REFINEMENT OF NUTRIENT MANAGEMENT PRACTICES IN *Dendrobium* ORCHIDS

By

SHILPA P

(2015-12-010)

THESIS

Submitted in partial fulfillment of the

requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

Kerala Agricultural University, Thrissur

Department of Floriculture and Landscaping

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2017

DECLARATION

I hereby declare that the thesis entitled "**Refinement of nutrient management practices in *Dendrobium* orchids**" 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, associate ship, fellowship or other similar title of any other university or society.

Vellanikkara, **Shilpa P**

Date: 22.08.2017 (2015-12-010)

CERTIFICATE

Certified that this thesis entitled **"Refinement of nutrient management practices in *Dendrobium* orchids"** is a record of research work done independently by Shilpa. P (2015-12-010) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara

22-08-2017

Dr. Mini Sankar

Assistant Professor

Department of Floriculture and Landscaping

College of Horticulture, Vellanikkara

CERTIFICATE

We, the undersigned members of the advisory committee, **Shilpa P** a candidate for the degree of **Master of Science in Horticulture**, with major field in **Floriculture and Landscaping**, agree that the thesis entitled **"Refinement of nutrient management practices in *Dendrobium* orchids"** may be submitted by **Shilpa P**, in partial fulfilment of the requirement for the degree.

21/10/2017

Dr. Mini Sankar
(Chairman, Advisory Committee)
Assistant Professor
Department Floriculture and Landscaping,
College of Horticulture, Vellanikkara

21/10/2017

Dr. C. K. Geetha
(Member, Advisory committee)
Professor and Head
Dept. Floriculture and Landscaping
College of Horticulture, Vellanikkara

Sudhadevi 21/10/17

Dr. P. K. Sudhadevi
(Member, Advisory committee)
Professor and Head of AFCIP
Dept. Floriculture and Landscaping
College of Horticulture, Vellanikkara

21/10/17

Dr. Reshmy Vijayaraghavan
(Member, Advisory Committee)
Assistant Professor
Dept. of Plant Pathology

21/10/17

EXTERNAL EXAMINOR
Dr. M. Ganga
Associate Professor (Horticulture)
Horticulture Research Station (TNAU)
Ooty – 643 001, the Nilgiris Dt., Tamil Nadu

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF APPENDICES

ACKNOWLEDGEMENT

Before I say a word of acknowledgement I owe to bow my head before the ***Almighty God*** *, whose unbound rays of blessings always enshrined in my thoughts, deeds and so also bestowed with good health, strength , confidence and enthusiasm.*

It is with great respect and devotion, I place on record my deep sense of gratitude and indebtedness to my chairperson ***Dr. Mini Sankar,*** *Assistant Professor (Floriculture & Landscaping), for her sustained and valuable guidance, unflinching support, constructive professional criticism, motherly approach and whole hearted cooperation rendered throughout the conduct of my research work and preparation of thesis. I gratefully remember her knowledge and wisdom which nurtured this research project in right direction without which fulfillment of this endeavour would have not been possible.*

Words fall short in expressing my profound sense of gratitude and sincere thanks to ***Dr. C. K. Geetha,*** *Professor and Head, Department of Floriculture & Landscaping and member of my advisory committee for her expert advice, constant inspiration and meticulous help throughout the course of study.*

I am deeply obliged to ***Dr. P. K. Sudhadevi*** *Professor and Head, Department of Floriculture & Landscaping, a member of my advisory committee for her kind help, valuable suggestions and cooperation throughout the research program and critical scrutiny of the manuscript.*

I sincerely thank ***Dr. Reshmy Vijayaraghavan,*** *Assistant Professor, Department of Plant Pathology for her support, ever willing help and scholarly suggestions and cooperation throughout my study period.*

I consider it as my privilege to express my deep felt gratitude to ***Dr. S. Krishnan****, Professor and Head, Department of Agricultural Statistics for his support, critical comments and valuable advice during the preparation of manuscript.*

I express my heartiest gratitude to ***Dr. K. Surendra Gopal****, Assistant Professor of Department of Microbiology for his valuable suggestions and cooperation throughout the course of study.*

I am grateful to all the non-teaching staff of the Departmentof Plantation Crops and Spices especially ***Pushpa chechi, Rajani chechi, Roshmi Chechi, Neethu chichi and Abida chichi*** *for the help rendered by them during the course of my study and also to all laborers especially* ***Unniettan, Rama chichi, Usha chichi and Rathicechi*** *for their whole hearted help during the research study*

I owe a lot to all my dear friends especially ***Deepa. T, Arjun Mohan, Umesh Lashkar, Surya Raj , Geethumol Thankappan and Manisha Elza Jacob*** *for their whole hearted cooperation. I am also indebted to my Seniors and Juniors viz* ***Priya Philip, Amal, Athira, Muammed Murthala and Lakshmi*** *for their timely help when I needed the same.*

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

Above all, I am forever beholden to ***my parents and sister*** *for their selfless sacrifice, moral support, constant prayers and inspiring encouragement in all my endeavours.*

Vellanikkara

SHILPA. P

Introduction

1. INTRODUCTION

Floriculture is an international multi billion dollar industry which includes the cultivation and marketing of various cut flowers, loose flowers, potted flowering and foliage plants, cut greens and various floricultural products. Nowadays commercial floriculture has increased its credibility by providing excellent self-employment for youth and good remuneration for small and marginal farmers due to increased returns per unit area. India is bestowed with varied agro climatic conditions and enormous genetic diversity which offer inimitable scope for expansion of floriculture industry in our country. Floriculture sector is considered as one of the esteemed focus segments by the government of India. Traditional flowers have been the main stay in the country but in recent times commercial production of cut flowers like rose, carnation, gerbera, orchids, anthurium, etc. under protected condition for export purpose is gaining momentum day by day.

Orchids are elegant cut flowers that capture the title of 'highest selling flower' in Indian cut flower industry due to their unique shape, size, colour and characteristic aroma of certain species. As orchids symbolize wealth, beauty and social status, a tremendous increase in use of these flowers for social functions was noticed in the past few years (De and Pathak, 2015).

Among the diversified species of orchids, *Dendrobium* is the most commonly grown species in India, especially in Kerala. These species are either epiphytic or occasionally lithophytic, adapted to wide variety of habitats. Floriferousness, year round availability and long vase life make it an excellent cut flower of international importance (Sunil *et.al,* 2015). The combined tropical and subtropical climate of Kerala due to the peculiar elevation from sea level is highly congenial for growing of *Dendrobium* orchids than any other place. Commercial growers of *Dendrobium* in Kerala are facing many problems including inclement weather, pest and disease attack as well as lack of technical knowhow. They are unaware of the scientific nutrient management practices and in order to obtain a profitable yield, growers are adopting their own methods like use of homemade organic mixtures, readymade formulations available in the market, etc. Injudicious nutrient management practices lead to a decline in crop yield and a higher incidence of pests and diseases besides affecting the production of export quality flowers.

Orchids are highly specific in their nutrient requirement and in addition to the environmental factors, quality and quantity of nutrients, their quantity and time of application are also very important for the production of quality spikes.

Bioinoculants are single or multiple species of microorganisms which improve the crop growth and yield through various mechanisms like enhanced nutrient absorption, production of phytohormones, biotic and abiotic stress tolerance, etc. Commercial formulations of bioinoculants are widely used in crop production programmes of various ornamentals. Application of exogenous growth hormones has also been reported to increase the yield and quality attributes in ornamental crops.

Positive influence of organic and inorganic sources of nutrients, growth hormones and plant growth promoting micro-organisms, the rate and frequency of application, for improving growth and yield of *Dendrobium* have been well documented in various experiments conducted at Department of Floriculture and Landscaping of the College of Horticulture, KAU during different periods. Still there is a need to develop a package which integrates the benefits from all possible sources of organic, inorganic and biological components that could be recommended to orchid growers.

Hence the present study was conducted with the following objectives

1. To Evaluate the response of *Dendrobium* to organic and inorganic nutrients, growth regulators and bioinoculants
2. To refine the existing nutrient management practices for *Dendrobium* orchids.

14

Review of literature

2. REVIEW OF LITERATURE

Orchids occupy a prime position among the top ten cut flowers in the world. Due to exquisite beauty and wide adaptability, *Dendrobium* orchids are most commonly cultivated in tropical regions throughout the world. Kerala has great potential for cultivation of *Dendrobium* orchids, since the prevailing climatic conditions are highly congenial for the growth and flower production of this species. Like any other crop, integrated use of organic and inorganic sources of nutrients was proved to be an effective tool for yield enhancement in orchids. Incorporation of various bio-inoculants in nutrient management schedule of ornamentals is one of the current approaches for improvement of growth and yield. Bio-inoculants are inoculum of a single species or assemblage of different species of microorganisms which exert direct or indirect benefits on plant growth through various mechanisms. It has also been observed that exogenous application of growth substance like cytokinines positively influence the flower production in orchids. Literature pertaining to the effect of various inorganic and organic nutrients, bioinoculants and growth regulators on orchids and other ornamental crops are reviewed hereunder.

2.1 EFFECT OF INORGANIC NUTRIENTS

Orchids have specific nutrient requirements. Quantity of nutrients, rate of application and stage of crop growth at which they are applied have a major role in deciding ultimate growth and yield. Mineral requirement of orchids and their supply through various inorganic sources of nutrients have been studied by various scientists.

Swapna (2000) reported that application of NPK 10:20:20 in the form of ammonium nitrate, orthophosphoric acid and potassium nitrate at the rate of 0.2 per cent resulted in highest spike production in *Dendrobium* variety Sonia 17 and the plant height was

significantly superior when NPK 30:10:10 was applied at the rate of 0.2 per cent weekly twice.

Studies conducted by Shobhana (2000) revealed that out of different nutrient solutions, application of NPK mixture 30:10:10 at the rate of 0.2 percent on alternate days resulted in an improvement in vegetative characters in terms of number of leaves and shoots in *Dendrobium*. Nandini (2000) reported an effective improvement in the leaf length, leaf width and leaf perimeter in *Dendrobium* cv. Sonia 17 when treated with NPK 30:10:10 at a concentration of 0.2 per cent along with 200 ppm BA.

Maximum chlorophyll content in leaves was observed during shoot emergence in *Dendrobium* 'Sonia 17' when sprayed with NPK 13:27:27 at the rate of 0.1 per cent at twice in a week + 97 (Nair, 2001). He also observed a positive influence of nitrogen concentration on shoot emergence in *Dendrobium* cv. Sonia 17 and reported that the application of fertilizer mixture of NPK in the ratio of 3:6:1 during vegetative and 1:2:2 during flowering phase significantly improved the growth and yield in *Dendrobium*. Application of NPK 30:10:10 at the rate of 0.2 per cent twice a week was found to be very effective for improving vegetative parameters like leaf length and leaf width in *Dendrobium* Sonia 17 (Devi and Chezhian, 2001).

A significant increase in plant height was observed in *Dendrobium* 'Sonia 17' when 0.2 per cent NPK 20:10:10 of was applied in combination with 100 ppm BA (Anon, 2003). Rajeevan and Swapna (2003) recorded maximum length of spike, number of spikes, number of florets, rachis length and intermodal length in *Dendrobium* when sprayed with NPK 10:20:10 at the rate of 0.2 per cent.

Maximum number of flowers per spike was observed in *Cymbidium* cv. 'Show girl Cook's Bridge' when the plants were applied with NPK 15:5:5 at the rate of 0.3 per cent and

17

a reduction in number of flowers per spikes was noticed when the concentration was above 15:5:5 (Naik and Barman, 2006).

Ramachandrudu (2008) observed maximum spike length and floret diameter in *Dendrobium* 'Mona Red'; more number of florets per spike in *Dendrobium* 'Burena Zeb' and highest number of spikes per plant in *Dendrobium* 'Singapore' with the application of NPK 10:30:30 at 0.2 per cent twice in a week.

According to Dinesh (2009) application of NPK 3:1:1 during vegetative phase and 1:2:2 during flowering phase at 0.2 per cent weekly twice resulted in maximum chlorophyll content in *Dendrobium* cv. Earsakul. He also observed maximum number of roots per plant when they were applied with NPK 3:1:1 during vegetative and 1:2:2 during flowering phase along with organic mixture, *Piriformospora indica* and bone meal in the same variety.

Application of NPK 30:10:10 at the rate of 0.1 per cent gave the best results in terms of various growth attributes like leaf length, width, number of pseudobulbs per clump, pseudobulb length and pseudobulb girth in one year old plants of *Cymbidium* (Naik *et al.*, 2010). According to Nair and Sujatha (2010) the maximum number of productive canes per plant in *Dendrobium* var. Sonia 17 was with the treatment of NPK 1:6:1 at the rate of 0.2 per cent at biweekly intervals and maximum number of leaves per plant was when treated with NPK 3:1:1 combination. Foliar application of NPK in the ratio of 3:1:1 during vegetative and 1:2:2 during flowering stage at the rate of 0.2 per cent weekly twice in combination with supernatant liquid of cowdung slurry can be recommended for orchids (KAU, 2011).

Tiwari and Kumar (2011) recorded maximum root length and number of roots per clump in *Cymbidium iriodiodes* when the plants were applied with NPK 19:19:19 at the rate of 0.1 per cent once in a week.

18

In *Dendrobium* cv. Medameuraiwan, the highest leaf area was recorded when the plants were grown under fan and pad polycarbonate covered green house and sprayed with NPK 3:1:1 during vegetative and 1:2:2 during flowering stage at the rate of 0.2 per cent weekly twice (Sugapriya, *et al.*, 2012).

Kabir *et al.*, (2012) recorded a relatively higher leaf area in *Dendrobium* when the plants were sprayed with NPK 10:15:20, 15:20:25, 10:25:25 and 10:25:30 at 45, 90, 135 and 180 days respectively after planting. Another study conducted by Kabir *et al.*, (2012) in three *Dendroium* cultivars namely Red Bull, Kasim Gold and White 5 N revealed that, the plants treated with NPK 10:25:30 produced maximum number of leaves, leaf length, leaf width, leaf area and leaf area index, while the NPK combination of 15:20:20 resulted in maximum plant height and stem diameter and they concluded that, low level of nitrogen with high levels of P and K will favour the leaf growth and number of leaves while high level of nitrogen along with low level of potassium will favour the plant height and stem girth. .

The experiment conducted by Ali *et al.*, (2014) in *Mokara* sp. resulted in maximum plant height and leaf area index when the plants were treated with NPK 4:3:2 and maximum leaf length and leaf number, when treated with NPK 3:2:1.

2.2 EFFECT OF BIO-INOCULANTS

2.2.1 *Azospirillum*

Azospirillum is a plant growth promoting bacteria that exert stimulatory effect on plant growth through different mechanisms like secretion of phytohormones, biological nitrogen fixation and enhancement of mineral uptake by plants. Remarkable changes in terms

of increase in plant height, leaf size, root length and volume and plant biomass were observed in various crops as a result of inoculation with *Azospirillum* (Gadagi *et al.*, 2004).

Study conducted by Preethi *et al.*, (1999) revealed that the combined application of N at the rate of 37.5 kg/ha and ascorbic acid 1000 ppm along with *Azospirillum* gave the highest number of flowering shoots, maximum pedicel length, flower diameter, flower weight, nutrient content of the plant and lowest number of blind shoots in Edward rose (*Rosa bourboniana*).

Results of the study in *Chrysanthemum morifolium* with different treatments of 0,50,75 and 100 per cent recommended dose of nitrogenous fertilizers in combination with *Azospirillum* inoculation revealed a significant improvement in plant growth, flower yield and nutrient uptake in all the treatments, compared to treatments without inoculation (Ravi *et al.*, 2002).

Inoculation of *Azospirillum* along with different levels of nitrogenous fertilizers resulted in a significant increase in plant growth, yield and in different biochemical attributes like chlorophyll content, total sugars and total free amino acids in *Tagetes erecta.* (Bhaskaran *et al.*, 2002).

Binisha (2003) reported an improvement in vegetative characters like plant height, number of shoots, girth of shoots and number of leaves in *Dendrobium* when NPK 10:5:10 at the rate of 0.2 per cent was applied along with *Azospirillum.* Highest root volume, root length and dry matter production were observed in plants treated with NPK 20:10:10 at the rate of 0.2 per cent in combination with *Azospirillum*, Phosphobacteria and AMF.

A treatment combination consisting of *Azospirillum* and Phosphobacteria as root dip method, followed by foliar application of NPK 10:5:10 at the rate of 0.2 per cent at weekly

twice has resulted in significant improvement of growth parameters like plant height and number of shoots in *Dendrobium* variety Sonia 17 (Anon, 2003).

Study conducted by Gadagi *et al.* (2004) in Gaillardia pulchella revealed that the plants inoculated with *Azospirillum* strain OAD -2 resulted in increased in plant height, number of leaves per plant, branches per plant and total dry mass accumulation than other inoculants and control.

An experiment consisting of three growing media along with *Azospirillum* inoculation and application of water soluble fertilizers through fertigation significantly improved the characters like bud diameter, stem length and vase life of the flowers in carnation cv. Sunrise (Bhatia *et al.*, 2004).

Srinivasa (2006) conducted a study to evaluate the effect of biofertilizers on growth and flowering of anthurium. The results revealed that the parameters like plant height, number of leaves, leaf length and width, number of suckers per plant, flower yield and quality of flowers were maximum in treatments consisted of NPK at the rate 30:20:40g/m² along with *Azospirillum*.

Remarkable increase in yield was observed in *Jasminum auriculatum* when *Azospirillum* was applied in combination with recommended dose of NPK fertilizers and FYM (Jayamma *et al.*, 2008). It was also reported that the application of lignite based cultures of *Azospirillum, Pseudommonas striata, Pseudomonas fluorescence and Trichoderma viridae,* each at the rate of 8 kg/ha, could substitute the recommended NPK fertilizers to an extent of 50 per cent without affecting the growth parameters, floral characteristics and flower yield.

Dalve *et al.* (2009) observed a positive influence on plant height, number of leaves, days required for spike emergence and opening of first pair of florets, number of florets per spike, number of spikes per plot, number of corms and cormels per plant in *Gladiolous sp.* when the plants were treated with *Azospirillum* and *Azotobacter* along with the application of NPK at the rate of 375: 200: 200 kg/ ha.

Khan *et al.* (2009) observed maximum plant height, wrapper leaf area, tepal diameter and bulb yield in *Tulipa gesneriana* with the application of thick slurry of *Azospirillum* and 100 per cent recommended dose of N fertilizer in the form of urea.

Chaudhari *et al.*, (2010) reported that the treatment consisting of N at the rate of 50g/ plant, *Azospirillum* and *Azotobacter* at the rate of 1ml/plant resulted in maximum plant height, plant spread, number of branches and stem diameter and the treatments consisting of N at the rate of 25g/ plant, *Azospirillum* and *Azotobacter* each at the rate of 1ml/ plant gave maximum leaf area in *Rosa damscena.*

A significant improvement in plant height, plant spread, leaf area and relative growth rate were observed in *Chrysanthemum coronarium* when the plants were treated with 175 kg/ha N in combination with *Azospirillum* and *Azotobacter* (Panchal *et al.*, 2010). Moutia *et al.* (2010) reported an improvement in shoot length and root dry weight when a group of ornamental species were treated with a consortia consisting different strains of *Azospirillum.*

Verma *et al.* (2012) conducted a study to analyse the effect of integrated nutrient management on growth, yield and quality of flowers in *Chrysanthemum morifolium* cv. Raja. From the experiment, they observed that the plants under the treatments with *Azospirillum* and PSB along with 50 per cent recommended dose of NPK (150: 100: 100 kg/ ha) had maximum plant height, plant spread, number of branches, dry matter accumulation and flower yield.

Handaragall *et al.*, (2013) reported an improvement in flower yield and quality in *Anthurium andreanum* cv. 'Tropical Red' with the combined application of *Azospirillum*, AMF and PSB at the rate of 2g/ plant, GA_3 1000 ppm and NPK 30:10:10 0.2 per cent as foliar spray.

Qasim *et al.* (2014) reported a maximum plant growth and flower yield in *Gladiolus grandiflorus* cv. White prosperity when the corms were treated with *Azospirillum* and the maximum chlorophyll content, nutrient accumulation and total soluble sugars by the application of *Azospirillum* followed by *Azotobacter.*

Study conducted by Ali *et al.*, (2014) in *Gladiolus grandiflorus* to assess the effect of different bio fertilizers on growth and flower quality revealed that the treatment consisting of *Azospirillum* gained highest values in terms of plant height, spike length, number of florets per spike, fresh weight of the spike and earlier spike emergence when compared to other biofertilizers

An investigation was carried out by Kumar (2015) in *Gladiolous hybridus* cv. Peater Pears in which the plants were supplied with different sources of nutrients like vermicompost, FYM and NPK along with *Azospirillum* and PSB. The results revealed that the plants under the treatments consisting of 75 per cent recommended dose of NPK, 50 per cent vermicompost along with application of *Azospirillum* and PSB at the rate of 2g per plant had maximum plant height, number of leaves per plant, leaf length, early spike emergence and maximum longevity of spikes compared to other treatments.

2.2.2 AMF

Arbuscular Mycorrhizal Fungi are one of the dominant type mycorrhizae that usually colonize the root cortex of plants acquiring carbon from them and helping the host plants for

the enhanced and rapid uptake as well as translocation of phosphorous and other nutrients and minerals, especially of low mobility, from the soil (Harrison and Buuren, 1995).

Lin *et al.,* (1999) could observe the best promotion in growth of Asian hybrid lily when the plants were inoculated with AMF and Phosphate Solubilizing Bacteria.

Rajadurai *et al.*, (2000) conducted an experiment in French marigold to analyse the effect of biofertilizers (AMF and *Azospirillum*) in growth and flowering of the plant and observed that the plants treated with NPK (45:45:37.5mg/kg) in combination with *Azospirillum* and AMF showed the best response in terms of plant height, number of leaves, laterals per plant and early flowering.

Scagel (2001) reported that the application of AMF, into the rooting medium of miniature rose cultivars produced the best response in terms of number of rooted cuttings and roots per cuttings.

Kumar (2002) conducted an experiment to evaluate the effect of AMF and growth regulators in *gladiolous* cv. Jessica and observed that the plants under the treatment of pre-soaking and foliar application of kinetin 50 ppm in combination with AMF had maximum plant growth and flower yield while the treatment consisting of pre-soaking and foliar application of GA_3 100 ppm along with AMF inoculation gave the best result in terms of corm yield.

Varshney *et al.*, (2002) conducted an experiment to investigate the effect of AMF with four different levels of phosphorous in Asiatic hybrid *Lilium*. It was observed that, bulblets inoculated with AMF had better response in terms of shoot length, number of leaves, leaf area, bulblet size and weight and P uptake than the uninoculated ones.

An experiment was conducted in china aster with inoculation of AMF and phosphobacteria in combination with N and P applied at the rate of 25, 50, 75 and 100 per cent of the recommended dose and full dose of K. The results revealed that the plants applied with 75 percent of recommended dose of N and full dose of K along with AMF and phosphobacteria produced maximum plant height, number of branches, number of leaves, leaf area, fresh weight of flowers and flower yield (Prabhat *et al.*, 2003).

Madhaiyan *et al.* (2003) observed an increase in the root and shoot length, shoot and root dry weight in vanilla seedlings when the plants were inoculated with mycorrhizal fungi in combination with AMF, over the control and they could also observe the mycorrhizal infection in the cortical cells of inoculated vanilla seedlings.

The study conducted by Sohn *et al.* (2003) in chrysanthemum plants inoculated with AMF revealed that, the plants with root inoculation had a greater response in terms of plant height, root length, fresh and dry weight of shoot, leaf area and early flowering compared to control.

Peterson and Massicotte (2004) reported that the mycorrhizae are having significant role to play in orchids by way of nutrient uptake and translocation for germination, development of protocorm and initial root development. Another study conducted by Padmadevi *et al.* (2004) in anthurium revealed that the plants inoculated with AMF and PSB along with inorganic nutrients, N, P and K exhibited maximum growth rate compared to uninoculated plants. Cameron *et al.* (2006) reported that the inoculation of tissue cultured plantlets of orchids with AMF helped in the supply of organic nitrogen to the plants.

A significant increase in number of bulbs per plant and advanced sprouting was observed in tuberose when inoculated with AMF, *Azotobacter* and PSB followed by the application of N at the rate of 100 kg/ha and P at the rate of 50 kg/ha (Chaudhary, 2007).

Effect of Arbuscular Mycorrhizal colonization on nutrient uptake and flowering of pelargonium plants grown in peat based substrate with two levels of compost and with or without AMF inoculation was studied by Perner *et al.* (2007). Results revealed that the plants inoculated with AMF had root colonization rate upto 36 per cent of total root length and resulted in improved nutrient status and flower development compared to non-inoculated plants.

Inoculation of AM Fungi and *Pseudomonas fluorescens* along with lower concentration of superphosphate in gerbera resulted in greater root and shoot biomass, root colonization per cent, maximum leaf area and number of flowers per plant over the control (Karishma *et al.*, 2013).

2.2.3 PGPR Mix - I

Plant Growth Promoting Rhizobacteria (PGPRs) are microbial populations in the rhizosphere that promote the plant growth by way of synthesis of phytohormones, increasing availability of mineral nutrients to the plants and exerting antagonism against plant pathogens through competition or due to the production of antimicrobial metabolites. Use of PGPRs as potential bioinoculants had been reported in many crops. Currently there has been a shift from use of a single species of PGPR as bioinoculant to the use of multi microbial consortia. PGPR Mix – 1 is a microbial consortia developed at KAU specifically for improving crop growth and yield. Since the reports pertaining to beneficial effects specifically due to PGPR - Mix I on ornamental crops are very meagre, cited here are the effect of similar types of consortia on growth and yield enhancement of various ornamental crops.

A significant increase in the biomass production was observed in *Sedum alfredii* due to application of PGPR consortia containing strains of *Burlcholderia sp.* (Guo *et al.*, 2011).

Shilev *et al.*(2012) reported an increment in plant height, number of leaves and root volume in *Helianthus annus* as a result of inoculation with a consortia containing different strains of *Pseudomonas fluorescens.*

Inoculation with a multi strain consortia of *Pseudomonas sp.* was found to be highly effective for increasing root and shoot dry weight of *Helianthus annus* in metal contaminated soils (Yang *et al.*, 2013).

2.3 Effect of BA

Benzyl adenine (BA) comes under the group of synthetic cytokinin which have been found to play a major role in various physiological and biochemical processes like cell division, organ formation, regeneration, seed germination and suppression of apical dominance. Besides all these actions, this hormone was also found to act as one of the multifactorial components which act as a floral stimulus. This stimulus reaches the shoot apical meristem through vascular tissues and thereby induces the flower initiation (Bernier and Perlleux, 2005). Based on this action, many studies have been conducted and it was proved that the exogenous application of cytokinins on plants will result in an induction or early flowering in many ornamental species.

Swapna (2000) observed that application of NPK 30:10:10 at the rate of 0.2 per cent twice in a week along with 200 ppm BA resulted in earlier flowering, maximum number of shoots and biomass in *Dendrobium* 'Sonia 17'. She also reported an improvement in number of spikes per plant and number of florets per spike by the application NPK 10:20:10 at the rate of 0.2 per cent along with 100 ppm BA. Study conducted by Nandini (2000) in *ex vitro* established six months old plantlets of *Dendrobium* cv. Sonia 17 revealed that, the plants treated with NPK 30:10:10 at the rate of 0.2 per cent along with 200 ppm BA resulted in a significant improvement in vegetative parameters like leaf length and leaf width.

Shobhana (2000) observed maximum number of pseudobulbs and leaves in *Dendrobium* orchids under the treatments consisting of foliar application of BA 50 ppm at fortnightly intervals. Nandini (2000) reported an effective improvement in the leaf parameters like leaf length, leaf width and leaf perimeter in the *Dendrobium* cv. Sonia 17 when treated with NPK 30:10:10 at the concentration of 0.2 percent along with 200 ppm BA.

In order to study the effect of foliar nutrition on chlorophyll content in *Dendrobium* orchids, Devi and Chezhiyan (2002) conducted an experiment under greenhouse condition using inorganic fertilizer (NPK) in combination with benzyl adenine (BA). The results showed that the plants supplied with NPK 30:10:10 and 20:10:10 at the rate of 0.2 per cent along with 200ppm BA gave the highest level of total chlorophyll content in the plants.

Matsumoto (2006) conducted a study in *Miltoniopsis* orchid hybrids to analyse the influence of plant growth regulators BA and GA_3 on plant growth and flowering and reported that the plants receiving benzyl adenine (25-50 mM) promoted the new vegetative shoots compared to other treatments.

An experiment conducted by Blanchard and Runkle (2008) in *Phalaenopsis* orchids using BA and GA_3 revealed that, the plants treated with BA alone at the concentration of 200 or 400 ppm showed an early spike emergence, more number of inflorescence and flowers per plant compared to untreated and GA_3 treated plants

Wu and Chang (2009) reported remarkable increase in number of flowers per spike in *Phalaenopsis* cultivars Luchia pink '244' and Sogo Yukidian 'V3' when plants were treated with 70 ppm and 150 ppm BA respectively. Another comparitive study conducted by Wu *et al.* (2012) in *Phalaenopsis* cultivars Sogo Yukidian and Tai Lin Redangel 'V31' with two additional cytokinins, kinetin, 2- iso-pentenyl adenine and BA revealed that out of different hormonal sprayings the treatment with BA gave the maximum spikes per plant and number of

flowers per spike in cultivar Sogo Yukidian V3 and an increase in number of spikes per plant and number of flowers per spike were observed in cultivar Lin Redangel 'V31' when the plants were treated with 100 ppm BA.

Sakai and Ichihara (2010) reported that spraying of 6-benzyladenine at different concentrations *viz.*, 450, 900 and 4500 ppm induced maximum number of flower per spike within 10 weeks of treatment without cool temperature in *Dendrobium nobile* orchids which prefer a cooler temperature to flower. They also observed a significant increase in flower spray production in *Dendrobium* Jaquelin Thomas Uniwai 'princess' when one year old pseudobulbs were injected with 100 ppm BA. In *Dendrobium* Red Emperor 'Prince', a higher production of inflorescence per plant and more number of flowers per plant were observed when the plants were treated with 900 ppm BA.

Barman *et al.* (2014) observed an improvement of growth and yield in terms of plant height, number of shoots, number of leaves per cane, girth of cane, number of spike per cane and number of flowers per spike in *Dendrobium* hybrid 'Thongchai Gold' when the plants were sprayed with GA_3 100 ppm in combination with 100 ppm BA followed by 200 ppm GA_3.

Materials and methods

3. MATERIALS AND METHODS

The present study entitled "Refinement of nutrient management practices in *Dendrobium* orchids" was conducted to evaluate the effect of various sources of nutrients, bio-inoculants and different levels of benzyl adenine on growth and yield of *Dendrobium* orchids. The experiment was carried out in the polyhouse of All India Co-ordinated Research Project on Floriculture in the Department of Floriculture and Landscaping, College of Horticuture, Vellanikkara, Thrissur, Kerala from August 2016 to July 2017. Vellanikkara is situated at a latitude of $10^0$31'N and longitude of $76^0$3'E and lies 22.25 m above MSL. The area enjoys a warm humid tropical climate. The meteorological data during the period of study are given in Appendix I.

3.1 MATERIALS

3.1.1 Planting material

Six months old tissue cultured plants of *Dendrobium* variety Yellow Splash were used for the study

3.1.2 Pot and Potting media

Mud pots of size 16.00 cm length, 16.00 cm width and 13.50 cm depth with perforations filled with a media containing tile pieces, charcoal and coconut husk pieces were used for growing the plants.

3.1.3 Growing structure

The plants were grown in a shade house with 35 – 40 % shade

3.1.4 Treatments

Design : Completely Randomised Design (CRD)

No. of treatments : 10

No. of replications : 3

No. of plants/ treatment : 5

T_1 - POP + PGPR Mix-1 + BA 50 ppm

T_2 - POP + PGPR Mix-1 + BA 100 ppm

T_3 - POP + PGPR Mix-1 + BA 150 ppm

T_4 - POP + AMF (Arbuscular Mycorrhizal Fungi) + BA 50 ppm

T_5 - POP + AMF + BA 100 ppm

T_6 - POP + AMF + BA 150 ppm

T_7 - POP + *Azospirillum* + BA 50 ppm

T_8 - POP + *Azospirillum* + BA 100 ppm

T_9 - POP + *Azospirillum* + BA 150 ppm

T_{10} - Control (KAU POP recommendation for orchids)

All the treatments were superimposed over the existing package of practices recommendations of KAU for orchids

The adhoc nutrient recommendation for orchids as per Package of Practices recommendations of KAU is foliar application of $N:P_2O_5:K_2O$, 3:1:1 during period of vegetative growth and 1:2:2 during flowering period at the rate of 0.2 per cent twice a week. Three bio-inoculants *viz*; PGPR Mix – 1, AMF and *Azospirillum* and three levels of benzyl adenine *viz*; 50, 100 and 150 ppm were superimposed on this recommendation.

3.2 Growth regulator application

Benzyl adenine (BA) was applied as foliar spray in three different levels *viz*; 50,100 and 150 ppm at monthly intervals.

3.3 Biofertilizer application

PGPR Mix-1 was applied through root dipping of plants in loose water slurry at the rate of 500g/2.5l of water for 20 minutes prior to planting. AMF was directly applied at the rate of 50g/kg of potting media at the time of planting. Likewise *Azospirillum* was applied through root dipping of plants in a slurry of 500g of the inoculum in 50 ml of water for 30 minutes prior to planting.

Plate. 1. Variety used for the study – *Dendrobium* Yellow Splash

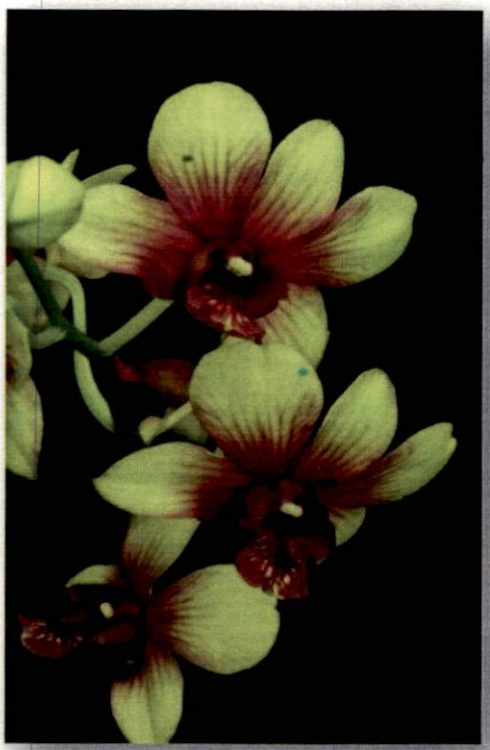


Plate. 2. Six months old tissue cultured plantlets used for the study

Plate. 3. General view of the experimental field - at the time of planting

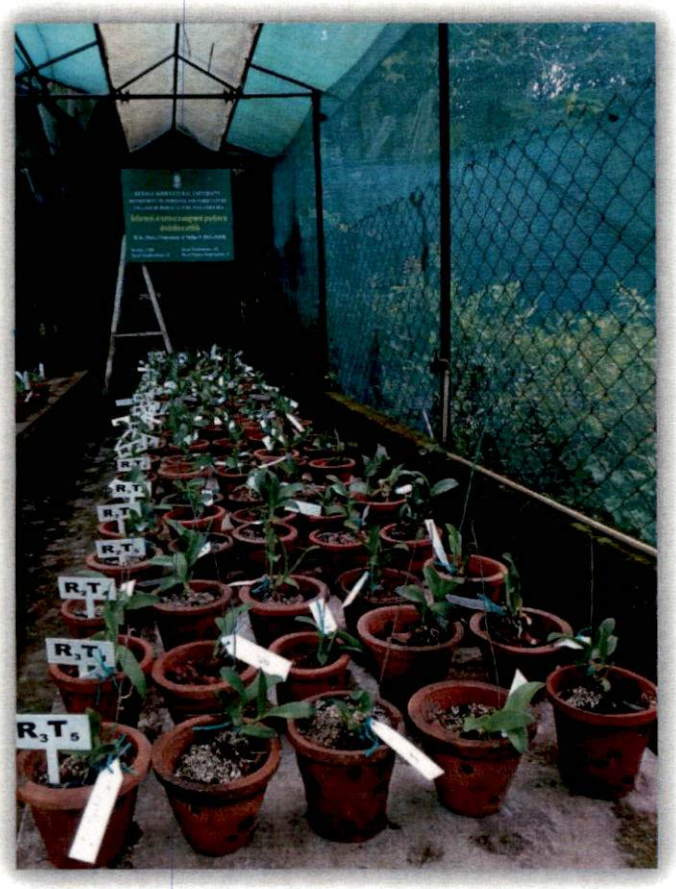


Plate. 4. General view of the experimental field - Six months after planting

3.4 OBSERVATIONS

Observations were taken at monthly intervals and data were recorded.

3.4.1. Vegetative characters

3.4.1.1 Plant height

The height of the plant was measured from the base of the plant upto the tip of newly emerging leaf and expressed in cm.

3.4.1.2 Plant spread

The plant spread in East-West and North-South directions were taken and recorded in cm.

3.4.1.3. Number of leaves per plant

Total number of leaves on the plant was counted and recorded

3.4.1.4 Leaf length

Length of the leaf was measured and expressed in cm.

3.4.1.5 Leaf breadth

Width of the leaf at the middle point was measured and expressed in cm.

3.4.1.6. Leaf area

Leaf area was calculated using the following regression equation

Leaf area (a) = -25.857 + 8.95 x breadth + 2.184 x length (Naik, 2014)

3.4.1.7. Internodal length

The distance between two adjacent internodes were measured and expressed in cm.

3.4.1.8. Number of pseudobulbs per plant

Number of pseudobulbs produced per plant was counted and recorded

3.4.2. Floral characters

3.4.2.1 Days to flowering

Total number of days taken from the time of planting to appearance of spike in each treatment was recorded.

3.4.2.2 Days to first floret opening

Total number of days taken from spike formation to opening of first flower in the spike was recorded

3.4.2.3 Days to last floret opening

Total number of days taken from spike formation to opening of last flower in the spike was recorded

3.4.2.4 Length of spike

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

3.4.2.5 Length of stalk

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

3.4.2.6 Number of flowers per spike

Total number of flowers per spike was counted and recorded

3.4.2.7 Size of flower

Length and breadth of individual flowers were taken and expressed in cm^2.

3.4.2.8 Number of spikes per plant

Number of spikes emerged during the period of observation in each plant was counted and recorded

3.4.2.9 Longevity of spike on the plant

Total number of days taken for the wilting of first flower of the spike from the emergence of inflorescence was calculated and recorded

3.4.2.10 Interval of spike production

Time interval between the production of two successive spikes within a plant was observed and recorded

3.4.3 Post harvest studies

Flowers for post-harvest studies were harvested during commercial stage of harvest i.e., with 1 – 2 unopened buds in the spike and kept in known volume of water and observations were recorded

3.4.3.1 Fresh weight of spike

Weight of spikes immediately after harvesting was recorded and expressed in grams.

3.4.3.2 Days taken for first flower to wilt

Total number of days taken for the wilting of first flower of the spike in vase was noted and recorded.

3.4.3.3 Physiological loss in weight

The physiological loss in weight was recorded as the difference between fresh weight of the spike and weight of the spike at the end of vase life and expressed in gram.

3.4.3.4 Total water uptake

Total water uptake was measured as the difference between initial volume of water in the vase and volume of water at the end of vase life and expressed in ml.

3.4.3.5 Vase life

Number of days taken for the first flower of the spike to show the signs of wilting in vase was counted and recorded.

3.4.4 Root parameters

Root parameters were observed and recorded after uprooting the plants at the end of the study

3.4.4.1 Number of roots

Total number of roots per plant was counted and recorded

3.4.4.2 Root length

Length of the longest root was measured and expressed in cm.

3.4.4.3 Root volume

The total roots from each plant were collected, root volume was measured by displacement method and expressed in cm^3.

3.5 Root colonization

3.5.1 *Azospirillum*

Using Most Portable Number (MPN) technique, the population of *Azospirillum* was enumerated with the use of Okon's semisolid malic acid medium. After the incubation of two days at 37° C, presence of *Azospirillum* can be confirmed by the appearance of a blue coloured dense white pellicles of 1 -2 mm below the upper surface of medium (Okon *et al.*, 1977).

3.5.2 AMF (Arbuscular Mycorrhizal Fungi)

Feeder roots were collected, washed and made into bits of 1 cm and transferred to test tubes containing 10 per cent KOH solution. Then boiled the material at 121^{0}C at 15 pounds for 10 minutes followed by washing of the bits in tap water for two to three times and dipped in 2 per cent HCl for 10 – 30 minutes for acidification. After draining excess acid, the bits were dipped in trypon blue dye (0.05 per cent) and kept in the water bath at 90^{0}C for 10 – 30 minutes. After removing excess stain the roots were dipped in acidified glycerol overnight and then observed under microscope for AMF colonization. The colonization percentage was calculated as follows.

$$\text{Percentage colonization} = \frac{\text{Number of root segments colonized} \times 100}{\text{Total number of segments observed}}$$

3.6 Nutrient analysis

After the experiment, the plants were uprooted and analysis was done for the major nutrients and nutrient uptake by the plant. The plants were dried in the shade for two weeks followed by oven drying at 60 - 70^0 C for six hours. The dry weight of the plants was recorded. Then the dried samples were ground and chemically analysed for nitrogen, phosphorous, potassium, calcium, magnesium and sulphur

3.6.1 Nitrogen

One gram dried sample was digested using concentrated sulphuric acid and nitrogen content was estimated by Microkjeldhal method (Jackson, 1958)

3.6.2 Phosphorous

Dried sample of 0.5g was digested using diacid mixture of nitric acid and perchloric acid taken in a ratio of 9:4 and finally phosphorous content was estimated using vanadomolybdophosphoric yellow colour method. The intensity of yellow colour was read in Spectronic – 20 at 470 nm (Johnson and Ulrich, 1959).

3.6.3 Potassium

An aliquot was prepared from the above mentioned digested sample and estimated for potassium using flame photometer (Jackson, 1958).

3.6.4 Calcium

From the extract obtained by digestion with diacid mixture, calcium content was determined using flame emission spectrophotometer (Black, 1965).

3.6.5 Magnesium

Total Mg content was determined from digested sample with diacid mixture using atomic absorption spectrophotometer (AAS – 4129) (Jackson, 1958).

3.6.6 Sulphur

Total sulphur content in the plants was determined using turbidometric method from diacid mixture digested samples (Black, 1965).

3.6.7 Dry matter production

The whole plant sample including psuedostem, leaves and roots were dried in to a constant weight at 70 – 80^{0}C in hot air oven and dry weight of the sample gave total dry matter production and expressed in g/ pot.

3.6.8 Nutrient uptake

From the values of concentration of nutrients and dry weight of the parts sampled, the nutrient uptake was computed and expressed in g/pot.

3.7 Physiological parameters

3.7.1 Chlorophyll content

The chlorophyll content in the leaves were estimated using 80 per cent acetone (Porra, 2002). Recently developed fully matured leaves were taken and made into small pieces of 100 mg and then ground well using mortar and pestle along with 10 ml of 80 per cent acetone. Then the ground material was centrifuged at 5000 rpm for 10 minutes. The supernatant solution thus obtained was poured into small vials and the absorbance was read at 646.6 nm and 663.6 nm using distilled water as blank with spectrophotometer. From the values obtained from the spectrophotometer, Chlorophyll a, b and total chlorophyll content were estimated using the following formula and expressed in mg g^{-1} fresh weight.

Chlorophyll a = 12.25 ($A_{663\cdot6}$) – 2.55 ($A_{646\cdot6}$) x 10 ml acetone / 100 mg leaf tissue

Chlorophyll b = 20.31 ($A_{646\cdot6}$) – 4.91 ($A_{663\cdot6}$) x 10 ml acetone / 100 mg leaf tissue

Total Chlorophyll = 17.76 ($A_{646\cdot6}$) + 7.34 ($A_{663\cdot6}$) x 10 ml acetone / 100 mg leaf tissue

3.7.2 Stomatal characters

A thin layer of glue (quick fix) was applied on the lower surface of the leaves and the stomatal impressions were taken. The number of stomata per square millimetre of microscopic field (0.11 mm^2) was counted and recorded as per square millimetres (number of stomata/ 0.11 mm^2).

3.8 STATISTICAL ANALYSIS

WASP software was used to statistically analyse data pertaining to vegetative, floral, physiological and root parameters as well as nutrient content and nutrient uptake by the plants

Results

4. RESULTS

An experiment entitled "Refinement of nutrient management practices in *Dendrobium* orchids" was conducted at the Department of Floriculture and Landscaping to study the influence of different bio-inoculants and benzyl adenine on growth and yield of Dendrobium orchids. Observations on morphological and yield parameters were recorded, analysed and results are presented below.

4.1 VEGETATIVE CHARECTERS

Data pertaining to the effect of treatments on vegetative characters of *Dendrobium* var. Yellow Splash are presented in Tables 1 to 9.

4.1.1 Plant height

Influence of treatments on plant height was not significant up to 5th month after planting (MAP). At 6 MAP, treatment T_5 (POP + AMF + 100 ppm BA) recorded maximum plant height (24.30 cm) followed by the treatments T_6 (POP + AMF + 150 ppm BA) and T_4 (POP + AMF + 50 ppm BA) which were performing on par with each other (22.13 and 21.97 cm respectively). Minimum plant height was observed in T_7 (POP + Azospirillum + 50 ppm BA) and T_{10} which were statistically on par (15.12 and 16.03 cm respectively).

The same trend was noticed upto 10 MAP. Maximum plant height of 33.09 cm was observed in T_5 followed by T_6 (30.43 cm) and T_4 (28.91cm) and plant height was minimum T_{10} (21.93 cm) at 10 MAP. Treatment consisted of AMF were superior in terms of plant height throughout the growth period. It could be inferred that the treatment T_5 recorded maximum plant height compared to all other treatments during the period of observation (Table 1 and Plate. 5).

4.1.2 Plant spread

Plant spread in East-west and North-South directions were recorded upto 10 MAP. Regarding the plant spread in East-West direction, there was no significant difference among the treatments up to 6 MAP. During 7 MAP the highest value of 25.50 cm was obtained in treatments T_9 (POP + *Azospirillum* + 150 ppm BA) followed by T_8 (POP + *Azospirillum* + 100 ppm BA) and T_7 (POP + *Azospirillum* + 50 ppm BA) and the treatment T_6 (POP + AMF + 150 ppm BA) had the minimum plant spread in East-West direction (19.37 cm).

Treatments T_9 and T_8 exhibited superiority in terms of plant spread in East –West direction throughout the growth period (Table 2).

Table 1. Influence of bioinoculants and benzyl adenine on plant height (cm)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	13.5	13.50	14.93	17.95	20.85	22.98	21.50	22.88	24.98	25.98	27.75
T_2 (POP + PGPR Mix-1 + BA 100ppm)	11.19	12.92	16.35	19.25	20.83	22.83	18.08	19.80	21.98	24.77	25.88
T_3 (POP + PGPR Mix-1 + BA 150ppm)	11.82	13.40	16.45	19.08	21.43	22.40	18.29	22.00	23.17	23.02	24.33
T_4 (POP + AMF + BA 50ppm)	12.67	14.18	15.28	17.50	20.23	22.88	21.97	23.22	26.33	28.60	28.92
T_5 (POP + AMF + BA 100ppm)	10.87	13.05	14.37	18.05	19.72	23.23	24.30	25.77	30.45	33.17	33.09
T_6 (POP + AMF + BA 150ppm)	12.17	14.45	15.73	17.35	22.25	25.47	22.13	23.60	26.03	30.43	30.43
T_7 (POP + *Azospirillum* + BA 50ppm)	11.20	13.87	13.93	16.87	18.07	21.82	15.12	18.43	20.50	22.82	22.25
T_8 (POP + *Azospirillum* + BA 100ppm)	14.07	15.78	16.15	17.77	20.75	22.52	18.50	20.82	23.20	24.17	25.45
T_9 (POP + *Azospirillum*+ BA 150ppm)	11.58	13.25	15.05	17.53	20.80	23.13	19.15	20.99	22.56	24.45	25.38
T_{10} (POP)	12.75	15.03	15.75	17.23	22.13	23.57	16.03	17.73	19.97	23.10	21.93
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**4.225**	**4.350**	**3.743**	**5.406**	**5.389**

Plate. 5. Influence of bioinoculants and benzyl adenine on plant height

(1) **(2)**

(1) – Control (T_{10})
(2) - POP + AMF + BA 100PPM (T_5)

Table 2. Influence of bioinoculants and benzyl adenine on Plant spread in E – W direction (cm).

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	23.32	14.91	11.25	15.28	17.90	18.59	18.42	20.47	21.30	21.17	21.67
T_2 (POP + PGPR Mix-1 + BA 100ppm)	12.08	12.92	16.50	15.29	19.23	19.25	17.79	22.05	18.98	20.05	20.17
T_3 (POP + PGPR Mix-1 + BA 150ppm)	10.00	12.67	14.75	15.87	19.17	18.77	18.21	21.38	20.27	22.85	21.50
T_4 (POP + AMF + BA 50ppm)	11.67	14.92	17.25	15.71	19.29	19.25	18.21	20.30	22.67	23.05	21.17
T_5 (POP + AMF + BA 100ppm)	10.08	9.08	12.00	14.71	18.12	19.46	18.08	20.10	21.92	21.57	21.42
T_6 (POP + AMF + BA 150ppm)	12.10	13.33	15.42	15.33	18.96	19.49	19.83	19.37	21.25	22.90	21.42
T_7 (POP + *Azospirillum* + BA 50ppm)	13.04	12.62	14.83	14.92	17.53	18.17	18.17	23.05	23.48	21.75	19.50
T_8 (POP + *Azospirillum* + BA 100ppm)	10.83	13.33	14.50	16.87	16.71	18.62	18.75	23.77	26.50	26.70	28.98
T_9 (POP + *Azospirillum*+ BA 150ppm)	12.72	12.27	11.83	15.17	17.50	17.25	18.42	25.50	28.43	29.00	28.53
T_{10} (POP)	9.07	13.53	15.50	14.96	17.92	18.58	17.92	21.97	19.87	20.5	19.67
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**2.615**	**4.524**	**5.338**	**4.663**

Considering the plant spread in North–South direction, no significant variation between the treatments could be observed upto 6 MAP. At 7 MAP, the treatment T_9 recorded maximum plant spread (25.10 cm) in North-South direction, which was on par with T_8 (24.03 cm). Same pattern of variation was observed up to 10 MAP. Plant spread in North-South direction was maximum in T_9 (25.97 cm) followed by T_8 and T_5 which were performing on par with each other (25.23 cm and 23.17 cm respectively) and minimum value for this parameter was noticed in T_1 during 10 MAP (Table 3).

In general it could be observed that the treatment T_9 was superior in terms of plant spread in East – West and North – South directions, throughout the period of observation.

4.1.3 Number of leaves per plant

Regarding the number of leaves per plant, there was no significant difference among the treatments throughout the period of observation, however the more number of leaves in treatments T_9 and T_5 could be observed during this period (Table 4).

4.1.4 Leaf length

As far as the leaf length is concerned, no significant difference among the treatments was observed during the initial seven months. Treatment T_9 recorded maximum length of the leaves (14.88 cm) during 8 MAP which was on par with T_7 (14.20cm). Minimum leaf length was observed in T_{10} which was on par with all other treatments. During subsequent months also T_9 was superior in terms of leaf length followed by T_7. In general it could be observed that T_9 was superior in terms of length of leaves compared to all other treatments (Table 5).

4.1.5 Leaf breadth

The breadth of leaves in plants upto seven months of planting did not vary among the treatments. But a steady progressive increase in the leaf breadth was observed in all the treatments up to 7 months of planting.

The maximum value for leaf breadth was observed in treatment T_8 (5.27 cm) at 8 MAP which was found to be on par with T_9 (4.88 cm). This was followed by T_7, T_2 and T_6 which were performing on par with each other (4.68 cm, 4.67 cm and 4.63 cm respectively). Minimum leaf breadth was observed in T_{10} (3.97 cm). The same trend was observed in

Table 3. Influence of bioinoculants and benzyl adenine on plant spread in N – S direction (cm)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	10.17	8.62	9.75	7.83	11.12	10.68	10.96	19.48	20.67	18.17	19.33
T_2 (POP + PGPR Mix-1 + BA 100ppm)	8.25	9.75	12.50	8.62	10.12	11.17	10.12	19.03	22.27	21.78	22.17
T_3 (POP + PGPR Mix-1 + BA 150ppm)	8.67	11.13	13.47	8.87	11.31	11.19	10.33	19.87	18.85	20.77	22.50
T_4 (POP + AMF + BA 50ppm)	8.67	9.83	14.83	8.62	10.96	11.19	10.79	18.27	19.00	17.43	20.50
T_5 (POP + AMF + BA 100ppm)	8.00	11.70	13.58	8.96	10.96	11.92	10.83	19.58	21.38	16.48	23.17
T_6 (POP + AMF + BA 150ppm)	7.27	9.67	12.67	8.62	10.57	11.13	11.37	18.40	22.12	22.40	22.75
T_7 (POP + *Azospirillum* + BA 50ppm)	9.38	10.10	7.98	8.79	9.67	9.62	9.42	19.53	22.47	19.28	19.6
T_8 (POP + *Azospirillum* + BA 100ppm)	12.22	10.47	12.54	12.08	9.62	10.37	10.37	24.03	24.50	26.70	25.23
T_9 (POP + *Azospirillum*+ BA 150ppm)	7.88	9.15	7.00	8.08	10.71	10.12	10.50	25.10	25.77	28.83	25.97
T_{10} (POP)	11.48	13.50	9.67	8.00	10.07	10.42	9.50	16.93	19.08	13.17	22.67
CD (0.05)	**NS**	**NS**	**4.891**	**NS**	**NS**	**NS**	**NS**	**3.604**	**3.101**	**4.575**	**3.511**

Table 4. Influence of bioinoculants and benzyl adenine on number of leaves per plant

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	7.67	7.83	7.83	9.67	9.83	8.83	9.17	10.33	10.17	11.17	9.33
T_2 (POP + PGPR Mix-1 + BA 100ppm)	7.00	6.67	8.50	9.83	8.67	9.83	8.67	9.67	12.33	11.33	8.33
T_3 (POP + PGPR Mix-1 + BA 150ppm)	8.33	9.00	8.33	10.83	11.17	11.00	7.83	9.67	11.83	11.83	10.17
T_4 (POP + AMF + BA 50ppm)	7.50	7.7	7.83	10.17	10.83	11.50	8.50	9.50	11.00	11.83	9.50
T_5 (POP + AMF + BA 100ppm)	7.83	8.33	8.17	11.67	12.33	9.33	9.50	10.17	12.17	11.33	11.50
T_6 (POP + AMF + BA 150ppm)	6.33	7.67	6.50	10.17	10.33	10.50	8.00	9.50	11.33	12.17	9.83
T_7 (POP + *Azospirillum* + BA 50ppm)	7.67	7.67	8.33	9.33	10.17	8.67	5.83	8.00	9.33	9.33	7.50
T_8 (POP + *Azospirillum* + BA 100ppm)	6.83	6.83	7.17	9.17	9.83	9.33	7.50	7.67	9.50	12.00	9.00
T_9 (POP + *Azospirillum*+ BA 150ppm)	7.33	7.50	8.50	9.33	11.00	10.00	8.67	10.33	12.33	13.33	10.67
T_{10} (POP)	9.00	9.17	8.67	10.17	11.67	10.83	8.33	9.33	10.40	11.00	9.67
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 5. Influence of bioinoculants and benzyl adenine on leaf length (cm)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	8.83	9.27	9.23	9.32	10.42	11.75	11.43	12.97	12.12	13.02	13.47
T_2 (POP + PGPR Mix-1 + BA 100ppm)	8.47	9.02	8.98	9.17	10.37	11.38	11.65	13.03	12.38	12.70	13.52
T_3 (POP + PGPR Mix-1 + BA 150ppm)	7.19	8.03	8.13	8.25	10.15	28.13	12.47	11.93	11.97	11.87	12.43
T_4 (POP + AMF + BA 50ppm)	8.52	9.02	8.88	9.07	10.63	11.82	11.12	11.72	12.47	12.37	13.43
T_5 (POP + AMF + BA 100ppm)	7.65	8.48	8.52	8.77	10.75	11.98	10.88	11.23	12.35	13.13	13.33
T_6 (POP + AMF + BA 150ppm)	8.52	8.88	8.98	8.83	10.65	11.45	10.78	11.40	12.85	12.70	13.73
T_7 (POP + *Azospirillum* + BA 50ppm)	7.90	8.52	8.53	8.68	9.53	10.65	9.97	12.15	14.20	14.05	14.30
T_8 (POP + *Azospirillum* + BA 100ppm)	8.78	9.50	9.45	9.65	9.93	11.20	11.45	12.58	12.67	12.75	13.23
T_9 (POP + *Azospirillum*+ BA 150ppm)	8.32	8.52	8.75	8.95	10.17	10.98	10.95	11.97	14.88	14.52	15.78
T_{10} (POP)	8.42	8.42	8.32	8.38	10.00	11.67	11.30	11.58	11.92	12.88	12.55
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**1.348**	**1.431**	**1.313**

subsequent months also and it could be concluded that the treatments T_8 and T_9 were superior in leaf breadth during the observation period (Table 6).

4.1.6 Leaf area

The treatments did not show any significant difference regarding the leaf area till 7th month after planting. Later, from 8th month of planting onwards, significant variation among the treatments was observed with a progressive increase in the leaf area.

During 8th month of planting, plants under the treatment T_9 showed maximum leaf area (50.35 cm^2) which was on par with T_8 (48.73 cm^2). During this period the least leaf area was observed in the plants under treatment T_{10} (35.67 cm^2)

During 9th and 10th month of planting T_8 recorded maximum leaf area (51.81 cm^2 and 57.94 cm^2 respectively) which was on par with T_9. Generally the superiority of treatments under *Azospirillum* inoculation was observed as far as leaf area is concerned and it could be inferred that the treatment T_8 was the best among the treatments in terms of this parameter (Table 7).

4.1.7 Internodal length

The data recorded till the 10th month of planting showed that there was no significant difference among the treatments throughout the growth period. Still there observed a progressive increase in the intermodal length with peak value exhibited by T_3 (4.98 cm) followed by T_5 (4.93 cm), during 10th month of planting (Table 8).

4.1.8 Number of pseudobulbs per plant

The number of pseudobulbs per plant did not show any significant variation till the 6th month of planting. During the 7th month the maximum number of pseudobulbs was observed in the treatment T_9 (5.67) followed by T_8 (4.67). The least value in this period was noticed in the treatment T_2 (3.83). The same trend was observed upto 10th month of planting (Table 9). Generally the treatment T_9 was observed to have maximum effect on number of pseudobulbs per plant compared to other treatments (Table 9 and Plate 6.).

Table 6. Influence of bioinoculants and benzyl adenine on leaf breadth (cm)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	2.85	3.12	3.20	3.12	3.80	4.22	4.20	4.60	4.60	4.73	5.13
T_2 (POP + PGPR Mix-1 + BA 100ppm)	2.68	3.13	3.20	3.35	4.18	4.53	4.57	4.73	4.67	4.75	5.12
T_3 (POP + PGPR Mix-1 + BA 150ppm)	2.88	3.00	2.90	2.92	4.20	4.55	4.62	4.45	4.15	4.90	5.05
T_4 (POP + AMF + BA 50ppm)	2.90	3.30	3.10	3.23	4.10	4.58	4.68	4.73	4.42	4.73	5.03
T_5 (POP + AMF + BA 100ppm)	2.78	2.83	2.85	2.97	3.98	4.15	4.33	4.48	4.52	4.70	4.68
T_6 (POP + AMF + BA 150ppm)	2.88	3.13	3.33	3.38	4.15	4.47	4.37	4.67	4.63	4.68	5.25
T_7 (POP + *Azospirillum* + BA 50ppm)	2.92	3.20	3.25	3.37	3.63	4.18	4.42	4.63	4.68	4.80	5.23
T_8 (POP + *Azospirillum* + BA 100ppm)	3.17	3.55	3.67	3.72	3.83	4.68	4.57	4.78	5.27	5.57	6.13
T_9 (POP + *Azospirillum*+ BA 150ppm)	3.02	3.05	3.15	3.40	4.12	4.52	4.53	4.37	4.88	5.08	5.45
T_{10} (POP)	2.90	3.02	2.95	3.02	3.68	4.85	4.95	4.43	3.97	4.27	4.43
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.640**	**0.504**	**0.657**

Table 7. Influence of bioinoculants and benzyl adenine on leaf area (cm^2)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	22.97	28.78	29.68	30.00	31.13	40.45	36.69	43.63	41.77	44.89	49.49
T_2 (POP + PGPR Mix-1 + BA 100ppm)	20.10	23.73	23.99	25.85	30.48	38.38	40.46	44.97	42.95	44.39	49.46
T_3 (POP + PGPR Mix-1 + BA 150ppm)	18.53	19.89	21.24	21.68	29.00	39.21	40.28	40.03	37.41	43.91	46.49
T_4 (POP + AMF + BA 50ppm)	18.06	20.44	21.86	22.94	33.11	38.55	40.33	42.09	40.89	43.51	48.53
T_5 (POP + AMF + BA 100ppm)	17.08	22.53	23.04	24.30	31.70	37.76	36.69	38.81	41.54	44.89	45.18
T_6 (POP + AMF + BA 150ppm)	16.52	19.01	18.12	19.31	28.95	32.39	36.77	40.81	43.67	43.79	51.12
T_7 (POP + *Azospirillum* + BA 50ppm)	16.99	17.60	18.26	19.14	31.58	38.28	34.09	42.15	47.07	47.79	52.21
T_8 (POP + *Azospirillum* + BA 100ppm)	19.02	22.62	20.47	21.24	33.40	41.54	39.95	44.43	48.73	51.81	57.94
T_9 (POP + *Azospirillum*+ BA 150ppm)	19.64	21.30	21.52	22.31	35.83	43.09	38.63	39.36	50.35	51.34	57.39
T_{10} (POP)	15.05	17.36	17.85	19.74	35.47	37.88	43.12	39.12	35.67	43.00	41.23
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**7.448**	**5.612**	**6.933**

Table 8. Influence of bioinoculants and benzyl adenine on intermodal length (cm)

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	1.03	1.612	1.87	2.43	3.30	4.13	4.35	4.40	4.18	4.72	4.75
T_2 (POP + PGPR Mix-1 + BA 100ppm)	1.08	1.40	2.10	2.80	3.43	3.92	4.08	4.33	4.53	4.62	4.83
T_3 (POP + PGPR Mix-1 + BA 150ppm)	1.00	1.70	2.45	2.72	3.52	4.37	4.32	4.97	4.22	4.45	4.98
T_4 (POP + AMF + BA 50ppm)	1.22	1.73	2.20	2.67	3.70	4.15	4.03	4.12	4.13	4.50	4.83
T_5 (POP + AMF + BA 100ppm)	1.05	1.93	2.20	2.97	3.57	4.00	3.93	4.08	4.05	4.43	4.93
T_6 (POP + AMF + BA 150ppm)	0.98	1.48	1.43	2.73	3.62	4.10	4.43	4.48	4.28	4.43	4.63
T_7 (POP + *Azospirillum* + BA 50ppm)	0.83	1.48	1.95	2.32	3.05	3.55	3.47	4.22	4.72	4.50	4.25
T_8 (POP + *Azospirillum* + BA 100ppm)	4.18	2.05	1.97	2.60	3.45	4.38	3.98	4.37	4.30	4.27	4.73
T_9 (POP + *Azospirillum*+ BA 150ppm)	1.13	1.63	2.38	2.75	3.53	4.03	4.30	4.75	4.38	4.53	4.92
T_{10} (POP)	1.23	1.83	2.40	2.62	3.23	4.03	4.00	4.37	4.62	4.78	4.47
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

Table 9. Influence of bioinoculants and benzyl adenine on number of pseudobulbs per plant

Treatment	Month of observation										
	At the time of planting	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP	10 MAP
T_1 (POP + PGPR Mix-1 + BA 50ppm)	3.50	3.50	3.67	4.33	4.33	3.83	4.33	4.33	4.50	4.67	4.00
T_2 (POP + PGPR Mix-1 + BA 100ppm)	3.33	3.33	3.50	3.83	3.50	3.67	4.00	3.83	4.17	4.33	3.50
T_3 (POP + PGPR Mix-1 + BA 150ppm)	3.83	3.83	3.67	4.50	4.67	4.67	3.83	4.17	4.50	4.17	3.67
T_4 (POP + AMF + BA 50ppm)	3.83	3.83	3.83	4.17	4.17	4.33	4.33	4.17	4.67	4.83	4.33
T_5 (POP + AMF + BA 100ppm)	4.00	4.00	4.00	4.67	4.67	4.50	4.00	4.00	4.17	4.67	3.83
T_6 (POP + AMF + BA 150ppm)	2.67	2.67	3.33	4.17	3.67	4.17	3.50	4.17	3.83	4.00	3.33
T_7 (POP + *Azospirillum* + BA 50ppm)	3.33	3.50	3.50	4.17	4.33	3.67	3.33	4.17	4.00	3.67	3.33
T_8 (POP + *Azospirillum* + BA 100ppm)	3.33	3.33	3.17	3.50	3.50	3.33	3.33	4.67	5.33	5.00	4.83
T_9 (POP + *Azospirillum*+ BA 150ppm)	3.33	3.33	3.50	3.67	4.00	4.17	3.67	5.67	5.83	6.00	5.00
T_{10} (POP)	3.50	3.50	3.67	4.50	4.33	4.17	4.00	4.17	3.33	3.33	3.00
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**0.762**	**0.946**	**0.777**	**0.713**

Plate. 6. Influence of bioinoculants and benzyl adenine on number of pseudobulbs per plant

(1) **(2)**

(1) – Control (T_{10})
(2) - POP + *Azospirillum* + BA 150ppm (T_9)

4.2 FLORAL CHARACTERS

Data pertaining to the effect of treatments on floral characters of *Dendrobium* var. Yellow Splash are presented in Table 10.

4.2.1 Days to flower

No significant difference among the treatments could be observed regarding this parameter. However early flower formation was observed under the treatment T_8 (POP + Azospirillum + BA 100 ppm) compared to all others.

4.2.2 Days to first flowering opening

Significant differences could be observed among the treatments regarding number of days to first flower opening. Plants under the treatment T_2 took maximum number of days for first flower to open (67.33), which was found to be on par with all other treatments except T_3. The treatment T_3 (POP + PGPR Mix – 1 + BA 150 ppm) showed least number of days for first flower opening (29.67).

4.2.3 Days to last flower opening

Considering the number of days taken for the last flower to open, treatment T_6 (POP + AMF + BA 150 ppm) took maximum days (75.00), followed by T_2 (73.67) which was found to be on par with T_1. Plants under the treatment T_3 (29.00) exhibited least number of days for this parameter compared to others.

4.2.4 Spike length

Treatments did not vary significantly regarding the data pertaining to the spike length. Maximum spike length observed was 29.4 cm in treatment T_6 followed by T_4, T_9, T_2, T_5, T_7 and T_8 which were on par with each other (22.03, 21.80, 21.33, 20.43, 20.43 and 20.30 cm respectively). Plants under the treatment T_3 (POP + PGPR Mix – 1 + BA 150 ppm) produced shortest spike (14.00 cm) (Plate 9).

In general it could be observed that the plants inoculated with AMF and *Azospirillum* produced longest spikes, when compared to other treatments.

Table 10. Influence of bioinoculants and benzyl adenine on floral characters

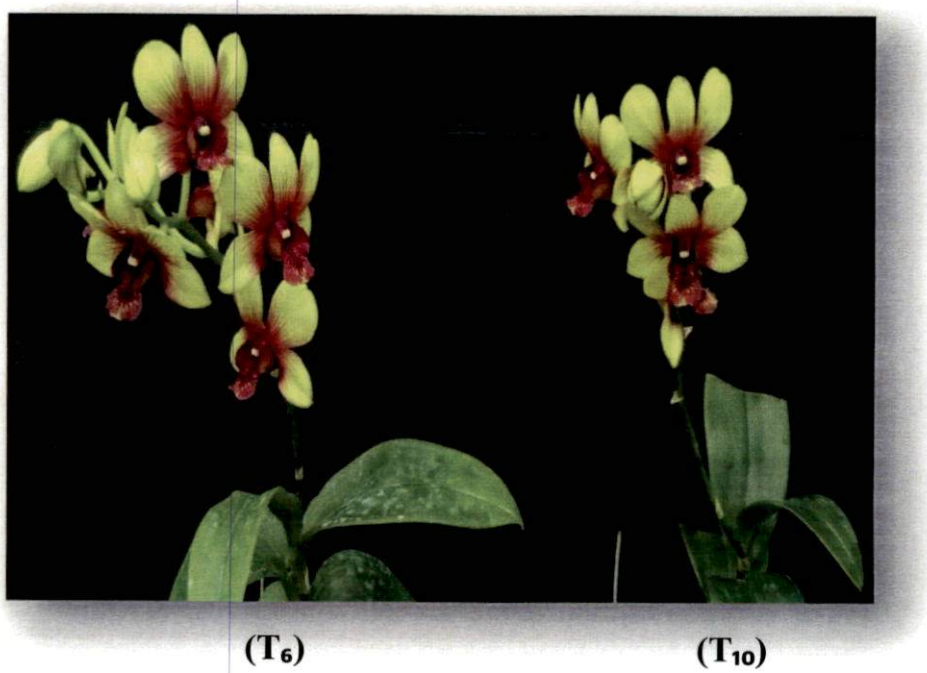
Treatment	Days to flower	Days to 1st flower opening	Days to last flower opening	Spike length (cm)	Stalk length (cm)	Number of floret per spike	Internodal length (cm)	Number of spikes per plant	Floret size (cm^2)	Longevity in the field (days)
T_1 (POP + PGPR Mix-1 + BA 50ppm)	289.00	47.33	56.67	17.27	7.33	6.00	5.07	1	17.00	63.33
T_2 (POP + PGPR Mix-1 + BA 100ppm)	308.67	67.33	73.67	21.33	10.23	6.00	4.10	1	18.389	60.33
T_3 (POP + PGPR Mix-1 + BA 150ppm)	262.67	29.67	29.00	14.00	6.40	7.00	5.63	2	18.43	50.33
T_4 (POP + AMF + BA 50ppm)	264.33	44.67	41.67	22.03	10.63	9.00	5.83	1	20.51	56.33
T_5 (POP + AMF + BA 100ppm)	274.00	41.33	50.33	20.43	12.97	8.67	5.17	2	20.35	57.67
T_6 (POP + AMF + BA 150ppm)	283.33	41.00	75.00	29.40	16.27	9.33	7.17	2	27.41	72.67
T_7 (POP + *Azospirillum* + BA 50ppm)	296.67	46.33	51.33	20.43	13.83	5.00	5.13	1	17.72	63.67
T_8 (POP + *Azospirillum* + BA 100ppm)	256.00	52.33	49.33	20.30	12.23	6.67	5.43	1	19.02	66.00
T_9 (POP + *Azospirillum*+ BA 150ppm)	304.33	46.67	50.67	21.80	12.37	7.33	5.03	2	21.35	64.33
T_{10} (POP)	300.33	41.67	56.00	17.80	10.00	5.33	3.67	1	17.33	47.67
CD (0.05)	**NS**	**11.68**	**7.11**	**2.41**	**2.84**	**2.13**	**1.44**	**NS**	**3.11**	**7.04**

Plate. 7. Influence of bioinoculants and benzyl adenine on plant spread

(1) (2) (3) (4)

(1) - POP + AMF + BA 150 ppm (T_6)
(2) - POP + *Azospirillum* + BA 150 ppm (T_9)
(3) - POP + PGPR Mix -1 + BA 100 ppm (T_2)
(4) - Control (T_{10})

Plate. 8. Comparison of AMF inoculated plant with control



(T_6) (T_{10})

T_{10} - Control
T_6 - POP + AMF + BA 150 ppm

4.2.5 Stalk length

Plants inoculated with AMF as well as *Azospirillum* showed superiority in terms of stalk length

Maximum stalk length was observed in the treatment T_6 (POP + AMF + BA 150 ppm) (16.27 cm) followed by T_7 and T_5 with stalk length 13.83 and 12.97 cm, respectively and the least stalk length was observed with the treatment T_3 (6.40 cm).

4.2.6 Number of flowers per spike

A remarkable influence could be observed in the number of flowers per spike among the treatments. Plants under the treatment T_6 produced maximum number of flowers (9.33) followed by T_4 (9.00) and T_5 (8.67) which were found to be on par with each other. Among the ten treatments minimum number of flowers was observed in treatments T_7 (5.00) and T_{10} which were statistically on par (5.00 and 5.33).

In general it could be observed that the treatments consisting of AMF with different levels of benzyl adenine was superior in terms of number of flowers per spike with maximum value in T_6 (POP + AMF + BA 150 ppm).

4.2.7 Internodal length

Considering the intermodal length of spikes, a significant difference among treatments could be observed resulting highest internodal length of spike by the plants under the treatment T_6 (7.16 cm) followed by T_4 (5.83 cm) and T_3 (5.63 cm. Least internodal length was observed in plants under the treatment T_{10} (3.66 cm).

4.2.8 Number of spikes per plant

Regarding number of spikes per plant no significant variation could be observed among the treatments. However the treatment T_6 (POP + AMF + BA 150 ppm) showed superiority in terms of this parameter during the period of observation.

4.2.9 Flower size

Treatment T_6 (POP + AMF + BA 150 ppm) produced significantly largest flowers of size 27.41 cm^2 followed by T_9 (21.35 cm^2) and T_4 (20.51 cm^2). Minimum flower size was

observed under the treatment T_1 (17.00 cm^2) and T_{10} (17.33 cm^2) which were statistically on par (Plate 8).

4.2.10 Longevity of spike

Significant difference among the treatments was observed regarding this parameter. Maximum spike longevity was observed in T_6 (72.67). This was followed by T_8 and T_9 (66.00 and 64.33 days respectively). Among the treatments, minimum spike longevity was observed under the treatments T_{10} (47.67) and T_3 (50.33) were performing on par.

4.3 POST HARVEST STUDIES

Regarding the post-harvest parameter, highest fresh weight of the spike (13.63 g) was exhibited by the treatment T_6 (POP + AMF + BA 150 ppm) and it was minimum in the treatment T_{10} (POP).

The treatment T_6 took more number of days for the wilting of first flower in the spike (7.00 days) and minimum number of days for the wilting of fist flower in the spike was observed in T_{10}.

Physiological loss in weight was highest in treatment T_2 (POP + PGPR Mix – 1 +BA 100 ppm) and T_6 which were found to be on par (1.09 and 1.083 respectively). No significant difference among the treatments could be observed regarding the water uptake.

Maximum vase life was observed in treatment T_6 and T_5 which were performing on par with each other (25.33 and 23.33 days respectively) and and minimum vase life of 11.67 days was observed in T_{10} (Table. 11 and Plate. 9)

4.4 ROOT PARAMETERS

After the experiment, the observations regarding the root parameters were taken and presented in table 12.

4.4.1 Number of roots

Among the various treatments, significant variation was observed in terms of number of roots and the maximum number of roots were produced by the plants under the treatment T_9 (48.33) which was found to be on par with T_1(47.00). The least number of roots were observed in treatment T_2 (31.00) (Plate. 10).

Table 11. Influence of bioinoculants and benzyl adenine on post - harvest parameters

Treatments	Fresh weight of spikes (g)	Days to wilt first flower	PLW (g)	Total water uptake (ml)	Vase life (days)
T_1 (POP + PGPR Mix-1 + BA 50ppm)	8.817	4.667	0.573	7.267	17.667
T_2 (POP + PGPR Mix-1 + BA 100ppm)	9.367	5.333	1.090	9.967	20.000
T_3 (POP + PGPR Mix-1 + BA 150ppm)	6.633	4.667	0.360	7.000	17.333
T_4 (POP + AMF + BA 50ppm)	9.513	5.000	0.583	9.567	17.333
T_5 (POP + AMF + BA 100ppm)	9.010	4.333	0.943	7.467	23.333
T_6 (POP + AMF + BA 150ppm)	13.633	7.000	1.083	10.567	25.333
T_7 (POP + *Azospirillum* + BA 50ppm)	8.160	4.667	0.497	7.367	18.000
T_8 (POP + *Azospirillum* + BA 100ppm)	8.223	5.667	0.563	8.667	19.000
T_9 (POP + *Azospirillum*+ BA 150ppm)	9.950	5.333	1.007	9.000	21.667
T_{10} (POP)	5.410	3.667	0.433	7.000	11.667
CD (0.05)	**2.296**	**1.282**	**0.204**	**NS**	**3.546**

62

Plate. 9. Comparison of superior treatments with control

(T_6) **(T_5)** **(T_9)** **(T_{10})**

T_6 - POP + AMF + BA 150 ppm
T_5 - POP + AMF + BA 100 ppm
T_9 - POP + *Azospirillum* + BA 150 ppm
T_{10} - Control

4.4.2 Root length

When the data regarding the root length was considered, a momentous variation was observed among the treatments where the maximum root length was exhibited by treatments T_5, T_8, T_9 and T_4 (24.18, 24.07, 23.85 and 23.67 respectively) which were statistically on par. A minimum root length of 16.00 cm was observed in T_3 (Table 12).

In general the treatment POP + AMF + BA 150ppm (T_5) was observed to be superior in terms of this parameter (Plate. 11).

4.4.3 Root volume

A notable variation was observed among the treatments. The maximum root volume of 30.25 cm^3 was observed in T_9 (POP + *Azospirillum* + BA 150 ppm) which was on par with the treatment T_4 (22.90 cm^3). Minimum root volume was observed in the treatments T_2 (Table 12).

4.4.4 Root colonization

AMF

Microbial analysis was conducted at the end of 10^{th} month after planting. From the various treatments, maximum root colonization of percentage 83 per cent was observed for the treatment T_6 which consisted of AMF inoculation along with 150ppm BA (Table 13 and Plate. 12).

Azospirillum

Even though the plants inoculated with *Azospirillum* exhibited remarkable improvement regarding growth, yield and physiological parameters, it could not be re isolated from the plants when subjected to microbial analysis.

PGPR Mix – 1

PGPR Mix – 1 is a consortia of various kinds of micro-organisms and its composition is not yet revealed. Hence root colonization study could not be conducted for this bio-inoculant. However the plants under the treatment with PGPR Mix -1 showed superiority in almost all characters compared to control.

Table 12. Influence of bioinoculants and benzyl adenine on root parameters

Treatments	Root parameters		
	Number of roots	Root length (cm)	Root volume (cm^3)
T_1 (POP + PGPR Mix-1 + BA 50ppm)	47.00	20.80	15.45
T_2 (POP + PGPR Mix-1 + BA 100ppm)	31.00	18.75	15.40
T_3 (POP + PGPR Mix-1 + BA 150ppm)	36.67	16.00	20.33
T_4 (POP + AMF + BA 50ppm)	46.33	23.67	22.90
T_5 (POP + AMF + BA 100ppm)	37.00	24.18	15.43
T_6 (POP + AMF + BA 150ppm)	44.00	18.50	20.20
T_7 (POP + *Azospirillum* + BA 50ppm)	35.67	17.77	5.73
T_8 (POP + *Azospirillum* + BA 100ppm)	44.67	24.07	19.73
T_9 (POP + *Azospirillum*+ BA 150ppm)	48.33	23.85	30.25
T_{10} (POP)	39.00	22.00	10.30
CD (0.05)	**10.808**	**4.835**	**9.29**

Table 13. Influence of bioinoculants and benzyl adenine on AMF colonization

Treatment	Colonization percentage (%)
T_4	73
T_5	79
T_6	86

*T_4 - POP + AMF + BA 50 ppm

T_5 - POP + AMF + BA 100 ppm

T_6 - POP + AMF + BA 150 ppm

Plate.10. Influence of bioinoculants and benzyl adenine on number of root

T_{10} - Control
T_9 - POP + *Azospirillum* + BA 150 pp

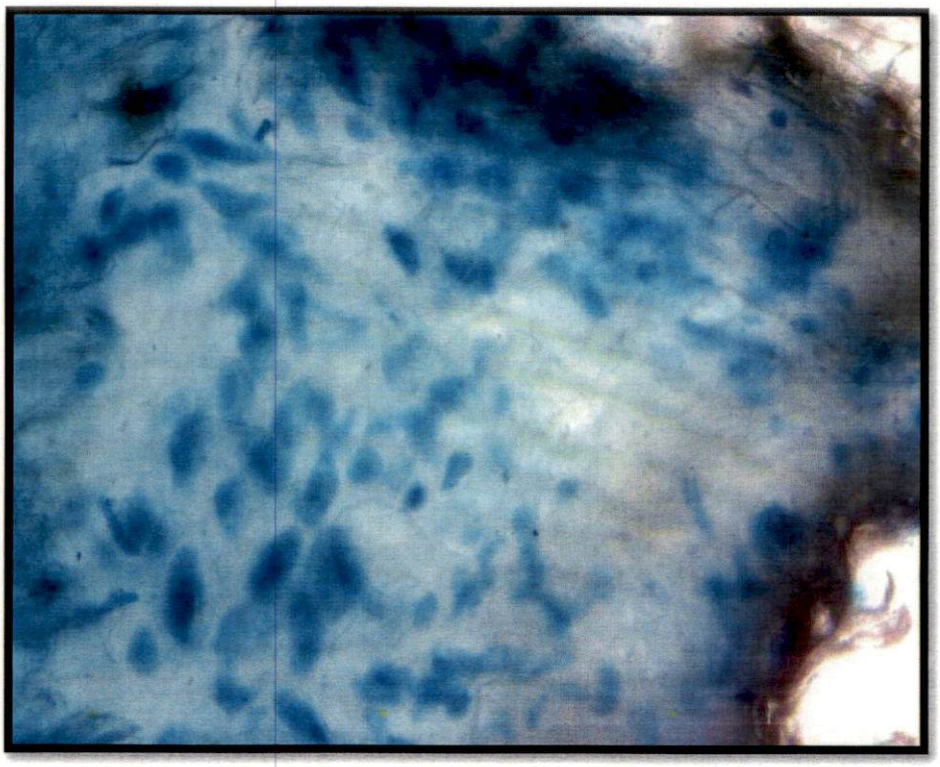
(T_9) **(T_{10})**

Plate. 11. Influence of bioinoculants and benzyl adenine on root length

T_{10} - Control
T_5 - POP + AMF + BA 100 ppm

(T_5) **(T_{10})**

Plate. 12. Microphotograph of roots colonized with AMF in various treatments



4.5 NUTRIENT ANALYSIS

4.5.1 Nutrient content

A significant variation was observed among the treatments when the plants were subjected to the analysis of nutrients (Table 14). From the results, it could be observed that the plants under the treatment T_5 (POP + AMF + BA 100 ppm) showed the maximum value in N and P content (1.24 and 0.87 per cent respectively). This was followed by T_9 (1.17 per cent) in case of N and T_4 (0.86 per cent) and T_7 (0.85 per cent) in case of P content. Treatment T_{10} recorded least value in both N and P content (0.73 and 0.16 per cent respectively).

When K content was estimated, highest value of 0.53 per cent was observed in the treatment T_3 (POP + PGPR Mix – 1 + BA 150 ppm) followed by T_1 (0.46 per cent) and T_2 (0.45 per cent). The least value was observed in T_9 (0.21 per cent). Maximum content of Ca (2.60 per cent) was observed in the treatment T_3 (POP + PGPR Mix - 1 + BA 150 ppm) followed by T_2 (2.57 per cent) and the least value was noted in T_9 (1.82 per cent). Considering the data pertaining to Mg and S, maximum value was observed in the treatment T_3 (0.54 and 1.72 per cent respectively) and the minimum value was observed under the treatment T_7 in case of Mg (0.29 per cent) and T_8 in case of S (1.11 per cent).

In general treatment consisting of AMF and *Azospirillum* were superior in terms of N and P content with maximum value in treatment T_5 (POP + AMF + BA 100 ppm). Treatment consisting of PGPR Mix – 1 had highest amount of K, Ca, Mg and S with T_3 (POP + AMF + BA 150 ppm) having the maximum content of these nutrients.

4.5.2 Nutrient uptake

Regarding nutrient uptake, maximum uptake of almost all the nutrients was found in T_3 (POP + PGPR Mix -1 + BA 150 ppm) (Table 15). Considering the data regarding N uptake, maximum values were observed in T_8 (POP + *Azospirillum* + BA 100 ppm) and T_1 (4.18 and 4.14g/ pot) which were statistically on par. Least N uptake was observed in the treatment T_7 (0.860g/ pot).

In the case of P uptake, maximum value of 0.980g/ pot was observed in T_5 and the least uptake was observed in the treatment T_{10} (0.54g/ pot). Considering K, Ca, Mg and S

Table 14. Influence of bioinoculants and benzyl adenine on nutrient content (%)

Treatment	N	P	K	Ca	Mg	S
T_1 (POP + PGPR Mix-1 + BA 50ppm)	1.12	0.17	0.46	2.35	0.39	1.16
T_2 (POP + PGPR Mix-1 + BA 100ppm)	0.89	0.16	0.45	2.57	0.44	1.60
T_3 (POP + PGPR Mix-1 + BA 150ppm)	0.79	0.22	0.53	2.60	0.54	1.72
T_4 (POP + AMF + BA 50ppm)	0.89	0.87	0.41	1.95	0.42	1.30
T_5 (POP + AMF + BA 100ppm)	1.24	0.87	0.42	2.43	0.42	1.33
T_6 (POP + AMF + BA 150ppm)	0.89	0.16	0.31	1.90	0.39	1.23
T_7 (POP + *Azospirillum* + BA 50ppm)	0.88	0.85	0.37	2.39	0.29	1.33
T_8 (POP + *Azospirillum* + BA 100ppm)	1.08	0.19	0.28	2.49	0.48	1.11
T_9 (POP + *Azospirillum*+ BA 150ppm)	1.17	0.18	0.21	1.82	0.41	1.27
T_{10} (POP)	0.73	0.16	0.39	2.13	0.39	1.33
CD (0.05)	**0.144**	**0.058**	**0.067**	**0.127**	**0.071**	**0.108**

70

Table 15. Influence of bioinoculants and benzyl adenine on nutrient uptake (g/ pot)

Treatment	N	P	K	Ca	Mg	S
T_1 (POP + PGPR Mix-1 + BA 50ppm)	4.68	0.71	1.92	9.82	1.64	4.84
T_2 (POP + PGPR Mix-1 + BA 100ppm)	3.61	3.51	1.82	10.45	1.78	6.46
T_3 (POP + PGPR Mix-1 + BA 150ppm)	7.10	1.98	4.70	23.30	4.78	15.38
T_4 (POP + AMF + BA 50ppm)	5.45	1.00	2.53	11.96	2.55	7.99
T_5 (POP + AMF + BA 100ppm)	8.62	10.35	4.90	28.89	5.05	15.89
T_6 (POP + AMF + BA 150ppm)	6.29	0.78	1.59	9.57	1.99	6.21
T_7 (POP + *Azospirillum* + BA 50ppm)	2.69	2.59	1.15	7.36	0.89	4.05
T_8 (POP + *Azospirillum* + BA 100ppm)	4.13	0.72	1.07	9.45	1.84	4.19
T_9 (POP + *Azospirillum*+ BA 150ppm)	6.87	1.06	1.25	10.64	2.36	7.49
T_{10} (POP)	3.10	0.54	1.34	7.40	1.37	4.60
CD (0.05)	**2.488**	**1.834**	**1.186**	**7.01**	**1.370**	**4.376**

uptake, maximum nutrient uptake was observed in the treatment T_3 (2.29, 11.30, 2.33 and 7.49g/ pot respectively).

It could be concluded that the treatment consisting of PGPR Mix-1 with 150ppm benzyl adenine was superior in terms of uptake of the major nutrients except N and P while *Azospirillum* and AMF inoculated plants were exhibited higher uptake of N and P.

4.6 PHYSIOLOGICAL PARAMETERS

4.6.1 Chlorophyll content

There was a significant influence for the various treatments on chlorophyll content of the plants. The highest total chlorophyll content of was observed in T_5 (POP + AMF + BA 100 ppm) and T_1 which were performing on par with each other (7.22 and 6.71mg/ g respectively). The least value for total chlorophyll content was estimated for the treatment, T_{10} (1.34mg/ g).

From the estimated value of chl *a*, the best result of 3.13mg/ g and 3.04 mg/ g were obtained for the treatments T_5 and T_1 respectively followed by the treatments T_4 and T_6. The minimum chl *a* content was noted in the treatments T_{10} (0.73mg/ g) and T_7 (0.77mg/ g) which were statistically on par.

During the estimation of chl b also, concomitant results were obtained showing the highest values for the treatment T_5 (4.09mg/ g) and T_6 (4.02mg/ g) and T_1 (3.91mg/ g). The least value of chl b was exhibited by the treatment T_{10} (0.80mg/ g).

The results on estimation of chlorophyll content showed that the plants receiving the treatment T_5, consisting of AMF and 100 ppm BA along with POP, produced high amount of chlorophyll pigment than any other treatments (Table16).

4.6.2 Stomatal characters

Regarding the stomatal density, the treatment combination of *Azospirillum* along with 100 ppm benzyl adenine (T_8) exhibited highest density of stomata (44.33) which was found

Table 16. Influence of bioinoculants and benzyl adenine on chlorophyll content (mg/ g)

Treatment	Chlorophyll		
	Chl a	*Chl b*	Total chlorophyll content
T_1 (POP + PGPR Mix-1 + BA 50ppm)	3.04	3.91	6.76
T_2 (POP + PGPR Mix-1 + BA 100ppm)	1.85	2.63	4.48
T_3 (POP + PGPR Mix-1 + BA 150ppm)	1.55	1.91	3.45
T_4 (POP + AMF + BA 50ppm)	2.63	3.37	6.01
T_5 (POP + AMF + BA 100ppm)	3.13	4.09	7.22
T_6 (POP + AMF + BA 150ppm)	2.27	4.02	5.62
T_7 (POP + *Azospirillum* + BA 50ppm)	0.77	1.33	2.10
T_8 (POP + *Azospirillum* + BA 100ppm)	1.58	2.26	3.84
T_9 (POP + *Azospirillum*+ BA 150ppm)	1.47	2.07	3.54
T_{10} (POP)	0.73	0.80	1.34
CD (0.05)	**1.381**	**1.639**	**2.90**

to be statistically on par with T_7 (41.00). The least stomatal count of 30.00 was observed in the treatment T_1 which was found to be on par with the treatment T_{10} (31.33) (Table 17 and Plate. 13).

4.7 Incidence of pest and diseases

During early stages of growth, dark brown, sharply defined sunken leaf spots were observed and later it was identified as leaf spot caused by *Colletotrichum* sp. Foliar spray with carbendazim 12 % + mancozeb 63 % (Saaf) @ 2g/ l was given during the initial stage itself for the effective control of this leaf spot. Later, during the flowering phase of the plants, *Alternaria* flower blight was observed in which small light brown spots were observed in the buds and flowers, which later enlarged and covered the entire flowers. The infected flowers in the entire spikes were finally decayed and wilted. As a control measure against *Alternaria* sp. foliar application with copper hydroxide (Kocide 2000) and propineb 70 WP (antracol) were given each at the rate of 2g/l. No pest attack was observed in the plants except snail attack during the early stages of the plant which could be effectively controlled by application of salt in the periphery of pots (Plate. 14).

Table 17. Influence of bioinoculants and benzyl adenine on stomatal characters

Treatment	Stomatal density	Stomatal index
T_1 (POP + PGPR Mix-1 + BA 50ppm)	30.00	13.512
T_2 (POP + PGPR Mix-1 + BA 100ppm)	40.67	13.790
T_3 (POP + PGPR Mix-1 + BA 150ppm)	40.67	13.553
T_4 (POP + AMF + BA 50ppm)	38.33	13.733
T_5 (POP + AMF + BA 100ppm)	37.67	13.003
T_6 (POP + AMF + BA 150ppm)	36.00	12.793
T_7 (POP + *Azospirillum* + BA 50ppm)	41.00	13.523
T_8 (POP + *Azospirillum* + BA 100ppm)	44.33	12.490
T_9 (POP + *Azospirillum*+ BA 150ppm)	36.00	13.150
T_{10} (POP)	31.33	12.517
CD (0.05)	**5.440**	**NS**

Plate. 13. Microscopic view of orchid stomata

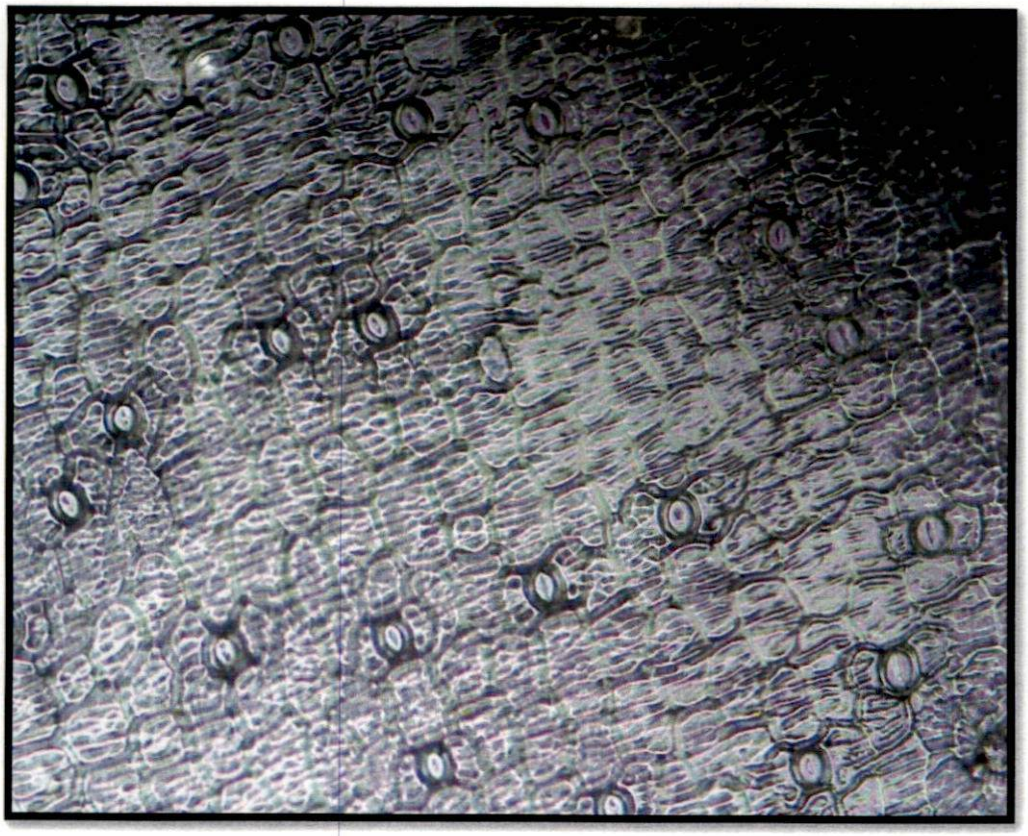
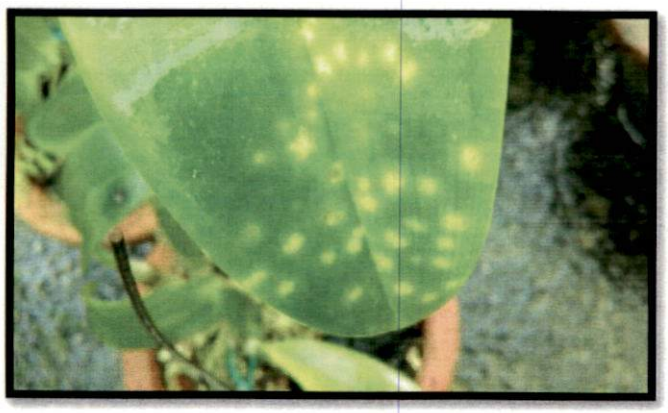
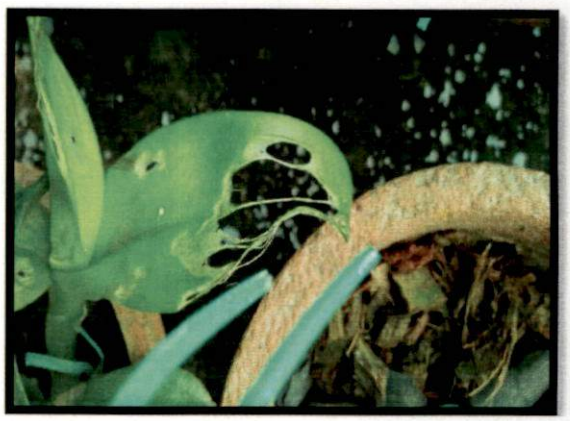


Plate. 14. Incidence of pests and diseases

Alternaria **blight on mature flower spikes**



Colletotrichum **leaf spot**



Snail attack on leaves

Discussion

5. DISCUSSION

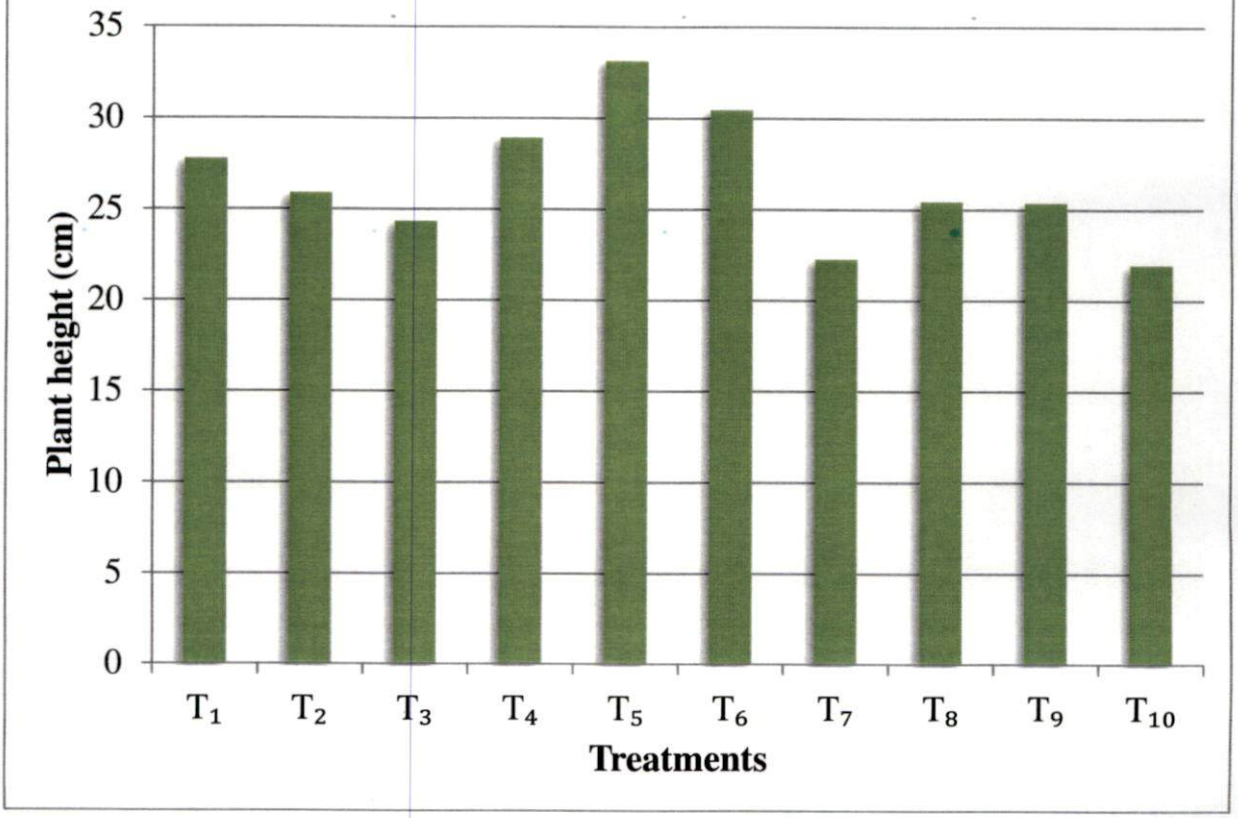
A study was conducted at Department of Floriculture and Landscaping, college of Horticulture, Vellanikkara to evaluate the influence of various bioinoculants and different levels of benzyl adenine on morphological and yield parameters of *Dendrobium* var. Yellow Splash. The experiment was conducted with different treatment combinations involving various bioinoculants *viz*; PGPR Mix – 1, *Azospirillum* and AMF and different levels of benzyl adenine, which were superimposed on the existing KAU Package of Practices Recommendations for orchids. The result obtained from the study are briefly discussed in this chapter

5.1 INFLUENCE OF BIO-INOCULANTS AND BENZYL ADENINE ON VEGETATIVE CHARACTERS

Significant improvement in vegetative characters was observed in treatments with bio-inoculants and benzyl adenine, compared to control. Treatments with AMF and different concentrations of benzyl adenine were superior in terms of plant height and maximum plant height was observed in T_5 (POP + AMF + BA 100 ppm). Increased plant height of AMF inoculated plants may be due to efficient endomycorrhizal association of the fungus with roots of *Dendrobium* orchids which helps in better uptake and mobilization of nutrients. It also secretes various phytohormones and vitamins which may act as antagonists and supress various pathogens. The mycorrhizal extramatrical hyphal strands extend into the medium, absorb water and minerals effectively and make it available to the plants (Fig. 1).

Increased levels of N and P were observed in plants inoculated with AMF. Both of these minerals are having a major role in several metabolic processes. Nitrogen is a vital component in the plant body which is essential for cell division, photosynthesis and plant growth and it also act as a major component of amino acids, vitamins and chlorophyll molecule while phosphorous play a major role in protein synthesis, protoplasm formation and is known to regulate many enzymatic processes leading to enhancement of metabolism and formation of new cells. Better availability of nutrients along with the production of phytohormones and antibiotics might have contributed to the morphological and physiological changes resulting in a significant improvement of height in plants inoculated with AMF. An improvement of plant height as a result of AMF inoculation has been reported

Fig. 1. Influence of bioinoculants and benzyl adenine on plant height (cm)



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

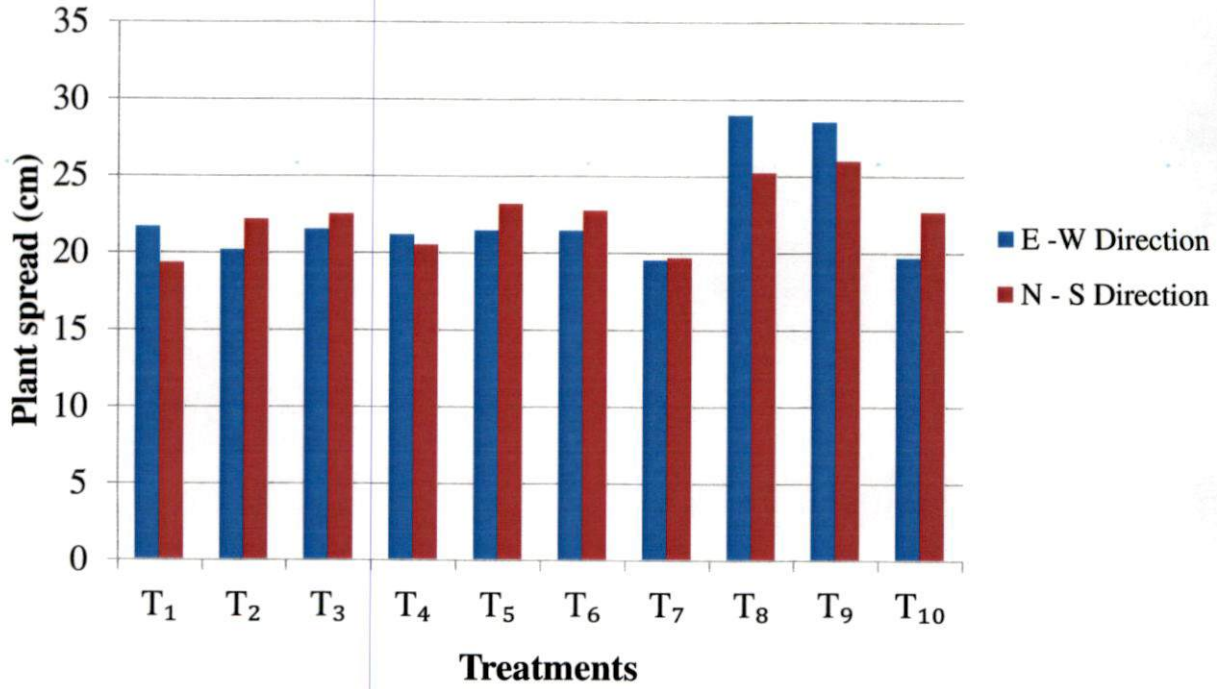
T_{10} - POP

in various flower crops (Varshney, 2002; Prabhat *et al.*, 2003; Bhatia and Guptha, 2007; Verma *et al.*, 2012; Karishma *et al.*, 2013 and Bhatia *et al.*, 2016)

A perusal of the data with respect to other vegetative characters like plant spread, number of leaves, leaf length, leaf breadth, leaf area and number of shoots per plant (Fig. 4) revealed that there was a significant improvement of all these parameters in plants inoculated with *Azospirillum* along with different levels of benzyl adenine and maximum values for all these parameters were observed in T_9 (POP + AMF + BA 150 ppm). Beneficial effect of *Azospirilum* might be the result of versatile mechanisms like increased nutrient uptake, enhanced stress resistance, vitamin production, siderophores and biocontrol, all operating simultaneously or sequentially in *Azospirillum* inoculated plants (Cohen *et al.*, 2015). *Azospirillum* species are having the capacity of self-production of phytohormones like auxin, gibberlins, cytokinins and nitric oxide and inducing the synthesis of these compounds in plant tissues (Gadagi, 1999). Cytokinin regulates several processes such as cell division, leaf expansion, shoot and root morphogenesis, etc. Gibberlins are responsible for cell division and elongation of plant cells. *Azospirillum* present in the rhizosphere are able to produce metabolites which act as signals for the production of phytohormones in the plant system. In the present study, eventhough *Azospirillum* could not be reisolated from the inoculated plants, the organism present in the rhizosphere might have produced exudates that could hasten the production of phytohormones and other metabolites in inoculated plants. Action of phytohormones like cytokinins and gibberlins coupled with increased nutrient status of these plants might have contributed to the improvement of vegetative characters like plant spread, length, breadth and area of leaves. These results are in conformity with findings of Bhaskaran *et al.* (2002) in marigold, Gadagi *et al.* (2002), Anon (2003) in dendrobium, Gadagi *et al.* (2004) in gaillardia, Srinivasa (2006) in *Anthurium andreanum*, Ravi *et al.* (2003) in chrysanthemum, Choudhari *et al.* (2013) in gladiolous and Hoda and Mona (2014) in petunia (Fig. 2, 3 and 4)

In both the cases of inoculation with AMF and *Azospirillum*, positive results were obtained when the plants were supplied with benzyl adenine along with these bioinoculants. Benzyl adenine is characterised as a synthetic cytokinin which highly helps in the cell division, cell elongation, organ formation and regeneration and also play an important role in translocation of assimilates to growing cells. Application of exogenous cytokinin has been reported to increase the synthesis of endogenous cytokinin in plants (Letham, 1994). In the

Fig. 2. Influence of bioinoculants and benzyl adenine on plant spread (cm)



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

Fig. 3. Influence of bioinoculants and benzyl adenine on on leaf area

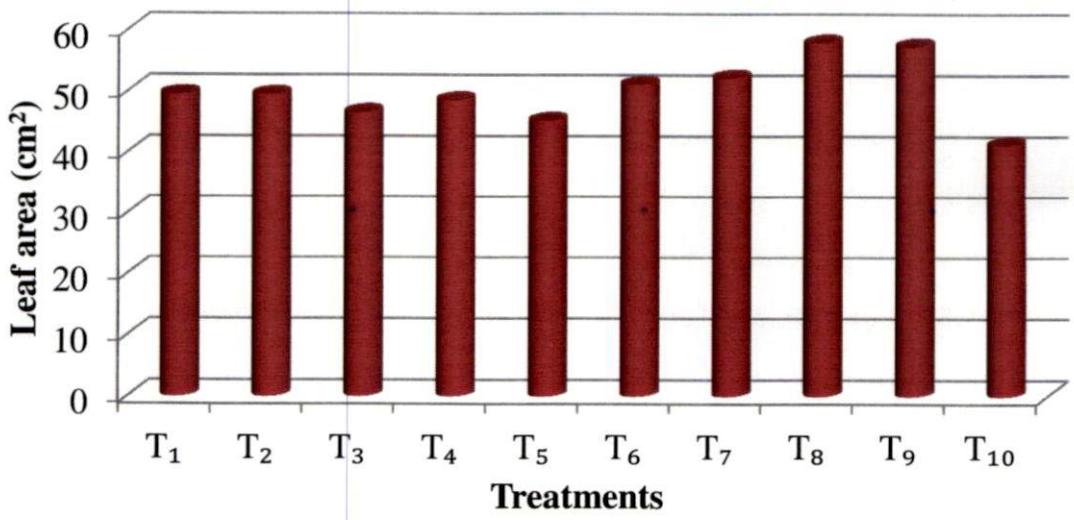
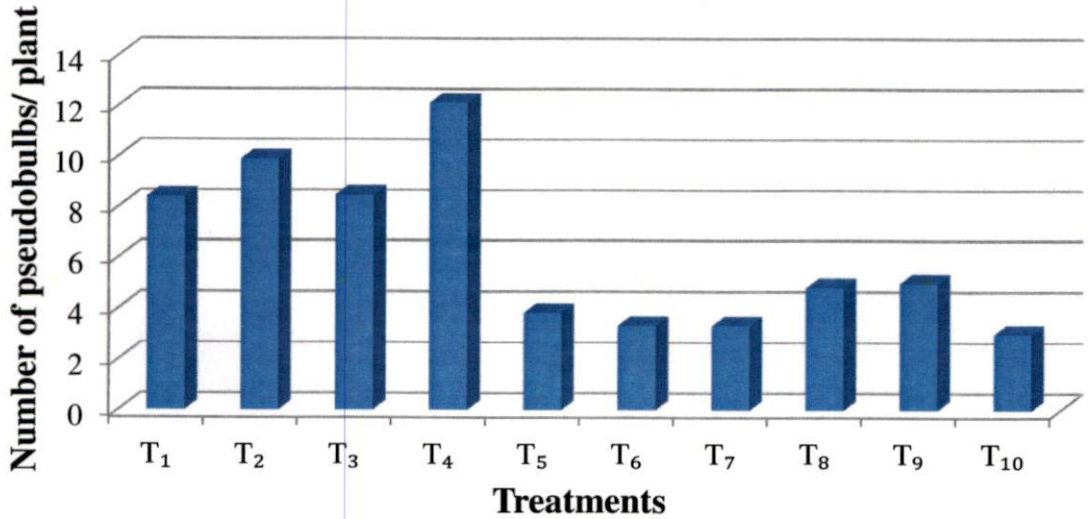


Fig. 4. Influence of bioinoculants and benzyl adenine on on number of pseudobulbs/ plant



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

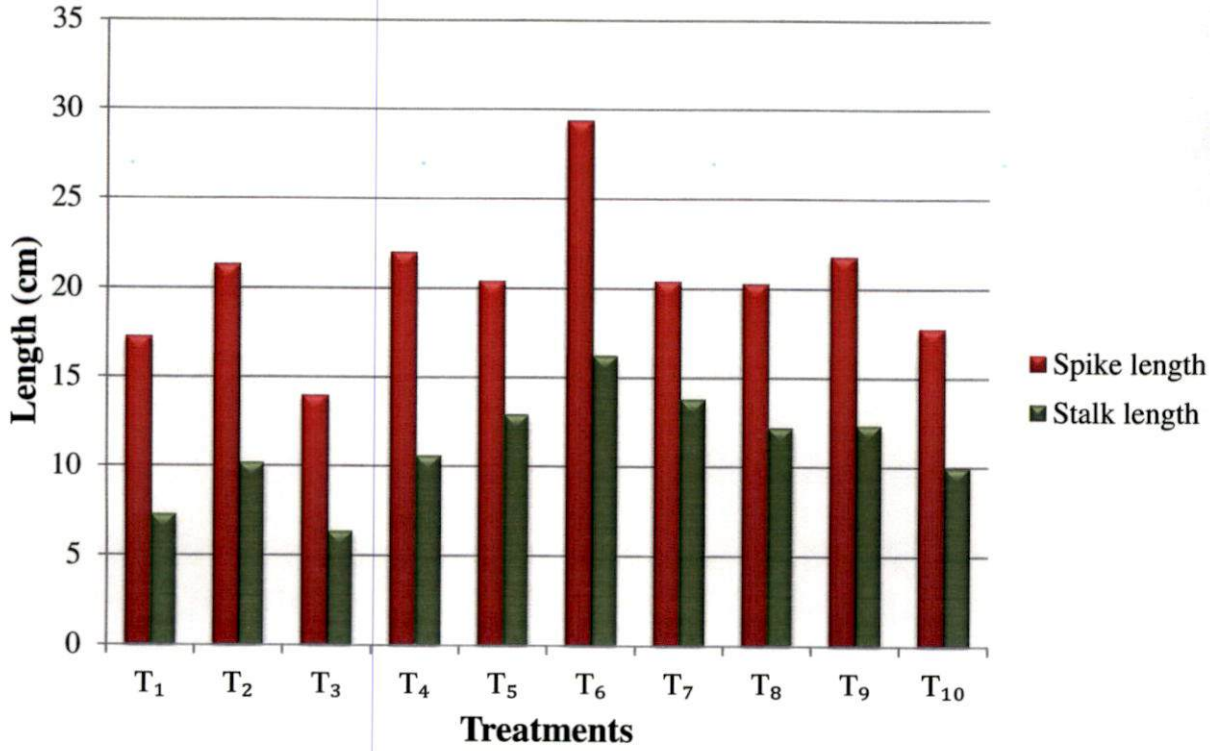
present study the exogenous application of benzyl adenine might have increased the level of cytokinin in the plants. Elevated cytokinin level and morphophysiological changes due to bioinoculant treatments might have resulted in an improvement of vegetative growth in plants under these treatments.

5.2 INFLUNCE OF BIOINOCULANTS AND BENZYLADENINE ON FLORAL CHARACTERS

Length of the spike, stalk length, number of flowers per spike and flower size are the important floral attributes which determine the quality of orchid spikes. In the present study an improvement in all these parameters was observed under the treatments consisting of AMF and *Azospirillum* along with different levels of benzyl adenine (Fig 5, 6 and 7). Regarding the days taken for the initiation of flowering even though there was no significant variation, plants under the treatments of AMF and *Azospirillum* took lesser number of days for flower emergence compared to other treatments. Minimum number of days for opening of first flower in the spike were observed under the treatment T_3 (POP + PGPR Mix- 1 + BA 150 ppm). Longevity of spike in the field is an important parameter while growing it as a pot plant. Treatment consisting of AMF along with 150 ppm benzyl adenine (T_6) had maximum longevity of the spike on the plant compared to other treatments (Fig. 8).

The superiority of treatments consisting of AMF in combination with different levels of benzyl adenine was well documented in floral characters. However the treatment T_6 (POP + AMF + BA 150ppm) was found to be the best among all the treatments. In the present study, an endomycorrhizal association could be observed with the roots of inoculated plants. This type of association might have resulted in morphological and physiological changes in roots. A significant improvement in root length and root volume was also observed in AMF inoculated plants. Even though epiphytic orchids like *Dendrobium* are characterised by the presence of areal roots covered with velamen tissue which can be functioned as absorbing roots, once got attached to a substrate (Dycus *et al.*, 1957). Hence in addition to the nutrients supplied through foliar spray, there might have been an additional exploration of nutrients, especially phosphorous, from the growing substrate also. AMF forms a symbiotic association with host plant and it also liberates growth promoting substances, vitamins and suppresses several pathogens. The positive influence of AMF inoculated plants in floral characters might be due to increased availability of nutrients to the plants combined with efficient translocation of phytohormones resulting in early flower initiation and production of quality

Fig. 5. Influence of bioinoculants and benzyl adenine on spike length and stalk length



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

Fig. 6. Influence of bioinoculants and benzyl adenine on number of flowers per spike

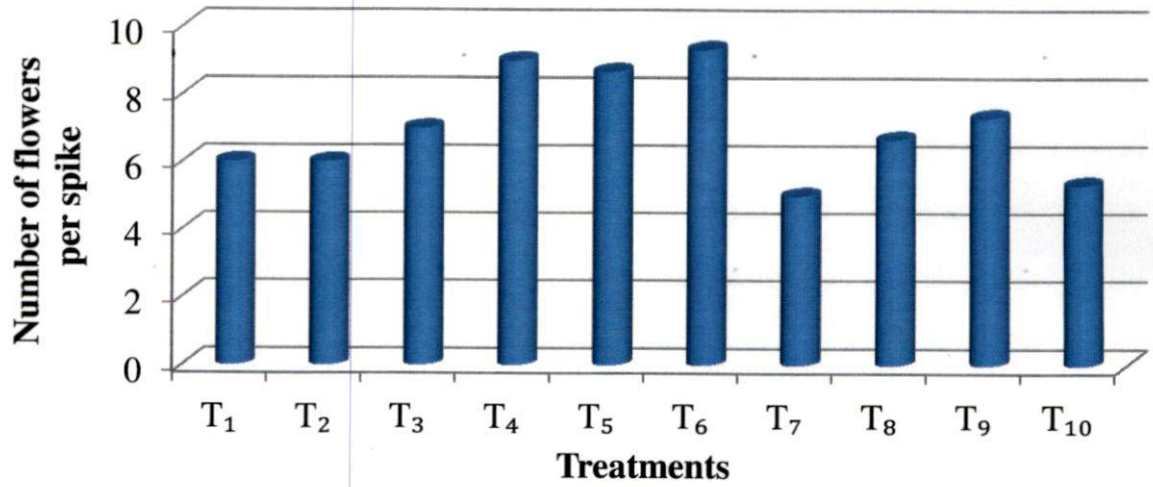
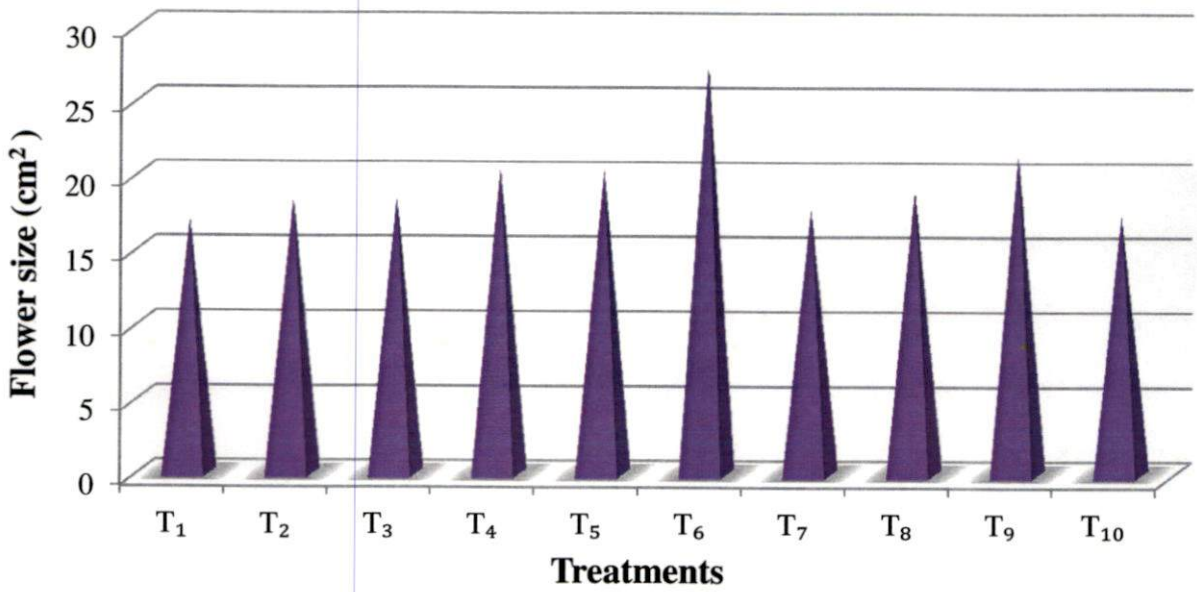


Fig. 7. Influence of bioinoculants and benzyl adenine on flower size (cm^2)



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

Fig. 8. Influence of bioinoculants and benzyl adenine on longevity of spike in the field

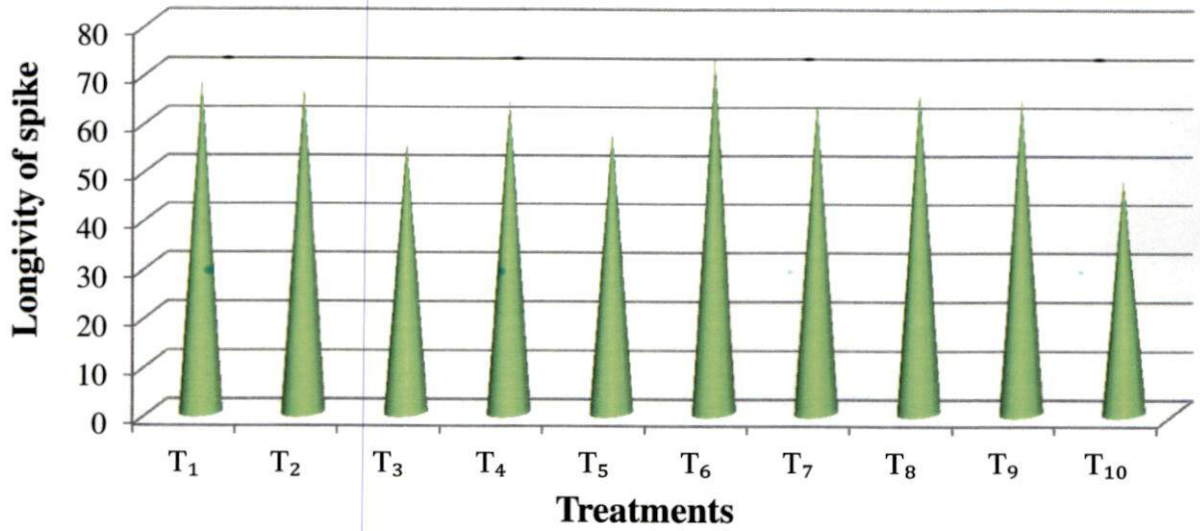
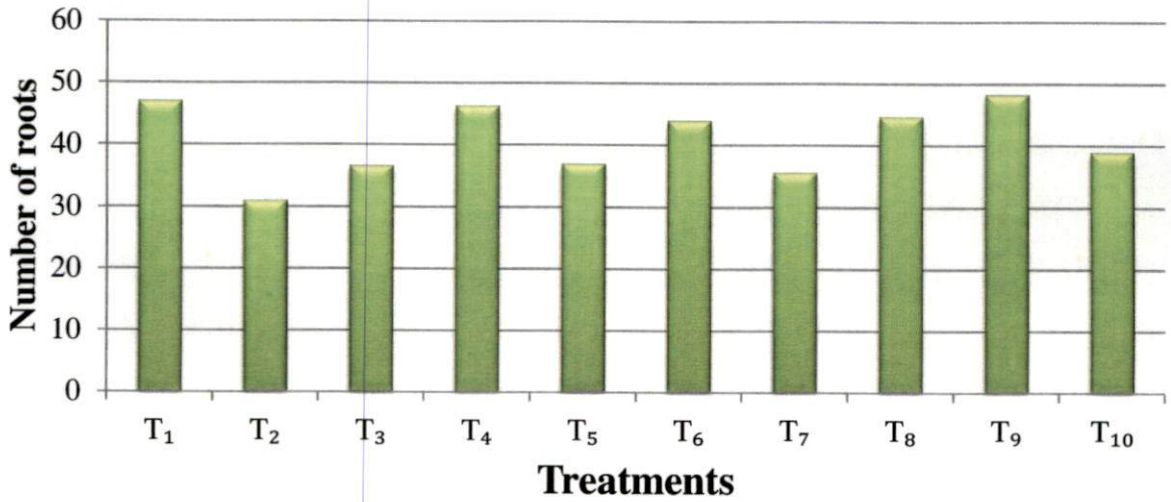


Fig. 9. Influence of bioinoculants and benzyl adenine on number of roots



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

spikes. In the present experiment maximum content of N and P were observed in treatment T_6 (POP + AMF + BA 150ppm). Nitrogen helps in improved vegetative growth by way of its role in synthesis of amino acids, chlorophyll formation and carbohydrate translocation. It also plays a key role in translocation of cytokinins which are highly essential for flower initiation and development. It was reported that cytokinins are synthesised in the aerial roots of orchids and translocated to shoots, which depends upon the nitrogen status of the plant (Zhang *et al.* 1995). In the case of phosphorous, it also plays significant role in respiration, photosynthesis, cell division and enlargement, as well as energy storage and transfer which promote root formation, flowering and growth of the plants (Uchida, 2000).

An increased root volume coupled with high nitrogen content might have exerted a positive influence in the production and translocation of cytokinins which might have positively influenced the floral characters of these plants. An improvement in flower production and quality of flowers by inoculation of AMF had been reported in many flower crops (Bhaskaran *et al.* 2002; Prabhat *et al.* 2003; Yadav *et al.* 2013 and Bhatia *et al.* 2016).

Regarding the interaction effect of AMF and benzyl adenine, application of 150 ppm BA along with AMF inoculation was found to be effective. Benzyl adenine is a synthetic cytokinin. Cytokinins are reported to be one of the multifactorial components that functions as floral stimulus and exogenous application of cytokinin was reported to hasten the rate of flower initiation and flower production due to an increase in endogenous level of cytokinin in plant system (Blanchard and Runkle, 2008). The result of the study is in conformity with the findings of Swapna (2000),Prabhat *et al.* (2003), Perner et al (2007), Willium and Ichihara (2010), Bohra *et al.*, (2014) and Barman *et al.* (2014) who reported a positive influence of benzyl adenine on floral characters in various flower crops.

5.3 INFLUENCE OF BIOINOCULANTS AND BENZYLADENINE ON POST HARVEST CHARACTERS

The superiority of treatments consisting of AMF was well documented in post-harvest characters. Maximum fresh weight of the spike was observed in the treatment T_6 (POP + AMF + BA 150 ppm). Superiority regarding this parameter in AMF inoculated plants might be due to easy uptake of nutrients and better nutrient content in these plants resulting in faster mobilization of photosynthates from vegetative parts to floral parts and thereby increasing the fresh weight of the spikes.

The same treatment T_6 took maximum number of days for the wilting of first flower in the spike. Vase life is most important post-harvest character and the treatment consisting of AMF (T_6 and T_5) exhibited maximum vase life (25.33 and 23.33 respectively). The superiority of AMF inoculated plants in terms of vase life may be due to better vascular development and thereby enhancing the absorption of holding solution which helps to maintain the turgidity of the spike. There are also reports that AMF play an important role in increasing the vase life of cut flowers by reducing ethylene production.

The role of AMF in improving post – harvest character of cut flowers was reported by Besmer and Koide (1999) in snapdragon, Wen and Chang (1995) in gerbera and Karishma *et al.* (2011) in chrysanthemum.

5.4 INFLUENCE OF BIO-INOCULANTS AND BENZYLADENINE ON ROOT PARAMETERS

From the result obtained, it could be clearly observed that, maximum root length and root volume were exhibited by the plants inoculated with AMF (Fig. 10 and Fig. 11). This could be mainly attributed to higher phosphorous uptake in the plants by extraradical mycelium leading to higher shoot and root growth in inoculated plants (Smith and Read, 1997). AMF was reported to produce metabolites that can alter the plant's ability to produce roots and to alter root regeneration and root morphology resulting in an increased absorptive surface area and feeder root longevity (Linderman, 1988). A diffusible symbiotic signal produced by AMF which is recently identified as lipo chito oligosaccharides (LCOs), designated as *My factor*s that help in root growth and branching (Maillet *et al.* 2011). This could be considered as another reason for the high rate of root growth in AMF inoculated plants, which is in accordance with the findings of Poulton *et al.* (2002); Liu *et al.* (2004), Martin and Stutz (2004); Perner *et al.* (2007) and Karishma *et al.* (2013).

Maximum number of roots were produced by the plants under the treatment T_9 (POP + *Azospirillum* + 150 ppm BA) (Fig. 11). *Azospirillum* is a gram negative diazotrophic rhizobacteria which exhibits chemotactic response to plant exudates, i.e., the ability to sense and navigate towards the most favourable niches for growth (Rodrigues *et al.* 2009). When plants are inoculated with *Azospirillum*, signalling molecules will be produced by the bacteria and the plant will produce lateral roots and root hairs which are the source of exudates to maintain bacterial population in rhizosphere (Gadagi *et al.* 2004). Significant changes in root

Fig. 10. Influence of bioinoculants and benzyl adenine on root length

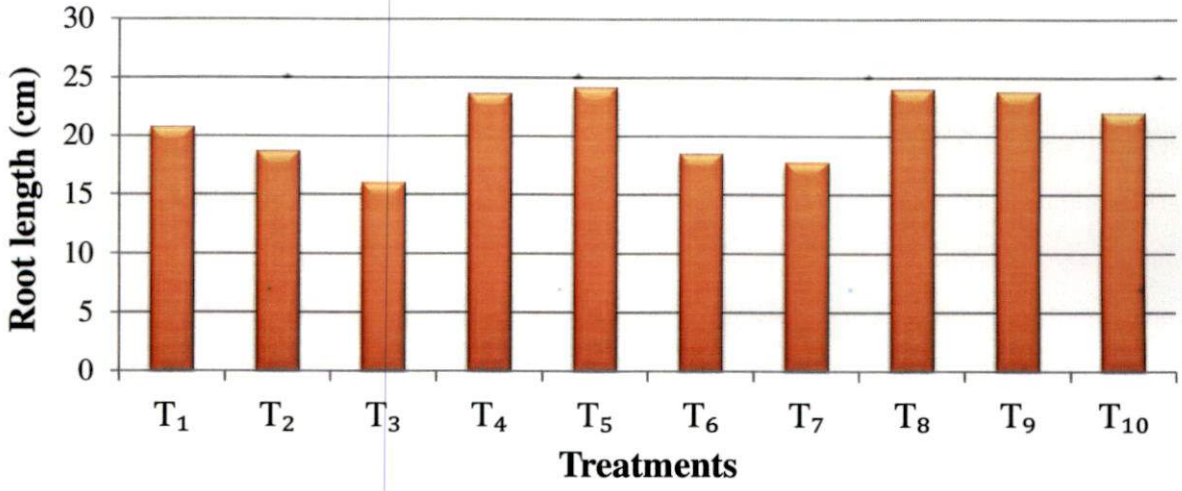
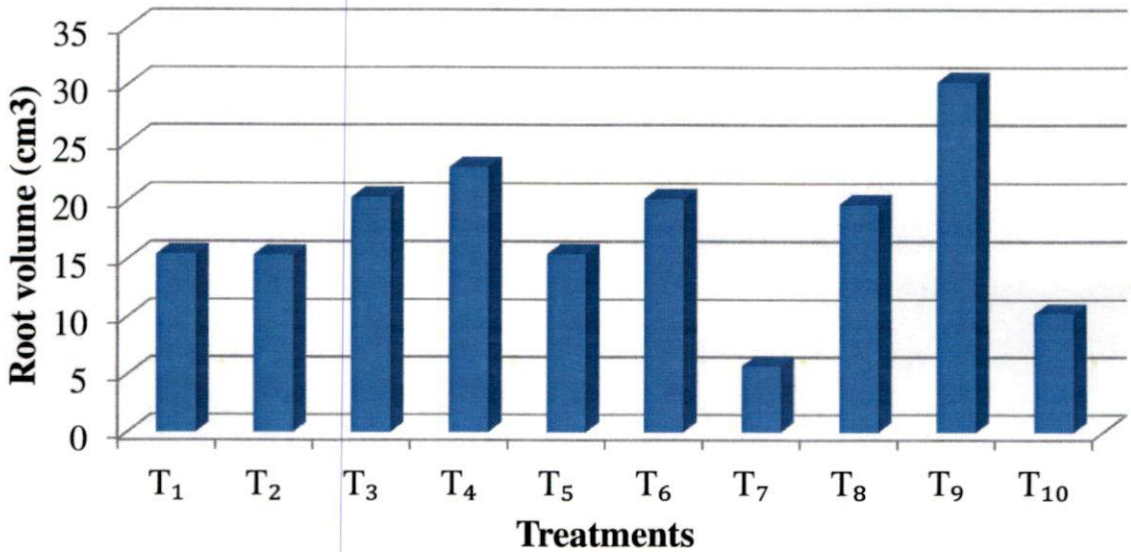


Fig. 11. Influence of bioinoculants and benzyl adenine on root volume



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

length, root volume and number of roots were reported as a result *Azospirillum* inoculation in different cereals (Wani, 1990; Okon and Itzigsohn, 1995). Production and secretion of phytohormones like auxin, cytokinin and gibberlins by *Azospirillum* itself might be the reason for the production of more number of roots in the inoculated plants. Similar findings were reported by Molla *et al.* (2001) and Remans *et al.* (2008).

With respect to root parameters considered, bioinoculants along with 100 ppm benzyl adenine exhibited maximum values for all the root characters. Even though benzyl adenine has least effect on root growth, sometimes through autoinductive cytokinin regulation, this effect may reverse, favouring better root production in the plants. In this autoinductive regulation, degradation of cytokinin in plants occur when its amount rises than the optimum level (Kaminek *et al.*, 1997). So, here, in addition to the phytohormone produced inside the plant due to *Azospirillum* inoculation, exogenous application of BA might have elevated the level of cytokinin concentration within the plants and higher root production might have occurred due to autoinductive cytokinin regulation. Similar findings were also reported by Wroblewska (2013) in *Gaura lindheimeri*.

5.5 INFLUENCE OF BIO-INOCULANTS AND BENZYLADENINE ON NUTRIENT CONTENT

5.5.1 Nutrient content

At the fag end of the experiment, analysis for N, P, K, Ca, Mg, and S was done to compare nutrient content as well as nutrient uptake by plants under different treatments. It was observed that, nitrogen and phosphorous content were highest in the plants inoculated with AMF (T_5 - POP + AMF + BA 100 ppm) compared to other treatments while maximum K, Ca, Mg and S content were observed in plants treated with PGPR Mix-1 along with 150 ppm BA (T_3).

The reason for maximum N and P content in AMF inoculated plants (T_5) may be due to highest intraradical and extraradical mycelial system extension, which improved the absorption area for nutrient uptake in these plants. These mycorrhizal fungi also secrete acid phosphates and organic acids which will facilitate the release of P from organic complexes. All these factors might have resulted in better accumulation of N and P in AMF inoculated plants (Ezawa *et al.*, 2005; Cameron *et al.*, 2006 and Perner *et al.*, 2007). In addition to the root colonization effect of AMF, exogenous application of phytohormone may also be a

reason for higher N and P content of the plants, while K, Ca, Mg and S were analysed, their contents were found comparatively lower in AMF inoculated plants, and highest amount of these nutrients were observed under plants inoculated with PGPR Mix - 1. This result is in line with the findings of Poole and Seeley (1978) who reported a decreased concentration of K, Ca and Mg in *Cymbidium* orchids due to increase in the level of nitrogen. Similar studies in accordance with these results were conducted by Guo *et al.* (2011) and Shilev *et al.* (2012).

5.5.2 Nutrient uptake

Uptake of N and P was maximum in plants inoculated with *Azospirillum* and AMF respectively (Fig. 12). This may be due to the efficient association of both of the bioinoculants with plant roots, which may alter the morphology and physiology of roots resulting in an increased absorptive surface area and feeder root longevity (Linderman, 1988) thereby resulting in higher uptake of these nutrients. These results are in close conformity with the findings of Sarawgi *et al.* (1999) who reported an increased P uptake in *Cicer arietinum* by AMF inoculation. In the case of K, Ca, Mg and S uptake, maximum uptake was recorded in the treatment T_3 (POP + PGPR Mix -1 + BA 150ppm) and this could be due to the higher dry matter content of the plants under this treatment.

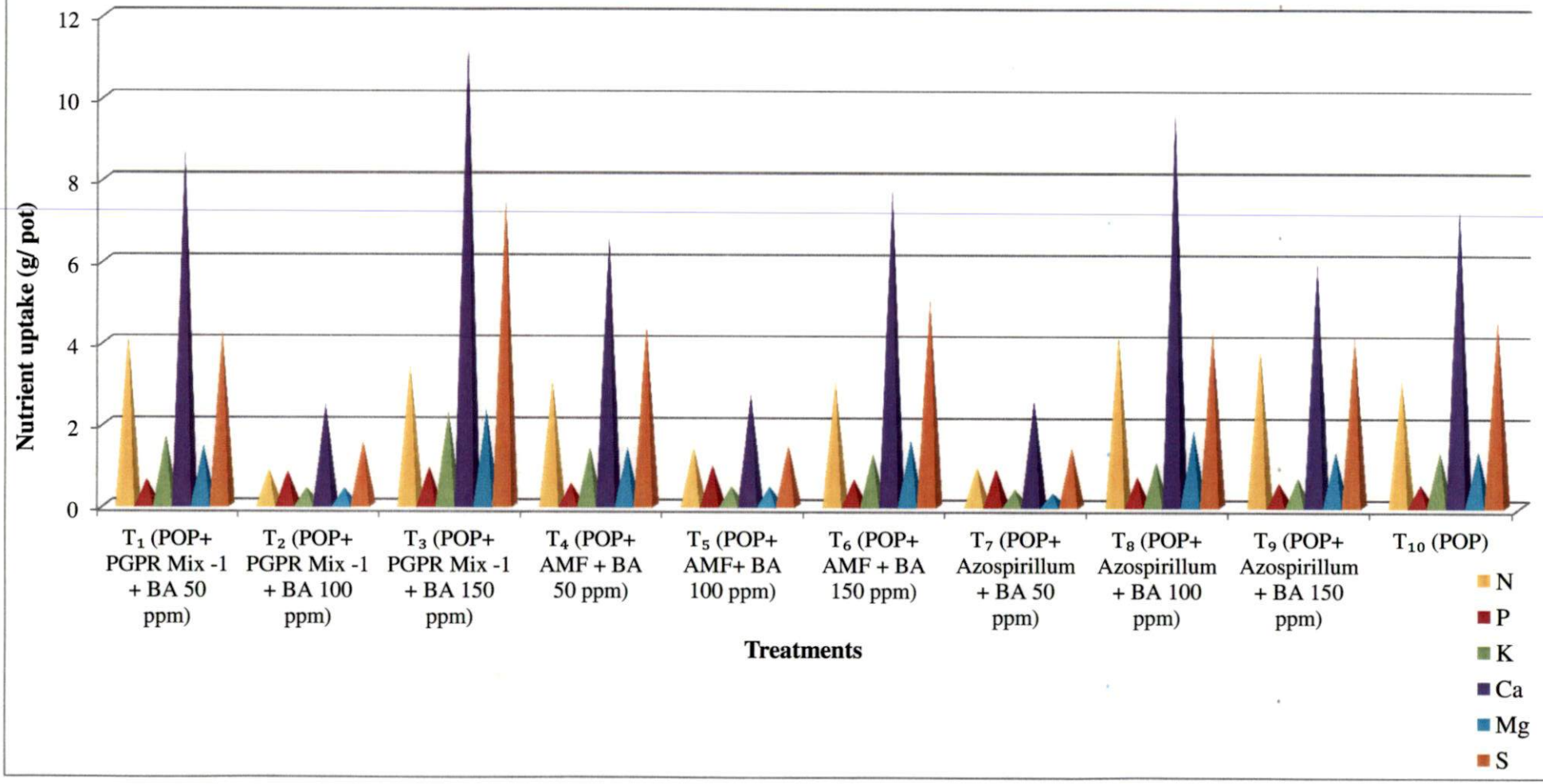
5.6 INFLUENCE OF BIO-INOCULANTS AND BENZYL ADENINE ON PHYSIOLOGICAL PARAMETERS

5.6.1 Chlorophyll content

Regarding chlorophyll content, maximum chl a, chl b and total chlorophyll content (Fig. 13) were observed in treatment T_5 (AMF + 100 ppm benzyl adenine) . This might be due to an elevated level of stomatal conductance, transpiration, photosynthesis and plant growth. Formation of larger and more number of bundle sheath chloroplast could be another reason for maximum production of chloroplast in inoculated plants (Arumugan *et al.* 2010).

Nitrogen and Magnesium are major nutrients which act as an essential part of chlorophyll molecule (Brady and Weil, 2010). So higher concentration of these nutrients in the inoculated plants might be another reason for an increase in chlorophyll content. Similar results were obtained by Crespo (2015) in Maize genotypes and Beltrano *et al.* (2013) in pepper.

Fig. 12. Influence of bio-inoculants and benzyl adenine on nutrient uptake by the plants



In addition to the effect of AMF, benzyl adenine also has an important role for higher chlorophyll production. Cytokinin encourages the protease activity and results in the release of cations like Ca^{2+}, Mg^{2+} and Zn^{2+} from their nature bound or complexed state (Fletcher and McCullagh, 1971) and the release of these ions may also be the possible reason for higher chlorophyll production in this treatment. The result of the present study is found to be in accordance with the findings of Fletcher and McCullagh (1971) in cucumber.

5.6.2 Stomatal characters

Considering the stomatal density, maximum number of stomata was obtained in the treatment consisting of *Azospirillum* and 100 ppm benzyladenine applied along with the recommended dose of NPK and cowdung slurry (POP) (Fig. 14). Since the maximum leaf area was observed in the same treatment, it could be the reason for high stomatal density in this treatment.

Fig. 13. Influence of bioinoculants and benzyl adenine on chlorophyll content

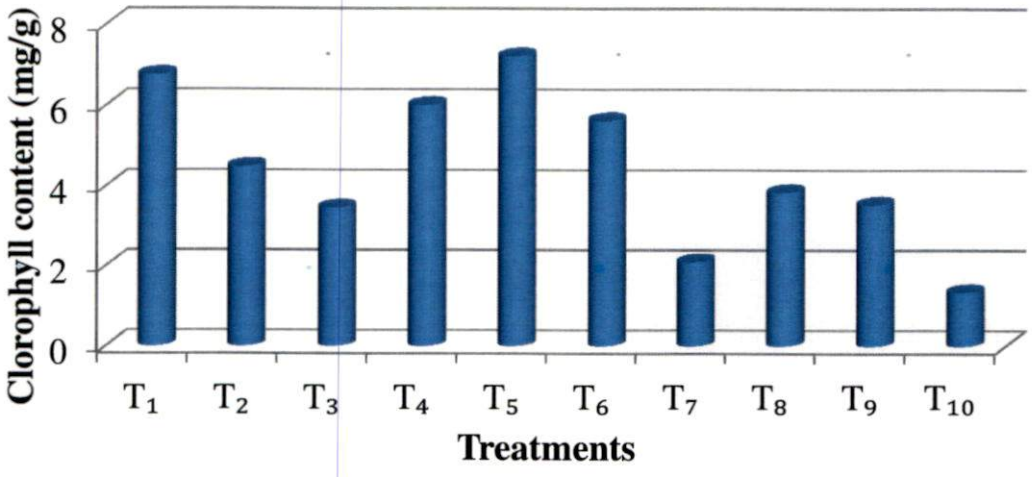
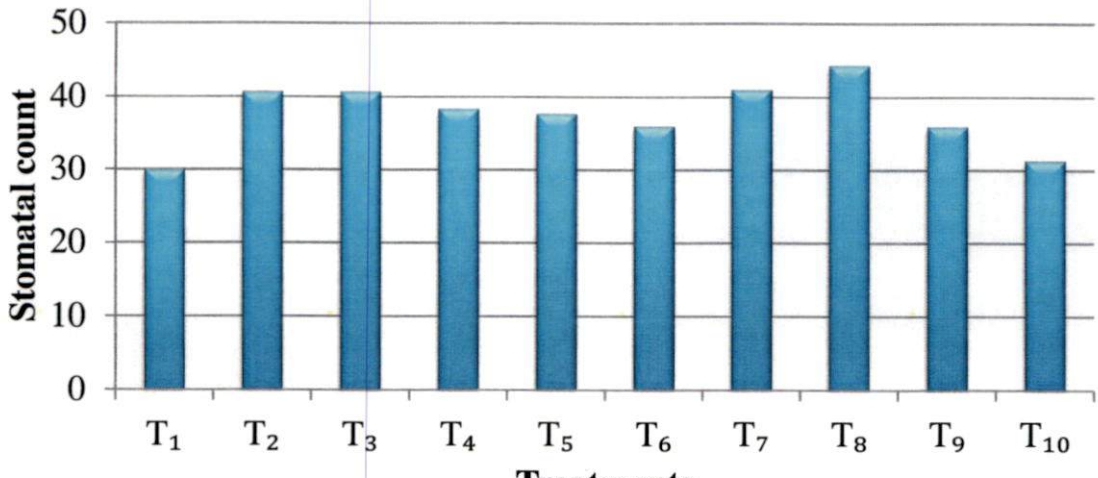


Fig. 14. Influence of bioinoculants and benzyl adenine on stomatal density



T_1 - POP + BA (Benzyl adenine) 50ppm + PGPR Mix 1

T_2 - POP + BA 100ppm + PGPR Mix 1

T_3 - POP + BA 150ppm + PGPR Mix 1

T_4 - POP + BA 50ppm + AMF (Arbuscular Mycorrhizal Fungi)

T_5 - POP + BA 100ppm + AMF

T_6 - POP + BA 150ppm + AMF

T_7 - POP + BA 50ppm + *Azospirillum*

T_8 - POP + BA 100ppm + *Azospirillum*

T_9 - POP + BA 150ppm + *Azospirillum*

T_{10} - POP

Summary

6. SUMMURY

An experiment entitled 'Refinement of nutrient management practices in *Dendrobium* orchids' was conducted during August 2015 to July 2016 in the Department of Floriculture and Landscaping, College of Horticulture, Kerala Agricultural University, Thrissur in order to evaluate the the response of *Dendrobium* to organic and inorganic nutrients, growth regulators and bio-inoculants and to refine the existing nutrient management practices for *Dendrobium* orchids. Six months old Yellow Splash variety of *Dendrobium* orchid was used for the study. The experiment consisted of ten treatments with five replication and 15 plants under each treatment. The KAU Package of Practice Recommendations for orchids is foliar application of $N:P_2O_5:K_2O$, 3:1:1 during period of vegetative growth and 1:2:2 during flowering period at the rate of 0.2 per cent twice a week. Three bio-inoculants *viz.*, PGPR Mix – 1, AMF (Arbuscular Mycorrhizal Fungi) and *Azospirillum* and three levels of benzyl adenine *viz.*, 50, 100 and 150 ppm were superimposed on this recommendation. The observations on various vegetative, floral and post-harvest parameters, root characters and physiological parameters were taken.

Among the vegetative characters significant variation was observed for the characters like plant height, plant spread, leaf length, breadth , leaf area and number of pseudobulbs per plant.

Regarding the plant height, no significant difference was observed upto fifth months after planting. From sixth month onwards significant difference was observed with maximum value under the treatment T_5 (POP + AMF + BA 100 ppm) and the least height was observed in plants under control (33.09 and 21.94 respectively).

The treatment consisting of *Azospirillum* along with POP and different doses of benzyl adenine were superior in terms of other vegetative parameters, like plant spread, number of pseudobulbs per plant, leaf length, breadth and leaf area. Plant spread in EW and NS directions were maximum under the treatment T_8 (POP + *Azospirillum* + BA 100 ppm) and T_9 (POP + *Azospirillum* + BA 150 ppm) throughout the growth period (28.98 and 25.97 cm respectively). Highest value for leaf length and number of pseudobulbs per plant was obtained for the treatment T_9 (15.78 and 5.00 respectively) while maximum leaf breadth and leaf area were observed under the treatment T_8 (6.13 cm and 57.94 cm^2 respectively). All these parameters were minimum under control.

Number of leaves per plant and intermodal length did not show any significant variation throughout the growth period.

Regarding various floral characters, early emergence of spike was observed under the treatment T_8. The treatment T_6 (POP + AMF + BA 150 ppm) was superior in terms of other floral attributes *viz.,* days to last flower opening, length of spike, stalk length, number of flowers per spike, intermodal length, flower size and longevity of spike in the field. No significant variation between treatments could be observed regarding the number of spikes per plant.

Among the post – harvest parameters, fresh weight of the spike, days to wilt of the first floret and vase life were found to be highest under the treatment T_6 (13.63g, 7.00 and 25.33 days respectively) while all these parameters were found to be minimum under control. Considering physiological loss in weight, maximum value was obtained under the treatment consisting of PGPR Mix -1 + BA 100 ppm while minimum value was obtained under the treatment T_3 (POP + PGPR Mix -1 + BA 150 ppm). There was no variation obtained in the parameter total water uptake of the spike during the post- harvest period.

Number of roots, root length and root volume were the parameters considered under the root characters in which number of roots and root volume were observed maximum under the treatment T_9 (48.33 and 30.25 cm^3 respectively) and maximum root length was observed in the treatment T_5 (24.18 cm). From the root colonization study conducted for AMF, among three treatments maximum colonization percentage was obtained in the treatment T_6 (POP + AMF + BA 150 ppm) followed by T_5 and T_4.

Regarding the nutrient content, the highest N and P content were observed under the treatment consisting of POP + AMF + BA 100 ppm (T_5) while content of K, Ca, Mg and S were found to be highest in the treatment T_3. In the case of nutrient uptake, plants under the treatment T_8 (POP + *Azospirillum* + BA 100 ppm) showed highest N uptake and the P uptake was found to be maximum in T_5 (POP + AMF + BA 100ppm). Uptake of K, Ca, Mg and S were found to be maximum in the plants inoculated with PGPR Mix -1 (T_3).

While physiological parameters were considered, maximum chlorophyll content could be observed in the plants inoculated with AMF (T_5). Regarding stomatal characters, highest stomatal density was observed under the treatment T_8 (POP + *Azospirillum* + BA 100 ppm) and stomatal index was found to be non significant.

References

7. REFERENCES

[Anonymous]. 2003. *Orchids*. Biennial Progress Report, Indian Council of Agricultural Research, New Delhi, pp. 204 – 243.

Ali, H., Dash, P.K., Islam, M., Ahmed, M. and Mondal, T. 2014. Effect of NPK spray formulations on growth of two cultivars of orchid (*Mokara* sp.). *J. Agri. Vet. Sci.* 7(5): 31 – 42.

Arumugam, R., S. Rajasekaran and S.M. Nagajan. 2010. Response of Arbuscular mycorrhizal fungi and Rhizobium inoculation on growth and chlorophyll content of *Vigna unguiculata* (L) Walp Var. Pusa 151. *J. Appl. Sci. Environ. Manag.* 14:113-115.

Barman, D., Usha Bharathi, T., Pokhrel, H., Naik, S. K., and Medhi, R. P. 2014. Influence of concentration and mode of application of different growth regulators on *Dendrobium* hybrid Thongchai Gold. *J. Crop and Weed.* 10(3): 223 – 230.

Beltrano, J., Ruscitti, M., Arango, M. C. and Ronco, M. 2013. Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and P levels. *J. soil science and plant Nutr.* 13(1): 123-141.

Bernier, G., and Périlleux, C. 2005. A physiological overview of the genetics of flowering time control. *Plant Biotechnol. J. 3*(1): 3-16.

Besmer, Y.L. and R.T. Koide, 1999. Effect of mycorrhizal colonization and P on ethylene production by snapdragon (*Antirrhinum majus*. L) flower. Mycorrhiza, 9: 161-166.

Bhaskaran, P., Ambrose, G., and Jayabalan, N. 2002. Usefulness of biofertilizers in economizing nitrogenous fertilizers in *Tagetes erecta* L. *J. Phytological Res. 15*(2): 155-160.

Bhatia, S. and Gupta, Y. C. 2007. Studies on use of biofertilizer in carnation (*Dianthus caryophyllus* Linn.) flower production. *J. Ornamental Hortic.* 10(2): 131-132.

Bhatia, S., Gupta, Y. and Dhiman, S. 2016. Influence of biofertilizers on leaf nutrient status and flower quality of carnation cv. Sunrise. *Int. J. Farm Sci.* 6(3): 1-6.

100

Bhatia, S., Gupta, Y. C. and Dhiman, S. R. 2004. Effect of growing media and fertilizers on growth and flowering carnation under protected condition. *J. Ornamental Hortic.* 7(2): 174-178.

Binisha, S. 2003. Supplementary effect of bio-fertilizers in Dendrobium. MSc (Ag) thesis, Kerala Agricultural University, Thrissur, 89p.

Black, C. A. 1965. *Methods of soil analysis Part 1and 2.* American Society of Agronomy, Inc., USA.

Blanchard, M. G. and Runkle, E. S. 2008. Benzyl adenine promotes flowering in *Doritaenopsis* and *Phalaenopsis* orchids. *J. plant growth reg.* 27(2): 141 - 150.

Bohra, M. and Kumar, A. 2014. Studies on effect of organic manures and bioinoculants on vegetative and floral attributes of Chrysanthemum cv. Little Darling. *The Bioscan.* 9(3): 1007-1010.

Brady, N.C. and R.R. Weil. 2010. Elements of the nature and properties of soils. Prentice 37 Hall, Secaucus, NJ. 624p.

Cameron, D. D., Leake, J. R. and Read, D. J. 2006. Mutualistic mycorrhiza in orchids: evidence from plant fungus carbon and nitrogen transfers in green – leaved terrestrial orchid *Goodyera repens. New Pytol.* 171: 405 – 416.

Chaudhari, C. K., Jadav, R. G. and Masu, M. M. 2010. Effect of biofertilizers and their combinations with nitrogen fertilizer on growth, yield and quality of rose (*Rosa damascene* L.). *Asian J. Flort. 4*: 373-376.

Chaudhary, N., Swaroop, K., Janakiram, T., Biswas, D. R., and Singh, G. 2013. Effect of integrated nutrient management on vegetative growth and flowering characters of gladiolus. *Indian J. Hort.* 70(1): 156-159.

Chaudhary, S. S. 2007. Effect of nitrogen, phosphorus and biofertilizer application on plant growth and bulb production in tuberose. *Haryana J. Hortic. Sci.* 36(2): 82-85.

Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanlandro, H. and Piccoli, P. N. 2015. *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiologia plant.* 153(1): 79-90.

Crespo, R. J. 2015. Impact of Arbuscular Mycorrhizal Fungi on the physiology of maize genotypes under variable nitrogen and phosphorus levels. pp. 92–117. PhD Thesis, Agro. Hort. Dep. Nebraska Univ. Lincoln 2015.

Dalve, P. D., Mane, S. V. and Ranadive, S. N. 2009. Effect of biofertilizers with reduced doses of nitrogen on flower quality of gladiolus. *J. Maharashtra Agric. Univ.* 34(1): 122-123.

De, L. C., and Pathak, P. Value addition in orchids. *J. Orchid Soc.* 29: 31 – 37.

Devi, H. and Chezhiyan, N. 2001. Response of nitrogen and growth hormones on leaf parameter of *Dendrobium* cv. Sonia 17. *Orissa J. Hortic.* 29: 24 – 28.

Devi, H. U. N. and Chezhiyan, N. 2002. Foliar nutrition as a tool to increase chlorophyll content in *Dendrobium*. *J. Ornamental Hortic.* 10(2): 128 – 130.

Dinesh, D. 2009. Influence of nutrients and Plant Growth Promoting Root Endophyte (PGPRE) on growth and development of *Dendrobium* cv. Earsakul. MSc (Ag) thesis, Kerala Agricultural University, Thrissur, 68p.

Dycus, Augustus M., and Lewis Knudson. 1957. The role of the velamen of the aerial roots of orchids. *Bot. Gazette*. 119(2): 78-87.

Ezawa T, Hayatsu M, Saito M. 2005. A New Hypothesis on the Strategy for Acquisition of Phosphorus in Arbuscular Mycorrhiza: Up-Regulation of Secreted Acid Phosphatase Gene in the Host Plant. *Mol. Plant-Microbe Interactions*. 18(10): 1046-53

Fletcher, R. A. and McCullagh, D. 1971. Benzyl adenine as a regulator of chlorophyll synthesis in cucumber cotyledons. *Can. J. Bot.* 49(12): 2197-2201.

Gadagi, R. 1999. Studies on *Azospirillum* isolates of ornamental plants and their effect on *Gaillardia pulchella* var. picta Fouger. M.Sc. (Ag) Thesis, University of Agricultural Sciences, Dharwad, 72p.

Gadagi, R., Chauhan, M., and Sa, T. 2002. Influence of diazotrophicus *Azospirillum* on growth, flower yield and N-uptake of chrysanthemum. In: *17th World cong. soil sci.* pp.14-21.

Gadagi, R.S., Krishnaraj, P. U. Kulkarni, J.H. and Sa,T. 2004. The effect of combined *Azospirillum* inoculation and nitrogen fertilizer on plant growth promotion and yield response of the blanket flower *Gaillardia pulchella*. *Sci. Hortic.* 100(1): 323-332.

Guo, J., Tang, S., Ju, X., Ding, Y., Liao, S. and Song, N. 2011. Effects of inoculation of a plant growth promoting rhizobacterium *Burkholderia* sp. D54 on plant growth and metal uptake by a hyperaccumulator *Sedum alfredii* Hance grown on multiple metal contaminated soil. *World J. Microbiol. and Biotechnol.* 27(12): 2835-2844.

Handaragall, A. G., Jayanthi, R. and Rajendra, B. N. 2013. Effect of GA3 and foliar nutrients along with biofertilizers on growth and flowering of anthurium (*Anthurium andreanum* Lind) cv. Tropical red. *Asian J. Hortic.* 8(1): 71-74.

Harrison, M. J. and van Buuren, M. L. 1995. A phosphate transporter from the mycorrhizal fungus *Glomus versiforme*. *Nat.* 378(6557): 626p.

Hoda, E. and Mona, S. 2014. Effect of bio and chemical fertilizers on growth and flowering of Petunia hybrida plants. *Am. J. Plant Physiol.* 9: 68–77.

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

Jayamma, N., Jagadeesh, K. S. and Patil, V. S. 2008. Growth and flower yield of jasmine (*Jasminum auriculatum*) as influenced by biofertilizers and graded doses of chemical fertilizers. *J. Ornamental Hortic.* 11(4): 275-280.

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

Kabir, M. I., Mortuza, M. G. and Islam, M. O. 2012. Morpological features, growth and development of *Dendrobium* sp. orchid as influenced by nutrient spray. *J. Environ. Sci. Nat. Resour.* 5(1): 309 – 318.

Kamínek, M., Motyka, V. and Vaňková, R. 1997. Regulation of cytokinin content in plant cells. *Physiol. Plant.* 101: 689–700.

Karishma, Aggarwal. A., Tanwar, A. and Neetu. (2011). Efficacy of bioinoculants plant growth regulators and nutrients in prolonging vase life of *Chrysanthemum indicum* L. *American-Eurasian J. Agric. Environ. Sci.* 11(4): 593-599.

Karishma, Y. K., Tanwar, A. and Aggarwal, A. 2013. Impact of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* with various levels of superphosphate on growth enhancement and flowering response of Gerbera. *J. Ornamental Plants*. 3(3): 161-170.

KAU [Kerala Agricultural University]. 2011. Package of Practices Recommendations: crops. Directorate of Extension, Kerala Agricultural University, Thrissur, 360p.

Khan, F. U., Siddique, M. A. A., Khan, F. A. and Nazki, I. T. 2009. Effect of biofertilizers on growth, flower quality and bulb yield in tulip (*Tulipa gesneriana*). *Indian J. Agric. Sci. 79*(4): 248-251.

Kumar, M. 2015. Flower and corm production in gladiolus (*Gladiolus hybridus* Hort.) cv." Peater pears" as influenced by different nutrients sources. *Ann. Hortic.* 8(1): 99-102.

Kumar, S. 2002. Studies on the effect of VAM and growth regulators on *Gladiolus grandiflorus L. cv. Jessica*. PhD diss., Chaudhary Charan Singh Haryana Agricultural University, Hisar, 198p.

Letham, D. S. 1994. Cytokinins as phytohormones-sites of biosynthesis, translocation and function of translocated cytokinin. *Cytokinins: chemistry, activity and function*, pp. 57-80.

Lin, S. C., Huang, S. N. and Houng, K. H. 1999. Inoculation effect of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth of Asian hybrid lilly. *J. agric. Res. China*. 48: 135 – 142.

Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. *Phytopathol*. 78(3): 366-371.

Liu, A., Wang, B. and Hamel, C. 2004. Arbuscular mycorrhiza colonization and development at suboptimal root zone temperature. *Mycorrhiza*. 14(2): 93-101.

Madhaiyan, M., Krishnan, P. S. and Pragatheswari, D. 2003. Rapid detection and assessment of orchid mycorrhizal colonization in *Vanilla planifolia* Andr. Roots. *Mycorrhiza news*. 14: 10 – 13.

Maillet, F., Poinsot, V., Andre, O., Puech-Pagès, V., Haouy, A., Gueunier, M., Cromer, L., Giraudet, D., Formey, D., Niebel, A. and Martinez, E.A. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nat.* 469(7328): p.58.

Martin, C. A. and Stutz, J. C. 2004. Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L. *Mycorrhiza*. 14(4): 241-244.

Matsumoto, T. K. 2006. Gibberellic acid and benzyl adenine promote early flowering and vegetative growth of *Miltoniopsis* orchid hybrids. *HortScience*. 41(1): 131-135.

Molla, A. H., Shamsuddin, Z. H., and Saud, H. M. 2001. Mechanism of root growth and promotion of nodulation in vegetable soybean by *Azospirillum brasilense*. *Commun. Soil Sci. Plant Anal.* 32: 2177–2187.

Moutia, J. F. Y., Saumtally, S., Spaepen, S. and Vanderleyden, J. 2010. Plant growth promotion by *Azospirillum* sp. in sugarcane is influenced by genotype and drought stress. *Plant and soil*. 337(2): 233-242.

Naik, R. 2014. Growth and physiological response of *Dendrobium* cv. Earsakul in different growing conditions. Ph.D. thesis, Kerala Agricultural University, Thrissur, Kerala, 238p.

Naik, S. K. and Barman, D. 2006. Influence of foliar application of nitrogen on flowering of Cymbidium hybrid. *J. Ornamental Hortic.* 9: 270 – 273.

Naik, S. K., Barman, D. and Medhi, R. P. 2010. Response of Cymbidium 'Pine Clash Moon Venus' to major nutrients at vegetative growth stage. *J. Ornamental Hortic.* 13(3): 182 – 188.

Nair, S. A. and Sujatha, K. 2010. Effect of varying levels of foliar nutrients on round the year production and quality of *Dendrobium* cv. Sonia 17. *J. Ornamental Hortic.* 13(2): 87 – 94.

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

Nandini, V. H. 2000. Influence of nitrogen and hormones on growth and development of *Dendrobium* cv. Sonia 17. MSc thesis, Tamil Nadu Agricultural University, Coimbatore. 117p.

Okon, Y. and Itzigsohn, R. 1995. The development of *Azospirillum* as a commercial inoculant for improving crop yields. *Biotechnol. Advances*. 13(3): 415-424.

Okon, Y., Albrecht, S. L. and Burris, R. H. 1977. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Appl. and Environ. Microbiol*. 33(1): 85-88.

Padmadevi, K., Jawaharlal, M. and Vijayakumar, M. 2004. Effect of biofertilizers on floral characters and vase life of anthurium (*Anthurium andreanum* Lind.) cv. Temptation. *S. Indian Hortic. 52*(6): 228p.

Panchal, R. V., Parekh, N. S., Parmar, A. B., & Patel, H. C. (2010). Effect of biofertilizers and nitrogenous fertilizer on growth, flowering and yield of annual white chrysanthemum (Chrysanthemum coronarium L.) under middle Gujarat agroclimatic conditions. *Asian J. Hortic. 5*(1): 22-25.

Perner, H., Schwarz, D., Bruns, C., Mäder, P. and George, E. 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. *Mycorrhiza*. 17(5): 469-474.

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.

Poole, H.A. and J.G. Seeley. 1978. Nitrogen, potassium and magnesium nutrition of three orchid genera. *J. Amer. Soc. Hortic. Sci*. 103:485–488.

Porra, R. J. 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynth. Res.* 73(3): 149-156.

Poulton, J. L., Bryla, D., Koide, R.T. and Stephenson, A.G. 2002. Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. *New Phytol.* 154: 255–264

Prabhat. K., Raghava, S. P. S. and Misra, R. L. 2003. Effect of biofertilizers on growth and yield of China aster. *J. Ornamental Hortic.* 6(2): 85-88.

Preethi, T. L., Pappiah, C. M. and Anbu, S. 1999. Studies on effect of *Azospirillum* sp., nitrogen and ascorbic acid on the growth and flowering of Edward rose (*Rosa bourboniana* Desp.). *S. Indian Hortic.* 48: 83 – 87.

Qasim, M., Younis, A., Zahir, Z. A., Riaz, A., Raza, H. and Tariq, U. 2014. Microbial inoculation increases the nutrient uptake efficiency for quality production of *Gladiolus grandiflorus*. *Pakist. J. Agric. Sci.* 51(4): 875 - 880.

Rajadurai, K. R., Manivannan, K., Jawaharlal, M. and Beaulah, A. 2000. Effect of *Azospirillum* and VAM on growth characters of African marigold (*Tagetes errecta* L.). *S. Indian Hort.* 48: 83 – 87.

Rajeevan, P. K. and Swapna, P. 2003. Regulation of flower yield and quality in Dendrobium 'Sonia 17'. *J. Orchid Soc. India.* 17: 17 – 26.

Ramachandrudu, K. 2008. Performance of *Dendrobium* orchids under agro-climatic conditions of Goa. *J. Ornamental. Hortic.* 11(3): 232 – 234.

Ravi, G., Mukesh, C. and Tongmin, S. 2002. Influence of diazotrophicus *Azospirillum* on growth, flower yield and N-uptake of chrysanthemum. In: *17th World cong. soil sci.* pp.14-21.

Remans, Roseline, Beebe, S., Blair, M., Manrique, G., Tovar, E., Rao, I. and Croonenborghs, A. 2008. Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). *Plant and soil.* 302(2): 149-161.

Rodríguez-Salazar,J., Suárez, R., Caballero-Mellado, J., and Iturriaga, G. 2009. Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. *FEMS Microbiol. Letters.* 296(1): 52-59.

Sakai, W. S. and Ichihara, K. 2010. N6-Benzyladenine Induced Flowering of Potted Nobile-Type *Dendrobium* Red Emperor 'Prince' Orchid Plants. In: *Int. Orchid Symp.* 878: 317-320.

Sarawgi, S.K., Tiwari, P.K. and Tripathi, R.S. 1999. Uptake and balance sheet of nitrogen and phosphorus in gram (*Cicer arietinum*) as influenced by phosphorus, biofertilizer and micronutrients under rainfed conditions. *Indian J. Agron.* 44(4): 768-772

Scagel, C.F. 2001. Cultivar specific effects of mycorrhizal fungi on rooting of miniature rose cuttings. *J. Environ Hort.* 19: 15 – 20.

Shilev, S., Sanch, E. D. and Benlloch, G. M. 2012. Rhizospheric bacteria alleviate salt – produced stress in sunflower. *J. Environ. Manag.* 95: 37 – 41.

Smith, S. E. and Read, D. J. 1997. Mycorrhizal symbiosis 2. ed. *Academic, London,* 605p.

Sobhana, A. 2000. Improvement of *Dendrobium* through hybridization and *in vitro* mutagenesis. Ph.D thesis, Kerala Agricultural University, Thrissur.

Sohn, B. K, Kim, K. Y., Chung, S. J., Kim, W. S., Park, S. M., Karg, J. G., Rim., Y. S., Cho, J. S., Kim, T. H. and Lee, J. H. 2003. Effect of different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. *Scientia Hort.* 98: 173 – 183.

Srinivasa, V. (2006). Effect of Media on Growth and Flowering of *Anthurium andreanum. Environ. and ecol.* 24(2), 257p.

Sugapriya, S., Mathad, J. C., Patil, A. A., Hedge, R. V., Lingaraju, S. and Biradar, M. S. 2012. Evaluation of *Dendrobium* orchids for growth and yield grown under greenhouse. *Karnataka J. Agric. Sci.* 25(1): 104 – 107.

Sunil, K., Momin, B. C., and Niki, D. 2015. Response of nutrition on growth and flowering of Dendrobium orchids under eastern Himalayan region. *HortFlora Res. Spectrum*, 4(3): 214-219.

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

Tiwari, A. K. and Kumar, V. 2011. Effect of potting medium on Cymbidium species. *Prog. Agric.* 11 (2): 276 – 281.

Uchida, R. 2000. Essential nutrients for plant growth: nutrient functions and deficiency symptoms. *Plant nutrient manag. in Hawaii's soils*: 31-55.

Varshney, A., Sharma, M. P., Adholeya, A., Dhawan, V. and Srivastava, P. S. 2002. Enhanced growth of micropropagated bulblets of *Lilium* sp. inoculated with arbuscular mycorrhizal fungi at different P fertility levels in an alfisol. *The J. Hortic. Sci. and Biotechnol.* 77(3): 258-263.

Verma, S. K., Angadi, S. G., Patil, V. S., Mokashi, A. N., Mathad, J. C. and Mummigatti, U. V. 2012. Growth, yield and quality of chrysanthemum (*Chrysanthemum morifolium* Ramat.) cv. Raja as influenced by integrated nutrient management. *Karnataka J. Agric. Sci.* 24(5): 681 - 683.

Wani, S.P. 1990. Inoculation with associative nitrogen fixing bacteria: role in cereal grain production improvement. *Indian J. Microbiol.* 30: 363–393.

Wen, C.L. and D.C.N. Chang, 1995. Effects of temperature and *Glomus* sp. on the cut flower quality of micropropagated *Gerbera jamesoni*. Memoirs of the College of Agriculture, National Taiwan University, 35: 75-91.

Wróblewska, K. 2013. Benzyl adenine effect on rooting and axillary shoot outgrowth of Gaura lindheimeri Engelm. A. Gray cuttings. *Acta Scientiarum Polonorum. Hortorum Cultus*. 12(3): 127 – 136.

Wu, P. H. and Chang, D. C. 2009. The use of N-6-benzyladenine to regulate flowering of *Phalaenopsis* orchids. *HortTechnol.* 19(1): 200-203.

Wu, P. H. and Chang, D. C. 2012. Cytokinin treatment and flower quality in Phalaenopsis orchids: Comparing N-6-benzyladenine, kinetin and 2-isopentenyl adenine. *Afr. J. Biotechnol.* 11(7): 1592-1596.

Yadav, K., Aggarwal, A. and Singh, N. 2013. Arbuscular mycorrhizal fungi induced acclimatization and growth enhancement of *Glycyrrhiza glabra* L.: a potential medicinal plant. *Agric. Res.* 2(1): 43-47.

Yang, R., Luo, C., Chen, Y., Wang, G., Xu, Y. and Shen, Z. 2013. Copper-resistant bacteria enhance plant growth and copper phytoextraction. *Int. J. Siol. Biol.* 46: 49 – 54.

Zhang R, Zhang X, Wang J, Letham DS, Mckinney SA, Higgins T.J.V. 1995. The effect of auxin on cytokinin levels and metabolism in transgenic tobacco tissues expressing an *ipt* gene. *Planta.* 196(1): 84–94.

Appendices

APPENDICES

Appendix No. 1. Meteorological data during the period of research from September 2016 to July 2017

Months	Temperature (°C)		Relative Humidity (%)	Rainfall (mm)	Rainy days	Sunshine hours	Mean sunshine hours
	Mean Max	Mean Min					
September	30.3	23.6	82	086.0	10	144.5	4.8
October	31.5	22.7	81	037.3	4	170.3	5.5
November	32.9	22.2	69	013.8	1	174.7	5.8
December	32.4	22.3	69	0529	3	200.7	6.5
January	34.1	22.9	53	0.0	0	235.2	7.6
February	36.0	23.2	51	0.0	0	243.0	8.7
March	36.1	24.7	67	13.2	1	229.9	7.4
April	39.7	23.0	70	13.2	1	194.4	6.5
May	38.3	22.4	72	167.5	11	170.0	5.5
June	33.0	21.2	87	630.2	25	58.9	2.0
July	32.9	21.0	85	385.5	22	89.4	2.9

REFINEMENT OF NUTRIENT MANAGEMENT PRACTICES IN *Dendrobium* ORCHIDS

By

SHILPA P.

(2015-12-010)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

requirement for the degree of

Master of Science in Agriculture

(FLORICULTURE AND LANDSCAPING)

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR- 680656

KERALA, INDIA

2017

Abstract

A study entitled 'Refinement of nutrient management practices in *Dendrobium* orchids' was carried out at Department of Floriculture and Landscaping, College of Horticulture, Vellanikkara from August 2016 to July 2017. The objective of the study was to evaluate the influence of different bioinoculants and growth regulators on growth and yield and to refine the existing nutrient management practices of *Dendrobium* orchids. The experiment consisted of ten different treatments which included different combinations of three bioinoculants *viz.,* Arbuscular Mycorrhizal Fungi (AMF), *Azospirillum* and PGPR Mix-I and three levels of benzyl adenine (BA) *viz.,* 50, 100 and 150 ppm all super imposed on existing KAU package of practice (POP) recommendations for orchids. Six months old tissue cultured plants of *Dendrobium* variety 'Yellow Splash' were used for the study. Observations regarding the morphophysiological characters, yield, postharvest aspects and nutrient uptake were recorded.

Significant variation was observed among the treatments regarding the vegetative characters. Plant height was maximum in plants treated with POP + AMF + BA 100 ppm (T_5),whereas treatment T_9 (POP + *Azospirillum* + BA 150 ppm) was found superior in terms of other vegetative parameters like plant spread, leaf length and number of pseudobulbs per plant. Maximum leaf breadth and leaf area were observed under the treatment T_8 (POP + *Azospirillum* + BA 100 ppm). No significant variation could be observed among the treatments in terms of number of leaves and internodal length.

Among the treatments, early flower emergence was observed under the treatment T_8 (POP + *Azospirillum* + BA 100 ppm). Treatment T_3 (POP + PGPR Mix-I + BA 150 ppm) recorded minimum number of days for the opening of first flower. Maximum field life of the spike was observed in plants under the treatment T_6 (POP + AMF + BA 150 ppm).The treatments consisting of AMF and *Azospirillum* with different levels of bioinoculants were superior in terms of length of the spike, stalk length, number of flowers per spike and flower size and the best performance was observed under the treatment T_6 (POP + AMF + BA 150 ppm). Among the postharvest characters the treatment T_6 (POP + AMF + BA 150 ppm) showed superiority in vase life as well as fresh weight of the spike. Treatment T_9 (POP + *Azospirillum* + BA 150 ppm) exhibited highest value in terms of root parameters namely, number of roots per plant and root volume whereas T_5 (POP + AMF + BA 100 ppm) was

superior in terms of root length. Regarding the nutrient content in the tissues, treatment T_5 was having maximum content of N and P whereas highest content of K, Ca, Mg and S were observed with T_3 (POP + PGPR Mix-I + BA 150 ppm). Uptake of N was highest under the treatment T_8 (POP + *Azospirillum* + BA100ppm) while P uptake was found highest in T_5 (POP + AMF + BA 100 ppm). Plants under the treatment T_3 exhibited the maximum uptake of all other nutrients *viz.*, K, Ca, Mg and S.

Chlorophyll a, b and total chlorophyll content were significantly higher in plants treated with AMF, 100 ppm BA along with POP (T_5). The treatment combination of *Azospirillum* along with 100 ppm BA exhibited highest stomatal density (T_8) and regarding stomatal index, there was no significant difference observed among the treatments.

From the present study it could be observed that the treatments consisting of AMF as well as *Azospirillum* in combination with 100-150 ppm BA significantly improved both vegetative as well as floral characters in *Dendrobium*. The treatment POP + AMF +150 ppm BA can be recommended for enhancing the growth and yield of *Dendrobium* orchids.