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Influence of Nutrient Combinations and Growing Systems on Root Growth and Symbiotic Association of Piriformospora indica (PGPRE) in Dendrobium cv. Earsakul

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ABSTRACT

Dendrobium is an important orchid for cut flower and potted plant production. The present experiment was designed to work out a suitable treatment combination and growing system for better root growth of Dendrobium. The treatments were replicated thrice in Completely Randomized Design. Experimental results clearly indicated that, among plant growth promoters, the treatment NPK + GR + OM + VW + PGPRE + Bone meal (T_6) recorded higher number of roots (91.00, 79.72), root volume (16.34 m^3, 14.19 m^3) in six month and three year old plants. Significantly longer roots (34.01 cm) and higher root colonization (66.63 per cent) was resulted in POP + OM + VW + PGPRE + Bone meal + GR (T_4) in six month old plants. Among three growing systems, top ventilated polyhouse (S_2) recorded significantly higher number of roots (89.00, 94.75), longer roots (31.44 cm, 43.33 cm), root volume (17.14 m^3, 19.16 m^3) and root colonization (63.34, 41.30 per cent). In interaction, the combination of POP + OM + VW + PGPRE + Bone meal + GR (T_4) and top ventilated polyhouse (S_2) had maximum influence on root parameters. In anatomical studies, after inoculation, in Dendrobium cv. Earsakul roots, hyphae of the Piriformospora indica fungus entered into the tissue of the root through the root tip. In the cortical cells of the roots, development of intracellular hyphal coils and round bodies could be observed. Hyphae multiplied within the cortical tissues and never traversed through endodermis.

Key words : Anatomical studies, Dendrobium cv. Earsakul, Inorganic nutrients, Organic, Piriformospora indica (PGPRE), Root growth, Three growing systems.

Among the orchid genera, Dendrobium is a very complex and extremely large genus widely used in the commercially cut flower production. It is the second largest genus in the family with nearly 1600 species, is one of the commercially important species. Most Dendrobium species are epiphytic and are from tropical and sub-tropical regions. It is a popular genus for cut flower production. Many growers in the states of Kerala, Tamil Nadu and Coastal Karnataka are cultivating Dendrobium on a commercial scale.

The type of nutrients, their quality and frequency of application play an important role on the growth and quality of flower (Naik et al., 2010). In orchids, growth and floral initiation is determined by the genotype and its interaction with the environmental conditions. Temperature, humidity, light and photoperiod are some of the important environmental conditions that influence reproductive biology of orchids. Regulation of light intensity is essential for successful orchid culture. During plant development, the transition from vegetative to reproductive growth is triggered by a number of environmental and endogenous signals. Under controlled conditions of greenhouse, the flowers exhibit the best quality attributes required for the market. For better growth, yield and quality of the flowers, the system of growing is very important. Micro climate inside the growing system may drastically influence the growth, flowering and quality of flowers (Femina et al., 2006). In their natural habitat, epiphytes usually meet with a greater degree of environmental stress.

The root-colonizing fungal mutualist Piriformospora indica was discovered in the rhizosphere of the woody shrubs Prosopsis juliflora and Zizyphus nummularia in the Indian Thar desert and it was named according to its characteristic pear-shaped chlamydospores (Verma et al., 1998).

Piriformospora indica AM fungi – like fungus, showed prominent positive influence on a wide range of plants of agriculture, forestry and flori-horticultural importance. Fungus has a wide host range of monocots and dicots including

legumes, terrestrial orchids (Dactylorhiza maculata) and members of the bryophytes (Aneura pinguis). The fungus showed potential as an agent for biological control of disease against soil-borne root pathogens. ^{32}P experiments suggest that this fungus is important for phosphorus acquisition by the roots, especially in the arid and semi-arid regions. Mycelium could utilize a wide variety of inorganic and organic phosphate chemicals and produced acid phosphatases at the tip of the hyphae (Singh et al., 2003a, b).

The major constraints encountered in Dendrobium orchid cultivation are growing conditions, long pre blooming period. In addition to that root growth in orchids is very important for proper growth and development of the plant. These are very important for growth and development of orchids. Plant nutrition is often dependent on mutualistic associations with other organisms. Mycorrhizal associations (mutualistic interaction between vascular plant roots and fungi, whereby the roots benefit from enhanced water and nutrient uptake and the fungi gain ready access to translocating photosynthates) are likely to favour nutrient uptake in epiphytic plants (Lesica and Antibus, 1990).

It has been reported that mycorrhizal orchids can acquire more N, P and water than non-mycorrhizal controls (Alexander et al., 1984; Yoder et al., 2000; Cameron et al., 2006, 2007). Thus, understanding the relationship between orchids and mycorrhizal fungi is of great important.

Hence, the present study was undertaken to study the influence of nutrient combinations and growing systems on root growth and symbiotic association of Piriformospora indica in Dendrobium cv. Earsakul orchid to know the structural linkage between the host and PGPRE.

MATERIAL AND METHODS

Experiment I

The experiments were carried out at the orchidarium of All India Coordinated Floriculture Improvement Project (AICFIP) in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, Kerala. Studies were conducted over a period from April 2011 to March 2013 in three types of growing systems viz., two level shade house (S_1), top ventilated polyhouse (S_2) and fan and pad system (S_3). Commercially cultivated orchid hybrid variety Dendrobium cv. Earsakul was used for the study. Plants having two stages of growth viz., six months and three year old plants were used. Plants were grown under 50 per cent shade in two level shade house (size: 21.00 m x 6.00 m x 3.50 m x 2.00 m, top one layer shade net, lower one layer poly film 200 micron with misting system), top ventilated polyhouse (size: 21.00 m x 6.00 m x 3.50 m x 2.00 m, poly film 200 micron covering with shade net and misting system) and in 75 per cent shade in fan and pad system (size: 12.50 m x 8.00 m x 6.00 m x 4.00 m, poly film 200 micron covering, UV stabilized shade net with fan and pad for cooling system). The major nutrients $N:P_2O_5:K_2O$ at two different ratios, viz., 3:1:1 and 1:2:2 @ 0.2 per cent were applied as foliar sprays during vegetative and flowering stages, respectively. The frequency of application was weekly twice. Nutrient combinations were made using ammonium nitrate, ortho-phosphoric acid and potassium nitrate.

The treatments consisted of T_1- POP recommendations of KAU (foliar feeding with fertilizer mixture of $N:P_2O_5:K_2O$ 3:1:1 during vegetative period and 1:2:2 during flowering period @ 0.2 per cent, spraying at weekly twice as ammonium nitrate, ortho-phosphoric acid and potassium nitrate respectively), T_2- POP + PGPRE (the fungal culture of Piriformospora indica was mixed with vermiculite @ 1 g per 100 g of vermiculite and applied near the root zone at the time of planting) + bone meal (15 g per plant applied near root zone at the time of planting), T_3- POP + OM (bone meal, neem cake and ground nut cake 100 g each, soaked in water for 3-4 days and diluted to 10-15 times with water, filtered and sprayed over plants at 15 days interval) + vermiwash (diluted to 3 per cent and sprayed at 15 days interval) + PGPRE + bone meal, T_4- POP + OM + VW + PGPRE + bone meal + GR (BA 50 mg l^{-1} and GA_3 10 mg l^{-1} sprayed at monthly intervals), T_5- 10:20:10 NPK + GR and T_6- NPK + GR + OM + VW + PGPRE + bone meal. The experiment was laid out in completely randomized design comprising six treatments, three replications and five plants per treatment for recording observations. Observations were recorded on number of roots per plant, root length, root volume and root colonization.

Table 1. Influence of plant growth promoters (T), growing systems (S) and T x S interaction on root parameters in six month old plants of Dendrobium cv. Earsakul at the time of flower bud formation.

Treatments	Number of roots per plant				Root length (cm)				Root volume (m^3)				Root colonization (%)			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
T_1	62.67	68.17	57.66	62.83	21.50	17.68	26.73	21.97	13.50	11.17	9.00	11.22	0.00	0.00	0.00	0.00
T_2	85.17	79.33	52.56	72.35	32.42	31.90	18.73	27.68	12.58	19.83	10.00	14.14	31.10	48.90	22.22	34.07
T_3	101.10	85.17	61.83	82.70	30.47	29.57	21.53	27.19	13.97	20.50	8.67	14.38	51.10	57.77	15.55	41.48
T_4	68.50	106.83	48.00	74.44	27.76	41.73	32.56	34.01	12.83	26.67	6.67	15.40	79.91	84.44	35.55	66.63
T_5	73.17	92.67	56.33	74.05	21.20	35.90	17.10	24.73	11.88	17.33	10.67	13.29	0.00	0.00	0.00	0.00
T_6	83.67	101.83	87.50	91.00	25.25	31.83	21.40	26.16	21.00	7.37	20.67	16.34	71.10	62.23	51.17	61.48
Mean	79.04	89.00	60.65		26.43	31.44	23.00		14.29	17.14	10.94		58.30	63.34	31.11	
CD		T: 6.71			T: 2.68					T: 2.79				T: 8.26		
(P= 0.05)		S: 4.75			S: 1.89					S: 1.97				S: 7.16		
		T x S: 11.63			T x S: 4.64					T x S: 4.83				T x S: 14.32		

Table 2. Influence of plant growth promoters (T), growing systems (S) and T x S interaction on root parameters in three year old plants at the time of flower bud formation.

Treatments	Number of roots per plant				Root length (cm)				Root volume (m^3)				Root colonization (%)			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
T_1	56.00	67.50	53.71	59.07	27.33	39.68	28.62	31.88	7.17	9.40	4.67	7.08	0.00	0.00	0.00	0.00
T_2	95.67	87.67	37.33	73.56	32.83	39.88	38.38	37.03	13.33	21.67	6.67	13.89	24.45	30.37	11.11	21.98
T_3	88.00	106.17	34.33	76.17	46.73	67.87	26.58	47.06	8.33	25.07	7.33	13.58	30.36	28.15	22.22	26.91
T_4	56.83	119.00	37.93	71.25	27.73	48.73	24.72	33.73	6.83	21.50	6.33	11.56	55.55	50.37	37.77	47.90
T_5	37.57	86.50	29.00	51.02	31.50	38.62	28.88	33.00	7.00	20.83	7.67	11.83	0.00	0.00	0.00	0.00
T_6	67.83	101.67	69.67	79.72	27.70	25.17	17.75	23.54	14.40	16.50	11.67	14.19	49.62	56.30	42.22	49.38
Mean	66.98	94.75	43.66		32.30	43.33	27.49		9.51	19.16	7.39		39.99	41.30	28.33	
CD		T: 11.30				T: 6.04				T: 2.21				T: 4.85		
(P=0.05)		S: 7.99				S: 4.27				S: 1.56				S: 4.20		
		T x S: 19.57				T x S: 10.47				T x S: 3.83				T x S: 8.40		

The experimental data were analyzed by the ANOVA (Analysis of Variance technique (Panse and Sukhatme, 1985). MSTATC and MS-Excel software were used for computation of data.

Experiment II

Study on symbiotic interactions

Anatomical studies

Roots from the treated plants were washed thoroughly in running tap water, cut into 1.0 cm pieces and treated overnight with 10 per cent KOH solution at room temperature and the root segments were boiled for 10 minutes. Thereafter, the root segments were washed 3-5 times with sterilized distilled water and then treated with 1 per cent HCl for 3-4 minutes before staining with 0.05 per cent trypan blue in lactophenol (Phillips and Hayman, 1970). Then the root bits were de-stained with fresh lactophenol and fine thin, transparent, cross and longitudinal section of the root segments were put on a glass slide and gently pressed with cover slip, examined microscopically (40X and 100X), photographed and the growth and nature of attachment of hyphae in root cells was observed and the information on structural linkage between the host and Piriformospora indica (PGPRE) was recorded.

RESULTS AND DISCUSSION

Experiment I

Number of roots per plant

The data on root growth have been presented in Table 1 and 2 as influenced by various plant growth promoters (treatments), growing systems and their interaction. Results revealed that, the treatment NPK + GR + OM + VW + PGPRE + Bone meal (T_6) resulted in significantly higher number of roots in both stages of plants (91.00, 79.72). This might be due to the phosphorus nutrient in the early stages of growth is beneficial for producing more number of roots per plant. The plants having higher number of shoots and beneficial effects of P. indica which enhanced the better root system which in turn helps in rapid growth of the plant and ultimately plants are having maximum number of roots per plant. These results are in conformity with the findings of Swapna (2000), Binisha (2003) and Dhinesh (2009) in Dendrobium.

It is clear from the data (Table 1, 2) that among systems of growing, top ventilated polyhouse (S_2) had significant influence on number of roots per plant irrespective of the age of the plants (89.00, 94.75). This might be due to plant height, number of leaves per plant and number of shoots per plant was high in plants grown under top ventilated polyhouse. Hence in the same corollary, this result could be explained that system of growing influenced the number of roots per plant.

Data on influence of plant growth promoters (treatments) and growing systems on number of roots per plant indicated that the combination of POP + OM + VW + PGPRE + Bone meal + GR and top ventilated polyhouse (T_4S_2) recorded significantly higher number of roots per plant (106.83, 119.00) in both stages of plants (Table 1, 2). The result of the study clearly indicated that application of P. indica influenced the production of number of roots per plant under top ventilated polyhouse.

Root length

A perusal of data presented in Table 1 and 2 revealed that the treatment POP + OM + VW + PGPRE + Bone meal + GR (T_4) in six month old plants (34.01 cm) and POP + OM + VW + PGPRE + Bone meal (T_3) in three year old plants (47.06 cm) recorded significantly longer roots. The reason might be due to that the P. indica made the P available to the plants which in turn increase the length of the roots. These results are supported by Tiwari and Kumar (2011) in Dendrobium.

Results showed that top ventilated polyhouse (S_2) recorded significantly longer roots (31.44 cm, 43.33 cm) irrespective of the age of the plants (Table 1, 2). Plants under top ventilated polyhouse exhibited increased plant height, more number of leaves and number of shoots. This might be the reason for more root length observed in top ventilated polyhouse.

In TxS interaction, the combination of POP + OM + VW + PGPRE + Bone meal + GR in six month old plants and POP + OM + VW + PGPRE + Bone meal in three year old plants recorded significantly higher root length in top ventilated polyhouse (41.73 cm, 67.87 cm), respectively (T_4S_2, T_3S_2).

Plate 1. Anatomical studies in roots for structural linkage between Dendrobium cv. Earsakul and Piriformospora indica (a PGPRE).

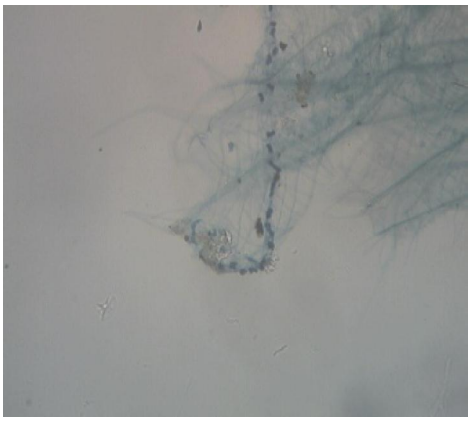


Plate 1 a. Fungus entry through root tip (60x)

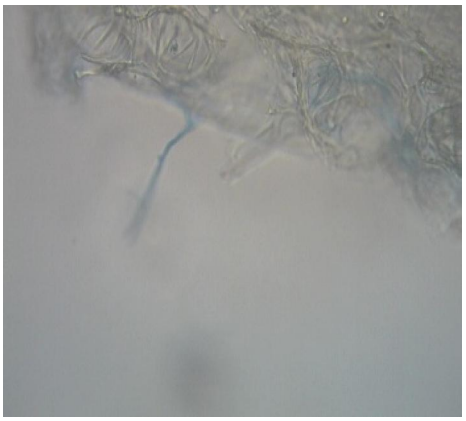


Plate 1b. Hyphae touching the root surface (60x)

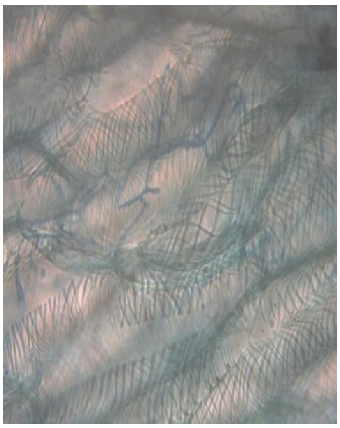


Plate 1 c. Fungus entry through velamen of the root tissue (60x)

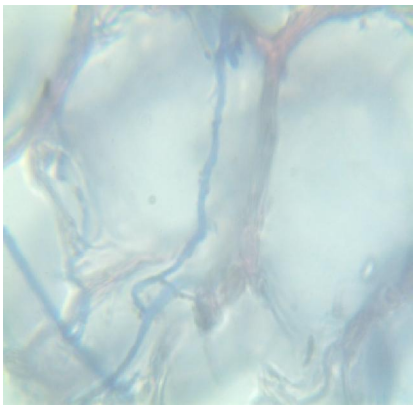


Plate 1 d. Hyphae of the fungus was detected in cortical cells (100x)

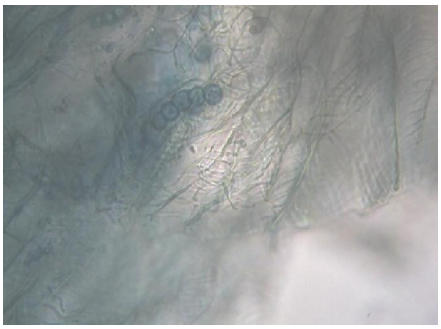


Plate 1 e.The fungus produced chlamydospores at the apex of undifferentiated hyphae (60x)

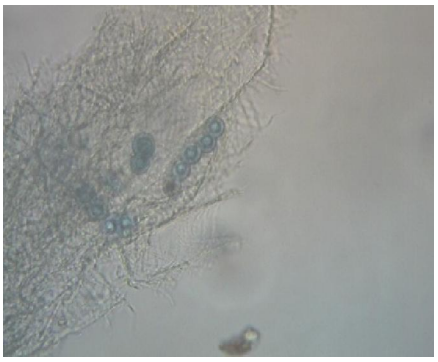


Plate 1 f. Pear-shaped chlamydospores in root tissue (60x)

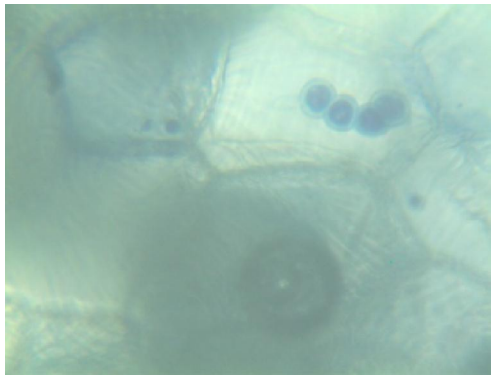


Plate 1 g. Cortical cells showing round bodies (60x)

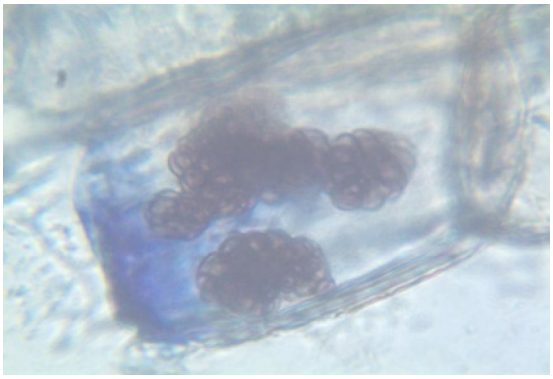


Plate 1 h. Cortical cells with highly coiled intracellular structures (60x)

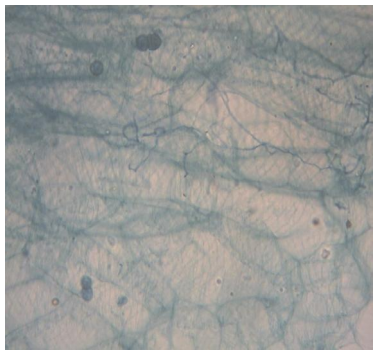


Plate 1 i. Fungus hyphae in cortical cells (40x)

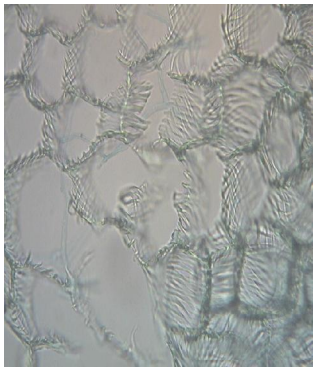


Plate 1 j. Fungus hyphae in cortical cells (60x)

Root volume

The information made available in Tables 1 and 2 showed that among various plant growth promoters, NPK + GR + OM + VW + PGPRE + Bone meal (T_6) resulted in significantly higher root volume (16.34 m^3, 14.19 m^3) in both stages of plants. The possible reason might be due to that the treatment NPK + GR + OM + VW + PGPRE + Bone meal recorded higher number of roots per plant in both stages of plants and this could be the reason for higher root volume in six month and three year old plants, respectively. These results are in conformity with the findings of Dhinesh (2009) in Dendrobium.

Significantly highest root volume of 17.14 m^3 and 19.16 m^3, respectively was recorded in top ventilated polyhouse (S_2). Congenial environmental conditions (Fig. 1, 2, 3) favoured better root growth which in turn more roots volume. Plants grown under top ventilated polyhouse recorded maximum number of roots and root length. This resulted in high root volume in top ventilated polyhouse.

In TxS interaction, the combination of POP + OM + VW + PGPRE + Bone meal + GR (26.67 m^3) in six month old plants and POP + OM + VW + PGPRE + Bone meal (25.07 m^3) in three year old plants recorded significantly higher root volume in top ventilated polyhouse (T_4S_2 and T_3S_2).

Root colonization of Piriformospora indica

Root colonization study revealed that the treatment POP + OM + VW + PGPRE + Bone meal + GR (T_4) in six month old plants (66.63 per cent), NPK + GR + OM + VW + PGPRE + Bone meal (T_6) in three year old plants (49.38 per cent) recorded highest root colonization of P. indica. This might be due to enhanced root production, root volume and root colonization of P. indica was observed in treatment combination involving P. indica. The positive influence of P. indica for the above root parameters was clearly evident from this study. In the present study, P. indica had a positive effect on root parameters, which conform with the observations of Dhinesh (2009) in Dendrobium.

Fig 1. Yearly mean temperature (^{0}C) in different growing systems

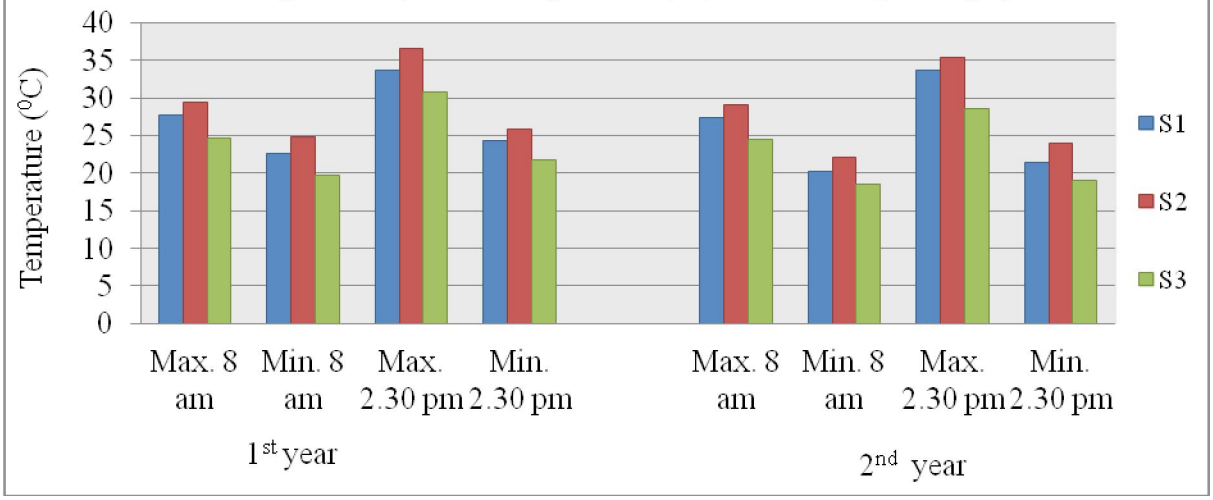


Fig 2. Yearly mean relative humidity (%) in different growing systems

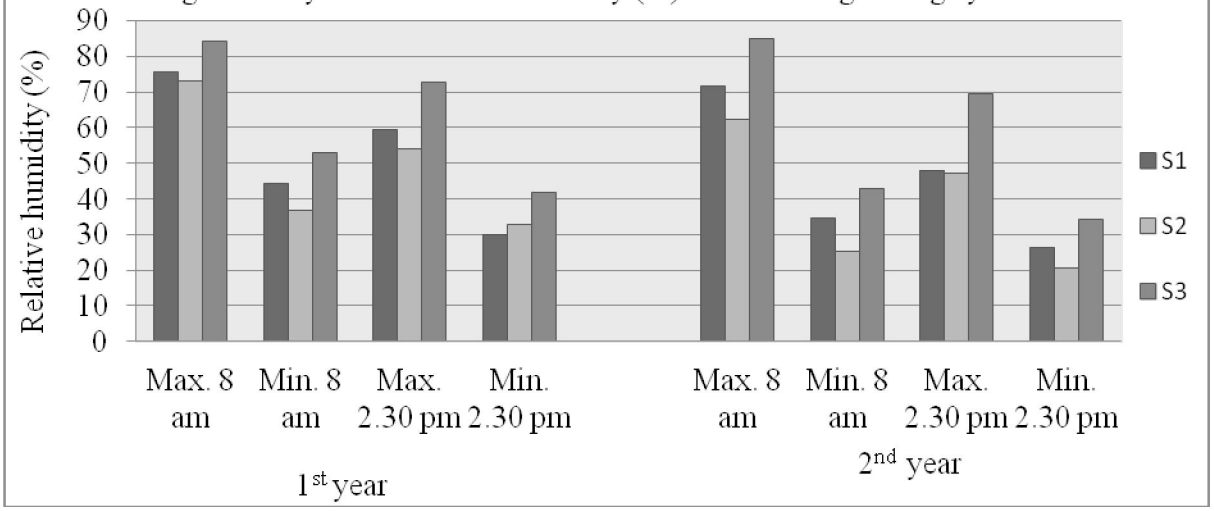
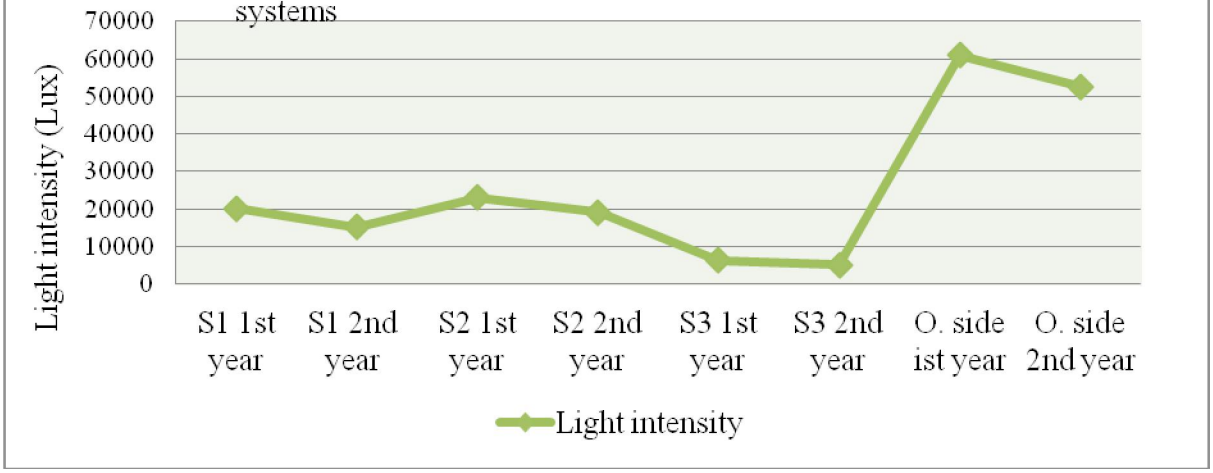


Fig 3. Yearly mean light intensity (lux) inside and outside of the growing systems



It was found from the Tables 1 and 2 that, top ventilated polyhouse (S_2) had significant influence on root colonization of P. indica irrespective of the age of the plants (63.34, 41.30 per cent). This could due to higher temperature is the main reason for higher root colonization in the system of growing (Fig. 1). It was reported that P. indica at higher temperature (25-35 ^{0}C) resulted in higher mycelial growth (Varma et al., 1999) and the higher temperature prevailing in the top ventilated polyhouse (29.25 to 35.95 ^{0}C) might be the possible reason for higher root colonization. Similar type of findings is reported by Leonhardt (2000) in Dendrobium.

In TxS interaction, data showed that the the combination of POP + OM + VW + PGPRE + Bone meal + GR and NPK + GR + OM + VW + PGPRE + Bone meal had more positive influence on root colonization of P. indica in top ventilated polyhouse irrespective of age of the plants (84.44, 56.30 per cent). The results inferred and further conformed the results of experiments of plant growth promoters and systems of growing independently.

Experiment II

Anatomical studies in roots for structural linkage between the host (Dendrobium cv. Earsakul) and PGPRE (Piriformospora indica)

After inoculation, in Dendrobium cv. Earsakul roots, hyphae of the P. indica entered into the tissue of the root through the root tip (Plate 1a). Hyphae, first touching the root surface (Plate 1b) and entered through velamen tissue (Plate 1c). The hyphal growth of the fungus was detected on the root surface between the outer cell layers of the cortex and within the cortical cells (Plate 1d). The fungus produced chlamydospores at the apex of undifferentiated hyphae (Plate 1e) and within the cortical cells of the root tissue (Plate 1f). The fungal hyphae invade the cortical cells and form tightly interwoven coils called 'pelotons' characteristic of orchid mycorrhizae. In the cortical cells of the roots, development of intracellular hyphal coils and round bodies could be observed (Plate 1g, h). Hyphae multiplied within the cortical tissues and never traversed through endodermis (Plate 1i, j). These findings are in agreement with findings of Karimi et al. (2011) in barley, Verma et al. (1998) in maize, Varma et al. (2001) in maize and Stein et al. (2008) in Arabidopsis. P. indica did not invade the stellar tissue or traverse upwards into the shoot because stellar tissue is a hardier one and the fungus is an endosymbiont.

Hence, from the present findings, it was concluded that the treatment POP + OM + VW + PGPRE + Bone meal + GR (T_4) and top ventilated polyhouse (S_2) i.e. T_4S_2 was found better treatment combination for better root growth and development in Dendrobium cv. Earsakul. In anatomical studies, hyphae of the fungus multiplied within the cortical tissues and never traversed through endodermis. P. indica did not invade the stellar tissue or traverse upwards into the shoot because stellar tissue is a hardier one and the fungus is an endosymbiont.

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