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Studies on Effect of Different Propagating Media and Plant Growth Promoting Rhizobacteria on Seed Germination and Seedling Vigour of *Rauvolfia tetraphylla* Linn.

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ABSTRACT

The experiment entitled "Effect of different propagating media and PGPRs on seed germination and seedling vigour of Rauvolfia tetraphylla" was conducted at College of Horticulture, UHS campus, Bengaluru during August 2015 to February 2016, with eight treatments in CRD design replicated for four times. The results showed that germination per cent (16.5), germination rate(0.29), seedling height (17.37 cm), girth (0.23 cm). seedling vigor (412.27), root length (6.5 cm) and total biomass were significantly higher in the plants raised in media having Red soil + sand + vermicompost + Rhizobium + PSB + Pseudomonas fluroscens and least was found in Red soil + sand + FYM. Hence, from the above experiment it is inferred that the plants raised in media having Red soil + sand + vermicompost + Rhizobium + PSB + Pseudomonas fluroscens were better compared to all other propogating media combinations.

Key words: *Rauvolfia tetraphylla, Seedling vigor, Vermicompost, Rhizobium, PSB, Pseudomonas*

INTRODUCTION

Rauvolfia tetraphylla Linn. is an evergreen woody shrub or a small tree, belongs to the family Apocyanaceae. It is introduced from South America, which has been used in Indian System of Medicine since long back. It is a small branched, woody shrub grows upto 1.2 m height, leaves are simple, 7.5 -10 cm long and 3.5 -5 cm broad, root is prominent, tuberous, usually branched, 0.5 to 2.6 cm diameter, up to 40 to 60 cm deep into soil. The root bark, which constitutes 40-60 per cent of the whole root, rich in alkaloids. The roots are frequently used as a substitute of *Rauvolfia serpentina* for medicinal purposes. The plant is a good source of reserpine. The roots also yield the alkaloid deserpidine, which is an antihypertensive and tranquilizer. Roots are sedative, tonic and febrifuge, valuable remedy in high blood pressure, used for treatment of insomnia, madness, painful affections of the bowels, hypochondria and irritative conditions of the central nervous system.

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Seed germination in *Rauvolfia* is highly variable from 5 to 30 per cent even when only heavy seeds are chosen for sowing. Thus, seed germination and growth of seedlings is a major problem. Propagation media is a basic need and being a store house of water, air and mineral supply, ensuring easy germination of seed and their further growth. Hence, proper selection of media is one of the important criteria for obtaining better germination, vigorous seedlings[9] .The plant growth promoting rhizobacteria plays an important role in increasing metabolic activity in germinating seeds and protecting seeds and seedlings by various mechanisms such anti-biotics production and siderophores production thus, resulting in early and higher rate of germination. Further, these rhizobacteria stays in association with root, fix atmospheric nitrogen, increases the availability and uptake of phosphorus and other nutrients in addition to production of plant growth regulators [16.39] Hence by keeping this point in view the following objective is proposed. The increasing demand of *Rauvolfia* in national and international markets and decreasing availability has encouraged many innovative growers to cultivate this useful herb. But, poor germination leads to paucity of planting material and increased cost of propagules.

MATERIAL AND METHODS

To study the effect of media and PGPR on seed germination and seedling vigour in sarpagandha

Source of seeds, media and plant growth promoting rhizobacteria used

The fully matured blackish red coloured fruits of *Rauvolfia tetraphylla* were collected from medicinal garden, College of Horticulture, Bengaluru. The seeds were extracted from fruits, shade dried for two days and used for the experiment. The Vermicompost, Rhizobium, VAM, *Pseudomomas fluroscens* and PSB were procured from University of Agricultural Sciences, Bengaluru. Whereas, Red earth, Sand, Cocopeat and Farm Yard Manure were obtained from the college itself. The coir pith was purchased from Prashanth Coir Products (private firm.

Inoculation of PGPR and sowing of seeds

The PGPR inoculum was applied at the rate of 5 g per protray having media at 1 cm depth before seeds are sown and Rhizobium was pre treated with seeds and shade dried for 12 hours before sowing. The seeds were sown in the portrays at the rate of one seed per. The observation on seedling growth were recorded at 30 days after transplanting.

Design and layout of experiment and treatment details

Number of treatments: 8; Number of replication: 4; Design: CRD (Completely randomized design).

T_1 Red soil + Sand + FYM (1:1:1)
T_2 Red soil + Sand + Vermicompost (1:1:1)
T_3 Red soil + Sand + Coco peat (1:1:1)
T_4 Red soil + Sand + Coir pith (1:1:1)
T_5 Red soil + Sand + FYM (1:1:1) + Rhizobium + PSB + Pseudomonas fluroscens
T_6 Red soil + Sand + Vermicompost (1:1:1) + Rhizobium + PSB + Pseudomonas fluroscens
T_7 Red soil + Sand + Coco peat (1:1:1) + Rhizobium + PSB + Pseudomonas fluroscens
T_8 Red soil + Sand + Coir pith (1:1:1)+ Rhizobium + PSB + Pseudomonas fluroscens

Observations recorded

Germination per cent

Germination per cent was recorded by counting the germinated seeds till germination process completed (upto 15 days of sowing) and germination per cent was calculated by using the following formula

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Rate of germination

Rate of germination was calculated as described by the Association of Official Seed Analyst (1983) which is as follows:

$$\text{Germination rate} = \frac{\text{Number of germinated seed} + \ldots + \text{Number of germinated seed}}{\text{Days of first count} + \ldots + \text{Days of final count}}$$

Seedling height (cm)

The randomly selected five plants were labelled and height was measured from the collar region to the base of the last fully opened leaf on the main stem at 30 days after transplanting. The mean value was computed and expressed in centimeter (cm).

Seedling vigour

The seedling vigour was calculated as per the formula given by Abdul Baki and Anderson[2].

Seedling Vigour = Germination (%) × Mean seedling length (cm)

Seedling girth (cm)

The stem girth was measured with the help of Vernier Calipers at marked point above 1 cm of the media at 30 days after transplanting from tagged five plants. The average value was compared and expressed in centimeter (cm)

Number of leaves per seedlings

Number of fully opened leaves produced in five tagged seedlings were counted at 30 days after transplanting and the average was computed and expressed in number.

Leaf area (cm^2)

All the leaves are collected from labeled plants at 30 days after transplanting. Leaf area was measured by using leaf area meter. The mean value was computed and expressed in square centimeter (cm^2).

Fresh weight of shoot (g)

The tagged plants were uprooted after 30 days of transplanting, shoots are separated and washed with water to remove adhering media. The fresh weight of shoot of tagged plants were weighed by using electronic weighing balance the mean value was computed and expressed in grams (g).

Fresh weight of root (g)

The fresh weight of roots of tagged plants were weighed after washing with water by using electronic weighing balance the mean value was computed and expressed in grams (g).

Root volume (cc)

Volume of the root is measured by using water displacement technique and expressed in cubic centimeter.

Root length (cm)

The root length was measured from the collar region of the plant to the tip of the longest root and expressed in centimeter.

Dry weight of shoot (g)

The fresh shoots were kept in hot air oven at 50º C for 48 hours till constant weight is obtained. The weight of dried shoot was weighed by using electronic weighing balance and expressed in grams (g).

Root dry weight (g)

The fresh roots were kept in hot air oven at 50° C for 48 hours till constant weight is obtained. The weight of dried roots was taken by using Electronic weighing balance and expressed in grams.

Total biomass (g)

The total weight of shoot and root was computed and mean value was expressed as Total biomass in grams.

Statistical analysis

The experimental data collected were subjected to Fisher's method of analysis of variance (ANOVA) as per methods outlined by Panse and Sukhatme (1967). The Critical difference (CD) was calculated wherever the 'F' test was found significant. The data were analyzed and presented at 5 per cent level of significance.

EXPERIMENTAL RESULTS

The results of this experiment are as mentioned.

Germination (%)

The maximum seed germination (16.50%) was noticed in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* and this was *on par* with Red soil + Sand + Coir pith + Rhizobium + PSB + *Pseudomonas fluroscens* (16.00%). The lowest seed germination (6.50 %) was recorded in the Red soil + Sand + FYM.

Rate of Germination (Days)

Significant differences were observed among the treatments with respect to rate of germination. Maximum rate of germination (0.29) was noticed in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* and this was *at par* with Red soil + Sand + Coir pith + Rhizobium + PSB + *Pseudomonas fluroscens* (0.28). The minimum rate of germination (0.11) was recorded in the Red soil + Sand + FYM and Red soil + Sand + FYM+ Rhizobium + PSB + *Pseudomonas fluroscens* (Table 1).

Seedling Height (cm)

The media containing Red soil + Sand + Vermicompost + Rhizobium + PSB +

Pseudomonas fluroscens recorded maximum height of seedling (17.37 cm) and was *at par* Red soil + Sand + Coco peat + Rhizobium + PSB + *Pseudomonas fluroscens* (16.97 cm). The lesser seedling height (9.87 cm) was recorded in the Red soil + Sand + FYM.

Seedling Girth (cm)

The seedling girth was found maximum (0.23 cm) in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* and *was on par* with Red soil + Sand + Coco peat + Rhizobium + PSB + *Pseudomonas fluroscens* 0.21 cm and Red soil + Sand + Coir pith + Rhizobium + PSB + *Pseudomonas fluroscens* (0.18 cm). The minimum seedling girth (0.10 cm) was recorded in the Red soil + Sand + Coco peat and Red soil + Sand + Coir pith (Table.1).

Number of leaves per seedling

The maximum number of leaves per seedling (6.40) was noticed in the seedlings grown in media containing Red soil + Sand + FYM + Rhizobium + PSB + *Pseudomonas fluroscens* and *was at par* with treatment Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* (5.93) and Red soil + Sand + Coir pith + Rhizobium + PSB + *Pseudomonas fluroscens* (5.70). The minimum number of leaves per seedling (4.07) was observed in the Red soil + Sand + Coir pith.

Leaf area (cm^2)

Significant differences were observed among the treatments with respect to leaf area (Table.1). The maximum leaf area (28.14 cm^2) was recorded in the seedlings grown in media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. The lowest leaf area (18.85 cm^2) was recorded in the Red soil + Sand + Coir pith.

Seedling Vigour

Seedling vigour was found to be maximum (412.27) in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* and was *at par* with Red soil + Sand + Coir puth + Rhizobium + PSB + *Pseudomonas fluroscens* (358.40). The minimum seedling vigour was recorded in the Red soil + Sand + FYM (110.47).

Fresh Weight of Shoot (g)

The maximum fresh weight of shoot (4.37 g) was noticed in the media seedlings grown in containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. The lowest fresh weight of shoot (2.13 g) was recorded in the Red soil + Sand + Coco peat.

Fresh Weight of Root (g)

The media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* recorded maximum fresh weight of root 0.54 g. Whereas, the minimum of 0.19 g fresh weight of root was recorded in the Red soil + Sand + Coco peat (Table 2).

Total Fresh Biomass (g)

The total fresh biomass (4.91 g) was found to be maximum in the seddlings grown in media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. The lowest total fresh biomass (2.32 g) was obtained in the seedlings grown in Red soil + Sand + Coco peat (Table 2).

Dry Weight of Shoot (g)

The maximum dry weight of shoot (1.52 g) was recorded in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. While, the minimum dry weight of shoot (0.75 g) was recorded in the Red soil + Sand + Coco peat (Table 2).

Dry Weight of Root (g)

Significant differences were observed among the treatments with respect to dry weight of root. The maximum dry weight of root (0.19 g) was recorded in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. The minimum dry weight of root (0.07 g) was noticed in the Red soil + Sand + Coco peat.

Total Dry Biomass (g)

The media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* recorded highest total dry biomass (1.71 g). The minimum total dry biomass (0.82 g) was observed in the Red soil + Sand + Coco peat (Table 2).

Root Length (cm)

The root length of 6.50 cm was noticed to be maximum in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonasfluroscen*s and this was *at par* with Red soil + Sand + FYM + Rhizobium + PSB + *Pseudomonas fluroscens* (6.47 cm) and Red soil + Sand + Coco peat (6.30 cm). The minimum of 5.40 cm root length was recorded in the Red soil + Sand + FYM.

Root Volume (cc)

The maximum root volume was observed in the media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* (3.13 cc). While, the minimum root volume (1.13 cc) was recorded in the Red soil + Sand + FYM.

Table 1: Effect of different propagating media and plant growth promoting rhizobacteria on germination and seedling growth of Sarpagandha (*Rauvolfia tetraphylla*)

Treatments	Germination %	Germination rate	Seedling height (cm)	Seedling girth (cm)	No. of leaves per seedling	Leaf area (cm^2)	Seedling vigour
T_1	6.50	0.11	9.87	0.11	4.27	20.18	110.47
T_2	8.00	0.13	12.17	0.14	5.07	19.51	152.73
T_3	9.50	0.16	11.93	0.10	4.60	19.48	175.60
T_4	9.50	0.15	12.50	0.10	4.07	18.85	185.07
T_5	7.00	0.11	13.87	0.16	6.40	28.14	142.80
T_6	16.50	0.29	17.37	0.23	5.93	26.12	412.27
T_7	11.00	0.20	16.97	0.21	5.47	25.48	281.33
T_8	16.00	0.28	15.55	0.18	5.70	26.36	358.40
S.Em ±	0.90	0.016	0.21	0.019	0.30	0.33	18.95
F test	*	*	*	*	*	*	*
C.D at 5%	2.65	0.04	0.62	0.056	0.88	0.97	55.44

* = Significant at 5% probability level

T_1 = Red soil + Sand + FYM
T_2 = Red soil + Sand + Vermicompost
T_3 = Red soil + Sand + Coco peat
T_4 = Red soil + Sand + Coir pith
T_5 = Red soil + Sand + FYM + Rhizobium + PSB + Pseudomonas fluroscens
T_6 = Red soil + Sand + Vermicompost + Rhizobium + PSB + Pseudomonas fluroscens
T_7 = Red soil + Sand + Coco peat + Rhizobium + PSB + Pseudomonas fluroscens
T_8 = Red soil + Sand + Coir pith+ Rhizobium + PSB + Pseudomonas fluroscens

Table 2: The biomass, root length and volume in Sarpagandha (*Rauvolfia tetraphylla*) seedlings as influenced by different propagating media and plant growth promoting rhizobacteria

Treatments	Fresh weight of shoot (g)	Fresh weight of root (g)	Total fresh biomass	Dry weight of shoot (g)	Dry weight of root (g)	Total dry biomass (g)	Root length (cm)	Root volume (cm^3)
T_1	2.40	0.24	2.64	0.87	0.08	0.95	5.40	1.13
T_2	2.47	0.25	2.72	0.99	0.08	1.07	6.00	1.53
T_3	2.13	0.19	2.32	0.75	0.07	0.82	6.30	2.07
T_4	2.73	0.25	2.98	0.96	0.09	1.05	5.93	1.33
T_5	3.53	0.37	3.90	1.24	0.13	1.37	6.47	2.33
T_6	4.37	0.54	4.91	1.52	0.19	1.71	6.50	3.13
T_7	3.87	0.48	4.35	1.35	0.17	1.52	5.73	2.33
T_8	3.55	0.34	3.89	1.24	0.12	1.36	5.90	2.20
S.Em ±	0.11	0.01	0.12	0.04	0.003	0.043	0.15	0.09
F test	*	*	*	*	*	*	*	*
C.D at 5%	0.33	0.03	0.36	0.11	0.01	0.12	0.45	0.27

* = Significant at 5% probability level

T_1 = Red soil + Sand + FYM
T_2 = Red soil + Sand + Vermicompost
T_3 = Red soil + Sand + Coco peat
T_4 = Red soil + Sand + Coir pith
T_5 = Red soil + Sand + FYM + Rhizobium + PSB + Pseudomonas fluroscens
T_6 = Red soil + Sand + Vermicompost + Rhizobium + PSB + Pseudomonas fluroscens
T_7 = Red soil + Sand + Coco peat + Rhizobium + PSB + Pseudomonas fluroscens
T_8 = Red soil + Sand + Coir pith+ Rhizobium + PSB + Pseudomonas fluroscens

DISCUSSION

Germination per cent & Rate of germination

The media and PGPRs composition showed significant differences with respect to germination percentage and rate of germination in 'Sarpagandha' seeds. The highest germination (16.50 %) and rate of germination (0.29) was recorded in media having Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* and was *on par* with Red soil + Sand + Coir pith + Rhizobium + PSB + *Pseudomonas fluroscens* . The highest seed germination and rate of germination may be due to good physical and chemical properties by decreasing compactness and increasing the porosity of the medium. The applied PGPR's might have attributed for creating favourable condition through secretions of vitamins and growth promoting substances. The results are in line with those obtained by[22], in Kalmegh and[1], in Isabgol amd[18], in *Withania coagulans*, Verma and Singh[35] in Pyrethrum and Krishna[16] in kalmegh.

The plant growth promoting rhizobacteria plays an important role in increasing metabolic activity in germinating seeds and protecting seeds by various mechanisms such as antifungal compound production, antibiotics production and siderophores production. The lowest germination percentage and rate was recorded in media having red soil, sand and coir pith.

Seedling Height (cm) and Girth (cm)

The seedling height and girth was found significantly maximum when the seeds were sown in media having red soil, sand and vermicompost with PSB and *Pseudomonas fluroscens*. This may be due to combined effect of media and PGPRs favoured the plant growth. Vermicompost is a rich source of nutrients, helps in better water uptake and maintains good physical property of the soil and PGPR's help in better growth. Similar results were found by Verma and Singh.,in Pyrethrum[7], in *Terminalia bellarica*[3] in Nutmeg[35], in Aonla and[13], in Jamun. The minimum height and girth was observed with red soil, sand and FYM.

Number of leaves per seedling and leaf area (cm^2)

The seeds sown in media having Red soil + Sand + FYM + Rhizobium + PSB + *Pseudomonas fluroscens* recorded maximum number of leaves and leaf area. The maximum number of leaves and leaf area may be due to the influence of proprietary compound which might have decomposed and released nutrients. Further, these PGPRs may have made good availability of the nutrients present in the media, thus, helped in production of more number of leaves which in turn increased leaf area. Similar findings were observed in Aonla and Jamun by Aseri and Rao[5].

Seedling vigour

The seedling vigour was maximum when the seeds were sown in media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. The maximum seedling vigour was attributed to enlarged embryos, higher rate of metabolic activity and respiration, better utilization and mobilization of metabolites to growing points and higher activity of enzymes. Enzymatic and hormonal mechanism by production of phytoharmones by PGPR's stimulated that metabolic process such as sugar mobilization, protein hydrolysis, oxidation etc., which leads to increase in root length, shoot length and seedling dry weight, in turn increase in seedling vigour. Further, these rhizobacteria stays in association with seedling root, fix atmospheric nitrogen, increases the availability and uptake of phosphorus and other nutrients, and also enhances the production of plant growth regulators thus, resulted in higher seedling vigour. These findings are in conformity with the results reported by Krishna[16] in *Andrographis paniculata*. Similar findings were observed in Nutmeg by Abirami[3], in Periwinkle by Lenin[19], in Ashwagandha by Preeti[27], and Kalmegh by Mishra, S and Jain[22]. The lowest seedling vigour was observed in media having red soil, sand and FYM.

Root length (cm) and volume (cc)

The maximum root length and volume was found when the seeds were sown in media containing Red soil + Sand + Vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens*. This may be due to restorer effect of apical dominance from PGPR's which promotes root initiation through more nutrient uptake and root cell elongation and initiation. The vermicompost being a rich source of N, P, K and other mico nutrients which might have helped in gainig better root length and volume. PGPRs not only helps in making availability of nutrients but, also produce certain growth promoting phytoharmones which may have been found helpful for root elongation and biomass accumalation as revealed by Abdolshakoor[1], in Isabgol, Verma and Singh[35] in Pyrethrum[37], in Tamarind[3], in Nutmeg. The least root length and volume was noticed when seeds were sown in media having red soil, sand and FYM.

Fresh and dry weight of shoot, root and total biomass

Rhizobium, PSB and *Pseudomonas fluroscens* inoculated in media having red soil, sand and vermicompost recorded maximum weight of shoot, root and total biomass. The maximum fresh and dry weight is mainly due to the role of vermicompost through increased the water holding capacity and release of available nutrients to the growing plant which inturn increases the production of auxin, gibberellins, cytokinins. Further, increased fresh and dry weight of shoots and roots may be due to several mechanisms of plant growth promoting rhizobacteria through phytohormone production, Nitrogen fixation, stimulation of nutrient uptake and bio-control of pathogenic microorganisms. These findings are in line with Aruw kayina and Reddy[4] in Senna, in Tamarind and[3], in Nutmeg. The least fresh and dry weight of biomass was recorded with red soil, sand and cocopeat.

CONCLUSION

Concluded from above experiment it is inferred that the plants raised in media having Red soil + sand + vermicompost + Rhizobium + PSB + *Pseudomonas fluroscens* were better compared to all other propogating media combinations.

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