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Studies on seed germination of *Stereospermum suaveolens* with respect to different parameters

Darshini R. Trivedi, Aruna G. Joshi*

The Maharaja Sayajirao University of Baroda, Faculty of Science, Department of Botany, Vadodara 390002, India

*Corresponding author, E-mail: arunajoshimsu@gmail.com

Abstract

Stereospermum suaveolens DC. is a valuable medicinal tree species, now placed in the threatened category due to its overexploitation. This species is usually propagated through seeds but has a very poor rate of germination. The right choice of planting substrates and pretreatments given to seeds are important factors influencing germination and growth of the seedlings. The present study was conducted to determine suitable planting substrate(s) and an effective pretreatment for germinating the seeds. The moisture content of one year old seeds was high (15 ± 1.3) and that of the current year (fresh) seeds were low (8.1 ± 0.3). Fresh seeds were pre-soaked overnight in water and then grown in various substrates like coco peat, soil, sand, coco peat / sand (1:1) and coco peat / soil (1:1), filter paper, Murashige and Skoog's medium (MS) and Woody Plant Medium. Results revealed that the optimum seed germination (68%) was recorded in coco peat substrate followed by MS medium (66 %) and lowest rate was in soil (6%). The other germination parameters studied were mean daily germination, which was same in coco peat as in MS medium (2.2), followed by filter paper (2.0). The germination rate was faster in coco peat (5.4) when compared to that on other substrates, while germination index was highest in MS medium (9.9). Coco peat was suitable in terms of germination. The effect of different pretreatments on germination were therefore studied for this substrate. Seeds soaked in distilled water showed maximum percent of germination compared to other pretreatments.

Key words: coco peat; germination; *Stereospermum suaveolens*; substrate.
Abbreviations: IAA, indole-3-acetic acid; MDG, mean daily germination; MS, Murashige and Skoog's medium.

Introduction

Many tree species are propagated by seeds, but reports on seed germination are limited due to insufficient research (Mng'omba et al. 2007). AOSA (1970) defines germination as the emergence and development from the seed of those essential structures which are indicative of the ability to produce a normal plant under favourable conditions. In the germination test rules, two germination counts are mentioned: the 'first day count' which is the day when approximately two thirds of the germinable seeds are expected to germinate and the 'final count' is the end of the test period (Bedell 1998). In tree species seed germination is difficult due to hard seed coats and dormant seed embryos (Jaiswal, Chaudhary 2005) and they often fail to germinate even under favourable moisture, oxygen and soil conditions (Urgenc, Cepel 2001). Moisture content of seeds is one of the factors influencing germination, as it is a test in retention of viability of seeds (McDonald, Copeland 1999) and it also depicts the seed quality and storagibilty of seeds. Pretreatments like mechanical scarification ie. soaking in cold or hot water, acid scarification with sulphuric or hydrochloric acid are known to overcome dormancy of seeds (Bedell 1998). Pre-soaking of seeds in growth regulator like gibberellic acid and indoleacetic acid (IAA) can also enhance seed germination percentage (Chauhan et al 2009). The type of planting substrate is another factor that influences germination and can be species-specific; therefore in nurseries importance is given to substrate for raising seedlings (Jaiswal, Chaudhary 2005). A suitable substrate is the one which contain numerous air spaces, provides adequate aeration (Thomson 1992), non toxic, free from moulds and microorganisms, cost effective and is easy to handle (Agrawal 1997).

Stereospermum suaveolens DC. is an important medicinal tree belonging to the family Bignoniaceae (Anonymous 1998). It contains lapachol and lapachonone, which act against dermatitis (Anonymous 1998). Its roots are used in preparation of the ayurvedic formulations Dasmoolarisht and Chywanprash (Yashoda et al. 2004). Destructive harvesting practices have seriously reduced seed production and caused gradual erosion of its natural populations. Now this species is placed under the threatened category. The species is mainly propagated through seeds and collecting them becomes a laborious process as their pericarps are winged. Another difficulty it faces is poor germination rate and thus propagation through seeds in the wild is limited (Baul 2006). Hence, steps have to be taken to conserve this tree of great economic value by finding suitable methods for its large scale propagation.

The present study was planned with an objective to find a suitable planting substrate and an effective pretreatment for germination of *S. suaveolens* seeds.

Materials and methods

Seed source and substrates for germination

The seeds of *Stereospermum suaveolens* DC. were collected from the Rajpipla Forest Division.

Different substrates used for seed germination were pure coco peat, soil, sand, a mixture of coco peat / sand (1:1) and coco peat / soil (1:1), a Petri dish containing filter paper (single layer), Murashige and Skoog's (MS) and Woody Plant Medium (WPM). Coco peat and its mixtures were filled in plastic root trainers (98 wells) while soil was filled in pots and flasks were used for the medium.

To prepare coco peat substrate, 150 g of cocopeat (dry mass) was soaked in distilled water (four times dry mass) overnight. Coco peat and a mixture of coco peat with soil / sand (1:1) were prepared and autoclaved at 121 °C and 103.4 kPa for 45 min.

MS and WPM (one litre) basal medium was prepared with all macronutrients, micronutrients and vitamins. The media and petridishes containing filter paper were sterilized by autoclaving at 121 °C and 103.4 kPa for 25 min.

Seed germination

The winged pericarp was removed from the seeds and then they were soaked for 24 h in distilled water before placing in the different substrates.

For coco peat-containing substrates, seeds were treated with 0.1% $HgCl_2$ for 2 min and inoculated singly in wells of the root trainer containing a specific type of the substrate. Bavistin (200 mg L^{-1}) was added to the substrate to avoid contamination and then the root trainers were housed in a culture room at 25 ± 2 °C. All the manipulations were carried out in a Laminar Air Flow Cabinet.

Garden soil was filled in pots and the seeds (five per pot) were placed in it. All the pots were housed in the M.S.University Botanical Garden.

Sand was filled in polyethylene bags and one seed per bag was placed in them. All the polybags were maintained in the Botanical Garden.

For germination of filter paper, seeds were treated with 0.1% $HgCl_2$ for 2 min, washed three times with sterile water, and then five seeds were placed in each Petri dish containing 5 to 10 mL of sterile water.

For germination in MS and WPM seeds were treated with 0.1% $HgCl_2$ for 2 min, washed three times with sterile water and five seeds were inoculated in each flask.

There were two replicates with 50 seeds in each. The substrates coco peat and its combination with sand and soil in root trainers were irrigated every alternate day and sprayed with Bavistin fungicide with five days interval to avoid fungal contamination. Sand and soil substrate kept outside were also watered every day.

Observations were made every day for development of seedlings for a period up to 30 days.

Germination parameters

The first count, as well as final count of germinating seedlings, was done according to germination test rules (AOSA 1981). The first count was made after one week (10 days) and final count by the end of the 4th week (30 days).

Moisture content (%) of seeds was determined on fresh mass basis after drying samples at 103 ± 2 °C for 17 h (ISTA 1993) by using the following formula:

$$\text{Moisture content} = FM - DM / FM \times 100,$$

where FM is fresh mass of seeds and DM is dry mass.

Final germination percentage (FG) and mean daily germination (MDG) were calculated as follows (Panwar, Bhardwaj 2005):

$$FG = n / N \times 100,$$

$$MDG = FG / D,$$

where n is the number of germinated seeds, N is the total number of seeds and D is the number of days to final germination.

The germination rate (GR) as well as germination index (GI) were calculated as follows (Panwar, Bhardwaj 2005):

$$GR = \sum_{i=1}^{n} n_i / t_i,$$

$$GI = \sum_{i=1}^{n} n_i t_i / N,$$

where n_i is the number of germinated seeds in day t_i.

Pretreatments before germinating in coco peat

For treatment with plant hormones, seeds were soaked for 24 h in gibberellic acid (GA_3; 15 µM) or indole-3-acetic acid (IAA; 5 µM) before germinating in coco peat substrate. Seeds were scarified with concentrated and diluted sulphuric acid (20%) for 10 min and 1 h, respectively, later washed thoroughly with water and then placed in the substrate. For hot water treatment, seeds were soaked in hot water (100 °C) for 15 min, then transferred to distilled water at room temperature and thereafter placed in substrate for germination.

Observations were recorded only for final germination in different treatments and data were statistically analyzed using ANOVA.

Results

Before seed germinaton experiments were carried out, moisture content of old and fresh seeds of *S. suaveolens* were determined. It was observed that old seeds retained high percent moisture (15 ± 1.3) whereas fresh seeds had low moisture content (8.1 ± 0.3).

Observations on seed germination experiments

revealed that of the different planting substrates that were used, coco peat gave higher percent germination (68%) of *S. suaveolens* seeds, followed by MS medium (66%), filter paper (60 %), least in soil (6%), and failure to germinate in WPM medium (Table 1). The maximum first count percent was recorded in coco peat, which indicates this substrate is quick and effective for seed germination, as compared to other substrates (Table 1).

Germination rate of seeds was highest in coco peat (5.4) as seed emergence occurred within 10 days and all seedlings developed by the end of third week. In other substrates the germination rate was poor and the growth of seedling was also slow, as it took more than 15 days for seed emergence and seedlings developed after a month (Fig. 1).

The mean daily germination (MDG) had the same value (2.2) for both coco peat and MS medium, followed by filter paper and sand. The other substrates, like soil, mixture of coco peat with sand and soil had lower MDG (Fig. 2).

The germination index was greater in MS medium (9.9) compared to coco peat (8.9), and lowest in soil (Fig. 3).

The results show that coco peat was the most suitable substrate for germination of *S.suaveolens* seeds; therefore, further study on effect of different pretreatments on seed germination were carried out only in this substrate. The test showed that pretreatments of seeds, excepting distilled water, were not effective and that they did not have any additional effect on germination. Soaking seeds in distilled water induced the highest germination when compared to other pretreatments, like dilute sulphuric acid, gibberellic acid, IAA and hot water, where percent germination was poor, and no germination was observed in concentrated sulphuric acid (Fig.4). ANOVA showed that the seed germination significantly differed among the six different pretreatments ($p < 0.05$).

Discussion

Moisture content of seeds was calculated before carrying out seed germination. In our studies on *S. suaveolens* the fresh seeds had a low moisture content, indicating high permeability of the seed coat which was lower in old seeds. An ideal planting substrate should be sufficiently porous, and be able to retain moisture, which plays an important role in seed germination (Richard et al. 1964) and growth of seedlings (Jaiswal, Chaudhary 2005). There are several reports on effect of substrates on germination on plants like *Jatropha curcas* (Gairola et al. 2011), *Gonystylus bancanus*

Table 1. Percentage of germination at first count and final count of seeds of *Stereospermum suaveolens* soaked in distilled water for 24 h and germinated in different substrates

Substrate	Temperature (°C)	First count (%)	Final count (%)
Sand	38	20	35
Soil	40	0	6
Coco peat	25	56	68
Coco peat / sand	25	8	11
Coco peat / soil	25	6	16
Filter paper	25	44	60
MS medium	25	23	66
WPM	25	0	0

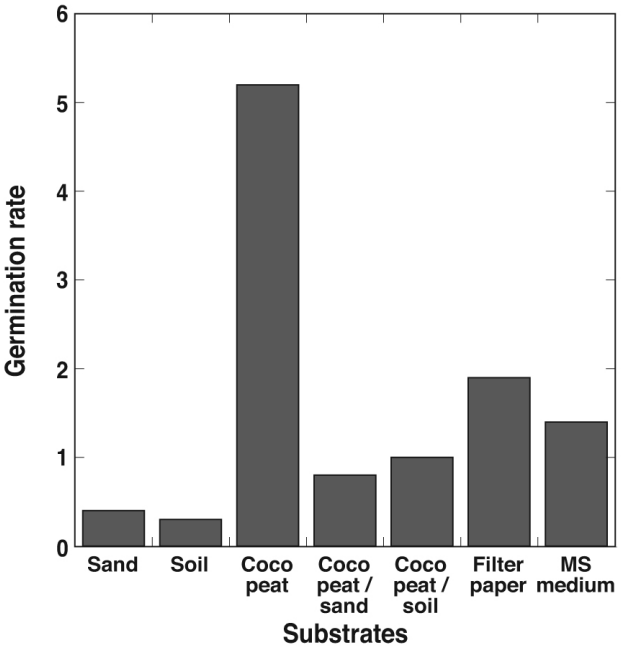

Fig. 1. Germination rate of *Stereospermum suaveolens* seeds in different planting substrates.

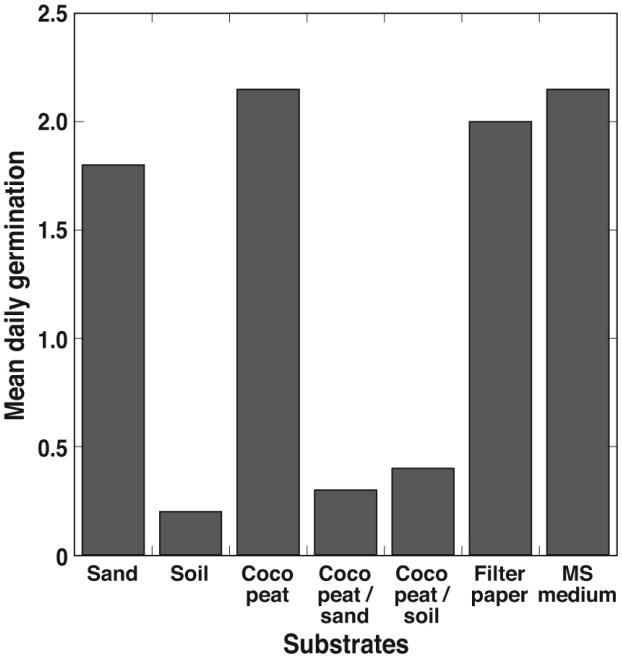

Fig. 2. Mean daily germination of *Stereospermum suaveolens* seeds in different planting substrates.

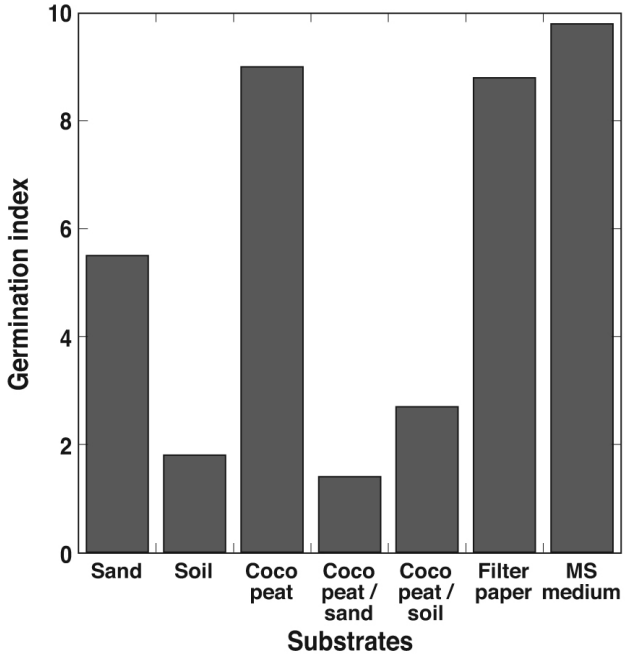

Fig. 3. Germination index of *Stereospermum suaveolens* seeds in different planting substrates.

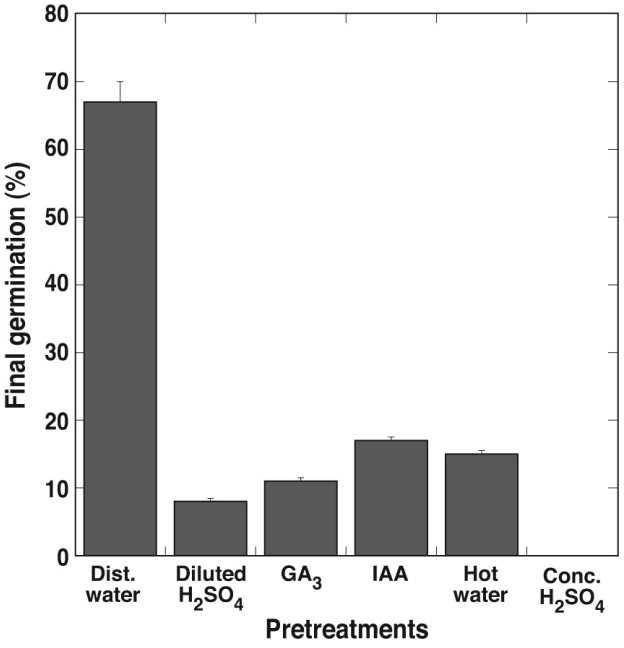

Fig. 4. Final germination of *Stereospermum suaveolens* seeds in coco peat with different pretreatments.

(Utami et al. 2006) and *Calendula officinalis* (Ming et al. 1999).

Coco peat is a commonly used substrate in horticultural practices for growing seedlings (Yau, Murphy 2000). This substrate is beneficial for germinating seeds, as it is 100% organic with high water holding capacity, good aeration, has nutrient absorption qualities and is non toxic. Improved growth of seedlings using coco peat has been reported in a number of species like *Pterocarpus macrocarpus* (Kijkar 1991), *Eucalyptus tereticornis* (Kumar, Marimuthu 1997) and Swietenia macrophylla (Woods et al. 1998). The present work also showed coco peat as a suitable substrate for germination, as a maximum number of seedlings can be generated within three weeks. Similar observations were recorded for *Gonystylus bancanus* (Utami et al 2006), and *Oroxylum indicum* (Trivedi, Joshi 2012) which had the highest percent of seed germination in coco peat and least in soil.

Filter paper can hold sufficient amount of water and dissipate water evenly all over the surface quickly and thus has effect on germination of seeds (Jaiswal, Chaudhary 2005). In our experiments a number of seeds germinated in petridish containing filter paper but the rate of germination was less and growth of seedlings was also uneven. Other substrates like sand and soil did not favour germination of seeds, as sand has high permeability and soil has a very high tendency of compression, resulting in poor rate of germination (Ming et al. 1999).

MS and WPM medium have different mineral salt concentrations which greatly affects *in vitro* germination. It has been successful for seeds of *Senna macranthera* (Faria et al. 2012), while MS medium in *Pterocarpus marsupium* (Mishra et al. 2013) and *Salvia sclarea* (Ghanbari et al. 2012). Different substrates are species specific. For *S. suaveolens* MS medium was equally effective but the rate of germination was slow, while WPM medium was ineffective.

Different pretreatments are also essential for effective germination and are species specific. Acid pretreatment is advantageous for seeds with a very hard seed coat (Hosseini et al. 2013). Soaking seeds in hot water significantly improved seed germination percentage in *Bobgannia malagascarionsis* (Thokozani et al. 2011). In contrast to this, acid and hot water treatment proved to be least effective in terms of germination in our experiments. Similar observations were recorded for *Flacourtia indica* and *Parinari curatellifolia* seeds, where different pretreatments did not enhance the percent germination (Prins, Maghembe 1994). Acid and mechanical treatments have failed in germinating seeds of *Arceuthobium abietinum* (Scharpf 1970). Treatment of *Prunus mahaleb* seeds with gibberellic acid has been reported to overcome dormancy and ensure uniform germination (Cetinbas, Koyunko 2006; Al-Absi 2010). Seeds of horse gram and black gram when treated with IAA and gibberellic acid showed enhanced percent germination (Chauhan et al. 2009), whereas *S. suaveolens* seeds did not respond to these hormones.

The studied species had maximum seed germination when soaked in distilled water, as similar for seeds of Strychnous spinosa (Prins, Maghembe 1994). Soaking in water prior to sowing is also known to enhance germination percent and rate in species like *Cedrus deodara, Hardwickia binata, Pongamia pinnata, Cinnamomum camphora, Melia*

azadarach, Terminalia chebula and *Terminalia tomentosa* (Bedell 1998).

Conclusions

In order to generate a large number of seedlings of *S. suaveolens* in a short period, studies on seed germination in different substrates along with pre-treatment's were carried out. From our studies it can be concluded that coco peat was effective for germination as the rate of germination was faster in comparison to filter paper and MS medium. The seeds were simply soaked in distilled water without any special pretreatments before placing in the substrate. A large number of seeds germinated into healthy seedlings in this substrate, more than in the other substrates tested and hence coco peat was suitable for germinating seeds of this species.

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