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Selection of suitable potting media for the propagation of *Evolvulus alsinoides* L. (slender dwarf morning glory) through seeds and stem cuttings

Niluka Nakandalage[1]* and H.I.G.K Anuruddi[1]

[1]*Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka*

ABSTRACT

Evolvulus alsinoides Linn (Vishnukranthi) is a multipurpose medicinal herb used to treat various ailments in traditional Ayurveda medicine. Less availability of quality planting materials exist as a major bottleneck for its commercial scale cultivation. Therefore, a series of experiments were undertaken to evaluate the most promising media for seed and vegetative propagation of Evolvulus alsinoides. Seed viability was tested by Tetrazolium test (TZ test). Suitable seed germination media was tested using seed trays filled with eight potting mixtures in a completely randomized design with five replicates. The effect of auxin (Indole Acetic Acid (IAA) and Indole Butyric Acid (IBA) at 3000 ppm) and rooting media on soft wood and semi hardwood cuttings of Evolvulus alsinoides was tested. Data were analyzed using ANOVA with a statistical analysis system at 5% significance level, and means were separated using Duncan Multiple Range Test. TZ test exhibited the seed viability percentage as 87%. Early and significantly (P≤0.05) higher seed germination (79%) was observed in the soil: sand (1:1) nursery media. Sand was the best media to propagate stem cuttings of E. alsinoides. The highest plant height of 20.3 cm was observed with IBA treated top wood cuttings. Semi hardwood cuttings treated with IAA reported the best performance in terms of number of leaves, number of branches, shoot dry weight, root dry weight and whole plant dry weight of E. alsinoides. Therefore, seed germination in soil: sand (1:1) media and semi hardwood cuttings established in sand media treated with 3000 ppm of IAA were most suitable for propagation of E. alsinoides in mass scale. Potential farmers could easily adopt these findings for cultivation of this valuable medicinal plant.

Keywords: *Auxin, Evolvolus alsinoides, growing media, propagation, rooting, seeds, stem cuttings*

INTRODUCTION

Evolvulus alsinoides L. (slender dwarf morning glory) belonging to family convolvulaceae is a flowering perennial herb which is distributed in dry tropical and sub-tropical regions in the world (Singh, 2008). This plant is identified by various names in different cultures. In Sinhala, it is called as *Vishnukranti* and in Indian region it is known as *shankhapushpi* which is a Sanskrit based common name (Austin, 2008). Branches of the herb are more than 30 cm long, slender and wiry with long hairs and leaves are small and elliptic to oblong with an entire margin and dense hairs (Singh, 2008). Solitary flowers with blue petals appear in upper axils of the branches of the herb. Four seeds are enclosed in globose-shaped capsules (Austin, 2008).

Evolvulus alsinoides has been used as a multipurpose traditional medicine in the treatment of various ailments (Siraj *et al*., 2019). Extract of this plant exhibit strong anti-inflammatory properties (Lekshmi and Reddy, 2011). Different parts of this plant are used as a remedy for cough, cold, fever and

Corresponding author:* niluka@crop.ruh.ac.lk https://orcid.org/0000-0003-0715-1429

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venereal diseases (Singh, 2008). It is mainly used as a brain tonic while several other uses reported were as a memory booster, improving intellect, used in disorders like insanity and nervous debility in conventional medical practices (Nahata *et al*., 2010). This plant also contains alkaloids, flavonoids and cardiac glycosides (Singh, 2008; Lekshmi and Reddy, 2011).

Evolvulus alsinoides herb is subjected to overexploitation due to unplanned, unmanaged and unsustainable harvesting to cater the increasing demand (Naikawadi *et al*., 2012). Thus, establishment of commercial cultivation with normalized agro techniques is important in order to ensure the production and continuous supply of quality of fresh and dry raw materials. Lack of quality planting materials exists as one of the major problems in *E. alsinoides* production. Thus, it is very much important to study its propagation (Naikawadi *et al*., 2012). Seed dormancy of *E. alsinoides* exists for several months (Jayasuriya *et al*., 2008). Although, there are some reports on nodal propagation through tissue culture, it is not an economically viable solution (Naikawadi *et al*., 2012). Hence, it is imperative to develop suitable and cost effective propagation techniques for this invaluable species (Naikawadi *et al*., 2012). Present study investigated suitable potting media for propagation of *E. alsinoides* through seeds and stem cuttings, the effect of plant growth promoting hormones and cutting types for successful production of the crop.

MATERIALS AND METHODS

Three experiments were conducted at the plant house of the Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka, the most suitable media for seed germination and survival, to select the best potting media for rooting and to select the most promising cutting type and auxin combination for propagation of *E. alsinoides*.

Experiment 1

Experiment 1 was carried out to test the most promising media for seed germination and seedling survival.

The seeds were collected from already established mother plants in a plant house. First, the seeds were tested for viability using Tetrazolium test (TZ test) (Hartman *et al*., 1997).The most promising seed germination media was tested using seed trays filled with eight nursery media prepared with different sand, compost, and coir dust ratios. The experiment was laid out in a Completely Randomized Design (CRD) with five replicates. Percentages of seed germination and seedling survival were calculated for each treatment.

Table 1: The different potting media used for seed germination of *E. alsinoides*

Treatments	Potting media
M_1	Soil: sand (1:1)
M_2	Soil: compost (1:1)
M_3	Soil: coir dust (1:1)
M_4	Sand: coir dust (1:1)
M_5	Soil: sand: compost (1:1:1)

M_6	Soil: sand: compost (1:1:2)
M_7	Soil: coir dust: compost (1:1:1)
M_8	Soil: coir dust: compost (1:1:2)

Experiment 2

Experiment 2 was conducted to select the best potting media for rooting of *E. alsinoides* cuttings.

Semi hardwood cuttings of *E. alsinoides* (20 cm long with 3-4 nodes) were collected from well maintained, healthy and vigorously grown mother plants. The stem cuttings were established in three growing media combinations prepared with different ratios of sand, compost and coir dust. The growing media used were sand (P_1), sand: coir dust 1:1 (P_2) and sand: compost: coir dust 1:1:1 (P_3). Then these potting media were drenched using fungicide and filled in transparent polythene (Gauge 150) bags (25cm width and 40 cm height) to make single propagators. The cuttings were established to a depth of 3 cm in the propagators after saturating the media with water. A Factorial Completely Randomized Design (FCRD) with three replicates was carried out. Data on plant height (cm), number of leaves, number of branches, shoot and root dry weight (sdw/ rdw) (g) were recorded at six weeks after planting. Data were analyzed using SAS statistical software at 5% significance level. Duncan Multiple Range test was employed for mean separation.

Experiment 3

After identifying the suitable potting medium with experiment 02, the promising cutting types (softwood and semi hardwood cuttings) and the auxin (IBA and IAA 3000ppm) combination for the propagation of *E. alsinoides* was tested.

In here, the selected medium (sand) was filled into single propagators prepared with transparent polythene of gauge 150. softwood and semi hardwood cuttings of *E. alsinoides* (20 cm long with 3-4 nodes) were treated by dipping in the solutions of 3000 ppm IAA, IBA and mixture of IAA and IBA growth hormones before planting. Treatment combinations used for the experiment were as indicated in Table 02.

Table 2: Treatment combinations of different cutting types and auxin used for *E. alsinoides* propagation through stem cuttings

Treatment codes	**Treatments**	
	Cutting Type (C)	**Auxin (H)**
T_1	Softwood	IAA
T_2	Softwood	IBA
T_3	Softwood	IAA & IBA

T_4	Semi hard wood	IAA
T_5	Semi hard wood	IBA
T_6	Semi hard wood	IAA & IBA

Single stem cutting per propagator was planted to a depth of 3 cm after saturating the media with water. Each propagator was kept under the same condition to avoid the effects from environmental variations. A Factorial Completely Randomized Design (FCRD) with three replicates were practiced for the experiment. Data on plant height (cm), number of leaves, number of branches, shoot and root dry weight (sdw/rdw) (g) were recorded at six weeks after planting. Data were analyzed using SAS statistical software at 5% significance level. Duncan Multiple Range test was employed for mean separation.

RESULTS AND DISCUSSION

Experiment 1: Seed viability and the selection of most promising nursery media for seed germination and seedling survival of *Evolvulus alsinoides*

In the present study, Tetrazolium test (TZ test) exhibited the seed viability percentage as 87%. A seed dormancy stage in several months is observed in E. alsinoides which affects the seed viability and germination (Jayasuriya *et al.*, 2008). As indicated by Naikawadi *et al.* (2012), the yellowish green seeds of *E. alsinoides* were more viable (80%) and brown color seeds showed about 40% viability whereas black color seeds did not show any positive result in the seed viability test.

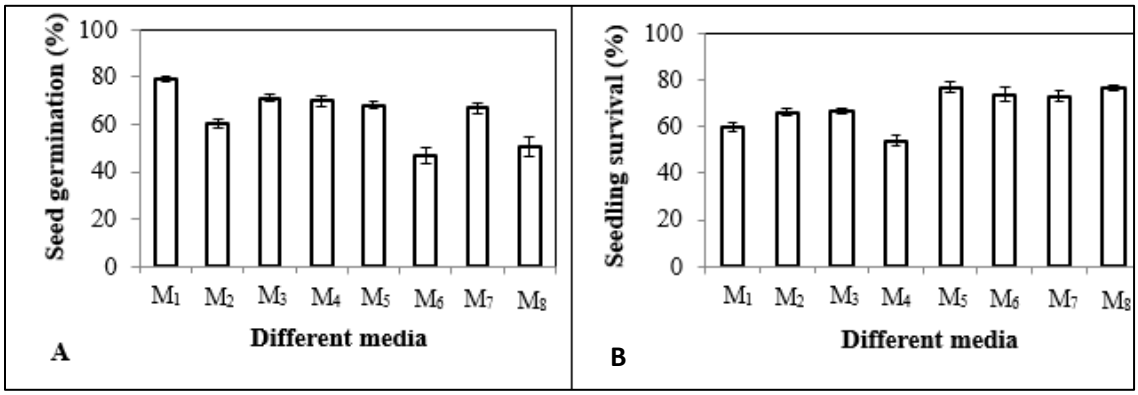

Figure 1: Changes in seed germination percentage (%) (A) and Seedling survival percentage (%) (B) of *E. alsinoides* seeds grown in eight different potting media

According to Figure 01, the results indicate that soil: sand (M1) is the best medium for germination of *E. alsinoides* seeds. Early seed germination was observed in the soil: sand (1:1) (M_1) nursery media at 5 days after seed sowing. Significantly ($P \leq 0.05$) higher seed germination (79%) was also observed in the same nursery media (Figure 1 A). Growing media with soil and sand has induced the germination and survival percentage of certain other medicinal plants. Seedlings of *Andrographis paniculata*, *Barleria prionitis*, and *Rhinacanthus polonnaruwensis* showed high growth performances in sand: garden soil potting media (Jayawardhane *et al.*, 2021). In

contrast to this, Dharmveer *et al*. (2016), suggests that optimum growing media for germination and growth of *Angelica glauca*, an endangered medicinal herb in India, was sand: soil: cocopeat: vermicompost (1:1:1:1). Kumar (2013) suggested that soil is the most superior media for seed germination purposes. But the seed germination results of different plant species may vary depending on the different textures of soil or combinations of soil and other components (Travlos, *et al*., 2007; Zaller, 2007; Celik, 2001). According to the present study, the higher seedling survival percentage (77%) was observed in the nursery mixture of soil: sand: compost (1:1:1)(M5) followed by soil: coir dust: compost (1:1:2)(M8) (76.6%) and soil: sand: compost (1:1:2)(M6) (73.8%) (Figure 1 B). According to Jayawardhane *et al* (2021), 14% of *R. polonnaruwensis* plants died when they were planted in sand: garden soil media while *A. paniculata* and *B. prionitis* showed a 60% and 10% mortality when they are grown in sand: compost medium (1:1). These findings emphasis the importance in selecting species specific media for seed germination.

Experiment 2: Selection of most promising growing media for *Evolvulus alsinoides* stem cuttings

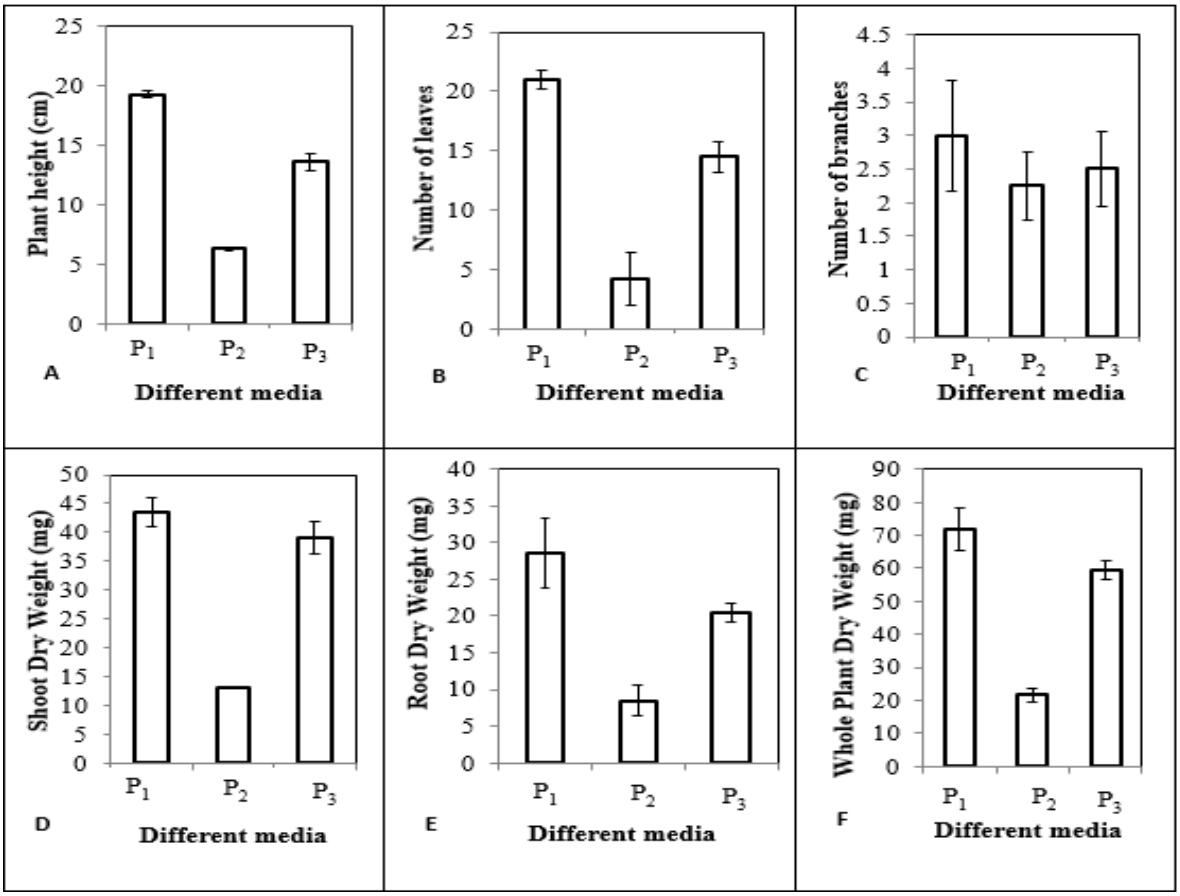

Figure 2: Effect of different growth media on plant height (cm) (A), number of leaves (B), number of branches (C), shoot dry weight (mg) (D), root dry weight (mg) (E) and whole plant dry weight (F) of *E. alsinoides*

Figure 02 indicates that sand media (P_1) performed well in terms of all the growth parameters considered in *E. alsinoides.* Therefore, sand media was selected for further analysis of any combination effect of cutting type and hormone type on above parameters of *E. alsinoides.* Akinyele, (2010) reports that sand media was too porous and did not retain enough moisture required by *Buchholzia coriacea Engler* cuttings. Therefore, they suggest that cuttings need a medium that is not too porous but still provides with good drainage avoiding water retention and facilitate proper rooting. However, the widespread use of sand as a medium for rooting of cuttings suggests that aeration and aseptic conditions are more crucial for the development of roots by cuttings than nutritious elements present in soil. Growth performances of *Ipomea pes-carpe* which is another medicinal plant in family convolvulaceae was better with soil: compost media (1:1) under proper shade (Shinde *et al*., 2018). Also Dickens *et al* (2009) reports that fine sand as the appropriate media for optimum rooting of *Irvingia wombolu* (bitter bush- mango) stem cuttings which is a valuable multipurpose tree in West and Central Africa.

Experiment 3: Selection of most promising hormone type and cutting type combination for *Evolvulus alsinoides* stem cuttings

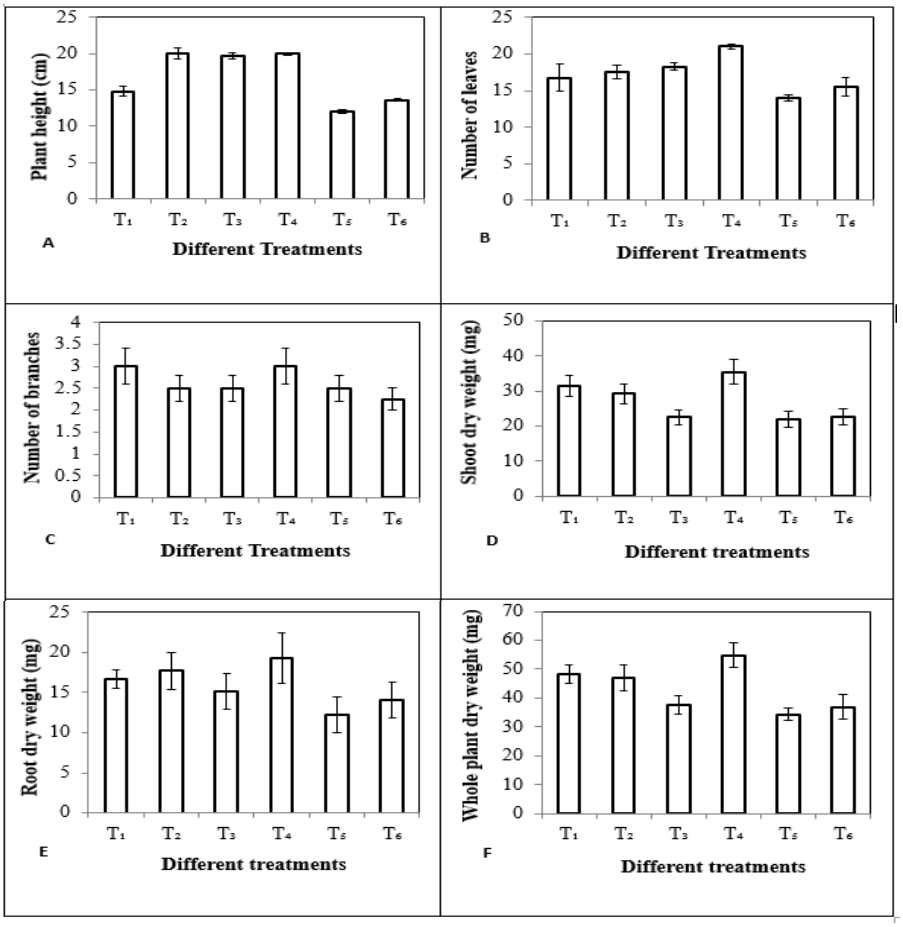

Figure 3: Effect of hormone and cutting type combination on plant height (cm) (A), number of leaves (B), number of branches (C), shoot dry weight (mg) (D), root dry weight (mg) (E) and whole plant dry weight (F) of *E. alsinoides*

According to figure 3, effect of auxins (IAA, IBA and IAA+IBA) with different cutting types in sand media on growth parameters of *E. alsinoides* showed differences. All the considered growth parameters of *E. alsinoides* were significantly higher ($p \leq 0.05$) in semi hardwood cuttings treated with IAA in sand media (T_4) except to the plant height. The plant height was significantly higher in top wood cuttings treated with IBA (T_2). However, the plant height of T_4 was not significantly different from T_2. Therefore, in this research, application of IAA positively affected the leaf development of *E. alsinoides* cuttings as IAA helped to establish a stronger root system that was very effective in nutrient absorption. According to Kristiansen *et al*. (2005), the establishment and growth of stem cuttings depend on factors like diameter and portion of the stem, moisture level, temperature and nutrient level of the growing media and on seasonal variations. There are numerous research findings which prove the effectiveness of different concentrations of IAA on root proliferation and number of roots in different species. According to Tien *et al* (2020) *Solanum procumbens* which is a precious medicinal plant has responded successfully to 500 ppm of IAA treatment in terms of maximum root number, root length, and root weight among the different IAA treatments and a remarkable inhibition of above parameters were observed when dosage is increased. In contrast to our findings, Majeed *et al* (2009) reports that, plant height of *Aesculus indica* cuttings were optimum with IBA with 2000ppm concentration. Concentrations of 500ppm IBA and 400ppm IAA gave maximum shoot length of *Rhinacanthus nasutus* which is a medicinally important shrub (Alagesaboopathi *et al*., 2018). In a study conducted to observe the effect of growth regulator application on field grown *Salvia sclarea L*, the maximum number of leaves were observed in application of 50 µL L-1 IAA (Singh *et al*., 2008). According to Tiwari *et al* (2015) better sprouting was observed in thick stem cuttings than thin cuttings in different medicinal plants. According to the current research, IBA either with semi hardwood cuttings or top wood cuttings has not performed well in comparatively to IAA. This finding align with a prior study done with *Cordiu alliodora* cuttings which did not show a significant difference in shoot dry weight even when the cuttings were subjected to different IBA concentrations during six weeks after planting (Mesen *et al.*, 1997). Most of those research findings suggest using high dosages of hormone concentrations in contrast to this research. According to previous studies, the sprouting and leaf initiation of the cuttings are due to the accumulation of carbohydrates of the cuttings with the application of auxins (Shahzad *et al.*, 2019). Findings of this study are consistent with previous research on the role of auxin in the sprouting and leaf growth of cuttings (Kontoh *et al*., 2016).

Figure 4: **A & B**- *E. alsinoides* mother plants maintained for planting material, **C**- Early seed germination in the soil: sand (1:1), **D**- Higher seedling survival percentage in soil: sand: compost (1:1:1) nursery mixture, **E**- *E. alsinoides* semi hardwood cuttings treated with mixture of IAA and IBA planted in coir dust: sand: compost (1:1:1), **F**- *E. alsinoides* softwood cuttings treated with IAA planted in sand media, **G**- *Evolvulus alsinoides* at 6 weeks after planting

CONCLUSIONS

It can be concluded that soil: sand (1:1) is the best media for *E. alsinoides* seed germination. Semi hardwood cuttings treated with 3000 ppm IAA in sand media would be a suitable protocols for mass scale propagation. Knowledge on seed germination and propagation of *E. alsinoides* could be utilized to support large-scale commercial production of this valuable medicinal plant, or even as ornamental plant in home gardens. This will also aid in the reduction of over exploitation of the crop.

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