INFLUENCE OF NUTRIENTS AND PLANT GROWTH PROMOTING ROOT ENDOPHYTE (PGPRE) ON GROWTH AND DEVELOPMENT OF *Dendrobium* CV. EARSAKUL

By

DHINESH. D

THESIS

Submitted in partial fulfillment of the

requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

Department of Pomology and Floriculture

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2009

DECLARATION

I hereby declare that the thesis entitled **"Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other university or society.

Vellanikkara

Dhinesh. D

(2007-12-105)

CERTIFICATE

Certified that this thesis, entitled **"Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul"** is a record of research work done independently by **Mr. Dhinesh. D,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

Dr. K. Ajith Kumar
Chairperson, Advisory committee
Associate Professor
AICFIP, Dept. of Pomology and Floriculture
College of Horticulture
Vellanikkara

Vellanikkara

CERTIFICATE

We, the under signed members of the Advisory Committee of **Mr. Dhinesh. D.,** a candidate for the degree of **Master of Science in Horticulture** with major in **Pomology and Floriculture**, agree that this thesis entitled **"Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul"** may be submitted by Mr. Dhinesh. D, in partial fulfillment of the requirement for the degree.

Dr. K. Ajith Kumar
Chairperson, Advisory Committee
Associate Professor
AICFIP, Department of Pomology and Floriculture
College of Horticulture
Vellanikkara

Dr. P. K. Rajeevan
(Member)
Professor Associate Dean and Head
Dept. of Pomology & Floriculture
College of Horticulture

Dr. A. Sobhana
(Member) Professor
Cashew Research Station
Madakkathara
Vellanikkara

Dr. K.N. Anith
(Member)
Associate Professor
Dept. of Agricultural Microbiology
College of Agriculture, Vellayani
Thiruvanathapuram

ACKNOWLEDGEMENT

In connection with this venture, I sincerely place on record my profound sense of gratitude and indebtedness to numerous people who have helped me in this project. And so comes the time to look back on the path traversed during the endeavours and to remember the faces behind the action with a sense of gratitude. Nothing of significance can be accomplished without the acts of assistance, words of encouragement and gestures of helpfulness from others.

First and foremost I bow my head to the ***God*** *who enabled me to successfully complete the thesis work in time.*

I avail this opportunity to express my deep sense of reverence, gratitude and indebtedness to my major Advisor ***Dr. K. Ajith Kumar****, Associate Professor, AICFIP, Department of Pomology and Floriculture and Chairperson of my Advisory Committee for him sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the course of study, research work and preparation of the thesis. I really consider it my fortune in having him guidance for the thesis work.*

I place a deep sense of obligation to ***Dr. P.K. Rajeevan****, Professor and Head, Department of Pomology and Floriculture, College of Horticulture and member of my Advisory Committee for his unstinted support, critical comments and valuable suggestions during the preparation of this manuscript.*

I express my deep sense of gratitude to ***Dr. A. Sobhana****, Professor, Kashew Research Station, Madakkathara, and member of my Advisory Committee for her friendly help, unwavering encouragement, unflagging perseverance and well timed support which made the successful completion of this thesis.*

My heartfelt thanks are expressed to ***Dr. K.N. Anith****, Associate Professor, Department of Agricultural Microbiology, College of Agriculture, Vellayani, Trivandrum. For his whole hearted cooperation and immense help extended for the successful completion of this thesis.*

I extend my sincere thanks to ***Dr. P.K. Valsalakumari****, Professor, Department of Pomology and Floriculture, College of Horticulture, for her valuable suggestions.*

I express my deep sense of gratitude to ***Dr. C.K. Geetha, Dr. Sudhadevi****,* ***Dr. N.P. Parameswaran, Dr. Lyla Mathew, Dr. Sarah T .George, Dr. T. Radha,*** *the teaching staff of*

the Department of Pomology and Floriculture, for their kind co-operation and guidance during entire period of my study.

With profound respects, I place my thanks to ***Dr. Suresh Kumar, Dr. Jim Thomas, Dr. Beena, Dr. Salikutty Joseph, Dr. Sujatha, Dr. Surendra Gopal,*** *for them wholehearted cooperation, help and valuable suggestions during various stages.*

I extend my heartfelt thanks to ***Sampath, Sivaji, Prathamesh and Tamarai*** *for their valuable help while recording observation and thesis writing.*

I have no words to express my deep sense of gratitude to all my senior friends ***Rathish, Koteswararao Yadav, Manoj, Femina, Manikandan, Thiyagu, Unnikrishnan, Madhu*** *and* ***Hemalatha,*** *for their valuable assistance and guidance extended throughout the investigation.*

Words cannot really express the true friendship that I relished from my batch mates ***Kiran, Vijit, Guru, Grish, saranya, Srividya, Kanimozhi, Lamina and Jisha*** *for the heartfelt help, timely suggestions and back-up which gave me enough mental strength to get through all mind-numbing circumstances.*

I have no words to express my deep sense of gratitude to my junior friends ***Tamarai, Navaneeth, Arya, Sunil, Gajanan, Randeep*** *and* ***Shijini*** *for their moral support and encouragement.*

I am deeply indebted to my ***Parents*** *and* ***family members*** *without whose moral support, blessings and affection this would not have been a success. It would be impossible to list out all those who have helped me in one way or another in the successful completion of this work. I, once again express my heartful thanks to all those who helped me in completing this venture in time.*

The award of fellowship by KAU is greatfully acknowledged.

Dhinesh. D

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIX

Dedicated to

My Parents

Introduction

1. INTRODUCTION

Orchids, the most spectacular among flowering plants, are unique with their versatility in colour, form, size, shape, and longer life span of the plant and flower. They belong to family Orchidaceae, which is the largest family of the flowering plants. Taxonomically, it represents the most highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species. Orchids have a wide range of growing habit, from terrestrial to epiphytic.

Orchids constitute an order of royalty in the world of ornamental plants and they have immense horticultural importance. They play a very useful role to balance the natural ecosystem (Kaushik, 1983). They are most pampered of the plants and occupy top position among all the flowering plants valued for cut flower production and as potted plants. They are known for their long lasting flowers of myriad shapes, sizes and colours and bewitchingly beautiful flowers which fetch a very high price in the international market (Rajeevan *et al*., 2002). Orchids are major players in the multibillion dollar floriculture trade of the world. Today, orchids such as, *Dendrobium*, *Cymbidium*, *Phalaenopsis* and *Oncidium* are marketed globally and the orchid industry has contributed substantially to the economy of many ASEAN (Association of the South East Asian Nations) countries. The orchid cut flower industry is growing at the rate of 10-20 per cent annually and presently is a US $ 44 billion industry (Pradhan, 2001).

India being one of the mega biodiversity regions in the world is endowed with a rich heritage of orchids with about 1300 species in 167 genera distributed in various parts of the country especially in the Eastern Himalayas and the Western Ghats which forms two major biodiversity Hot-Spots. These Hot-Spots are the real treasure homes of orchids-the loveliest of all the flowers. The diverse climatic belts in India are highly suitable for growing orchids of various types. Sikkim, West Bengal (Darjeeling and Kalimpong) and Kerala are leading states in India for orchid cut-flower production.

The climatic condition of Kerala is highly congenial for growing orchids and is one of the leading states in India in the commercial cultivation of orchids. Tropical orchids like *Dendrobium*, *Arachnis*, *Cattleya*, *Phalaenopsis* and *Vanda* thrive well under Kerala conditions. Among the cultivated Epiphytics, *Dendrobiums* and *Vandas* are the most popular. *Dendrobium* is considered as the second largest genus of orchids. About 900-2000 species are reported in the genus with estimate of 1340 species (Baker and Baker, 1996). In Kerala, *Dendrobiums* occupy more than 75 per cent of cultivated orchids. The popular hybrids here are Earsakul, Sonia 17, Sonia 28, Renappa, Sabine Red, Emma White, Kasem White, Pink, Fairy White and Pravit White. These hybrids are highly floriferous and occur in all possible shape, size and colour. Being sympodial in growth, they are easy to house and maintain. These hybrids differ not only with respect to morphological features of plants and blooms but also in their response to various external factors. However, the various species and hybrids do not have synchronous flowering habit. Many of them do not have year round flowering.

Orchid hybrids require copious amount of nutrients since their growth and flowering rates are faster. The type of nutrients, their quality and frequency of application play an important role on the quality of flower. Conventional nutritional application in liquid form has been found to be very effective in orchids. The major constraints encountered in orchid cultivation are growing conditions, long pre blooming period, susceptibility to pest and diseases, short vase life *etc*.

The members of orchidaceae are characterized by a novel form of mycorrhizal interaction. *Piriformospora indica* is a newly described axenically cultivable phytopromotional endosymbiont, which mimics the capabilities of Arbuscular Mycorrhizal Fungi (AMF). *P. indica* acts as plant growth promoter (auxin), biofertilizer, bioregulator, immunomodulator, phytoremediator, biological hardening agent for micro propagated plantlets; imparts resistance against heavy metals, biocontrol against insects and pathogens, stress tolerance - both salt and

temperature; works as stimulator with Plant Growth Promoting Rhizobacteria (PGPRs) (Varma *et al.*, 1999; Waller *et al.*, 2005; Deshmukh *et al.*, 2006; Shahollari *et al.*, 2005, Sherameti *et al.*, 2008). The axenic cultivability of *P. indica* on economically viable synthetic media makes it suitable for mass scale inoculam production for application in agro-forestry and horticulture.

In this context, present investigation on Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul was taken up with the objective,

- To study the influence of organic and inorganic nutrients and *Piriformospora indica* (PGPRE) on the growth and development of *Dendrobium* cv. Earsakul.

Review of literature

2. REVIEW OF LITERATURE

The investigations on the influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul were taken up to study the effects of organic and inorganic nutrients and *Piriformospora indica* (PGPRE) on the growth and development of *Dendrobium* cv. Earsakul.

The relevant literature on the effect of organic manures, inorganic nutrients, orchidaceous mycrrhizae, Plant Growth Promoting Root Endophyte (PGPRE), *Piriformospora indica* on orchids is reviewed. Research information on other crops is also reviewed wherever pertinent literature in orchids is lacking.

2.1 Effect of organic manures

Fresh as well as dry cow dung, sheep, chicken, pig or fish manure, dried leaves, oil cakes, bone meal are some of the common manures used for feeding for orchids. Application of dilute urine, one part in ten parts of water is beneficial, as it contains all necessary nutrient elements and also certain growth promoting substances. Abraham and Vatsala (1981) found that beer at the strength of one quart per 10 gallons of water is beneficial for the growth of the orchids.

Growth of *Phalaenopsis* in sugarcane bagasse was reported to be improved significantly by increasing the nitrogen dose from 70 to 200 ppm and also by inclusion of 10-40 per cent organic fertilizer (Wue *et al.*, 1994).

Organic mixtures like cow dung, neem cake, poultry manure, etc. are also used for orchids. These may be soaked in water 4-5 days for fermentation, diluted 10-15 times with water, filtered and sprayed over the plants, fresh cows urine (1:20-25, with water) is also useful as foliar spray (Singh, 2006).

The growth of young plants of *Dendrobium* hybrids was found to improve when they were provided with a complete inorganic fertilizer in 20 per cent coconut milk. This treatment increased leaf number and height of pseudobulb, although when treatment without coconut milk application more pseudobulbs were found (Sagarik and Siripong, 1963).

In a two–year pot trial with *Oncidium* Golden Shower and *Dendrobium* Louisae Dark, dried chicken manure at 50, 100 or 200 g per plant every three months was compared with a complete nutrient solution foliar spray at 25g in 4.5 litres of water every 10 days. Chicken manure increased flower yield in *Dendrobium* and increased inflorescence length and flowers per spray in both orchids, but the highest rate was sometimes detrimental. The intermediate rate was the best for *Dendrobium* and the lowest rate for *Oncidum* (Chua, 1974).

2.1.1 Cow dung

Singh (1996) recorded that cowdung contained 82 % water and 18 % solid matter (minerals 0.1 %, ash 2.4 %, organic matter 14.6 %, Ca and Mg 0.4 %, SO_3 0.05 %, silica 1.5 %, N 0.5 %, P 0.2 % and K 0.5 %).

Nene (1999) reported that cowdung contained undigested fibre, epithelial cells, pigments and salts, rich in N, P, K, S, micronutrients and intestinal bacteria and mucous. Cow dung is rich in bacteria, fungi and other microbial organisms.

2.1.2 Vermiwash

Vermiwash is a transparent, pale yellow, liquid biofertilizer. It is a mixture of excretory products and mucous secretion of earthworms (*Lampito mauritii* and *Eisenia fetida*) and organic micronutrients of soil, which may be promoted as a potent fertilizer for better growth and yield of plants (Shweta *et al.*, 2005).

Vermiwash contains N 0.020 %, P 0.007 %, K 0.100 %, and Zn 170 ppm and some nitrogen fixing bacteria, nitrosomonas and nitrobacter. Nitrogen present in vermiwash helps to promote vegetative growth (Ramachandran and Thimmaraju, 1983). Higher uptake of P in rice treated with vermiwash was observed (Kale *et al.*, 1992).

A well decomposed farm yard manure or vermicompost application as well as spraying of vermiwash encourages good growth in vanilla (Thomas *et al.*, 2004).

2.2 Effect of inorganic nutrients

2.2.1 Growth

Plants, whether grown in the field or in pots, take their nutrition from inorganic minerals. Nitrogen is a vitally important plant nutrient and is the most frequently deficient of all nutrients. An adequate supply of nitrogen is associated with high photosynthetic activity and vigorous vegetative growth indicated by dark green leaves. Excess of nitrogen in relation to other nutrients like P and K can improve the vegetative growth. If nitrogen is used properly in conjunction with other major nutrient inputs, it can speed up the maturity of crops. Orchids, like any other plant, require nitrogen, phosphorus, potash and trace elements for healthy growth (Kang, 1979; Fitch, 1981).

Effectiveness of foliar application of nutrients in orchids is a debatable issue. Poole and Sheehan (1970) observed nutrient uptake by orchid leaves. However, the study by Benzing and Pridgeon (1983) revealed that the rate of absorption by leaves was less than one eighth of that observed roots. Sinclair (1990) was also of the opinion that under natural conditions, the absorption of water or nutrients by orchid leaves was rarely significant. Nitrogen has a positive response on vegetative growth. Phosphorus is related with root growth and early

maturity of the crop. Potassium is involved in enzyme activation, translocation of assimilates, protein synthesis and nitrogen uptake (Tisdale *et al*., 1995).

The plants require macronutrients, nitrogen, phosphorus and potassium in varied amounts. It is noticed that cultivated orchids needed a little supplementary nutrition other than provided by their growing medium (Sanford, 1974).

In *Dendrobium* Lim Hepa, increasing nitrogen dose from 50 ppm to 300 ppm and potassium from 25 ppm to 150 ppm showed clear effects on vegetative growth. Nitrogen at 300 ppm delayed flowering and enhanced stem length (Uesato *et al*., 1987).

Mukherjee (1990) recommended a formulation containing calcium nitrate, magnesium sulphate, potassium nitrate and ammonium sulphate as major components in addition to trace elements for ideal growth of pot grown orchids.

In comparison study of different levels of N in growing medium of *Phalaenopsis* and *Cattleya* seedlings, Sheehan (1960) reported increase in leaf growth with increase in nitrogen application. Penningsfield and Fast (1962) reported the effect of deficient and excess uses of nitrogen in *Cattleya*. Increased use led to rotting of roots and leaves and deficiency resulted in yellowing and wilting of leaves.

An increase in N was found to have a favourable effect on plant height as reported by Sagarik and Siripong (1963) in young *Dendrobium* hybrids. Northen (1970) observed that excess nitrogen to increased vegetative growth and delayed flowering in orchids.

Bhattacharjee (1977) reported that spraying N, P_2O_5 and K_2O 100 ppm each at fortnightly intervals was beneficial in *Bulbophyllum*. In *Cymbidium* and *Phalaenopsis* seedlings, 100 ppm N together with 50 ppm P and 25 ppm K was

found to be optimal (Poole and Seeley, 1978). Based on their studies on nutrient culture of *Cattleya, Cymbidium* and *Phalaenopsis,* they concluded that N concentration was the most important factor determining growth of all the three orchid genera. Khaw and Chew (1980) reported that the *Aranda* 'Noorah Alsagoff', the nutrient requirement found optimum for enhancing growth parameters was 20.9 mg N, 5.0 mg P, and 21.8 mg K, applied at weekly intervals. Along with that, 3.4 mg magnesium was also found to give good results.

According to Arditti and Ernst (1981), ammonium nitrate is the best source of N during the early *ex-vitro* stage of orchids. Bhattacharjee (1981) studied that the effect of N, P, K of 0, 500 and 1000 ppm on *Dendrobium moschatum* and concluded that markedly improved vegetative growth and flowering. Addition of P_2O_5 also influenced all vegetative characters and flower characters. Concentration of 500 ppm of all the three nutrients was best.

The application of liquid fertilizers, containing 500 ppm potassium nitrate, 500 ppm ammonium nitrate and 100 ppm ammonium sulphate on seedlings of *Cymbidium* 'Pharoah Pathfinder' resulted in overall increase in vegetative growth when applied as spray at weekly intervals for a period of 6 months (Bik and Berg, 1983). Spraying of nutrient solution of N, P and K 100: 20: 75 ppm, respectively, showed improvement in growth characters in *Cymbidium* and *Cattleya* (Johnson, 1984).

Higher dose of nitrogen was found to be beneficial under outdoor cultivation of orchids and longer pseudobulbs were produced when nitrogen was applied at 48 mg l^{-1} (Sakai *et al*., 1985). Schum and Fisher (1985) obtained higher number of leaves and fresh weight with N and K applied in the ratio 1:1.

Yadav and Bose (1989) reported that spraying of N, P and K at 1000 ppm each, enhanced length and number of leaves in *Aerides multiflorum*, but plants deficient in nitrogen showed stunted growth and early maturity. In study

conducted by Uesato *et al.* (1987) in *Dendrobium* 'Lim Hepa' reported that by increasing N dose from 50 to 300 ppm and K dose from 25 to 150 ppm gave positive results with respect to vegetative growth. Nitrogen at 300 ppm delayed flowering and enhanced stem length.

A fertilizer mixture of NPK (30:10:10) rich in nitrogen was found to be good for vegetative growth of orchids as reported by Boodley (1981); Linda (1987); Stewart (1988); Marguerite (1989) and Peter (1990). Abraham and Vatsala (1981) and Singh (1992) reported that N has significant influence on the vegetative growth of orchids. Stewart (1988) recommended a combination of 3:1:1 NPK for better vegetative growth and 1:1:1 for sustained growth respectively.

Seeni and Latha (1990) suggested that combination of diammonium phosphate and potassium nitrate (20:10:10 NPK) was by far the most effective in terms of rapid leaf and root growth in *Phalaenopsis*. A trial conducted to study the uptake of nitrate and ammonium by *Cymbidium sp*., *Bombidia sp* and *Dendrobium* cv. 'White Fairy' resulted in higher uptake rate for ammonium (Hew, 1993).

NPK 17:17:17 complex sprayed at weekly intervals at 10g l^{-1} could increase the number of clumps and leaves in *Cymbidium traceanum* (Sobhana and Rajeevan, 1995). Wadasinghe and Hew (1995) suggested that the leaves of backshoots of *Dendrobium* cv. Jashika Pink were an important source of photosynthates for the growth and flower production. Hence adequate nutrition in the current season becomes beneficial for the succeeding vegetative growth as well as flowering.

Wang (1996) observed that higher N rate produced wider leaf spread, more and larger leaves and greater total leaf area, regardless of the type of fertilizer used in young seedlings of *Phalaenopsis* cv. Tam Butterfly. In

Phalaenopsis 'Pink Chiffon', an increment of nitrogen from 50 to 1000 ppm, showed positive results on vegetative aspects, especially on the number of leaves and leaf area (Sheehan, 1996). Nair (2001) reported that the concentration of nitrogen significantly influenced the shoot emergence in Sonia 17.

Thekkayam (1996) reported that application of 300 ppm N, 400 ppm P and 300 ppm K from the time of planting to nine months after planting and thereafter a dosage of 400 to 500 ppm N, 400 ppm P and 500 ppm K showed positive results in *Arachnis* 'Maggie Oei Red Ribbon' grown in trenches .

Taejung *et al.* (1998) reported that healthy compact plants were produced in *Cymbidium* with NPK combination having high content of K. Leaf analysis showed high N content when 30:10:10 NPK was applied and least, when 6:40:6 NPK was given.

Studies conducted by Umamaheswari (1999) indicated that nutrient recommendation of 2.0 mg NPK each from three to six months, 6:2:2 NPK from six to nine months and 6:2:6 NPK from nine to twelve months were noticed good for growing orchids.

Mixture of N: P_2O_5: K_2O 3:1:1 applied during vegetative period and 1:2:2 applied during flowering period is very effective in orchids (KAU, 2007). The growth of orchids is markedly improved by regular schedule of fertilizing the plants in liquid form (Bose and Bhattacharjee, 1980).

Tsai *et al.* (1996) reported that nutrient uptake rate in *Cymbidium sinense* was highest with 20:10:15 NPK rate and with 0.5 per cent organic fertilizer. *Phalaenopsis* plants provide with higher N rate, regardless of which fertilizer was used produced wider leaf spread, more and longer leaves and greater total leaf area.

Binisha *et al.* (2003) found that the treatment combination of NPK 10:5:10 at 0.2% concentration along with *Azospirillum* proved to be very effective in improving the vegetative characters like plant height, number of leaves, number of shoots with leaves and girth of shoot, where as NPK 20:10:10 at 0.2 % concentration along with *Azospirillum* was found to improve the floral characters like days to first flowering, number of flowers per spike and spike length.

Shanker *et al.* (2003) reported that the effect of potting media *viz*., charcoal and gravel chips and nutritional spray of two concentrations @ 1 g l^{-1} and 1.5 g l^{-1} from 19:19:19 NPK based water soluble fertilizer at an interval of once in 4 and 8 days on the growth and yield of *Dendrobium* hybrid white hairy was investigated. The vegetative parameters like plant height, number of leaves, leaf area and flowering characters like flower bud appearance, flower bud opening, number of flower stalks per plant, length of flower stalk, flower diameter, longevity of flower stalk on the plant and vase life of flower stalk were found to be favourably influenced by both potting media and spraying of nutrients.

2.2.2 Flowering

Pradhan (1976) recommended the use of 1:1:1 of NPK mixture for flowering season. Boon (1982) and Merriman (1987) recommended N, P and K in the ratio 11:13:6 at weekly intervals for increased flower production during summer and autumn in *Oncidium* and *Cymbidium*, respectively. Stewart (1988) suggested that use of a high K content combination of NPK 1:1:3 was beneficial for flowering.

Influence of different levels of N, P, and K fertilization was also investigated by Bhattacharjee (1982) on *Aerides multiforum*. It showed that marked improvement in the production of more number of flower stalks per plant with maximum number of large sized flowers on longer stalks was recorded with

100 ppm each of N and P_2O_5 spray. Increased doses of P_2O_5 and K_2O also prolonged the vase life of flower spikes.

Longman (1989) recommended foliar feeding of mature flowering plants using NPK at 18:18:18. Yin-Tungwang (2000) studied the long–term effect of reduced fertilizer application and use of low N, high P and K fertilizer in the fall on reproductive performance and flower longevity of hybrid *Phalaenopsis* orchid.

Swapna (2000) noticed that application of NPK as ammonium nitrate, orthophosphoric acid and potassium nitrate 10:20:20 at 0.2 percent was significantly superior to all other nutrient treatments for highest spike production in *Dendrobium* var. Sonia 17.

Yoneda *et al.* (1999) studied the effect of macro element concentration on growth, flowering and nutrient absorption in *Odontoglossum* hybrid. They observed that low N rates resulted in shorter and thinner stalks, fewer flowers and advanced flowering date.

Wang (1996) reported that fertilizer application using 20.0, 8.6 and 16.6 NPK @ 1 gl^{-1} in *Dendrobium* 'Renappa' resulted in more number of inflorescence and flowers. Wang and Gregg (1994) reported that increasing the nitrogen application from 0.25 to 1.00 g l^{-1} increased the flower number, stalk diameter and stalk length. In *Cattleya* and *Phalaenopsis*, with an increase of NPK dose from 77:15.5:39.1 ppm to 308:62:156.40 ppm resulted in early flowering, increased fresh weight and increased N and K content in leaves (Tanaka *et al.*, 1981, 1988a, 1988b and 1989).

Bose (1978) suggested that growth and flowering in orchids are improved by a regular schedule of fertilizers in liquid form. Increased nitrogen supply in *Phalaenopsis* up to 1000 ppm produced longer flower spike and spike with increased girth. With an increase in the NPK dose of 3:2:2 to 5:5:2 improvement

in flowering of *Dendrobium* Madame Pompadour was observed (Vachorotayan and Keethapirom, 1975).

2.2.3 Chlorophyll content

Sanford (1974) reported that the amount of chlorophyll content present in leaves was adequate for photosynthesis. Kirichenko *et al.* (1989) found that the pigment system of flowers had low chlorophyll a:b ratio compared to that of leaves in *Dendrobium* and several other species.

Chae-Soochan *et al.* (1998) expressed that the ratio of chlorophyll a,b was in the ratio of 2:1 in several *Dendrobium nobile* cultivars. Based on a study in *Dendrobium*, Khoo and Hew (1999) reported that the chlorophyll a:b ratio was same in the case of flowers and leaves where as the total chlorophyll content in flowers declined as they matured.

2.3 Orchidaceous mycorrhizae

The association between orchids and mycorrhizal fungi is included in this category. These fungi enter plant cells by invaginating the cell membrane and forming hyphal coils within cells of the protocorm and developing root. These coils are active for only a few days, after which they loose turgor and degenerate while nutrient contents are absorbed by the developing orchid. The fungi participating in this type of symbiosis are Basidiomycetes similar to those involved in decaying wood (e.g., *Coriolus* sp., *Fomes* sp., *Marasmius* sp.) and pathogenesis (e.g., *Armillaria* sp. and *Rhizoctonia* sp.). In mature orchids, mycorrhizae also have roles in nutrient uptake and translocation. Orchid mycorrhizas support orchid development and initial root development by delivering nutrients for germination, protocorm and initial root development (Peterson and Massicotte, 2004).

2.3.1 Orchid mycorrhiza association

Mycorrhiza is highly evolved, symbiotic associations between soil fungi and plant roots. The mycorrhiza plays an important role in the life cycle of plants of the family Orchidaceae. Mycorrhiza in vanilla roots was first recorded by Decordenoy (1904), who observed the infection of fungi on the roots adhering to their nutrients *via* the fungus. The mycorrhizal association is reproduced by Tonnier (1954) in flasks by growing vanilla seedlings in agar with *Rhizoctonia*. Warcup and Talbot (1967) found *R. repens* as endomycorrhizal fungi on many orchidaceous plants.

Smith (1966) observed that orchid mycorrhizal fungi like *Rhizoctonia repens* and *R. solani* were able to utilize and translocate carbohydrates to the orchid plants and there by increasing the plant growth. Numerous isolates of genus *Rhizoctonia* have been obtained from orchid root growth in culture and shown to establish a mycorrhizal relationship when allowed to infect germinating orchid seeds (Warcup, 1975).

Alexander and Hardley (1983) has shown that mycorrhizal infections in the orchid *Goodyera repens* bring about enhanced growth rate and increased P concentration with in tissues. Orchid mycorrhiza showed improved resistance to drought and environmental stress (Allen and Boosalls, 1983). Terashita (1982) and Masuhara and Katsuya (1992) observed that this adult orchid form mycorrhizal association often with *R. repens* and occasionally with *R. solani.*

Ogoshi *et al.* (1983) reported that some isolates of binucleate *Rhizoctonia* induce symbiotic germination of some of the Australian orchids. Warcup (1983) has shown that some *R. solani* isolates induced symbiotic germination of seeds of *Microtis unifolia, Prasophyllum regiium* and *orchis moria*. Abbot and Robson (1984) reported that mycorrhizal inoculation increased the growth and yield of different crop plants by improving P uptake and yield of different crop

particularly when these nutrients are sparingly available orchid mycorrhizal fungi improved nitrogen nutrition in orchid plants by facilitating the use of certain nitrogen forms that were difficult for the non mycorrhizal plants to exploit (Press, 1986).

Association of orchid mycorrhiza and arbuscular mycorrhizal fungi in vanilla was studied in detailed by Madhaiyan (1999). It was observed that inoculation of orchid mycorrhiza and AMF significantly increased the shoot and root dry weight of vanilla.

Significant increase in shoot and root length of vanilla seedlings was observed due to the inoculation of mycorrhizal fungi, AMF or combination of both over control. Madhaiyan *et al.* (2003) observed that infection of orchid mycorrhiza and peletion formation in cortical cells, particularly in the outer cortical cells of vanilla seedlings inoculated with orchid mycorrhiza.

2.4 Plant Growth Promoting Root Endophyte (PGPRE)

Taken literally, the word endophyte means 'in the plant' (endon-within; phyton-plant). The usage of this term is as broad as its literal definition and spectrum of potential plant hosts and inhabitants, including bacteria (Kobayashi and Palumbo 2000), fungi (Stone *et al.,* 2000), algae (Peters 1991), and insects (Feller 1995). Any organ of the host can be colonized. Equally variable is the life-history strategy of the symbiosis, ranging from facultatively saprobic, to parasitic, to exploitive, to mutualistic. However, common to all endophytic interactions is the provision of nutrients and a buffer from external environmental stresses and microbial competition. Unlike mycorrhizal fungi, endophytes reside entirely within host tissues and emerge during host senescence. These fungi comprise a phylogenetically diverse group that are members of the Dikarya (Schardl and Leuchtmann, 2005; Van Bael *et al.,* 2005; Girlanda *et al.,* 2006; Arnold and Lutzoni, 2007). While most endophytes belong to the ascomycota clade, some

belong to the Basidiomycota. Although these fungi are often grouped together, they can be discriminated into different functional groups just as has been done with mycorrhizal fungi (Brundrett, 2006).

2.4.1 Root symbioses

Previously, only mycorrhizal fungi were considered mutualistic symbionts of plant roots. In this definition, fungi that colonize plants without causing visible disease symptoms at any specific moment (Petrini, 1991, Wilson, 1995) are called fungal endophytes. In all ecosystems, many plant parts are colonized by fungal endophytes.

Non-mycorrhizal microbes such as *Piriformospora indica* (Verma *et al.*, 1998), *Fusarium* spp. and *Cladorrhinum foecundissimum* (Gasoni and Gurfinkel, 1997; Kuldau and Yates, 2000; Sieber, 2002) *Chaetomium* spp. (Vilich *et al.*, 1998) have been shown to improve the growth of their hosts after root colonisation. In these symbioses, fungi most probably benefit by obtaining a reliable nutritional source while hosts may acquire multiple advantages beside an improved growth. Various hosts inoculated with root endophytes displayed an increased tolerance to abiotic stresses and induced resistance.

The nature of endophytic colonization of plants does not depend only on its adaptation to a particular host or organ but also on innate but variable virulence patterns encountering host defense responses and environmental conditions (Schulz and Boyle, 2005).

2.4.2 Uptake of nutrients

Endophytes promote the growth of plants in various ways, through secretion of plant growth regulators; e.g. indole-acetic acid (Lee *et al.* 2004), phosphate solubilizing activity (Wakelin *et al.*, 2004), by enhancing hyphal

growth and mycorrhizal colonization (Will and Sylvia 1990), production of siderophores (Costa and Loper 1994) and by supplying biologically fixed nitrogen (James *et al.*, 1994). Other effects of endophyte infection on the host plant include osmotic adjustment, stomatal regulation, modification of root morphology, enhanced uptake of minerals and alteration of nitrogen accumulation and metabolism (Malinowski and Belesky, 1999; Belesky and Malinowski, 2000).

Endophyte–infected plants often grow faster than non infected ones (Cheplick *et al.*, 1989). This effect is at least in part due to the production of phytohormones such as indole-3-acetic acid (IAA), cytokinins, and other plant growth promoting substances (Zou and Tan, 1999) and or partly owing to the fact that endophytes could have enhanced the hosts uptake of nutritional elements such as nitrogen and phosphorus (Gasoni and Gurfinkel, 1997; Malinowski and Belesky, 1999).

Endophytes may enhance growth by producing phytohormones without any apparent facilitation of host nutrient uptake or stimulation of host nutrient metabolism. The endophytic fungi may enhance biomass by producing growth hormones or inducing the host hormone production (Petrini, 1991; Schulz and Boyle, 2005).

2.4.3 Induction of resistance

The systemic and foliar endophytes have received particular attention and can reduce herbivory by producing alkaloids toxic to insects and vertebrates (Schardl, 2001).

Mycorrhizal fungi are also capable of inducing resistance, and a number of mechanisms have been proposed for this resistance induction (Azcon and Barea, 1996).

Many such mechanisms of mycorrhiza-induced resistance are related to the nutritional status of the host, often correlated with mycorrhizal colonization, although some non-nutritional alternatives have also emerged (Borowicz, 2001).

Mycorrhizas can also mitigate the effects of herbivores, although these effects are highly variable (Gehring and Whitman, 2002).

2.5 *Piriformospora indica*

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). Depending on the ultra structure of hyphae (presence of dolipore septa) and 18s-rRNA gene sequence, *P. indica* was grouped in the class Hymenomycetes (Basidiomycota) (Verma *et al.*, 1998). Serological classification showed close antigenic properties with mycorrhizal fungi (Varma *et al.*, 2001).

In contrast to mycorrhizal fungi, this fungus can be cultured axenically on various synthetic simple and complex media at 25-35^{o}C (Varma *et al.*, 1999).

Morphologically, *P.indica* hyphae are white and almost hyaline. They are thin walled irregularly septated and 0.7 to 3.5 µm in diameter. Septate hyphae often show anastmosis. Each hyphal segment is multinucleate with variable numbers of nuclei. Hyphal tips differentiate into chlamydospore of 16- 25 µm length and 10-17 µm in width, which emerge individually or in clusters. Each spore contains 8-25 nuclei. So far, neither clamp connections nor sexual structures could be observed (Varma *et al.*, 2001).

2.5.1 Growth promotional effect

P. indica tremendously improves the growth and overall biomass production of diverse hosts, including legumes (Varma *et al.*, 1999, 2001; Singh *et al.*, 2002a), medicinal and other plants of economic importance (Rai *et al.* 2001; Singh *et al.*2003 a, b).

Pronounced growth promotional effects were also seen with terrestrial orchids (Blechert *et al.*1999; Singh and Varma 2000; Singh *et al.* 2000, 2002 b). The fungus promises to be a potential source for colonizing the orchids, their better growth and higher rate of survival of seeds (Rexer *et al.*, 2000).

The medicinal plants *Spilanthes calva* and *Withania somnifera* were inoculated with *Piriformospora indica*, in nurseries and subsequently transferred to the field. A significant increase in growth and yield of both plant species was recorded relative to uninoculated controls. Shoot and root length, biomass, basal stem, leaf area, overall size, number of inflorescences and flowers and seed production were all enhanced in the presence of the fungus. Net primary productivity was also higher than in control plants. (Rai *et al.*, 2001).

This fungus has great potential in forestry, horticulture, agriculture, viticulture and especially for establishment of tissue culture-raised plants much needed in the plant industry (Singh *et al.*, 2003a).

P. indica acts as plant growth promoter (auxin), biofertilizer (solid and liquid engineering), bioregulator, immunomodulator, phytoremediator, biological hardening agent for micro propagated plantlets; imparts resistance against heavy metals, biocontrol against insects and pathogens, stress tolerance- both salt and temperature; works as stimulator with Plant Growth Promoting Rhizobacteria (PGPRs), antioxidant and drug enhancer, anti-ageing agent; promotes synthesis of anti-cancer drugs and possesses potential for its use in bionanomedicines (Varma

et al., 1999; Rai *et al.*, 2004; Waller *et al.*, 2005; Deshmukh *et al.*, 2006; Shahollari *et al.*, 2005, Serfling *et al.*, 2007; Sherameti *et al.*, 2008, 2008b).

Inoculation dramatically enhanced the number and length of the adventitious roots in pelargonium and poinsettia. *P. indica*-based biotechnology is proposed as a new tool for improving plant propagation systems of plant species or cultivars with low to moderate capacity of adventitious root formation (Druege *et al.*, 2007).

Rai and Varma (2005) observed a profuse proliferation of root of *Adhatoda vasica* after inoculation of *P.indica* and root colonization of *A. vasica* by *P.indica* increased with time from 53% after 2 months to 95% after 6 months.

2.5.2 Phosphate mobilization

Studies on *P. indica* have shown fungal-mediated uptake of radio-labelled phosphorus from the medium and its translocation to the host in an energy-dependent process, evident by a sharp increase in its content in the shoot. *P. indica* produces significant amounts of acid phosphatases for the mobilization of insoluble phosphates and translocates the phosphorus to the host energy-dependent process (Singh *et al.*, 2000).

2.5.4 Root colonization

P. indica infests roots of a broad range of monocotyledonous and dicotyledonous plants (Verma *et al.*, 1998; Pham *et al.*, 2004). Endophytic root colonization by this fungus confers enhanced growth to the host plant (Varma *et al.*, 1999; Peskan-Bergheofer *et al.*, 2004) and provides protection against biotic and abiotic stresses and it requires host cell death for barley root colonization (Deshmukh *et al.*, 2006).

2.5.5 Plant protection

Waller *et al.* (2005) reported that the potential of *P. indica* to induce resistance to fungal diseases, tolerance to salt stress and grain yield elevation in the monocotyledonous plant barley.

As endophytic growth of *P. indica* is restricted to the root, the fungus is able to provide systemic protection due to a yet unknown mechanism of induced resistance. As *P. indica* can easily be cultured without a host plant (Varma *et al.*, 1999), it is suitable as a model system to study compatible plant-microbe interactions.

Materials and methods

3. MATERIALS AND METHODS

The investigation envisages to study the influence of organic and inorganic nutrients and *Piriformospora indica* (PGPRE) on growth and development of *Dendrobium* cv. Earsakul. The procedures adopted are discussed below.

3.1 Experiment site

Studies were conducted over a period from February 2008 to January 2009 at the orchidarium of All India Co-ordinated Floriculture Improvement Project in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, Kerala.

3.1.1 Location

The College of Horticulture, Vellanikkara is situated at a latitude of 10° 31' N and longitude of 76° 3' E. The area lies 22.25 m above MSL and enjoys the typical warm humid tropical climate of Kerala.

3.1.2 Climate

The climatalogical data during the period of investigation are given in Appendix I.

3.2 Materials

3.2.1 Variety

Commercially cultivated orchid hybrid variety *Dendrobium* cv. Earsakul was used for the experiment (Plate 1).

Plate 1. Variety used for the study - *Dendrobium* cv. Earsakul

3.2.2 Planting material

Three months old tissue cultured plants of the *Dendrobium* cv. Earsakul were used.

3.2.3 Potting media

On media containing tile pieces, charcoal and coconut fibre were used.

3.2.4 Shading

The plants were grown under 50 per cent shade provided by green coloured shade nets.

3.2.5 Inorganic nutrients

The major nutrients N:P_2O_5:K_2O at two different ratios, viz., 3:1:1 and 1:2:2 @ 0.2 % 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, orthophosphoric acid and potassium nitrate.

3.2.6 Organic manures

Organic manures such as organic mixture (groundnut cake contains N 6.5%, P 1.3%, K 1.5%, neem cake contains N5.5%, P 1.1%, K 1.5%, and bone meal contains N 2 to 4%, P 20 to 25%), vermiwash, bone meal, fresh cow dung slurry were used and they were applied at fortnightly intervals.

3.2.7 *Piriformospora indica* (PGPRE)

The fungal culture (*P. indica*) was mixed with vermiculite @ 1g per 100g of vermiculite and filled in the pots (Plate 2).

3.3 Methods

3.3.1 Design of the experiment

The experiment was laid out in CRD with nine treatments, three replications and three plants per replication for recording observations. .

3.3.2 Treatments

Treatments comprised of organic manures, inorganic and *Piriformospora indica* (PGPRE). Treatment details of the experiment are furnished in Table 1.

Table 1. Treatment details of the experiment

Sl. No	Treatments	Notations
1	Package of practices (POP) recommendation of KAU for orchids	T_1
2	POP+ Organic mixture	T_2
3	POP+ Vermiwash	T_3
4	POP+ Bone meal	T_4
5	POP+ *Piriformospora indica* (PGPRE)	T_5
6	POP+ Organic mixture+ *P. indica*+ Bone meal	T_6
7	POP+ Vermiwash+ *P. indica*+ Bone meal	T_7
8	POP+ Organic mixture+ Vermiwash+ *P. indica*+ Bone meal	T_8
9	Organic mixture+ Vermiwash+ *P. indica*+ Bone meal	T_9

Fungal culture

Fungal culture+ Vermiculite
(@ 1g per 100g of vermiculite)

Plate 2. *Piriformospora indica* fungal culture

3.3.2.1 POP recommendation

Spraying with supernatant liquid of cow dung slurry (1 Kg of fresh cow dung in 5 l of water), 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 (dose of the mixture is 2-3 g per litre of water, spraying weekly twice). NPK as ammonium nitrate, ortho phosphoric acid and potassium nitrate respectively are spraying twice/weekly intervals.

3.3.2.2 Organic mixture

Bone meal, neem cake, groundnut cake 100 g each, soaked in water for 3-4 days and diluted to 10-15 times with water, filtered and sprayed over the plants at 15 days intervals.

3.3.2.3 Vermiwash

Vermiwash diluted to 3 per cent and sprayed at 15 days intervals.

3.3.2.4 *Piriformospora indica* (PGPRE)

The *P. indica* was mixed with vermiculite @ 1g per 100g of vermiculite and applied near the root zone at the time of planting.

3.3.2.5 Bone meal

Applied near the root zone at the time of planting @ 15 g per plant.

3.3.3 Plant protection

Streptomycin sulphate (0.20 g l^{-1}) was applied once in three months for the control of the bacterial soft rot disease. Snails were controlled by application of metaldehyde bait 2.5 per cent.

3.4 Observations

The observations on vegetative and flowering characters were taken from three sample plants in each replication and recorded at monthly intervals from May 2008 to June 2009.

3.4.1 Growth characters

3.4.1.1 *Plant height*

The height of the plant was measured and recorded from the base of the plant to tip and was expressed in cm.

3.4.1.2 *Number of leaves*

Number of leaves on each shoot was counted and recorded.

3.4.1.3 *Number of shoots*

Number of shoots per plant was counted and recorded.

3.4.1.4 *Girth of the shoots*

Girth of the biggest shoot was measured and expressed in cm.

3.4.1.5 ***Internodal length***

The length of the last developed internode from the mature shoot was taken and expressed in cm.

3.4.2. Root parameters

Root parameters were conducted at two times, one at six months after planting and another at the time of flower bud formation.

3.4.2.1 ***Number of roots***

Number of roots per plant was counted at the time of destructive sampling and expressed in number.

3.4.2.2 ***Root length***

Root length was taken at the time of sampling and expressed in cm.

3.4.2.3 ***Root volume***

Root volume was measured at the time of sampling and expressed in ml.

3.4.2.4 Root colonization

The method proposed by Giovannetti and Mosse (1980) was followed for assessment of root colonization of *P. indica*. The root-pieces (1 cm) were selected at random from the stained samples and mounted on grid-intersects glass slide. In all, 15 root pieces per sample were observed and per cent infection was calculated as follows

$$\text{Per cent colonization} = \frac{\text{No. of root segments colonized}}{\text{Total no. of segments observed}} \times 100$$

3.4.3 Physiological parameters

The physiological parameters were recorded at six months after planting.

3.4.3.1 *Leaf area*

The length and breath of leaf was recorded and the area of leaf was computed using the formula, (Swapna, 2000)

$$a = 2.78 + 0.688\ lb$$

Where, a = leaf area in cm^2

l = length of the leaf in cm

b = maximum width of the leaf in cm.

3.4.3.2 *Chlorophyll content*

The chlorophyll content of the leaves was determined using Dimethyl sulphoxide (DMSO) (Shoaf and Livm, 1976). The most recent, fully developed leaf was taken and cut into small pieces. Incubated the sample in 7.0 ml of DMSO at 65°C for 30 minutes. At the end of the incubation period the supernatant solution was decanted and the leaf tissue was discarded. The volume was made up to 10 ml with DMSO. The absorbance was read at 645 and 663 nm using DMSO as blank. Chlorophyll a, b and total ratio was calculated using the formula, and expressed in mg g^{-1} leaf wt.

$$\text{Chlorophyll 'a'} = 12.7\ (A_{663}) - 2.69\ (A_{645}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Chlorophyll 'b'} = 22.9\ (A_{645}) - 4.68\ (A_{663}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Total chlorophyll} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000 \times W \times a}$$

Where,

A = Absorbance at specific wave lengths 645 and 663 nm.

V = Final volume of the chlorophyll extract (ml),

W = Fresh weight of the sample (g),

a = Path length of light (1 cm).

3.4.3.3 ***Relative growth rate***

Relative growth rate (RGR) is the rate of increase in dry weight per unit dry weight per unit time expressed in g g^{-1} day. It is calculated by the formula suggested by Blackman (1919).

$$RGR = \frac{\text{Loge } W_2 - \text{Loge } W_1}{(t_2 - t_1)}$$

Where, W_1 and W_2 are the dry weight of the whole plant at time t_1 and t_2 respectively.

3.4.3.4 ***Net assimilation rate***

Net assimilation rate (NAR) refers to the change in dry weight of the plant per unit leaf area per unit time. NAR can be determined by measuring plant dry weight and leaf area periodically during the growth and is commonly expressed in g m^{-2} day^{-1} (Williams, 1946).

$$NAR = \frac{W_2 - W_1}{(t_2 - t_1)} \times \frac{\text{Loge } L_2 - \text{Loge } L_1}{L_2 - L_1}$$

Where, L_1 and L_2 are the LAI of plant and W_1 and W_2 as the whole plant dry weight at time t_1 and t_2 respectively.

3.4.3.5 ***Crop growth rate***

Crop growth rate (CGR) was calculated using the formula of Watson (1958) and expressed in g m^{-2} day^{-1}.

$$CGR = NAR \times LAI$$

3.4.3.6 ***Dry matter production***

Pseudo stems, leaves and roots of the uprooted plants were dried to a constant weight at 70°C–80°C in a hot air oven. The sum of the dry weights of component parts gave total dry matter production and expressed as g $plant^{-1}$.

3.4.4 Floral characters

3.4.4.1 ***Days to flowering***

Total number of days taken for the flower to open was recorded.

3.4.4.2 ***Days to first flower opening***

Total number of days taken for the first flower to open was recorded.

3.4.4.3 ***Days to last flower opening***

Total number of days taken for the last flower to open was recorded.

3.4.4.4 ***Length of the spike***

Length of spike was measured from the point of emergence to the tip of flower pedicel emergence and expressed in cm.

3.4.4.5 ***Number of flowers per spike***

Number of flowers per spike was counted and recorded.

3.4.4.6 ***Size of flower***

Size of individual flowers was recorded by noting down the length and breadth of the flowers, expressed in cm.

3.4.4.7 ***Number of spike per plant***

Number of spikes initiated in each plant, during the period of observation was counted and recorded.

3.4.4.8 ***Vase life***

The number of days that the flowers can be kept fresh, after removing from the plant was observed and recorded.

3.5.5 Nutrient analysis

Nutrient analysis was conducted at two times, one at six months after planting and another at the time of flower bud formation.

Plant parts like shoot, leaf and root were taken separately at six MAP and at the time of flower bud formation, washed and dried in shade for one week and then dried in oven at 65-70^{o} C for 6 hours. The dried samples were ground, mixed and then chemically analysed for major nutrients, *viz*., nitrogen, phosphorus, potassium, calcium and magnesium and micro nutrients, *viz*., Cu, Fe, Zn, Mn.

3.5.5.1 *Nitrogen*

One gram dried leaf sample was digested using concentrated sulphuric acid; oxidized using 30 % H_2O_2 and the N content was estimated by Microkjeldahl method (Jackson, 1958).

3.5.5.2 *Phosphorus*

The leaf sample (0.5 g) was digested using diacid mixture of nitric acid and perchloric acid taken in the ratio of 9:4 (Johnson and Ulrich, 1959). Finally phosphorus was estimated using vanadomolybdophosphoric yellow colour method (Jackson, 1958). The intensity of yellow colour was read in Spectronic-20 at 470 nm.

3.5.5.3 *Potassium*

From the digested sample as mentioned above, an aliquot was prepared and K content was estimated using a flame photometer. Micro nutrients (Cu, Fe, Zn, Mn) and Secondary nutrients (Ca, Mg,) were estimated by Atomic Absorption Spectrophotometer (Jackson, 1958).

3.5.6 Incidence of pest and diseases

Incidence of pest and diseases was also observed during the period of study and proper control measures were taken.

3.5 Statistical analysis

The experimental data were analysed by the ANOVA (Analysis of Variance technique (Panse and Sukhatme, 1985) and DMRT (Duncan's Multiple Range Test) technique. MSTATC and MS-Excel software were used for computation of data.

Results

4. RESULTS

The results of the experiments entitled "Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul" are presented in this chapter.

4.1 Growth characters

Monthly observations on growth parameters of the cultivar Earsakul upto 14 MAP are presented in Tables 2-6 and plates 3& 4.

4.1.1 Plant height

Table 2 shows the influence of nutrients and *Piriformospora indica* on plant height.

Upto 4 MAP, no significant difference in plant height was observed among the treatments.

From 5 month onwards there was significant difference among the treatments. During fifth month the treatment T_5 recorded the highest value (17.26 cm) followed by T_2 and T_6 and all these treatments were found to be statistically on par. The minimum plant height was recorded by the treatment T_3 which was on par with all other treatments.

At 6 MAP, treatment T_5 recorded maximum plant height (17.66 cm) followed by T_6, T_2, T_8, T_1 and T_7. The minimum value was recorded by the treatment T_3 which was on par with treatments T_9 and T_4. From seven to twelve months, no significant difference was observed among the treatments.

Six months after planting

At the time of flower bud formation

Plate 3. View of the experimental plot at different stages of growth

Table 2. Influence of nutrients and *Piriformospora indica* on plant height (cm)

Treatment	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP	14 MAP
T_1	6.69^{a}	8.69^{a}	9.61^{a}	12.63^{a}	13.31^{b}	14.09^{ab}	15.09^{a}	15.82^{a}	16.69^{a}	17.33^{a}	17.98^{a}	18.75^{a}	20.10^{ab}	20.79^{ab}
T_2	6.35^{a}	9.72^{a}	10.94^{a}	14.23^{a}	14.40^{ab}	14.73^{ab}	14.65^{a}	14.82^{a}	15.17^{a}	15.09^{a}	15.53^{a}	17.17^{a}	20.47^{ab}	20.37^{ab}
T_3	7.51^{a}	9.60^{a}	10.95^{a}	11.53^{a}	11.80^{b}	12.00^{b}	12.25^{a}	12.29^{a}	13.50^{a}	13.64^{a}	15.09^{a}	15.77^{a}	16.43^{b}	18.12^{b}
T_4	7.21^{a}	9.47^{a}	11.07^{a}	12.83^{a}	12.34^{b}	12.91^{b}	12.84^{a}	12.97^{a}	12.67^{a}	13.18^{a}	14.29^{a}	14.98^{a}	19.75^{ab}	20.89^{ab}
T_5	8.85^{a}	10.61^{a}	11.49^{a}	14.83^{a}	17.26^{a}	17.66^{a}	18.39^{a}	16.02^{a}	17.78^{a}	17.43^{a}	18.20^{a}	18.78^{a}	25.52^{a}	25.33^{a}
T_6	7.94^{a}	10.11^{a}	12.22^{a}	14.42^{a}	14.22^{ab}	15.28^{ab}	16.37^{a}	16.87^{a}	17.07^{a}	17.11^{a}	17.72^{a}	18.20^{a}	22.40^{ab}	24.27^{ab}
T_7	7.73^{a}	11.14^{a}	12.13^{a}	13.38^{a}	13.60^{b}	14.00^{ab}	14.19^{a}	15.01^{a}	15.78^{a}	15.05^{a}	16.10^{a}	17.32^{a}	22.39^{ab}	24.29^{ab}
T_8	6.00^{a}	9.00^{a}	10.72^{a}	12.87^{a}	13.70^{b}	14.58^{ab}	15.50^{a}	16.13^{a}	16.33^{a}	16.73^{a}	18.08^{a}	17.74^{a}	24.55^{a}	25.98^{ab}
T_9	7.39^{a}	9.33^{a}	10.56^{a}	11.81^{a}	12.88^{b}	13.39^{b}	14.78^{a}	15.12^{a}	15.52^{a}	15.54^{a}	16.76^{a}	16.00^{a}	17.51^{b}	20.82^{b}

During 13 MAP, significant difference in plant height was observed and treatments T_5 and T_8 showed maximum plant height of 25.52 cm and 24.55 cm respectively. During 14 MAP significant differences was observed among treatments and T_8 and T_5 showed maximum value of 25.98 cm and 25.33 cm respectively. The minimum value was recorded by the treatment T_3 and on par with all other treatments. In general treatment T_3 registered the lowest plant height in all the growth stages.

4.1.2 Number of leaves per plant

Data pertaining to number of leaves per plant are presented in Table 3.

The treatments did not record significant difference with respect to number of leaves produced during first and second month of plant growth.

During third MAP maximum number of leaves was recorded in treatment T_5which was on par withal other treatments except T_9.

From 4 to 14 MAP all treatments were found to be statistically on par with respect to the number of leaves per plant.

4.1.3 Number of shoots per plant

Data related to number of shoots per plant are presented in Table 4.

The treatments were on par with each other and they did not significantly differ with respect to number of shoots produced during one MAP to 2 MAP and 4 MAP to 13 MAP.

Table 3. Influence of nutrients and *Piriformospora indica* on number of leaves

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP	14 MAP
T_1	5.00^{a}	6.11^{a}	6.89^{ab}	5.67^{a}	6.44^{a}	6.56^{a}	3.56^{a}	4.11^{a}	5.34^{a}	5.11^{a}	6.11^{a}	7.00^{a}	8.89^{a}	9.89^{a}
T_2	6.22^{a}	7.67^{a}	7.56^{ab}	6.22^{a}	6.11^{a}	6.56^{a}	4.00^{a}	5.67^{a}	6.66^{a}	6.11^{a}	7.33^{a}	7.67^{a}	10.89^{a}	12.22^{a}
T_3	4.67^{a}	5.56^{a}	7.56^{ab}	6.67^{a}	5.11^{a}	5.44^{a}	3.11^{a}	4.21^{a}	4.89^{a}	5.45^{a}	5.78^{a}	6.78^{a}	7.34^{a}	8.11^{a}
T_4	4.89^{a}	7.56^{a}	6.89^{ab}	5.33^{a}	6.22^{a}	7.00^{a}	3.55^{a}	4.22^{a}	5.32^{a}	5.56^{a}	6.45^{a}	6.10^{a}	7.99^{a}	10.11^{a}
T_5	6.22^{a}	8.34^{a}	8.78^{a}	6.00^{a}	6.67^{a}	7.00^{a}	3.78^{a}	5.00^{a}	5.89^{a}	6.22^{a}	7.11^{a}	8.67^{a}	10.78^{a}	12.33^{a}
T_6	4.78^{a}	6.67^{a}	6.78^{ab}	5.22^{a}	5.78^{a}	6.54^{a}	3.78^{a}	3.89^{a}	5.22^{a}	4.66^{a}	5.55^{a}	6.44^{a}	7.78^{a}	10.11^{a}
T_7	5.32^{a}	6.89^{a}	6.78^{ab}	5.33^{a}	4.66^{a}	5.67^{a}	3.22^{a}	3.44^{a}	4.55^{a}	4.53^{a}	5.44^{a}	6.56^{a}	7.68^{a}	9.11^{a}
T_8	4.56^{a}	6.44^{a}	7.22^{ab}	5.00^{a}	5.99^{a}	6.78^{a}	4.66^{a}	5.00^{a}	6.33^{a}	5.78^{a}	6.89^{a}	8.22^{a}	9.89^{a}	11.78^{a}
T_9	4.67^{a}	6.11^{a}	5.89^{b}	5.11^{a}	5.43^{a}	5.78^{a}	3.89^{a}	4.00^{a}	5.67^{a}	5.22^{a}	6.22^{a}	5.78^{a}	8.68^{a}	9.77^{a}

Table 4. Influence of nutrients and *Piriformospora indica* on number of shoots per plant

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP	14 MAP
T_1	3.66^{a}	6.11^{a}	5.11^{b}	4.00^{a}	3.78^{a}	3.89^{a}	3.89^{a}	6.11^{a}	7.22^{a}	6.67^{a}	7.43^{a}	8.22^{a}	8.78^{a}	10.00^{ab}
T_2	4.00^{a}	5.78^{a}	6.43^{ab}	4.78^{a}	3.56^{a}	3.89^{a}	3.11^{a}	6.43^{a}	7.43^{a}	7.22^{a}	7.89^{a}	9.67^{a}	9.78^{a}	11.22^{a}
T_3	3.33^{a}	5.11^{a}	5.78^{ab}	4.11^{a}	3.22^{a}	3.67^{a}	3.00^{a}	5.22^{a}	5.67^{a}	5.78^{a}	6.56^{a}	6.67^{a}	8.11^{a}	6.57^{b}
T_4	3.44^{a}	5.78^{a}	6.89^{a}	4.22^{a}	3.33^{a}	4.00^{a}	3.11^{a}	5.44^{a}	6.33^{a}	7.44^{a}	8.22^{a}	8.78^{a}	9.67^{a}	10.44^{ab}
T_5	3.22^{a}	5.33^{a}	5.33^{ab}	4.76^{a}	3.55^{a}	3.89^{a}	3.11^{a}	7.00^{a}	7.89^{a}	7.56^{a}	8.67^{a}	10.11^{a}	11.22^{a}	12.11^{a}
T_6	3.33^{a}	5.11^{a}	5.33^{ab}	3.56^{a}	3.55^{a}	4.00^{a}	2.78^{a}	4.89^{a}	5.55^{a}	5.44^{a}	6.31^{a}	6.89^{a}	7.44^{a}	8.55^{ab}
T_7	4.45^{a}	4.78^{a}	5.66^{ab}	3.89^{a}	3.77^{a}	4.11^{a}	2.53^{a}	5.11^{a}	5.45^{a}	5.89^{a}	6.89^{a}	6.44^{a}	7.31^{a}	8.22^{ab}
T_8	3.67^{a}	5.44^{a}	6.44^{ab}	4.22^{a}	3.88^{a}	3.88^{a}	3.00^{a}	5.44^{a}	6.11^{a}	6.22^{a}	7.22^{a}	9.22^{a}	9.33^{a}	10.89^{ab}
T_9	3.67^{a}	5.00^{a}	5.67^{ab}	4.56^{a}	3.22^{a}	3.77^{a}	2.78^{a}	5.67^{a}	6.44^{a}	5.77^{a}	6.89^{a}	8.56^{a}	10.22^{a}	9.78^{ab}

During 3 MAP, significant difference in production of number of shoots per plant was observed among the treatments. The treatment T_4 (6.89) recorded the maximum shoots per plant and which was on par with all other treatments except T_1 (5.11).

At 14 MAP, significant difference was noticed with respect to number of shoots. Maximum number of shoots was recorded in the treatment T_5 (12.10) followed by T_2 (11.22) which was on par with T_8 (10.89), T_4 (10.44), T_1 (10.00), T_9 (9.78), T_6 (8.55). The lowest number of shoots was recorded in the treatment T_3 (6.56).

4.1.4 Girth of the shoot

No significant difference was noticed with respect to girth of the shoot produced from first to six MAP (Table 5).

During seventh and eighth MAP significant difference among treatments was noticed. The treatment T_9 recorded the maximum value of 2.27 cm and 2.42 cm at seventh and eighth MAP respectively. The treatment T_3 recorded the minimum value of 1.27 cm and 1.48 cm at seventh and eighth MAP respectively. All other treatments were found to be on par with each other.

Highest value for the shoot girth was recorded in the treatment T_8 followed by T_9 at 9 and 10 MAP. This was on par with T_7, T_5, T_4, T_2 and T_1.

At 11 MAP significant variation was obtained due to the influence of treatments. During the month, the treatment T_8 again recorded the highest value of 2.92 cm followed by treatments T_5 (2.26 cm), T_1 (2.24 cm), T_7 (2.20 cm), T_4 (2.04 cm)

Table 5. Influence of nutrients and *Piriformospora indica* on girth of the shoot (cm)

Treatment	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP	14 MAP
T_1	0.89 a	1.24 a	1.29 a	1.71 a	1.59^{a}	1.51^{a}	1.66^{a} b	1.68^{a} b	1.84^{a} b	1.90^{a} b	2.24^{a} bc	2.57 b	2.96^{b} cd	3.11 ab
T_2	1.03 a	1.03 a	1.08 a	1.15 a	1.31^{a}	1.49^{a}	1.53^{a} b	1.53 b	1.62^{a} b	1.72 b	1.93^{b} c	3.02 ab	3.23^{a} bc	3.46 ab
T_3	0.90 a	1.04 a	1.04 a	1.19 a	1.21^{a}	1.27^{a}	1.27 b	1.48 b	1.47 b	1.57 b	1.62^{c}	2.38 b	2.50^{d}	2.97 b
T_4	0.71 a	1.22 a	1.33 a	1.34 a	1.42^{a}	1.44^{a}	1.65^{a} b	1.67^{a} b	1.85^{a} b	2.01^{a} b	2.04^{a} bc	2.55 b	2.76^{c} d	3.02 b
T_5	1.15 a	1.16 a	1.40 a	1.42 a	1.56^{a}	1.70^{a}	1.80^{a} b	1.88^{a} b	1.95^{a} b	2.10^{a} b	2.26^{a} bc	2.72 ab	3.29^{a} bc	3.38 ab
T_6	1.00 a	1.23 a	1.38 a	1.53 a	1.53^{a}	1.53^{a}	1.53^{a} b	1.55 b	1.58 b	1.84^{a} b	1.93^{b} c	2.62 b	3.27^{a} bc	3.23 ab
T_7	0.87 a	1.01 a	1.20 a	1.48 a	1.52^{a}	1.66^{a}	1.72^{a} b	1.80^{a} b	1.80^{a} b	1.88^{a} b	2.20^{a} bc	2.57 b	3.08^{b} c	3.37 ab
T_8	1.02 a	1.32 a	1.51 a	1.54 a	1.54^{a}	1.58^{a}	1.63^{a} b	1.67^{a} b	2.48^{a}	2.73^{a}	2.92^{a}	3.37 a	3.72^{a}	3.96 a
T_9	0.88 a	1.27 a	1.41 a	1.53 a	1.79^{a}	1.86^{a}	2.27^{a}	2.42^{a}	2.44^{a}	2.62^{a}	2.84^{a} b	3.33 a	3.46^{a} b	3.62 ab

Plant height (T_8)

Number of leaves (T_5)

Number of shoots (T_5)

Girth of the shoots (T_8)

Internodal length (T_5)

Plate 4. Influence of nutrients and *Piriformospora indica* on growth characters in *Dendrobium* cv. Earsakul

and T_2 (1.93 cm). The lowest value was recorded by T_3 (1.62 cm) which was on par with T_6 (1.93 cm), and T_2 (1.93 cm).

At 12 MAP, maximum girth of shoots was recorded by T_8 (3.37 cm) followed by T_9 (3.33 cm) which was on par with T_5 (2.72 cm) and T_2 (3.02 cm).

At 13 MAP, T_8 recorded the highest value of 3.72 cm which was on par with T_9 (3.46 cm), T_5 (3.29 cm), T_6 (3.27 cm) and T_2 (3.23 cm).

Significant difference in girth of shoots was observed during 14 MAP. During this period, treatment T_8 registered maximum girth of 3.56 cm which was on par with T_5 (3.38 cm), T_9 (3.32 cm), T_7 (3.30 cm), T_6 (3.23 cm), T_2 (3.56 cm) and T_1 (3.11cm).

4.1.5 Internodal length

Data regarding influence of different treatments on internodal length are presented in Table 6.

At one MAP, internodal length shows significant difference among the treatments. Maximum internodal length was recorded in the treatment T_4 (0.65 cm) followed by T_5 which is on par with T_4. The minimum value was noted in T_7 (0.40 cm) which was on par with all other treatments.

No significant difference in internodal length was observed in all treatments during 2 to 7 MAP.

At 8 MAP, the treatment T_8 recorded the maximum value for internodal length and significantly superior to all other treatments. No significant difference in internodal length was observed during 9 to 12 MAP.

Table 6. Influence of nutrients and *Piriformospora indica* on internodal length (cm)

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP	11 MAP	12 MAP	13 MAP	14 MAP
T_1	0.53^{ab}	0.73^{a}	1.11^{a}	1.59^{a}	1.62^{a}	1.86^{a}	1.68^{a}	1.97^{ab}	2.09^{a}	2.29^{a}	2.47^{a}	2.90^{a}	3.28^{ab}	3.74^{a}
T_2	0.51^{ab}	0.69^{a}	1.18^{a}	1.77^{a}	1.81^{a}	1.90^{a}	2.01^{a}	2.15^{ab}	2.23^{a}	2.32^{a}	2.49^{a}	2.84^{a}	3.24^{ab}	3.80^{a}
T_3	0.58^{ab}	0.72^{a}	1.09^{a}	1.47^{a}	1.50^{a}	1.98^{a}	1.50^{a}	1.61^{b}	1.88^{a}	1.93^{a}	2.09^{a}	2.51^{a}	2.72^{b}	3.15^{a}
T_4	0.65^{a}	0.76^{a}	1.31^{a}	1.64^{a}	1.67^{a}	1.67^{a}	1.71^{a}	1.91^{ab}	1.95^{a}	2.19^{a}	2.32^{a}	2.73^{a}	2.94^{ab}	3.33^{a}
T_5	0.61^{a}	1.02^{a}	1.15^{a}	1.86^{a}	1.93^{a}	2.33^{a}	2.34^{a}	2.45^{ab}	2.49^{a}	2.54^{a}	2.69^{a}	2.85^{a}	3.63^{a}	4.11^{a}
T_6	0.58^{ab}	0.73^{a}	1.30^{a}	1.72^{a}	1.85^{a}	2.07^{a}	2.37^{a}	2.37^{ab}	2.52^{a}	2.60^{a}	2.62^{a}	2.95^{a}	3.21^{ab}	4.01^{a}
T_7	0.39^{b}	1.09^{a}	1.28^{a}	1.77^{a}	1.89^{a}	2.19^{a}	2.26^{a}	2.25^{ab}	2.45^{a}	2.51^{a}	2.55^{a}	2.78^{a}	3.14^{ab}	3.77^{a}
T_8	0.51^{ab}	1.07^{a}	1.65^{a}	1.74^{a}	1.78^{a}	1.87^{a}	2.45^{a}	2.67^{a}	2.82^{a}	2.85^{a}	2.87^{a}	3.05^{a}	3.53^{a}	3.97^{a}
T_9	0.53^{ab}	0.90^{a}	1.18^{a}	1.62^{a}	1.74^{a}	1.76^{a}	2.13^{a}	2.20^{ab}	2.40^{a}	2.24^{a}	2.50^{a}	3.09^{a}	3.24^{ab}	3.46^{a}

At 13 MAP, significant difference in internodal length was obtained, T_5 recorded the high internodal length of 3.64 cm which was on par with T_8 (3.54 cm). Treatment T_5 recorded the maximum internodal length of 4.11cm at 14 MAP, but no significant difference was observed among the treatments.

4.2 Root parameters

4.2.1 Number of roots per plant

Data on number of roots per plant are presented in Table 7 and Plate 5.

The data corresponding to number of roots per plant revealed that the plants showed significant variation among the treatments at six months after planting due to the influence of nutrients and *Piriformospora indica*.

Highest number of roots (33.00) was produced by the plants which received the treatment T_6, which was on par with T_1 (32.67) and T_5 (31.35). The lowest number of roots per plant (17.67) was recorded by T_3 and which was on par with T_9 (19.00). All other treatments were found to be statistically on par with each other. However, at the time of flower bud formation no significant difference was observed with respect to number of roots per plant.

4.2.2 Root length

Data pertaining to root length are presented in Table 7 and Plate 5.

At 6 MAP, no significant difference in root length was observed among treatments. Comparison of data on root length at the time of flower bud formation indicated that all the treatments had significant influence and the treatment T_9

Number of roots (T_6) at six MAP

Number of roots (T_5) at flower bud formation stage

Root length (T_2) at six MAP

Root length (T_9) at flower bud formation stage

Root volume (T_5) at six MAP

Root volume (T_5) at flower bud formation stage

Plate 5. Influence of nutrients and *Piriformospora indica* on root parameters in *Dendrobium* cv. Earsakul

Table 7. Influence of nutrients and *Piriformospora indica* (PGPRE) on root parameters

Treatments	Number of roots/ plant		Root length (cm)		Root volume (ml)	
	6 months after planting	At the time of flower bud formation	6 months after planting	At the time of flower bud formation	6 months after planting	At the time of flower bud formation
T_1	32.67^{a}	52.67^{a}	11.33^{a}	18.50^{ab}	3.333^{a}	11.670^{ab}
T_2	25.33^{ab}	58.00^{a}	11.43^{a}	15.97^{b}	2.667^{a}	11.000^{abc}
T_3	17.67^{b}	46.33^{a}	9.77^{a}	14.33^{b}	2.000^{a}	6.333^{c}
T_4	26.00^{ab}	55.67^{a}	8.43^{a}	18.00^{ab}	2.667^{a}	6.333^{c}
T_5	31.33^{a}	64.33^{a}	10.30^{a}	19.50^{ab}	3.667^{a}	13.330^{a}
T_6	33.00^{a}	48.33^{a}	8.80^{a}	16.30^{b}	3.333^{a}	8.000^{bc}
T_7	24.67^{ab}	58.33^{a}	10.10^{a}	15.67^{b}	3.000^{a}	7.000^{bc}
T_8	29.33^{ab}	59.67^{a}	10.43^{a}	15.20^{b}	3.000^{a}	9.000^{abc}
T_9	19.00^{b}	53.33^{a}	10.73^{a}	23.33^{a}	2.667^{a}	8.000^{bc}

recorded the maximum root length (23.33 cm) followed by T_5 (19.50 cm), T_1 (18.50 cm) and T_4 (18.00 cm) which were statistically on par. The treatment T_3 recorded minimum root length (14.33 cm) which was on par with T_6, T_2, T_7 and T_8. Gradual increment in root length was observed from 6 MAP to at the time of flower bud formation.

4.2.3 Root volume

Data pertaining to root volume are presented in Table 7 and Plate 5.

Similar to root length, at six months after planting no significant difference was observed in root volume and all treatments were statistically on par with each other.

The treatments had significant effect on root volume at the time of flower bud formation. The treatment T_5 recorded a maximum root volume of 13.330 ml which was followed by T_1 (11.670 ml). Treatment T_3 recorded the lowest root volume of 6.333 ml. Similar to other root characters, marked increment in root volume was observed at the time of flower bud formation.

4.2.4 Root colonization of *P. indica*

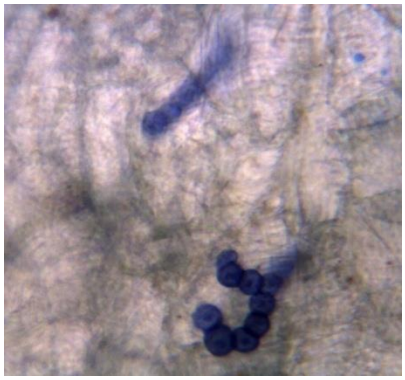
Data pertaining to root colonization of *P. indica* are presented in Table 8 and Plate 6.

At 6 MAP, no significant difference was observed among treatments with respect to root colonization of *P. indica*.

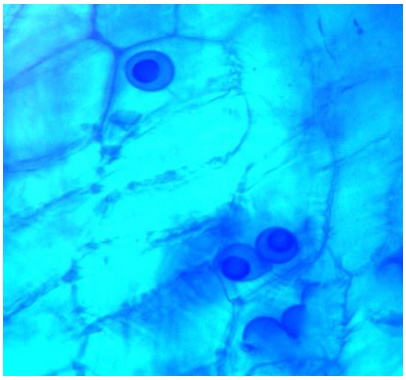
While comparing the percentage of root colonization in different treatments (T_5 to T_9) at the time of flower bud formation, it was found that they were

Table 8. Influence of nutrients and *Piriformospora indica* on root colonization of *P. indica* (%)

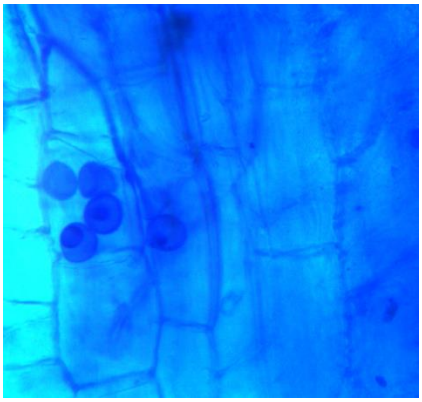
Treatments	6 Months after planting	At the time of flower bud formation
T_5	60.00[a]	79.89[a]
T_6	48.89[a]	68.89[ab]
T_7	53.33[a]	73.33[ab]
T_8	55.55[a]	64.44[b]
T_9	57.78[a]	71.11[ab]



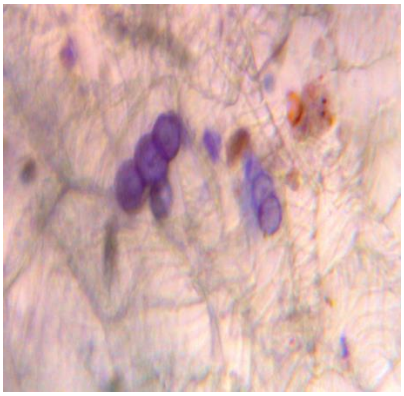
POP+ *P. indica* (T_5)



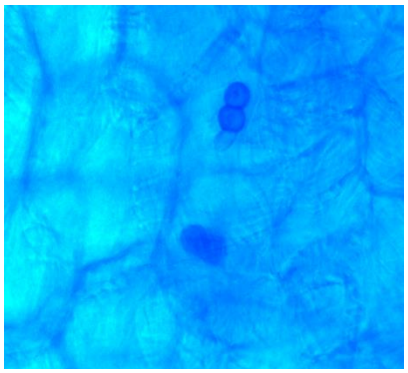
POP+ OM+ *P. indica*+ BM (T_6)



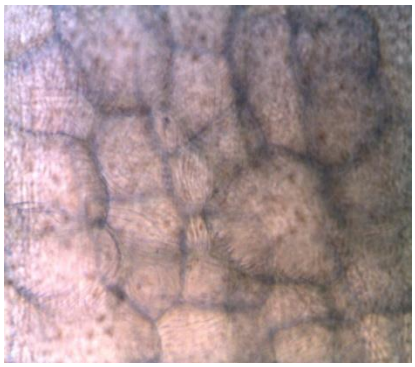
POP+ VW+ *P. indica*+ BM (T_7)



POP+ OM+ VW+ *P. indica*+ BM (T_8)



OM+ VW+ *P. indica*+ BM (T_9)



Control

Plate 6. Influence of nutrients and *Piriformospora indica* on root colonization of *P. indica* (40x) in *Dendrobium* cv. Earsakul

significantly different and the treatment T_5 recorded the maximum value (79.89 per cent). The treatment T_8 recorded minimum value (64.44 per cent) and treatments T_7, T_9 and T_6 were found to be statistically on par with T_8. Gradual increment in root colonization was observed from 6 MAP to at the time of flower bud formation.

4.3. Physiology parameters

4.3.1 Leaf area

Data pertaining to leaf area are presented in Table 9.

Nutrients along with *Piriformospora indica* showed detectable variations in leaf area throughout the experimental period. The maximum leaf area was recorded in the treatment T_8 (26.27 cm^2) which was significantly superior to all other treatments and the minimum value recorded in T_3 (16.82 cm^2).

3.2 Dry matter production

Data pertaining to dry matter production are presented in Table 9.

No significant difference in dry matter production was observed among the treatments at 6 MAP.

4.3.3 Crop growth rate

Data pertaining to crop growth rate are presented in Table 9.

Significant difference was observed with respect to crop growth rate at six MAP. Treatment T_5 recorded the higher crop growth rate of 0.1017 g m^{-2} day^{-1} which

Table 9. Influence of nutrients and *Piriformospora indica* (PGPRE) on physiological parameters at six MAP

Treatments	Leaf Area (cm^2)	Dry Matter Production (g $plant^{-1}$)	Crop Growth Rate (g m^{-2} day^{-1})	Relative Growth Rate (g g^{-1} day)	Net Assimilation Rate (g m^{-2} day^{-1})
T_1	20.62^{ab}	23.20^{a}	0.0517^{ab}	0.0133^{a}	0.0257^{a}
T_2	21.09^{ab}	28.23^{a}	0.0830^{ab}	0.0167^{a}	0.0400^{a}
T_3	16.82^{b}	17.07^{a}	0.0297^{b}	0.0090^{a}	0.0167^{a}
T_4	20.16^{ab}	21.17^{a}	0.0467^{ab}	0.0123^{a}	0.0280^{a}
T_5	20.92^{ab}	31.87^{a}	0.1017^{a}	0.0190^{a}	0.0363^{a}
T_6	20.59^{ab}	27.83^{a}	0.0427^{ab}	0.0120^{a}	0.0263^{a}
T_7	23.14^{ab}	24.87^{a}	0.0593^{ab}	0.0080^{a}	0.0427^{a}
T_8	26.27^{a}	22.37^{a}	0.0437^{ab}	0.0090^{a}	0.0150^{a}
T_9	18.70^{ab}	23.23^{a}	0.0503^{ab}	0.0143^{a}	0.0233^{a}

was significantly superior to all other treatments. All other treatments were found to be statistically on par with each other.

4.3.4 Relative growth rate

Data pertaining to relative growth rate are presented in Table 9.

The relative growth rate did not vary significantly among different treatments.

4.3.5 Net assimilation rate

Data pertaining to net assimilation rate are presented in Table 9.

No significant difference in net assimilation rate was observed at 6 MAP.

4.3.6 Chlorophyll content

Data pertaining to chlorophyll content are presented in Table 10.

The chlorophyll 'a' content in the plant varied significantly among different treatments. The highest chlorophyll 'a' content was recorded in treatment T_4 (0.2560 mg g^{-1}) which was on par with T_1 (0.2363 mg g^{-1}). Treatments T_4 and T_1 were on par with T_3, T_6, T_2 and T_5. The lowest value was recorded from treatment T_9 (0.1447 mg g^{-1}).

No significant difference with respect to the chlorophyll 'b' content was observed at 6 MAP.

Table 10. Influence of nutrients and *Piriformospora indica* (PGPRE) on chlorophyll content at six MAP

Treatments	Chlorophyll a (mg g^{-1} leaf wt)	Chlorophyll b (mg g^{-1} leaf wt)	Total Chlorophyll (mg g^{-1} leaf wt)
T_1	0.2363^{a}	0.0760^{a}	0.3123^{a}
T_2	0.2013^{abc}	0.0320^{a}	0.2333^{abc}
T_3	0.2077^{ab}	0.0423^{a}	0.2500^{abc}
T_4	0.2560^{a}	0.0553^{a}	0.3113^{a}
T_5	0.2010^{abc}	0.0320^{a}	0.2330^{abc}
T_6	0.2067^{ab}	0.0590^{a}	0.2657^{ab}
T_7	0.1483^{bc}	0.0303^{a}	0.1787^{c}
T_8	0.1737^{bc}	0.0353^{a}	0.2090^{bc}
T_9	0.1447^{c}	0.0560^{a}	0.2007^{bc}

Total chlorophyll content showed significant difference among different treatments. Treatment T_1 and T_4 recorded maximum value of 0.3123 mg g^{-1} and 0.3113 mg g^{-1} respectively. Treatment T_6 (0.2067 mg g^{-1}), T_5 (0.2010 mg g^{-1}), T_3 (0.2077 mg g^{-1}), and T_2 (0.233 mg g^{-1}) were on par with T_1 and T_4. The lowest total chlorophyll content was recorded in the treatment T_7 (0.1787 mg g^{-1}).

4.4 Flower characters

The influence of nutrients and *Piriformospora indica* on floral characters are presented in Table 11 and Plate 7**.**

4.4.1 Days to flowering

Data regarding days to flowering are presented in Table 11.

The results indicated that, the different treatments markedly influence the days to flowering. Early flowering was observed in the treatment T_5 (393 days) followed by T_7 (398 days). Plants at different treatments were under flowering stage, except T_8 and T_9.

4.4.2 Days to first flower opening

Data regarding days to first flower opening are presented in Table 11.

With respect to first flower opening, treatment T_5 took 41 days followed by T_7 (47 days).

Flowering in treatment POP+ *P. indica*

Spike obtained from the treatment POP+ *P. indica*

Plate 7. Influence of nutrients and *Piriformospora indica* on flowering in *Dendrobium* cv. Earsakul

4.4.3 Days to last flower opening

Data pertaining to last flowering are presented in Table 11.

With respect to days to last flower opening, treatment T_5 and T_7 recorded 11days and 12 days respectively.

4.4.4 Length of spike

The lengths of spike are presented in Table 11.

Treatments had marked influence on spike length and the maximum length of spike (22.33 cm) was recorded in T_5 followed by T_7 (20.10 cm).

4.4.5 Number of flowers per spike

Data regarding the number of flowers per spike are presented in Table 11.

Maximum number of flowers per spike was recorded in T_5 with five flowers followed by four flowers per spike in treatment T_7.

4.4.6 Size of the flower

Data regarding the size of the individual flowers are presented in Table 11.

Plants received the treatment T_5, produced large flower size (7.0 x 6.5 cm) followed by T_7 (7.0 x 6.1 cm).

Table 11. Influence of nutrients and *Piriformospora indica* (PGPRE) on flower characters

Treatments	Days to flowering	Days to first flower opening	Days to last flower opening	Length of the spike (cm)	Number of flowers per spike	Size of the flower (cm)	Number of spike per plant	Vase life (days)
T_1	462	-	-	-	-	-	1	-
T_2	465	-	-	-	-	-	1	-
T_3	467	-	-	-	-	-	1	-
T_4	469.5	-	-	-	-	-	1	-
T_5	393	41	11	22.3	5	7.0 X 6.5	1	29
T_6	470	-	-	-	-	-	1	-
T_7	398	47	12	20.1	4	7.0 X 6.1	1	27
T_8	-	-	-	-	-	-	-	-
T_9	-	-	-	-	-	-	-	-

4.4.7 Number of spikes per plant

Data related to number of spikes per plant are presented in Table 11.

During the period of study, one spike per plant was recorded in treatment T_1, T_2, T_3, T_4, T_5, T_6 and T_7.

4.4.8 Vase life in water

Due to limited number of spike produced during the period, the study on the vase life of flowers in water could not be carried out for all treatments (Table 11). However, the treatments T_5 recorded higher vase life of 29 days followed by T_7 (27 days).

4.5 Nutrient content

4.5.1 Nitrogen

Influences of treatments on nitrogen content are presented in table 12.

The nitrogen content in the plant did not have significant differences among the treatments during six MAP and flower bud formation stage.

4.5.2 Phosphorus

Data pertaining to phosphorus content are presented in Table 12.

No significant difference in phosphorus content was observed at six MAP.

Table 12. Influence of nutrients and *Piriformospora indica* on N, P, K, Ca, and Mg content (%)

Treatments	N		P		K		Ca		Mg	
	6 MAP	Flower bud formation	6 MAP	Flower bud formation	6 MAP	Flower bud formation	6 MAP	Flower bud formation	6 MAP	Flower bud formation
T_1	2.427^{a}	2.322^{a}	0.1603^{a}	0.3457^{ab}	1.360^{a}	1.493^{a}	0.929^{a}	0.613^{a}	0.285^{ab}	0.223^{a}
T_2	2.497^{a}	2.730^{a}	0.1910^{a}	0.3670^{a}	1.340^{a}	1.430^{a}	1.239^{a}	0.545^{a}	0.264^{abc}	0.241^{a}
T_3	2.602^{a}	2.928^{a}	0.1793^{a}	0.3560^{a}	1.390^{a}	1.330^{a}	1.074^{a}	0.408^{a}	0.230^{bc}	0.214^{a}
T_4	2.147^{a}	2.065^{a}	0.2037^{a}	0.3157^{ab}	1.220^{a}	1.173^{a}	1.384^{a}	0.594^{a}	0.254^{abc}	0.234^{a}
T_5	2.392^{a}	2.590^{a}	0.1663^{a}	0.2517^{abc}	1.287^{a}	1.253^{a}	1.224^{a}	1.378^{a}	0.273^{ab}	0.222^{a}
T_6	2.368^{a}	2.310^{a}	0.1920^{a}	0.2193b^{c}	1.657^{a}	1.873^{a}	1.351^{a}	3.283^{a}	0.212^{c}	0.280^{a}
T_7	1.960^{a}	2.497^{a}	0.1513^{a}	0.3727^{a}	1.567^{a}	1.500^{a}	1.911^{a}	0.858^{a}	0.311^{a}	0.270^{a}
T_8	1.808^{a}	2.252^{a}	0.1760^{a}	0.2737^{abc}	1.653^{a}	1.440^{a}	1.423^{a}	1.069^{a}	0.292^{a}	0.272^{a}
T_9	1.832^{a}	2.158^{a}	0.1083^{a}	0.1803^{c}	1.260^{a}	1.410^{a}	1.427^{a}	2.788^{a}	0.284^{ab}	0.287^{a}

The phosphorus content in the plant had significantly difference among treatments at flower bud formation stage. Higher P content of 0.3727 per cent was recorded in T_7.Treatments T_2, T_3, T_1 and T_4 were found to be statistically on par with T_7. The treatment T_9 (without POP) recorded lowest P content of 0.1803 per cent.

4.5.3 Total potassium

Data on uptake of potassium are presented in Table 12.

The results revealed that no significant difference in potassium content was observed among different treatments during six MAP and at flower bud formation stage.

4.5.4 Calcium

Data on calcium content are presented in Table 12.

The calcium content in the plant did not have significant differences among the treatments during six MAP and flower bud formation stage.

4.5.5 Magnesium

Data on magnesium content are presented in Table 12.

At 6 MAP, significant difference was observed in all treatments with respect to magnesium content. Highest magnesium content of 0.3113 per cent recorded in treatment T_7 followed by T_8 (0.2920 per cent). T_7 and T_8 were on par and

significantly superior to all other treatments. The lowest magnesium content of 0.2120 per cent was noted in treatment T_6.

The magnesium content did not have significant difference among the treatments at the time of flower bud formation.

4.5.6 Iron

Data on iron content are presented in Table 13.

Significant difference was observed with respect to iron content at six MAP. The treatment T_5 recorded the highest iron content of 2484 ppm followed by T_7 (2191 ppm) which was on par with T_8 and T_4. The lowest value of 1218 ppm was recorded in T_1.

Significant difference was observed with respect to iron content at flower bud formation stage. Treatment T_7 recorded the maximum iron content of 2231.00 ppm at flower bud formation stage followed by T_5 (1410.33 ppm). The lowest iron content (679.00 ppm) was recorded T_3. All treatments were on par with each other except T_7.

4.5.7 Copper

Data on copper content are presented in Table 13.

No significant difference in copper content was observed among different treatments at six MAP and flower bud formation stage.

4.5.8 Zinc

Data on zinc content are presented in Table 13.

Table 13. Influence of nutrients and *Piriformospora indica* (PGPRE) on Fe, Cu, Zn and Mn content (ppm)

Treatments	Fe		Cu		Zn		Mn	
	6 MAP	Flower bud formation	6 MAP	Flower bud formation	6 MAP	Flower bud formation	6 MAP	Flower bud formation
T_1	1218.33^{c}	813.00^{b}	20.97^{a}	14.97^{a}	58.83^{a}	34.27^{a}	115.93^{a}	61.17^{a}
T_2	1340.00^{c}	709.33^{b}	14.17^{a}	14.50^{a}	44.27^{bc}	41.40^{a}	86.07^{a}	63.97^{a}
T_3	1691.33b^{c}	679.00^{b}	28.53^{a}	16.67^{a}	55.10^{ab}	47.57^{a}	96.57^{a}	57.87^{a}
T_4	1768.00^{abc}	775.00^{b}	15.97^{a}	23.53^{a}	45.10^{bc}	39.83^{a}	98.73^{a}	59.63^{a}
T_5	2484.00^{a}	1410.33^{ab}	20.70^{a}	22.17^{a}	39.67^{cd}	35.43^{a}	104.90^{a}	68.80^{a}
T_6	1546.67^{bc}	859.33^{b}	16.77^{a}	11.67^{a}	42.10^{bcd}	96.67^{a}	90.00^{a}	64.47^{a}
T_7	2191.33^{ab}	2231.00^{a}	21.47^{a}	20.47^{a}	46.77^{abc}	41.60^{a}	99.20^{a}	73.33^{a}
T_8	1750.33^{abc}	1165.33^{b}	17.20^{a}	18.30^{a}	33.30^{cd}	33.00^{a}	88.63^{a}	64.27^{a}
T_9	1416.67^{c}	1104.33^{b}	26.17^{a}	17.33^{a}	30.20^{d}	36.23^{a}	60.73^{a}	61.90^{a}

Estimation of zinc at six MAP revealed that there were significant difference was present among the treatments. The treatment T_1 recorded the highest zinc content (58.83 ppm) followed by T_3 (55.10 ppm) and the lowest zinc content of 30.20 ppm was obtained from T_9.

No significant difference in zinc content was observed among the treatments at flower bud formation stage.

4.5.9 Manganese

Data on manganese content are presented in Table 13.

No significant difference was observed among different treatments at 6 MAP and flower bud formation stage.

4.6 Pest and disease incidence

During the entire period of study the commonly noticed pest were snails (*Ariophanta* sp.) and slugs (*Arion* sp. and *Linox* sp.). These pests were controlled to the maximum extend using Metaldihyde @ 2.5% pellet (snail kill). No other pest was noticed during the period of study.

Regarding diseases, the most commonly observed disease was bacterial soft rot which was characterized by the yellowing of leaves, later turning to water soaked lesions with characteristic smell. Streptomycin sulphate was applied @ 0.20g l^{-1} for controlling the disease.

Discussion

5. DISCUSSION

Orchids can be found anywhere in the world, except in the polar region. Considering their wide distribution around the globe, it is understood that different orchids from different parts of the world experience numerous sets of macro and micro climate. Critical factors for successful orchid growing include temperature, light, humidity, photoperiod, watering, nutrients, potting media etc. Due consideration to each of the factors is vital for orchid growing.

Orchids, like any other plant, require nitrogen, phosphorus, potassium, and trace elements for healthy growth (Kang, 1979; Fitch, 1981). In general, orchids grow satisfactorily when fed with a balanced fertilizer during early phases of growth. The effectiveness of application of one element depends on the presence of the other elements. There is evidence to indicate that a combination of organic and inorganic fertilizers gives better growth.

The orchidaceae has an intimate association with fungi. In adult orchids mycorrhiza is assumed to be important for mineral nutrition of nitrogen and phosphorus, largely because the root systems of many terrestrial orchids are poorly developed (Smith and Read, 1997; Brundrett, 2002). Fungus (*Piriformospora indica*) having a broad host spectrum shows pronounced growth-promotional effects in many plants. It mobilizes the insoluble phosphates and translocates the phosphorus to the host in an energy-dependent process. The axenic cultivability of *P. indica* on economically viable synthetic media makes it suitable for mass scale inoculum production for application in agro-forestry and horticulture. Pronounced growth promotional effects were also seen with terrestrial orchids (Singh *et al.*, 2000).

The present investigation was envisaged with objective to studying the growth and development of *Dendrobium* cv. Earsakul at different combinations of organic

and inorganic nutrients and *P. indica* (PGPRE) and analyze of the influence on growth parameters, root characters, physiological aspects, floral characters, uptake of nutrients and incidence of pest and diseases.

5.1 Influence of nutrients and *Piriformospora indica* on growth parameters

Height, in general, indicates the overall growth and different treatments did not show appreciable difference in plant height. But during the earlier stages of growth, significantly higher plant height was recorded in treatment combination of POP recommendations of KAU for orchids (POP) along with *P. indica* and also in POP+ organic mixture+ vermiwash+ *P. indica* + bone meal (Fig.1 to 5).

In general, results on number of leaves and number of shoots showed that no significant difference was present among treatments throughout the growth stages. But in all the stages of growth, treatment combination of POP + *P. indica* showed superior results compared to others.

The results indicated that the action of *P. indica* had significant influence in the later stage of plant growth. The increase in growth characters like plant height and internodal length may be due to root endophyte inoculation which had added nitrogen to crop growth through associative symbiosis and increased production of growth hormones like NAA, GA and cytokinins. These phytohormones might have caused morphological changes in roots thereby causing an increase in uptake of nutrients resulting in better growth. The possible reason for such an effect could be that *P. indica* increases the cell division and cell elongation in the region of auxiliary buds. This is in conformation with the results obtained by Balasubramanian (1989) in French marigold, Preethi (1990) in Edward rose, Mariappan (1992) in marigold and Manonmani (1992) in *Jasminum sambac*.

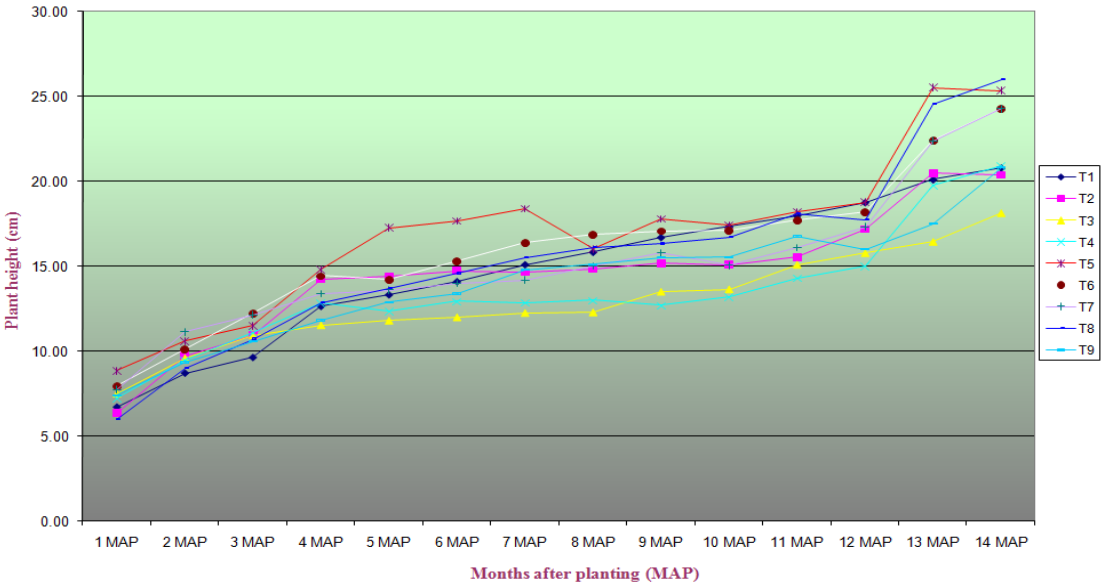


Fig. 1. Influence of nutrients and *Piriformospora indica* (PGPRE) on plant height

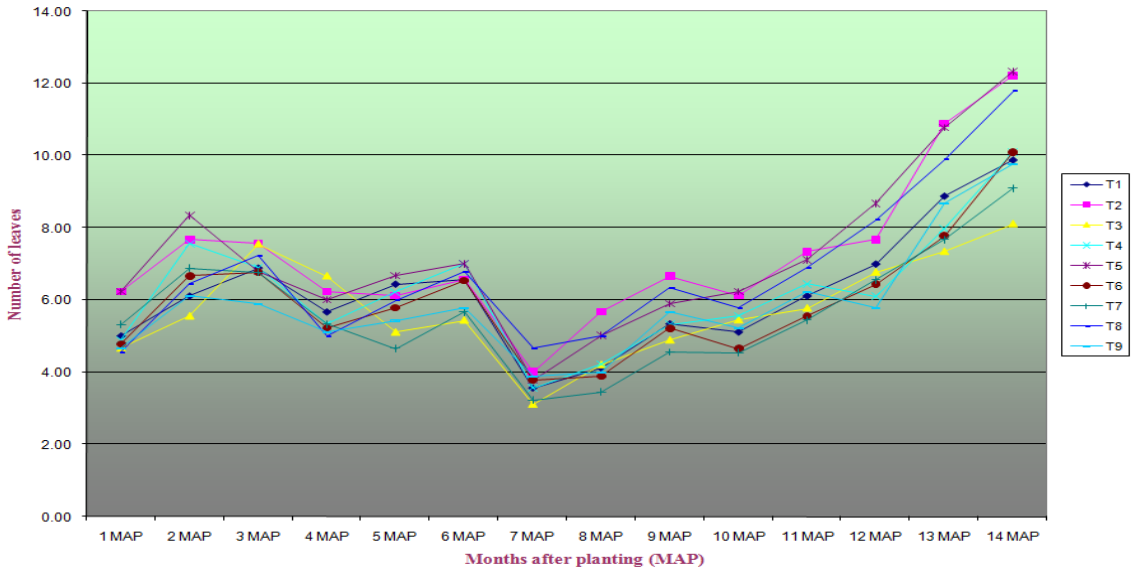


Fig. 2. Influence of nutrients and *Piriformospora indica* (PGPRE) on number of leaves

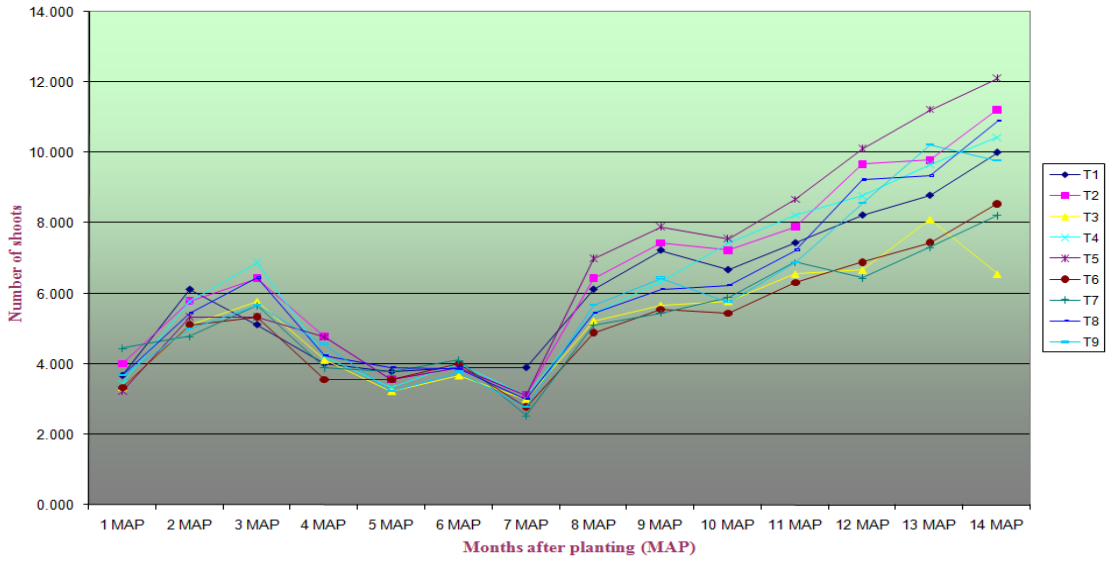


Fig. 3. Influence of nutrients and *Piriformospora indica* (PGPRE) on number of shoots

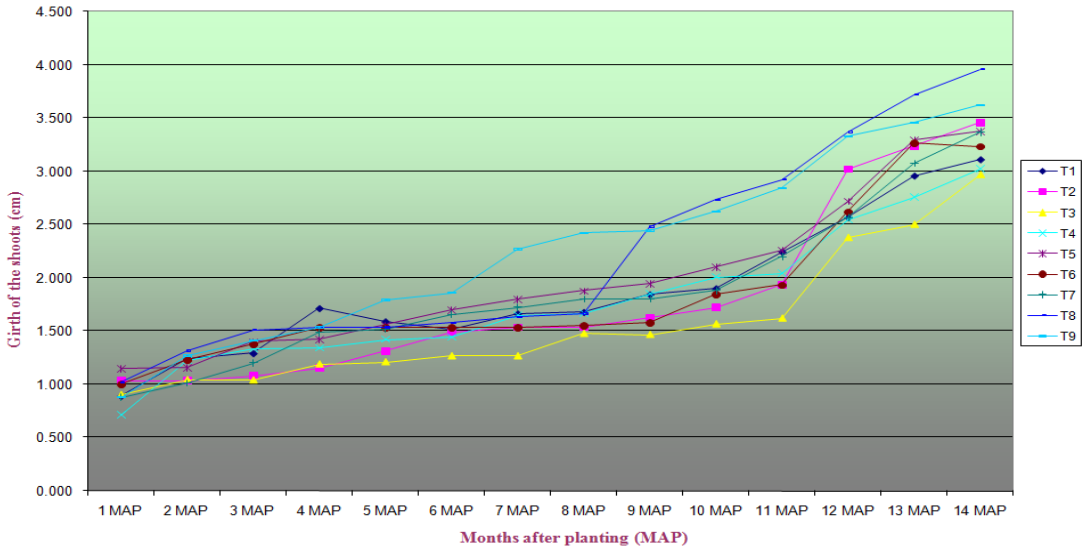


Fig. 4. Influence of nutrients and *Piriformospora indica* (PGPRE) on girth of the shoot

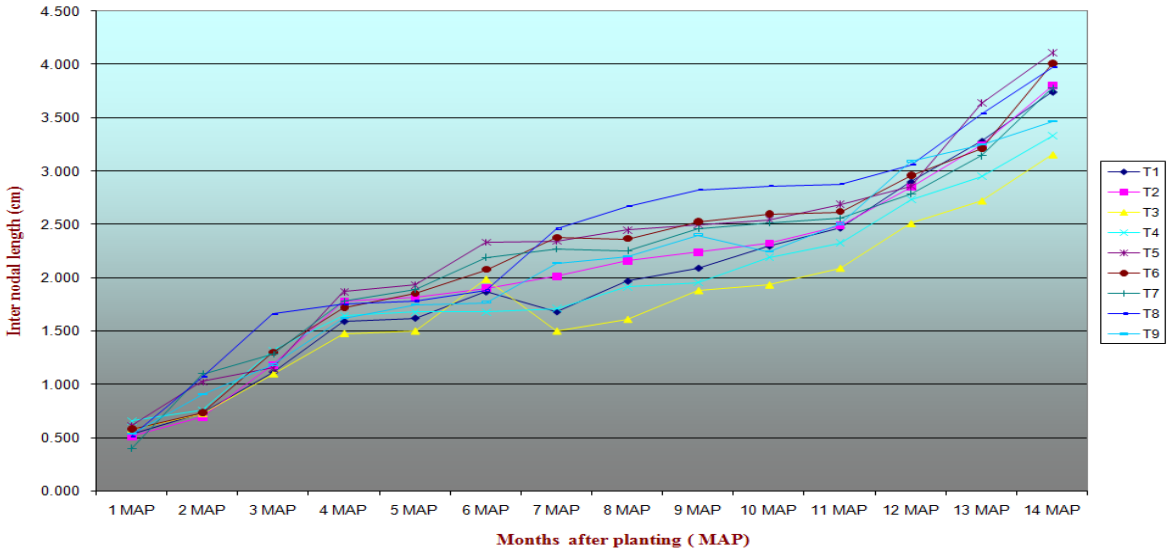


Fig. 5. Influence of nutrients and *Piriformospora indica* (PGPRE) on internodal length

During the early stages of growth, in general, the influence of nutrients and *P. indica* on girth of the shoots and internodal length was not evident. However, during later stages of growth, the treatments POP+ organic mixture+ vermiwash+ *P. indica*+ bone meal and POP+ *P. indica* promoted the more girth of shoots and internodal length. The possible reason for this acceleration might be the influence of nitrogen, chief constituent of protein, essential for the formation of protoplasm which leads to cell division and cell enlargement. Moreover, nitrogen is an important component of amino acids and co-enzymes which are of considerable biological importance (Bakly, 1974). Similar views were expressed by Rathore *et al.* (1985) in marigold.

Many types of organic manure including dilute form of cowdung, dilute urine, groundnut cake were reported to the good for the growth of orchids (Bhattacharjee, 1981; Bose and Bhattacharjee, 1980). Bhattacharjee (1982) recorded that chemical fertilizer mixture containing both macro and micro nutrients produced response in orchids. It is evident that, application of inorganic fertilizers along with organic manures (bone meal, neem cake, ground nut cake), vermiwash, bone meal applied along with *P. indica* significantly influenced the growth parameters. Singh *et al.* (2000) reported the growth promotional effects of *P. indica* on terrestrial orchids. The significant improvement in the production of tallest plant, with more number of leaves, shoots *etc*. may be due to the influence of the *P. indica*.

5.2 Influence of nutrients and *Piriformospora indica* on root parameters

Influence of nutrients and *Piriformospora indica* on number of roots per plant was significant at 6 MAP. Maximum number of roots per plant was observed from the treatment POP + organic mixture + *P. indica* + bone meal which gave statistically superior results. Root production was not influenced by the different treatment at flower bud formation stage (Fig. 6 to 9).

During the initial stages of the study, the influence of treatments on root length was not evident. But significant difference was observed in root length at the time of flower bud formation. The treatment with organic mixture + vermiwash + *P. indica* + bone meal showed superior results followed the treatment with POP + *P. indica*.

In the present investigation, nutrients and *P. indica* did not influence root volume at 6 MAP. Significant variation was found at the time of flower bud formation. The treatment with POP+ *P. indica* recorded the maximum root volume during both the periods. The role of nutrients along with *P. indica* in increasing root volume is established in this study.

Root colonization studies revealed that more homogenous results were present among treatments having *P. indica* at 6 MAP. Significant difference existed among *P. indica* applied treatments at the time of flower bud formation. Maximum root colonization was recorded in POP + *P. indica* during both the periods. Hence it is evident that POP + *P. indica* was the best treatment combination for root colonization of *P. indica*.

Enhanced root production, root volume and root colonization of *P. indica* was observed in treatment involving POP+ *P. indica*. The positive influence of *P. indica* for the above root parameters was clearly evident from this study.

Hardly (1969) reviewed the process of nutrient exchange between plant and fungus. It has been shown that vitamins, amino acids and sugar are translocated from the fungus to the orchid. In the present study it is evident that the treatment with *P. indica* had beneficial effect on production of roots. This may be due to its ability to mobilize the insoluble phosphates and translocates the phosphorus to the host in an

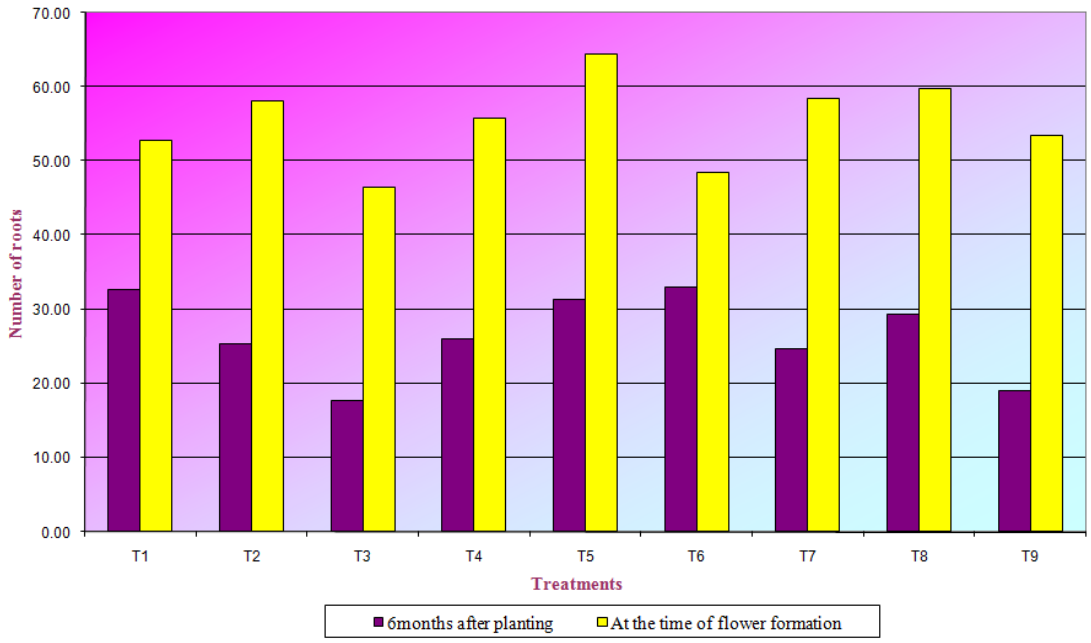


Fig. 6. Influence of nutrients and *Piriformospora indica* (PGPRE) on number of roots

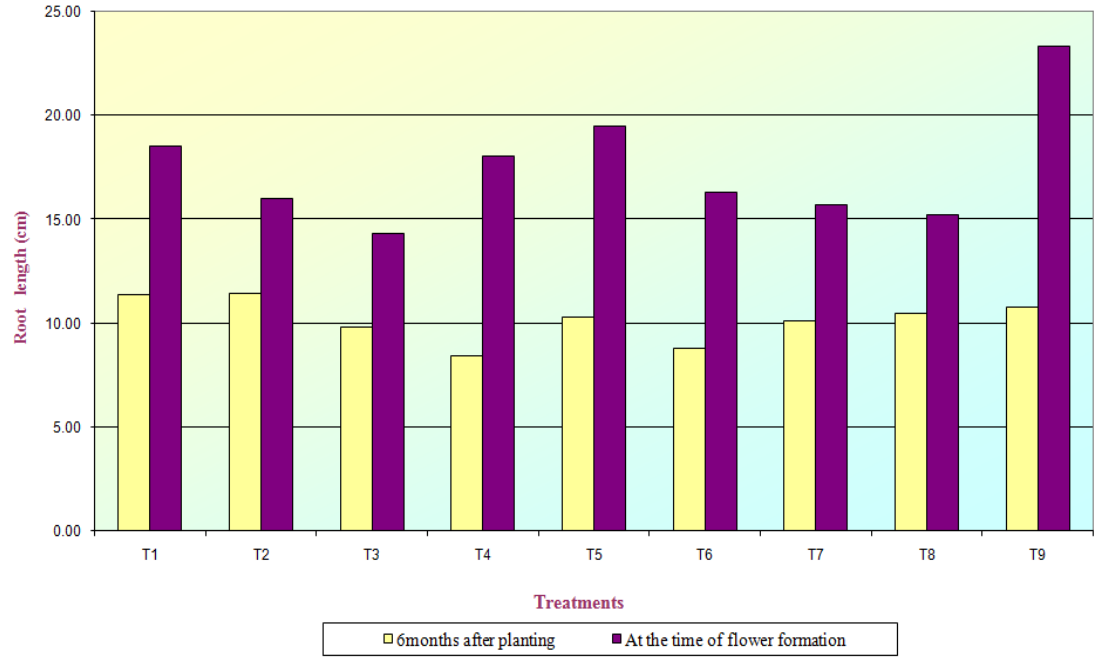


Fig. 7. Influence of nutrients and *Piriformospora indica* (PGPRE) on root length

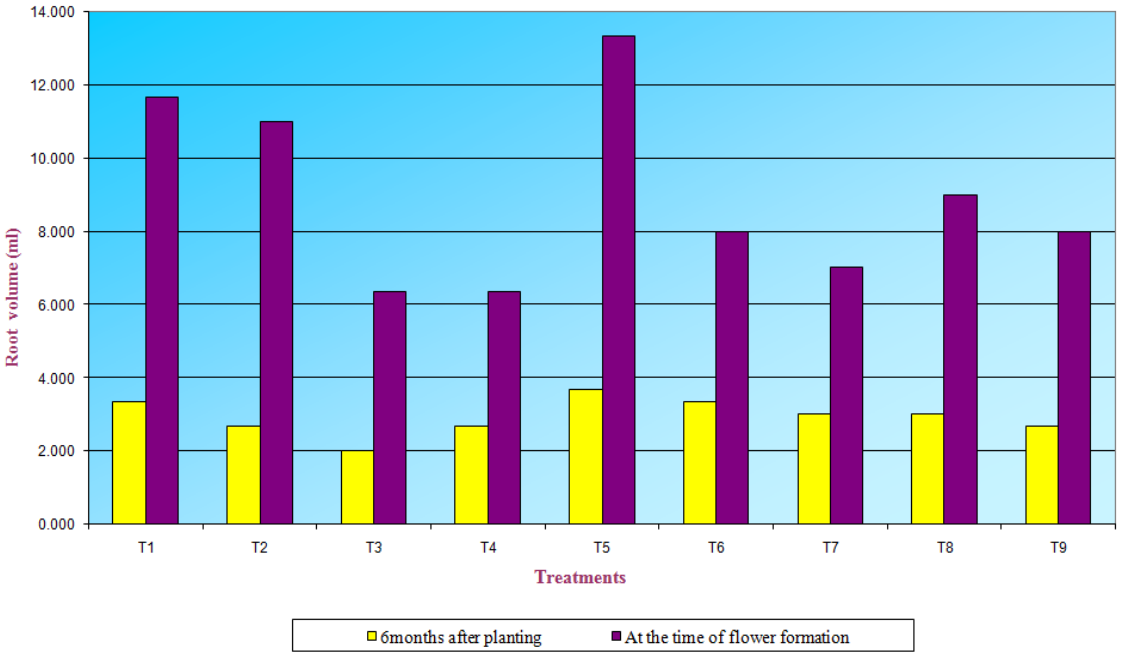


Fig. 8. Influence of nutrients and *Piriformospora indica* (PGPRE) on root volume

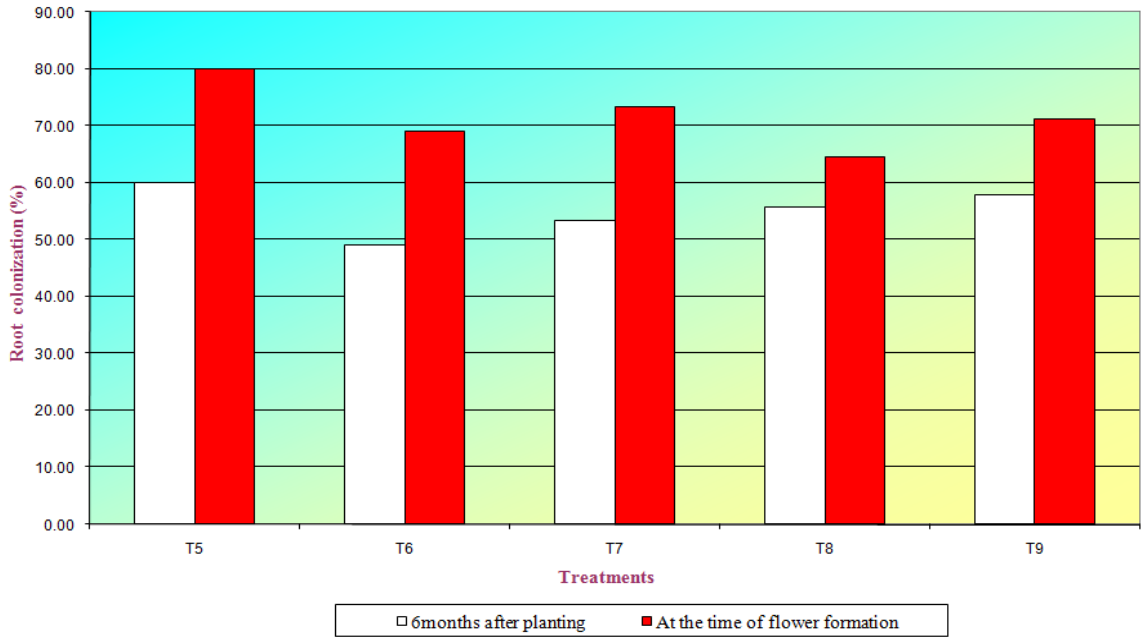


Fig. 9. Influence of nutrients and *Piriformospora indica* (PGPRE) on root colonization of *P. indica*

energy-dependent process. The phosphorus nutrient in the early stages of growth is beneficial for producing more roots in plants.

In the present study, *P. indica* had a positive effect on root parameters, which confirm with the observation of Malinowski and Balesky (1999); Balesky and Malinowski (2000); Gasoni and Garfinkel (1997) in *Spilanthes calva*, Geranium, *Pinus* sp, *Withania somnifera*, Pelargonium, and *Arabidopsis thaliana*.

5.3 Influence of nutrients and *Piriformospora indica* on physiological parameters

Physiological parameters indicate the efficiency of the plant in terms of yield. The present study took into consideration the physiological parameters like, leaf area, relative growth rate, crop growth rate, net assimilation rate and dry matter production and chlorophyll content of the plants (Fig 10 to15).

Leaf area varied significantly among different treatments. Maximum leaf area was observed in treatment POP+ organic mixture+ vermiwash+ *P. indica* + bone meal due to the interaction of all applied inputs and adaptation of plants to expose larger photosynthetic surface under 50 per cent shade level. In general, combinations of organic and inorganic nutrients along with *P. indica* gave maximum leaf area.

Highest relative growth rate of 0.0190 g g^{-1} day^{-1} was recorded in treatment POP+ *P. indica* at six MAP. It was observed that, treatment did not influence the relative growth rate of the plants. Crop growth rate varied significantly among different treatments at flower bud formation stage. Highest crop growth rate was recorded in those plants which received POP + *P. indica*. Net assimilation rate and dry matter production were not influenced by the different treatment combinations.

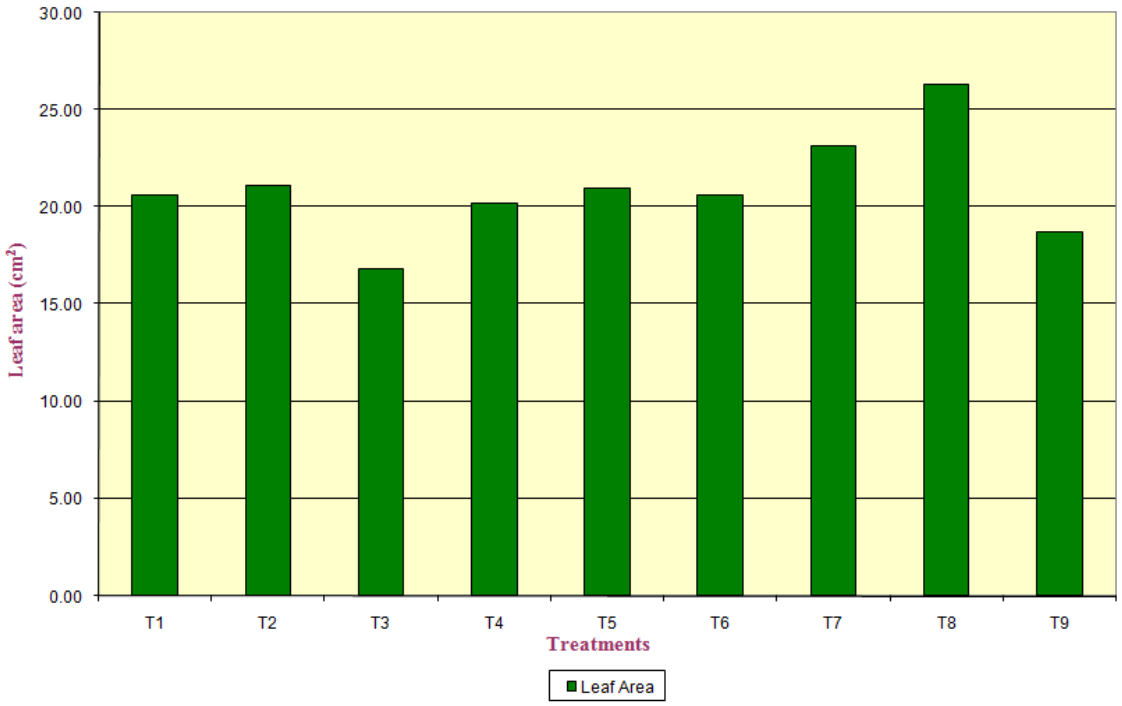


Fig. 10. Influence of nutrients and *Piriformospora indica* (PGPRE) on leaf area

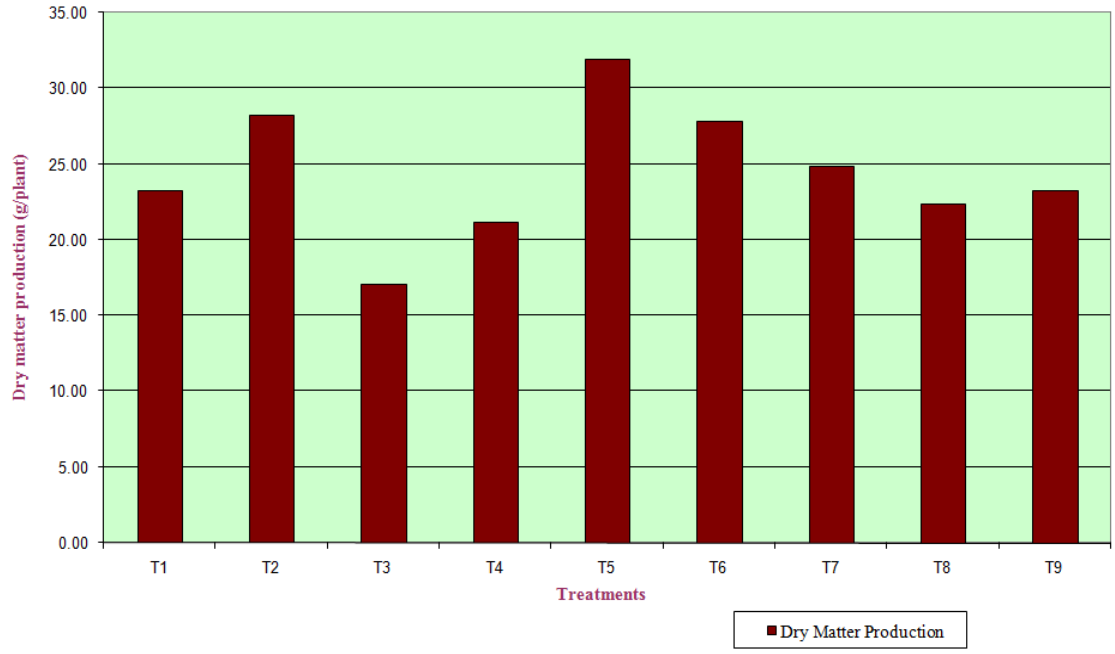


Fig. 11. Influence of nutrients and *Piriformospora indica* (PGPRE) on dry matter production

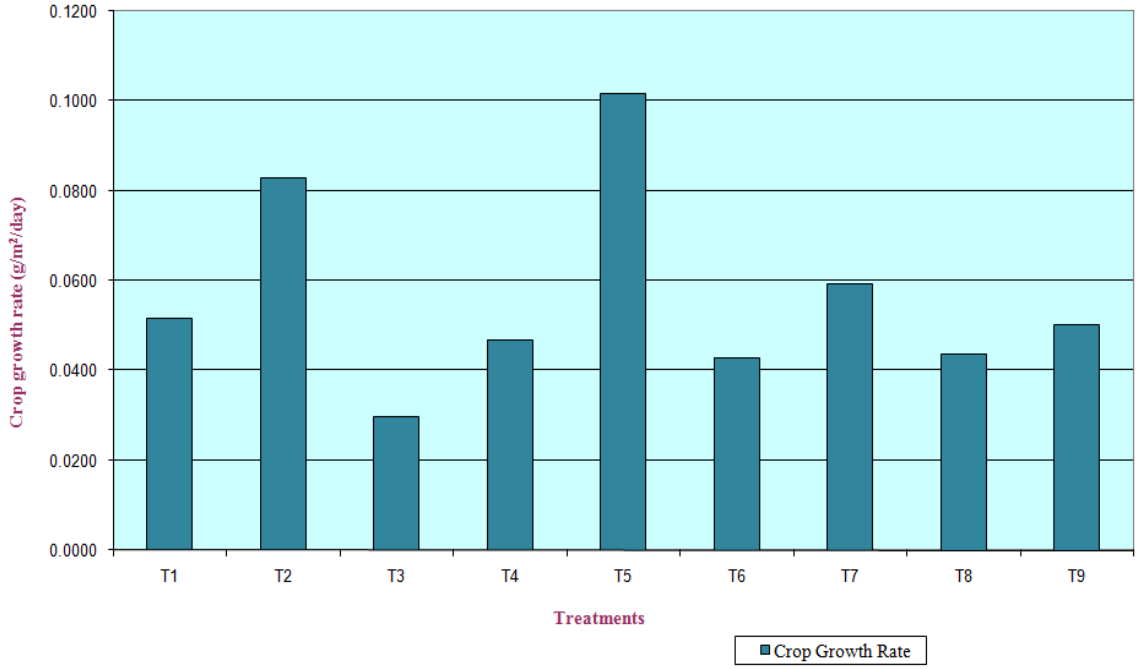


Fig. 12. Influence of nutrients and *Piriformospora indica* (PGPRE) on crop growth rate

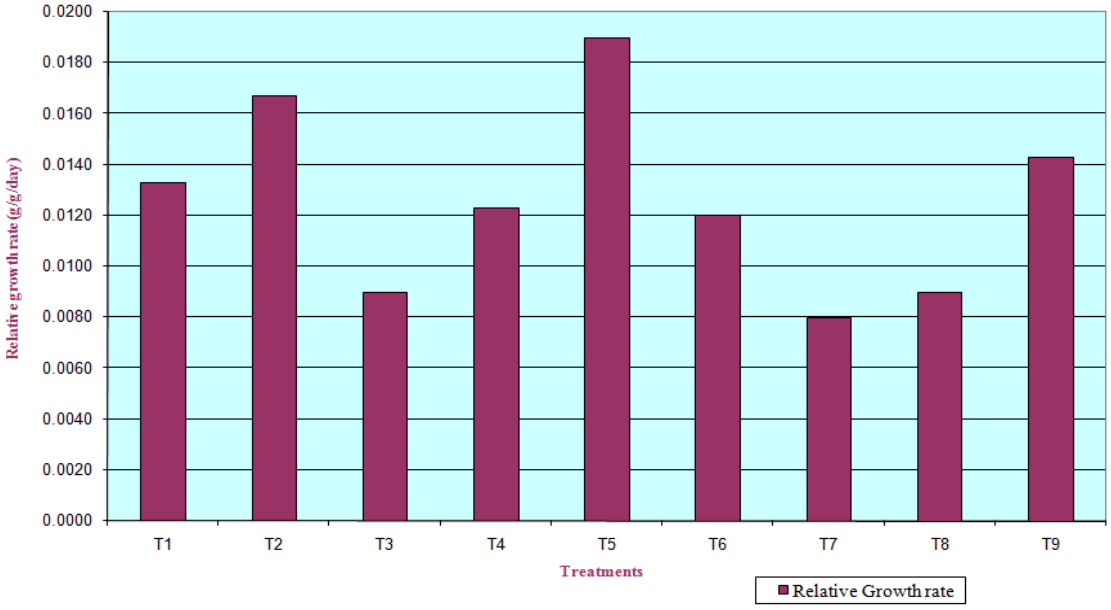


Fig. 13. Influence of nutrients and *Piriformospora indica* (PGPRE) on relative growth rate

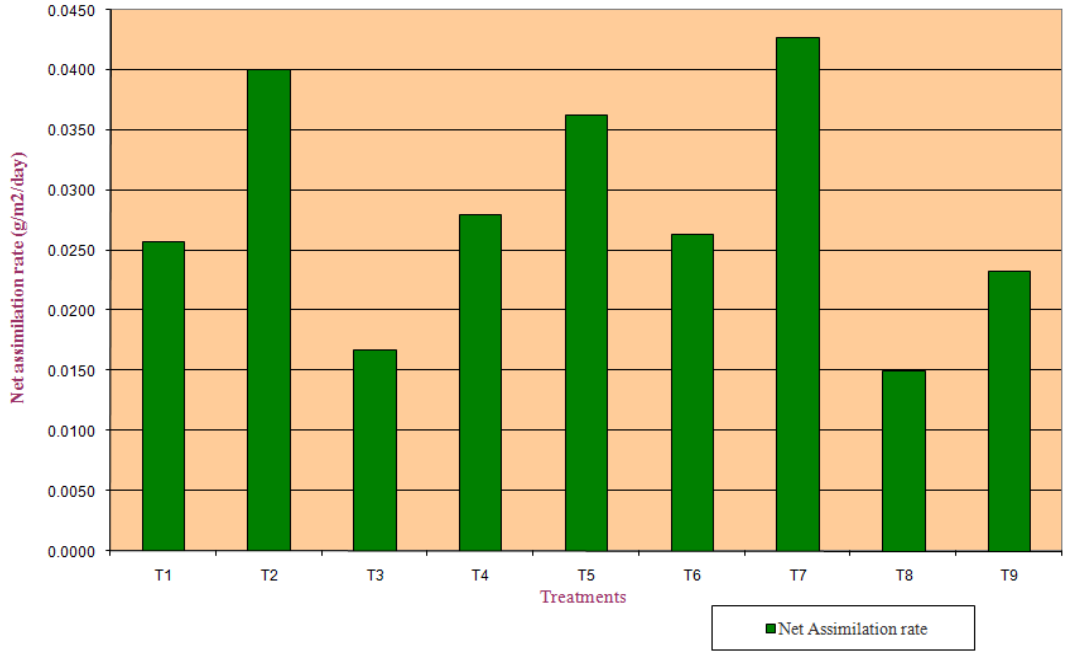


Fig. 14. Influence of nutrients and *Piriformospora indica* (PGPRE) on net assimilation rate

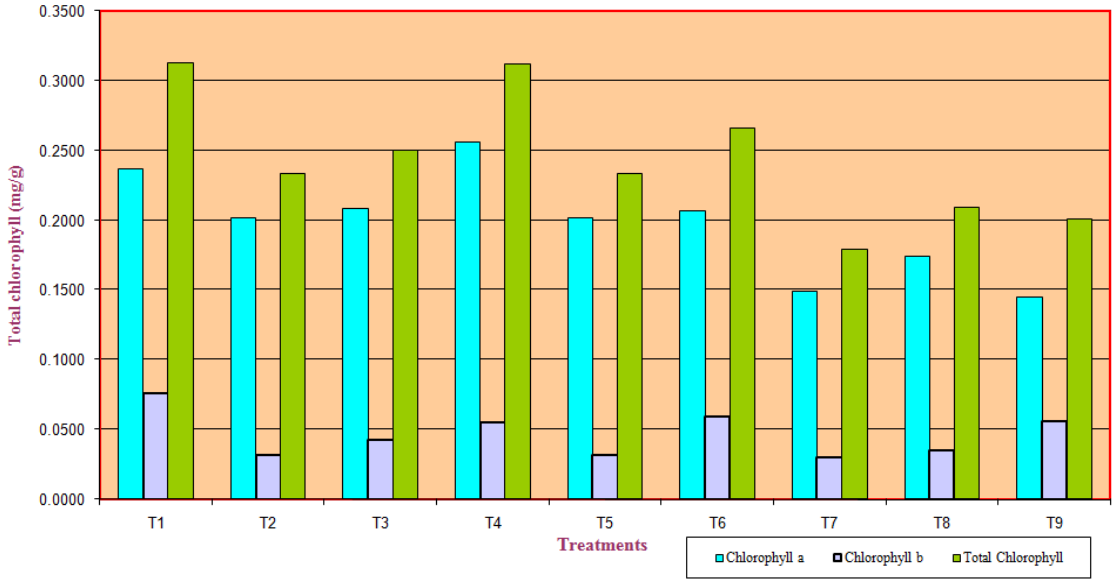


Fig. 15. Influence of nutrients and *Piriformospora indica* (PGPRE) on chlorophyll content

The study revealed that highest chlorophyll 'a', chlorophyll 'b' and total chlorophyll content were in the treatment receiving POP recommendation for orchids.

For most plants, healthy fully expanded leaves are the major source or net producer of photoassimilates. Other green organs (chlorophyll-containing organs) such as green stems, roots and floral and fruiting organs may also provide additional carbon through photosynthesis for growth. Orchids have many types of storage organs that are peculiar to their habitat; pseudobulbs, swollen roots and underground tubers (Arditti, 1992). Improvement in this harvestable yield of tropical orchids grown for its cut-flowers should adopt a two-prolonged approach that seeks to increase both the photosynthetic capacity of the source leaves and the ability of the inflorescence sink to important assimilates (Hew *et al*., 1996). This may be the reason for having no significant variation in physiological parameters in *Dendrobium* among different treatment combinations.

5.4 Influence of nutrients and *Piriformospora indica* on flower parameters

Cut flowers have always been the commonest form of commercial cultivation of orchids. Flowering in orchids depends on specific temperature, light intensity, nutrients, potting media, humidity etc. In this trial, all the plants did not come to flowering during the period of study. Flower characters in orchid genus *Dendrobium* cv. Earsakul are found to be influenced by nutrients and *P. indica*. Early flowering was resulted when plants was given foliar spraying with supernatant liquid of cowdung slurry, $N:P_2O_5:K_2O$ @ 3:1:1 during vegetative period and 1:2:2 during flowering period as 0.2 per cent foliar spraying in weekly twice (POP) along with *P. indica*. The positive influence of nutrients along with root endophyte may be the reason for early flowering. Favourable effect of POP + *P. indica* was also evident in the case of flower parameters like days to flowering, days to first flower opening, days to last flower opening, spike length, number and size of flowers.

Maximum vase life of 29 days was recorded in treatment POP + *P. indica* which is followed by treatment POP+ vermiwash+ *P. indica*+ bone meal. Plants receiving treatment with POP recommendation along with *P. indica* produced favourable floral characters.

Applications of nutrients along with *P. indica* resulted in maximum plant height, number of leaves, number of shoots and internodal length of the plants. This in turn resulted in maximum relative growth rate, crop growth rate and dry matter production. It is observed that, the combined effect of POP + *P. indica* resulted in higher number of roots, root volume and maximum root colonization of *P. indica*.

The enhanced growth parameters like plant height, number of leaves, number of shoots, as well as number of roots and root volume might have contributed to the early flowering and improved floral characters like spike length, number and size of the flowers as well as field longevity in T_5, this being due to the increased absorption of the nutrients as a result of the fungal association.

Considering the favourable effect of POP+ *P. indica* on growth, physiological parameters, root and flowering characters, conclusion can be drawn that in the treatment combination of spraying with supernatant liquid of cowdung slurry (1 kg of fresh cowdung in 5 litres of water)+ foliar spray with $N:P_2O_5:K_2O$ @ 30:10:10 during vegetative period and 10:20:20 at the flowering period at 0.2 per cent applied weekly twice+ basal application of *P. indica* fungal culture (20 g per plant) near root zone, resulted in significant positive influence on the growth and floral characters of *Dendrobium* cv. Earsakul.

5.5 Influence of nutrients and *Piriformospora indica* on nutrient content

Growth and flowering in orchids are improved by a regular scheduling of nutrients spray to the plants in liquid form. Nutrient concentration of plants is a good indication of the overall growth of the plant, assimilate accumulated in the sink of the plant body and the potential of that plant to effectively utilize the nutrient sources provided. The nutrients applied if properly absorbed and assimilated only can contribute to the development of any plant. Hence it is essential to study whether the supplied nutrients are absorbed and *P. indica,* has any role in enhancing the absorbance of nutrients by plants. Nutrient content in the plant parts as well as the uptake are also of great importance.

The study revealed that the nitrogen content in the plant was not influenced by the different treatments at six MAP and at the time of flower bud formation. Significant increase in phosphorus content was recorded in treatments POP+ organic mixture, POP+ vermiwash+ *P. indica*+ bone meal at the time of flower bud formation. In general, potassium, calcium and magnesium content were not influenced by the nutrients and *P. indica* during all growth stages (Fig. 16 to 24).

Studies on *P. indica* have shown fungal-mediated uptake of radio-labelled phosphorus from the medium and its translocation to the host in an energy-dependent process, evidenced by a sharp increase in its content in the shoot (Varma, 2000). *P. indica* produces significant amounts of acid phosphatases for the mobilization of insoluble phosphates and translocates the phosphorus to the host energy-dependent process (Singh, 2000). However, the positive effect of *P. indica* in increasing in phosphorus content was not visible in this study.

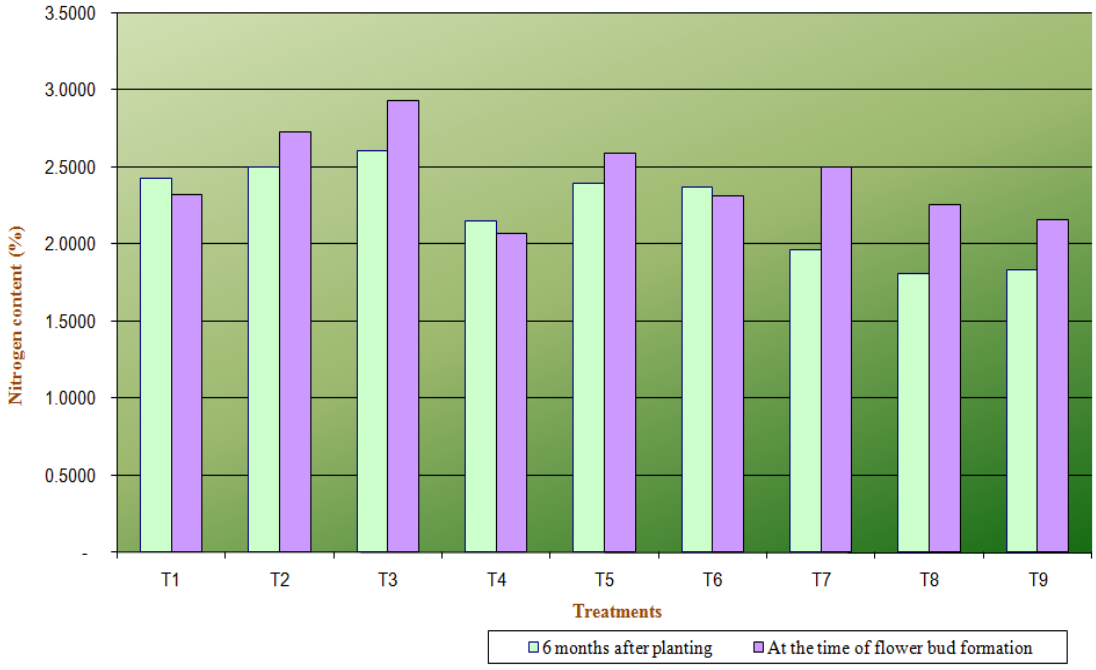


Fig. 16. Influence of nutrients and *Piriformospora indica* (PGPRE) on nitrogen content

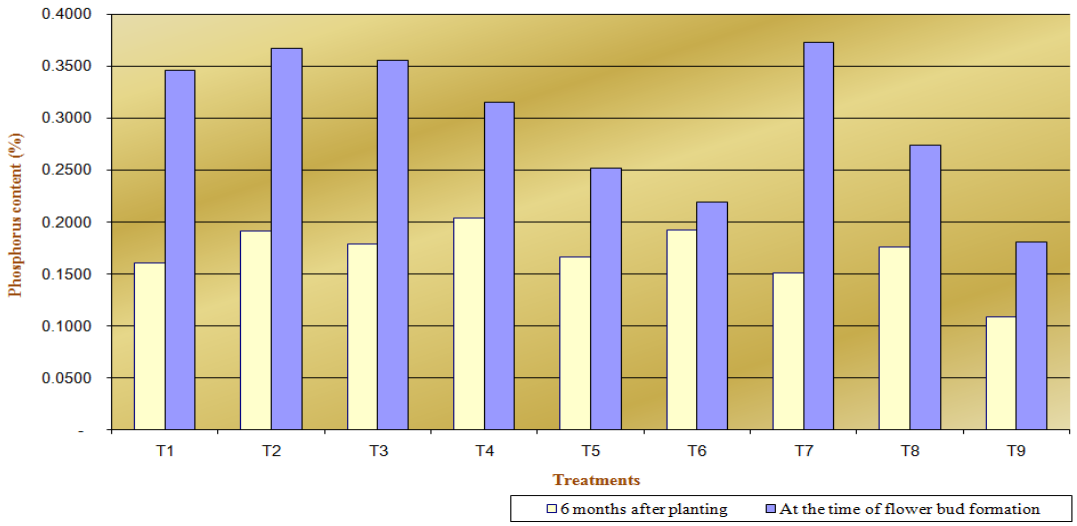


Fig. 17. Influence of nutrients and *Piriformospora indica* (PGPRE) on phosphorus content

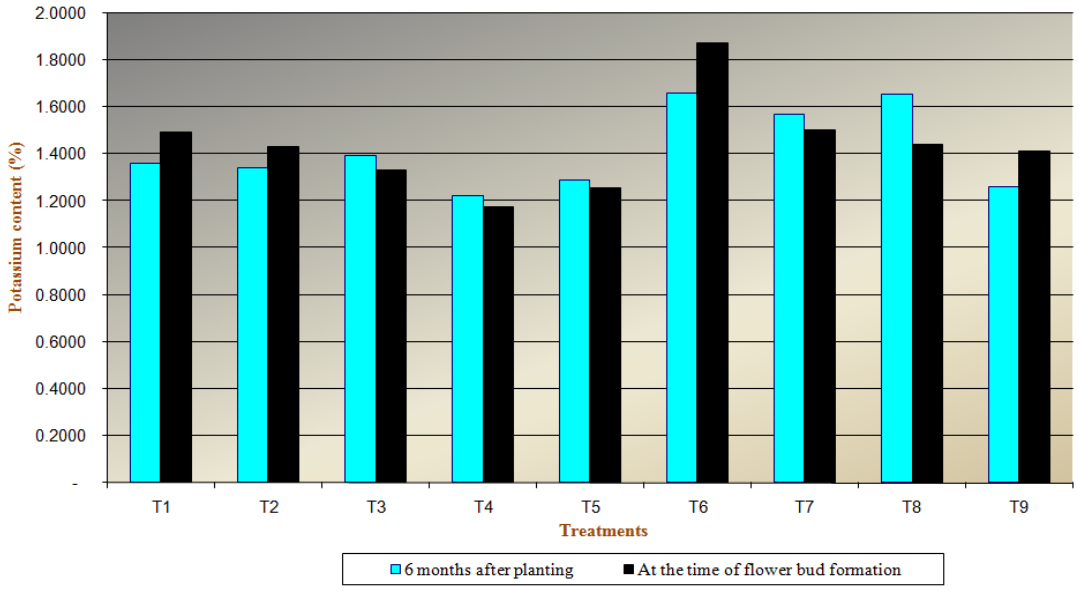


Fig. 18. Influence of nutrients and *Piriformospora indica* (PGPRE) on potassium content

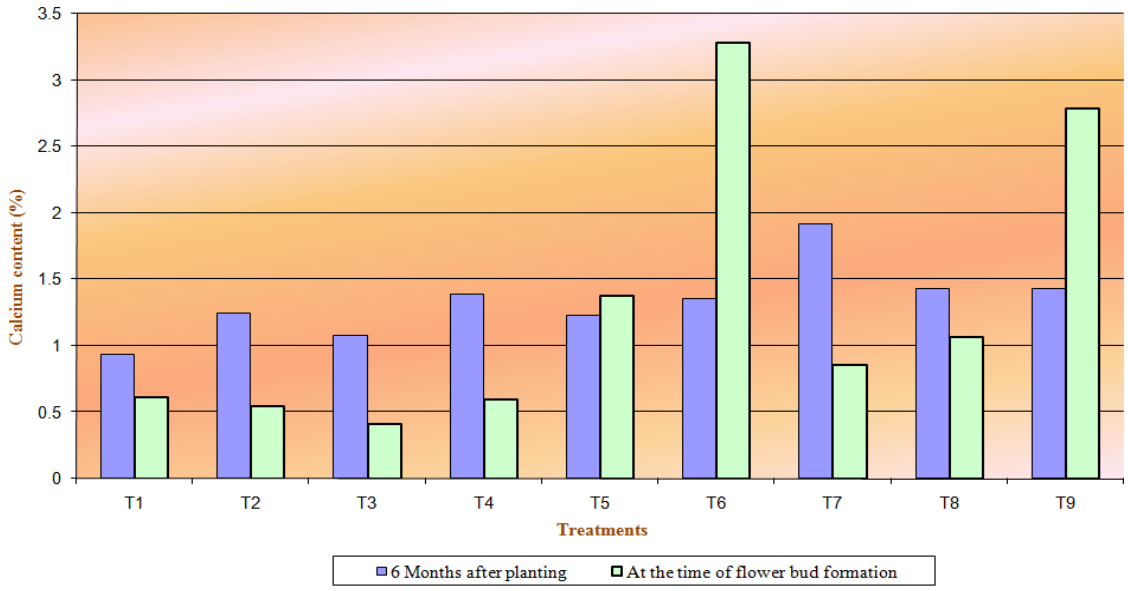


Fig. 19. Influence of nutrients and *Piriformospora indica* (PGPRE) on calcium content

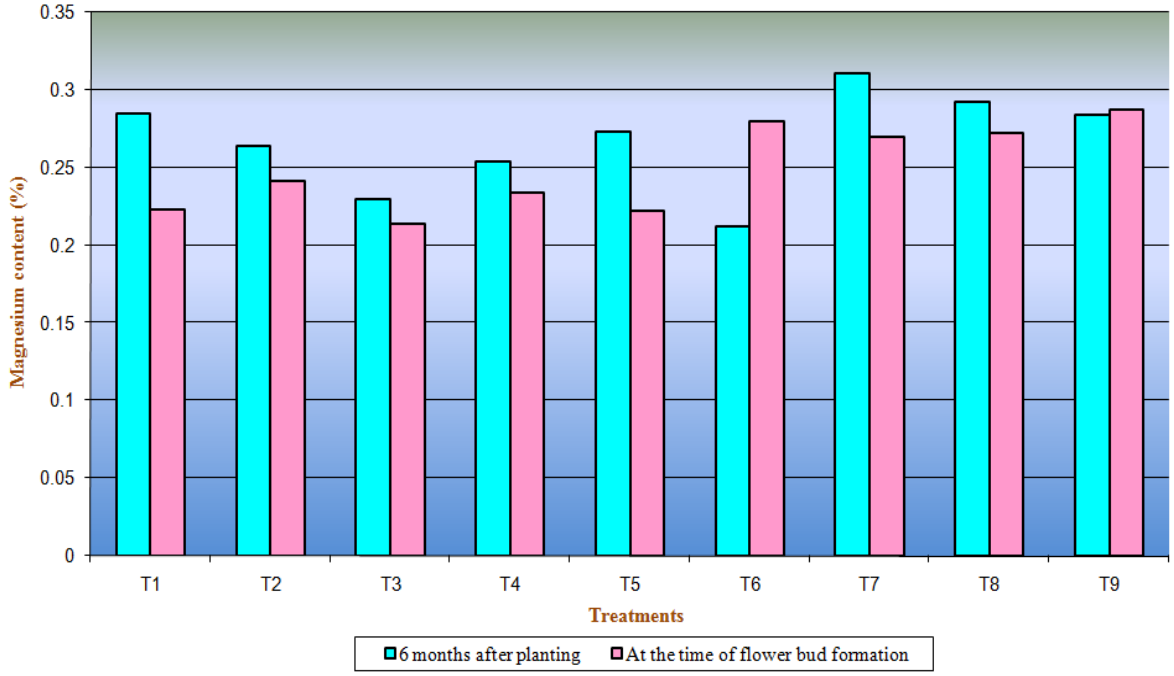


Fig. 20. Influence of nutrients and *Piriformospora indica* (PGPRE) on magnesium content

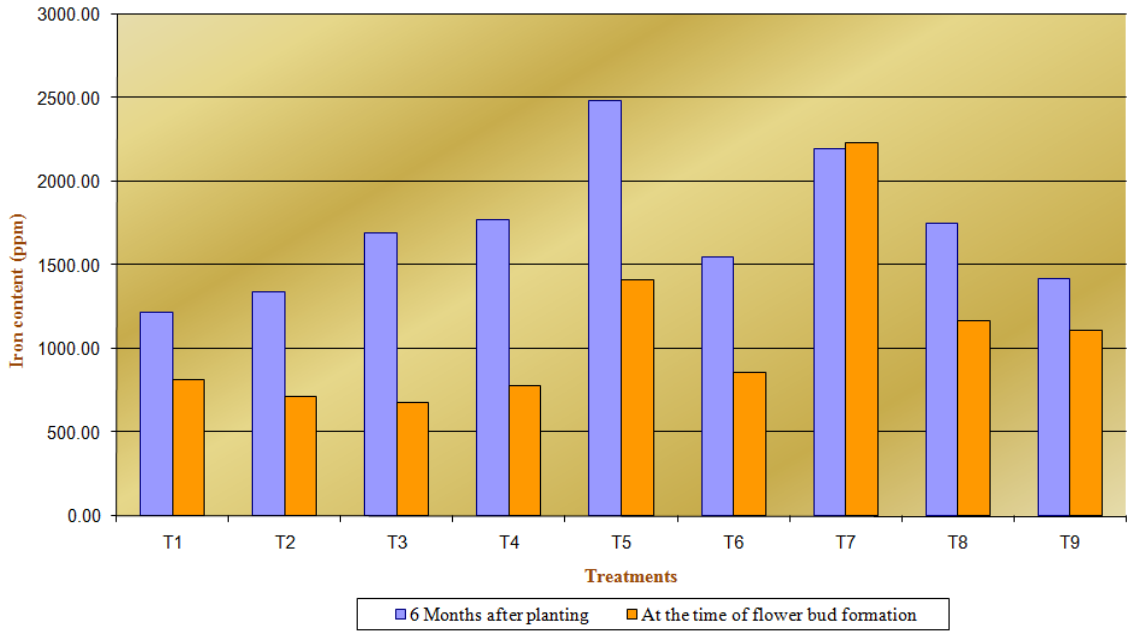


Fig. 21. Influence of nutrients and *Piriformospora indica* (PGPRE) on iron content

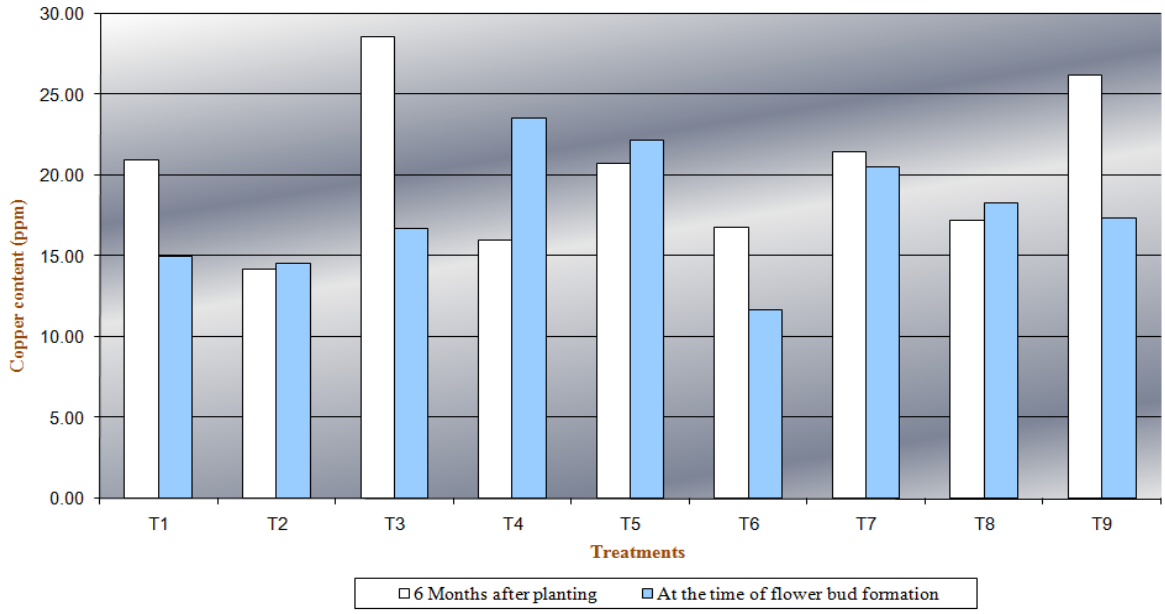


Fig. 22. Influence of nutrients and *Piriformospora indica* (PGPRE) on copper content

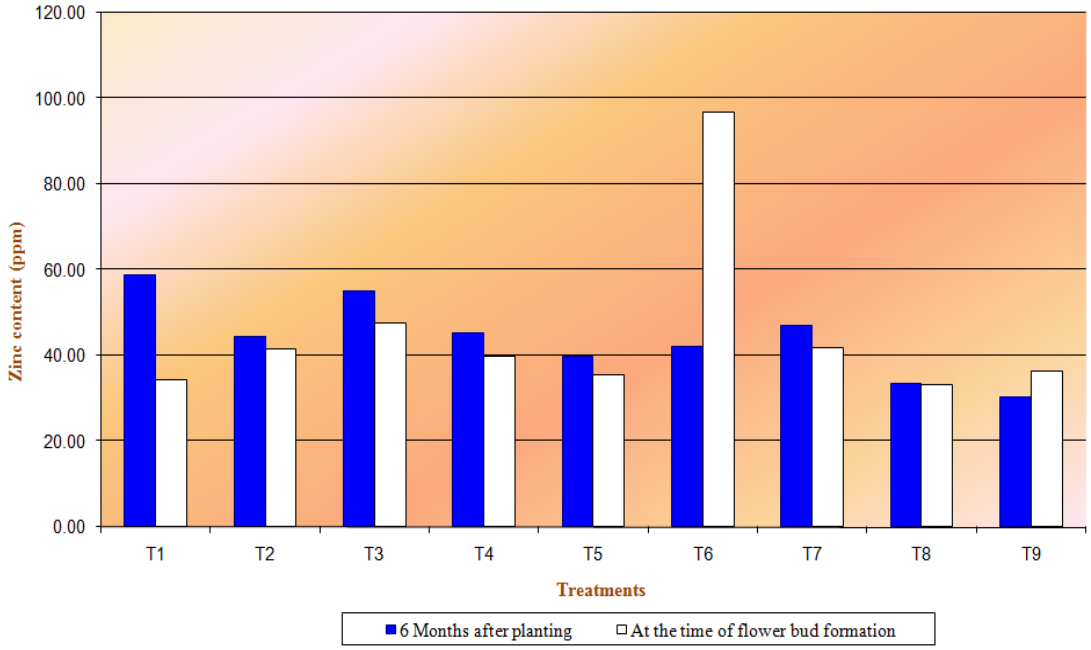


Fig. 23. Influence of nutrients and *Piriformospora indica* (PGPRE) on zinc content

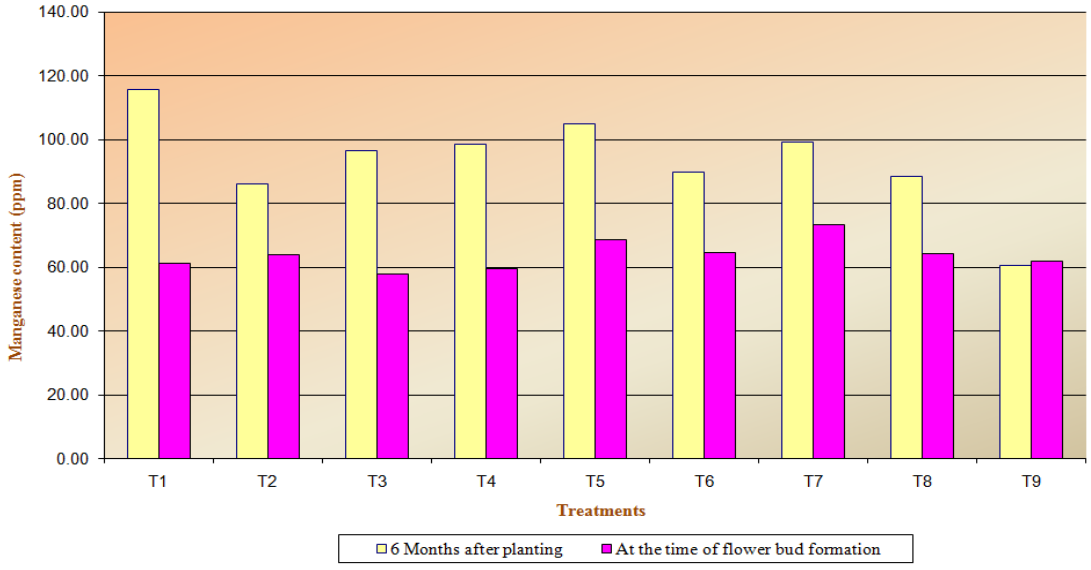


Fig. 24. Influence of nutrients and *Piriformospora indica* (PGPRE) on manganese content

Considering effects on various micronutrients like copper, zinc and manganese by the plants were not influenced by the nutrients and *P. indica*. However, maximum iron content was influenced by treatment involving POP+ *P. indica* during early stages of growth and POP+ vermiwash+ *P. indica*+ bone meal in later stages of growth.

5.6 Influence of nutrients and *Piriformospora indica* on incidence of pests and diseases

During the entire period of study, there was not much incidence of pests and diseases. But snails and slugs were observed during rainy season which is a common phenomenon in orchids cultivated in Kerala. Bacterial diseases were also observed during early stages of growth which was controlled timely by using suitable prophylactic measures. Waller *et al.* (2005) observed that the potential of *P. indica* to induce resistance to fungal diseases, in monocotyledonous barley. *P. indica* is able to provide systemic protection due to a yet unknown mechanism of induced resistance (Varma *et al.*, 1999) tolerance to salt stress and grain yield elevation in the monocotyledonous plant barley.

Future line of work

The present studies on *Dendrobium* cv. Earsakul cannot be considered conclusive. Hence, investigations have to be undertaken to refine the results of the present study. Since the treatment combination POP+ *P. indica* was found to enhance the growth, physiological parameters, root characters and flowering characters it requires further studies on identification of compounds responsible for plant promotional and elicitation effects in plants, time and methods of application and their colonization patterns during the symbiotic status.

Summary

6. SUMMARY

An experiment was conducted during February 2008 to June 2009 in the orchidarium of All India Co-ordinated Floriculture Improvement Project at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, to study the influence of nutrients along with *Piriformospora indica* on growth and development of *Dendrobium* cv. Earsakul. The study was undertaken using the commercial cultivar Earsakul belonging to the genus *Dendrobium*. *Piriformospora indica* was applied near the root zone at the time of planting after mixed with vermiculite @ 1 g per 100 g of vermiculite. The organic nutrients consisted of various manures like, vermiwash (3 per cent spray at 15 days intervals), bone meal (15g per plant) applied near the root zone at the time of planting and organic mixture (neem cake, bone meal, 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 the plants at fortnightly intervals). The inorganic nutrients consisted of $N:P_2O_5:K_2O$ 30:10:10 applied during vegetative period and 10:20:20 applied during flowering period @ 0.2 per cent spraying at weekly twice. The salient findings of the study are summarized below.

Among the growth characters, upto 12 MAP different treatments did not show appreciable difference in plant height. But during later stages of growth, significantly higher plant height was recorded in treatment combinations of POP recommendations of KAU for orchids (POP) + *P. indica* and in POP+ organic mixture+ vermiwash+ *P. indica* + bone meal. In general, no significant variation in number of leaves and number of shoots was observed among different treatments. Maximum girth of the shoots was resulted by providing POP+ organic mixture+ vermiwash+ *P. indica* + bone meal from 9 MAP. Among the different treatments, maximum internodal length was recorded by providing POP + *P. indica* towards the later stages of growth.

Among the root parameters studied, highest number of roots was recorded in treatment combinations POP+ organic mixture+ *P. indica* + bone meal at 6 MAP. No significant difference in number of roots was observed at the time of flower bud formation. At 6 MAP, no significant difference in root length was observed among treatments. At the time of flower bud formation, treatment receiving organic mixture+ vermiwash+ *P. indica* + bone meal recorded the maximum root length. Highest root volume was resulted by providing POP + *P. indica* at flower bud formation stage.

Percentage of root colonization of *P. indica* was maximum in treatment POP + *P. indica* at 6 MAP and flower bud formation stage.

Among the physiological parameters, significant variation in leaf area was observed among different treatments. Higher leaf area was resulted by providing POP+ organic mixture+ *P. indica* + vermiwash+ bone meal. Relative growth rate, net assimilation rate and dry matter production did not vary significantly among different treatments. Crop growth rate varied significantly due to different treatments. Highest CGR was recorded in the treatment POP + *P. indica*.

Chlorophyll 'a' content varied significantly among treatments. Highest chlorophyll 'a' content was recorded in the treatment POP + bone meal. No significant variation in chlorophyll 'b' was observed among treatments. Total chlorophyll content showed a significantly difference among treatments. Application of POP alone resulted in maximum total chlorophyll content.

Flower characters, earliest flowering, days to first flower opening, days to last flower opening, spike length, flower size and number of flowers per spike were recorded in treatment receiving POP + *P. indica*.

With respect to nutrient content, no significant difference in nitrogen content, potassium content and calcium content was obtained at 6 MAP and at the time of flower bud formation. Significant difference in phosphorus content was observed at flower bud formation stage. Maximum phosphorus content was recorded in treatment receiving POP+ vermiwash+ *P. indica* + bone meal. Significant variation in magnesium content was noticed in different treatments at 6 MAP. Maximum magnesium content was recorded in treatment receiving POP+ vermiwash+ *P. indica*+ bone meal. No significant difference in magnesium content was observed in different treatments during flower bud formation stage.

Significant difference with respect to iron content was noticed among different treatments at 6 MAP and flower bud formation stage. The maximum iron content was recorded in treatment receiving POP+ *P. indica* and treatment receiving POP+ Vermiwash+ *P. indica*+ bone meal, at 6 MAP and flower bud formation stage, respectively. No significant variation in copper and manganese content was observed among different treatments at 6 MAP and flower bud formation stage. Significant variation in uptake of zinc was noticed among different treatments at 6 MAP. Maximum zinc content was recorded in treatments receiving POP at 6 MAP. No significant difference in zinc content was noticed at the time of flower bud formation stage.

Incidences of pests and diseases were negligible during the entire period of study.

References

7. REFERENCES

Abbot, L.K and Robson, R.D. 1984. The effect of mycorrhizae on plant growth. In: Powell, C.L and Bagyaraj, D.J. (eds.), *VA Mycorrhizae*. CRC Press, Boca Retrn, pp.13-130

Abraham, A. and Vatsala, P. 1981. *Introduction to Orchids*. Tropical Botanical Garden and Research Institute, Trivandrum, 533p.

Alexander, C. and Hardley, G. 1983. Variation in symbiotic activity of *Rhizoctonia* isolates from *Goodyera repens* mycorrhizas. *Trans. Br. Mycol. Soc*. 3: 99-106

Allen, M.F. and Boosalls, M.C. 1983. Effect of two species of VAM mycorrhizal fungi on drought tolerance of winter wheat. *New Phytol*. 93: 967-976

Arditti, J. 1992. *Fundamentals of Orchid Biology*. John Wiley and Sons. New York, U.S.A.

Arditti, J. and Ernst, R. 1981. Physiology of germination of orchid seed. In: Arditti, J. (ed.), *Orchid Biology II- Reviews and Perspectives.* Comstock Publishing Associates, Cornell University Press, Ithava, 320p.

Arnold, A.E. and Lutzoni, F. 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology*. 88: 541–549

Azcon-Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. *Mycorrhiza*. 6: 457–464

Baker, M.L. and Baker, C.O. 1996. *Orchid Species Culture- Dendrobium*. Timberi Press, Singapore, p.852

Bakly, S.A. 1974. Effect of fertilization treatments on the yield of chrysalis imperial rose plants. *Agric. Res. Rev*. 52: 95-99

Balasubramanian, J. 1989. Studies on the combined effect of Azospirillum, VA mycorrhizal and inorganic fertilizers on growth and performance of French marigold (*Tagetes patula* L.). M.Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, p.180

Belesky, D.P. and Malinowski, D.P. 2000. Abiotic stresses and morphological plasticity and chemical adaptations of *Neotyphodium*-infected tall fescue plants; In: Bacon, C.W. and White, J.F. (eds.), *Microbial Endophytes*. New York, Marcel Dekker, pp. 455-484

Benzing, D.H. and Pridgeon, A.M. 1983. Foliar trichomes of Pleurothallidinae (*Orchidaceae*): functional significance. *Am. J. Bot*. 70(2): 173-180

Bhattacharjee, S.K. 1977. Cultivation notes on monotypic orchid genera of India. *Orchids Digest*. 40: 223-226

Bhattacharjee, S.K. 1981. The effect of nitrogen, phosphorus and potassium on growth and flowering of *Dendrobium moschatum* Willd, *Cymbidium moschatum* Willd and *Thicuania moschata* Rafin. *Gartenbauwssenschaft*. 46: 178-181

Bhattacharjee, S.K. 1982. Effect of nutrition on growth and flowering of *Aerides multiflorum* Rchb. *Lalbaugh J*. 27(3): 13-18

Bik, R.A. and Berg-Van-den, T.J.M. 1983. Effect of substrate and nitrogen supply on yield and quality of *Cymbidium*. *Acta Hort*. 150: 289-295

Binisha, S. 2003. Supplementry effect of bio-fertilizers in *Dendrobium*. M.Sc. thesis, Kerala Agricultural University. p.49

Blackman, V.H. 1919. The compound interest law and plant growth. *Am. Bot*. 33: 353-360

Blechert, O., Kost, G., Hassel, A., K. Rexer, K.H. and Varma, A. 1999. First remarks on the symbiotic interaction between *Piriformospora indica* and terrestrial orchids. In: Varma, A. and Hock, B. (eds.), *Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology,* (2nd ed.), Springer-Verlag, Germany, pp.683-688

Boodley, J.W. 1981. *The Commercial Green House Handbook*. Van Nostrand Reinhold Company, New York, 480p.

Boon, T.E.A. 1982. Growing variegated *Oncidiums*. *Aust. Orchid Rev*. 47(4):34-36

Borowicz, V.A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology*. 82: 3057–3068

Bose, T. K. 1978. *Commercial Flowers*. Naya Prakash, Calcutta, 635p

Bose, T.K. and Bhattacharjee, S.K. 1980. *Orchids in India*. Naya Prakash, Calcutta, p.237

Brundrett, M.C. 2002. Coevolution of roots and mycorrhizas of land plants. *New Physiol*. 154: 275-304

Brundrett, M.C. 2006. Understanding the roles of multifunctional mycorrhizal and endophytic fungi. In: Schulz, B.J.E., Boyle, C.J.C. and Sieber, T.N. (eds.), *Microbial Root Endophytes*. Berlin: Springer- Verlag, pp.281–293

Chae, S.C., Cheon, S., Son, K.C., Yun, J.G., Son, S.C., Yun, J.G. 1998. Photosynthetic pattern of *Dendrobium nobile* cultivars and their photosynthetic abilities as affected by temperature, light intensity and CO_2 concentration. *J. Korean Soc. Hort. Sci.* 39(6): 756-760

Cheplick, G.P., Clay, K. Marks, S. 1989. Interactions between infection by endophytic fungi and nutrient limitation in the grasses *Lolium perenne* and *Festuca arundinacea*. *New Phytol*. 111: 89-97

Chua, S.E. 1974. The effects of different levels of dried chicken manure on growth and flowering of *Oncidium* Golden Shower (var. Caldwell) and *Dendrobium* louisae Dark: *Singapore. J. Primary Ind.* 4(1): 16-23

Costa, J. Mand-Loper J.E. 1994. Characterization of siderophere production by the biological control agent *Enterobactor cloaca. Plant Microbe Interaction.* 7: 440-448

Decordenoy, T.H. 1904. Biological contribution to two vanillier. *J. Trop. Agric.* 4: 104-106

Deshmukh, S., Huckelhoven, R., Schafer, P., Imani, J., Sharma, M. and Wei, M. 2006. The root endophytic fungus *Piriformospora indica* requires host cell death for proliferation during mutualistic symbiosis with barley. In: *Proceedings of National Academy of Science*. USA. pp.18450–18457

Druege, U., Baltruschat, H. Franken, P. 2007. *Piriformospora indica* promotes adventitious root formation in cuttings. *Hort. Sci.* 112: 422-426

Feller, I.C. 1995. Effects of nutrient enrichment on growth and herbivory of dwarf red mangrove (*Rhizophora mangle*). *Ecol. Monogr*. 65: 477-505

Fitch, C.M. 1981. *All about Orchids*. Doobleday and company. Inc. New York, U.S.A. p 113

Gasoni, L. and Gurfinkel, B.S. 1997. The endophyte *Cladorrhinum foecundissimum* in cotton roots: phosphorus uptake and host growth. *Mycol. Res*. 101: 867-870

Gehring, C.A. and Whitman, T.G. 2002. Mycorrhizae- herbivore interactions: population and community consequences. In: Van-Der-Heijden, M. and Sanders, I. (eds.), *Mycorrhizal Ecology*. Springer, Berlin, pp.295–320

Giovannetti, M. and Mosse, B. 1998. An evauation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*. *Vol. 84*, pp.489-500

Girlanda, M., Perotto, S. and Luppi, A.M. 2006. Molecular diversity and ecological roles of mycorrrhiza-associated sterile fungal endophytes in mediterranean ecosysems. In: Boyle, C.J.C. and Sieber, T.N. (eds.), *Microbial root endophytes*. Berlin: Springer-Verlag, pp.207–226

Hadley, G. 1969. Cellulose as carbon source for orchid mycorrhiza. *New Phytol*. 68: 933-939

Hew, C.S. 1993. Orchid cut flower production in Asian countries. In: Arditti, J. (ed.), *Orchid Biology Review and Perspective*. John Wiley, New York, 238p.

Hew, C.S., Clifford, P.E. and Young, J.W.H. 1996. Aspects of carbon portioning in tropical orchids. *J. Orchids Soc. India*. 10(1-2): 53-80

Jackson, M. L. 1958. Soil Chemical Analysis. Prentic Hall Inc., USA, p.363

James, E.K., Reis, V.M., Olivares, F.L., Baldani, J.I. and Dobereiner, J. 1994. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. *J. Exp. Bot*. 45: 757-766

Johnson, C.M. and Ulrich, A. 1959. Analytical methods for use in plant analysis. *Calif. Agric. Exp. Station Bull*. 766: 26-76

Johnson, W.R.B. 1984. A simple liquid nutritional programme for orchids. *Aust. Orchid Rev*. 49(3): 197-204

Kale, R.D. Mallesh, B.C. Bano, K and Bagyaraj, D.J. 1992. Influence of vermicompost application on the available macronutrients and selected microbial populations in paddy field. *Soil Biol. Chem*. 24: 1317-1320

Kang, L.C. 1979. *Orchids: Their cultivation and hybridization*. Singapore: Eastern University Press, SDN, p.569

KAU, 2007. *Package of Practices Recommendation Crops*, Kerala Agricultural University, Director of Extension, KAU, Mannuthy, Thrissur, Kerala. 190p.

Kaushik, P. 1983. *Ecological and Anatomical Marvels of the Himalayan Orchids.* Today and Tomorrow's Printers and Publishers, New Delhi, India, pp.123-139

Khaw, C.H. and Chew, P.S. 1980. Preliminary studies on the growth and nutrient requirements of orchids. In: Singh, K.G. (ed.), *Proceedings of Third ASEAN Orchid Congress*. Ministry of Agriculture, Malaysia, pp.49-64

Khoo, G.H. and Hew, C.S. 1999. Developmental changes in chloroplast ultra structure and carbon fixation of *Dendrobium* flowers (Orchidaceae). *Inter. J. Plant Sci*. 160: 699-705

Kirichenco, E.B., Chernyad, N.I., Voronkova, T.V., Sokolova, R.S. and Doman, N.G. 1989. The activity of photosynthetic apparatus in orchids during flowering. *Fiziologiya Ratstenii*. (Azerbaijani). 36(4): 710-716

Kobayashi, D.Y. and Palumbo, J.D. 2000. Bacterial endophytes and their effects on plants and uses in agriculture. In: Bacon, C.W and White, J.F. (eds.), *Microbial Endophytes*. Marcel Dekker, New York, pp.199-236

Kuldau, G.A. and Yates, I.E. 2000. Microbial Endophytes. In: White, C.W.B.J.F. (ed.), *Evidence for Fusarium Endophytes in Cultivated and Wild Plants*. 120, Marcel Dekker, New York and Basel, pp.85-95

Lee, S., Flores-Encarnacion, M., Contreras-Zentella, M., Garcia-Flores, L., Escamilla, J.E. and Kennedy, C. 2004. Indole-3-Acetic acid biosynthesis is deficient in *Gluconacetobacter diazotrophicus* strains with mutations in *Cytochrome-c* biogenesis genes. *J. Bact*. 186: 5384–5391

Linda, K. 1987. The culture of Anguloas tulips by quite another name. *Am. Orchid Soc. Bull*. 56: 15-17

Longman, H. 1989. *Orchid growing*. Van Nosts and Runhold Co., New York, 183p.

Madhaiyan, M, 1999. Studies on the effect of Orchid Mycorrhizal fungi in *Vanilla planifolia* and *Dendrobium* spp. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 134p.

Madhaiyan, M., Krishnan, P.S. and Pragastheswari, D. 2003. Rapid detection and assessment of orchid mycorrhizal colonization in *Vanilla planifolia* Andr. roots. *Mycorrhiza News*. 14:10-13.

Malinowski, D.P. and Belesky, D.P. 1999. Neotyphodium coenophialum- endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. *J. Plant Nutr*. 22: 835–853

Manonmani, R. 1992. Effect of soil inoculation of Azospirillum and Phoshobacteria and graded dose of nitrogen and phosphrous on growth and yield of *Jasminum sambac Ait., cv. Gundumalli.* M.Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, P.105

Marguerite, W. 1989. The care and feeding of Draculas. *Am. Orchid Soc. Bull*. 58: 987-993

Mariappan, R. 1992. *Studies on the effect of Azospirillum and cycocel (CCC) on marigold (Tagetes erecta* L.*) cv. MDU-1*. M.Sc. thesis, Tamil Nadu Agricultural University, Coimbatore, p.111

Masuhara, G. and Katsuya, K.1992. *In situ* and *invitro* specicity between *Rhizoctonia* spp. and *Spiranthes sinensis* (Persoon) Ames. Var. *Amoena* (M. Bieberstein) Hara (Orchidaceae). *New Phytol*. 127: 711-718

Merriman, A.J. 1987. Factors that influence the growing and flowering of Cymbidiums to perfection. *Aust. Orchid Rev*. 40(1): 45-49

Mukherjee, S.K. 1990. *Orchids*. Indian Council of Agricultural Research, New Delhi, pp.9-16

Nair, U.S. 2001. Endogenous and exogenous regulation of growth and development in *Dendrobium* var. Sonia 17 and Sonia 28. M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, Kerala, 71p.

Nene, Y.L. 1999. Seed health in ancient and medicinal history and its elegance to present dry agriculture. *Asian Agric. Hist*. 3(3): 332-345

Northen, R.T. 1970. *Home Orchid Growing*. Van Nostrand Reinhold, New York, 165p.

Ogoshi, A., Oniko, M., Araki, T. and Ui, T. 1983. Anastamosis groups of binucleate *Rhizoctonia* in Japan and North America and their perfect states. *Mycol. Soc. Jpn*. 24: 79-87

Panse, V.G. and Sukhatme, P.V. 1985. Statistical methods for agricultural workers, ICAR, New Delhi, pp.97-164

Penningsfield, F. and Fast, G. 1962. Effect of deficiency and excess of nitrogen in *Paphio pedilum*. *Am. Orchid Soc. Bull*. 31: 301-304

Peskan-Berghofer, T., Shahollari, B., Giong, P.H., Hehl, S., Markert, C. and Blanke, V. 2004. Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant– microbe interactions and involves early plant protein modifications in the endoplasmatic reticulum and at the plasma membrane. *Physiol. Plant*. 122: 465–477

Peter, T. 1990. Cattleya culture. *Orchid Rev*. 54: 104-107

Peters, S. 1991. Field and culture studies of *Streblonema macrocystis* new species *Ectocarpales phaeophyceae* from chile, a sexual endophyte of giant kelp. *Phycologia*. 30: 365-377

Peterson, R.L. and Massicotte, H.B. 2004. Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. *Can. J. Bot*. 82: 1074-1088

Petrini, O. 1991. Fungal endophytes of tree leaves. In: Hirano, J.A.S. (ed.), *Microbial Ecology of Leaves*. Springer Verlag, New York, pp.179-197

Pham, G.H., Singh, A., Malla, R., Kumari, R., Prasad, R. and Sachdev, M. 2004. Interaction of *Piriformospora indica* with diverse microorganisms and plants. In: Varma, A., Abbott, L.K., Werner, D. and Hampp, R., (eds.) *Plant surface microbiology*. Berlin, Heidelberg, New York, Springer, pp. 237–265

Poole, H.A. and Seeley, T.J. 1978. Nitrogen, potassium and magnesium nutrition of three orchid genera. *J. Am. Soc. Hort. Sci*. 103(4): 485-488

Poole, T. and Sheehan, T.J. 1970. Effects of media and supplementary microelement fertilization on growth and chemical composition of Cattleya. *Am. Orchid Soc. Bull.* 46: 155-160

Pradhan, U.C. 1976. *Indian Orchids: Guide to identification and culture. Vol.I.* Udai C. Pradhan, Kalimpong, India, p.12

Pradhan, U.C. 2001. Orchid commerce in India- guidelines for a new century. In:Pathak, P., Singh, B. and Singh, M.P. (eds.), *Orchids Science and Commerce*. Dehraun, India, pp.509-514

Preethi, T.L. 1990. Studies on the effect of nitrogen, Azospirillum and ascorbic acid on growth and flowering of Edward rose (Rosa bourboniana Desp.). *S. Indian Hort*. 48: 83-87

Press, M.C. 1986. The parasite Habit: trends in metabolic reduction. In: Ter-Berg, S.J. (ed.). *Proceedings of a Workshop on Biology and Control of Orobanche*. Agriculture University, Wageningen, pp.96-106

Rai, M. and Varma, A. 2005. Arbuscular mycorrhiza like biotechnological potential of *Piriformospora indica*, which promotes the growth of *Adhatoda vasica* Nees. *J. Biotech*. 52: 643–650

Rai, M., Acharya, D. and Singh, A. 2001. Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. *Mycorrhiza*. 11: 123–128

Rai, M.K., Varma, A. and Pandy, A.K. 2004. Enhancement of antimycotic potential in *Spilanthes calva* after inoculation of *Piriformospora indica*, a new growth promoter. *Mycoses*. 47: 479-481

Rajeevan, P.K., Sobhana, A., Baskar, J., Swapna, S. and Bhattacharjee, S.K. 2002. *Orchids*. ICAR, New Delhi, p.62

Rathore, S.V.S., Dera, D.K. and Chand, V. 1985. Studies on nutrition through foliar sprays of urea on the performance of African Marigold (*Tagetes erecta*). *Udanyanika*. 5: 37-40

Rexer, K.H. Blechert, O. Kost, G and Varma, A. 2000. Potential growth of the fungus. *New Phytol*. 12: 56-87

Sagarik, R. and Siripong, S. 1963. A study of some orchid fertilizers. *Am. Orchid Soc. Bull.* 32: 174-176

Sakai, K., Osuga, M. and Yonemura, K. 1985. Effect of fertilizer application on growth and flowering in *Dendrobium* sp. II. *Res. Bull. Aichi. Agric. Res. Centre*. 17: 239-247

Sanford, W.W. 1974. The ecology of orchids. In: Whithner, C.I. (ed.), *The orchids: Scientific Studies*. John Wiley and Sons, New York, p.59

Schardl, C, Leuchtmann, A. 2005. The epichloe endophytes of grasses and the symbiotic continuum. In: Dighton, J., White, J.F., Oudemans, P., Boca- Raton, F.L. and Taylor, F. (eds.), *The Fungal Community: Its Organization and Role in the Ecosystem*. pp.475–503

Schardl, C.L. 2001. *Epichloe festucae* and related mutualistic symbionts of grasses. *Fungal Gen. Biol.* 33: 69–82

Schulz, B. and Boyle, C. 2005. The endophytic continuum. *Mycol. Res.* 109: 661- 686

Schum, A. and Fisher, P. 1985. The N: K_2O ratio in *Phalaenopsis*. *Deutsacher Hort*. 39(36): 1704-1706

Seeni, S. and Latha, P.G. 1990. Post transplantation growth of *Phalaenopsis* hybrid seedlings in community pots. *J. Orchid Soc. India*. 4: 127-135

Serfling, A. Stefan, G.R. Lind, V. and Deising, H.B. 2007. Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field conditions. *Phyto Path*. 97: 523-531

Shahollari, B. Varma, A. and Oelmuller, R. 2005. Expression of a receptor kinase in roots is stimulated by the basidiomycete *Piriformospora indica* and the protein accumulates in Triton-X-100 insoluble plasma membrane microdomains. *J. Plant. Physiol.* 162: 945-958

Shankar, K.S., Manivannan, K. and Kamalakannan, S. 2003. Effect of potting media and nutrients on flowering of *Dendrobium* hybrid 'White Fairy'. In: National symposium on Recent Advances in Indian Floriculture, pp.97-100

Sheehan, T.J. 1960. Effects of nutrition and potting media on growth and flowering of certain epiphytic orchids. *Am. Orchid Soc. Bull.* 30: 289-292

Sheehan, T.J. 1996. The fertilization of orchids. In: Garmo, L.R. (ed.), *Proceedings of Fifth World Orchid Conference*, California, pp.95-97

Sherameti, I, Shahollari, B., Venus, Y., Altschmied, L., Varma, 2008 b. The root colonizing endophyte *Piriformospora indica* confers drought tolerance in Arabidopsis by stimulating the expression of drought stress related genes in leaves. *Mol. Plant Microbe Int*. 21:799-807.

Sherameti, I., Shahollari, B., Venus, Y., Altschmied, L., Varma, A. and Oelmuller, R. 2008. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch degrading enzyme glucan- water dikinase in tobacco and *Arabidopsis* roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J. Bot. Chem*. 280: 26241-26247

Shoaf, T.W. and Livm, B.W. 1976. Improved extraction of Chlorophyll 'a' and 'b' from algae using dimethyl sulfoxide. Limnol. *Oceanogr*. 2: 926-928

Shweta, Depika and Sonal. 2005. Organic carbon transformation in different organic wastes by microbial vermicomposting. In: Kumar, A. (ed.), *Worms and Vermitechnology*. APH Publishing Corporation, New Delhi, pp.71-74.

Sieber, T.N. 2002. In The Hidden Half. In: Waisel, A.E.U.K.Y. (ed.), *Fungal Root Endophytes*. Marcel Dekker, New York, pp.887-917

Sinclair, R. 1990. Water relations in orchids. In: Arditti, V.J. (ed.), *Orchid Biology- Reviews and Perspectives*. pp.63-120

Singh, A. and Varma, A. 2000. Orchidaceous mycorrhizal fungi. In: Mukerji, K.G., Chamola, B.P. and Singh, J. (eds.), *Mycorrhizal Biology*. Kluwer Academic/ Plenum Publishers, New York, pp.265-288

Singh, A., Sharma, J., Rexer, K.H. and Varma, A. 2000. Plant productivity determinants beyond minerals, water and light: *Piriformospora indica* - A revolutionary plant growth promoting fungus. *Curr. Sci.* 79: 1548–1554

Singh, A.K. 2006. *Orchids in Flower Crop Cultivation and Management*. New India Publishing Agency, 101, Vikas Surya Plaza, New Delhi, p.463

Singh, A.N., Singh, A.N., Kumari, M., Kumar, S., Rai, M.K., Sharma, A.P. and Varma, A. 2003a. Biotechnological importance of *Piriformospora indica* Verma *et al*.- a novel symbiotic mycorrhiza- like fungus: an overview. *Indian J. Biotech*. 2: 65-75

Singh, A.N., Singh, A.R., Kumari, M., Kumar, S., Rai, M.K., Sharma, A.P. and Varma, A. 2003b. Unmassing the accessible treases of the hidden unexplored microbial world. In: Prasad, B.N. (ed.), *Biotechnology in Sustainable Biodiversity and Food Security*. Science Publishers, Enfield, NH, pp.101-124

Singh, A.R., Singh, A.N., Rexer, K.H., Kost, G., Varma, A. 2002b. Root endosymbiont: *Piriformospora indica*- a boon for orchids. *J. Orchid Soc. India.* 15: 89-102

Singh, A.R., Singh, A.N., Varma, A. 2002a. *Piriformospora indica*- invitro raised leguminous plants: a new dimension in establishment and phytomotion. *Indian J. Biotech.*1: 371-376

Singh, F. 1992. Orchids. In: Chadha, K.L. and Chowdhari, B. (eds.), *Ornamental Horticulture in India*. ICAR, New Delhi, pp.127-136

Singh, S.S. 1996. *Soil Fertility and Nutrient Management*, Kalyani Publishers, Ludhiana, pp.126- 131

Smith, S.E. 1966. Physiology and ecology of orchid mycorrhizal fungi with reference to seedling nutrition. *New Phytol.* 65: 488-497

Smith, S.E. and Read, D.J. 1997. *Mycorrhizal Symbiosis.* (2nd ed.) New York, Academic Press, p.172

Sobhana, A. and Rajeevan, P.K. 1995. Foliar application of nutrient formulations in *Cymbidium traceanum*. *J. Orchid Soc. India*. 9(1-2): 45-50

Stewart, J. 1988. *Orchids*. The Royal Botanic Garden, The Hamlyn Publishing Group Ltd., England, 45p.

Stone, J.K., Bacon, C.W. and White, J.F. 2000. An overview of endophtic microbes: endophytism defined. In: Bacon, C.W. and White, J.F. (eds.), *Microbial Endophytes.* Dekker, New York, pp.3-30

Swapna, S. 2000. Regulation of growth and flowering in *Dendrobium* var. Sonia 17. Ph.D. thesis, Kerala Agricultural University, Thrissur, Kerala, 235p.

Taejung, K. Hoon, S.J. and Yoeoup, P.K. 1998. Effect of NPK ratios on the growth and mineral content of temperate *Cymbidium*. *J. Korean Soc. Hort. Sci.* 39(4): 469-474

Tanaka, T., Kanya, Y., Masuda, M. and Gomi, K. 1989. Growth and nutrient uptake of *Cattleya* hybrid grown with different composts and fertilizers. *J. Jpn. Soc. Hort. Sci*. 57(4): 674-684

Tanaka, T., Matsuno, T., Masuda, M. and Gomi, K. 1988a. The effect of concentration of nutrient solution and potting media on the growth and chemical composition of *Cattleya* hybrid. *J. Jpn. Soc. Hort. Sci.* 57(1): 85-90

Tanaka, T., Matsuno, T., Masuda, M. and Gomi, K. 1988b. The effect of concentration of nutrient solution and potting media on the growth and chemical composition of *Phalaenopsis* hybrid. *J. Jpn. Soc. Hort. Sci.* 57 (1): 78-84

Tanaka, T., Ogino, Y. and Gomi, K.1981. Fertilizer application for *Cattleya* hybrid. *Bull. Faculty Agric .Miyazaki Univ*. 28(1): 129-135

Tearashita, T. 1982. Fungi inhabiting wild orchids in japan II. Isolation of symbionts from *S.sinensis* var. *Amoena*. *Mycol. Soc. Jpn*. 23: 319-328

Thekkayam, S.G. 1996. Performance of selected orchids under varying light regions, culture methods and nutrition. Ph.D. thesis, Kerala Agricultural University, Thrissur, Kerala, 221p.

Thomas, J., Kuruvilla, K.M. and Vadivel, V. 2004. Tips on quality planting materials production of vanilla. *Spice India*. 17(9): 37-41

Tisdale, S.L., Nelson, W.L., Beaton, J.D. and Havlin, J.L. 1995. *Soil Fertility and Fertilizers*, (5th ed.). Prentice Hall of India Pvt. Ltd, New Delhi, 634p.

Tonnier, J.P. 1954. Effect of *Noveanx assais* on germination of seeds of *Vanilla planifolia* Andrew. *Int. Bot. Sci*. 12: 412-418

Tsai, Y.F., Hsiang and Huang, H.C. 1996. The improvement of fertilizers techniques by utilization of nutrient uptake rate in *Cymbidium sinense*. *Bull. Taichung. District Agric. Improv. Stn*. 53: 13-24

Uesato, K., Yagi, N. and Odo, S. 1987. Effects of nitrogen and phosphate on the growth of *Ceratobium Phalaenanthe* types. *Dendrobium Sci. Bull. Agric. Rjukys Univ*. 34: 11-19

Umamaheswari, R. 1999. Nutrition of tissue culture plants of *Dendrobium* Sonia 17. M.Sc. thesis, Kerala Agricultural University, Thrissur, Kerala, 124p.

Vachorotayan, S. and Keethapirom, S. 1975. Effects of fertilizers upon growth and flowering of *Dendrobium* 'Pompodour. In: *Report of the First ASEAN Orchid Conference*, Bangkok, Thailand, pp.138-156

Van-Bael, S.A., Maynard, Z., Rojas, E., Mejia, L.C., Kyllo, D.A., Herre, E.A., Robbins, N., Bischoff, J.F. and Arnold, A.E. 2005. Emerging perspectives on the ecological roles of endophytic fungi in tropical plants. In: Dighton, J., White, J.F., Oudemans, P., Boca-Raton, F.L. and Taylor, F. (eds.), *The Fungal Community: Its Organization and Role in the Ecosystem*. pp.505–518.

Varma, A., Singh, A., Sahay, N.S., Sharma, J., Roy, A., Kumari, M., Rana, D., Thakran, S., Deka, D., Bharti, K., Franken, P., Hurek, T., Blechert, O., Rexer, K.H., Kost, G., Hahn, A., Hock, B., Maier, W., Walter, M., Strack, D. and Kranner, I. 2001. Mycota. In: Hock, B. (ed.), *Piriformospora indica*: a cultivable Mycorrhiza- like endosymbiotic fungus. Springer series, Heidelberg, Germany. pp.123–150

Varma, A., Verma, S., Sudha, S., Sahay, N.S. and Franken, P. 1999. *Piriformospora indica*: a cultivable plant growth promoting root endophyte with similarities to arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* 65: 2741-2744

Verma, S., Varma, A., Rexer, K.H., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B. and Franken, P. 1998. *Piriformospora indica*, a new root-colonizing fungus. *Mycologia*. 90: 896-903

Vilich, V., Dolfen, M. and Sikora, R.A. 1998. *Chaetomium* spp. colonization of barley following seed treatment and its effect on plant growth and *Erysiphe graminis* f. sp. *hordei* disease severity. *J. Plant Dis. Prot*. 105: 130-139

Wadasinghe, G. and Hew, C.S. 1995. The importance of back shoots as a source of photoassimilates for growth and flower production in *Dendrobium* cv. Jashika pink (Orchidaceae). *J. Hort. Sci.* 70(2): 207-214

Wakelin, S.A., Warren, R.A., Harvey, P.R. and Ryder, M.H. 2004. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biol. Fertil. Soils.* 40: 36–43

Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., Wettstein, D., Franken, P. and Kogel,

K.H. 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance and higher yield. In: *Proceedings of National Academy of Science*. USA, pp.13386–13391

Wang, Y.T. 1996. Media and fertilization affect performance of potted *Dendrobium* and *Phalaenopsis*. *Hort*. *Tech*. 5(3): 234-237

Wang, Y.T. and Gregg, L.L. 1994. Medium and fertilizer affect the performance of *Phalaenopsis* orchids during flowering cycles. *Hort*. *Sci*. 29: 269-271

Warcup, J.H. 1975. Factors affecting symbiotic germination of orchid seed. In: sanders, F.E., Mose, B. and Tinker, P.B. (eds.), *Endomycorrhizae*. Academic Press. Londan, pp.87-104

Warcup, J.H. 1983. Pathogenic *Rhizoctonia* and orchids. In: Parker, C.A., Rovira, A.D., Moore, K.J. and Wong, P.T.W. (eds.), *Ecology and Management of Soil Borne Pathogens*. APS press, St, Paul, Minnesota, pp.69-70

Warcup, J.H. and Talbot, P.H.B. 1967. Perfect states of *Rhizoctonia* associated with orchids. *New Phytol*. 66: 631-639

Watson, D.J. 1958. The dependence of net assimilation rate on leaf area index. *Ann*. *Bot*. 23: 431-439

Will, M.E. and Sylvia, D.M. 1990. Interaction of rhizosphere bacteria, fertilizer and vesicular-arbuscular mycorrhizal fungi with *zea* oats. *Appl*. *Environ*. *Microbiol*. 56: 2073–2079

Williams, R.F. 1946. The physiology of plant growth with special reference to the concept of net assimilation rate. *Ann*. *Bot*. 10: 41-72

Wilson, D. 1995. Endophyte - the evolution of a term, and clarification of its use and definition. *Oikos.* 73: 274-276

Wue, G.D., Chen, W.H., Chen, J.B., Chyou, M.S. and Cheng, Y.Y. 1994. Effect of applying nitrogen and organic fertilizer to bagase medium on growth of *Phalaenopsis.* In: *Report on the Taiwan Sugar Research Institute,* Department of Horticulture, Taiwan sugar research Institute, No.146, pp.1-8

Yadav, L.P. and Bose, T.K. 1989. Orchids. In: Bose, T.K. and Yadav, L.P. (eds.), *Commercial Flowers*. Naya Prakash, Calcutta, p.208

Yin-Tungwang. 2000. Impact of high phosphorus fertilizer and timing of termination of fertilization on flowering of a hybrid moth orchid. *Hort. Sci.* 35(1): 60-62

Yoneda, K., Suzuki, N. and Hasegawa, I. 1999. Effect of macro element concentrations on growth, flowering and nutrient absorption in an *Odontoglossum* hybrid. *Hort. Sci.* 80: 259-265

Zou, W.X. and Tan, R.X. 1999. Fungal endophytes. *Adv. Plant Sci*. 2: 183-198

Appendix

Appendix-1

Monthly distribution of weather parameters during the experiment February 2008 to June 2009

Month	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	Mean Sunshine (hrs)	Mean Wind speed (Km/hr)
	Maximum	Minimum	Morning	Evening			
February	33.6	22.9	80	41	29.7	8.2	4.5
March	33.2	23.4	78	49	205.3	6.9	4.8
April	33.2	24.9	89	60	65.6	6.3	3.2
May	33.0	24.7	87	58	11.5	6.1	3.5
June	29.9	23.5	93	77	636.7	2.0	3.0
July	29.3	23.2	93	75	416.3	2.7	3.1
August	29.0	23.6	93	71	321.9	3.4	2.9
September	29.4	23.2	92	68	314.2	5.4	2.4
October	30.5	23.4	87	64	380.8	5.7	3.3
November	31.7	23.1	84	56	21.7	6.0	4.0
December	31.6	22.5	73	47	2.6	7.7	7.1
January	32.8	21.9	70	38	0.0	9.4	8.0
February	35.1	22.1	78	35	0.0	9.9	5.1
March	35.1	24.4	87	53	29.0	7.9	3.4
April	34.5	25.3	89	58	16.5	5.8	1.8
May	33.0	24.8	89	64	199.5	5.5	1.9
June	30.0	23.7	94	73	565.0	13.9	3.4

INFLUENCE OF NUTRIENTS AND PLANT GROWTH PROMOTING ROOT ENDOPHYTE (PGPRE) ON GROWTH AND DEVELOPMENT OF *Dendrobium* CV. EARSAKUL

By

DHINESH. D

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the

requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

Department of Pomology and Floriculture

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR- 680 656

KERALA, INDIA

2009

ABSTRACT

Investigations on the "Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul" was carried out in the orchidarium of All India Co-ordinated Floriculture Improvement Project at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, during 2008 to 2009. The specific objective was to study the influence of organic and inorganic nutrients and *Piriformospora indica* (PGPRE) on the growth and development of *Dendrobium* cv. Earsakul.

Foliar sprays of supernatant liquid of cowdung slurry, inorganic nutrients of $N:P_2O_5:K_2O$ 3:1:1 during vegetative period and 1:2:2 during flowering period @ 0.2 per cent, weekly twice (Package of Practices recommendations of KAU for orchids) along with *P. indica* resulted in increased growth parameters like number of leaves, number of shoots and internodal length of the plants. However, maximum plant height and girth of the shoots was obtained in the treatment combination Package of Practices (POP) + organic mixture + vermiwash + *P. indica* + bone meal.

Maximum number of roots and root volume was resulted from the treatment combinations of POP along with *P. indica*. However, maximum root length was recorded in treatment receiving POP + organic mixture at 6 Months after Planting (MAP) and organic mixture + vermiwash + *P. indica* + bone meal at flower bud formation stage.

Physiological parameters like dry matter production, crop growth rate and relative growth rate were promoted by POP along with *P. indica* application. POP + vermiwash + *P. indica* + bone meal produced maximum net assimilation rate. Application of POP recommendations resulted in maximum chlorophyll 'b' and total

chlorophyll content. POP along with bone meal application resulted in maximum chlorophyll 'a' content.

Early flowering and lengthy spikes were obtained from the treatment receiving POP along with *P. indica*.

The treatment combination POP with vermiwash resulted in increased uptake of Nitrogen at 6 MAP and at the time of flower bud formation. In general, at 6 MAP and at the time of flower bud formation, maximum uptake of Phosphorus was recorded in the treatments POP + bone meal and POP + vermiwash + *P. indica* + bone meal. During both the periods, POP + organic mixture + *P. indica* + bone meal resulted in maximum uptake of Potassium. In general, uptake of Calcium and Magnesium was maximum in the treatment receiving POP + vermiwash + *P. indica* + bone meal during both the stages of growth. Application of organic mixture + vermiwash + *P. indica* + bone meal along with POP gave beneficial effect on uptake of micro nutrients, *viz*., Fe, Cu, Zn and Mn.

Incidence of pests and diseases was negligible during the entire period of study.