EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON GROWTH AND YIELD OF BITTER GOURD (*Momordica charantia* L.)

By

Naveen Kumar, K.S.

THESIS

Submitted in partial fulfilment of the Requirement for the degree of

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Faculty of Agriculture
Kerala Agricultural University

Department of Olericulture
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA.
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DECLARATION

I, hereby declare that this thesis entitled **"Effect of Plant Growth Promoting Rhizobacteria (PGPR) on growth and yield of bitter gourd *(Momordica charantia* L.*)*"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
23-07-07

Naveen Kumar K.S.
(2005-12-110)

CERTIFICATE

Certified that this thesis, entitled **"Effect of Plant Growth Promoting Rhizobacteria (PGPR) on growth and yield of bitter gourd *(Momordica charantia* L.)"** is a record of research work done by Mr. Naveen Kumar, K.S. 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 him.

Date: 29.09.07
Place: Velaanikkara

Dr. P.G. Sadhan Kumar
Chairman, Advisory Committee
Professor and PI (AICVIP)
Department of Olericulture,
College of Horticulture,
Vellanikkara.

CERTIFICATE

We, the undersigned members of the advisory committee of **Mr. Naveen Kumar K.S.** a candidate for the degree of **Master of Science in Horticulture**, with major field in Olericulture, agree that the thesis entitled **"Effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of bitter gourd (*Momordica charantia* L.)"** may be submitted by **Ms. Naveen Kumar K.S,** in partial fulfillment of the requirement for the degree.

Dr. P.G. Sadhan Kumar
Professor and PI (AICVIP)
Department of Olericulture
College of Horticulture
Vellanikkara.
(Major Advisor, Advisory Committee)

Dr. T.E.George
Professor and Head
Department of Olericulture
College of Horticulture
Vellanikkara.
(Member)

Dr. Sally K Mathew
Professor
Department of Plant Pathology
College of Horticulture
Vellanikkara
(Member)

Mr. S. Krishnan
Assistant professor (Sr.Scale)
Department of Agri. Statistics
College of Horticulture
Vellanikkara
(Member)

(EXTERNAL EXAMINER)

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Introduction

I. INTRODUCTION

Vegetables are rich source of vitamins, proteins, carbohydrates and minerals, which constitute an important component in human nutrition. Vegetables play an important role in human diet as providers of wide range of nutrients that supply energy, promote growth and sustain the metabolic functions essential for life. Besides the nutritional value of vegetables, increased interest is being bestowed on the functional and therapeutic benefits of vegetables in human health.

Among the various vegetables, cucurbits are the largest group of summer vegetables grown all over India. Cucurbit vegetables are fair source of thiamine and riboflavin. Bitter gourd is the leading vegetable crop of Kerala. The higher yield and maximum returns make it the most preferred vegetable crop of Kerala farmers.

Agriculture is highly dependent on the use of chemical fertilizers, growth regulators, fungicides and pesticides for obtaining increased yield. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecology, nutrient recycling and destruction of biological communities that otherwise support crop production.

The use of bioresources to replace these chemicals is gaining importance. In this context, plant growth promoting rhizobacteria (PGPR) are often considered as novel and potential tool to provide substantial benefits to agriculture. In recent years due emphasis has been paid towards the use of beneficial microorganisms harboring rhizospere as bioinputs. During the past one decade, scientists are looking back at the rhizospere system and have identified PGPR, which have a great potential and promise for promoting plant growth and improving soil health (Tilak *et al.,* 2003).

PGPR are free living bacteria that have the ability to improve plant growth through suppression of deleterious root colonizing microorganisms and by production of plant growth regulators (Kloepper and Schroth, 1981). PGPR are present in large number on the root surface where the plants exudates and lyrates provide nutrients (Nelson, 2004).

In India, beneficial effects of PGPR have been reported by various workers on a wide range of crops including cereals, pulses, vegetables and oil seeds and have been comprehensively reviewed. The beneficial response of crops to inoculation with these organisms is attributed to better seed germination and seedling emergence, improved nutrition, and reduction in disease incidence and increased crop production. However, such comprehensive studies on the influence of PGPR are at present lacking in bitter gourd. Hence, the present study was undertaken with the following objectives.

- To test the effect of PGPR on growth and growth parameters of bitter gourd.
- To study the effect of PGPR on yield and yield attributes of bitter gourd.
- To assess the influence of PGPR on quality and quality attributes of bitter gourd.

Review of Literature

II. REVIEW OF LITERATURE

There are several beneficial microorganisms in the rhizosphere, which can improve soil quality, enhance plant growth and conserve natural resources for sustainable agricultural production. Among the beneficial microorganisms, plant growth promoting rhizobacteria (PGPR) are major groups of soil microorganisms, which have a potential use in plant growth enhancement and biocontrol of diseases.

2.1 Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (PGPR) are free-living bacteria that have beneficial effect on plants by root colonization which stimulate the growth (Kloepper and Schroth, 1981). Intensive research work has been carried out on use of various PGPR as plant growth promoters on various crops. There are several reports which show that PGPR promoted the growth and reproductive parameters of plants ranging from cereals, pulses, ornamentals, vegetable crops, plantation crops and even in tree species. The important PGPR are *Azospirillum, Pseudomonas fluorescens*, phosphate solubilising bacteria (PSB) and *Bacillus subtilis.*

The literature on the effect of inoculation of *Azospirillum Spp., Pseudomonas fluorescens*, phosphate solubilising bacteria (PSB) and *Bacillus subtilis* on growth, yield, shelf life and quality attributes is being reviewed with emphasis on vegetable crops.

2.2 Effect of PGPR on growth and growth attributes

Vanpeer and Schipper (1988) observed that the increased root and shoot fresh weight of tomato, cucumber, lettuce and potato were due to of bacterization with *Pseudomonas* strains. Plant height, number of primary, secondary and tertiary branches and days to 50 per cent flowering of chilli were significantly increased due to the inoculation of *Azospirillum* to seed, soil and seedling (Amirthalingam, 1988).

Plant height, shoot girth and fruit yield of bhendi were significantly improved due to soil application of *Azospirillum* at 2.5 kg/ha (Parvatam *et al.,* 1989). Highest root length and plant height were recorded for bhendi with 50% recommended dose of nitrogen + *Azospirillum* (2 kg/ha) (Sankaranarayanan *et al.,* 1995).

Verma *et al.* (1997) studied the effect of *Azospirillum* and *Azotobacter* cultures on cabbage and results showed significantly higher values for the growth parameters like number of outer leaves and head volume. The *Azospirillum* was alone significant for all the parameters except number of outer leaves. The interaction effects of both the cultures were found to be non significant for all the traits except head volume of cabbage.

Chattoo *et al.* (1997) reported that, in knolkhol, both *Azospirillum* and *Azotobacter* resulted in a non significant increase in leaf number per plant when compared with control during rabi season. *Azospirillum* increased the leaf area significantly over control only in rabi season and weight per plant in both rabi and kharif season. The increase in growth attributes could be because of certain growth promoting substances secreted by the inoculated microbes which in turn might have lead to better root development, transportation of water uptake and deposition of nutrients.

Selvi *et al.* (1997) reported foliar (inceptisol) or soil (alfisol) application of micronutrients to okra, with or without composted coir pith (CCP) and *Azospirillum* increased the dry matter yield at all stages of growth (vegetative, flowering and harvest) when compared with the other treatments.

The number of leaves and dry weight of seedlings were maximum in chilli with the inoculation of *Azospirillum* (2 kg/ha). Single or combined application of *Azospirillum* with *Azotobacter* resulted in increased growth of tomato seedlings (Barakart and Gabr, 1998).

Bambal *et al.* (1998) studied the effect of different treatments of two PGPR (*Azospirillum* and *Azotobacter* alone and in combination) and three nitrogen rates (100%, 75% and 50%) on cauliflower. N was applied as urea. *Azospirillum* + *Azotobacter* + 100% N resulted in the highest chlorophyll content (1.48 mg/g) and leaf area (634.5 cm2/plant) when compared with no PGPR application.

The three ways mixture of PGPR strains *Bacillus subtilis*, *Bacillus pumilis* and *Curtobacterium flaccumfaciens* as a seed treatment showed intensive plant growth promotion in tomato (Raupach and Kloepper, 1998).

Thilakavaty and Ramaswamy (1999) reported that, in onion, significant improvement in growth parameters like plant height, number of leaves, root length and neck girth were recorded in the treatment NPK 45:45:30 kg/ha with inoculation of *Azospirillum* and phosphate solubilising bacteria (PSB) when compared over plants applied with inorganic fertilizers alone.

Nirmala and Vadivel (1999) observed that in cucumber, application of neem cake at 0.25 kg/plant, *Azospirillum* at 2 kg/ha and phosphobacteria 2 kg/ha gave the longest vine (330 cm), tender fruit (22 cm), highest number of leaves per plant (170), leaf area (29396 cm^2), dry matter production (335 g/plant) and the minimum number of days to appearance of first female flower (28 days).

The combined application of farmyard manure (12.5 t/ha) and *Azospirillum* + phosphobacteria (2 kg/ha) with inorganic fertilizers (recommended dose of N, P and 100% of K) favourably influenced the growth parameters of brinjal with a maximum yield of 36.48 t/ha (Nanthakumar and Veeragavathatham, 2000).

Ganeshe *et al.* (2000) found that *Azospirillum* and *Azotobacter* were not effective on okra at 0 N level but were superior to the control when applied with 20 and 40 kg N/ha. Nitrogen at 20 and 40 kg/ha was equally effective with *Azospirillum* and *Azotobacter*.

Naidu *et al.* (2000) reported that in okra, the plant height, number of leaves per plant, number of nodes per plant, internodal length and number of fruits per plant were maximum under 35 tonnes FYM + *Azospirillum* + phosphorus solubilizing bacteria (PSB).

At 27 and 70 days after seeding, there were significant differences between treatments for number of leaves, shoot height, fresh shoot weight, root length, and fresh root weight. These growth variables were greatest with the 2.5% carrier control and treatment *Bacillus subtilis* (LS257). The untreated control had significantly lower values for the measured growth variables than all other treatments (Bonnie, *et al.*, 2000).

Gulati *et al.* (2001) reported that in cowpea, highest number of nodules (18-29) and nodule weight were recorded by the inoculation of both PGPR and *Rhizobium* followed by PGPR alone.

Anburani and Manivannan (2002) observed that in brinjal, farm yard manure + poultry manure along with NPK and *Azospirillum* + phosphate solubilising bacteria (PSB) recorded the highest plant height, number of primary branches and number of leaves. However, farm yard manure along with NPK and *Azospirillum* + phosphate solubilising bacteria (PSB) recorded the highest values for stem girth, number of secondary branches and leaf area. These treatments also recorded the lowest number of days for appearance of first flower, days for 50% flowering and highest number of flowers.

At the time of harvest, tomato plants inoculated with *Pseudomonas fluorescens* showed maximum height when compared with uninoculated plants. Maximum root length, shoot and fruit dry weight were observed in plants inoculated with *Pseudomonas fluorescens* + *Azospirillum* + *Glomus mosseae* (Muthuraju *et al.*, 2002).

Application of organic manures with and without inoculation of *Azospirillum* significantly increased the growth and vigour of cabbage plants over application of inorganic fertilizers alone. The treatment with 50% N (urea) + 50% N (vermi compost) + *Azospirillum* showed maximum growth in terms of increase in plant height, number of leaves and dry matter production (Shalini *et al.*, 2002a).

Sharma (2002) studied the beneficial effect of *Azospirillum* and *Azotobacter* on growth parameters of cabbage and reported significant increase in the number and weight of non wrapper leaves per plant over without inoculation. Significantly higher length and width of the cabbage head were obtained with *Azospirillum* inoculation. However, maximum values were obtained with *Azospirillum* inoculation.

The bacterial treatments were highly effective in increasing the seedling length and root length of okra. The highest length was noticed in phosphate solubilising bacteria (PSB) + *Azospirillum* and the same inoculants recorded highest vigour index of the seedlings (Sajindranath *et al.*, 2002).

Naidu *et al.* (2002) reported that in brinjal, NPK at 100:60:50 kg/ha + farmyard manure at 25 t/ha recorded the highest values for plant height, number of leaves and primary branches per plant. But, no effect of *Azospirillum* and phosphate solubilising bacteria (PSB) was noticed.

Seed treatment of watermelon with *Pseudomonas fluorescens* showed better germination and plant vigour compared to untreated plants (Nallathambi *et al.,* 2003).

Kokalis-Burelle, *et al.* (2003) reported that *Bacillus subtilis* significantly increased shoot weight and stem diameter of muskmelon and watermelon seedlings as well as transplants.

Singh and Khare (2003) reported that the cabbage plants inoculated with *Azospirillum* and *Azotobacter* along with the 75% recommended doses of nitrogen (135 kg/ha) resulted in significant increase in the plant height, number of unfolded leaves, leaf area and leaf area index (LAI) when compared with the plants with inorganic fertilizers alone.

Application of *Azospirillum* and PSB at 2 kg/ha with the 50 percent recommended dose of fertilizers and vemicompost at 2 t/ha increased the vine length, earliness in flowering, yield and yield components. Beneficial response of crops to inoculation with *Azospirillum* was attributed to better seed germination and seedling emergence, improved nitrogen nutrition and increased crop production (Tilak *et al.*, 2003).

Azospirillum + phosphorus solubilizing bacteria (PSB) inoculation in okra showed that the number of nodes per plant, number of leaves, leaf area, fresh and dry weight of the plant increased significantly with the increase in fertilizer. Maximum leaf area was found at 75% and full dose of recommended fertilizers dose (Prabu *et al.,* 2003).

Nuruzzaman *et al.* (2003) reported that the morpho-physiological characters of okra could be modified by the application of PGPR + cowdung. However, PGPR + cowdung treatments were comparable to treatment with 60% nitrogen. This suggests that *Azotobacter* + cowdung 5 t/ha, or *Azotobacter* +

Azospirillum + cowdung 5 t/ha or *Azospirillum* + cowdung 5 t/ha were more beneficial in eco friendly okra cultivation and may be used as an alternative of inorganic N by saving cost of production and sustaining productivity.

Badawy *et al.* (2003) studied the effect of *Azospirillum lipoferum* and *Bacillus polymyxa* [*Paenibacillus polymyxa*] and revealed that significantly increased in shoot and root fresh and dry weights of tomato plants.

Prabu *et al.* (2003) reported that phytohormones produced by the *Azospirillum* stimulated the root growth and induced changes in root morphology, which in turns improved the assimilation of nutrients and yield in okra.

Assessment of growth by the PGPR (*Bacillus subtilis* stains SE34 and IN937b) isolates under both green house and field conditions revealed enhancement in emergence rate, germination percentage, plant height, fresh weight and dry weight of the chilli, brinjal and okra plants (Amruthesh *et al.*, 2003).

Pardeep and Sharma (2004a) observed that in tomato, the maximum plant height was obtained when plants were supplied with FYM 25 t/ha + SSP at 469 kg/ha and PSB. The inoculation of bacterium *Bacillus licheniformis* significantly increased the height of plants and the leaf area of both tomato and pepper and the effects were greater on pepper than on tomato (Lucas-Garcia *et al.*, 2004).

Wange and Kale (2004a) reported that in brinjal, the parameters viz., plant height (44.8 cm), number of leaves per plant (43) and shoot dry weight (30.8 g) were significantly highest in the seedling inoculated with mixed culture of *Azospirillum* and *Azotobacter* followed by the application of 75 kg N/ha .

Navale and Wani (2004) showed that the onion plants inoculated with *Azospirillum* resulted significantly higher dry weight of shoot and bulb (4.55 g and 6.19 g/plant respectively) than uninoculation.

Bahadur *et al.* (2004) revealed that in cabbage significantly higher fresh weight of outer leaves was recorded under press mud + PSB, Digested sludge + PSB and digested sludge + *Azospirillum*. Where as significant improvement in number of inner leaves and head weight was observed when press mud was applied in soil and seedlings were inoculated with phosphate solubilising bacteria (PSB).

In lettuce, maximum number of leaves (44), average leaf area (200.6 cm^2), fresh shoot weight (125 g), dry matter weight (10.6 g) and root length (10.7 cm) per plant were recorded in plants inoculated with *Azospirillum* + *Azatobacter* + N at150 kg/ha when compared with plants applied with N at 50 kg/ha alone (Wange and Kale, 2004b).

Singh and Singh (2005) reported that in cauliflower highest plant height (63.93 cm), number of leaves per plant (24.22) and gross weight of the plant with out root (3.50 kg) were significantly superior in plants inoculated with *Azospirillum* + recommended dose of NPK at 120:60:60 kg/ha. Combined inoculation of *Pseudomonas fluorescens* and *Azospirillum* recorded maximum value of plant height, number of branches, canopy spared and dry matter production when compared with uninoculated plants.

Jha *et al.* (2006) reported that number of leaves per plant, plant height, shoot thickness, yield and bulb characteristics in onion showed a significant increase with the application of half the recommended dose of nitrogen and phosphorous along with PGPR and VAM. The better PGPR strains were *Azospirillum, Pseudomonas fluorescens* and *Azotobacter*.

Nirmala *et al.* (2006) observed significantly higher plant height, fresh shoot and root weight when the pea crop grown under the combined application of press mud as seed application at 200g per 10 kg seeds and soil inoculation of PSB at 5 kg per hectare than uninoculated plants.

Prabu *et al.* (2006) revealed that there were significant difference on vine length due to application of different levels of organic manures, inorganic fertilizers and biofertilizers. Application of 50% recommended dose of fertilizers (RDF) + vermicompost at 2t/ha + biofertilizers recorded the maximum vine length in cucumber and the same treatment recorded earliness in flowering. This might be due to better nutritional status of the plants.

2.3 Effect of PGPR on yield and yield attributes

The *Azospirillum* and *Azotobacter* inoculation in cabbage significantly increased the head volume, head weight and yield/ha when compared with the uninoculated plants (Verma *et al.*, 1997).

de Freitas *et al.* (1997) reported that the canola plants inoculated with *Bacillus thurengensis* increased the number and weight of pods and seed yield without rock phosphate.

Ganeshe *et al.* (1998) reported that nitrogen 40 kg/ha in combination with soil inoculation of *Azospirillum* gave a yield (56.33 q/ha) that was statistically equivalent to that obtained with 80 kg nitrogen/ha (56.78 q/ha), and also gave similar fruit nutritional quality in okra.

Yield related attributes of knolkhol showed a favourable response to bacterial inoculation. Inoculation of *Azospirillum* showed significantly increase in bulb weight, bulb volume, bulb diameter and yield in both rabi and kharif season than uninoculated plants. The increase in yield and yield related attributes may be due to better root proliferation, nutrients and water uptake, higher leaf number and leaf area (Chattoo *et al.*, 1997).

Single or combined application of *Azospirillum* with *Azotobacter* resulted in an increase in the total fruit yield (Barakart and Gabr, 1998).

Bambal *et al.* (1998) reported that *Azospirillum* + *Azotobacter* + 100% N resulted in the highest yield (29.64 t/ha) and early maturity of curds in cauliflower when compared with the other treatments. Inoculation of PSB and *Azotobacter* to seed was more useful than soil application and it gave 8.2% higher curd yield over soil inoculation. When both bacteria's inoculated together, higher curd yield (11.4%) was noticed than inoculated separately.

Thilakavaty and Ramaswamy (1999) reported that the application of *Azospirillum* + PSB in combination with 45:45:30 kg NPK/ha was much more remarkable as compared with the application of recommended dose (60:60:30 kg NPK/ha) alone. Inoculation of *Azospirillum* and PSB to the seed bulb of onion and the soil inoculation gave an increased yield of 18.3 per cent.

Nirmala and Vadivel (1999) observed that in cucumber application of neem cake at 0.25 kg/plant, *Azospirillum* at 2 kg/ha and phosphobacteria 2 kg/ha recorded minimum day to appearance of first female flower (28 days), more girth of the tender fruit (18 cm), highest tender fruit yield per vine (2053 g) and increased dry matter production (335 g/plant).

Nanthakumar and Veeraragavathatham (1999) observed that the brinjal plants treated with a combined application of organic fertilizers, *Azospirillum*, PSB and inorganic NPK had increased the number of flowers per plant, plant yield and fruit weight when compared with the crops treated with inorganic fertilizers alone.

There were significant differences between treatments in percentage of plants blooming at 71, 74, 77, and 81 days after seeding. The 2.5% carrier *Bacillus subtilis* treatments LS255, LS256, and LS257 had the highest percentages of flowering plants on the first four evaluation dates than control. On day 83, there were no significant differences between treatments and controls for percentage of plants blooming (Bonnie, *et al.,* 2000).

Ganeshe *et al.* (2000) reported that in okra the fruit yield of (56.78 q/ha) was obtained with the application of recommended N rate (40 kg N/ha), followed by *Azospirillum* + 40 kg N/ha (56.33 q/ha) when compared with control. Same treatment gave the highest net return (Rs 16 293/ha) and cost:benefit ratio (2.37).

Naidu *et al.* (2000) reported that in okra the number of fruits per plant, weight of fruits per plant and fruit yield were maximum in treatment 35 tonnes FYM + *Azospirillum* + PSB.

Gaikwad and Wani (2001) reported that the inoculation of PSB significantly increased the plant dry matter, N and P uptake and yield of brinjal over uninoculated control. Inoculants either with single superphosphate or rock phosphate increased the fruit yield of brinjal in the range of 3.00 to 39.25% over uninoculated control.

Nanthakumar and Veeraragavathatham (2001) reported that the yield of brinjal plant significantly increased due to application of FYM, *Azospirillum* and phosphobacteria (*Bacillus sp.*) in addition with NPK when compared with the application of inorganic fertilizers alone.

Bahadur and Manohar (2001) reported that in okra, higher number of pods per plant and average fruit weight than control was noticed in plants, which was inoculated with *Azospirillum* and supplied with 75% recommended dose of nitrogen. Similarly, *Azospirillum* + 50% recommended dose of nitrogen exhibited a highly significant increase in pod yield per plant and pod yield per hectare. These treatments recorded 15% and 14% more yield per plant respectively over control.

The okra fruit yield was significantly higher for *Azospirillum* inoculation (141.94 q/ha) than control (129.74 q/ha). Increase in yield by *Azospirillum* inoculation had a cumulative effect of various positive roles played by *Azospirillum* (Asha Raj and Geetha Kumari, 2001).

Baronia (2001) reported that the maximum green pod yield/ha in the french bean plants was noticed with PSB and *Rhizobium* along with the nitrogen 20 kg/ha + phosphorous 80 kg/ha (132 q/ha) followed by the treatments inoculated with same bacteria's with nitrogen 10 kg/ha and phosphorous 40 kg/ha (122.50 q/ha).

Kamali *et al.* (2002) reported that both *Azospirillum* and *Azotobacter* enhance dry matter content of okra plants significantly over control at all growth stages. However, *Azospirillum* proved superior to *Azotobacter*, increase in dry matter content could be due to more leaf number, more photosynthetic area and better accumulation of food deposits in the plant.

Devi *et al.*, (2002) reported that the greatest fruit girth, fruit weight, number of fruits per plant, and fruit yield were recorded for 50% N+25% poultry manure, *Azospirillum brasilense* and *Bacillus polymyxa*.

The maximum yield of fresh tomato fruit weight was recorded in plants treated with *Pseudomonas flourescens* + *Azospirillum* + *Glomus mosseae* (Muthuraju *et al.*, 2002).

Application of organic manures with and without inoculation of *Azospirillum* significantly increased the growth and vigour of cabbage plants over application of inorganic fertilizers alone. The treatment with 50% N (Urea) + 50% vermin compost + *Azospirillum* showed maximum growth in terms of increase in plant height, number of leaves and dry matter production (Shalini *et al.,* 2002a)

Prabu *et al.* (2002) observed that the okra plants inoculated with *Azospirillum* + VAM along with the NPK and FYM produced highest yield with highest dry matter, highest organic carbon, highest NPK content in soil and highest NPK content in plants and fruit when compared with uninoculated plants.

Anburani and Manivannan (2002) reported that FYM at 25 t/ha along with 100% NPK and *Azospirillum* + Phosphate solubilising bacteria (PSB) recorded the highest fruit set percentage (65%), number of fruits (26.64), fruit yield per plot (62.92 kg) and estimated fruit yield (31.67 t/ha).

Bhagavantagoudra and Rokhade (2002) reported that in cabbage, application of *Azospirillum* through soil + seedling dipping along with 100% nitrogen recorded the highest C:B ratio of 4.29 followed by inoculation of *Azospirillum* through soil + seedling dipping along with 75% nitrogen (4.05). Whereas, least C:B ratio was found in 50% nitrogen without application of *Azospirillum* (2.67). Among the methods, *Azospirillum* application through soil + seedling dipping was the best. However, in terms of N level, 100 and 75% nitrogen were at par and superior to 50% nitrogen. Application of *Azospirillum* through soil + seedling dipping along with 100% nitrogen recorded 31.75% higher yield over the recommended dose of fertilizer without the application of *Azospirillum*.

Naidu *et al.* (2002) reveled that in brinjal NPK at 100:60:50 kg/ha + farmyard manure at 25 t/ha recorded the highest values for fruit length, fruit girth, number of fruits per plant and fruit yield with no effect of *Azospirillum* and PSB.

The highest fruit yield (54.32 t/ha) in tomato was recorded upon the treatment with recommended dose of FYM and NPK with *Azospirillum* and lowest yield (30.13 t/ha) was recorded upon treatment with FYM alone (Harikrishna *et al.,* 2002).

Sengupta *et al.* (2002) reported that inoculation with *Azospirillum* improved the crop growth and yield compared to control treatments in tomato.

Sharma (2002) observed that the gross and net weight of cabbage head per plant and yield per hectare were significantly enhanced by inoculation of *Azospirillum* over no bacterial inoculation. A treatment combination of *Azospirillum* inoculated with 60 kg N/ha resulted in maximum yield per hectare.

The combined application of two third recommended dose of fertilizer, FYM and *Azospirillum* + VAM produced maximum yield with highest dry matter, highest organic carbon and NPK content in the soil and highest NPK content in plant and fruits of okra (Prabu *et al.,* 2002).

A treatment combination of *Azospirillum* + 60 kg N/ha recorded the highest head weight per plant, head weight per ha and yield per ha, with a benefit:cost ratio of 2.9 in cabbage. The treatment with 50% N (urea) + 50% N (vermicompost) + *Azospirillum* showed the maximum yield (37 tonnes/ha) (Sharma, 2002).

Application of 75% nitrogen of recommended dose (135kg/ha) in combination with *Azospirillum* + *Azotobacter* inoculation significantly increased the yield attributes like weight of head, diameter of head and number of folded leaves and yield of cabbage (Singh and Khare, 2003).

Phosphate-dissolving bacterial (PDB), sawdust compost and N application significantly enhanced shoot dry weight, fruit fresh and dry weights, fruit yield, and N and P uptake. Thus, sawdust compost and acidic N fertilizer application, along with PDB inoculation, can enhance okra yield and nutrient uptake in calcareous soil (Abou-Hadid and Sawan, 2003).

Among the plant growth promoting rhizobacteria, *Bacillus pumilus* (SE-34), *B. pasteurii* (T4), *B. subtilis (IN937*-b) and *B. subtilis* (GBO3) strains significantly improved the okra crop both under greenhouse and field conditions. The potential strains increased the biomass of plants, total number of leaves, fruits, mean length, girth and biomass of the fruits. The total number of seeds per fruit and 1000-seed weight was also enhanced (Mashooda-Begum, 2003).

Raut *et al.* (2003) reported that in tomato, the maximum number of fruits per plant (20.96) was recorded with 20 tonnes poultry manure + 5 kg *Azospirillum* + 5kg PSB per hectare.

Wange and Kale (2003) noticed that the treatment of 75 kg N/ha + *Azotobacter* + *Azospirillum*, significantly improved the shoot dry weight, fruit number, fruit weight per plant as well as yields of both okra and bitter gourd crops over the recommended dose of inorganic nitrogen alone. The next best treatments were 75 kg N/ha + *Azospirillum* and 75 kg N/ha + *Azotobacter*.

SuYing *et al.* (2003) compared with conventional fertilizer application, 98% of the trials on biofertilizer application was documented to increase crop yield, from which 87.4% showed positive results of more than 5% and 56.6% showed positive results of more than 10%. Average yield increase was also found to vary with different types of biofertilizers.

Devi *et al.* (2003) reported that the cabbage yield was highest (55.82 t/ha) with the application of 50% recommended N + 25% poultry manure + *Azospirillum brasilense or Azotobacter chroococcum*, whereas the highest benefit cost ratio (4.30) was recorded with the application of 75% N + biofertilizers.

Omar and El-Kattan (2003) reported that *Bacillus polymyxa* inoculation had positive effect on enzymatic activities under cucumber plants and positive and significant on cucumber yield (variety Pasandra). The increase reached about 25% over the uninoculated control.

Swani *et al.* (2003) showed that the integrated treatments had better crop growth, higher nutrient uptake and fresh fruit yield as compared to fertilizer alone. The yield due to fertilizer N (50%) integrated with *Azospirillum* and *Azotobacter* were statistically at par with 100% fertilizer N alone. However, integration of *Azospirillum* and *Azotobacter* with higher levels of fertilizer N (75 and 100%) doses yielded significantly higher yields (76.7 and 87.0 q/ ha) of okra crop. The highest yield was obtained in plots with full dose of RDF and 75% RDF. *Azospirillum* + PSB significantly increased the yield. *Azospirillum* + VAM were statistically superior over *Azospirillum* + PSB and both being superior to the control.

Wange and Kale (2004b) observed that in lettuce significantly highest yield (12.7 t/ha) was recorded in the plants inoculated with *Azospirillum* + *Azotobacter* + 150 kg nitrogen/ha when compared in plants applied with nitrogen at150 kg/ha alone.

Bahadur *et al.* (2004) reported that the significant improvement in head weight, head length, head diameter and number of outer leaves were noticed in cabbage plants supplied with digested sludge and seedlings inoculated with PSB.

Selvi *et al.* (2004) reported that in okra the increase in pod yield due to the combined application of microfood (MF), composted coir pith (CCP) and *Azospirillum* ranged from 35 to 38% over the application of NPK alone. Highest pod yield (20.67 t/ha) was recorded for NPK + foliar MF + CCP + *Azospirillum*. The application of foliar (MF) and foliar straight micro nutrients (SMNS) gave the highest benefit cost ratio (29.0 and 27.3, respectively).

The integrated use of organic and inorganic sources of nutrients, *Azospirillum* and PSB increased the N, P and K concentrations in the plants (including fruits) of okra, pea and tomato. The integrated nutrient management also significantly increased shoot dry matter yield of tomato and fruit yields of okra and tomato (Singh, *et al.*, 2004).

Lucas-Garcia *et al.* (2004) reported that the inoculation of bacterium *Bacillus licheniformis* resulted in significantly increased number and diameter of tomato fruits in both sand and hydroponic medium.

Navale and Wani (2004) reported that the combined inoculation of *Azospirillum* + *Glomus* both for diameter and yield of onion (5.93 cm and 29.28 MT/ha respectively) were found significantly superior to all inoculation treatments.

Singh and Singh (2005) revealed that the yield and yield attributes of cauliflower showed favourable response to microbial inoculation. The maximum average weight of curd (1.10 kg) and curd yield (28.2 t/ha) was recorded with the inoculation of *Azospirillum* + recommended dose of NPK @ 120:60:60 kg/ha, which was significantly superior over control. The increase in yield might be due to active and rapid multiplication of bacteria, hormone production and supply of anti bacterial and anti fungal compounds of which was unfavourable for growth and yield.

Narayanamma *et al.* (2005) observed in cauliflower the curd diameter, curd height and curd yield were significantly increased with inoculation of *Azospirillum* + 75% N + 100% PK or PSB + 75% P + 100% NK over control. Bacterial inoculation in combination with inorganic fertilizers was efficient in yield increase over the exclusive application chemical fertilizers and can be attributed to increase in uptake of nutrients resulting in faster synthesis and translocation of photosynthetic from source (leaves) to sink (curd).

Mahanthesh *et al.* (2005) reported that the onion plants provided with *Azospirillum* + 100% N + P K produced highest bulb yield, highest dry matter production maximum up take of N, P and K. Among all the treatments, application of *Azospirillum* + 100% N + P K (*Azospirillum* 125:50:125 NPK kg/ha) was found to be most remunerative considering the uptake, bulb yield and total dry matter production and cost benefit ratio, which gave the highest return.

Rabindra and Srivastava (2006) reported that the highest yield of tomato was obtained when plants were inoculated with Phosphate Solubilizing Bacteria (PSB) along with the 75 per cent of P and full dose of N and K.

Jha *et al.* (2006) reported that the significant increase in the yield and bulb characters was recorded in onion when plants were inoculated with PGPR and VAM along with the recommended dose of nitrogen and phosphorous fertilizers over plants uninoculated. Appreciable increase in both vertical and horizontal bulb diameter was obtained by inoculation of PGPR like *Azospirillum*, *Pseudomonas fluorescens* and *Azotobacter* along with recommended dose of nitrogen and phosphorous.

Prabhu *et al.* (2006) reported that in cucumber there was significant difference on vine length due to application of different levels of organic manures, inorganic and biofertilizers. The number of fruits per plant was increased by the application of 50% recommended dose of fertilizers + vermicompost @ 2t/ha + biofertilizers. Yield attributing parameters such as length, girth and weight of fruit were found highest in treatments with the combination of organic manures, inorganic and biofertilizers.

2.4 Effect of PGPR on Quality attributes

Quality of knolkhol showed good response to bacterial inoculants. *Azospirillum* resulted in nonsignificant increase in the dry matter and vitamin C content over control. Increase in dry matter content could be due to more leaf number and better accumulation of food deposit in edible part. While increase in vitamin C content could be due to increased efficiency of microbial inoculants to fix atmospheric nitrogen and secrete growth promoting substances which alter the physiological process like synthesis of carbohydrates (Chattoo *et al.,* 1997).

Thilakavathy and Ramaswamy (1999) observed that in onion significant improvement of quality parameters like shape index, colour of the bulb and pyruvic acid content was recorded in the plants inoculated with *Azospirillum* and Phosphate solubilising bacteria (PSB) + 45:45:35 kg NPK/ha.

Ganeshe *et al.* (2000) reported that the longest shelf life was recorded in okra for the control (6.67 days) and *Azospirillum* soil inoculation with no inorganic N (6.17 days).

Naidu *et al.,* (2000) reported that the crude protein percentage of okra was highest under 35 tonnes FYM + *Azospirillum* + Phosphorus Solubilizing Bacterium (PSB)

Naem *et al.* (2000) reported that the simultaneous inoculation of both biofertilizers was more effective in increasing fruit protein and ascorbic acid contents than the inoculation of either of the biofertilizers. The levels of fruit total phenolic compounds were highest with Phosphorein + Microbein singly or in combination with the recommended N and P rates, and with poultry manure + Phosphorein. The inoculation of Microbein together with the application of 80 kg N + 45 kg P or 15 m3 poultry manure was optimum for tomato production.

Total soluble solid (TSS) in tomato was significantly improved under the influence of *Azotobacter* while phosphate solubilising bacteria (PSB) did not influence significantly (Chaurasia *et al.,* 2001).

Singh *et al.* (2001) reported that the treatment having farmyard manure and dense organic manure gave yield at par or higher than the conventional methods and plant products had higher protein content and better shelf life. Although the treatment having PGPR + dense organic manure gave low yield in all the crops except peas but, it excelled all the treatments in case of higher vitamin C content and lower nitrate level.

Asha Raj and Geetha Kumari (2001) observed that quality of okra fruits showed good response to *Azospirillum* inoculation. Crude protein content (17.73%) was significantly increased by *Azospirillum* inoculation than control. *Azospirillum* inoculation had resulted in significant reduction in crude fiber content as compared to untreated plants. As a result plants became more succulent and the crude fiber content and higher ascorbic acid content might have improved the keeping quality of *Azospirillum* inoculated okra plants compared to control.

Chaurasia *et al.* (2001) revealed that the combined application of phosphate-solubilizing bacteria and *Azotobacter* was not as effective in enhancing the shelf life of tomato as the individual application of *Azotobacter.* Total soluble solid (%) was significantly improved under the influence of *Azotobacter,* while Phosphate Solubilizing Bacteria did not influence significantly.

Sengupta *et al.* (2002) mentioned that in tomato the highest contents of total soluble solids (TSS; 6.41 and 6.42 brix) and ascorbic acid (24.15 and 24.19 mg) were recorded with 120 kg N/ha. *Azospirillum* treatment recorded the highest TSS (5.95 and 5.90) and ascorbic acid content (22.76 and 22.56 mg).

Raut *et al.* (2003) reported that the Vitamin C content in tomato fruits was highest (16.5 mg/100 g) in 30 tonnes FYM+5 kg *Azospirillum*, which enhanced storage life. The treatments having bio fertilizers and dense organic manure though recorded significantly low yield and gave low nitrate content but higher Vitamin C content.

Bahadur *et al.* (2003) revealed that in broccoli, significantly higher carotinoid contents over control were observed in treatments supplied with 20 t/ha digested sludge or FYM + seedling inoculation with PSB. Further, the organic manure and bacterial inoculation significantly reduced the fiber content of heads.

Chia-Hui and Kloepper (2003) mentioned that some PGPR strains increased the survival rate of tomato seedlings and enhanced the shoot weight even under heat stress condition. These results suggest that the PGPR treated plants subjected to heat stress mimicked the classic heat shock response.

Pardeep and Sharma (2004b) observed that in tomato the maximum total soluble solids (TSS) and ascorbic acid obtained by the plants treated with FYM 25 t/ha + SSP at 469 kg/ha and PSB.

Inoculation of cauliflower by *Azospirillum* with full dose of recommended dose of NPK application recorded significantly higher vitamin C content (59.9 mg/100g) when compared with the uninoculated cauliflower plants. The significant increase in the N, P and K content of curd was observed with the inoculation of *Azospirillum* + recommended dose of NPK (Narayanamma *et al.,* 2005).

Nirmal De *et al.* (2006) reported that significantly higher vitamin C content (30.0 mg/100g), carbohydrates (55%), protein (23.8%), phosphorous (0.67%) and sulphur (0.39%) in grains were obtained when the pea plants were grown under dual inoculation of rhizobium and PSB along with the press mud @ 5t/ha. Associative effect of rhizobium and PSB in dual inoculation treatment resulted in significant increase in nitrogen content, which directly reflected to protein content and protein level in grains.

Jha *et al.* (2006) showed that there was a marked increase in total soluble solids (TSS) of onion bulb in plants treated with *Azospirillum* which was closely followed by *Azotobacter* and dual inoculation of PGPR improved the shelf life of onion.

2.5 Nutrient uptake and availability

Asha and Kumari (2001) reported that the *Azospirillum* inoculation helps in promoting nitrogen fixing and in the release of various growth promoting agents which might have lead to better root development, better transportation of water, uptake and deposit of nutrients by okra.

Asha Raj and Geetha Kumari (2001) reported that the nutrient uptake and soil nutrient status were favourably influenced by inoculation of *Azospirillum* in okra.

Gaikwad and Wani (2001) reveled that the PSB inoculation resulted in significant increase in plant dry matter, N and P uptake and yield of brinjal when compared with the control.

Prasad *et al.* (2002) reported that the combination of *Azospirillum* with VAM was superior over the individual application of these two, mixed application reduced the 75 per cent of recommended chemical fertilizer dose which was found to be superior over all levels of bacteria and chemical fertilizer for growth and yield of tomato.

Shalini *et al.* (2002a) reported that the available soil nitrogen was significantly higher in cabbage plots receiving organic manure and *Azospirillum* than those in inorganically fertilized plots. The data on the population of *Azospirillum* in the rhizosphere revealed that the inoculated treatments showed significantly higher population.

Shalini *et al.* (2002b) noticed that the application of 50% (urea) + 50% N (vermi compost) + *Azospirillum* resulted in higher availability and uptake of nutrients by cabbage and thus produced the maximum yield (37 t/ha).

Harikrishna *et al.* (2002) mentioned that the application of FYM 25 t/ha + 75% N + 100% P + 100% K + *Azospirillum* resulted in the highest available N (299.9 kg/ha), P_2O_5 (44.2 kg/ha) and K_2O (321.9 kg/ha) in tomato. Although differences in K availability between this and other treatments were not significant in the highest fruit yield (54.32 t/ha) and N, P and K uptake at harvest. None of the treatments significantly influenced the K content in the plant.

Nanthakumar and Veeraragavathatham (2003) observed that in brinjal, N and P contents were highest in the treatments involving organic manure + inorganic fertilizers + PGPR. Potassium content of plant tissues increased from the vegetative stage to the flowering stage and declined during the harvesting stage in all three seasons irrespective of the treatments. Potassium content at the flowering stage did not show any significant variation among the treatments. At the harvesting stage K content was higher in the treatments involving organic manure + inorganic fertilizers + PGPR than the treatments with different levels of inorganic fertilizers alone.

Hernandez and Chailloux (2004) reported that in tomato the best results were achieved with *G. mosseae* + *Pseudomonas flourescens* and *G. mosseae* + *Azospirillum* combined with 50% nitrogen fertilizer. Arbuscular mycorrhizae inoculation with rhizobacteria stimulated nitrogen and phosphorus absorption.

Singh *et al.* (2004) reported that the integrated use of organic, inorganic sources of nutrients and biofertilizers increased the N, P and K concentrations in the plants (including fruits) of okra, pea and tomato. The integrated nutrient management also significantly increased shoot dry matter yield of tomato and fruit yields of okra and tomato.

Sunaina and Ajay (2005) reported that the large and heavily branched root system observed in potato plants arising from PGPR (*Bacillus* strains) treated strains lead to improved water and nutrient uptake. Increase in water uptake by excessive root branching of PGPR treated plants has ultimately revealed that it enhanced per plant yield to a significant level.

Jha *et al.* (2006) reported that in onion maximum residual nitrogen was observed with the inoculation of *Azospirillum* followed by *Azotobacter, which* was significantly different from control. Similar to available N and P was also more with dual inoculation of PGPR and VAM. Maximum sulphur was estimated with *Azospirillum* inoculation followed by *Azotobacter*, which was significantly higher than controled plot.

Narayanamma (2006) reported that in cucumber the available nitrogen (261.5 kg/ha), phosphorous (33.0kg/ha) and potassium (245.5) in soil after harvest of cucumber crop were significantly superior with the application of vermicompost in combination with biofertilizers and chemical fertilizers.

2.6 Effect of PGPR on pest and disease occurrence

Transcriptional activity of salicylic acid and pyochelin biosynthetic genes was detected during *Pseudomonas aeruginosa* 7NSK2 colonization of bean. Moreover, the iron nutritional state at inoculation influenced the transcriptional activity of salicylic acid and pyochelin biosynthetic genes in the same way as it influenced induction of systemic resistance to *Botrytis cinerea in* beans (de Meyer and Hofte, 1997).

Raupach and Kloepper (1998) studied the effect of PGPR mediated disease suppression in cucumber against angular leaf spot (1996) and against a mixed infection of angular leaf spot and anthracnose (1997). The three-way mixture of PGPR strains (*Bacillus subtilis* GB03 plus *Bacillus pumilus* INR7 plus

Curtobacterium flaccumfaciens ME1) as a seed treatment showed intensive plant growth promotion and disease reduction to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray.

PGPR are known to control a wide range of pathogens like fungi, bacteria, viruses etc., and they are known to control these pathogens by biocontrol mechanism which may be by composition or antagonism. However, the most studied phenomenon was the induction of systemic resistance by these bacteria in the host plant thereby containing the invading pathogens (van Loon *et al.*, 1998)

In cucumber, it was found that bacterized plants suppressed the development of mite population by more than 40% on the leaves of susceptible cultivar Corona. The plants with PGPR were less injured in relation to the plants without bacteria. Infested leaves of cultivar Aramis showed a lower level of inhibition in photosynthesis compared to injured Corona leaves and the presence of bacteria had a positive effect on this process (Tomczyk, 1999).

Zehnder *et al.* (1999) noticed that the tomato plants treated with PGPR demonstrated a reduction in the development of disease symptoms and often a reduction in the incidence of viral infection and increase in yield.

The plant growth promoting rhizobacteria (PGPR) were tested for there ability to control Rhizoctonia rot and damping-off caused by *Rhizoctonia solani* AG4, *Pythium aphanidermatum* and *P. ultimum* on cucumber. The presence of the PGPR reduced the incidence of disease (Chen-XiaoBin *et al.*, 1999).

Bansal *et al.* (1999) revealed that the PGPR inoculation reduced root-knot infection in comparison with the check and carbofuran treated bottle gourd plants. Among all the bacteria, CO-70 followed by HT-54 was effective in promoting plant growth and reducing nematode infection.

Singh *et al.* (2000) observed that the bacterization by both bacteria (*Pseudomonas fluorescens* and *Pseudomonas aeruginosa*) and aerial spray of Neemazal increased the dry weight of aerial parts, number of nodes and pods as well as seed weight of pea plants. Colonization of the rhizosphere and rhizoplane was more by *Pseudomonas aeruginosa* followed by strain Pf5 of *Pseudomonas fluorescens*. The lower most leaves supported better survival of these two plant growth-promoting rhizobacteria (PGPR) than the upper leaves.

PGPR treatments resulted in reduced ToMoV incidence and disease severity and in some cases, a corresponding increase in fruit yield. The use of PGPR could become a component of an integrated program for management of this virus in tomato (Murphy *et al.,* 2000).

In tomato, combination of three strains *viz., Pseudomonas fluorescens* strain CHA0, COP and COT was found to be more effective for the suppression of tomato spotted wilt virus when compared to individual strain application or all possible two strains combination (Kandan, 2000).

Tomczyk and Kiekiewicz (2001) reported that in cucumber and tomato the spider mite-feeding caused only a small decrease of protein content in Aramis (less susceptible), while the presence of PGPR in the root system caused an increase of soluble proteins in the leaves. In infested leaves of Romatos (highly susceptible) without PGPR an evident increase of amino acid content was found opposite to plants with PGPR.

Transplant vigour and survival in the field were improved by PGPR treatments in both tomato and pepper. Diseases of tomato caused by root-knot nematodes, *Fusarium, Phytophthora* and *Pythium* were not affected by PGPR treatments. PGPR formulation LS261 reduced the number of root-knot nematode galls on pepper. Incidence of wilt symptoms on tomato was significantly lower in PGPR treated plots and highest in the untreated plots. Yield of extra large tomato fruit and total yield increased with PGPR formulation LS256 (Kokalis-Burelle, 2002).

Pseudomonas fluorescens strain PF1 induced systemic resistance against soil born pathogens of solanaceous vegetables such as chilli and tomato (Ramamoorthy *et al.,* 2002).

Jetiyanon and Kloepper (2002) indicated that four mixtures of PGPR and one individual strain treatment significantly reduced the severity of all four diseases (CMV of cucumber, bacterial wilt of tomato, anthracnose of long cayenne pepper and damping off of green kuang futsoi) when compared to the nonbacterized control. Most mixtures of PGPR provided greater disease suppression than individual PGPR strains. These results suggest that the mixtures of PGPR can elicit induced systemic resistance to fungal, bacterial and viral diseases in the tomato, long cayenne pepper, green kuang futsoi and cucumber.

Shojai *et al.* (2003) reported that the use of antagonists like *Bacillus subtilis*, against mildews and application of milk and *Bacillus thuringiensis* (Bactospein) against Trichoplusia ni were effective. Results suggested that the possibility of the off-season cultivation of tomato and cucumber in the greenhouse through the proper implementation of a suitable IPM programme. Combination of PGPR strains *viz*., *Bacillus subtilis* GBO3 and *Bacillus amyloliquefaciens* IN937a with the carrier chitosan to the tomato leads to protection against CMV (Murphy *et al.,* 2003).

Sharma *et al.* (2003) reported that in okra the maximum reduction in number of galls was obtained with VAM, *Azotobacter* and *Azospirillum* alone. Combined application of phorate and microbes increased the root-knot galling, indicating the probable deleterious effect of phorate on microbes or vice versa. The *Azotobacter* with phorate at 1 kg a.i/ha showed higher galling, while *Azospirillum* and VAM with phorate at 1 kg a.i/ha showed little difference.

Shylaja *et al.* (2003) revealed that the activity of peroxidise was found to be better in the tolerant cultivar than in the susceptible. The susceptible cultivar seeds were treated with *Bacillus subtilis* and the peroxidase assay was repeated. Increase in the peroxidase activity was observed in the tomato roots of the susceptible cultivar treated with *Bacillus subtilis* when compared with untreated. On the 30th day seedlings obtained from the seeds of susceptible cultivar treated with *Bacillus subtilis* enzyme activity was significantly higher than the resistant cultivar not treated with bio agent. Suggesting the induction of systemic resistance in the susceptible cultivar was due to root colonization by *Bacillus subtilis.*

Nallathambi *et al.* (2003) mentioned that the seed treatment of watermelon with bacterial isolates aid better seed germination when compared to untreated control. As for management of virus disease is concerned none of the treatment could completely eliminate the disease. However, seed treatments followed by foliar sprays of bacterial isolates *Pseudomonas fluorescens* (CIAH-111) recorded fewer incidences when compared with untreated plants.

Kavitha *et al.* (2003) reported that the treatments with the isolate *Bacillus subtilis* recorded the lowest per cent chilli damping off diseases incidence of 15.66 % followed by 16.07 % in treatment with *Pseudomonas spp*, significant increase in plant growth was recorded because of treatment with these antagonistic bacteria. The combined Bacterization of tomato seeds with *Pseudomonas fluorescens* and *Bacillus subtilis* performed better in reducing the damping off incidence and increased the root and shoot length more effectively than individual application of PGPR strains.

Ramesh and Korikanthimath (2003) reported that the *Pseudomonas fluorescens* and *Trichoderma* could be combined for the management of damping off in nursery as both pocess different mode of action for pathogen suppression and growth promotion. For wilt management in brinjal, *Pseudomonas fluorescens* may be used as it induced systemic resistance in plant against the systemic pathogen.

Kloepper, (2003) reported that the *Bacilli* PGPR strains increased the plant growth in the absence of pathogens and if the plants are than inoculated with foliar pathogens, induced resistance is manifested by reduced disease severity compared to non bacterzied controls.

Anith *et al.* (2004) observed that when the PGPR and Actigard applications were combined, Actigard plus *Pseudomonas putida* 89B61 or BioYield reduced bacterial wilt incidence when compared with the untreated control. Incorporation of S-H mixture into infested soil 2 weeks before transplanting reduced the bacterial wilt incidence in one experiment. Combination of Actigard with the S-H mixture significantly reduced bacterial wilt incidence in tomato.

Siddiqui (2004) mentioned that the poultry manure with *Pseudomonas fluorescens* was the best combination for the management of *Meloidogyne incognita* on tomato but improved management of *M. incognita* can also be obtained if goat dung is used with *Pseudomonas fluorescence* or poultry manure with *Azospirillum brasilense*.

Sendhilvel *et al.* (2005) reported that the cowpea seed and the soil application of talc based formulation of *Pseudomonas fluorescens* (SVPF2) significantly reduced the root rot incidence, the growth parameters viz. germination percentage and vigour index were also increased in SVPF2 treated seeds.

Sally *et al.* (2005) reported that the seed treatment and soil application of *Trichoderma viride, T. harzianum, Pseudomonas fluorescens, Azotobacter chroococcum* reduced the dampingoff incidence considerably in brinjal, chilli and tomato. Application of these microbes also increased the per cent germination and promoted vigour of solanaceous seedlings as compared to control.

Several studies have indicated that PGPR may stimulate the production of biochemical compounds associated with host defense, massive accumulation of phytoalexins, phenolic compounds, increases in the activities of PR- proteins, defense enzymes and transcripts aNd enhanced lignification (Swamiyappan *et al.*, 2005).

Meena and Marimuthu (2005) reported that the potential of *Pseudomonas fluorescens* for the management of fruit of chillies was evaluated under green house and field conditions. Among the various strains of *Pseudomonas fluorescens* tested in *vitro*, the strain pf1 significantly inhibited the mycelial growth of *Colletotricum capsici*. Combined application of talc-based powder formulation of *Pseudomonas fluorescens* to seed (10g/kg seed) and foliage (1kg/ha) effectively controlled the fruit rot disease. Early and high induction of various defense compounds was observed due to *Pseudomonas fluorescens* treatment.

2.7 Mechanism of PGPR on growth and yield

The fertility of soil depends not only on its chemical composition but also on the qualitative and quantitative nature of microorganisms inhabiting it. Among different types of microorganism's bacteria are the most dominant group of microorganisms in the soil and probably equal one half of the bio mass in soil. These bacteria's will have a direct effect on growth and development of plants.

Brown (1970) suggested that the stimulation of plant growth by inoculating seeds with phosphatic solubilizers might be more due to the production of growth hormones rather than phosphate solubilization. The tested phosphate dissolving bacteria (*Bacillus polymyxa*, *Bacillus pulvifaciens* and

Pseudomonas striata) and fungi (*Aspergillus awamori*, *Aspergillus niger* and *Penicillum digitatum*) produced auxins and gibberellins to an appreciable extent and beneficial effects of these organisms may be due to the production of growth promoting substances besides phosphate solubilization..

Studies on oxygen uptake by cell free extracts of *Azospirillum brasilense* with malate, succinate, lactate and pyruvate have indicated that the tri-carboxylic acid cycle is operative in these organisms while the glycolytic and pentose phosphate pathway are of minor significance (Okon *et al.,* 1976b). It has been suggested that the PO_2 is low and that substrate carbon levels are limiting factor at the sites of nitrogen fixation in the roots (Dobereiner and Day, 1976).

The efficiency of nitrogen fixation by *Azospirillum brasilense* increased with the decreased carbon supply and low PO_2 (Okon *et al.,* 1976a). The optimum temperature for N_2 dependent growth by *Azospirillum* has been reported between 32 and 40 ^{0} C which similar to the optimum temperature reported for other nitrogen fixing bacteria from tropical environment (Day and Dobereiner, 1977). *Azospirillum* spp. required low phosphate for the expression of nitrogenase activity (Tarrand *et al.,* 1978).

Subba Rao (1982) suggested different mechanisms for the solubilization of inorganic phosphorous by phosphate solubilising microorganisms. Production of organic acids in the micro- environment around the root or in the culture media was considered to be the most important cause of phosphate solubilization and also reported that *Azospirillum* which lives on or in the roots can continuously release the phytohormones which improve the plant growth by inducing the proliferation of lateral roots. There was a marked increase in the root biomass of pearl millet and sorghum upon inoculation.

The significant increase in maize root biomass particularly indicated the role of growth hormones. The increased root surface might have resulted in a higher nutrient uptake and a better growth than N_2 fixation by the bacteria (Venkateswarulu and Rao, 1983).

The strains of *Azospirillum brasilense* are able to produce various growth regulating substances such as indole acetic acid (IAA) and gibberellin like substances. Seed and root exudates of pearl millet helped in the establishment of the successful association of pearl millet - *Azospirillum*, as these contain various biologically active compounds (organic acids, sugars and amino acids) which help in the multiplication of the bacterium (Venkateswarulu and Rao, 1985; Rao and Venkateswarlu, 1987).

Bhandari *et al.* (1989) reported that the nitric acids produced during the oxidation of inorganic compounds of nitrogen and sulphur react with insoluble phosphate (rock phosphate) brought about solubilization. Addition of rock phosphate coupled with inoculation with phosphate solubilizing microorganisms gave good response in many crops. These microorganisms solubilize not only insoluble soil phosphates and insoluble forms of phosphatic fertilizers such as rock phosphate, bone meal, etc., but also increase the efficiency of soluble forms of phosphatic fertilizers applied to soil (Somani *et al.*, 1990).

Soil microorganisms play a very significant role in making phosphorous available to plants by dephosphorilating phosphorous bearing organic compounds and also by bringing about favourable changes in soil reaction in the soil micro environment leading to solubilization of inorganic phosphate sources (Somani *et al.*, 1990).

Kloepper *et al.* (1991) reported that some bacteria’s solubilize organic phosphate by secreting phosphate or inorganic phosphate from soil particles by releasing organic acids and this could make phosphorous as well as micronutrients more readily available for plant growth in soil.

Nelson and Maloney (1992) reported about the competition for nutrients in biological control by *Pseudomonas fluorescens.* It was found that, *in vitro* antagonistic activity was based on competition and correlated with disease suppression.

Subba Rao (1995) gave conclusive evidence to show that the soil bacteria dissolved rock phosphate, bone meal, di-and tri-calcium phosphate, calcium phosphate and calcium carbonate. Most of the studies on phosphate solubilization were done first by isolating the microorganisms from the soil and then studying the solubilization under *in vitro* conditions.

The mechanism of PGPR involved in the growth promotion are due to increased production of auxin, gibberellin, cytokinin, ethylene (Kloepper and Schroth, 1981; Dubeikosky *et al.,* 1993; Glick, 1995), solubilization of phosphorous oxidation of sculpture, increases in nitrate availability, the extra cellular production of antibiotics, lytic enzymes, hydrocyanic acid, increas in root permeability, strict competition for available nutrients, root sites (Enebak and Carey, 2000), symbiotic N_2 fixation, mobilization of insoluble nutrients and volatile components (Ryu *et al.,* 2004).

The enhancement in yield with *Azospirillum* was due to production of growth substances like IAA and GA which might have increased the availability and uptake of nutrients through plant roots (Deka *et al.,* 1996).

Handelsman and Stabb (1996) reported that the antibiosis is now often implicated as an important mechanism of biological control, resulting from the fact that it was an attractive mechanism to study which provides a highly effective mode of action.

Subba Rao (1997) investigated that the source of extra phosphate taken up by growing mycorrhizal and non-mycorrhizal onions in soils was from the pool of 'liable' soil phosphate. Enzymes extracted by Polymorphic phospho microorganisms in intimate association with plant roots could be expected to mineralize phosphorous for plant uptake

Bashan and Holguin (1997) reported that the most reproducible effect on plants inoculated with *Azospirillum* was increased over all growth, greater production of root hairs and enhanced root surface area. These effects on roots were considered to be accounted for by bacterial production of IAA. In potato plantlets grown under *in vitro*, *Azospirillum* (P5JN) increased cytokinin content by inducing synthesis in the early stages of plant growth and development (Lazarovits and Nowak, 1997).

Anjaiah *et al.,* (1998) described about the antibiotics involved in disease suppression by *Pseudomonas fluorescens* as phenazine-1-carboxamide and anthranilate. For the biocontrol agent, *Bacillus cereus* (strain UW85), production of kanosamine and zwittermicin A was suggested to be important for biocontrol activity (Milner *et al.,* 1998).

Competition between pathogenic and saprophytic microorganisms for organic materials released from the roots can reduce growth and pathogenic activity of the pathogens. For this mode of action, the classical approach of comparing biocontrol activities of specific catabolic mutants with wild type strains is not simple, since anyone of a number of substrates could be utilized (Loper *et al.,* 1997).

Subsequently, *Azospirillum brasilense* was found to produce high quantities of IAA. This was most likely the explanation for the increased seedling root length, root hairs, root branching, and root surface areas (Kloepper, 2003).

Bashan and de-Bhasan (2004) reported that the gibberellins produced by *Azospirillum* contributed to increased root hair development. Application of gibberellin increased density of root hairs at levels similar to *Azospirillum* inoculation.

Inoculation of lettuce plants with bacteria increased the cytokinin content of both shoots and roots. Accumulation of zeatin and its riboside was highest in roots two days after inoculation, when their content was 10 times higher than in control. Changes in the content of other hormones (ABA and IAA) were observed at the end of experiments only. Accumulation of cytokinins in inoculated lettuce plants was associated with an increase in plant shoot and root weight of approximately 30% over 8 days. (Arkhipova *et al.,* 2005).

Materials and Methods

III. MATERIALS AND METHODS

The present investigation was carried out in the Department of Olericulture, College of Horticulture, Vellanikkara during 2006-2007 with an objective to study the effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of bitter gourd (*Momordica charantia* L.)

3.1 Materials

The site is located at 10°31' N and latitude, 76°13' E longitude and at an altitude of 22.25 m above MSL. The area experiences a typical warm humid climate and receives average rainfall of 2663 mm per year. The soil of the experimental site comes under the textural class of sandy clay loam and is acidic in reaction. Data on maximum and minimum temperature, rainfall and relative humidity during the entire cropping period were collected from Meteorological Observatory of College of Horticulture, Vellanikkara.

3.1.1 Season of Experiment

The crop was raised during the Rabi season from September 2006 to January 2007.

3.1.2 Variety

The high yielding variety Preethi, developed by Kerala Agricultural University was selected for the study.

3.1.3 Plant growth promoting rhizobacteria (PGPR)

Four plant growth promoting rhizobacteria (PGPR) viz., *Azospirillum*, Phosporous solubilising bacterias (PSB), *Pseudomonas fluorescens*, *Bacillus subtilis* were used along with a commercial product Aishwarya.

3.2 Methods

3.2.1 Layout and experiment design

The experiment was laid out in Randomized Block Design (RBD) with three replications (Plate I). There were six plants in a plot with two rows of three plants each with common border rows. Spacing adopted was 2 X 0.75 meters. The manurial and fertilizer doses were based on the POP recommendation (KAU, 2001) for bitter gourd. As per this the FYM and NPK were applied at the rate of 20 – 25 tonnes and 70:25:25 kg N:P_2O_5:K_2O ha $^{-1}$ respectively.

3.2.2 Treatments

T_1 - Manures and fertilizers as per POP recommandations

T_2 - T_1 + *Azospirillum*-5kg/ha (basal)

T_3 - T_2 + *Azospirillum*-5kg/ha (40DAS)

T_4 - T_1 + PSB - 5kg/ha (basal)

T_5 - T_4 + PSB - 5kg/ha (40 DAS)

T_6 - T_1 + *Pseudomonas flourescens* - 2.5kg/ha (basal)

T_7 - T_6 + *Pseudomonas flourescens* - 2.5kg/ha (40DAS)

T_8 - T_1 + *Bacillus subtilis* suspension - (10^8 cfu/ml) 2 l/plant (basal)

T_9 - T_8 + *Bacillus subtilis* - (10^8 cfu/ml) 2 l/plant (40DAS)

T_{10} - T_1 + Aishwarya - 30g/plant (basal)

T_{11} - T_{10} + Aishwarya - 30g/plant (40DAS)

3.2.3 Application of PGPR

The talc based PGPR viz., *Azospirillum*, Phosporous Solubilising Bacteria (PSB), and *Pseudomonas fluorescens* were applied to the soil and *Bacillus subtilis* suspension having concentration of 10^8cfu ml^{-1} was applied. Aishwarya was applied to the soil as granular application.

Plate 1. Field view of experimental plot

3.3 Field experiment

3.3.1 Land Preparation

In the experimental field weeds were removed and plot was laid out. Trenches were taken at 2 meters apart and full amount of FYM, half dose of N and full dose of P_2O_5 and K_2O of recommended dose were applied as a basal dose. Twigs of trees were given as support to the vines.

3.3.2 Sowing

Water soaked seeds were sown at 0.75 meter apart in the trenches.

3.3.3 After cultivation

The trenches were hand weeded regularly to keep the field weed free of weeds. Light earthing up was done along with the application of remaining half dose of N.

3.3.4 Plant protection

Plant protection chemicals were applied as and when required.

3.3.5 Harvesting

Fruits were harvested at vegetable harvest stage.

3.4 Biometrical observations

For taking observations five plants per plot were tagged and the following observations were recorded.

3.4.1 Growth and growth parameters

3.4.1.1 Days to germination

Number of days taken from sowing to germination was recorded.

3.4.1.2 Vine length

Vine length at 90 days after sowing (DAS) was recorded in five plants per plot and the average was taken. Measurement was taken from base of the vine to the growing tip of the plants.

3.4.1.3 Primary branches

Number of branches in five plants per plot was counted at 50 DAS and the average was worked out.

3.4.1.4 Root length

Root length was recorded in five plants per plot at the end of crop and average was taken. Measurement was taken from base of the vine to the tip of the mother root.

3.4.2 Flower characteristics

3.4.2.1 Days to first female flower

The number of days taken from sowing to opening of first female flower in each plot was recorded.

3.4.2.2 Number of female flowers

Total number of female flowers from five plants per plot was counted and their average was taken.

3.4.3 Biometrical characteristics of fruits

3.4.3.1 Days to first harvest

The number of days taken from sowing to first harvest of the fruits in each plot was recorded.

3.4.3.2 Number of fruits per plant

Total number of fruits from five plants at each harvest was counted and the average was calculated.

3.4.3.3 Fruit weight (g)

The weight of five randomly selected fruits in each plot from the second and third harvest was recorded and their average was worked out.

3.4.3.4 Fruit length (cm)

Length of five randomly selected fruits from second and third harvest was recorded and their average was calculated to get the average fruit length.

3.4.3.5 Fruit girth (cm)

Girth of five randomly selected fruits from second and third harvest was recorded and their average was calculated to get the average fruit girth.

3.4.3.6 Flesh thickness (cm)

Flesh thickness of five randomly selected fruits from second and third harvest was recorded. Their average was calculated to get the fruit flesh thickness.

3.4.3.7 Number of seeds per fruit

Number of seeds from randomly selected five fruits was counted and their average was taken as number of seeds per fruit.

3.4.4 Yield characteristics

3.4.4.1 Fruit yield per plant (g)

Weight of fruits from five plants was recorded and average was calculated to get fruit yield per plant.

3.4.4.2 Fruit yield per plot (g)

Weight of fruits from each plot after each harvest was recorded and added to get the total yield per plot.

3.4.5 Biochemical characteristics

3.4.5.1 Vitamin C content

Vitamin C content of fruit at vegetable harvest stage was estimated by titration with 2, 6 – dichlorophenol indophenol dye (Sadasivam and Manikantan, 1991). Five gram of the fresh sample was extracted in four per cent oxalic acid using a mortar and pestle and made up to 100 ml. Five ml of the extract was pipetted, added 10 ml of 4 per cent oxalic acid and titrated against the dye. Ascorbic acid content of the fresh sample was calculated from the titrated value and the value was expressed as mg per 100g of fresh fruit.

3.4.5.2 Iron content

The iron content was analyzed calorimetrically using ferric iron, which gives a blood red colour with potassium thiocynanate (Raghuramulu *et al.*, 2003). To an aliquot of the mineral solution, enough water was added to make up to a volume of 6.5ml followed by one ml of 30 per cent sulphuric acid, one ml of potassium per sulphate solution and 1.5ml of 40 per cent potassium thiocyanate solution. The intensity of the red colour was measured with in 20 minutes at 540nm. A standard graph was constructed using serial dilutions of standard iron solution. From the standard graph, the iron content of the sample was estimated and value was expressed as mg per 100g of fresh fruit.

3.4.5.3 Shelf life

Fruits were harvested at vegetable harvest stage, shelf life was recorded at room temperature under open condition, and average was taken.

3.5 Statistical analysis

Data were analyzed as per MSTATC package. To determine the influence of PGPR on each character pooled analysis over treatments was carried out.

Results

IV. RESULTS

Results of the investigations are presented under the following headings.

4.1 Effect of PGPR on vegetative characters of bitter gourd.

4.2 Effect of PGPR on flower characters of bitter gourd.

4.3 Effect of PGPR on biometrical characters of bitter gourd fruit.

4.4 Effect of PGPR on yield parameters of bitter gourd.

4.5 Effect of PGPR on quality parameters of bitter gourd.

4.1 Effect of PGPR on vegetative characters of bitter gourd.

Analysis of variance showed that there was significant difference among the treatments for the characters vine length (90DAS) and tap root length (Appendix I).

Means of various vegetative characters of bitter gourd at various stages of growth are given in Table 1.

4.1.1 Days to germination (days)

Days to germination was the earliest in Azospirillum treatment (T_3) followed by *Pseudomonas flourescens* application (T_7) which recorded 6.48 and 6.59 days respectively. Phosphorous solubilizing bacteria (PSB) treated seeds (T_4) took maximum days to germination (7.85days).

4.1.2 Vine length (90DAS)

The maximum vine length of bitter gourd was recorded in *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) and *Pseudomonas flourescens* (T_6) each recording 4.42 m. Control (T_1) recorded the minimum vine length (3.15 m).

4.1.3 Primary branches

The maximum number of primary branches was recorded in PSB treatment (T_5) followed by Aishwarya application (T_{10}) which recorded 4.80 and 4.40 respectively. *Azospirillum* (T_2) recorded minimum number of primary branches (3.23).

4.1.4 Tap root length (cm)

The maximum length of taproot was recorded in *Azospirillum* (double application) treatment (T_3) followed by *Azospirillum* (single application) treatment (T_2) which recorded 23.57 cm and 18.57 cm respectively. Tap root length was minimum in T_{10} (14.21 cm).

4.1.5 Secondary root length (cm)

The maximum secondary root length was recorded in PSB application (T_5) followed by *Azospirillum* (T_3) which recorded 39.88 cm and 37.12 cm respectively. Minimum secondary root length (20.18 cm) was recorded control (T_1).

4.1.6 Dry root weight (g)

The maximum dry root weight was recorded in *Bacillus subtilis* suspension (10^8 cfu/ml) treatment (T_8) followed by *Azospirillum* application (T_3) which recorded 4.64 g and 4.54 g respectively. Minimum dry root weight (1.73 g) was recorded in PSB application (T_5).

4.2 Effect of PGPR on flower characters of bitter gourd

Analysis of variance showed that there was significant difference among the treatments for the characters days to first female flower and number of female flowers (Appendix II).

Table 1: Effect of different treatments on vegetative characters of bitter gourd

Treatments	Days to germination	Vine length (m)	Primary branches (No)	Tap root length (cm)	Secondary root length (cm)	Dry root weight (g)
T_1	6.76	3.15	4.43	15.64	20.18	1.85
T_2	7.07	3.80	3.23	18.57	31.50	3.14
T_3	6.48	3.41	4.40	23.57	39.88	4.54
T_4	7.85	3.60	3.93	16.89	27.70	3.64
T_5	6.91	3.50	4.80	17.58	37.12	1.74
T_6	6.93	4.42	3.66	18.24	30.47	2.70
T_7	6.59	3.24	4.13	17.62	35.58	2.34
T_8	7.63	4.42	4.33	14.78	35.78	4.64
T_9	7.05	3.88	3.60	18.24	35.54	3.22
T_{10}	7.57	3.37	4.40	14.21	32.16	2.35
T_{11}	7.71	4.10	4.26	17.27	32.95	3.18
CD (0.05)	NS	0.56	NS	4.76	NS	NS

NS – Not significant

Means of various flower characters of bitter gourd at various stages of growth are given in Table 2.

4.2.1 Days to first female flower

The character, days to first female flower was lowest in control (T_1) followed by the treatment *Pseudomonas flourescens* (T_6) which recorded 43.93 and 44.95 days respectively. *Azospirillum* (T_3) application recorded the maximum days to appearance of first female flower (50.38 days).

4.2.2 Number of female flowers

The maximum number of female flowers was in *Bacillus subtilis* suspension (10^8 cfu/ml) treatment (T_9) followed by application of Aishwarya (T_{11}) which recorded 17.50 and 17.35 respectively. Control treatment (T_1) had minimum number of female flowers (14.06).

4.3 Effect of PGPR on biometrical characters of bitter gourd fruit.

Analysis of variance showed that there was significant difference among the treatments for the characters average fruit length, average fruit girth and average flesh thickness (Appendix III).

Means of various biometric characters of bitter gourd fruit at various stages of growth are given in Table 3.

4.3.1 Days to first harvest

Days to first harvest was minimum in control (T_1) followed by Aishwarya (T_{11}) which recorded 61.49 and 62.05 days respectively. *Pseudomonas flourescens* application (T_6) recorded maximum days to first harvest (65.11 days).

Table 2: Effect of different treatments on flower characters of bitter gourd

Treatment	Days to first female flower	Number of female flowers per plant
T_1	47.20	14.06
T_2	50.06	15.86
T_3	50.38	15.26
T_4	46.46	16.00
T_5	48.26	14.93
T_6	44.95	15.00
T_7	48.93	14.93
T_8	49.71	14.53
T_9	49.53	17.50
T_{10}	46.35	16.63
T_{11}	47.20	17.35
CD (0.05)	3.71	1.65

Plate 2. Field photo of control plot

Plate 3. Field photo of T_9 (*Bacillus subtilis* @ 2 l plant^{-1}, basal and 40 DAS)

4.3.2 Number of fruits per plant

There was no significant difference among the treatments for this character. The maximum number of fruits was recorded in treatment with Aishwarya (T_{11}) followed by *Pseudomonas flourescens* application (T_6) which recorded 13.60 and 12.68 respectively. Control plants (T_1) recorded minimum number of fruits (10.12).

4.3.3 Fruit weight (g)

Maximum fruit weight was recorded in *Bacillus subtilis* suspension (10^8 cfu/ml) treatment (T_9) followed by *Pseudomonas flourescens* application (T_6) which recorded 223.49 g and 185.55 g respectively. Minimum fruit weight (132.25 g) was recorded in control.

4.3.4 Fruit length (cm)

Maximum fruit length was recorded in *Bacillus subtilis* suspension (10^8 cfu/ml) treatment (T_9) followed by *Pseudomonas flourescens* application (T_7) which recorded 26.16 cm and 26.00 cm respectively. *Azospirillum* application (T_2) recorded minimum fruit length (18.20 cm).

4.3.5 Fruit girth (cm)

Maximum fruit girth was recorded in *Bacillus subtilis* suspension (10^8 cfu/ml) treatment (T_9) followed by *Pseudomonas flourescens* application (T_6) which recorded 22.93 cm and 20.66 cm respectively. Control treatment (T_1) recorded minimum fruit girth (16.73 cm).

4.3.6 Flesh thickness (cm)

Maximum flesh thickness was recorded in treatments *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9), Aishwarya (T_{11}) and PSB (T_5) each recording 0.73 cm. Control treatment (T_1) recorded minimum flesh thickness (0.43 cm).

\Table 3: Effect of different treatments on biometric parameters of bitter gourd

Treatments	Days to first harvest	Number of fruits per plant	Fruit weight (g)	Fruit length (cm)	Fruit girth (cm)	Flesh thickness (cm)	Number of seeds per fruit
T_1	61.49	10.12	132.25	19.90	16.73	0.43	32.66
T_2	64.33	11.53	143.55	18.20	19.66	0.56	37.00
T_3	64.55	11.15	138.66	23.26	17.96	0.56	41.66
T_4	62.61	12.37	139.08	19.43	19.20	0.63	41.00
T_5	63.16	12.60	147.72	23.56	18.83	0.73	38.66
T_6	65.11	12.68	185.55	23.03	20.66	0.70	38.66
T_7	63.33	11.43	157.44	26.00	18.73	0.53	28.00
T_8	63.94	11.89	144.27	25.70	18.10	0.60	39.00
T_9	62.94	12.16	223.49	26.16	22.93	0.73	45.33
T_{10}	62.33	12.52	159.05	25.00	18.90	0.63	43.00
T_{11}	62.05	13.60	181.83	23.06	18.83	0.73	49.33
CD (0.05)	NS	NS	NS	3.07	2.00	0.11	NS

NS – Not significant

4.3.7 Number of seeds per fruit

The maximum number of seeds per fruit was recorded in treatment Aishwarya (T_{11}) followed by *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9) which recorded 49.33 and 45.33 respectively. *Pseudomonas flourescens* (T_7) application recorded minimum number seeds per fruit (28).

4.4 Effect of PGPR on yield parameters of bitter gourd.

Analysis of variance showed that there was significant difference among the treatments for the characters fruit yield per plant and fruit yield per plot (Appendix IV).

Means of yield parameters of bitter gourd for different treatments are given in Table 4.

4.4.1 Fruit yield per plant (kg)

There was significant effect of application of PGPR on fruit yield per plant. Application of *Bacillus subtilis* suspension (10^8 cfu/ml) 2.l/plant as basal and also 40 days after sowing (T_9) significantly increased the yield in bitter gourd up to 2.72 kg/plant. The second best treatment was *Pseudomonas flourescens* (T_6) application (2.36 kg/plant) this was on par with Aishwarya (T_{11}) (Plate ii, iii, iv and v). The lowest yield (1.42 kg/plant) was recorded in control plot (T_1) which was on par with *Azospirillum* (T_2), *Azospirillum* ($T_{.3}$), PSB (T_4), PSB (T_5) and Aishwarya (T_{11}).

4.4.2 Fruit yield per plot (kg)

There was significant effect of application of PGPR on fruit yield per plot. Application of *Bacillus subtilis* suspension (10^8 cfu/ml) as basal and also 40 days

Table 4: Effect of different treatments on yield of bitter gourd

Treatment	Yield per plant (kg)	Yield per plot (kg)
T_1	1.42	8.55
T_2	1.47	8.85
T_3	1.43	8.54
T_4	1.52	9.08
T_5	1.51	9.08
T_6	2.36	14.19
T_7	1.94	11.61
T_8	1.72	10.30
T_9	2.72	16.33
T_{10}	1.61	9.65
T_{11}	2.06	12.35
CD (0.05)	0.29	1.74

Plate 4. Fruits harvested from control plot

Plate 5. Fruits harvested from T_9 (*Bacillus subtilis* @ 2 l plant^{-1}, basal and 40 DAS) plot

Table 5: Effect of different treatments on vitamin C, Iron content and shelf life of bitter gourd

Treatment	Vitamin C (mg/100g)	Iron content (mg/100g)	Shelf life (days)
T_1	95.33	0.808	2.00
T_2	90.50	0.799	3.66
T_3	114.33	0.884	3.66
T_4	105.00	1.072	3.66
T_5	114.00	0.915	3.00
T_6	123.83	1.251	4.00
T_7	76.00	1.899	3.33
T_8	128.50	1.289	4.00
T_9	105.00	0.962	3.00
T_{10}	138.16	0.979	3.66
T_{11}	114.16	1.307	3.00
CD (0.05)	33.56	0.54	0.25

after sowing (T_9) significantly increased the yield in bitter gourd (16.33 kg/plot), the second best treatment was *Pseudomonas flourescens* (T_6) application (14.19 kg/plot) which was on par with Aishwarya (T_{11}). The lowest yield (8.55 kg) was recorded in control plot (T_1) and was on par with *Azospirillum* (T_2), *Azospirillum* ($T_{.3}$), PSB (T_4), PSB (T_5) and Aishwarya (T_{11}).

4.5 Effect of PGPR on quality parameters of bitter gourd.

Analysis of variance showed that there was significant difference among the treatments for the characters vitamin C, iron content and shelf life (Appendix V).

Means of various quality parameters and shelf life of bitter gourd fruits are given in Table 5.

4.5.1 Vitamin C content (mg/100g)

The maximum vitamin C content was recorded in treatment Aishwarya (T_{10}) followed by *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) treatment which recorded 138.16 mg/100g and 128.5 mg/100g respectively. *Pseudomonas flourescens* (T_7) recorded minimum vitamin C content (76.00 mg/100g).

4.5.2 Iron content (mg/100g)

The maximum iron content was recorded in treatment *Pseudomonas flourescens* (T_7) followed by *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) treatment which recorded 1.889 mg/100g and 1.289 mg/100g respectively. *Azospirillum* application (T_2) recorded minimum iron content (0.799 mg/100g).

4.5.3 Shelf life (days)

The maximum shelf life of fruits was recorded in treatments *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) and *Pseudomonas flourescens* (T_6) each recording 4.00 days. Control treatment (T_1) recorded minimum days of shelf life (2 days).

Table 6: Performance of different treatments as percent increase/decrease over control on the growth characters of bitter gourd

Treatments	Days to germination	Vine length (90DAS)	Primary branches (No)	Tap root length (cm)	Secondary root length (cm)	Dry root weight (g)	Days to first female flower
T_2	4.58	20.63	- 27.08	`18.73	56.09	69.65	13.95
T_3	- 4.14	8.25	0.67	50.76	97.62	145.19	14.68
T_4	16.12	14.28	- 11.28	7.99	37.26	96.54	5.75
T_5	2.21	11.11	8.35	12.40	83.94	- 6.15	9.85
T_6	2.51	16.50	- 17.24	16.62	50.99	45.57	2.32
T_7	- 2.51	2.85	- 6.77	12.65	76.31	26.51	11.38
T_8	12.86	40.31	- 2.25	- 5.49	77.30	150.75	13.15
T_9	4.28	23.17	- 18.73	16.62	76.11	73.97	12.74
T_{10}	11.98	6.98	- 0.67	- 9.14	59.36	26.99	5.50
T_{11}	14.05	30.15	- 3.8	10.42	63.28	71.59	7.44

Table 7: Performance of different treatments as percent increase/decrease over control on the biometrical of bitter gourd

Treatments	Number of female flowers per plant	Days to first harvest	Number of fruits per plant	Average fruit weight (g)	Average fruit length (cm)	Average fruit girth (cm)	Average flesh thickness (cm)
T_2	12.80	4.61	13.93	8.54	- 8.54	17.51	30.23
T_3	8.53	4.97	10.17	4.84	16.88	7.35	30.23
T_4	13.79	1.82	22.23	5.16	- 2.36	14.76	46.21
T_5	6.18	2.71	24.50	11.69	18.39	11.53	69.76
T_6	6.68	5.88	25.29	40.30	15.72	23.49	62.79
T_7	6.18	2.99	12.94	19.06	30.65	11.95	23.25
T_8	3.34	3.98	17.49	9.08	29.14	8.18	39.53
T_9	24.46	2.35	20.15	68.99	31.45	37.05	69.76
T_{10}	18.27	1.36	23.71	20.26	25.62	12.97	46.51
T_{11}	23.39	0.91	34.38	37.48	15.87	12.55	69.76

Table 8: Performance of different treatments as percent increase/decrease over control on the yield and quality characters ofbitter gourd

Treatments	Number of seeds per fruit	Yield per plant (kg)	Yield per plot (kg)	Vitamin C (mg/100g)	Iron content (mg/100g)	Shelf life (days)
T_2	13.27	3.52	3.25	- 5.06	- 1.11	83
T_3	38.79	0.70	- 1.28	19.93	9.40	83
T_4	25.53	7.04	6.20	10.14	32.67	83
T_5	18.37	6.33	6.27	19.58	13.24	50
T_6	18.37	66.19	65.96	29.82	54.82	100
T_7	- 14.26	36.61	35.88	- 19.22	135.02	66.5
T_8	19.40	21.12	20.51	34.79	59.52	100
T_9	27.55	91.54	91.21	10.14	19.05	50
T_{10}	31.65	13.38	12.88	44.92	21.16	83
T_{11}	51.03	45.07	44.49	19.75	61.75	50

Discussion

V. DISCUSSION

Bitter gourd is the leading vegetable crop of Kerala. It is a rich source of thiamine and riboflavin. The highest yield and maximum returns make it the most preferred vegetable crop of farmers. PGPR are free living bacteria that had the ability to improve plant growth by production of plant growth regulators and through suppression of deleterious root colonizing microorganisms.

The results of the study entitled 'Effect of plant growth promoting rhizobacteria on growth and yield of bitter gourd (*Momordica charantia* L.)' are discussed in this chapter.

5.1 Field experiment

In the present study, the effect of *Azospirillum* (5 kg ha^{-1}), phosphorous solubilising bacteria (PSB) (5 kg ha^{-1}), *Pseudomonas fluorescens* (2.5 kg ha^{-1}), *Bacillus subtilis* (2 l plant $^{-1}$) and a commercial product Aishwarya (30 g $plant^{-1}$) were compared. The results were assessed in terms of vegetative characters (days to germination, vine length, number of primary branches, tap root length, secondary root length and dry root weight), flower characters (days to first female flower and number of female flowers $plant^{-1}$), biometrical characteristics (number of fruits, fruit length, fruit weight, fruit girth, flesh thickness and number of seeds), yield characters (yield $plant^{-1}$ and yield $plot^{-1}$) and quality attributes (vitamin C content, iron content, and shelf life).

5.1.1 Vegetative characters

The influence of different treatments in improving the vegetative characters of bitter gourd, in relation to days to germination, vine length, number of primary branches, tap root length, secondary root length and dry root weight are discussed (Fig 1, 2 and 3). Two time application of *Azospirillum* (T_3) was the

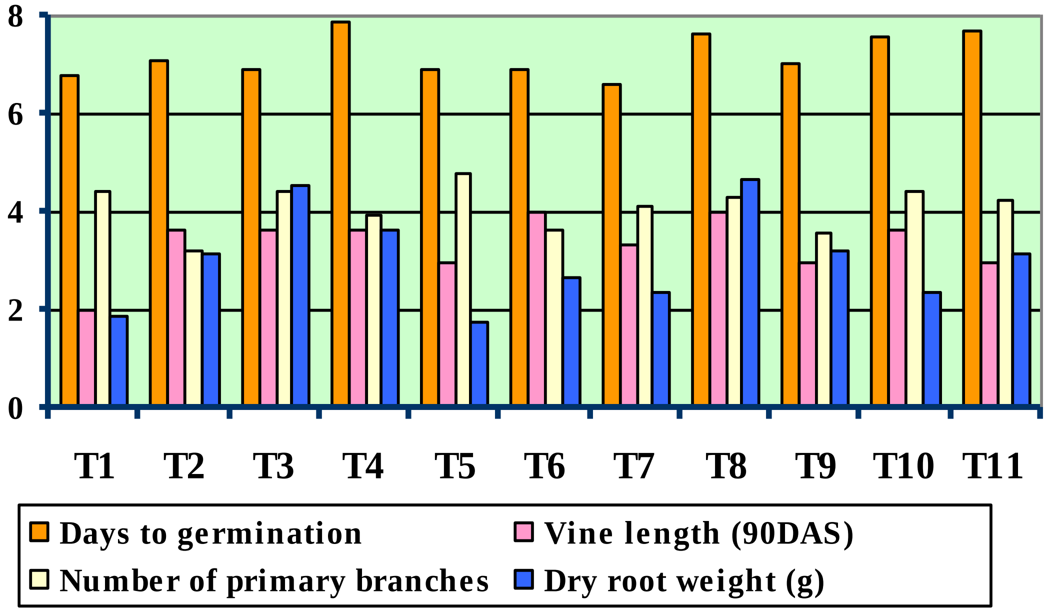

Fig 1: Effect of different treatments on days to germination, vine length, number of primary and dry root weight of bitter gourd.

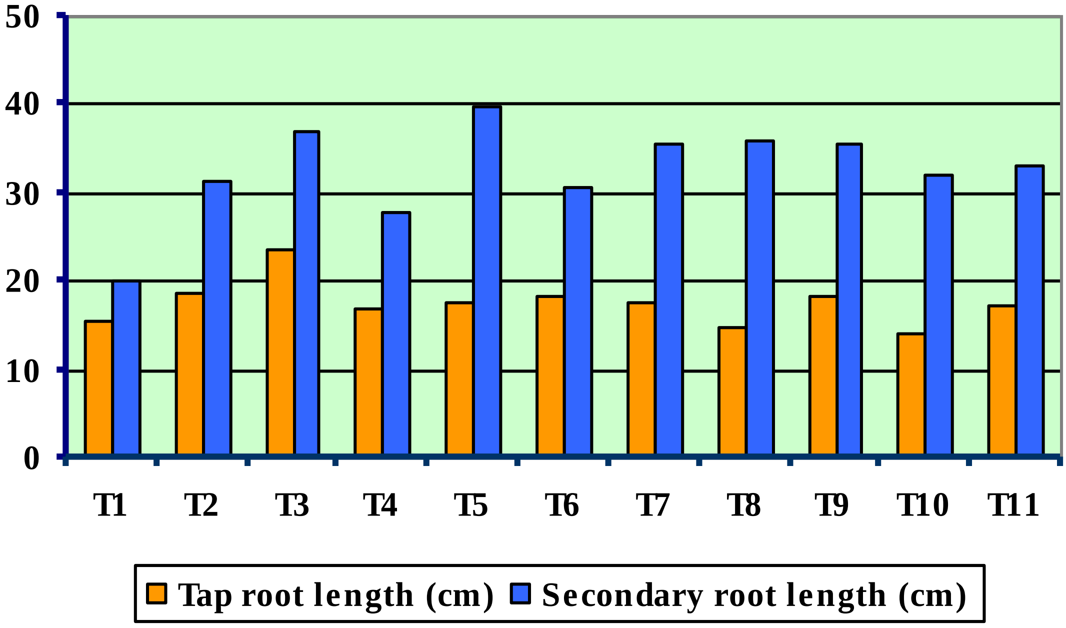

Fig 2: Effect of different treatments on tap root and secondary root length of bitter gourd.

best treatment which took 6.48 days for germination. The early germination could be due to the ability of *Azospirillum* to produce some growth promoting substances like auxins which might have led to enhance the physiological process in seeds, increased uptake of the nutrient and moisture (Kloepper, 2003). The colonization of this bacterium reduced the incidence of seed mycoflora which indirectly enhanced seed germination. Similar results have been obtained for *Azospirillum* on germination of watermelon and okra as reported by Nallathambi *et al.* (2003) and Prabu *et al.* (2003) respectively.

With respect to the vine length, single application of *Bacillus subtilis* (suspension (10^8 cfu/ml) (T_8) which recorded a length of 4.42 m which was found to be superior to other treatments. The probable, reason for the increase in the biometrical characters of the crop may be a blend of volatile organic compounds (Kloepper, 2003). Kevin (2003) reported that some of the strains of *Bacillus* were found to produce mixtures of lactic acid, isovalaric acid, isobutaric acid and acetic acid which might have directly or indirectly promoted the growth attributes. This finding is in confirmation with the results of Lucas-Garcia *et al.* (2004) in tomato and pepper, Seena (2006) in *Piper longum* and Govind (2005) in rice where *B. subtilis* promoted the plant growth.

PSB (T_5) as two time application was found to be effective in improving the number of primary branches. The growth promotion by PSB has been reported in various crops by various workers. The highest number of primary branches in bitter gourd due to inoculation of PSB may be because of the ability of PSB to solubilize and increase the availability of inorganic phosphorous from insoluble or other wise fixed form to soluble or readily available phosphorous (Bahadur *et al.,* 2004). The principle mechanism of PSB for mineral phosphorous solubilization is the production of organic acids and acid phosphates which play a major role in the mineralization of organic phosphorous in soil. Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization along with 2 keto gluconic acid, another organic acid identified in PSB strains with phosphorus solubilizing ability (Kevin, 2003). Anburani and Manivannan (2002) reported the increase in number of secondary branches in brinjal by PSB application. This result is also supported by the findings of Naidu *et al.* (2002) who has reported that the brinjal plants supplied with PSB produced the highest number of primary branches.

Plants treated with double application of *Azospirillum* (T_3) were statistically superior to all other treatments with a maximum tap root length (23.57cm) and secondary root length (39.88 cm) (Fig 2 and 3). There was an increase of 50.76% in tap root length and 97.62% in secondary root length over control. The result obtained is in confirmity with those of Sankaranarayanan *et al.* (1995); Thilakavathy and Ramaswamy (1999); Sajindranath *et al.* (2002); Muthuraj *et al.* (2002) and Bashan and de-Bashan (2004). Inoculation of seeds with *Azospirillum* results in growth promotion of roots, with increase in root length, number of root hairs, number of root branches and root surface area. These effects on roots are considered to be accounted for by bacterial production of IAA (Venkateswarulu and Rao, 1985). The most reproducible effect on plants inoculated with *Azospirillum* is greatly altered root architecture with increased overall root growth, greater production of root hairs and enhanced root surface area. These root alterations are most commonly seen at the seedling level and may or may not be present later in season (Kloepper, 2003). Bashan and de-Bashan, (2004) observed that gibberellins produced by *Azospirillum* have also been contributed to increased root hair development. Application of gibberellin increased density of root hairs at levels similar to *Azospirillum* inoculation.

With respect to the dry root weight one time application of *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) was found to be superior with a root weight of 4.644 g. It has been hypothesized for the mode of action of *Bacillus subtilis*, which acts as plant growth and health promoter and stress tolerance inducer, that the given bacterial production of auxin and auxin precursors during root colonization induces a push in the plant auxin synthesis with changing regulation of the appropriate mechanisms (Bochow *et al.,* 2001). These results are in conformity with the findings of Badawy *et al.* (2003) who has reported maximum dry root weight by *Bacillus subtilis* application in tomato. Inoculation of plants with *Bacillus subtilis* increased the cytokinin content of both shoots and roots. Accumulation of zeatin and its ribosids was the greatest in roots two days after inoculation, when their content was ten times higher than that in control. Changes in the content of other hormones (ABA and IAA) were observed at the end of experiment only. Accumulation of cytokinins in inoculated lettuce plants was associated with an increase in plant shoot and root weight. The increased root growth helps in enhanced assimilation of nutrients thus promoting more vegetative growth. Seena (2006) has reported maximum root length, fresh weight and dry weight of thippali by the application of *Bacillus subtilis*. Similar observation was recorded in a study conducted by Govind (2005), on the effect of *B. subtilis* in increasing the length and weight of roots in rice plants by Amruthesh *et al.* (2003) who have reported that *Bacillus subtilis* inoculation enhanced the emergence rate, germination percentage, plant height, fresh root weight and dry root weight of the chilli, brinjal and okra plants.

5.1.2 Flower characters

The effect of different treatments on days to first female flower and number of female flowers per plant are discussed here (Fig 4 and 5). Days to first female flower is an indication of earliness of the crop. Early crop fetches more returns to the farmers. In the present study there was significant difference among the treatments for the appearance of first female flower. The control plot produced first flower (43.93 days after sowing) which was on par with one time application of *Pseudomonas fluorescens* (T_6), Aishwarya (T_{10}) and PSB (T_4). The *Azospirillum* treated plants (T_2 and T_3) took maximum days for the appearance of first female flower (50.06 and 50.38 days respectively).

Number of female flowers can be taken as the indication of sex ratio in cucurbits especially bitter gourd. In the present study there was significant effect

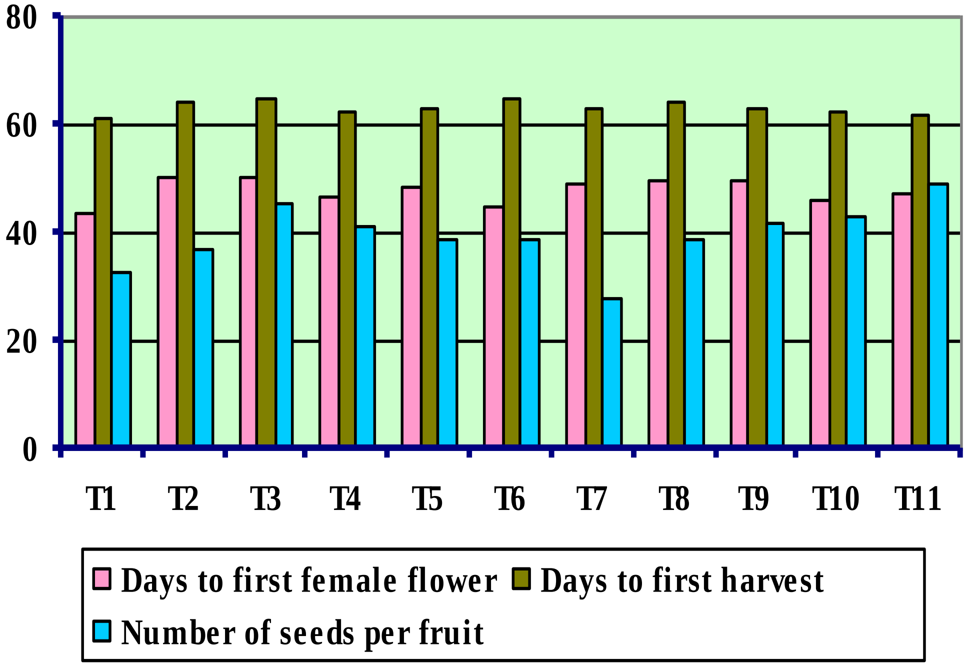

Fig 3: Effect of different treatments on days to first female flower, days to first harvest and number of seeds per fruits of bitter gourd

of application of different PGPR on the number of female flowers per plant. Maximum number of female flowers (17.50) was produced in two time application of *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9) which was on par with Aishwarya (T_{11}) and T_{10}, PSB (T_4) and *Azospirillum* (T_2). Minimum number of female flowers was produced in control (14.06). This finding is in conformity with that findings of Bonnie *et al.* (2000) who have reported the higher percentage of flowering in *Bacillus subtilis* treated tomato plants.

5.1.3 Biometrical characteristics

The influence of different treatments in improving the fruit characters of bitter gourd, in relation to number of fruits, fruit weight, fruit length, fruit girth, flesh thickness and number of seeds per fruit are discussed here (Fig 5, 6, and 7). With respect to the number of fruits per plant, treatment with two time application of Aishwarya (T_{11}) was found to be the best with 13.60 fruits $plant^{-1}$ followed by single application of *Pseudomonas fluorescens* (T_6) with 12.68 fruits. *Pseudomonas fluorescens* is known to influence plant growth by various mechanisms. Production of plant growth promoting substances, suppression of pathogens, nitrogen fixation and phosphorous solubilisation were attributed for plant growth promotion by *Pseudomonas fluorescens* (Pal *et al.*, 1999). The influence on number of fruits by *Pseudomonas fluorescens* has already been reported in various crops. Muthuraju *et al.* (2002) reported the production of more number of tomato fruits per plant by the plants supplied with *Pseudomonas fluorescens.*

Regarding fruit weight, fruit length and fruit girth, two time application of *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9) was found to be the best treatment with 223.49 g, 26.16 cm and 22.93 cm respectively. In case of average flesh thickness, double application of *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9), PSB (T_5) and Aishwarya (T_{11}) gave similar results with 0.73 cm. Research to elucidate mechanisms for growth promotion by *Bacillus subtilis* and

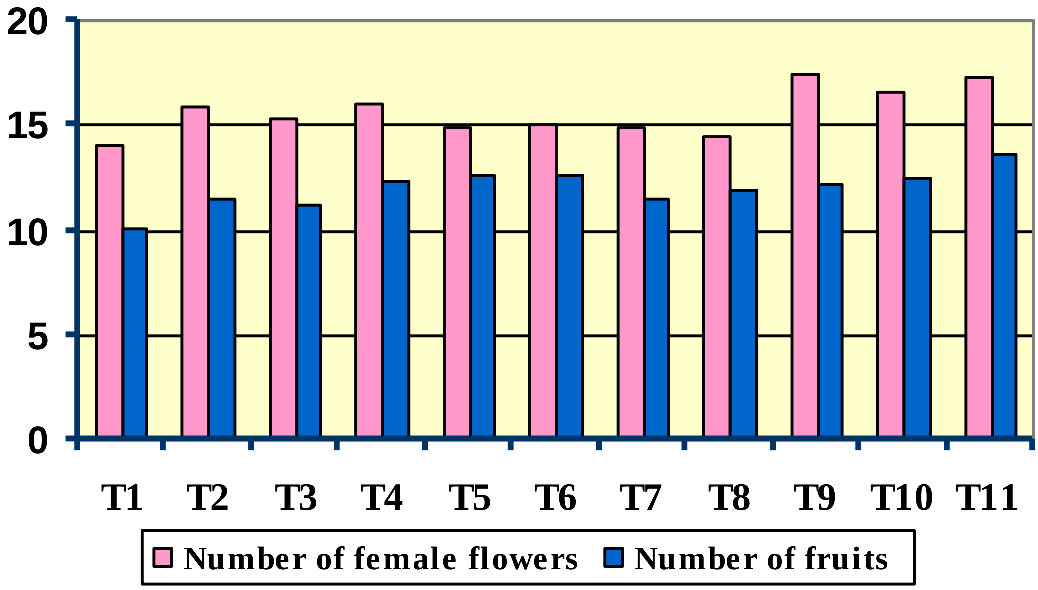

Fig 4: Effect of different treatments on number of female flower and number of fruits per plant of bitter gourd

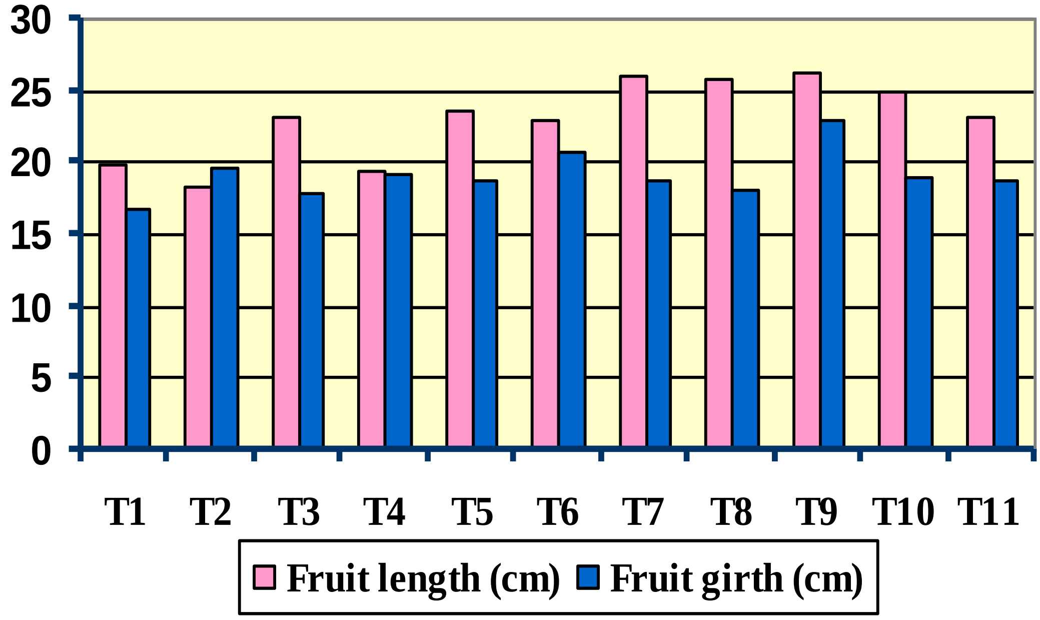

Fig 5: Effect of different treatments on fruit length and girth of bitter gourd.

B. amyloliquefaciens was reported by Kloepper (2003). *Bacillus subtilis and B. amyloliquefaciens* strains promoted plant growth by blend of volatile organic compounds. The role of volatiles in plant growth promotion was confirmed by several workers. In particular, the volatile compounds 2,3-butanediol and acetion, produced by both PGPR strains promoted growth of *Arabidopsis thaliana*. Volatile compounds other than ethylene have not previously been reported to increase plant growth. The discovery that specific volatile organic compounds produced by PGPR can promote plant growth opens a new research direction for their mode of action. Another reason that has been proposed for the promotion of plant growth by bacteria that colonize the rhizosphere is the production of phytohormones and phytohormonally active metabolites (Kloepper *et al.,* 1991). Berger *et al.* (1996) was able to show that the growth-promoting effect of culture filtrates of FZB24® *Bacillus subtilis* is not due to lipopeptides having an antibiotic action. This is supported by investigations with *Bacillus subtilis* mutants that no longer had the ability to form antibiotics, but still led to increased yields from peanut plants.

The influence of *Bacillus subtilis* on biometrical characteristics has already been reported in different crops by various workers. Devi *et al.* (2002) reported that plants supplied with *Bacillus polymyxa* had the highest fruit girth, fruit weight and number of fruits per plant. Similar results have been reported by Lucas-Garcia *et al.* (2004), who has reported that the tomato plants supplied with *Bacillus lichemaformis* recorded significantly increased number of fruits and diameter of fruits. Mashooda-Begum (2003) also got similar results in okra, where in *Bacillus pumilus* (SE-34), *B. pasteurii* (T4), *B. subtilis* (IN937-b) and *B. subtilis* (GBO3) strains significantly increased the biomass of plants, total number of leaves, fruits, mean length, girth, and biomass of the fruits. The findings of this experiment are also confirmed by de Freitas *et al.* (1997), who reported that the canola plants supplied with *Bacillus thuringiensis* increased the number and weight of pods.

With respect to the number of seeds per fruit, two time application of Aishwarya (T_{11}) was found to be the best treatment with 49.33 followed by *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9) with 45.33. This result confirmed the earlier reports of de Freitas *et al.* (1997), who reported that the canola plants inoculated with *Bacillus thuringiensis* had increased number and weight of pods and seed yield. The findings also supported by Mashooda-Begum (2003) who reported that the okra plants supplied with *Bacillus subtilis* had enhanced the total number of seeds per fruit and 1000-seed weight.

5.1.4 Yield characteristics

The yield plant^{-1} and yield plot^{-1} are discussed here (Fig 7 and 8). Among the different treatments both basal and 40 DAS application of *Bacillus subtilis* suspension (10^8 cfu/ml) (T_9) were found to be the best in increasing the yield plant^{-1} as well as yield plot^{-1} (2.72 kg and 16.33 kg respectively). Application of *Bacillus subtilis* suspension (10^8 cfu/ml) has shown positive effect on yield over the control (1.42 kg plant^{-1}). The yield increased to 1.72 kg plant^{-1} by the single basal application of *Bacillus subtilis* suspension (10^8 cfu/ml) (2 l plant $^{-1}$). It is also observed that additional application of *Bacillus subtilis* suspension (10^8 cfu/ml) (2 l plant $^{-1}$) at 40 DAS has got profound effect on yield which has increased up to 2.72 kg plant^{-1}. There was an increase of 21.12% (1.72 kg plant^{-1}) by the basal application and by the additional application of *Bacillus subtilis* suspension (10^8 cfu/ml) at 40 DAS there was an increase of 91.54% (2.72 kg plant^{-1}) (Plate vi and vii). This noticeable increase in yield might be due to the production of phytohormones such as zeatin, gibberellic acid and abscisic acid by *Bacillus subtilis* (Kilian *et al.*, 2000). Woitke *et al.* (2004) also observed that *Bacillus subtilis* isolate increased yield of plants in addition to inducing resistance to biotrophic fungal plant pathogens. Enhanced root growth is often accompanied by increased branching and a higher number of root tips. Their meristems are the most important sites for the synthesis of free cytokinins (Kilian *et al.*, 2000). These are presumably transported into the shoot via the xylem. Intensified and

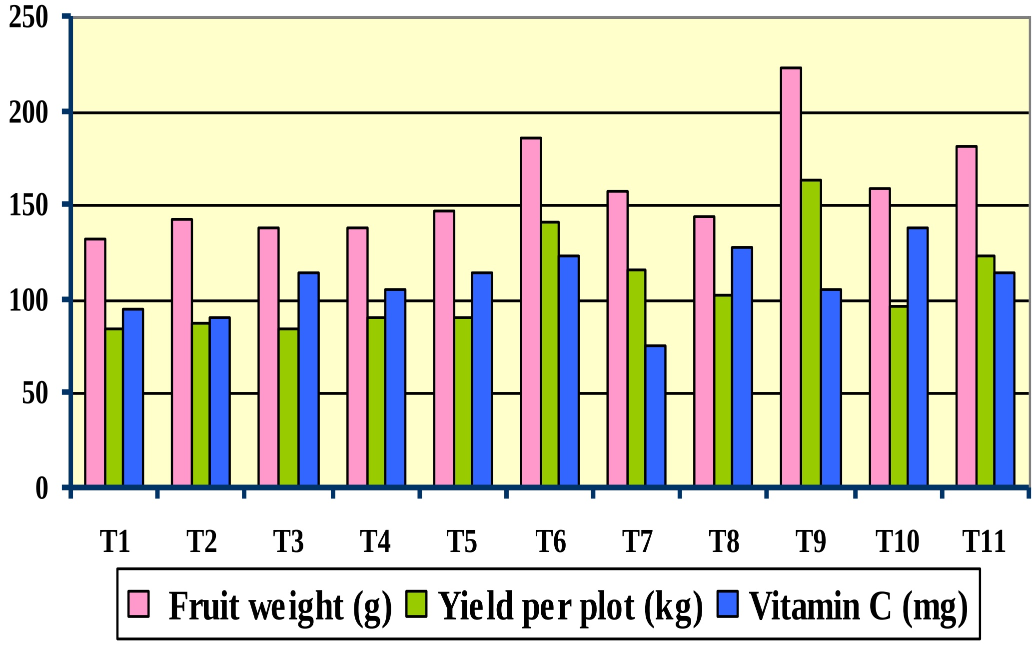

Fig 6: Effect of different treatments on fruit weight, yield per plot and vitamin C content of bitter gourd.

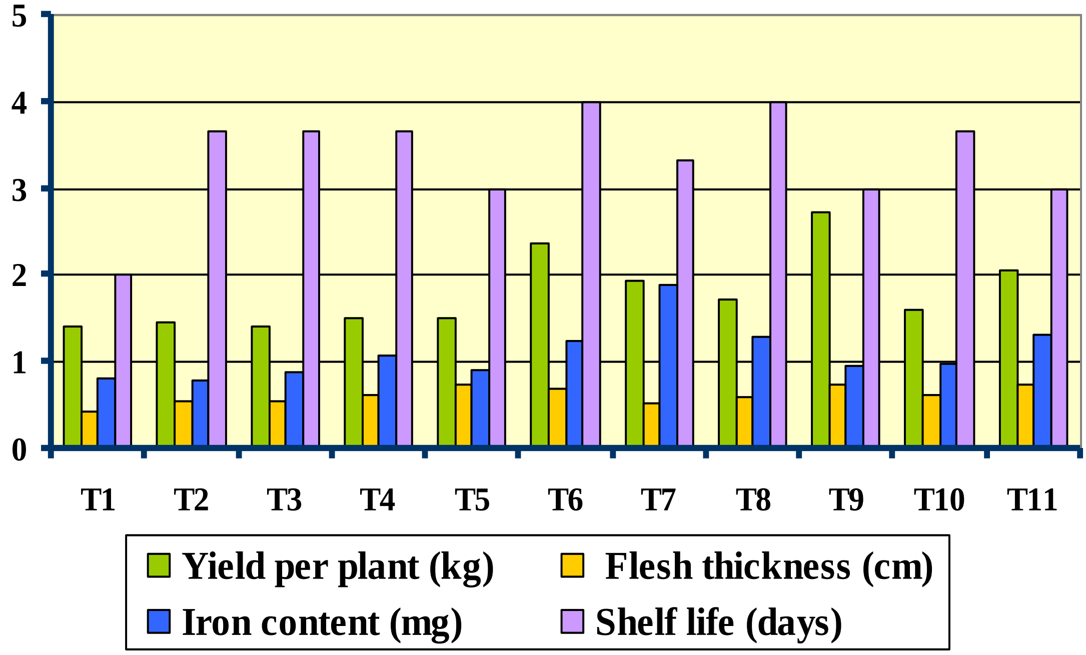

Fig 7: Effect of different treatments on yield per plant, flesh thickness, iron content and shelf life of bitter gourd.

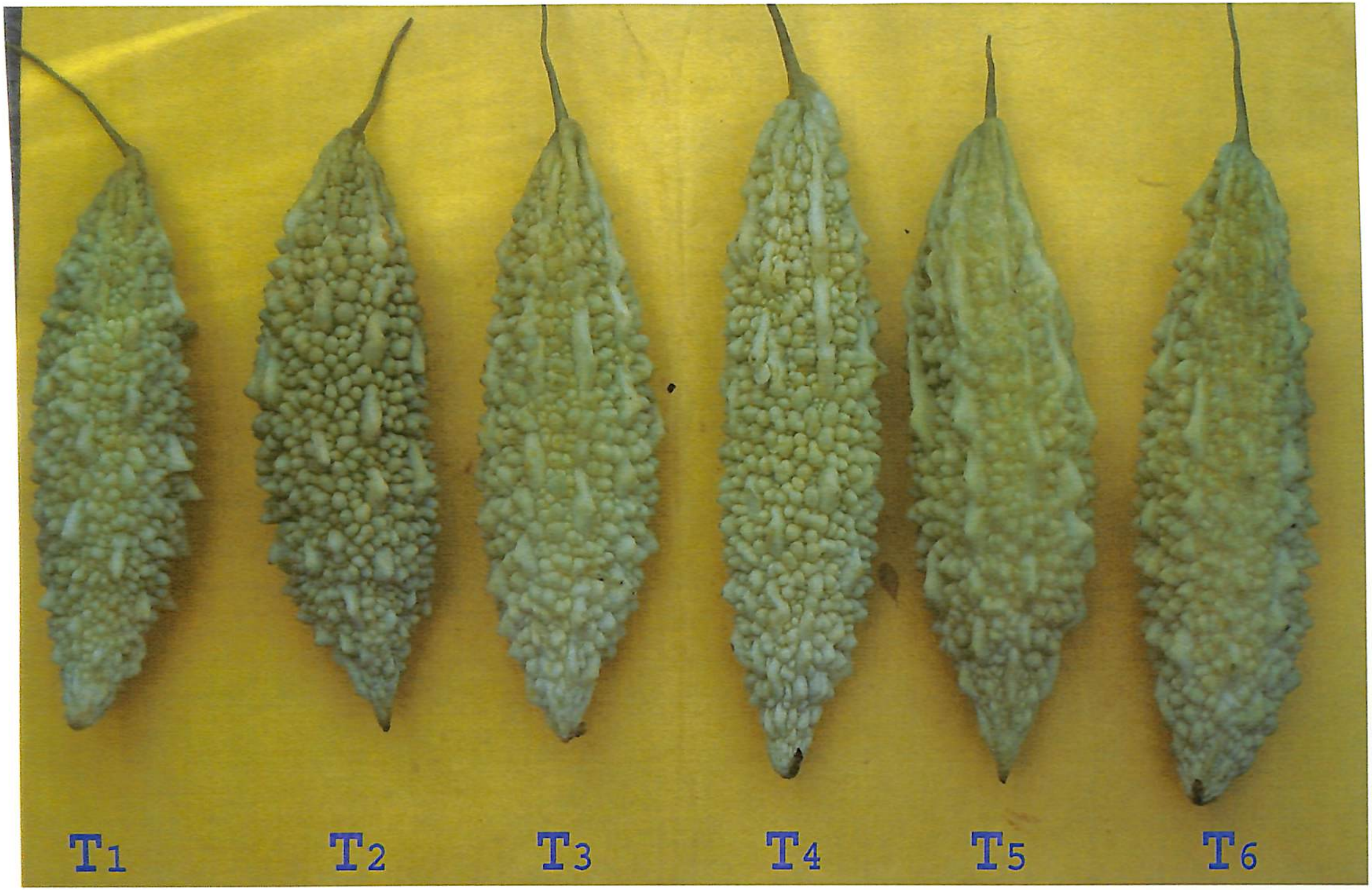

Plate 6. Comparison of fruits harvested from different treatments plots

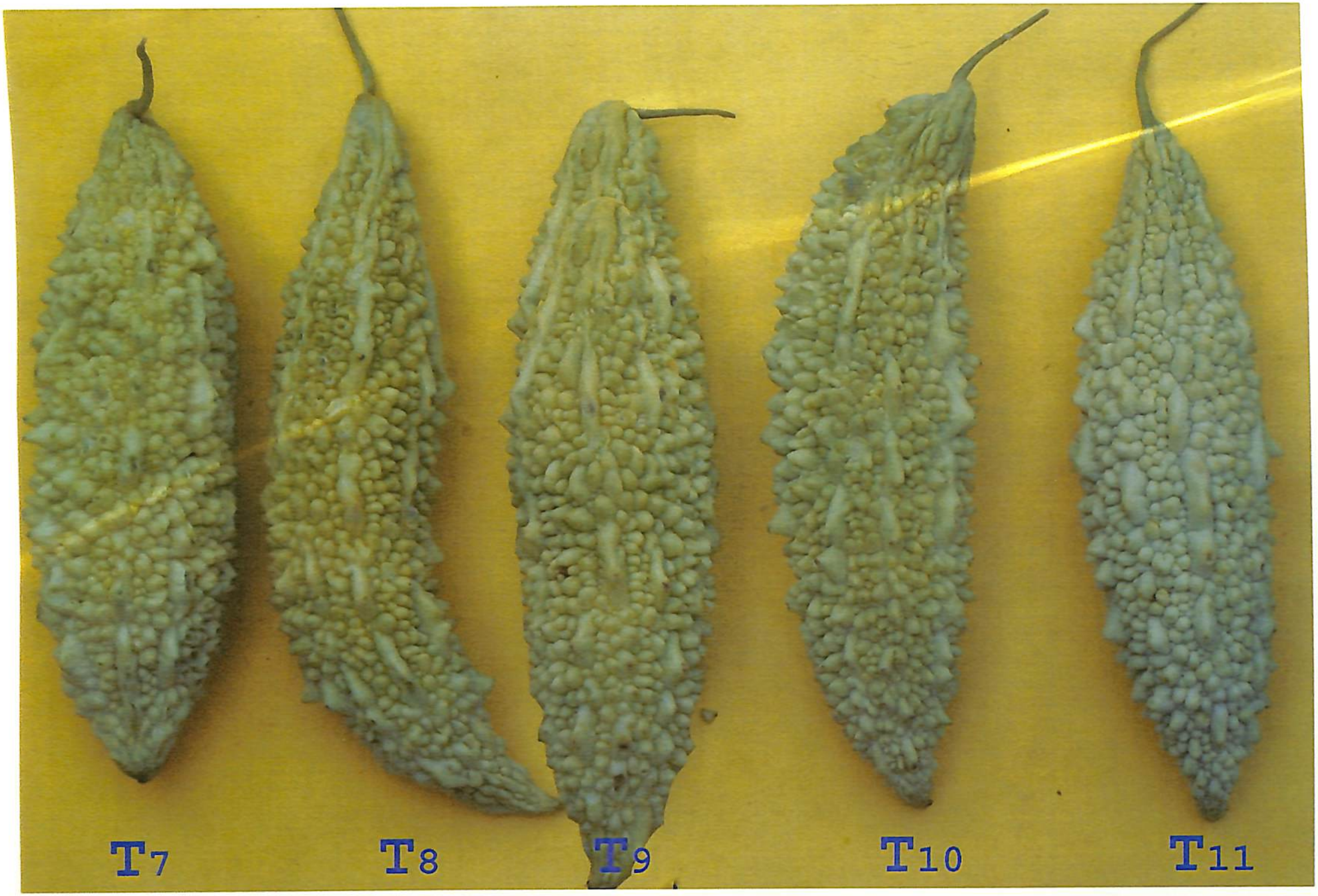

Plate 7. Comparison of fruits harvested from different treatments plots

prolonged synthesis of these phytohormones may be regarded as a cause of delayed senescence and improved yields (Kilian, *et al.,* 2000). Since the application of *Bacillus subtilis* leads to stronger root growth, there may also be an increased synthesis of plant cytokinins, which also cause delayed senescence and higher yields, as described above. The results of this experiment are supported by the findings of Yobo *et al.* (2004), who recorded significantly higher fruit yield in pepper by application of *Bacillus subtilis.* Omar and El-Kattan (2003) and Boehme *et al.* (2005) reported that cucumber plants inoculated with *Bacillus polymyxa* had positive effect on enzymatic activities of cucumber plants and had positive and significant effect on cucumber yield up to 25% more than control and produced good quality fruits.

5.1.5 Quality attributes

Bitter gourd is rich in vitamin C and iron content. Hence the effect of different PGPR treatments on quality characters as well as shelf life of fruits was also studied (Fig 7 and 8) and a significant response was noticed with PGPR inoculation. In the present investigation application of Aishwarya (T_{10}) 30 g plant^{-1} as basal increased the vitamin C content in bitter gourd fruits with 138.16 mg/100g followed by *Bacillus subtilis* suspension (10^8 cfu/ml) (T_8) with 128.50 mg/100g of fresh weight. There was an increase of 44.92% over control. The increase in vitamin C content might be due to growth promoting substances, which could have accelerated synthesis of carbohydrates, resulting in increase in vitamin C content which is a sugar acid (Kamali *et al.,* 2002). With respect to the iron content two time application of *Pseudomonas fluorescens* (T_7) was found to be the superior one with 1.889 mg/100g of fresh weight. Shelflife of the bitter gourd fruits got increased from 2 days to 4 days by the application of PGPR. Among the different PGPR, basal application of both PSB (T_6) and *Bacillus subtilis* (suspension (10^8 cfu/ml) (T_8) were found to be superior. This result confirmed the earlier report of Singh *et al.* (2003) who has reported the longer shelf life of vegetables at room temperature with the application of biofertilizers and dense organic manure.

Summing up the discussion so far, it can be concluded that the present study have enriched our knowledge on the application of different PGPR's in relation to growth and yield of bitter gourd. Among the different PGPR's *Bacillus subtilis* suspension (10^8 cfu/ml) performed well in improving various vegetative, biometrical and yield characteristics like vine length, dry root weight, average fruit weight, average fruit length, average fruit girth, average flesh thickness, number of female flowers, yield per plant, yield per plot and shelf life of fruits. From the present study it can be seen that application of *Bacillus subtilis* suspension (10^8 cfu/ml) 2 l/plant as basal dose and at 40 days after sowing has beneficial effects on growth and yield of bitter gourd.

Summary

VI. SUMMARY

A study entitled 'Effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of bitter gourd (*Momordica charantia* L.)' was carried out at Department of Olericulture, College of Horticulture, Vellanikkara during September, 2006 to January, 2007 to find out the effect of PGPR on growth parameters, biometrical parameters, yield characters and quality attributes of bitter gourd. The study resulted in the following findings.

1. Seed germination earliest in T_3 (*Azospirillum* @ 5 kg/ha basal + 40DAS) (6.48 days) followed by the T_7 (*Pseudomonas flourescens* @ 2.5 kg/ha basal + 40DAS) application (6.59 days).

2. Vine length 90 days after sowing was maximum (4.42 m) in both *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal) (T_8) and *Pseudomonas flourescens* @ 2.5/ha kg (basal) (T_6) applied plots.

3. The maximum number of primary branches (4.80) was produced in the plants applied with PSB @ 5 kg/ha (basal + 40DAS) (T_5) followed by the control.

4. Tap root length and secondary root length of bitter gourd were maximum (23.57 cm and 39.88 cm respectively) in plants applied with *Azospirillum* @ 5 kg/ha (basal + 40DAS) (T_3).

5. The highest dry root of bitter gourd (4.64 g) was recorded in plants treated with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal) (T_8) followed by the application of *Azospirillum* @ 5 kg/ha (basal) (T_3).

6. It was seen that there was significant difference among the different treatments for appearance of first female flower. It was the earliest in control (T_1) followed by *Pseudomonas flourescens* @ 2.5 kg/ha (basal + 40DAS) (T_6) application (43.93 and 44.95 days).

7. Plants applied with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal) (T_8) treatment had the highest (17.50) number of female flowers followed by the treatment T_{11} (Aishwarya @ 30 g/plant basal + 40DAS).

8. There was no significant difference among the treatments for days to first harvest. Among the different treatments days to first harvest was minimum (61.49 days) in control (T_1) followed by the treatment with Aishwarya @ 30 g/plant (basal + 40DAS) (T_{11}) application.

9. Maximum overall mean for number of fruits per plant (13.60) was recorded in plants applied with Aishwarya (T_{11}) @ 30 g/plant (basal + 40DAS) followed by the *Pseudomonas flourescens* @ 2.5 kg/ha (basal) (T_6) application (12.68). Number of seeds per fruit was also maximum (49.33) in plants treated with Aishwarya (T_{11}) @ 30 g/plant (basal + 40DAS) followed by *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal + 40DAS) (T_9) application (45.33).

10. Fruit weight, average fruit length and average fruit girth were maximum (223.49 g, 26.16 cm and 22.93 cm respectively) in the plants treated with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal + 40DAS) (T_9).

11. The maximum average flesh thickness was recorded in plants supplied with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal + 40DAS) (T_9),

PSB @ 5 kg/ha (basal + 40DAS) (T_5) and Aishwarya @ 30 g/plant (basal + 40DAS) (T_{11}) each with 0.73 cm.

12. There was a significant difference among the different treatments for yield per plant and yield per plot. Maximum yield per plant (2.72 kg) and yield per plot (16.33 kg) were produced in plants supplied with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal + 40DAS) (T_9) followed by the *Pseudomonas flourescens* @ 2.5 kg/ha (basal) (T_6) application (2.36 kg and 14.19 kg respectively).

13. Among the different treatments highest vitamin C content (138.16 mg) was recorded in the plants supplied with Aishwarya @ 30 g/plant (basal + 40DAS) (T_{10}) followed by the *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal) (T_8) application (128.50 mg).

14. Among the different PGPR applied the highest iron content (1.899 mg) was produced by the plants supplied with *Pseudomonas flourescens* @ 2.5 kg/ha (basal + 40DAS) (T_7) followed by Aishwarya @ 30 g/plant (T_{10}) application (1.307 mg).

15. Maximum shelf life of fruits under room temperature (4 days) was recorded in plants treated with *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant (basal) (T_8) and *Pseudomonas flourescens* @ 2.5 kg/ha (basal + 40DAS) (T_6).

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Appendix

APPENDIX –
General analysis of variance for vegetative characters in bitter gourd at different stages of growth

Source	df	Mean sum of squares					
		Days to germination	Vine length (90DAS)	Primary branches	Tap root length (cm)	Secondary root length (cm)	Dry weight of root (g)
Treatments	10	0.563	0.435**	0.624	18.386*	85.243	2.735
Error	20	0.425	0.110	0.723	7.794	55.243	1.181

** Significant at 1 per cent level

* Significant at 5 per cent level

APPENDIX – II

General analysis of variance for flower characters in bitter gourd at different stages of growth

Source	df	Mean sum of squares	
		Days to first female flower	Number of female flower
Treatments	10	12.747*	3.964**
Error	20	4.752	0.941

** Significant at 1 per cent level

* Significant at 5 per cent level

APPENDIX – III

General analysis of variance for biometrical characters of bitter gourd fruit

Source	df	Mean sum of squares						
		Days to first harvest	Number of fruits	Average fruit weight (g)	Average fruit length (cm)	Average fruit girth (cm)	Average flesh thickness (cm)	Number of seeds per fruit
Treatments	10	3.882	2.613	2248.664	22.456**	7.699**	0.028**	0.715
Error	20	6.822	1.232	132.314	3.249	1.379	0.004	0.366

** Significant at 1 per cent level

* Significant at 5 per cent level

APPENDIX – IV
General analysis of variance for yield parameters of bitter gourd

Source	df	Mean sum of squares	
		Yield per plant (g)	Yield per plot (g)
Treatments	10	557993.952	20050895.403
Error	20	29256.731	1045199.864

** Significant at 1 per cent level

* Significant at 5 per cent level

APPENDIX – V

General analysis of variance for quality parameters of bitter gourd

source	df	Mean sum of squares		
		Vitamin C (mg/100g)	Iron content (mg/100g)	Shelf life
Treatments	10	956.217*	0.308*	0.078**
Error	20	388.437	0.100	0.021

** Significant at 1 per cent level

* Significant at 5 per cent level

Abstract Of The Thesis

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) ON GROWTH AND YIELD OF ITTER GOURD
(*Momordica charantia* L.)

By

Naveen Kumar K.S.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirementfor the degree of

Master of Science in Horticulture

Faculty of Agriculture
Kerala Agricultural University

Department Of Olericulture
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR – 680 656
KERALA, INDIA

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ABSTRACT

Investigation on the effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of bitter gourd was carried out at Department of Olericulture, College of Horticulture, Vellanikkara, during September 2006 to January 2007 under field conditions. High yielding variety Preethi was used for the study.

The study revealed that seeds inoculated with *Azospirillum* (basal @ 5 kg ha^{-1} + 40 days after sowing (DAS) @ 5 kg ha^{-1}) recorded early germination (6.48 days) which was followed by the two time application of *Pseudomonas flourescens* (basal @ 2.5 kg ha^{-1} + 40 DAS @ 2.5 kg ha^{-1}). The maximum vine length (4.42 m) was recorded in both *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l/plant application (basal) and *Pseudomonas flourescens* @ 2.5 kg ha^{-1}. Number of primary branches was maximum (4.80) in plants applied with phosphorous solubilising bacteria (PSB) as basal @ 5 kg/ha and 40 DAS @ 5 kg/ha. Tap root length (23.57 cm) and secondary root length (39.88 cm) were highest in the plants supplied with *Azospirillum* (basal @ 5 kg ha^{-1} + 40 DAS @ 5 kg ha^{-1}) where as, dry root weight (4.64 g) was more in case of double application of *Bacillus subtilis* suspension (10^8 cfu/ml) basal @ 2 l plant $^{-1}$ + 40 DAS @ 2 l plant $^{-1}$.

Control plants were the earliest to flower and fruit. Plants inoculated with basal dose of *Bacillus subtilis* suspension (10^8 cfu/ml) produced the highest number of female flowers plant $^{-1}$ (17.50 flowers) than other treatments. Among the different treatments, double application of Aishwarya (basal @ 30 g plant^{-1} + 40 DAS @ 30 g plant^{-1}) recorded the highest number of fruits plant $^{-1}$ (13.60 fruits) and seeds plant $^{-1}$ (49.33 seeds) respectively which was followed (12.68 fruits and 45.33 seeds) by the inoculation of *Pseudomonas flourescens* (basal @ 2.5 kg/ha) and *Bacillus subtilis* suspension (10^8 cfu/ml) (basal @ 2 l plant $^{-1}$ + 40 DAS @ 2 l plant $^{-1}$) respectively. Plants treated with *Bacillus subtilis* (suspension (10^8 cfu/ml) basal + 40 DAS @ 2 l plant $^{-1}$ recorded maximum fruit weight, length

and girth (223.49 g, 26.16 cm and 22.93 cm respectively). Flesh thickness (0.73 cm) was highest in double application (basal @ 2 l plant $^{-1}$ + 40 DAS @ 2 l plant $^{-1}$) of the *Bacillus subtilis*, PSB and Aishwarya (basal @ 30 g plant^{-1}+ 40 DAS @ 30 g plant^{-1}) treatments.

There was a highly significant difference between all the treatments under study. Two time application of *Bacillus subtilis* suspension (10^8 cfu/ml) basal + 40 DAS @ 2 l plant $^{-1}$ produced the maximum yield plant $^{-1}$ and yield plot $^{-1}$ (2.72 kg and 16.33 kg respectively), which was followed by the single time application of *Pseudomonas flourescens* as basal @ 2.5 kg ha^{-1} (2.36 kg ha^{-1} and 14.19 kg ha^{-1}).

Maximum vitamin C content was recorded in the plants subjected to basal application of Aishwarya @ 30 g plant^{-1} (138.16 mg/100g) followed by single application of *Bacillus subtilis* as basal dose. Maximum iron content was recorded (1.89 mg/100g) in plants supplied with *Pseudomonas flourescens* (basal @ 2.5 kg ha^{-1} + 40 DAS @ 2.5 kg ha^{-1}). Storage time (4 days) was more for the plants treated with one time application of *Bacillus subtilis* suspension (10^8 cfu/ml) @ 2 l plant $^{-1}$ and *Pseudomonas flourescens* as basal @ 2.5 kg ha^{-1}.

It can be inferred that inoculation of bitter gourd with plant growth promoting rhizobactreia (PGPR) enhanced its growth, yield and quality attributes. Among the different PGPR studied, *Bacillus subtilis* suspension (10^8 cfu/ml) with two time application first as basal @ 2 l plant $^{-1}$ and second at 40 DAS @ 2 l plant $^{-1}$ performed best with respect to yield, flower and biometrical characters. The next best treatments were two time application of Aishwarya, *Pseudomonas flourescens*, *Azospirillum* and PSB respectively.