings of *Shorea leprosula* (Aminah *et al*., 1995), *Ulmus villosa* (Bhardwaj and Mishra, 2005), *Lippia javanica* (Soundy *et al*., 2008), *Buchholzia coriacea* (Akinyele, 2010), *Ficus hawaii* (Hassanein, 2013), *Massularia acuminate* (Usman and Akinyele, 2015), *Cyclopia subternata* (Mabizela *et al*., 2016) and *Toona ciliata* (Thakur *et al*., 2018).

Among the different concentrations of IBA, 5000 ppm showed the best result in both rooting and shootings of *Z. armatum* stem cuttings. This is in accordance with the findings of Daudi *et al*. (2016), who conducted propagation techniques in *Z. alatum* through stem cuttings and seed germination. They found that the cuttings treated with the concentration 5000 ppm of IBA exhibited better sprouting and rooting than the concentrations 4000 ppm and 6000 ppm of IBA. They also concluded that propagation from the stem cuttings is more suitable than seed sowing for *Z. armatum* because seed germination process is very slow in the species. Similar results were obtained by Singh and Rawat (2017) in *Z. armatum* semi-hard wood (SHW) and hard-wood (HW) branch cuttings. IBA at 0.3% and 0.4 % concentrations exhibited greater success in root and shoot growth, whereas lower concentrations completely failed to root.

Several studies have demonstrated the better rooting ability of IBA at higher concentrations. Majeed *et al.* (2009) proved that IBA at 4000 ppm concentration is an optimal plant growth regulator for rooting the cuttings of *Aesculus indica* . High rooting rate was obtained for *Celtis australis* cuttings treated with 3000 ppm IBA (Shameet *et al*.. 1989). *Dalbergia sisso* and *Dalbergia latifolia* also exhibited a very high rooting rate with the application of the concentration 5000 ppm of IBA (Sharma and Pandey, 1999). Maximum number of roots was produced in *Melissa officinalis* stem cuttings at 5000 ppm concentration of IBA (Sevik and Guney, 2013).

Thakur *et al.* (2018) concluded that the cuttings of *Toona ciliata* produced significantly maximum average length of sprouts, root and number of roots per cutting with the application of 8000 ppm of IBA in comparison to other formulations. The apical cuttings of *Berberis aristata* treated with 5000 ppm of IBA concentration demonstrated significantly better rooting and sprouting compared to other treatments (Ali *et al*., 2008). The higher concentration of IBA is required to compensate the low endogenous levels of auxin in the mature cuttings, otherwise difficult to root species like *Terminalia arjuna* (Banjara, 2017).

The growth medium or the rooting medium is one of the major factors affecting the rooting of stem cuttings (Ingram *et al.,* 1993). The rooting success in any cutting is affected by the interaction of a number of factors like water, oxygen, and nutrient availability in the growth media (Alikhani *et al*., 2011; Bhardwaj, 2014). The effect of growth media on the rooting ability of stem cuttings of several economically important plants have been demonstrated by several works (Wojtusik *et al*., 1994; Tchoundjeu *et al*., 2002; Akinyele, 2010; Jacygrad *et al*., 2012; Usman and Akinyele, 2015; Ibironke and Victor, 2016).

The results obtained in this study revealed that there was a significant effect of growth media on the root number, root length and the shoot length of *Z. armatum* stem cutings. The highest values were observed in neopeat medium and the lowest values in sand medium. Neopeat medium had more number of roots and the longest roots than sand and mix media. Sand is too porous and cannot retain water for a longer period of time as well as low in nutrient content whereas the neopeat consists of mixture of all the required nutrients, better aeration and adequate drainage (Akinyele, 2010). Poor aeration in waterlogged conditions may lead to decay of cuttings before root initiation (Schmitz *et al*., 2013).

This corroborated with the findings of Tchoundjeu *et al*. (1998) in the cuttings of *Prunus africana,* which rooted better in sawdust than in sand. Similar findings were described by Wojtusik *et al*., 1994 in *Prosopis juliflora* cuttings, which produced more number of roots and the longest roots in perlite medium than in compost medium. Therefore to enhance steady rooting, the best quality hormone and rooting media is crucial.

Conclusion

As the seed germination rate is very slow in *Z. armatum*, vegetative propagation through stem cuttings is a viable option for the mass production of elite plant materials. This study evaluated the effect of different growth hormone types, concentration and growth media on the rooting and shooting performance of *Z. armatum* stem cuttings. The results showed that growth media and hormonal concentration significantly affect the growth of root, shoot and the number of roots of *Z. armatum* stem cuttings. Both IBA and NAA responded well in rooting and shooting but IBA was found to be effective as compared to NAA. Among the various concentrations of IBA, 5000 ppm showed the best performance in terms of average root length, shoot length and number of roots per cutting. Similarly, the neopeat growth medium was found to be superior over the sand and mix media. So it can be concluded from this study that IBA 5000 ppm concentration with neopeat medium is the best treatment for rooting the stem cuttings of *Z. armatum*. The results obtained in this study could be of relative significance for the commercial production of quality plantlets as well as for improving agroforestry systems.

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