REASEARH ARTICLE

# Effect of maturity and potting media on vegetative propagation of *Salacia reticulata* (Kothalahimbatu) through stem cuttings

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**Abstract:** Medicinal plants could be conserved either through development of improved forms of controlled use and/or through development of cultivation practices. In this context, development of simple, cost effective propagation techniques for threatened species is highly important. *Salacia reticulata* (Kothalahimbatu) belongs to the family Hippocrateaceae is highly demanded medicinal plant species native to Sri Lanka. Due to over exploitation, the species has now been placed in the IUCN red list. The present investigation studied the effect of maturity stage of the cuttings and potting media on successful propagation of Kothalahimbatu. The investigation was carried out at the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka. In the first experiment, soft wood ($T_1$), semi hard wood ($T_2$) and hard wood ($T_3$) stem cuttings were planted in single plant propagator filled with a potting mixture of top soil and compost (1:1 by volume) to study the effect of maturity stage on rooting of cuttings. In the second experiment, double nodal semi hard wood stem cuttings were planted in black polythene bags filled with different potting mixtures; sand ($T_1$), top soil ($T_2$), sand:top soil (1:1) ($T_3$), sand:coir dust (1:1) ($T_4$), sand:top soil:coir dust (1:1:1) ($T_5$), coir dust ($T_6$), sand:compost (1:1) ($T_7$), top soil:coir dust (1:1) ($T_8$), top soil:compost (1:1) ($T_9$), sand:top soil:compost (1:1:1) ($T_{10}$) to study the effect of potting media on rooting of semi hard wood stem cuttings. Completely Randomized Design (CRD) was used with three replicates each consisted of thirty stem cuttings. Mean survival percentage at four, eight and twelve weeks after planting was significantly higher ($P \leq 0.05$) in soft wood and semi hard wood stem cuttings than hard wood stem cuttings. Furthermore, callus formation and emergence of new leaves were also higher in semi hard wood cuttings compared to other cutting types. In the second experiment, top soil:compost (1:1) exhibited significantly higher survival (66.67%) than other potting mixtures. Callus formation of the cuttings was found to be significantly higher ($P \leq 0.05$) in top soil:compost (1:1) ($T_9$). Based on the findings, semi hard wood stem cuttings planted in top soil:compost (1:1) could be recommended for vegetative propagation of *Kothalahimbatu*.

**Keywords:** *Salacia reticulata*, Semi hard wood cuttings, Potting media and Survival percentage.

## INTRODUCTION

*Salacia reticulata* (Wight) (Kothalahimbatu in Sinhala) is a plant native to sub-mountain forests in Sri Lanka and wild dense forest of coastal belts of India. (http://www.freshpatents.com/Glycosidase-inhibitors-and-methods-of-synthesizing-same). The plant is widely distributed in low country dry zone which consists mainly of flat and undulating land receiving <2000mm annual rainfall (http://www.agriinfotech.com/htmls/PDF- Files/Herbs/Liver%20Tonic.pdf).

In Ayurvedic system of medicine, *S. reticulata* has been used as a treatment for diabetes. In Ayurveda, it is advised that a person

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suffering from diabetes should drink water left overnight in a mug which has been made in Kothalahimbatu wood. There is a remedy of high blood content through the derivation of salacinol and kothalanol, the main active ingredients of dried roots and stems of *S. reticulata* (http://www.freshpatents.com/Glycosidase-inhibitors-and-methods-of-synthesizing-same). Other than diabetes, Kothalahimbatu is also used in the treatment of rheumatism, gonorrhea, asthma, amenorrhea and dyamenorrhea (Leakey, 2004). Salacinol and Kothalanol may potentially have fewer long term side effects than other existing oral antidiabetic agents.

The impacts of desirable agro ecological factors affect the lower distribution of Kothalahimbatu plant. Most of plants were of a secondary nature reflecting ancient and varied histories of chena (slash and burn) cultivation of dry zone, over harvesting and lack of care to their habitat when collecting plants from the wild. Over harvesting / unsustainable harvesting of plant is mainly due to the high demand for Ayurvedic medicines (Wijesundara, 2003). The technology to grow the plant on a commercial scale is not available at present and higher rates of extraction from the wild may lead to extinction of the species. Sri Lanka is currently confronted by this problem when it comes to *S. reticulata*.

Therefore greater attention needs to be given to *ex-situ* propagation of *S. reticulata*. Studies on the effect of maturity stage of cuttings and rooting media on vegetative propagation of *S. reticulata* were undertaken in the present study.

## MATERIALS AND METHODS

Planting material was collected from the bank of the lake in Denagama village at Matara district, Low Country Wet Zone of Sri Lanka (Elevation less than 100 m above mean sea level). Stem cuttings were transported by packing them in black polythene bags, with 100% Relative Humidity.

### Experiment 1: Effect of maturity stage of cuttings on vegetative propagation of Kothalahimbatu

Three types of cuttings were used ($T_1$ – Soft wood cutting, $T_2$ – Semi hardwood cutting and $T_3$ – Hard wood cutting). Kothalahimbatu stem cuttings were planted in individual propagators prepared with transparent polythene (12 cm width and 32 cm high). In this experiment, top soil: compost (1:1) mixture was used as potting media. Poly bags were filled with the media up to 15 cm from the bottom. After planting of stem cuttings, propagators were placed in a shade house (70%).

### Experiment 2: Effect of potting mixture on vegetative propagation of Kothalahimbatu stem cuttings

This experiment comprised of the following treatment combinations: Sand ($T_1$), Top soil ($T_2$), Sand : Top soil - 1 : 1 ($T_3$), Sand : Coir-dust - 1 : 1 ($T_4$), Sand : Top soil : Coir dust - 1 : 1 :1 ($T_5$), Coir dust ($T_6$), Sand : Compost - 1 : 1 ($T_7$), Top soil : Coir dust -1 : 1 ($T_8$), Top soil : Compost -1 : 1 ($T_9$) and Sand : Top soil : Compost -1 : 1 :1 ($T_{10}$). Double nodal semi hardwood stem cuttings were taken for the experiment. Cuttings were planted in black poly bags (250 gauge, 12.5 cm width and 15 cm height). Potting mixtures were filled up to 15 cm from the bottom. Watering was done on daily basis. The pots were kept in a shade house (70% shade).

Observations on survival percentage, number of cuttings with new leaves, number of cuttings with callus and number of roots were recorded after 4, 8 and 12 weeks of planting. To elicit data for particular time periods, each treatment was replicated thrice. Each treat-

ment consisted of thirty stem cuttings of which ten stem cuttings were considered in each data recordings. The experiments were arranged in a Completely Randomized Design. ANOVA procedure and Duncan Multiple Range Test were used for the mean separation.

## RESULTS AND DISCUSSION

### Experiment 1: Effect of maturity stage of cutting on vegetative propagation of Kothalahimbatu

Survival rate of cuttings was found to be significantly affected by the stage of maturity. The probability value was 0.028 (Pr>F 0.05). After four weeks of planting, soft wood cuttings showed the highest survival percentage of 62. At this stage, survival percentage of semi hardwood cuttings was proved to be better (51) over hard wood cuttings (34) (Fig. 1A).

Results indicated that soft wood cuttings had the highest survival percentage (53%) after 8 weeks of planting. But the survival percentage was lower than softwood cuttings after four weeks of planting. Semi hardwood cuttings gave better results than hard wood cuttings (Fig. 1B).

After twelve weeks of planting, the lowest survival percentage (12) was recorded from hard wood cuttings while no significant different was found between soft wood and semi hardwood stem cuttings (Fig. 1C).

Number of calluses produced in soft wood and semi hardwood cuttings was significantly different at 4, 8 and 12 weeks of planting (Fig. 2 A, B and C).

Semi hardwood cuttings produced the highest number of new leaves after 12 weeks of planting followed by softwood cuttings (Fig. 2D).

Amongst the three type of cuttings, semi hardwood stem cuttings were proved to be the best for all the parameters observed. Although, softwood cuttings had higher survival percentage after 12 weeks of planting, it had lower callus formation ability and lesser number of new leaves. Based on the above investigation, it could be recommended to multiply *S. reticulata* by semi hardwood cuttings.

### Experiment 2: Effect of potting mixture on vegetative propagation of Kothalahimbatu stem cuttings

The performance of Kothalahimbatu stem cuttings was measured in terms of survival percentage of stem cuttings, number of new leaves, callus formation, number of roots and root length. Different potting mixtures had varying responses to the parameters included in the study. The probability value was $P \leq 0.005$. Differences were significant for survival percentage after four weeks of planting (Fig. 3A). The highest survival percentage (93.33) was observed in Top soil: Coir dust (1:1) potting mixture. Whereas, the lowest survival percentage (13.33) was noted when top soil was used as potting media. After eight weeks of planting, the highest survival percentage was noticed in Top soil: Compost (1:1) media (Fig. 3B). This trend remained the same even after twelve weeks of planting (Fig. 3C). Semi hard wood cuttings did not survive in top soil after 8 and 12 weeks of planting. Cuttings planted in soil also showed 100 per cent mortality after 12 weeks of planting. New leaves and roots were not observed in four weeks old stem cuttings

The callus forming ability of semi hardwood cuttings in different potting mix-

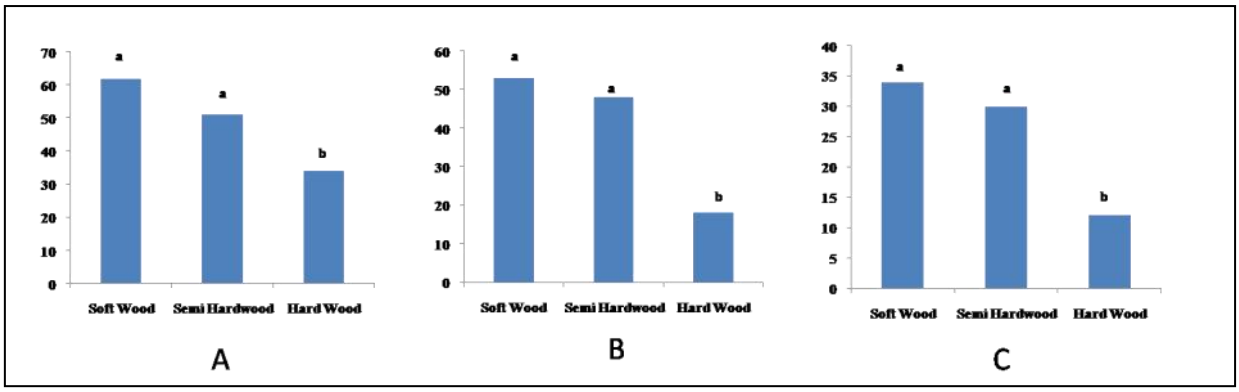


**Figure 1:** Effect of stem cuttings on survival ability of Kothalahimbatu after (A) 4 weeks (B) 8 weeks (C) 12 weeks of planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

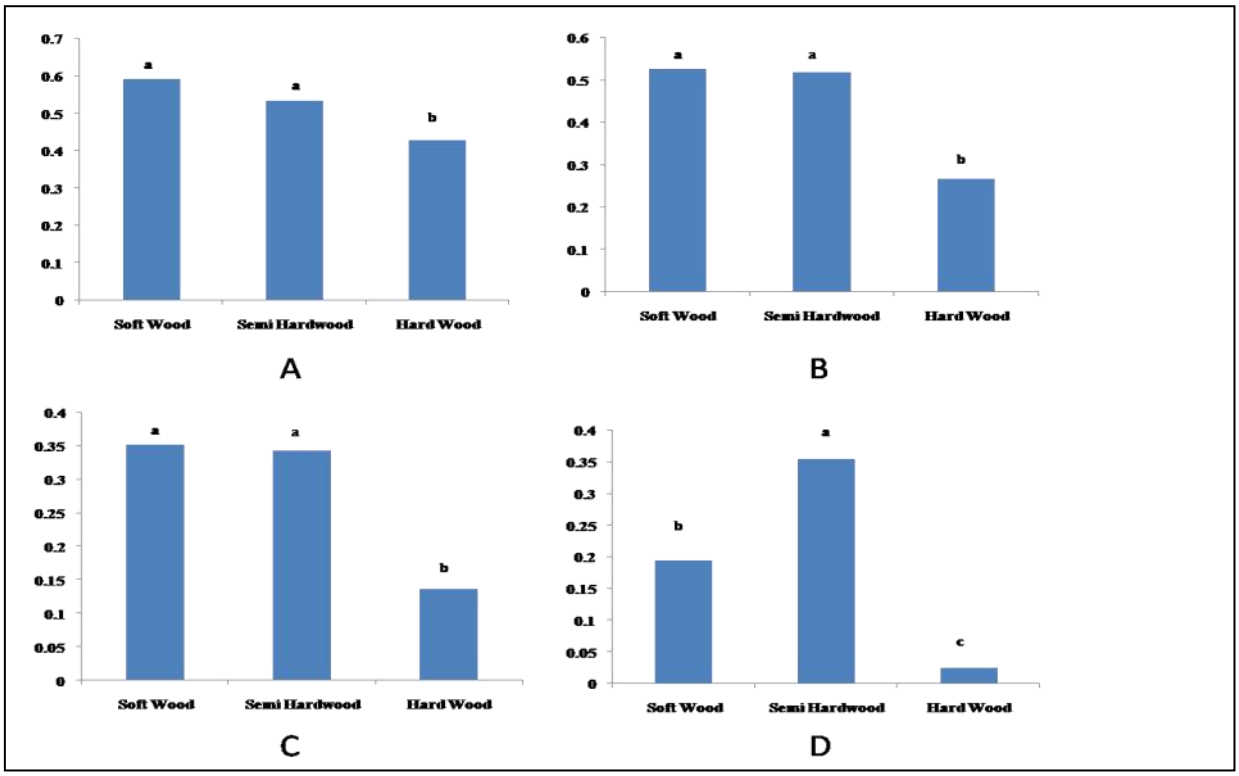


**Figure 2:** Effect of stem cuttings on callus formation ability of Kothalahimbatu cuttings after (A) 4 weeks, (B) 8 weeks, (C) 12 weeks of planting and (D) after planting and (D) New leaves forming ability Kothalahimbatu as influenced by stem cuttings at the age of twelve weeks. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

tures is mapped in Fig 4. According to fig. 4, stem cuttings in Top soil: Coir dust (1:1) medium, Coir dust medium and Sand: Top soil: Compost (1:1:1) medium showed better callus formation. Top soil: Coir dust (1:1) medium was the best among them. However, top soil: compost (1:1) media exhibited superior callus forming ability over all other treatments after 8 and 12 weeks of planting. Stem cuttings in top soil and sand media failed to produce callus after 12 weeks of planting.

Of the different treatments, only Top soil: Compost (1:1) medium produced new leaves.

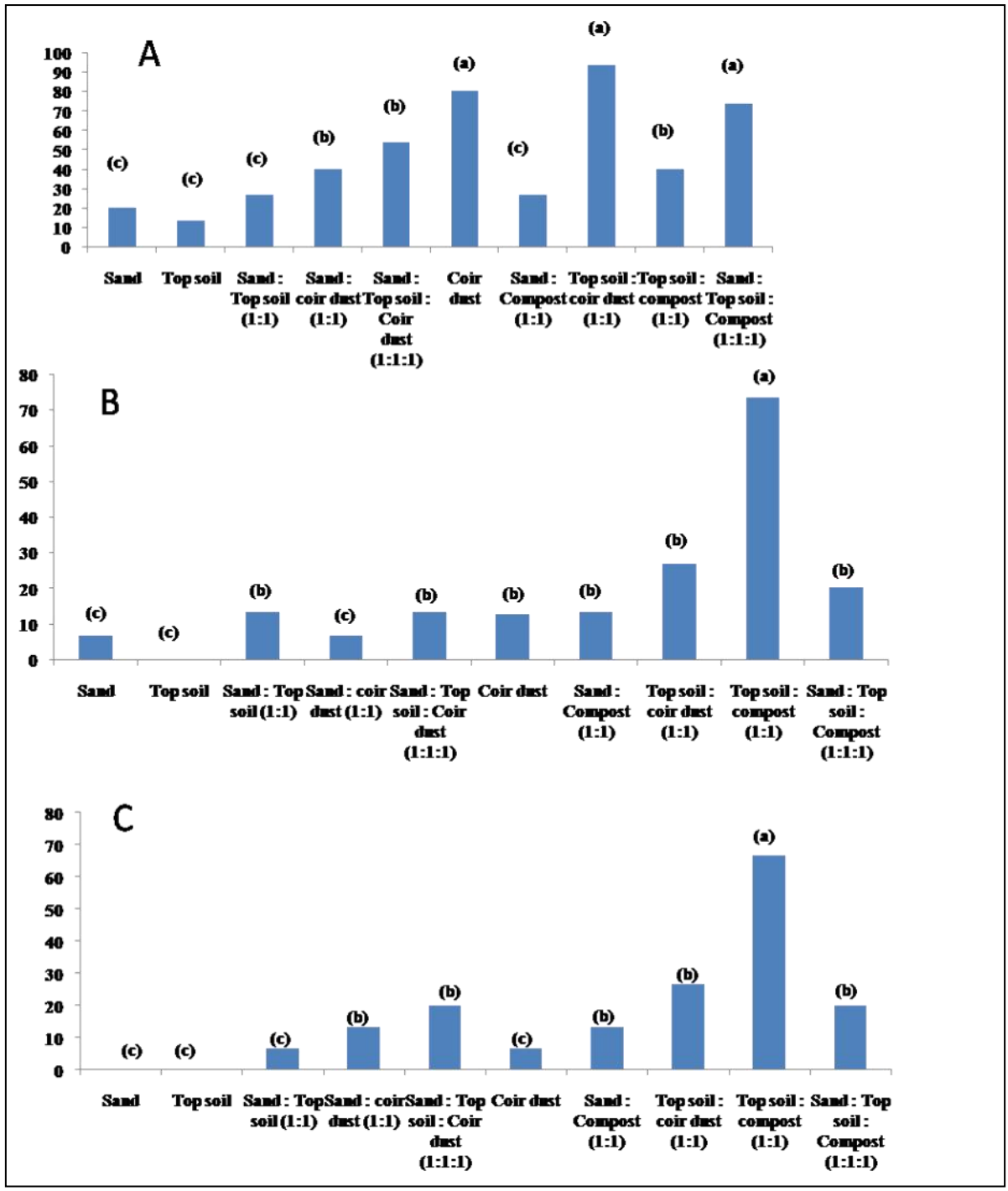


**Figure 3:** Effect of potting mixtures on survival percentage of *Salacia reticulate* stem cuttings after (A) 4 weeks, (B) 8 weeks and (C) 12 weeks of planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

Very few stem cuttings planted in Top soil: Compost (1:1) and Top soil: Coir dust (1:1) produced roots, however they were not enough for statistical analysis. Top soil and Sand should not be used as potting media for *S. reticulata.* In the above investigation, Top soil: Compost (1:1)

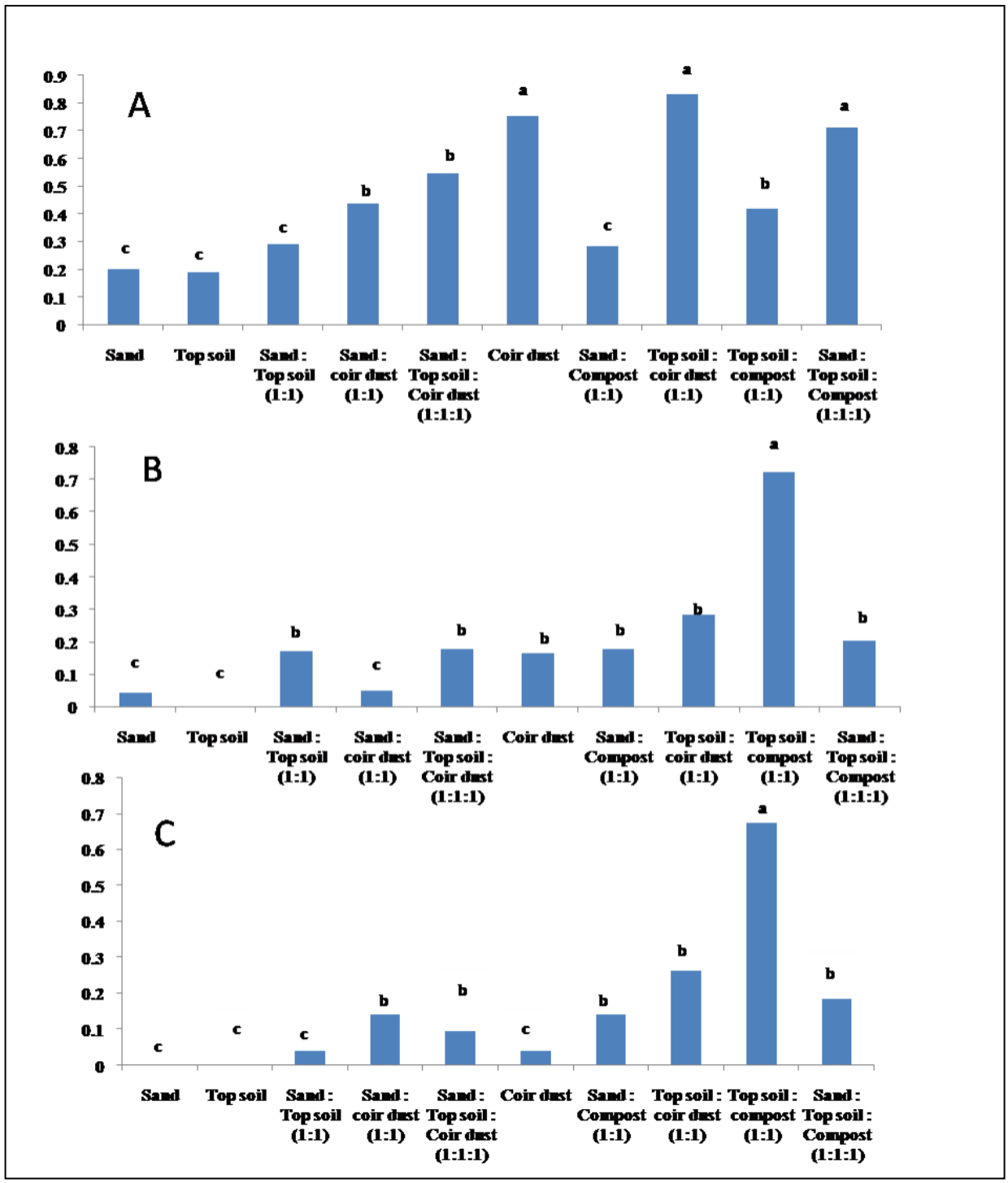


**Figure 4:** Effect of different potting mixtures on callus forming ability after (A) 4 weeks, (B) 8 weeks and (C) 12 weeks of planting in semi hardwood cuttings on Kothalahimbatu. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

emerged as the best potting mixture for the planting of semi hardwood cuttings of Kothalahimbatu.

Present results are in agreement with the previous reports of Subasinghe *et al.* (2008), where it has been mentioned that Kothalahimbatu could be vegetatively

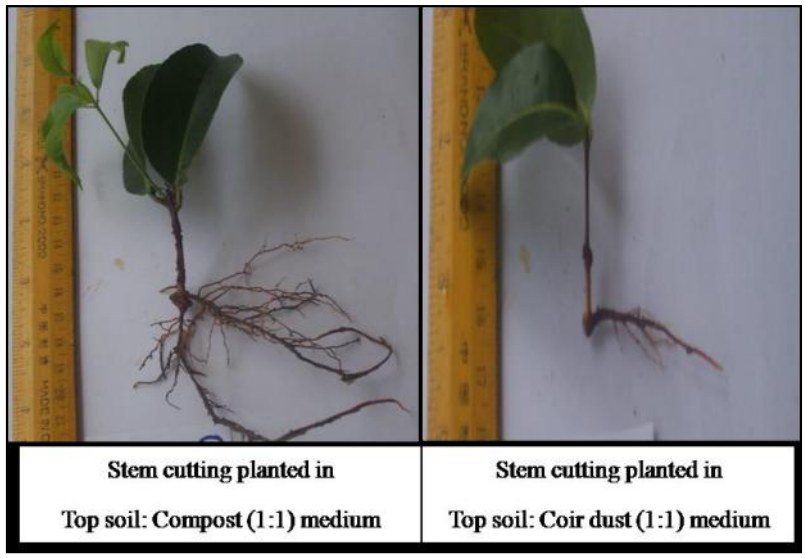


**Figure 5:** Pictures of stem cuttings planted in different potting mixtures

propagated by means of stem cuttings. Moreover, Oommen *et al*. (2000) reported that kothalahimbatu could be propagated through root cuttings as well. As reported by Hettiarachchi and Subasinghe (2010) soft wood cuttings of kothalahimbatu treated with hormone (Indol Butric Acid, 2000 ppm) and planted in sand: coir dust (1: 1) media recorded the highest root dry weight and root length. More recently, Dhanasri *et al*. (2013) studied the micropropagation of kothalahimbatu. Nodal segments have been cultured on MS supplemented with different growth regulators and the most efficient shoot multiplication have been observed with the supplementation of BA and IAA (3.5 + 0.5 mg/l). They further observed elongation of the microshoots in subcultures within 20 days. Based on the results, they concluded that high quality planting stocks of kothalahimbatu could be achieved through micropropagation. Taking all these reports into account, it could be concluded that vegetative propagation of kothalahimbatu could be done with varying degrees of success.

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