Studies on Vegetative Propagation of *Salacia reticulate* (Kothalahimbatu) through Stem Cuttings as Affected by Maturity of Cuttings and Potting Media

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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 reticulate* (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 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 (T1), semi hard wood (T2) and hard wood (T3) 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 reticulate, Semi hard wood, Potting media. Survival %, Callus formation*

INTRODUCTION

Salacia reticulate (Wight) (Kothalahimbatu in Sinhala) is a plant native to submontane 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 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 medicine system *Salacia reticulate* has been used as a treatment for diabetes. In Ayurvedic it is advised that a person 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 glucose content through the derivation of salacinol and kothalanol main active ingredients from dried roots and stems of *Salacia reticulate* (http://www.freshpatents.com/Glycosidase-inhibitors-and-methods-

of-synthesizing-same). Other than diabetes, Kothalahimbatu unique to treat 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 medicine (Wijesundara, 2003). The technology to grow the plant on a commercial scale is not available at present and it would lead to higher rates of extraction from the wild and that may lead to extinction of the species Sri Lanka is currently confronted with this problem with *Salacia reticulate.*

Therefore the greater attention needs to be given to *ex-situ* propagation for *Salacia reticulate.* Study on effect of cutting types and rooting media of *Salacia reticulate* (Kothalahimbatu) to fulfill the above objective.

MATERIALS AND METHODS

Planting materials were 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 put them in to black polythen bags, with 100% Relative Humidity.

Experiment 1: Determination of best cutting type for 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 on individual propagators. Single propagators were prepared using 12 cm width and 32cm high transparent polythene. In this experiment, top soil: Compost (1:1) mixture was used. Potting mixtures were filled up to 15cm from the bottom. After planting of stem cuttings air filled in to the individual propagator and tied-up open end of the single propagator. After that single propagators were placed in shade house. Observations on survival percentage, number of cuttings with new leaves, number of cuttings with callus, number of roots were taken when considering shoot growth and rooting performances of three times in 4, 8 and 12 weeks after planting. To obtain the data for particular time periods each treatment was replicated in three times. There were thirty stem cuttings consisted in each treatment. Ten cuttings were removed per treatment per replicate at once. Experiment was arranged in Completely Randomized Design. Anova procedure was used for analysis and Duncan multiple range test was used for mean separation.

Experiment 2: Determination of the best potting mixture for vegetative propagation of Kothalahimbatu semi hardwood stem cuttings.

Treatments of Sand (T_1), Top soil (T_2), Sand : Top soil - 1 : 1 (T_3), Sand : Coirdust - 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}) were used in the experiment. Double nodal semi hardwood stem cuttings were taken for the experiment. Ten cutting were consisted in each replicate. Cuttings were planted on black poly bags (Gage 250). Pot sizes were 12.5 cm width and 15cm height. Potting mixtures were filled up to 15 cm from the bottom. Water applied daily. The pots were kept in a shade house. Observations on survival percentage, number of cuttings with new leaves, number of cuttings with callus, number of roots were taken when considering shoot growth and rooting performances of three times in 4, 8 and 12 weeks after planting. To obtain the data for particular time periods each treatment replicated in three times. Each treatment consisted thirty stem cuttings. Therefore ten stem cuttings were

evaluated per treatment per replicate at once. Experiment was arranged in Completely Randomized Design. ANOVA procedure and Duncan Multiple Range Test were used for the mean separation.

RESULTS AND DISCUSSION

Experiment 1: Effect of cutting types on survival of *Salacia reticulate* stem cuttings

The analysis of survival ability of Kothalahimbatu stem cuttings with the effect of stem cutting type was measured at three age categories as 4, 8 and 12 weeks after planting. The variable (cutting type) was significantly affect for the survival percentage. There probability value was 0.028 (Pr>F 0.05). At the age of four weeks after planting, soft wood stem cuttings were highest in survival ability. The survival percentage was 62 % (Fig. 1A). At this stage semi hardwood stem cuttings were shown the second most survival ability than hard wood stem cutting type. The survival percentages were 51 % and 34 % respectively (Fig. 1A).

Results showed that soft wood stem cuttings were shown the highest survival percentage (53 %) at 8 weeks after planting (Fig. 1B). But the survival percentage was lower than the stem cuttings at four weeks after planting. Semi hardwood stem cuttings were shown the higher survival ability than hard wood stem cuttings as in following fig.1B

The twelve weeks age stem cuttings revealed significant effect for survival percentage. As in above eight weeks after planting stem cuttings, hard wood cutting type was shown the lowest in survival ability. The lowest survival percentage was 12 %. Soft wood and semi hardwood stem cuttings were not significantly different (Fig. 1C).

To find out survival ability of stem cutting types following parameters such as number of new leaves availability, callus formation ability, number of roots also measured. In this experiment soft wood and semi hardwood stem cuttings, significantly higher number of calluses produced at the considered three age levels (Fig. 2A, B and C).

When increasing the age of stem cuttings, which had ability to produce new leaves. T2 shows significantly highest number of semi hardwood stem cuttings with new leaves. There soft wood and hardwood stem cuttings also produced new leaves. With increasing age they were shows significantly low amount of cuttings with new leaves as in following Fig. 2D.

When comparing three types of stem cuttings, semi hardwood stem cuttings were performed in best and hard wood stem cuttings were shown the lowest survival percentage and callus formation. Decline the survival percentage and callus forming ability of soft wood stem cuttings with increasing of the age. But when increasing age of semi hardwood cuttings were shown better performances than others. Therefore successful survival stem cuttings could be obtained through the kept after at least three months.

Experiment 2: Effect of potting media on survival ability of *Salacia reticulate* semi hardwood stem cuttings

Survival ability of Kothalahimbatu stem cuttings were measured through the survival percentage of stem cuttings, number of new leaves availability, callus formation, number of roots and root length. The stem cuttings with four weeks age there were no new leaves as well as roots observed. Duncan multiple range test was used to test the experiment of "to determine the best potting mixture for vegetative propagation of Kothalahimbatu". The variables which considered were measured by the four, eight and twelve weeks after planting frequently. The stem cuttings at four weeks age were shown significantly different with potting mixture and survival percentage (Fig. 3A). Highest survival percentage shows Top soil: Coir dust (1:1) potting mixture (93.33 %). The lowest survival percentage shows Top soil (13.33 %) potting media.

Following Fig 4 express the mean value of stem cuttings with callus in different potting media. According to fig. 4 stem cuttings with Top soil: Coir dust (1:1) medium, Coir dust medium and Sand: Top soil: Compost (1:1:1) medium were the highest in callus formation. Top soil: Coir dust (1:1) medium was best among them. Stem cuttings in Top soil media bearded lowest number of cuttings with calluses. At the age of eight weeks cuttings were expressed the results as in fig. 4B.

The ANOVA test was significantly affect for the variables. The probability value was $P \leq 0.005$. Within the ten potting mixtures, highest survival percentage was shows in Top soil: Compost (1:1) media at the age of eight weeks after planting also (Fig. 3B). When increasing the stem cuttings uprooted age, Top soil: Coir dust (1:1) potting mixture was shown the lower survival percentage than Top soil: Compost (1:1) mixture. The lowest survival ability shows in Top soil applied potting media. At that age of stem cutting survival percentage was 0% in Top soil media. As same as survival percentage callus forming number of cuttings was highest in Top soil: Compost (1:1) media. That mean value was significant than the other potting media. At that age level was not success to generate new leaves and roots.

The stem cuttings with twelve weeks after planting also shows the highest (66.67 %) survival percentage in Top soil: Compost (1:1) media (Fig. 3C). The lowest survival percentage was zero as the age of four weeks after planting and eight weeks after planting. In this stage sand potting media also shows the zero percent survival ability.

Kothalahimbatu stem cutting at twelve weeks age, Top soil: Compost (1:1) mixture in front for callus formation as well as survival percentage (Fig 4C). There Sand and Top soil media were failed to formation of callus. And stem cuttings at twelve weeks, that age was not enough matured to produce roots. Only Top soil: Compost (1:1) medium bearded new leaves. Very few amount of stem cuttings planted in Top soil: Compost (1:1) and Top soil: Coir dust (1:1) were bared roots. The amounts of those cuttings were not enough for the analysis.

When comparing three types of uprooting ages, Top soil: Compost (1:1) potting mixture was the best. Top soil: Coir dust (1:1) potting media better than other potting mixtures except Top soil: Compost (1:1) media. As well as Top soil and Sand potting media were the worst for planting of stem cuttings. Therefore only Top soil medium and only Sand medium cannot be recommended for planting of Kothalahimbatu stem cuttings.

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Figures

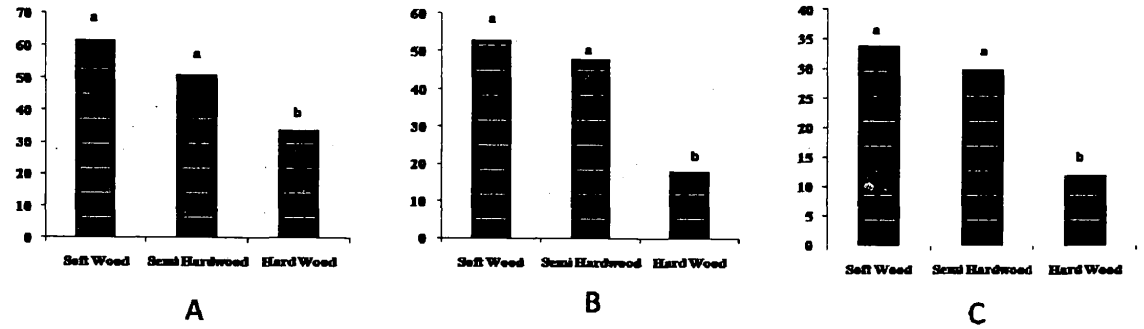


Figure 1: Survival ability of Kothalahimbatu due to the stem cutting types at (A) 4 weeks (B) 8 weeks (C) 12 weeks after planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

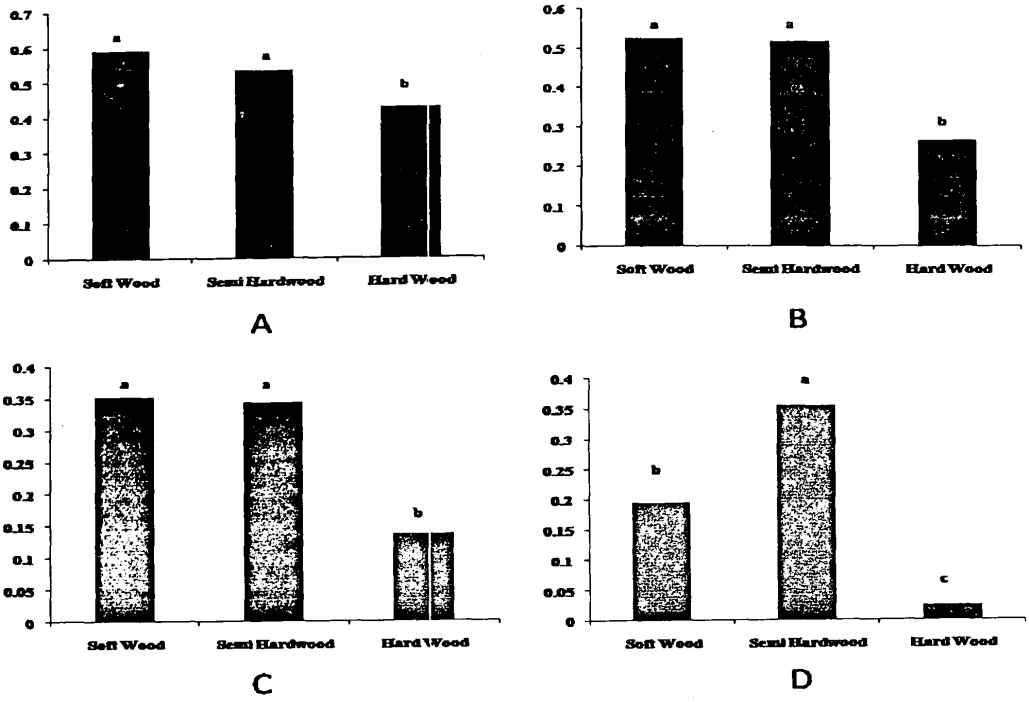


Figure 2: Callus formation ability Kothalahimbatu due to the stem cutting types at the age of (A) 4 weeks, (B) 8 weeks, (C) 12 weeks and (D) after planting and (D) New leaves forming ability Kothalahimbatu due to the stem cutting types at the age of twelve weeks after planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

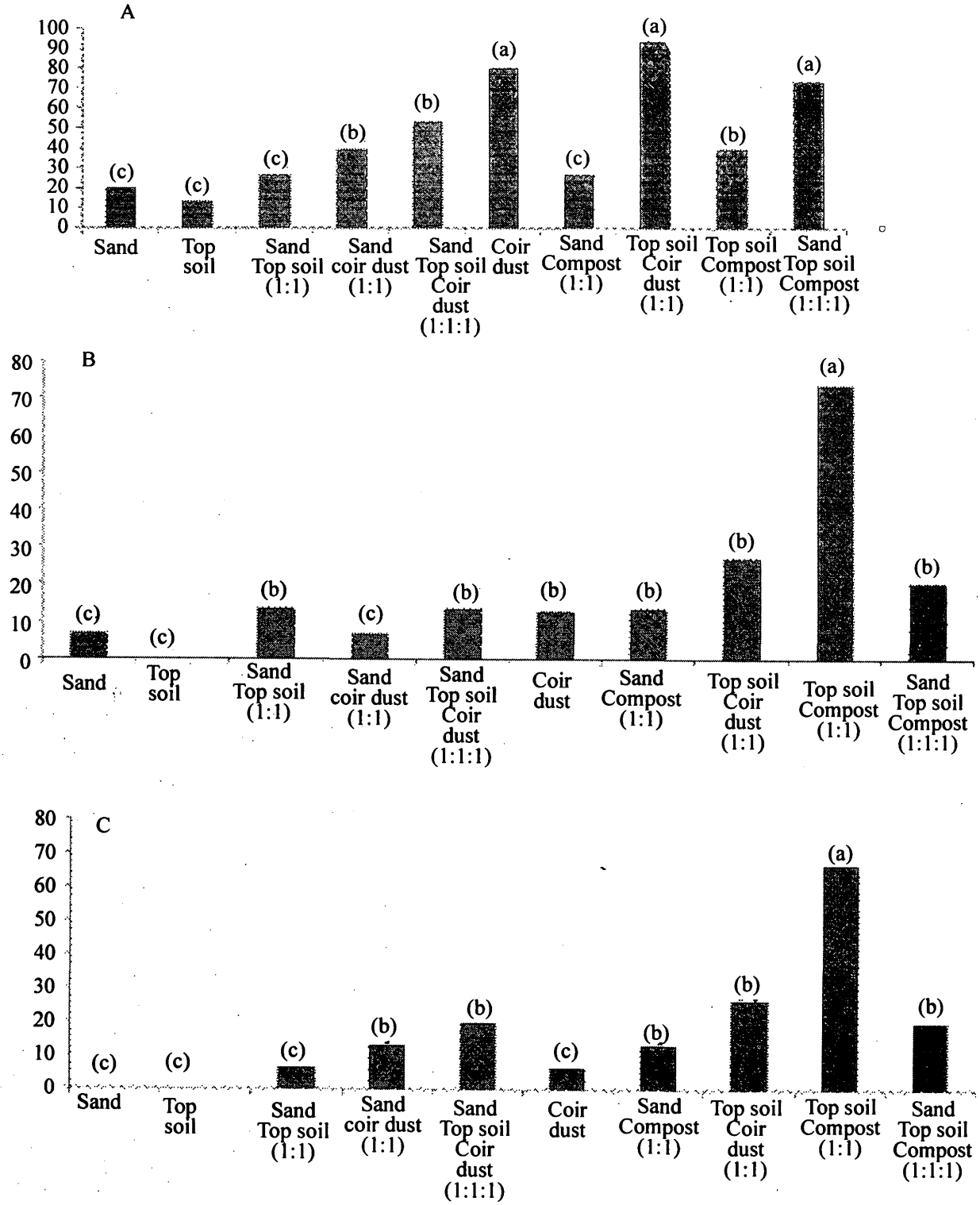


Figure 3: Effect of potting media on survival percentage of *Salacia reticulate* stem cuttings at (A) 4 weeks, (B) 8 weeks and (C) 12 weeks after planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$

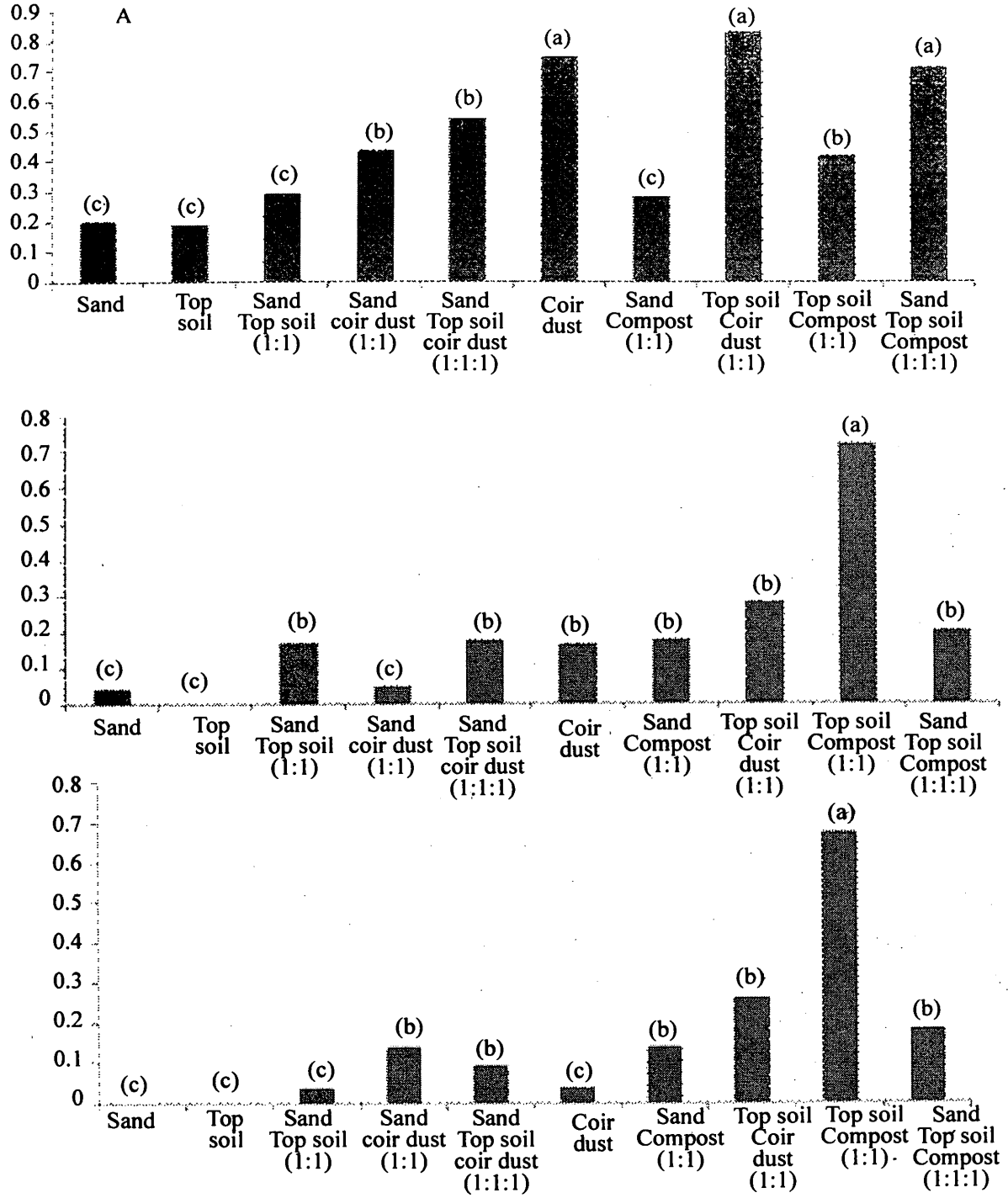
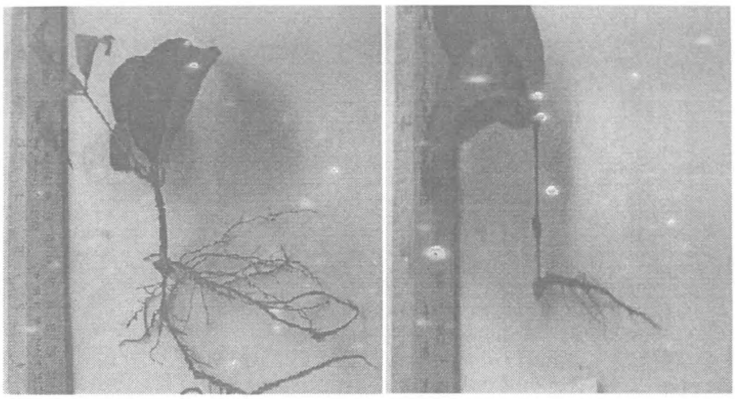


Figure 4: Callus forming ability with different potting mixture at (A) 4 weeks, (B) 8 weeks and (C) 12 weeks after planting. Means on the bars represent the same letter are not significantly different at $P \leq 0.05$



Stem cutting planted in Top soil: Compost (1:1) medium

Stem cutting planted in Top soil: Coir dust (1:1) medium

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

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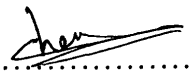
Signature : 

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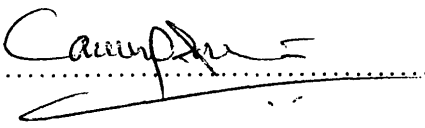
Signature : 

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