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Influence of growing media and seed treatment on seed germination and seedling vigour of Sarpagandha (*Rauvolfia serpentina* (L), Benth. ex Kurz)

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Abstract
A nursery experiment was carried out to study the effect of growing media, seed treatments on seed germination and seedling vigour of Sarpagandha. The experiment was conducted in a Factorial Completely Randomized Design with 18 treatment combinations and 3 replications. The treatment combinations consists of 2 types of growing media (M_1: Garden soil + FYM + Sand @2:1:1 and M_2: Coco peat + Vermiculite + Perlite @2:1:1) and 9 seed treatments (C_1: GA_3 50 ppm, C_2: GA_3 100 ppm, C_3: GA_3 150 ppm, C_4: NaCl 1%, C_5: NaCl 2%, C_6: Acid scarification by conc. sulphuric acid, C_7:Hot water treatment, C_8: Pre soaking in tap water, C_9: Control(without treatment). The results revealed that the seed germination and seedling vigour of Sarpagandha was significantly influenced by different growing media and seed treatments. Sarpagandha seeds, treated with GA_3 @ 150 ppm and sown in garden soil + FYM + sand @2:1:1 resulted in minimum days taken for initiation of germination (13.33), minimum days taken for completion of germination (53.00), maximum germination percentage (39.00%), maximum speed of germination (1.88) and maximum vigour index (1,153.60).

Keywords: Media, seed treatments, germination, seedling vigour, Sarpagandha

Introduction
Sarpagandha (*Rauvolfia serpentina* (L), Benth. ex kurz) belongs to the family-Apocynaceae is one of the most important native medicinal plants of India. There are approximately 85 species in the genus *Rauvolfia* found in tropical regions. Apart from *R. serpentina* there is another species, *R. tetraphylla* which is also cultivated on a commercial scale. Sarpagandha is an erect, evergreen perennial and under shrub. Fruits are drupe, single or generally didymous,7.5 mm in size, purple bluish to black in colour when ripe containing 1-2 stony seeds. The roots of plants are the principal source of alkaloids mainly used for medicinal purposes. The root of Sarpagandha has been used for the treatment of hypertension and as a sedative or tranquillizing agent, snake-bite, insect stings, nervous disorders, mania and epilepsy, intractable skin disorders such as psoriasis, excessive sweating and itching, gynecological ointments for menopause, toxic goiter and to promote uterine contraction in childbirth.

The Sarpagandha has enormous importance in the health care system. But after reports of its therapeutic properties, natural reserves of Sarpagandha have been declining due to over exploitation by the local and tribal people. This has led to listing of this species as "Endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) (Jain *et al*, 2003) [15]. In India, Government of India has prohibited the collection of plants growing in wild in forests and its export since 1969. For the fulfilment of the present and future demand, this plant needs to be cultivated scientifically at a commercial scale. Availability of good quality planting material is essential for commercial cultivation of Sarpagandha. Commercially, Sarpagandha is propagated by seeds. Irregular and low percentage of germination is the main obstacle in the seed propagation of Sarpagandha. The percentage of germination of seeds is quite variable, ranging from 10-60 per cent (Farooqui and Sreeramu, 2001) [12]. This is partly attributed to the adverse influence of the stony endocarp. Another serious factor is the absence of embryo, may be due to parthenocarpy or sterility. Irregular germination coupled with long germination period is also a major setback in seed propagation of Sarpagandha. To overcome the inhibitory effect of hard stony endocarp on dormancy, facilitate better germination and obtain higher quantity of quality planting

materials, a nursery experiment has been conducted to study the effect of growing media and seed treatments on seed germination and seedling vigour of Sarpagandha.

Materials and Methods

The experiment was carried out during 2017 and 2018 at All India Co-ordinated Research Project on Medicinal & Aromatic plants and Betelvine, Horticulture Research Station (HRS), Odisha University of Agriculture and Technology, Bhubaneswar. The average annual rainfall of Bhubaneswar is 1552 mm (based on average of preceding 10 years). Most of the rainfall i.e. 85% is received from July to September. The average temperature varies from 14 ^{0}C in winter to 40 ^{0}C in summer and relative humidity varies between 49 or 90% from June to December. The experiment was laid out in a Factorial Completely Randomized Design with 18 treatment combinations and 3 replications. The treatment combinations consists of 2 types of growing media (M_1: Garden soil + FYM + Sand @2:1:1 and M_2: Coco peat + Vermiculite + Perlite @2:1:1) and 9 seed treatments (C_1: GA_3 50 ppm, C_2: GA_3 100 ppm, C_3: GA_3 150 ppm, C_4: NaCl 1%, C_5: NaCl 2%, C_6: Acid scarification by conc. sulphuric acid, C_7:Hot water treatment, C_8: Pre soaking in tap water, C_9: Control(without treatment).

The ripened fruits were collected from mother block of AICRP on MAP and Betelvine, OUAT, Bhubaneswar. The fruits were pulped manually to extract the seeds. The extracted seeds were washed 2-3 times in clean water. The cleaned seeds are thoroughly dried and subjected to floating test by immersing in water. The heavy seeds which sink in water were selected for the experiment. Two types of growing media such as garden soil + farm yard manure + sand @ 2:1:1 and coco peat + vermiculite + perlite @ 2:1:1 were prepared by mixing the individual components on volume basis as per the requirement. The mixture of growing media was filled with protray having 100 cavities. The protray filled with above growing media were kept inside the naturally ventilated poly house.

The seeds after treatment with different plant bio-regulators and chemicals were sown in protrays during May, 2017 and 2018 as per different treatment schedule. One portray has been used in each treatment accommodating 100 seed. Regular watering was done as per the requirement. The prophylactic plant protection measures and weeding was taken during the course of investigation. Then, 20 seedlings of uniform growth were transplanted in the polythene bags of size 6"x4" filled with the aforementioned growing media after 50 days of sowing under each treatment in order to study the growth performance. The observation on germination was recorded from day of initiation up to 60 days of sowing. The germination %, speed of germination and vigour index were calculated by the following formula.

$$\text{Germination \%} = \frac{\text{No. of seedlings germinated}}{\text{Total numbers of seed sown}} \times 100$$

$$\text{Speed of germination index (SGI)} = \frac{\text{\% of germination}}{\text{Days to first count}} + \cdots + \frac{\text{\% of germination}}{\text{Days to final count}}$$

Vigour index = Standard Germination percentage x (Shoot length + Root length)

The data recorded on various characteristics of seed germination and seedling growth were subjected to Fisher's method of analysis of variance and interpretation of data was taken up as per Sukhatme and Amble (1995) [23].

Result and Discussion

Seed germination

Seed propagation in Sarpagandha is commercially accepted by the farmers because of higher root yield with thick tap roots. But, seed dormancy and lack of viable embryo are the major obstacles in seed propagation of Sarpagandha. Dormancy is an endogenously controlled but environmentally imposed temporary suspension of growth independent of ambient environmental conditions. In Sarpagandha, seed dormancy may be imposed by hard seed coat and presence of high ABA level. There are several instances where different kinds of chemicals and growth regulators were applied exogenously to overcome these obstacles. In light of the available information, different treatments were tried to obtain improved seed germination.

The results presented in Table 1 and 2 revealed that germination was significantly influenced by the different seed treatments. All the chemicals and other treatments tried were proved to be superior to the untreated seeds. Among different treatments, seeds treated with GA_3 @ 150 ppm recorded earliest germination (14.83 days), whereas seeds sown without any treatment (control) taken maximum days for initiation of germination (18.50). The seed treatment with GA_3@ 150 ppm resulted in 19.83% reduction in number of days taken to first germination over control. There was a significant difference among treatments for days to complete germination. The minimum days to complete germination was observed in seeds treated with GA_3 @ 150 ppm as compared to rest of the treatments. The earlier germination in GA_3 @ 150 ppm treatment might be attributed to the conversion of reserve food material into available simple sugar form by early induction of protein, α-amylase, in aleurone layer by forming m-RNA for protein (α-amylase) synthesis and inhibitory action of exogenously applied gibberellins on ABA present in seeds leading to early germination (Beweley and Black, 1994) [4]. The earliness in germination due to seed treatment of GA_3 has been reported by Phatak *et al.* (2017) [20] in Sarpagandha.

The germination % was recorded from 15 DAS to 60 DAS and the maximum germination % was observed with GA_3 @ 150 ppm (38.42%) and the minimum (25.50%) in control (without any treatment) at 60 DAS. The seed treated with GA_3 @ 150 ppm resulted 33.62% higher germination over control. The highest % of germination observed in GA_3 @ 150 ppm treatment might be due to efficient utilization of limited food reserve present in the seeds by early induction of α-amylase activity. Chetouani *et al.* (2017) [10] observed that *Thymus satureioides*. L seeds treated with 50 ppm GA_3 showed an increase of 27% germination compared to the control (10%). *Lavendula dentate* seeds treated with gibberellic acid at 1000 ppm showed maximum germination of 67% as compared to the control which did not exceed 1 percent. The present finding is in agreement with the results obtained by Bhuyar *et al*. (2000) [13], Ponkumar *et al.* (2008) [21], Hussain and Jha (2014) [14], Anonymous (2017) [1] and Phatak *et al.* (2017) [20] in Sarpagandha. The similar results were reported by Bhujbal (1975) [6], Dhankhar and Kumar (1996) [11] and Golap *et al*. (2000) [13] in Aonla, Bhuse *et al.* (2001) in Senna and Mithra and Ghosh (2004) [16] in Ashwgandha. However, Paul, *et al.* (2008) [19] reported that none of the chemical or acid seed treatments improved germination % significantly in Sarpagandha.

The high speed of germination is indication of high seed vigour. In the present study, the speed of germination index recorded was also significantly influenced by different seed

treatments. Among all the treatments, the maximum speed of germination index was recorded with GA_3 @ 150 ppm (1.77) and the minimum (1.02) was noticed in control (without any treatment). The improved speed of germination in GA_3 @ 150 ppm might be due to increased GA: ABA ratio in the seeds by exogenous application of GA_3 which could have overcome the inhibitory effect of ABA present in seeds and inhibition of mRNA synthesis which might have been accelerated be gibberellins (Beweley and Black, 1994) [4]. The enhancement of speed of germination due to seed treatment of GA_3 has been reported by Phatak *et al.* (2017) [20] in Sarpagandha. Aoyma *et al.* (1996) reported that soaking the seeds of *Lavandula angustifolia* in GA_3 at 200 ppm improved the germination percentage and also accelerated the speed of germination. Shetty *et al*. (2016) reported that seed treatment with GA_3 400 ppm followed by GA_3 300 ppm has recorded maximum value in germination and seedling growth parameters in *Celastrus paniculatus* Wild, a threatened medicinal plant.

The results of the studies revealed that germination was significantly influenced by the different growing media. The Sarpagandha seeds sown in garden soil + FYM + sand (2:1:1) recorded minimum days for initiation and completion of germination (15.44 and 62.89) which was lower than coco peat + vermiculite + perlite at 2:1:1 (17.93 and 62.89), respectively. The maximum germination was recorded with seeds sown in garden soil + FYM + sand @ 2:1:1 (34.52%) and the minimum (30.90%) was noticed with coco peat + vermiculite + perlite (2:1:1). The speed of germination index was also recorded with seeds sown in garden soil + FYM + sand @ 2:1:1 (1.59) and the minimum (1.30) was noticed with coco peat + vermiculite + perlite (2:1:1).

The interaction effect of growing media and seed treatments revealed that significantly minimum number of days for initiation of germination (13.33) was observed in the seeds treated with GA_3 @ 150 ppm and sown in garden soil + FYM + sand (2:1:1), whereas the maximum days of germination (19.67) was noticed with seeds sown in coco peat + vermiculite + perlite (2:1:1) without any treatment. The maximum germination (39.00%) was observed when seeds treated with GA_3 @ 150 ppm and sown in garden soil + FYM + sand (2:1:1) and the minimum (21.67%) was noticed in seeds sown in coco peat + vermiculite + perlite (2:1:1) without any treatment. The minimum days taken for initiation and completion of germination with higher germination of Sarpagandha seeds sown in garden soil + FYM + sand @ 2:1:1 reflected the fact that these combination might have provided favourable physical conditions needed for activating enzymatic and biochemical processes. The endogenous GA_3 present in the embryo might be at low concentration and therefore exogenous application of GA_3 through soaking of seeds in combination with garden soil + FYM + sand @ 2:1:1 might have enhanced the process of germination and given early and higher germination. Warakagoda and Subasinghe (2015) [25] reported that dipping the seeds in 2250 mg/1 GA_3 solution for 24 hours reduce the time taken for germination by removing inhibitory chemicals, facilitating embryo growth and reducing inherent ABA/GA_3 ratio. The present finding agrees well to the results obtained by Bharti *et al*. (2009) [5], Bisla *et al*., (1984) [9] and Awasthi *et al.* (1996) [3] who reported higher and early germination in Aonla, Ber and Peach, respectively.

Seedling growth and vigour

The results showed that the treatment of seeds with GA_3 150 ppm has recorded maximum growth in respect of shoot length (17.08 cm) and vigour index (1100.52) among all the treatments (Table 2). The minimum value in respect of the above parameters was recorded in control (seeds without treatment). GA_3 at 150 ppm played a major role in plant growth. The external application of GA_3 at higher concentration might have boosted the growth by increasing cell multiplication and cell enlargement ultimately resulting into higher plant growth. The rapid and early germination might have also resulted in giving more periods for vegetative growth of plants. The seed germinated earlier might have produced vigorous growth during later period. The increase in shoot and root length by pre sowing treatment of GA_3 is due to uniform germination, intensify hydrolytic process, better uptake of nutrients and moisture. The beneficial effect of GA_3 on vegetative growth of seedling has been reported by Ponkumar *et al.* (2008) [21] in Sarpagandha. The reports of Bhujbal (1975) [6] and Gholap *et al*. (2000) [13] as regards the seedling height, root growth and number of leaves in Aonla confirm the above findings. Palaniswamy and Ramamoorthy (1987) [17] in Papaya and Yelure (1992) [26] in Custard apple reported increase in growth of seedlings due to application of GA_3 solution. Prakash *et al*. (2017) [22] reported higher seedling vigour by application of GA_3 in Spinach. Wagh *et al*. (1998) [24] reported that seed treatment with GA_3 400 ppm solution prior to sowing was found helpful for increasing root growth in Aonla.

The results indicated that seedling shoot length, root length and vigour were found significantly maximum in growing media garden soil + FYM + sand @ 2:1:1 as compared to coco peat + vermiculite + perlite (2:1:1). This might be due to the favourable effect of proper combination of media having suitable pH, nutritional status and physical environment facilitate better growth and survival of Sarpagandha seedlings. Parasana *et al*. (2013) [18] reported that the growing media soil + sand + FYM (2: 1: 1) was found to be the most effective for better growth of mango seedling. Lopes *et al.* (2007) reported that the rooting media soil + sand (1:1) has the best one for higher dry weight of roots and dry weight of shoots compared to other treatments for all the observations. The growth and vigour index of Sarpagandha were not influenced by the interaction effect of media and seed treatments.

Table 1: Effect of growing media and seed treatment on seed germination of Sarpagandha

Treatment	Days taken for initiation of germination	Germination percentage (%)			
		15 DAS	30 DAS	45 DAS	60 DAS
Growing media					
M_1	15.44	0.21(1.50)	12.22(19.97)	26.67(30.79)	34.52(35.94)
M_2	17.93	0.49(3.09)	9.07(16.95)	21.40(27.37)	30.90(33.69)
SE(m)±	0.064	0.334	0.932	0.814	0.271
C.D. at 5%	0.182	0.962	2.684	2.344	0.781
Seed treatment					
C_1	16.17	0.33(2.31)	12.75(20.22)	24.42(29.40)	33.67(35.43)

C_2	15.50	0.50(3.26)	13.25(21.09)	28.75(32.40)	37.50(37.74)
C_3	14.83	1.00(5.21)	15.42(22.81)	29.00(32.17)	38.42(38.28)
C_4	16.83	0.33(2.70)	10.50(18.61)	25.00(29.90)	32.58(34.87)
C_5	16.33	0.61(4.49)	11.75(19.56)	28.08(31.75)	36.17(36.93)
C_6	17.33	0.17(1.35)	8.42(16.65)	21.50(27.28)	30.83(33.66)
C_7	17.00	0.17(1.35)	10.25(17.77)	23.83(29.06)	30.67(33.58)
C_8	17.67	0.00(0.00)	7.00(15.09)	18.92(25.75)	29.08(32.59)
C_9	18.50	0.00(0.00)	6.50(14.37)	16.83(24.01)	25.50(30.24)
SE(m)±	0.135	0.709	1.977	1.726	0.180
C.D. at 5%	0.387	2.041	NS	NS	0.520
Interaction (Growing media × Seed treatment)					
M_1C_1	15.33	0.17(1.35)	14.67(22.46)	29.33(32.69)	36.17(36.95)
M_1C_2	14.33	0.17(1.35)	15.33(22.79)	30.67(33.61)	37.50(37.74)
M_1C_3	13.33	0.67(3.83)	16.67(24.01)	28.00(31.17)	39.00(38.63)
M_1C_4	15.67	0.33(2.70)	12.50(20.42)	28.83(32.43)	35.17(36.35)
M_1C_5	15.00	0.57(4.30)	14.50(21.82)	29.17(32.22)	35.67(36.62)
M_1C_6	16.00	0.00(0.00)	9.33(17.54)	27.33(31.29)	34.50(35.94)
M_1C_7	15.67	0.00(0.00)	12.17(19.66)	28.50(32.19)	31.50(34.11)
M_1C_8	16.33	0.00(0.00)	7.83(16.24)	20.00(26.54)	31.83(34.33)
M_1C_9	17.33	0.00(0.00)	7.00(14.81)	18.17(24.98)	29.33(32.77)
M_2C_1	17.00	0.50(3.26)	10.83(17.97)	19.50(26.10)	31.17(33.92)
M_2C_2	16.67	0.83(5.18)	11.17(19.40)	26.83(31.19)	37.50(37.74)
M_2C_3	16.33	1.33(6.60)	14.17(21.60)	30.00(33.17)	37.83(37.94)
M_2C_4	18.00	0.33(2.70)	8.50(16.80)	21.17(27.38)	30.00(33.40)
M_2C_5	17.67	0.67(4.68)	9.00(17.31)	27.00(31.28)	36.67(37.24)
M_2C_6	18.67	0.33(2.70)	7.50(15.76)	15.67(23.27)	27.17(31.38)
M_2C_7	18.33	0.33(2.70)	8.33(15.89)	19.17(25.92)	29.83(33.06)
M_2C_8	19.00	0.00(0.00)	6.17(13.93)	17.83(24.97)	26.33(30.85)
M_2C_9	19.67	0.00(0.00)	6.00(13.93)	15.50(23.04)	21.67(27.71)
SE(m)±	0.191	1.002	2.796	2.441	0.298
C.D. at 5%	0.547	NS	NS	NS	0.863

(M_1:Garden soil+FYM+Sand, M_2:Cocopeat+Vermiculite+Perlite, C_1: GA_3 @ 50 ppm, C_2: GA_3 @ 100 ppm, C_3: GA_3 @ 150 ppm, C_4: NaCl @ 1%, C_5: NaCl @ 2%, C_6: Sulphuric acid scarification, C_7: Hot water treatment, C_8: Pre-soaking in tap water, C_9: Control (without treatment)

Table 2: Effect of growing media and seed treatment on germination and seedling vigour of Sarpagandha

Treatment	Speed of germination	Days taken for completion of germination	Shoot length(cm)	Root length (cm)	Vigour index
Growing media					
M_1	1.59	62.89	16.76	11.32	972.17
M_2	1.3	66.11	15.69	9.19	776.4
SE(m)±	0.040	0.324	0.085	0.245	27.228
C.D. at 5%	0.115	0.933	0.243	0.707	78.082
Seed treatment					
C_1	1.55	61.50	16.35	10.58	911.62
C_2	1.74	60.50	16.68	10.96	1,035.60
C_3	1.77	55.50	17.08	11.47	1,100.52
C_4	1.47	63.50	16.23	10.27	865.5
C_5	1.66	62.50	16.36	10.38	963.65
C_6	1.28	67.50	15.95	9.95	802.63
C_7	1.39	67.50	16.12	9.97	800.88
C_8	1.14	70.50	15.80	9.58	746.03
C_9	1.02	71.50	15.47	9.15	642.12
SE(m)±	0.085	0.687	0.180	0.521	57.759
C.D. at 5%	0.244	1.979	0.516	NS	165.636
Interaction (Growing media × Seed treatment)					
M_1C_1	1.76	60.00	17.03	11.67	1,038.83
M_1C_2	1.83	59.00	17.17	11.97	1,091.03
M_1C_3	1.88	53.00	17.30	12.27	1,153.60
M_1C_4	1.67	62.00	16.83	11.40	989.2
M_1C_5	1.76	61.00	16.93	11.50	1,007.90
M_1C_6	1.49	67.00	16.57	10.90	944.83
M_1C_7	1.56	65.00	16.77	11.00	875.35
M_1C_8	1.24	69.00	16.37	10.70	865.03
M_1C_9	1.13	70.00	15.90	10.50	783.72
M_2C_1	1.35	63.00	15.67	9.50	784.4
M_2C_2	1.65	62.00	16.20	9.95	980.17
M_2C_3	1.67	58.00	16.87	10.67	1,047.43

M_2C_4	1.28	65.00	15.63	9.13	741.8
M_2C_5	1.56	64.00	15.79	9.27	919.4
M_2C_6	1.07	68.00	15.33	9.00	660.43
M_2C_7	1.22	70.00	15.47	8.93	726.42
M_2C_8	1.04	72.00	15.23	8.47	627.03
M_2C_9	0.9	73.00	15.03	7.80	500.53
SE(m)±	0.120	0.972	0.254	0.736	81.683
C.D. at 5%	NS	NS	NS	NS	NS

(M_1:Garden soil + FYM + Sand, M_2:Cocopeat+Vermiculite+Perlite, C_1: GA_3 @ 50 ppm, C_2: GA_3 @ 100 ppm, C_3: GA_3 @ 150 ppm, C_4: NaCl @ 1%, C_5: NaCl @ 2%, C_6: Sulphuric acid scarification, C_7: Hot water treatment, C_8: Pre-soaking in tap water, C_9: Control (without treatment)

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