EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON GERMINATION, ANTIOXIDANT ENZYMES AND FRUIT QUALITY IN BRINJAL (*Solanum melongena* L.)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

INTEGRATED MASTER OF SCIENCE (HONS.)
in
BIOCHEMISTRY
(Minor Subject: Microbiology)

By

Karanpal Kaur
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LUDHIANA-141 004

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CERTIFICATE I

This is to certify that the thesis entitled, **"Effect of plant growth promoting rhizobacteria on germination, antioxidant enzymes and fruit Quality in brinjal (*Solanum melongena* L.)"** submitted for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Biochemistry** (Minor subject: **Microbiology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Karanpal Kaur (L-2010-BS-32-IM)** under my supervision and that no part of thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

Dr. (Mrs.) Neena Chawla
(Major Advisor)
Senior Biochemist,
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CERTIFICATE II

This is to certify that the thesis entitled, **"Effect of plant growth promoting rhizobacteria on germination, antioxidant enzymes and fruit Quality in brinjal (*Solanum melongena* L.)"** submitted by **Karanpal Kaur (L-2010-BS-32-IM)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Integrated Master of Science (Hons.)**, in the subject of **Biochemistry** (Minor subject: **Microbiology**) has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

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ABSTRACT

In the present study, the effect of PGPR was observed on germination, antioxidant enzymes and fruit quality in brinjal (*Solanum melongena* L.). Shoot length (46.37cm) and root length (51.07cm) were observed to be maximum with PGPR7. Treatment with PGPR2 showed maximum percentage germination (90.00%). PGPR9 showed maximum invertase activity in roots (19.23 µmole glucose/h/g FW), peroxidase activity (7.80change in absorbance/min/g FW), nitrogen content (2.85±0.09%), starch content (4.22±0.22%) and sucrose synthase activity (2.46±0.10 µg sucrose/min/mg FW). Seedlings treated with PGPR7 showed enhanced activity of superoxide dismutase (94.83 units/min/mg FW), amylase (0.42±0.42 mg maltose/mg protein/h), catalase (62.56 µmole H_2O_2/min/mg FW) and DPPH (94.80%). The maximum average fruit weight, plant height and number of fruits per plant were observed with PGPR7, PGPR2 and PGPR7 respectively. The treatment with PGPR10 showed more dry matter content (10.01±0.01%). The ortho-dihydroxy phenols varied from 11.40 to 70.78 mg/100g. The treatment with PGPR7 showed highest flavonol content (148.85±0.26 mg/100g), reducing sugars (8.78%), non-reducing sugars (8.78%) and proteins (3.64±0.07%). The total phenols content ranged from 22.62-234.46 mg/100g. It was observed that the treatment with PGPR6 gave maximum Zinc content (0.32±0.02%) and PGPR7 showed maximum iron content (0.76±0.76%) and maximum invertase activity in fruits (29.85±0.32 µmole glucose/h/g FW). Anthocyanin content was found in the range of 86.56 to 104.50mg/100g. It was concluded that the treatment given with PGPR7 led to higher activities of antioxidant enzymes and other plant growth parameters of brinjal (PBHL 51).

Keywords: *Solanum melongena* L., plant growth promoting rhizobacteria (PGPR), antioxidant enzymes, antioxidant capacity, phenols

Signature of Major Advisor **Signature of Student**

ਖੋਜ ਗ੍ਰੰਥ ਦਾ ਸਿਰਲੇਖ	:	ਬੈਂਗਣ (ਸੋਨੇਨਮ ਮੈਲੋਂਜਨਾ) ਵਿੱਚ ਅੰਕੁਰਣ, ਐਂਟੀਆਕਸੀਡੈਂਟ ਐਨਜ਼ਾਈਮਾਂ ਅਤੇ ਫਲਾਂ ਦੀ ਗੁਣਵੱਤਾ ਉੱਪਰ ਪਲਾਂਟ ਗ੍ਰੋਥ ਪ੍ਰਮੋਟਿੰਗ ਰਾਈਜ਼ੋਬੈਕਟੀਰੀਆ ਦਾ ਪ੍ਰਭਾਵ
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਮ ਅਤੇ ਦਾਖਲਾ ਨੰਬਰ	:	ਕਰਨਪਾਲ ਕੌਰ (ਐਲ-2010-ਬੀਐਸ-32-ਆਈਐਮ)
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਜੀਵ ਰਸਾਇਣ ਵਿਗਿਆਨ
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ਸਾਰ-ਅੰਸ਼

ਇਸ ਖੋਜ ਅਧਿਐਨ ਵਿਚ ਪੀ.ਜੀ.ਪੀ.ਆਰ. ਦਾ ਬੈਂਗਣ ਦੇ ਬੂਟਿਆਂ ਦੇ ਅੰਕੁਰਣ, ਐਂਟੀਓਕਸੀਡੈਂਟ ਐਨਜ਼ਾਈਮ ਅਤੇ ਫਲਾਂ ਦੀ ਗੁਣਵੱਤਾ ਉੱਪਰ ਅਸਰ ਵੇਖਿਆ ਗਿਆ। ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 ਨਾਲ ਟਾਹਣੀ ਦੀ ਲੰਬਾਈ 46.3 ਸੈ.ਮੀ. ਅਤੇ ਜੜ੍ਹ ਦੀ ਲੰਬਾਈ 51.07 ਸੈ.ਮੀ. ਸਭ ਤੋਂ ਵੱਧ ਪਾਈ ਗਈ, ਜਦਕਿ ਅੰਕੁਰਣ ਦੀ ਪ੍ਰਤੀਸ਼ਤ (90.00%) ਸਭ ਤੋਂ ਵੱਧ ਪੀ.ਜੀ.ਪੀ.ਆਰ-2 ਨਾਲ ਵੇਖੀ ਗਈ। ਪੀ.ਜੀ.ਪੀ.ਆਰ-9 ਨਾਲ ਜੜ੍ਹਾਂ ਵਿੱਚ ਐਨਜ਼ਾਈਮਾਂ ਦੀ ਗਤੀਵਿਧੀ ਹੇਠ ਲਿਖੀ ਦਰ 'ਤੇ ਪਾਈ ਗਈ: ਇਨਵਰਟੇਜ਼ (19.23 ਮਾਈਕ੍ਰੋਮੋਲ ਗਲੂਕੋਜ਼/ਘੰਟਾ/ਗ੍ਰਾਮ), ਪਰਆਕਸੀਡੇਜ਼ (7.80 ਯੂਨਿਟ ਓ.ਡੀ. ਵਿੱਚ ਤਬਦੀਲੀ/ਮਿੰਟ/ਗ੍ਰਾਮ), ਸੂਕਰੋਜ਼ ਸਿੰਨਥੇਜ਼ (2.46±0.10 ਮਾਈਕ੍ਰੋਗ੍ਰਾਮ ਸੂਕਰੋਜ਼/ਮਿੰਟ/ਮਿ.ਗ੍ਰਾਮ) ਜਦਕਿ ਨਾਈਟ੍ਰੋਜਨ ਅਤੇ ਸਟਾਰਚ ਦੀ ਮਾਤਰਾ 2.85±0.09% ਅਤੇ 4.22±0.22% ਕ੍ਰਮਵਾਰ ਪਾਈ ਗਈ। ਬੂਟਿਆਂ ਦੇ ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 ਨਾਲ ਛਿੜਕਾਉ ਕਰਨ 'ਤੇ ਸੁਪਰਆਕਸਾਈਡ ਡਿਸਮਿਊਟੇਜ਼ (94.83 ਯੂਨਿਟ/ਮਿੰਟ/ਮਿ.ਗ੍ਰਾਮ), ਅਮਾਈਲੇਜ਼ (0.42±0.42 ਮਿ.ਗ੍ਰਾਮ ਮਾਲਟੋਜ਼/ਮਿ.ਗ੍ਰਾਮ ਪ੍ਰੋਟੀਨ/ਘੰਟਾ), ਕੈਟਾਲੇਜ਼ (62.56 ਮਾਈਕ੍ਰੋਮੋਲ ਹਾਈਡ੍ਰੋਜਨ ਪਰਆਕਸਾਈਡ/ਮਿੰਟ/ਮਿ.ਗ੍ਰਾਮ) ਅਤੇ ਡੀ.ਪੀ.ਪੀ.ਐਚ ਰੈਡੀਕਲ ਨੂੰ ਘਟਾਉਣ ਦੀ ਖੂਬੀ 94.80% ਵੱਧ ਵੇਖੀ ਗਈ। ਸਭ ਤੋਂ ਵੱਧ ਔਸਤ ਫਲ ਦਾ ਭਾਰ, ਬੂਟੇ ਦੀ ਲੰਬਾਈ ਅਤੇ ਫਲਾਂ ਦੀ ਗਿਣਤੀ ਪ੍ਰਤੀ ਬੂਟਾ ਪੀ.ਜੀ.ਪੀ.ਆਰ-7, ਪੀ.ਜੀ.ਪੀ.ਆਰ-2 ਅਤੇ ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 (ਕ੍ਰਮਵਾਰ) ਵਿੱਚ ਵੇਖੀ ਗਈ। ਪੀ.ਜੀ.ਪੀ.ਆਰ-10 ਦੀ ਵਰਤੋਂ ਨਾਲ ਸੁੱਕਾ ਮਾਦਾ (10.01±0.01%) ਵੱਧ ਪਾਇਆ ਗਿਆ। ਔਰਥੋਡਾਈਹਾਈਡਰਾਕਸੀ ਫ਼ਿਨੋਲ ਦੀ ਮਾਤਰਾ 11.40 ਤੋਂ 77.78 ਮਿ.ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਦੇ ਵਿਚਕਾਰ ਰਹੀ। ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 ਦੀ ਵਰਤੋਂ ਨਾਲ ਫ਼ਲੇਵਾਨੌਲ (148.85±0.26 ਮਿ.ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ), ਰਿਡਿਊਸਿੰਗ ਸ਼ੂਗਰ (8.36%), ਨਾਨ-ਰਿਡਿਊਸਿੰਗ ਸ਼ੂਗਰ (8.78%) ਅਤੇ ਪ੍ਰੋਟੀਨ (3.64%) ਦੀ ਮਾਤਰਾ ਵਿਚ ਵਾਧਾ ਹੋਇਆ। ਕੁਲ ਫ਼ਿਨੋਲ ਦੀ ਮਾਤਰਾ 22.62-234.46 ਮਿ.ਗ੍ਰਾਮ ਜੀ.ਏ.ਈ/100 ਗ੍ਰਾਮ ਦੇ ਵਿਚਕਾਰ ਪਾਈ ਗਈ। ਇਹ ਵੇਖਣ ਵਿੱਚ ਆਇਆ ਕਿ ਪੀ.ਜੀ.ਪੀ.ਆਰ-6 ਦੀ ਵਰਤੋਂ ਨਾਲ ਜਿਸਤ (0.32%) ਅਤੇ ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 ਦੀ ਵਰਤੋਂ ਨਾਲ ਲੋਹੇ (0.76%) ਦੀ ਮਾਤਰਾ ਵਿਚ ਕਾਫੀ ਵਾਧਾ ਹੋਇਆ। ਨਾਲ ਹੀ ਫਲਾਂ ਵਿੱਚ ਇਨਵਰਟੇਜ਼ ਦੀ ਗਤੀਵਿਧੀ (29.85±0.32 ਮਾਈਕ੍ਰੋਮੋਲ ਗਲੂਕੋਜ਼/ਘੰਟਾ/ਗ੍ਰਾਮ) ਵੀ ਵੱਧ ਗਈ। ਐਂਥੋਸਾਈਨਿਨ ਦੀ ਮਾਤਰਾ 86.56-104.50 ਮਿ.ਗ੍ਰਾਮ/100 ਗ੍ਰਾਮ ਪਾਈ ਗਈ। ਇਸ ਖੋਜ ਨਾਲ ਇਹ ਨਤੀਜਾ ਨਿਕਲਿਆ ਕਿ ਪੀ.ਜੀ.ਪੀ.ਆਰ-7 ਦੀ ਵਰਤੋਂ ਨਾਲ ਬੈਂਗਣ (ਪੀ.ਬੀ.ਐਚ.ਐਲ-51) ਦੇ ਐਂਟੀਆਕਸੀਡੈਂਟ ਐਨਜ਼ਾਈਮਾਂ ਦੀ ਗਤੀਵਿਧੀ ਅਤੇ ਬੂਟੇ ਦੇ ਹੋਰ ਗੁਣਾਂ ਵਿਚ ਕਾਫੀ ਉੱਤਮਤਾ ਪ੍ਰਾਪਤ ਹੋਈ।

ਮੁੱਖ ਸ਼ਬਦ: ਸੋਲੇਨਮ ਮੈਲੋਂਜਨਾ, ਪੀ.ਜੀ.ਪੀ.ਆਰ, ਐਂਟੀਆਕਸੀਡੈਂਟ ਐਨਜ਼ਾਈਮ, ਐਂਟੀਆਕਸੀਡੈਂਟ ਸਮਰੱਥਾ, ਫ਼ੀਨੋਲ।

ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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LIST OF ABBREVIATIONS

%	–	Percentage
°C	–	Degree Centigrade
AA	–	Abscisic acid
AAS	–	Atomic absorption spectrophotometer
AOAC	–	Association of official analytical chemists
BNF	–	Biological nitrogen fixation
BSA	–	Bovine serum albumin
CAT	–	Catalase
cm	–	Centimetre
Conc.	–	Concentrated
DNA	–	Deoxyribonucleic acid
DW	–	Dry weight
FW	–	Fresh weight
g	–	Gram
GA	–	Gibberellic acid
GAE	–	Gallic acid equivalent
h	–	Hour
HCA	–	Hydroxycinnamic acid
IAA	–	Indole-3-acetic acid
Kcal	–	Kilocalorie
kg	–	Kilogram
l	–	Litre
M	–	Molar
mg	–	Milligram
min.	–	Minute
ml	–	Millilitre
mM	–	Millimolar
mol.	–	Mole

N	–	Normal
nm	–	Nanometre
PGPR	–	Plant growth promoting rhizobacteria
POX	–	Peroxidase
rpm	–	Revolutions per minute
SOD	–	Superoxide dismutase
spp.	–	Species
TPC	–	Total phenolic content
US	–	United States
USDA	–	United States department of agriculture
UV/VIS	–	Ultraviolet/Visible
V	–	Volume
W	–	Weight
µmole	–	Micromole

CHAPTER-I

INTRODUCTION

Brinjal or eggplant (*Solanum melongena* L.) is an important solanaceous crop of subtropics and tropics. The name Brinjal is popular in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. It is also called Aubergine in Europe.

The fruits of brinjal are an excellent remedy for liver problems. The green brinjal is good for diabetic patients (Shukla and Naik 1993). Brinjal is a warm season crop, therefore susceptible to severe frost. Low temperature during cool season causes deformation of fruits. A long and warm growing season is desirable for successful Brinjal production. Cool nights and short summers are unsuited to satisfactory yield. A daily mean temperature of 13 to 21 degree centigrade is favorable for optimum growth and yield (Kalloo *et al* 1990; Kumar *et al* 2000; Mohanty and Prusty 2000; Thapa 2002).

In hypercholesterolemic rabbits, the juice of brinjal reduces weight and plasma cholesterol content. According to USDA Nutrient database, the nutritional value of 100g of brinjal was estimated to contain 24 Kcal of energy, 5.7g carbohydrates, 0.19g fat, 1.01g proteins, 0.039 mg thiamin, 0.037 mg riboflavin, 0.0649 mg niacin, 0.281 mg pantothenic acid, 0.084 mg pyridoxine, 0.22 mg folate, 2.2 mg vitamin C, 9 mg calcium, 0.24 mg iron, 14 mg magnesium, 0.25 mg manganese, 25 mg phosphorus, 230 mg potassium and 0.16 mg zinc. The unripe fruit of brinjal is primarily used as a cooking vegetable for various dishes in different regions of the world. Brinjal is considered to be an important source of nutrition and cash income for many resource poor farmers (Bose *et al* 1993).

Plant growth promoting rhizobacteria (PGPR) are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or directly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of bio control agents. Various studies have documented the increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions (Ahemad and Kibret 2014).

Plant growth promoting rhizobacteria are beneficial bacteria which have the ability to colonize the roots and promote plant growth either through direct action or *via* biological

control of plant diseases (Kloepper and Schroth 1978). Strains with PGPR activity, belonging to genera *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and *Serratia* have been reported (Hurek and Reinhold-Hurek 2003). Among these, species of *Pseudomonas* and *Bacillus* are the most extensively studied (Kumar *et al* 2011). These bacteria competitively colonize the roots of plants and can act as biofertilizers and/or antagonists (biopesticides) or simultaneously both. These characteristics have highlighted the use of efficient microorganisms to improve plant growth and manage soil and plant health with the aim to achieve sustainability in agriculture, which amalgamates environmental health, economic feasibility and social equity ensuring long-term productivity of natural resources and improved livelihood.

Recently, plant growth-promoting rhizobacteria (PGPR)-mediated Induced Systemic Resistance (ISR) has received considerable attention which provides a highly effective defense response that is able to resist potential attack by microbial pathogens. Several mechanisms involved in the induction of systemic resistance include production of siderophores, salicylic acid, antibiotics and induction of pathogenesis related proteins (PR's). The production of enzymes related to pathogenesis (PR-proteins) such as chitinases and glucanases by strains of Rhizobacteria can lyse the fungal cell wall and are considered an important trait for antagonism (Saikia *et al* 2004). Apart from PR-proteins, the plants also produce other defense enzymes, including peroxidases. Metabolic changes involved in the defense mechanism of plants are correlated with changes in activity of key enzymes in primary and secondary metabolism. Peroxidase catalyses the formation of lignin (Figueiredo *et al* 2010).

With the aim to maximize crop productivity, chemical fertilizers are being used at an unprecedented rate posing grave threats to the inborn soil fertility which has reached a plateau from where fertility status could not be increased even by increasing their dosage (Kumar *et al* 2010). The emerging demand for chemical free food and the necessity to achieve sustainability in agriculture within a holistic vision to focus on environmental protection has encouraged exploitation of soil microorganisms for improving the crop health. Use of microorganisms presents an economically feasible and ecologically sound practice to enhance crop yield. Some of the mechanisms which are active simultaneously or sequentially at different stages of plant growth include mineral nutrient solubilization and increased nutrient availability to plants, nitrogen fixation; repression of soil-borne pathogens (by the production of hydrogen cyanide, siderophores, lytic enzymes, antibiotics, and/or competition for nutrients); improving plant tolerance to abiotic stresses by producing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants; production of phytohormones such as indole-3-acetic acid (IAA) and gibberellins (Gupta *et al* 2000). Thus,

PGPRs offer dual benefits by acting as potential bio fertilizers and bio control agents which would help to resolve the problem of enhancing crop productivity while retaining environmental soundness.

Objectives

To study effect of different PGPRs on:

- Germination in Brinjal.
- Antioxidant enzymes in roots and leaves of Brinjal at different stages of growth.
- Plant growth parameters, antioxidant capacity and fruit quality in Brinjal.

CHAPTER-II

REVIEW OF LITERATURE

Eggplant (*Solanum melongena* L.) is a plant native to India and many cultivars exhibiting different size, shape and color are cultivated in tropical, subtropical and temperate zones. Its fruit commonly known as aubergine, melanzana, garden egg or brinjal, also has same name and is widely used as vegetable in cooking. The most widely cultivated varieties are elongated, ovoid or slender type with dark purple skin. Eggplant or brinjal has a very low calorie value and a healthy nutrient profile. Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents (Cao *et al* 1996). Methanolic extracts (80%) of various parts (green crown, peel and flesh) of selected varieties of round and long aubergine were analyzed for total phenolic content (TPC) and antioxidant activity was measured using a number of colorimetric assays. The results showed that TPC methanolic extracts of different parts of selected varieties of aubergine, ranged from 16.72-25.00 mg GAE/100 g DW. The highest amounts (22.05-25.00 mg GAE/100 g DW) were obtained in round aubergine extracts and lower in long aubergine extracts (16.72-20.42 mg GAE/100 g DW).

PGPR affect plant growth through direct promotion by producing and secreting plant growth promoting substances or by eliciting root metabolic activities by supplying biologically fixed nitrogen and through indirect promotion by acting against phytopathogenic microorganisms. Moreover, the PGPR produced abundant exopolysaccharides (EPS) which reduce the toxic ion uptake including sodium and produce stress specific proteins in plants under salt stress (Geddie and Sutherland 1993, Nadeem *et al* 2006).

The present studies were undertaken to investigate the effect of Plant Growth Promoting Rhizobacteria (PGPR) on germination, antioxidant enzymes and fruit quality in brinjal. Pertinent literature is reviewed under the following sections:

2.1 The mechanisms PGPRs use to promote plant growth:

2.1.1 Mode of action of PGPR as bio fertilizers:

2.1.1.1 Significance of biological nitrogen fixation by PGPR

2.1.1.2 PGPR that increase the availability of nutrients in the rhizosphere

2.1.1.3 Production of phytohormones and regulation of ethylene levels in plant

2.1.1.4 Stimulation of legume–rhizobia symbioses

2.2 Mode of action of PGPR as bio control agents

2.3 Antioxidant enzymes

2.3.1 Peroxidase

2.3.2 Superoxide dismutase

2.3.3 Catalase

2.3.4 Amylase

2.3.5 Invertase

2.3.6 Sucrose synthase

2.4 Phenolic compounds

2.4.1 Phenolic compounds of *Solanum melongena* L.

2.5 Biochemical components

2.5.1 Anthocyanins

2.5.2 Other biochemical quality parameters

2.1 The mechanisms PGPRs use to promote plant growth

The mechanisms PGPRs use to promote plant growth are not fully understood, but they can be classified in four groups: bio fertilizers (solubilisation of mineral phosphates, asymbiotic N_2 fixation) (Salantur *et al* 2006, Cattelan *et al* 1999), phytostimulators (Egamberdiyeva, 2007), rhizoremediators (degrading organic pollutants) and bio pesticides (production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds). (Somers and Srinivasan 2004, Ahmad *et al* 2006, Bharathi *et al* 2004, Jeun *et al* 2004).

2.1.1 Mode of action of PGPR as bio fertilizers

Microorganisms having mechanisms that assist nutrient uptake or increase nutrient availability or stimulate plant growth are commonly referred to as biofertilizers. Biofertilizers are considered as an alternative or complement to chemical fertilization to increase the production of crops in low input agricultural systems. The means by which PGPR enhance the nutrient status of host plants can be categorized into four areas: (1) biological N_2 fixation, (2) increasing the availability of nutrients in the rhizosphere, (3) inducing increases in root surface area, (4) enhancing other beneficial symbioses of the host (Vessey 2003).

2.1.1.1 Significance of biological nitrogen fixation by PGPR

Nitrogen (N) is one of the major plant nutrients, and its low availability due to the high losses by emission or leaching is a limiting factor in agricultural ecosystems, hence the ability of some microorganisms to make atmospheric nitrogen available for plants by biological nitrogen fixation is of great importance (Martinez-Viveros *et al* 2010). Biological nitrogen fixation is synonymous with sustainability. Advances in agricultural sustainability will require an increase in the utilization of BNF as a major source of nitrogen for plants. The most studied and longest exploited PGPR (Plant growth promoting rhizobacteria) are the rhizobia (including the *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*) for their ability to fix atmospheric nitrogen in their legume

hosts (Vessey 2003). Non-symbiotic BNF carried out by free living diazotrophics can stimulate non-legume plants growth (Antoun *et al* 1998). There are studies showing that N-fixing bacteria, free-living as well as *Rhizobium* strains, can stimulate the growth of non-legumes such as radish (Antoun *et al* 1998) and rice (Mirza *et al* 2006), in this way contributing to reduced dependence on N-based fertilizers (Bhattacharjee *et al* 2008). Non-symbiotic N-fixing rhizospheric bacteria belonging to genera including *Azoarcus*, *Azospirillum*, *Burkholderias*, *Gluconacetobacter* and *Pseudomonas* have been isolated from different soils (Martinez-Viveros *et al* 2010).

The high energy requirement for N fixation and relatively low metabolic activity of free living organisms that compete for root exudates outside a nodule environment, has limited the ability of non-symbiotic bacteria to fix significant quantities of N. The presence of a diazotrophic bacterium in the rhizosphere of a certain plant is no longer considered to imply that such bacteria make a substantial contribution to N fixation and N supply for plant growth. Although the N fixing capacity of certain bacteria can easily be demonstrated under *in vitro* conditions, its demonstration in greenhouse and field studies is more complex and highly variable. Some observations suggest that Rhizobacteria can provide crops with significant quantities of N (Dobbelaere *et al* 2003). For example, associative symbioses between sugarcane and endophytic diazotrophs can contribute 20–60% of N requirements of the crop from the microsymbiont (Boddey *et al* 2001). In particular, *Gluconacetobacter diazotrophicus* has been shown to contribute significantly to the N-nutrition of sugarcane under controlled conditions (Sevilla *et al* 2001). Another N_2-fixing endophytic species of sugarcane, *Herbaspirillum seroepdicae*, has been shown to infect rice and result in increased $^{15}N_2$ incorporation (James *et al* 2002).

2.1.1.2 PGPR that increase the availability of nutrients in the rhizosphere

There is ample evidence that the mode of action of many PGPRs is by increasing the availability of nutrients for the plant in the rhizosphere. The mechanisms by which this happens involves solubilization of unavailable forms of nutrients and/or siderophore production which helps facilitate the transport of certain nutrients (notably ferric iron) (Rodriguez and Fraga 1999).

2.1.1.3 Production of phytohormones and regulation of ethylene levels in plant

The production of phytohormones by PGPR is one of the most important mechanisms by which many Rhizobacteria promote plant growth, these are signal molecules acting as chemical messengers and play a fundamental role as growth and development regulators in the plants. In most cases, these phytohormones are believed to assimilate partitioning patterns in plants and affect growth patterns in roots to result in bigger, more branched roots, and/or roots with greater surface area.

Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement (Salisbury 1994). Most commonly, IAA-producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from the soil. The reporting of root length and root surface area are important because increase in these parameters is more reflective of an increase in the volume of soil explored, than that which would be indicated by just increases in root weight. Tsavkelova *et al* (2007) reported an increase in the germination of orchid seeds (*Dendrobiummo schatum*) inoculated with *Sphingomonas* spp. and IAA producing *Mycobacterium* spp.

Production of other phytohormones by biofertilizing PGPR has been identified, but not nearly to the same extent as bacteria which produce IAA. Cytokinins are a class of phytohormones which are known to promote cell division, cell enlargement and tissue expansion in certain plant parts. Gibberellins (gibberellic acid; GA) are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury 1994). Evidence of GA production by PGPR is rare; however Gutierrez-Manero *et al* (2001) provided evidence that four different forms of GA were produced by *Bacillus pumilus* and *Bacillus licheniformis*.

Ethylene is essential for the growth and development of plants, but among its myriad of effects, it can elicit varied responses on plant growth depending on its concentration in root tissues. At high concentrations, it induces defoliation and cellular processes that lead to inhibition of stem and root growth as well as premature senescence, all of which lead to reduced crop performance (Ramamoorthy *et al* 2002). Under different types of environmental stresses, such as cold, draught, flooding, infection with pathogens, presence of heavy metals, plants respond by synthesizing 1-aminocyclopropane-1- carboxylate (ACC), which is the precursor for ethylene (Chen *et al* 2002, Glick *et al* 2007). Some of the ACC is secreted into the rhizosphere and is reabsorbed by the roots, where it is converted into ethylene. This accumulation of ethylene leads to a downward spiral effect, as poor root growth leads to a diminished ability to acquire water and nutrients, which, in turn leads to further stress. Thus, PGPR with the ability to degrade ACC in the rhizosphere can help to break this downward cycle and reestablish a healthy root system that is needed to cope with environmental stress. There are reports of ACC-deaminase producing bacteria that can diminish or prevent some of the harmful effects of high ethylene levels (Glick *et al* 1998). The ACC deaminase acts on ACC, an immediate ethylene precursor in higher plants, degrading this chemical to alpha keto butyrate and ammonium (Grichko and Glick 2001, Mayak *et al* 2004). Rhizosphere bacteria with ACC deaminase activity belonging to the *Achromobacter* (Govindasamy *et al* 2008),

Azospirillum (Ramamoorthy *et al* 2002), *Bacillus* (Ghosh *et al* 2003), *Enterobacter* (Ramamoorthy *et al* 2002), *Pseudomonas* (Govindasamy *et al* 2008) and *Rhizobium* (Duan *et al* 2009) genera have been isolated from different soils. Ghosh *et al* (2003) found ACC deaminase activity in three *Bacillus* species (*Bacillus circulans* DUC1, *Bacillus firmus* DUC2 and *Bacillus globisporus* DUC3), which stimulated root elongation of *Brassica campestris* plants. Mayak *et al* (2004) evaluated tomato plants inoculated with the bacterium *Achromobacter piechaudiis* under water and saline stress conditions.

As stated above, IAA production in PGPRs had been identified for a long time as a mode of action on the promotion of the growth of host plants. However, the more recent discoveries of the involvement of cytokinins, ACC deaminase, and possibly gibberellic acid producing PGPRs open the possibility that even more plant growth-regulating substances may be involved in the promotion of plant growth by some PGPRs. Many 'newer' plant growth-regulating substances have been identified in plants (e.g., brassino steroids and triacontanol (Salisbury 1994). Undoubtedly more plant growth-regulating substances have yet to be discovered. It is likely that the mode of action of currently identified and yet to be discovered PGPRs will involve production of substances which will mimic or influence the action of these newer plant growth-regulating substances.

2.1.1.4 Stimulation of legume–rhizobia symbioses

The biofertilizing PGPRs often act as 'helper bacteria' by promoting other host-symbiont relationship. There is evidence for a number of modes of action for PGPR stimulation of legume–rhizobia symbioses, but the most commonly implicated mode is phytohormone-induced (usually IAA) stimulations of root growth (Molla *et al* 2001, Vessey and Buss 2002). In this way, the stimulation of nodulation is most commonly an indirect effect; the PGPRs stimulate root growth, which provides more sites for infection and nodulation. Some PGPRs that stimulate legume–rhizobia symbioses appear to more directly influence the development of the symbioses. Burdman *et al* (1996) related *Azospirillum brasilense*-mediated stimulation in nodulation of common bean to an increased production of flavonoids by the legume host. These flavonoids are the initial chemical signals secreted by the legume host to induce *nod* genes in rhizobia and thereby initiate the legume–rhizobia symbiosis (Schultze and Kondorosi 1998). Andrade *et al* (1998) speculated that an increase in nodulation in pea mediated by inoculation with *Pseudomonas fluorescens was* due to an increase in flavonoid exudation by the host plant. Proposed alternative modes of action include toxin (i.e. tabtoxinine-β-lactam) release by *Pseudomonas syringae* stimulating the alfalfa–rhizobia symbiosis and B vitamins secretion by *Pseudomonas* spp. enhancing the red clover-rhizobia symbiosis (Marek Kozaczuk and Skorupska 2001).

2.2 Mode of action of PGPR as biocontrol agents

Phytopathogenic microorganisms have a great impact on crop yield and can significantly reduce plant performance and crop quality. The usual strategy for the control of phytopathogens is to apply chemical pesticides, but this has led to increased concerns over environmental contamination and resulted in the so-called pesticide treadmill in which pathogens develop resistance to individual chemical controls over time, needing a constant development of new pesticides (Fernando *et al* 2006). In this context, Rhizobacteria that can provide biocontrol of disease or insect pests (biopesticides) are considered an alternative to chemical pesticides (Zahir *et al* 2004). The widely recognized mechanisms of biocontrol mediated by PGPRs are competition for an ecological niche or a substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Compant *et al* 2005).

2.3 Antioxidant enzymes

Plants are subject to many forms of environmental stress. Some are abiotic, physicochemical, or density independent, such as temperature, drought, fire, and air pollution. Other sources of stress are biotic or density dependent, such as competition, herbivory, disease, and parasitism. Biochemical reactions produce many reactive oxygen species (ROS) such as hydrogen peroxide, superoxide and hydroxyl radicals which are considered to be the main cause of oxidative damage (Jimenez *et al* 2003). Moreover, limited water availability and heavy metals can intensify the processes of reactive oxygen species (ROS) production leading to oxidative stress. To cope with oxidative damage under extremely adverse conditions, plants have developed an antioxidant defense system by changes in the levels of antioxidants and antioxidant enzymes that includes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Foyer and Noctor 2005; Kumar *et al* 2011). These enzymes are key components in preventing the oxidative stress in plants as the activity of one or more of these enzymes is generally increased in plants when exposed to stressful conditions (Olugbeni *et al* 2007). Susceptibility of plants to oxidative stress may depend on the balance between ROS generated as well as on activity and availability of cellular antioxidants (Foyer *et al* 1994). Activities of various antioxidant enzymes are known to increase in defense responses (Anand *et al* 2009) and salinity (Hernandez *et al* 2000). In addition to antioxidative enzymes, phenolic and flavonoid compounds also possess antioxidant activity (Jin *et al* 2009).

Within a cell, the superoxide dismutase, catalase, peroxidase and other antioxidant enzymes constitute the first line of defense against ROS. Therefore their regulation depends mainly upon the oxidant status of the cell (Elstner and Hardgree 1982; Jung and Wyss 2004). Shim *et al* (2003) reported that increase in H_2O_2 in plant cell under oxidative stress may induce the activity of antioxidant enzymes to overcome stress effect (Lin and Kao 2000). A

study was carried out regarding the potential of PGPR on soil enzymes and plant growth in 2014. It was observed that PGPRs enhanced the urease and invertase activities. It was inferred that PGPR can supplement 50% chemical fertilizers for better plant growth and soil health (Nosheen and Bano 2014).

2.3.1 Peroxidases

Peroxidases [E.C.1.11.1.7] are heme-containing enzymes that use H_2O_2 to oxidise a large variety of hydrogen donors such as phenols (Klibanov *et al* 1981; Dordick *et al* 1986), aromatic amines (Van Haandel *et al* 1999), ascorbic acid, indole and certain inorganic ions. Peroxidase is widespread in plants and has frequently been found in fungi, bacteria and some invertebrate tissues.

Some peroxidases play a crucial role in delignification of lingo-cellulosic materials and in degradation of recalcitrant organic pollutants (Bumpus *et al* 1985).They catalyze H_2O_2-dependent oxidation of aromatic compounds, oxidation of heteroatoms, epoxidation, and enantioselective reduction of racemic hydroperoxides. Moreover, peroxidase (POD) and catalase (CAT) are enzymes that are known to be involved in metabolism of pesticides in plants (Laura *et al* 2003).

2.3.2 Superoxide dismutase

When plants are subjected to environmental stresses such as temperature extremes, drought, herbicide treatment and mineral deficiency, the balance between the production of ROS and the quenching of antioxidants is upset often resulting in oxidative damage. To control the level of ROS and to protect cells under stress conditions, plant tissues contain several enzymes scavenging ROS (superoxide dismutase {SOD; E.C.1.15.1.1}, Catalase {CAT; E.C.1.11.1.6} (Gossett *et al* 1994; Blokhina *et al* 2003). Superoxide dismutase is a major scavenger of O_2 and it catalyzes the conversion of superoxide radical into hydrogen peroxide. H_2O_2 is then scavenged by catalase and different classes of peroxidases (Gossett *et al* 1994; Lin and Kao 2000). Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage (Zhu *et al* 2004).

The superoxide released by a number of processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutase catalyzing the first step and then catalases and various peroxidases removing hydrogen peroxide.

2.3.3 Catalase

Catalase is universally present oxidoreductase enzyme and it is one of the key enzymes involved in the removal of toxic hydrogen peroxide. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. It catalyzes the decomposition of hydrogen peroxide to water and oxygen using either an iron or manganese cofactor. Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate. Catalase is usually located in a cellular organelle called the peroxisome. Peroxisomes in plant cells are involved in photorespiration and symbiotic nitrogen fixation (Scandalios *et al* 1997). It is a very important enzyme in protecting the cells from oxidative damage by reactive oxygen species (ROS). Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second. In addition, when excess hydrogen peroxide accumulates in the cell, catalase converts it to H_2O through this reaction, Catalase catalyzes the breakdown of H_2O_2 using one molecule of H_2O_2 as substrate donor and another molecule of H_2O_2 as oxidant or electron acceptor.

$$2H_2O_2^- \rightarrow 2H_2O + O_2$$

Catalase can also catalyze the oxidation by hydrogen peroxide, of various metabolites and toxins, including formaldehyde, formic acid, phenols, acetaldehyde and alcohols. It does so according to the following reaction:

$$H_2O_2 + H_2R \rightarrow 2H_2O + R$$

Hydrogen peroxide is highly toxic and produced in several reactions in the cell; hence it must be scavenged promptly to avoid injury to metabolic machinery of the tissues. To prevent damage to cells and tissues, it must be converted quickly into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less-reactive gaseous oxygen and water molecules.

2.3.4 Amylase

The amylases are very important enzymes to hydrolyze the stored polysaccharides required for the growth and development of seedlings (Muthusamy *et al* 2012). Amylases (glycoside hydrolases) break starch into glucose, maltose, maltotriose and dextrin by hydrolysis of glycosidic bonds. Therefore they are also called digestive enzymes. The first enzyme produced industrially was an amylase from a fungal source in 1894, which was used as a pharmaceutical aid for the treatment of digestive disorders (Panday *et al* 2000). *Bacillus*

subtilis and *B. mesentericus* were first used for the production of alpha amylase on commercial scale using large fermentors and LSF (Liquid State Fermentation). The employment of bacterial cultures for the production of commercial enzyme was pioneered by them Underkofler and Hickey (1954). Amylases contribute as a major class of industrial enzymes constituting approximately 25% of the enzyme market (Rao *et al* 1998). The most widely used thermostable amylases in the starch industry are produced from B. licheniformis (Morgan and Priest 1981).

Microbial production of amylase is more beneficial than that from other sources because it is economical; production rate is high and can be engineered to obtain enzymes of desired characteristics. The microbial amylases could be potentially useful in various pharmaceutical, fine-chemical industries, paper industries etc. With the emergence of biotechnology, the use of amylase has widened in clinical research, medical chemistry and starch analytical chemistry. These increased uses have placed greater stress on increasing indigenous amylase production and search for more efficient processes (Aiyer 2005). The major advantages of using microorganisms for production of amylases are the ability to produce in bulk and ease by which it can be manipulated for desired products (Lonsane and Ramesh 1990).

2.3.5 Invertase

Invertase is a key enzyme in carbohydrate metabolism. Invertase belongs to a multi-enzyme family with various forms classified in accordance with cellular localization, optimum pH, and solubility properties: two soluble forms (soluble acid invertase in the vacuole and neutral invertase in the cytoplasm) and a particulate form (cell-wall-bound invertase in the cell wall) (Doehlert and Felker, 1987). Little is known about the roles of neutral invertase, whereas acid invertases (soluble acid invertase and cell-wall-bound invertase) have been well characterized (Trouverie *et al* 2004). Although the predominant role of acid invertases is to provide glucose for cell energy production (Karuppiah *et al* 1989), they have several other physiological functions depending on their cellular localization. For example, invertases play a major role in sucrose partitioning and long-distance transport by modulating the sucrose gradient between the phloem and the surrounding cells, and in the control of the relative sink strength of plant tissues (Roitsch *et al* 1995; Godt and Roitsch 1997; Roitsch 1999). Invertase catalyzes the irreversible reaction to convert sucrose into glucose and fructose. Invertases are involved in cell elongation through the maintenance of cell turgor via osmotic potential control (Pfeiffer and Kutsuchera 1995). In addition, invertases play a role in the regulation of genes related to photosynthetic enzymes (Herbes *et al* 1996).

It was observed that the invertase activity was enhanced when the PGPRs (*Azospirillum brasilense and Azotobacter vinelandii*) were applied at 10^6 cells/mL (Nosheen and Bano 2014).

2.3.6 Sucrose synthase

In most plants, sucrose is the major end product of photosynthesis and the major form of carbohydrate transported in the phloem to non-photosynthetic organs such as the root system (Trouverie *et al* 2004). The transported sucrose cannot be used for metabolic processes, but must be cleaved into hexose by invertase (β-fructosidase, EC 3.2.1.26) or sucrose synthase (UDP-D-glucose:D-fructose 2-α-glucosyltransferase, EC 2.4.1.13) before entering into carbohydrate metabolism. Sucrose synthase plays a role in the respiratory pathway (Xu *et al* 1989), in energy production (Fukuda *et al* 2008), in the cell wall synthesis where membrane-associated callose synthase and cellulose synthase use UDP-glucose as a substrate (Delmer and Amor 1995), and for starch biosynthesis (Chourey and Nelson 1976; Claussen *et al* 1985; 1986; Doehlert 1990). Sugar is an important factor in root system formation; it is used as an energy source and for constructing structural components of cells and cell walls (Jarvis 1986). The extent of sugar import into many plant organs correlated with sucrose synthase activity (Claussen 1983; Sung *et al* 1988; Nguyen-Quoc *et al* 1990). Sucrose synthase catalyzes the reversible reaction that converts sucrose into fructose and UDP-glucose. Although the reaction is reversible, it is thought to be involved primarily in the breakdown of sucrose (Kruger 1990; Huber and Huber 1996).

2.4 Phenolic compounds

Phenolic compounds display a large range of structures and contribute to the nutritional qualities of vegetables. These phenolic compounds constitute one of the main classes of secondary metabolites. They are responsible for characteristic colour and taste properties. The family of secondary metabolites derived from aromatic metabolism includes phenolics, flavonoids, tannins and polyphenols or phenylpropane derivatives. Phenolics have important roles in plants such as in defence against herbivores and pathogens, mechanical support, attracting pollinators and most importantly act as antioxidant compounds. Antimicrobial activities of polyphenols were studied by Tranter *et al* (1993) and they were proposed as potential natural preservatives. Hollman *et al* (1995, 1996) studied the absorption of quercetin glycosides from onion on volunteers and they found that in healthy humans, these were absorbed and eliminated slowly through the day. Free radical scavenging (antioxidant) activity of phenolic compounds was determined by their reactivity as hydrogen or electron donating agents, the stability of the resulting antioxidant-derived radical, their reactivity with other antioxidants and their metal chelation properties (Rice-Evans *et al* 1996).

Plasma antioxidant activity was reported to increase after consumption of red wine (Cao *et al* 1998) and grape wine (Day *et al* 1997). Gee *et al* (1998) proposed that the quercetin glucosides are capable of interacting with glucose transporter receptors in the mucosal epithelium and, therefore, may be absorbed by the small intestine *in vivo.* Vinson *et al* (1998) determined that vegetables have antioxidant quality comparable to that of pure phenols and superior to that of the antioxidant vitamins A, C and E. Curir *et al* (2003) and Galeotti *et al* (2008) proposed that phenolic compounds such as flavonoids may act as phytoanticipins. Cacace and Mazza (2002) extracted the phenolics from black currant using different SO_2 concentrations and solvents. Potatoes with high amounts of phenyl propanoids were reported to reduce inflammation in human feeding studies (and immature potatoes were found to be responsible for lower blood pressure and to raise serum antioxidants (Vinson *et al* 2012).

2.4.1 Phenolic compounds of *Solanum melongena* L.

Out of 120 vegetables evaluated for antioxidant activity, brinjal ranked among the top ten for superoxide scavenging activity. Winter and Herrmann (1986) proposed that esters of hydroxycinnamic acids (HCAs) are the major class of phenolic compounds in brinjal fruit, in which chlorogenic acid is predominant. Sudheesh *et al* (1997) studied hypolipidemic beneficial effect of phenolic compounds extracted from eggplant fruit in normal and cholesterol fed rats. Stommel and Whitaker (2003) also studied the diversity of phenolic compounds found in eggplant. Huang *et al* (2004) reported antioxidant activity of eggplant and found that crude eggplant extract can inhibit low density lipids oxidation. Luthria and Mukhopadhyay (2006) optimized extraction of phenolic acids from eggplant. Sadilova *et al* (2006) reported that the flavonols isolated from eggplant exhibit antioxidant activity against chromosomal aberrations induced by doxorubicinlee. Singh *et al* (2009) reported the relationship between phenolic content and antioxidant activity of eggplant pulp. Due to higher content of phenolic compounds calyx part of plant has been used as a traditional medicine (Bruni *et al* 2004, Hirunpanich *et al* 2006).

2.5 Biochemical components

2.5.1 Anthocyanins

It is estimated that more than 635 anthocyanins have been found in nature which are constituted by anthocyanidins which are glycosylated and acylated differentially (He and Guisti 2010). Hedin *et al* (1983) showed that cyanidin-3-glucoside protects cotton leaves against the tobacco budworm. In addition to their functions in plants, anthocyanins have many other uses. Anthocyanins also possess known pharmacological properties and are used for therapeutic purposes. Cristoni and Magistretti (1987) reported the anti-ulcer activity of anthocyanins. Rice-Evans *et al* (1996) showed that the anthocyanins scavenging activity of

free radicals has been related to the catechol function of the B-ring. Sarma and Sharma (1999) studied the inter-reaction of anthocyanins and DNA and suggested that the cyanidin-DNA co-pigmentation may be a possible defence mechanism against the oxidative damage to DNA and may have *in vivo* physiological functions attributable to the antioxidant ability of anthocyanins. Li *et al* (2001) studied the regulation of anthocyanin biosynthesis during fruit development in 'Nyoho' strawberry and found that fruit pigmentation in most of the strawberry cultivars was affected by the amount of light falling on the fruit surface. Fruit colour was uniform in 'Nyoho' strawberry without any consideration of incident sunlight or shading. Stintzing *et al* (2002) suggested that whole pigment extracts from blackberry being devoid of acetylated structures afforded higher antioxidant values than those from black carrot and red cabbage that contain a high percentage of acylated structures. Nakajima *et al* (2003) found that introduction of an acyl moiety to anthocyanins and flavonols resulted into either retaining or decreasing the potential to scavenge the free radicals. Optiberry is the health-promoting product in the form of a synergistic combination of six selected extracts from wild blueberry, bilberry, cranberries, elderberries, raspberries and strawberries. Lee *et al* (2005) determined the monomeric anthocyanins in fruit juices, beverages, natural colorants and wines by using AOAC official method and reported that anthocyanin content expressed as cyanidin-3-glucoside equivalents ranged between 20 to 3000 mg/l. Zafra-Stone *et al* (2007) reported antioxidant, anticarcinogenic and antibacterial properties of optiberry.

2.5.1.1 Anthocyanins in *Solanum melongena* L.

Various types of anthocyanins have been extracted and identified from eggplant skin. Nasunin (delphinidin-3-p-coumaroylrutinosode-5-glucoside) is most commonly found, was first reported by Kuroda and Wada (1933, 1935). Tanchev *et al* (1970) identified delphidin-3-rutinoside and a smaller amount of delphidin-5-glucoside from the skin of Bulgarian eggplant. Guisti and Wrolstad (2005) reported anthocyanin content of 450.1 mg/kg fresh weight of eggplant as determined by pH differential method. Wu and Prior (2005) identified delphinidin-3-rutinoside as major anthocyanin in eggplant which was procured from US market. Similar to purple sweet potato, brinjal genotypes having purple coloured fruits consist of acylated anthocyanins (Ichiyanagi *et al* 2006). Wu *et al* (2006) reported that brinjal contain 85.70 mg anthocyanin content in 100g of fruit.

2.5.2 Other biochemical quality parameters

Plants are known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo 2011). Rate of assimilation, translocation and utilization of available nutrients indicates the growth potential of plants in the applied conditions. The nutrients are utilized and converted to organic matter, and so study of the organic contents is

important to evaluate the adaptability of plants to stressed environmental conditions (Fadtare and Mane 2008). Brinjal, being quite high in nutritive value, has been an important component of human diet since ancient times and can be well compared with tomato. It is low in fats, contains protein, fibre and carbohydrates. It is a good source of minerals and vitamins and is rich in total water soluble sugars, free reducing sugars and amide proteins. Eggplant is considered as a good source of fibre for reducing cholesterol (Jenkins *et al* 2012). Eggplant fruits are known for being low in calories but rich in minerals, which is good for human health. They are rich in potassium and are also a good source of magnesium, calcium and iron. Eggplant contains high moisture content and is a good source of some phytonutrients (Kandoliya *et al* 2015).

CHAPTER-III

MATERIAL AND METHODS

Procurement of samples

The present investigation, "Effect of plant growth promoting rhizobacteria on germination, antioxidant enzymes and fruit quality in Brinjal (*Solanum melongena* L.)" was carried out in the field and laboratories of Department of Vegetable Science, Punjab Agricultural University, Ludhiana. The materials and methods employed in the present study are described under the following headings:

3.1 Raising of crop and procurement of samples
3.2 Collection of samples
3.3 Extraction and assay of peroxidase
3.4 Extraction and assay of superoxide dismutase
3.5 Extraction and assay of catalase
3.6 Extraction and assay of amylase
3.7 Extraction and assay of invertase
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3.13.3 Number of fruits per plant

3.14 Statistical analysis

3.1 Raising of crop and procurement of samples and cultures

Seedlings were treated with different PGPR cultures procured from the microbiology laboratory of the Department of Plant Breeding and Genetics, PAU, Ludhiana. The crop of Brinjal was raised at the Vegetable Research Farm, Deptt. of Vegetable Science, PAU, Ludhiana. Samples of brinjal fruits were collected at maturity.

3.2 Collection of samples

The fruit samples were collected at 7^{th} day after anthesis (DAA), the subsequent second and third samples were taken at an interval of 7 days. For enzyme assays, various extracts were prepared and the enzymatic activities were determined in these extracts. The brinjal fruit samples were harvested at maturity and analyzed for germination, agronomic characters, biochemical plant growth parameters, antioxidant capacity and fruit quality.

3.3 Extraction and assay of peroxidase (EC 1.11.1.7) (Shannon *et al* 1966)

Reaction

Peroxidase catalyses the breakdown of H_2O_2 at the expense of electron acceptors such as ascorbate, quinones and cytochrome C. They are nonspecific in utilizing electron donor for oxidation of H_2O_2.

$$AH_2 + 2H_2O_2 \rightarrow 3H_2O + ½\ O_2 + A$$

AH_2 is a hydrogen donor, while A is its oxidized form.

(A) Extraction

i) The material was homogenized in ice-cold 0.1M phosphate buffer, pH 6.0 (1:10, w/v) in a chilled pestle and mortar using white sterile sand as abrasive.

ii) It was strained through two folds of muslin cloth and the homogenate was centrifuged at 16,000g for 20 min. at 4°C. The supernatant was used as enzyme source.

(B) Reagents

i) 0.05 M guaiacol prepared in 0.1 M potassium phosphate buffer (pH 6.5)

ii) 0.8 M H_2O_2 in distilled water

(C) Assay

The reaction mixture contained 3 ml of 50 mM guaiacol in 0.1 M phosphate buffer (pH 6.5), 0.1 ml of enzyme extract and 0.1 ml of 800 mM H_2O_2. The reaction mixture without H_2O_2 was taken as a blank. The reaction was initiated by adding H_2O_2 and change in

absorbance was recorded at 470 nm for 3 min at an interval of 30 seconds. Peroxidase activity has been defined as change in absorbance $min^{-1}mg^{-1}$ of protein.

3.4 Extraction and assay of superoxide dismutase (EC 1.15.1.1) (Marklund and Marklund 1974)

Reaction

Superoxide dismutase catalyzes the disproportionation of superoxide anion to H_2O_2 and molecular oxygen:

$$^{\bullet}O^{-}_{2} + {}^{\bullet}O^{-}_{2} + 2H^{+} \longrightarrow H_2O_2 + O_2$$

(A) Extraction

i) The material was homogenized in ice-cold 0.1M phosphate buffer, pH 6.0 (1:10, w/v) in a chilled pestle and mortar using white sterile sand as abrasive.

ii) It was strained through two folds of muslin cloth and the homogenate was centrifuged at 16,000g for 20 min. at 4°C. The supernatant was used as enzyme source.

(B) Reagents

i) 6 mM pyrogallol in water- Fresh solution was prepared for assay

ii) 6 mM EDTA in water

iii) 100 mM Tris-HCl buffer (pH 8.2)

(C) Assay

To the spectrophotometric cuvette, 1.5 ml of 100 mM Tris HCl buffer (pH 8.2), 0.5 ml of 6 mM EDTA, 1 ml of 6 mM pyrogallol solution and 0.1 ml of enzyme extract were added. Change in absorbance was recorded at 420 nm in a spectrophotometer at an interval of 30 seconds upto 3 min. One unit of SOD has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank. Specific activity is expressed as units $min^{-1}mg^{-1}$ of protein.

3.5 Extraction and assay of catalase (EC 1.11.1.6) (Chance and Maehly 1955)

Reaction

Catalase uses one molecule of H_2O_2 as substrate or electron donor and another molecule of H_2O_2 as oxidant or electron acceptor and performs the following reaction:-

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

(A) Extraction

The enzyme was extracted with 0.05 M sodium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrrolidone. Homogenate was centrifuged at 10000g at 4°C for 20 min and clear supernatant was used for enzyme assay.

(B) Reagents

i) 50 mM sodium phosphate buffer (pH 7.5)

ii) H_2O_2 solution (39 mM): 0.2 ml of 30% H_2O_2 diluted to 50 ml with sodium phosphate buffer (pH 7.5)

(C) Assay

In spectrophotometric cuvette, 1.8 ml of 50 mM sodium phosphate buffer (pH 7.5) and 0.2 ml of enzyme extract were added. The reaction was initiated by adding 1 ml H_2O_2 and utilization of H_2O_2 was recorded at an interval of 30 seconds for 3 min by measuring the decrease in absorbance at 240 nm. Extinction coefficient for H_2O_2 has the value of 0.0394 $mM^{-1}cm^{-1}$. Catalase activity was expressed as μmoles of H_2O_2 decomposed $min^{-1}g^{-1}$ of FW.

3.6 Extraction and assay of amylase (EC 3.2.1.1) (Bernfield 1955)

Reaction

Amylase is a hydrolytic enzyme which hydrolyzes starch and plays an important role in starch metabolism in plants.

(A) Extraction

i) 1g of sample material with 5-10 volumes of ice-cold 10mM calcium chloride solution was extracted overnight at 4°C.

ii) The extract was centrifuged at 54,000g at 4°C for 20 min. The supernatant was then used as enzyme source.

(B) Reagents

i) 0.1M Sodium acetate buffer, pH 4.7

ii) 1% starch solution: Dissolve 1g of starch in 100ml acetate buffer

iii) Dinitrosalicylic acid reagent

v) 40% potassium sodium tartarate

vi) Maltose solution: Dissolve 50mg maltose in 50ml distilled water

(C) Assay

1ml of starch solution and 1ml of properly diluted enzyme were taken in a test tube and incubated at 27°C for 15 min. The solution was heated in a boiling water bath for 5 min. 1ml of potassium sodium tartarate solution was added to the warm test tubes. After cooling the test tubes, the volume was made to 10ml by adding distilled water. The absorbance was measured at 560nm. Amylase activity was expressed as mg of maltose produced during 5 min. incubation with 1% starch solution.

3.7 Extraction and assay of invertase (EC 3.2.1.26) (Sridhar and Ou 1972)

Reaction

Invertase catalyzes the hydrolysis of cane sugar (sucrose):

Sucrose + H_2O → fructose + glucose

(A) Extraction

i) 5.0g of plant tissue was homogenized in a chilled mortar placed in an ice-bath with pre-cooled 20% glycerol
ii) The solution was filtered and the volume was made to 100ml with 20% glycerol
iii) 1-2ml of toluene was added

(B) Reagents

i) 2.5% Sucrose solution
ii) 1M Sodium acetate buffer, pH 5.0
iii) 20% glycerol
iv) Toluene
v) Dinitrosalicylic acid (DNS) reagent

(C) Assay

To 5ml of enzyme solution, 100ml of buffer and 5ml of sucrose solution were added and incubated at 37°C for 24h. 1ml of reaction mixture was pipetted out and the reaction was stopped by adding 1ml of DNS reagent. Reducing sugars present in the reaction mixture were estimated by DNS method and the protein content was also estimated by Lowry's method.

3.8 Extraction and assay of sucrose synthase (EC 2.4.1.13) (Wardlaw and Willenbrink 1994)

Reaction

The enzyme sucrose-6-phosphate synthase catalyzes the following reaction:

UDP-α-D-Glucose + α-D-Fructose-6-phosphate → Sucrose-6-phosphate

(A) Extraction

0.5g of material was homogenized in ice-cold extraction medium for 30 min and then centrifuged at 17,000g for 10 min. The supernatant was used for enzyme assay.

(B) Reagents

i) Extraction medium: 50mM HEPES buffer, pH 7.5, 7.5mM $MgCl_2$, 2mM EGTA, 5mM DTT, 2% PVP
ii) 100mM UDP-Glucose
iii) 100mM Fructose
iv) 1N NaOH

v) 30% HCl

vi) 1% Resorcinol (ethanolic)

(C) Assay

0.9ml extract, 0.5ml HEPES buffer, 0.2ml $MgCl_2$, 0.2ml UDP-Glucose and 0.2ml fructose were incubated for 30 min. at 30°C and the reaction was terminated by adding 2ml NaOH. Unreacted fructose was destroyed by heating to 100°C for 10 min. 0.5ml of 1% resorcinol (ethanolic) was then added followed by the addition of 1.5ml of 30% HCl and was incubated for 8 min. at 80°C. Centrifugation was carried out at 1500g for 5 min. and absorbance was measured at 520nm. The enzyme activity was expressed as µmole sucrose synthesized/h/0.5g sample.

3.9 Estimation of protein from enzyme extract (Lowry *et al* 1951)

Principle

The protein concentration is determined with Folin-Ciocalteau's phenol reagent, which couples redox reaction (Cu^+ ions formation, biuret method) with a second redox reaction (colour change). The protein forms a blue coloured complex with ions in alkaline solution. The optical density of solution is measured at 520 nm.

(A) Reagents

i) **Reagent A**: 2% sodium carbonate in 0.1N sodium hydroxide.

ii) **Reagent B:** 0.5% cupric sulphate in 1% sodium potassium tartarate.

iii) **Reagent C:** Alkaline copper tartarate. It was freshly prepared by mixing 50 ml of reagent A with 1ml of reagent B just before use.

iv) 1N Folin-Ciocalteau reagent.

(B) Procedure

To 0.1 ml of enzyme extract, 0.9ml of distilled water and 5 ml of reagent C was added. Shaken thoroughly and kept for 10 min. Then added 0.5 ml of Folin-Ciocalteau reagent and again shaken thoroughly. After keeping for 30 min., blue colour developed was read at 520 nm against the reagent blank. Bovine serum albumin (BSA) was used as standard in the range 10-100 µg. The concentration of protein was calculated from standard curve.

3.10 Extraction and estimation of biochemical quality parameters in brinjal

3.10.1 Extraction and estimation of anthocyanins (Rabino *et al* 1977)

Fruit samples were crushed and anthocyanins extracted with 10 ml of 1% HCl (w/v) in methanol for 24h at 4°C with occasional shaking. The absorbance of extracts which were clarified by filtration was measured at 530 nm and 637 nm with a LABINDIA 3000^+ UV/VIS Spectrophotometer. This formula was used to correct the contribution of chlorophyll and its degradation products in acid solution to the absorbance of the extracts at 530 nm.

3.10.2 Estimation of dry matter

50g of sliced fruit of each sample were dried in a pre-weighed petri-plate at 65 ± 2°C till-constant weight was obtained. The dried samples were cooled in a desiccator for 10 min. These were weighed and the dry matter percentage was then calculated.

3.10.3 Extraction and estimation of reducing sugars (mg/100g fresh weight) (Somogyi 1952)

Nelson's method: The colorimetric modification of Somogyi alkaline copper method is based on the determination of cuprous oxide produced by heating sugar with alkaline copper and reacting it with arsenomolybdate reagent.

Reagents: Copper reagent A- Twenty five gram of sodium carbonate (anhydrous), 25g Rochelle salt (sodium potassium tartarate), 20g sodium carbonate and 200g sodium sulphate (anhydrous) were dissolved in 800ml of water and diluted to 1 litre. The reagent was filtered and stored at room temperature. A sediment was formed after a few days which was filtered before use.

Copper reagent B: Fifteen percent $CuSO_4.7H_2O$ containing 1or 2 drops of conc. H_2SO_4/100ml was prepared. Distilled water was used for this purpose.

Arsenomolybdate colour reagent: Twenty five gram of ammonium molybdate was dissolved in 450 ml of distilled water. Twenty one ml of conc. H_2SO_4 was added and mixed. 3g of $Na_2SO_4.7H_2O$ was added and dissolved in 25 ml of water, mixed and placed in an incubator at 37°C for 24-48 h. This reagent was stored in a glass stoppered amber coloured bottle.

Method: One ml of sample was pipetted out into a narrow test tube graduated at 25 ml. One ml of a mixture (prepared on the day of use) of 25 parts of reagent A to 1 part of reagent B was added. The tubes were placed in a boiling water bath for 20 min. The tubes were cooled under running tap water and 1 ml of arsenomolybdate reagent was added to each tube. The colour developed very rapidly. The mixture was diluted to 25 ml after 15 min. and absorbance was measured in a digital spectrophotometer at 500 nm. Blank reagent was used to adjust the absorbance to zero. Amount of reducing sugars was determined using a standard curve prepared from glucose in the range 10-100µg/ml.

3.10.4 Extraction and estimation of non-reducing sugars

The content of non-reducing sugars was calculated by subtracting the reducing sugars from the total soluble sugars:

Total Sugars (Dubois *et al* 1956)

Principle

Sugars from furfurals and hydroxyl methyl furfurals (pentoses and hexoses respectively) in the presence of conc. H_2SO_4 which react with phenol with coloured compounds. All sugars give this test as oligo and polysaccharides will get hydrolyzed with conc. H_2SO_4 and some with furfurals.

Extraction procedure

(A) Reagents

i) Ethanol (80%)
ii) Lead acetate (saturated)
iii) Sodium oxalate

Weighed (500mg) dried samples and refluxed with 80% ethanol in a conical flask fitted with water condenser and centrifuged at 5000 rpm for 15 min. Supernatant was collected, the residue left over was given twice washings with 80% aqueous ethanol and the process was repeated. Supernatants were collected and pooled. Ethanol from pooled extract was removed at 50°C in a flash evaporator under vacuum. Then 1.0 ml of saturated lead acetate was added and volume of the extract made to 100 ml with distilled water. This was kept for 24 h till all the proteins in the extract get precipitated. To this a pinch of sodium oxalate was added to remove lead ions from extract. It was again kept for 24 h. Thereafter, the extract was filtered through Whatman no. 1 filter paper. This clear extract thus obtained was used for sugar estimation.

Estimation

(A) Reagents

i) 5% phenol (w/v) redistilled
ii) 95% H_2SO_4

(B) Procedure

To 0.1 ml of sugar extract, 1.0 ml of phenol was added followed by the addition of 5.0 ml of conc. H_2SO_4. The $H_2SO_4$was poured directly in the center of the test tube to ensure proper mixing. After 10 min. tubes were cooled to room temperature with running water. After 20 min. the absorbance was read at 490 nm against reagent blank. The concentration of total sugars was read from standard curve prepared by using glucose in the range of 10-80 µg.

The reducing sugars were estimated by Nelson Somogyi's method as mentioned above in 3.10.3.

3.10.5 Estimation of starch (McCready *et al* 1950)

(A) Reagents

a) **A:** Anthrone reagent: Anthrone reagent was prepared by dissolving 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid.

b) **B:** 52% Perchloric acid

(B) Extraction

The residue left after the extraction of sugars by was dissolved in 5 ml of 52% perchloric acid. The mixture was filtered through Whatman no. 1filter paper and volume of the filtrate was made to 100 ml with distilled water.

(C) Estimation

To 1 ml of perchloric acid extract, 4 ml of distilled water were added. To this, 10 ml of cold anthrone reagent were added. The contents were shaken vigorously and heated in the boiling water bath for 8 minutes. The tubes were cooled under running tap water and were shaken. The absorbance was measured at 630 nm against reagent blank in UV/VIS spectrophotometer. The blank was prepared by adding 10 ml anthrone reagent to 5 ml of distilled water.

3.10.6 Extraction and estimation of phenolic compounds

Extraction

Weighed (500mg) dried fruit samples and refluxed with 80% methanol for 2 h. The refluxed material was filtered and volume was made to 25 ml by washing with 80% methanol. The extract thus prepared was used for estimation of phenolic compounds viz. total phenols, ortho-dihydroxyphenols and flavonols.

3.10.6.1 Estimation of total phenols (Swain and Hillis, 1959)

(A) Reagents

i) **1N Folin-Ciocalteau's reagent:** 2N Folin-Ciocalteau's reagent diluted 1:1 (v/v) with distilled water.

ii) **Saturated solution of Na_2CO_3:** 17.5g of Na_2CO_3 was dissolved in 50 ml of distilled water.

(B) Procedure

Methanolic extract (5 ml) was evaporated to dryness and the residue was dissolved in 6.5 ml of distilled water. To this 0.5 ml of Folin-Ciocaltaeu's was added and shaken

thoroughly. After 3 min, 2 ml of saturated solution of Na_2CO_3 was added. After 1 h, the absorbance of blue colour was read in a spectrophotometer at 760 nm against the blank. The blank was prepared from water and reagents only. The concentration of total phenols was determined from standard curve prepared by using catechol (20-100µg).

3.10.6.2 Estimation of ortho-dihydroxyphenols (Nair and Vaidyanathan, 1964)

(A) Reagents

i) **10% Trichloroacetic acid (TCA)**
ii) **10% Sodium tungstate**
iii) **0.5N Hydrochloric acid (HCl):** 2.5 ml of HCl was diluted to 50 with water.
iv) **0.5% Sodium nitrite**
v) **0.5N Sodium Hydroxide:** 0.2g of sodium hydroxide was dissolved in water and volume was made to 100ml.

The extraction was repeated thrice, using 70% methanol in subsequent extractions.

B) Procedure

Methanolic extract (5.0ml) was evaporated to dryness and residue left behind was dissolved in 1.0 ml of distilled water. To this, 0.5 ml of 10% TCA, 1.0 ml of sodium tungstate, 0.5 ml of 0.5N HCl and 1.0 ml of freshly prepared 0.5% sodium nitrite were added. A Yellow colour developed. After 5 min 2.0 ml of 0.5N sodium hydroxide was added. The light cherry colour developed, whose absorbance was read after 15 min at 540 nm against the blank. The blank consisted of water and reagents only. The concentration of ortho-dihydroxyphenols was determined from standard curve prepared by using catechol (10-100µg).

3.10.6.3 Estimation of flavonols (Balabaa *et al*, 1974)

(A) Reagents

0.01M methanolic solution of Aluminium chloride ($AlCl_3$).

(B) Procedure

5.0 ml of methanolic extract was evaporated to dryness**.** The residue left was dissolved in 10 ml of 0.1 M methanolic solution of aluminium chloride. O.D was taken at 420nm against methanolic $AlCl_3$ as blank. The concentration of flavonols was determined from standard curve prepared by using rutin (50-250µg).

3.10.7 Estimation of nitrogen content (AOAC 1990)

(A) Reagents

a) **A:** Digestion mixture (K_2SO_4:$CuSO_4$ (10:1 w/w)

b) **B:** Conc. H_2SO_4

c) **C:** N/100HCl

d) **D:** 4% Boric acid

e) **E:** 40% NaOH

Mixed indicator- Bromocresol green (0.5 g) and methyl red (0.10g) were dissolved and pH was adjusted to 4.5 with 0.1N HCl.

(B) Estimation

0.5 g dry sample were taken in digestion flask and 2 g of digestion mixture, 10 ml of concentrated H_2SO_4 were added. The flasks were heated using digestion heater till the contents became clear. After cooling, volume was made upto 50ml in volumetric flasks with distilled water. 5 ml aliquot was distilled in micro kjeldahl distillation apparatus with 5 ml of 40% NaOH. The liberated ammonia was trapped in 20 ml of boric acid containing 2-3 drops of mixed indicator. About 10 ml of distillate was collected in 250 ml conical flask and titrated with N/100 HCl till the end point; a change in colour from blue to light pink appeared. A blank without sample was also run simultaneously and N was calculated.

3.11 Estimation of minerals (Fe, Zn)

3.11.1 Estimation of iron (Fe) (Gadzhieva 2014)

Reagents

(a) Standard

The standard solution of Iron: The stock standard solution of iron contained1000 mg/L of iron diluted with a pre-mixed solution of deionized water and analytical grade concentrated nitric acid to provide the working standard in 2% (w/v) HNO_3. The calibration blank solution used was a 2% (w/v) HNO_3 solution. The working standards were 1, 5 and 10 mg/L.

(b) Samples

Digestion was carried out by heating in a furnace. Homogenized samples (0.25 g) were placed in a beaker and heated at 825±25°C for 2h in a furnace. The cooled remnants were digested by addition of 10 ml of diluted HNO_3 (1:1). The resulting solutions were filtered and diluted to 25 ml with double-distilled water.

Procedure

An atomic absorption spectrophotometer (AAS) was used to measure the iron content in the samples. The AAS software contains pre-set spectrometer parameters for iron and these

were used to measure the iron content in samples. Each measurement was performed in triplicate.

3.11.2 Estimation of zinc (Zn) (Gadzhieva 2014)

(A) Reagents

All solutions were prepared from chemical and analytical grade reagents with double-distilled water.

i) **The standard solution of zinc:** ZnO (1.2447 g) was dissolved in 10ml of diluted HCl and then the solution was diluted to 1000 ml with double distilled water (1μg/ml Zn).
ii) **The standard working solution of zinc:** 1 ml of Zn standard solution was diluted to 100 ml with double-distilled water (10 μg/ml Zn).
iii) Conc. HCl
iv) Conc. HNO_3
v) Hydroxylamine hydrochloride solution (20%)
vi) Thiourea solution (10%)
vii) Potassium thiocyanate solution (20%)
viii) Rhodamine C solution (0.02%)
ix) Methyl orange solution (0.1%)
x) NH_3 solution (1:1)
xi) Acetate buffer (pH 5)

All these solutions were diluted to 50 ml with double-distilled water.

(B) Procedure

2.5 ml of the standard working zinc solution (10 μg/ml) and 5 ml of HCl (2mol/l) were placed in a volumetric flask and diluted to 50 ml with double-distilled water. Mineralization was carried out by heating in a furnace. Homogenized samples (0.25 g) were placed in a beaker and heated at 825±25°C for 2h in a furnace. The cooled remnants were digested by addition of 10 ml of diluted HNO_3 (1:1). The resulting solutions were filtered and diluted to 25 ml with double-distilled water. Zinc determination was carried out by direct aspiration into an air-acetylene flame atomic absorption spectrophotometer fit with a zinc-hollow cathode lamp. Then the sample was aspirated into the air-acetylene flame lower zinc detection limit. The AAS software contains pre-set spectrometer parameters for zinc and these were used to measure the iron content in samples. Each measurement was performed in triplicate.

3.12 Estimation of total antioxidant capacity: DPPH (1, 1-Diphenyl-2-picrylhydrazyl) assay (Shimada *et al* 1992)

(A) Reagents

i) 0.2 mM DPPH

ii) 80% methanol

(B) Procedure

0.0078 g DPPH was dissolved in methanol to make the volume 100 ml. An aliquot of 1.5 ml of sample solution was mixed with 1.5 ml methanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 minutes in dark at room temperature. The absorbance of resulting solution was measured at 517 nm. For the control, the assay was conducted in same manner but methanol was used instead of sample solution. DPPH scavenging capacity of tested sample was measured as a decrease in the absorbance and was calculated as:

$$\text{Scavenging activity \% (30min)} = \frac{\text{Control absorbance} - \text{extract absorbance}}{\text{Control absorbance}} \times 100$$

3.13 Agronomic characters

3.13.1 Plant height

Height of 3 plants in a sample was measured in cm.

3.13.2 Average fruit weight

Weight of three fruits of a sample was measured in grams.

3.13.3 Number of fruits per plant

Number of fruits per sample was counted at the final harvest.

3.14 Statistical analysis

Growth data and biochemical parameters have been presented as mean ± S.D. of three replicates. Data was statistically analysed by applying one-way analysis of variance (ANOVA) followed by post hoc analysis, the CD test.

CHAPTER-IV

RESULTS AND DISCUSSION

The present investigation was conducted to study the effect of plant growth promoting rhizobacteria on germination, antioxidant enzymes and enzymes of starch degradation in the seedlings in the laboratory experiment; and also their effect on various agronomic characters, fruit quality, various biochemical components and yield of brinjal after harvesting the crop at maturity.

The results of present findings have been presented under the following sub-headings:

4.1 Effect of different plant growth promoting rhizobacteria on germination percentage, root length and shoot length in brinjal

4.2 Effect of different plant growth promoting rhizobacteria on the activities of antioxidant enzymes and antioxidant capacity in brinjal at different stages of growth

4.3 Effect of different plant growth promoting rhizobacteria on the activities of the enzymes of starch degradation in brinjal

4.4 Effect of different plant growth promoting rhizobacteria on the biochemical plant growth parameters in brinjal

4.5 Effect of different plant growth promoting rhizobacteria on the fruit quality of brinjal (plant height, average fruit weight and number of fruits per plant)

4.1 Effect of different plant growth promoting rhizobacteria cultures on germination percentage, root length and shoot length in brinjal (*Solanum melongena* L.)

Table 1 represents the rhizobacteria cultures used for treating brinjal seedlings. The data on the germination percentage of brinjal (PBHL51) have been presented in table 2. Various plant growth promoting rhizobacteria cultures caused significant increase in germination percentage of brinjal seeds. PGPR7 significantly enhanced the seed germination to 90% and also increased the shoot length in brinjal to 46.37 cm as presented in table 2. Maximum root length (51.07cm) was observed in seedlings treated with PGPR7. The significant increase in shoot and root length in seeds may be due to the effect of PGPR cultures on the various physiological and biochemical responses during seed germination and it subsequently led to enhanced growth and development of brinjal seedlings. The values were significantly lower in untreated control. Seedlings treated with PGPR1 (*Serratia* spp.) showed minimum root length and shoot length. *Serratia* spp. emit high levels of ammonia, which during cultivation in petri dishes cause alkalization of the plant medium and subsequently reduces the growth of the plant (Weise *et al* 2013). In earlier studies, inoculation of *Bacillus cereus* (NPB6) was reported to highly enhance the root length and shoot length in brinjal

(Kumar *et al* 2014). Root length and shoot length were also improved by the application of PGPRs (Nosheen and Bano 2014).

Table 1: Rhizobacteria Cultures used for treating Brinjal seedlings

Treatment	Culture
T1 (PGPR1)	*Serratia* spp.
T2 (PGPR2)	*Pseudomonas* spp.
T3 (PGPR3)	*Bacillus* spp.
T4 (PGPR4)	*Bacillus* spp.
T5 (PGPR5)	*Pseudomonas* spp.
T6 (PGPR6)	*Pseudomonas* spp.
T7 (PGPR7)	*Bacillus* spp.
T8 (PGPR8)	*Pseudomonas* spp.
T9 (PGPR9)	*Pseudomonas* spp.
T10 (PGPR10)	*Pseudomonas* spp.

Table 2: Effect of PGPR on germination, root length and shoot length in *Solanum melongena* L.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
Control	53.33±0.23	25.33±9.75	5.73±2.58
T1	43.33±3.21	11.45±2.32	4.53±2.90
T2	90.00±2.47	49.93±5.77	37.43±2.86
T3	83.33±1.56	35.93±5.30	39.93±2.57
T4	40.00±3.47	14.4±8.15	8.9±6.39
T5	73.33±4.21	35.43±14.18	26.57±5.36
T6	83.33±2.31	28.63±2.31	37.07±3.95
T7	86.67±3.14	46.67±3.19	46.37±10.64
T8	63.33±0.68	22.63±13.14	12.9±8.69
T9	83.33±2.47	41.9±3.08	36.03±9.78
T10	83.33±1.42	51.07±3.09	28.07±2.22
CD (5%)	31.89	18.21	24.51

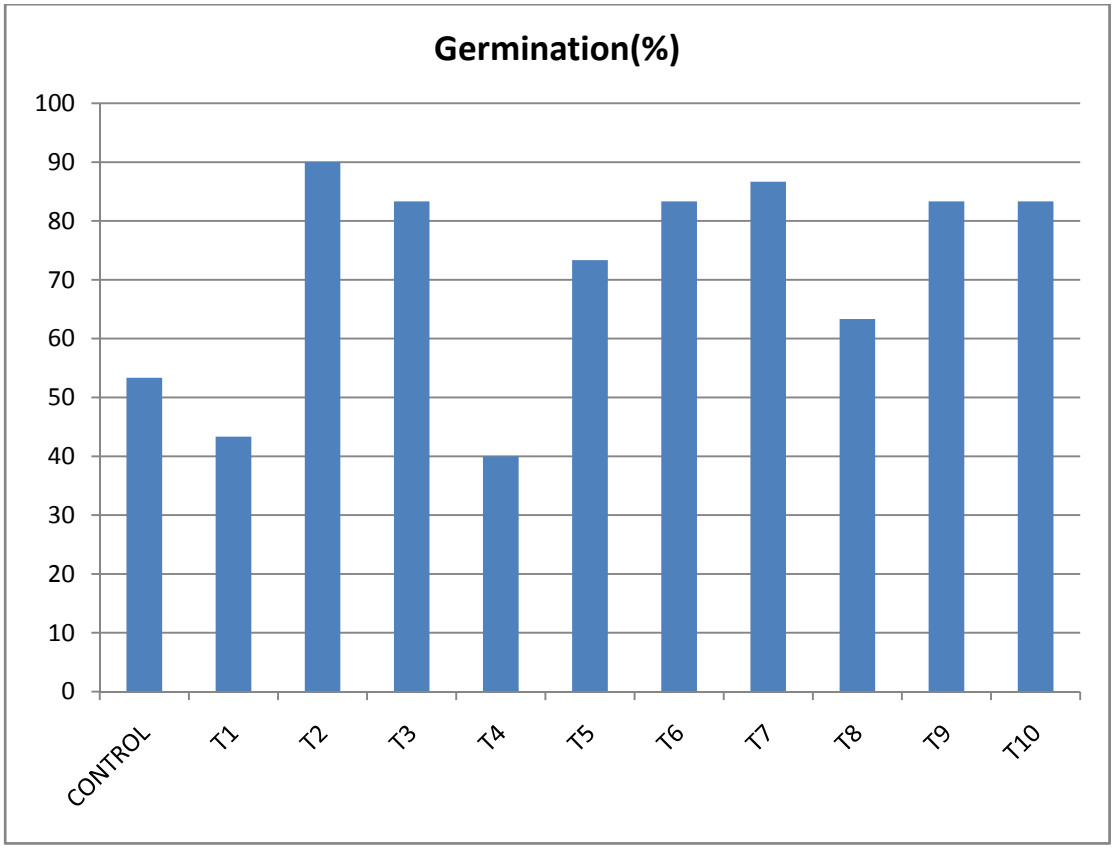

Fig 1: Percentage germination in the seedlings of *Solanum melongena* L.

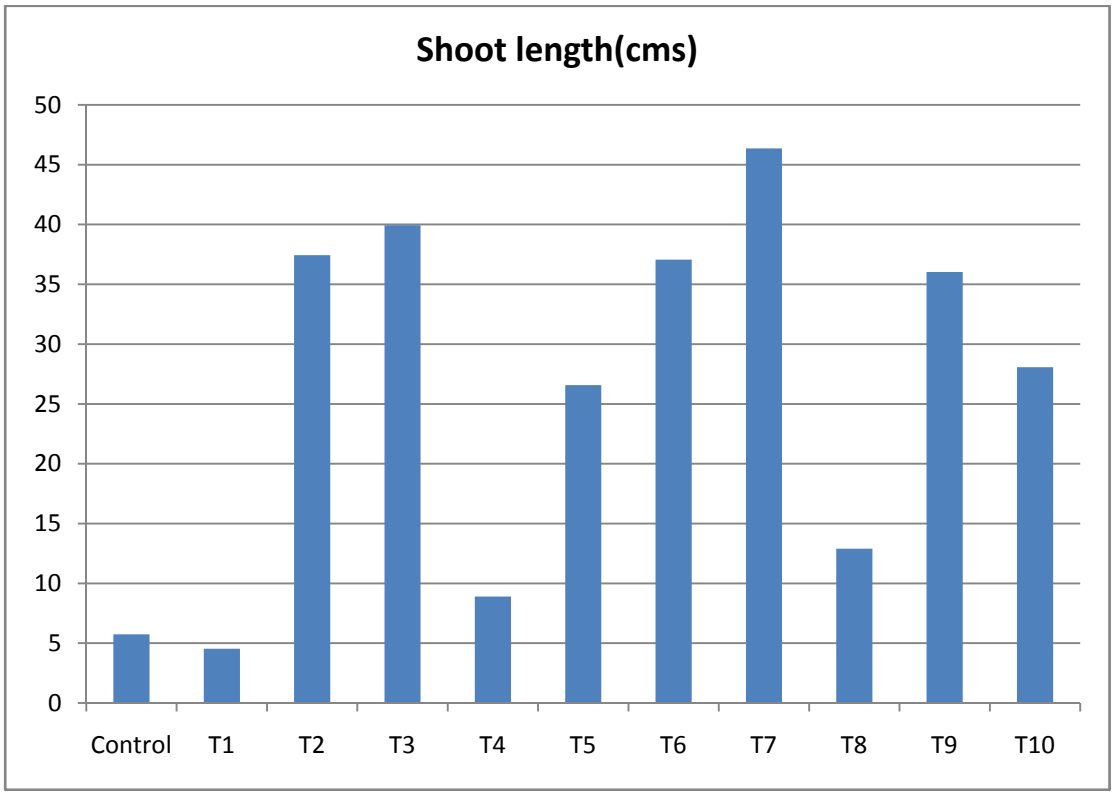

Fig 2: Shoot length (cms) in the seedlings of *Solanum melongena* L.

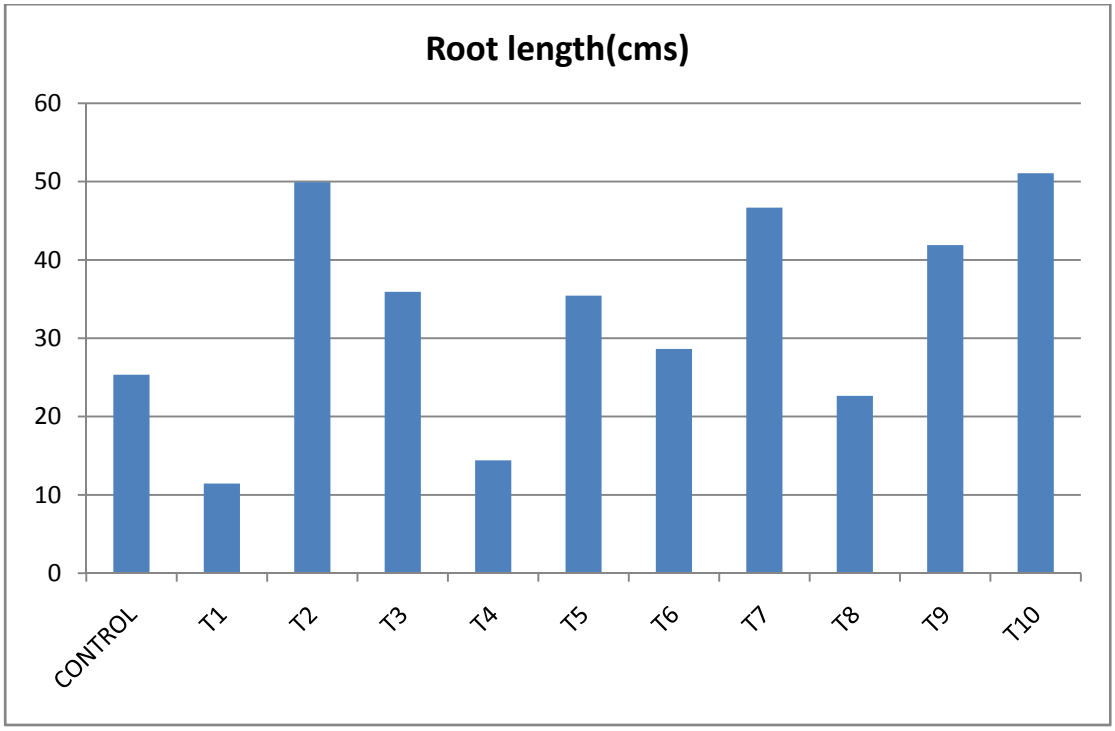

Fig 3: Root length (cms) in the seedlings of *Solanum melongena* L.

4.2 Effect of different plant growth promoting rhizobacteria on the activities of antioxidant enzymes and antioxidant capacity in brinjal at different stages of growth (*Solanum melongena* L.)

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria found on plant roots that induce growth by a wide variety of mechanisms. PGPR are used as inoculants for biofertilization, phytostimulation and biocontrol (Bloemberg and Lugtenberg 2001). Ten isolates of Pseudomonas and Bacillus were isolated and seedlings of brinjal were treated with these isolates. The present study was conducted to analyze the effect of these isolates on the antioxidant enzymes in brinjal (*Solanum melongena* L.). These isolates have the ability to colonize eggplant (*Solanum melongena* L.) roots. *Pseudomanas aeruginosa* (P07-1) and P. putida (P11-4) were determined to be successful colonizers in eggplant seedlings (Altinoc *et al* 2013).

Catalases are tetrameric heme containing enzymes which directly dismutate H_2O_2 into H_2O and oxygen (Scandalios *et al* 1997). Catalases play important role in removal of H_2O_2 generated in peroxisomes and glyoxysomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catalases. Various PGPR cultures caused significant increase in CAT activity. Seedlings treated with PGPR7 showed maximum SOD, catalase and amylase activities i.e. 94.83 units/min/mg FW, 62.56 µmole/min/mg FW, 0.42 units/min/mg FW respectively followed by PGPR9. The inhibition of catalase activity observed with many treatments as shown in table 3 can be linked to inhibition of enzyme synthesis or due to flux

of superoxide radicals that are known to inhibit catalase activity (Bashir *et al* 2006).

Peroxidases are heme containing enzymes that catalyze the one electron oxidation of several substrates at the expense of hydrogen peroxide. Peroxidases use hydrogen peroxide as an electron acceptor to catalyze a number of oxidation reactions. They are located in walls and vacuoles (Hameed *et al* 2009). Peroxidases have a role in biosynthesis of lignin and in defense against biotic/abiotic stress by consuming H_2O_2. Increased peroxidase activities are reported under abiotic stresses (Gill and Tuteja 2010). Data presented in Table 3 show that PGPR cultures had significant effect on POX activity. PGPR9 induced invertase activity (19.23 µmole glucose/h/g FW) and POX activity (7.80 change in absorbance min/g FW) in comparison with the control group (6.85µmole glucose/h/g FW and 5.49 change in absorbance min/g FW) respectively. Higher peroxidase activities have been reported to be related to drought tolerance in different plants (Guo *et al* 2006, Khanna-Chopra and Stelote 2007).

Table 3: Effect of PGPR cultures on antioxidant enzymatic activity in roots and antioxidant capacity in *Solanum melongena* L.

Treatments	Catalase activity (µmole/min/mg FW)	Peroxidase activity (change in absorbance/min./g FW)	SOD activity (Units/min/mg FW)	DPPH (%)
Control	30.21±0.29	5.49±0.15	77.29±0.74	86.43±0.76
T1	26.03±0.11	4.44±0.17	41.22±0.69	92.02±0.90
T2	28.37±0.34	2.81±0.10	55.57±0.07	89.27±0.84
T3	26.27±0.46	3.77±0.09	42.95±0.49	79.22±1.01
T4	18.45±0.42	4.56±0.10	74.29±0.16	90.04±0.17
T5	25.38±0.08	5.54±0.24	67.59±0.49	86.72±0.48
T6	20.93±0.06	3.42±0.12	56.67±0.11	84.84±0.17
T7	62.56±0.28	7.80±0.13	94.83±0.38	94.8±0.56
T8	20.97±0.12	3.16±0.07	26.41±0.15	87.16±0.30
T9	35.51±0.19	6.21±0.14	87.69±0.11	79.75±0.41
T10	27.58±0.27	4.84±0.12	78.56±0.10	86.63±0.86
CD (5%)	0.57	0.29	14.66	1.36

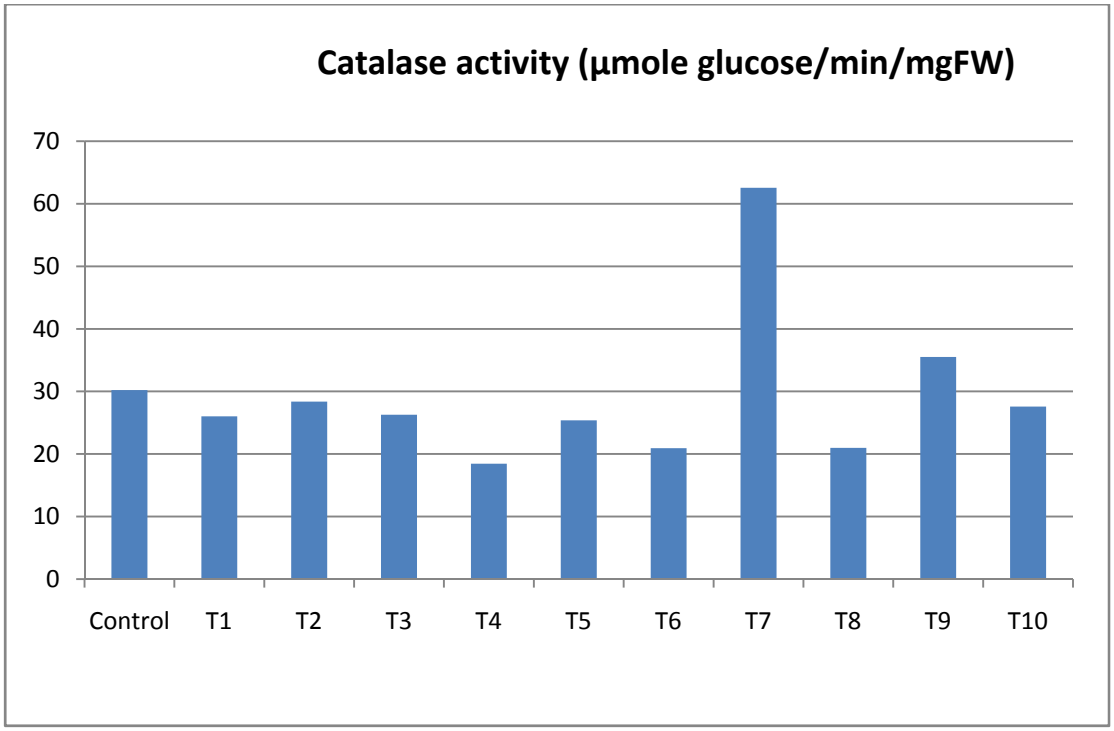

FIG 4: Catalase activity in the seedlings of *Solanum melongena* L.

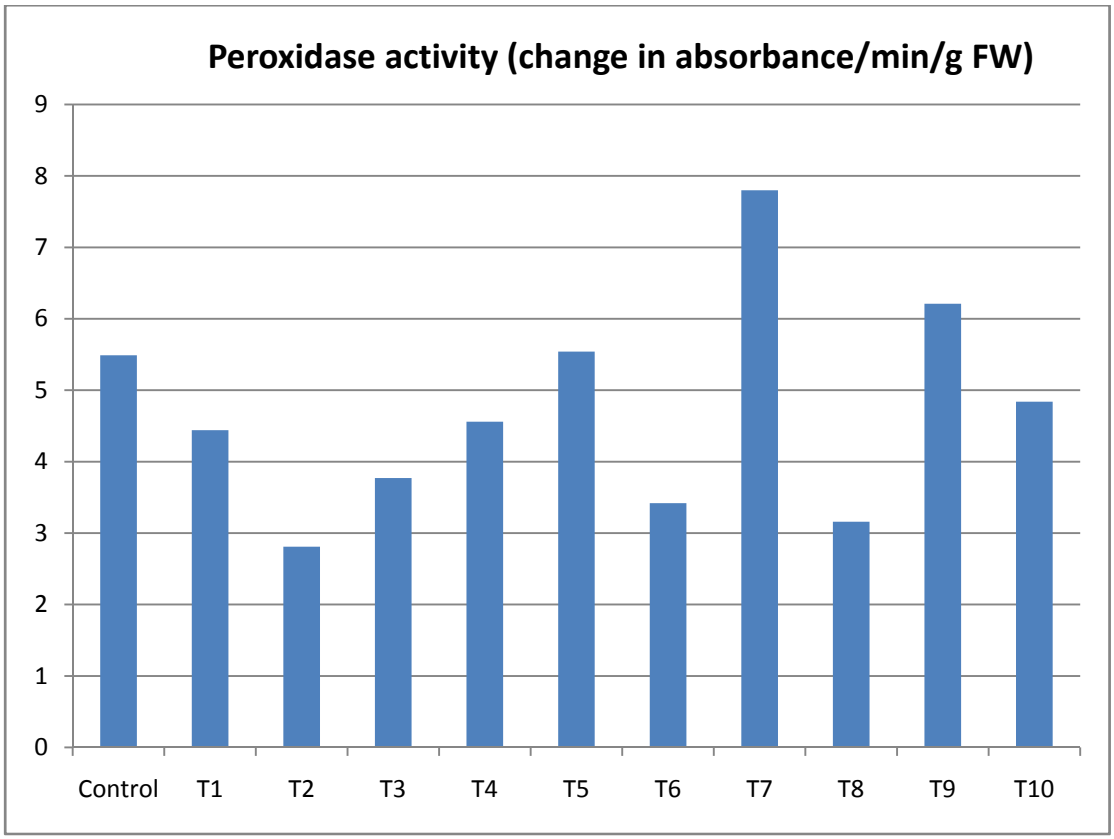

FIG 5: Peroxidase activity in the seedlings of *Solanum melongena* L.

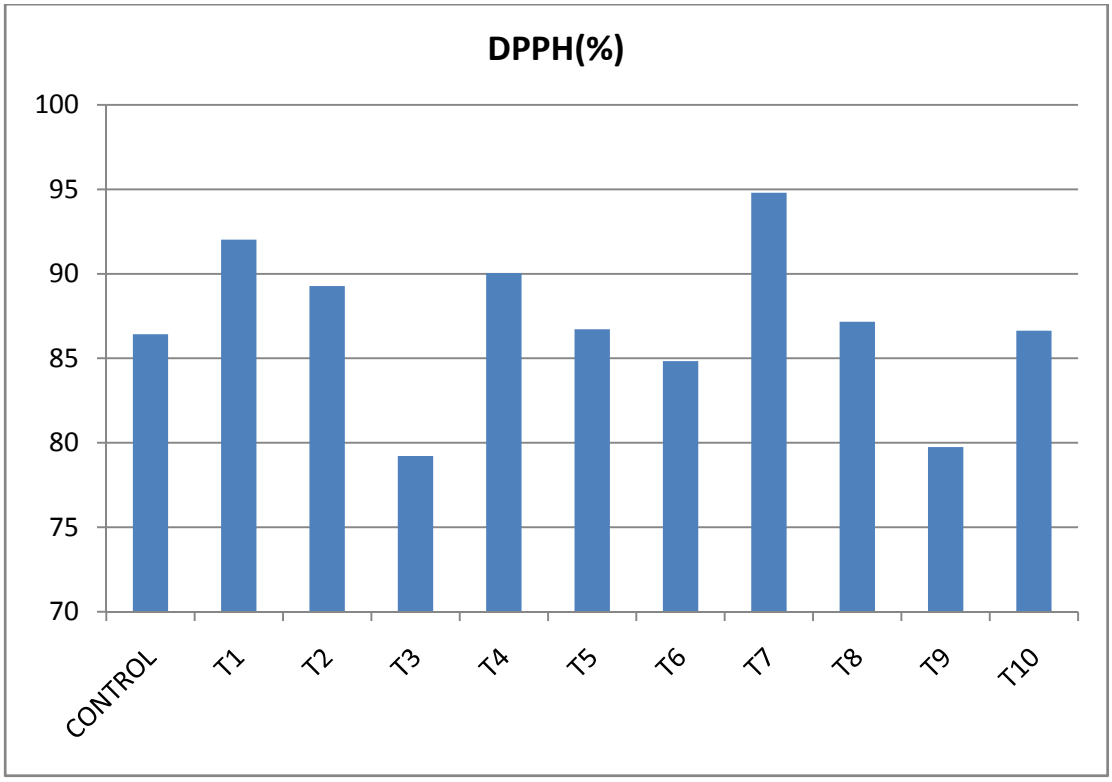

Fig 6: Total antioxidant capacity in the seedlings of *Solanum melongena* L.

The DPPH radical scavenging assays measure the relative antioxidant efficacy of natural extracts in scavenging free radicals generated in the assay medium (Apak *et al* 2007). The degree of discoloration is an indication of the scavenging capacity of the extracts. Somawathi *et al* (2014) reported that the extracts were able to reduce the stable violet DPPH radical to yellow DPPH-H over a range of concentrations (0.94-5.63 mg/ml). An increase in sample concentration significantly increased the DPPH radical scavenging activity of samples in a dose dependent manner. Difference in scavenging activities might be due to the presence of different phenolic compounds and the difference in the total phenolic content. In the present study, plant growth promoting rhizobacteria have been found to be effective in achieving higher antioxidant capacity as shown in plate 1. DPPH content in the seedlings treated with PGPR7 showed a higher DPPH content of 94.80% over the untreated control which had 86.43% DPPH content.

4.3 Effect of different plant growth promoting rhizobacteria on the activities of the enzymes of starch degradation in brinjal

The amylases are very important enzymes to hydrolyze the stored polysaccharides required for the growth and development of seedlings. The enhanced biochemical response has been observed in terms of improved activities of amylases which are responsible for the breakdown of the stored carbohydrates and to supply the energy to growing radical and plumule. In the present study, maximum amylase activity has been shown in the seedlings

Plate 1: DPPH radical scavenging activity of *Solanum melongena* L.

treated with PGPR7 (0.42±0.42 mg maltose/mg protein/h) followed by PGPR9 (0.40±0.40 mg maltose/mg protein/h). Several reports have demonstrated that such a treatment at appropriate dose significantly enhanced the activities of enzymes which are indispensable during germination of seeds and further growth of seedlings Muszynski and Gadyszewska 2008; Ashrafijiou *et al* 2010; Khalifa and Ghandoor 2011; Chen *et al* 2010; Podlesny (2002). This improved biological activity may be due to increased entropy and intrinsic energy of seeds during the germination process (Muthusamy *et al* 2012).

Sone and Fujikawa (1993) reported that the amylase synthesizing efficacy of bacterial strains in the brinjal pulp was much lower than that of other enzymes like pectinse and xylanase, which indicated that the relative amount of pectin and hemicellulose residues exceeds the amount of starch and cellulose granules present in brinjal pulp.

Table 4: Effect of PGPR on Starch hydrolyzing enzymes of *Solanum melongena* L.

Treatments	Invertase activity (µmole glucose/h/g FW)	Amylase activity (mg maltose/mg protein/h)
Control	6.85±4.12	0.39±0.39
T1	1.65±0.11	0.27±0.27
T2	3.4±0.29	0.27±0.27
T3	8.70±0.12	0.34±0.34
T4	1.55±0.23	0.30±0.30
T5	8.62±0.10	0.35±0.35
T6	10.38±0.12	0.25±0.25
T7	9.85±0.32	0.42±0.42
T8	8.94±0.15	0.29±0.29
T9	19.23±0.28	0.40±0.40
T10	1.59±0.35	0.375±0.38
CD (5%)	2.78	0.35

4.4 Effect of different plant growth promoting rhizobacteria on the biochemical plant growth parameters in brinjal

4.4.1 Anthocyanin content

Anthocyanins are naturally occurring pigments present in peel and to a lesser extent, in flesh of brinjal fruit (Mazza *et al* 2004). Anthocyanins are widely studied for their medical potential (Kong *et al* 2003). The present study was conducted to analyse the effect of different

plant growth promoting rhizobacteria on the anthocyanin content in brinjal.

Variation in anthocyanin content in brinjal under investigation is reported in Table 5. In present study the anthocyanin content was found in the range 86.56mg/100g to 104.50mg/100g. Treatment with PGPR7 gave the maximum anthocyanin content (104.50mg/100g). A high anthocyanin content is considered beneficial, regardless of how the fruit is to be used (Kandoliya *et al* 2015). The findings of Kandoliya *et al* 2015 revealed that the anthocyanin content in the brinjal fruit extracts varied from 32.89-39.12 mg/100g. In general, the higher phenol content was associated with higher antioxidant capacity (Santas *et al* 2008). Several studies have reported a good correlation between the total phenol content of plant extracts and antioxidant activity (Bahorun *et al* 2004).

Nitrogen is the only nutrient that is uptaken by plants in form of various ions and compounds. However, plants utilize NO_3^- or NH_4^+ (Marschner 1995). Diverse reactions of plants towards particular nitrogen ions types are observed, although definitely larger group of plant species prefers nitrates over ammonium, despite of much higher energetic costs for NO_3^- assimilation (Starck 2008).

The content in starch of eggplant is, in general, higher than the total soluble sugars, which is probably caused by the fact that the fruit of eggplant is harvested when physiologically immature, and therefore that the hydrolysis of starch has not been completed (Singh *et al* 2000).

Table 5: Effect of PGPR cultures on plant growth parameters of *Solanum melongena* L.: Anthocyanin content, Nitrogen and Starch content

Treatments	Anthocyanin Content (mg/100g)	Nitrogen (%)	Starch (%)
Control	86.81±0.13	0.17±0.01	3.26±0.23
T1	103.42±0.08	1.85±0.06	1.43±0.13
T2	93.33±0.18	0.19±0.01	2.25±0.23
T3	100.94±0.10	2.6±0.14	1.06±0.16
T4	87.85±0.16	0.09±0.01	2.2±0.17
T5	98.38±0.12	0.17±0.01	1.54±0.10
T6	105.38±0.14	2.20±0.01	2.29±0.14
T7	103.50±0.15	0.45±0.08	3.80±0.24
T8	86.56±0.06	1.58±0.16	1.14±0.83
T9	99.99±0.12	2.85±0.09	4.22±0.22
T10	104.49±0.13	0.90±0.10	1.37±0.07
CD (5%)	0.27	0.17	0.36

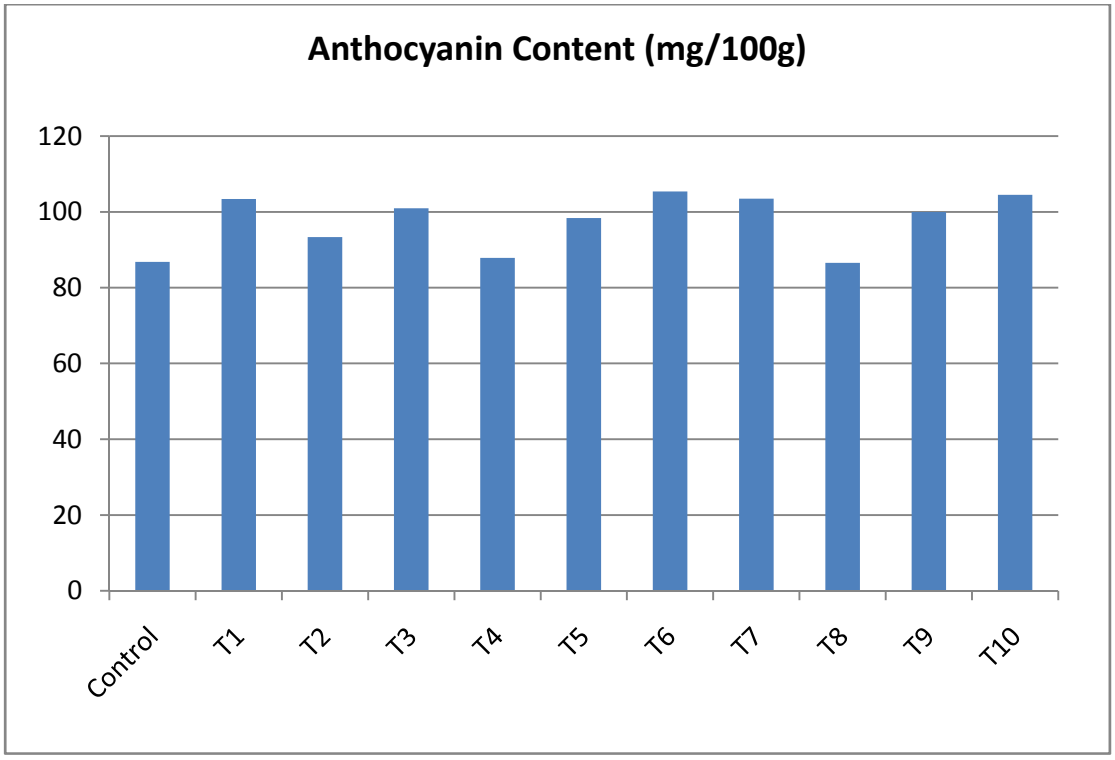

Fig 7: Anthocyanin content in the fruits of brinjal in response to different PGPRs

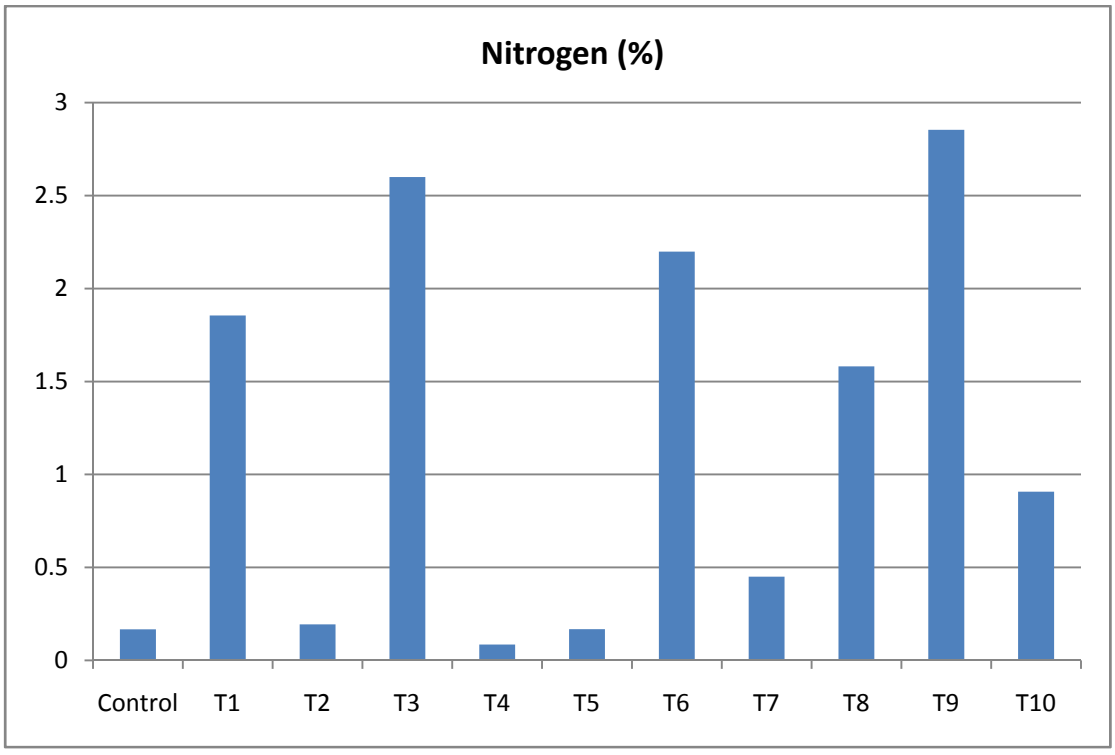

Fig 8: Variation in nitrogen content in the fruits of *Solanum melongena* L. in response to different PGPR cultures

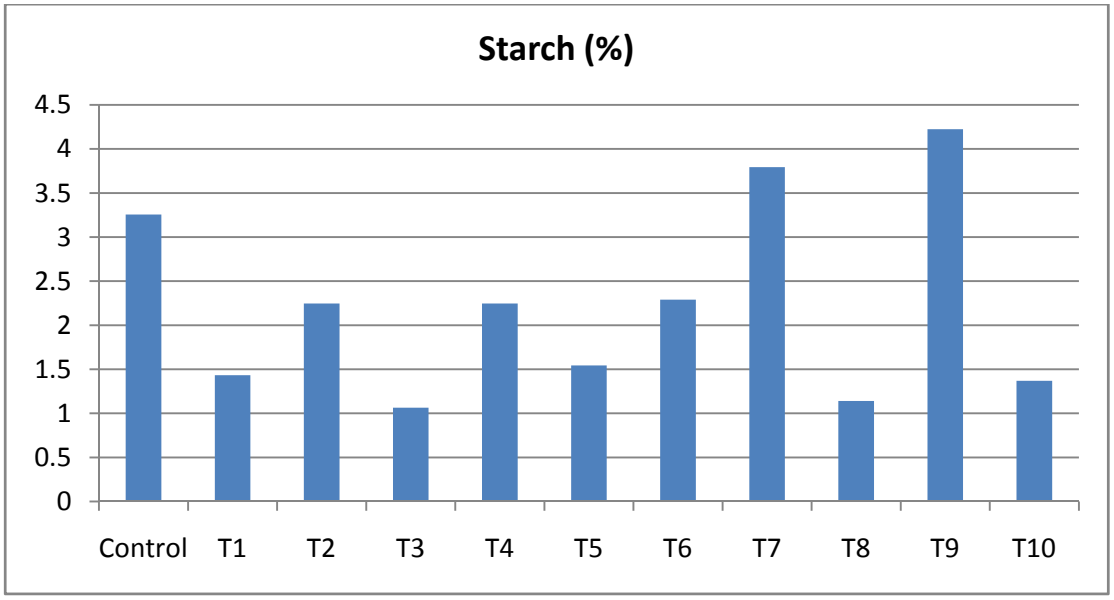

Fig 9: Starch content in the fruits of *Solanum melongena* L. in response to different PGPR cultures

4.4.2 Reducing and non-reducing sugars

Brinjal is known to have low sugar content and thereby it has low calorie value. Hanson *et al* (2006) analysed 35 brinjal genotypes and reported sugar content in the range of 20-30% on dry weight basis. In the present study, the reducing sugars content was found to be maximum with the treatment done with PGPR2 (8.36%) whereas the treatment done with PGPR4 showed the minimum content of reducing sugars (1.17%) as compared to the untreated control which showed 2.43% of reducing sugars. Table 6 shows the effect of different plant growth promoting rhizobacteria on the reducing sugars content, non-reducing sugars content and protein content in the fruits of brinjal. The content of non-reducing sugars was maximum with the treatment done with PGPR7 (8.78%) as shown in table 6. Fruits of brinjal showed low content of total soluble sugars.

Ghadsingh and Mandge (2012) also reported the value of soluble sugar ranged from 2.7 to 5.0g/100g. Basalah *et al* (1985) estimated soluble sugar level 0.02 to 0.24% dry weight. These amounts of sugar components have significant roles to human health. This is because, apart from the supply of energy, they are also needed in numerous biochemical reactions not directly concerned with energy metabolism. In addition, these carbohydrates may serve as substrates for the production of aromatic amino acids and phenolic compounds through the shikimic acid pathway and this may confer high phenolic and antioxidant potentials for brinjal (Kandoliya *et al* 2015).

In the present study, the protein content in brinjal fruits was estimated to be 0.61±0.20 to 3.64±0.07 % as represented in table 6. Shahnaz *et al* (2003) also estimated protein in brinjal which was 1.18g/100g. The higher protein indicates that its intake can

contribute to the formation of hormones which controls a variety of body functions such as growth, repair and maintenance (replacement of wear and tear of tissues) of body (Kandoliya *et al* 2015). The treatment with PGPR 10 showed a decrease in protein content as compared to the untreated control. This decrease in protein content might be because of the presence of chloride, carbonate and bicarbonate ions and nitrates present in soil. This decrease is also accompanied by bacterial ammonia emission which alters the pH of the rhizophsere and thereby influences organismal diversity and plant-microbe interactions (Weise *et al* 2013).

Table 6: Effect of different PGPR cultures on the reducing sugars, non-reducing sugars and protein content in brinjal

Treatments	Reducing sugars (%)	Non-reducing sugars (%)	Proteins (%)
Control	2.43±0.33	2.96±0.07	0.90±0.10
T1	1.48±0.30	2.72±0.12	1.53±0.11
T2	6.92±0.29	7.68±0.13	2.87±0.09
T3	3.95±0.48	3.85±0.22	1.05±0.05
T4	1.17±0.14	2.66±0.11	1.87±0.09
T5	6.50±0.22	7.23±0.24	2.67±0.09
T6	3.90±0.66	3.94±0.13	1.85±0.06
T7	8.36±0.12	8.78±0.17	3.64±0.07
T8	1.29±0.17	2.55±0.26	1.76±0.18
T9	5.69±0.20	6.84±0.10	3.10±0.09
T10	3.83±0.15	3.97±0.17	0.61±0.20
CD (5%)	0.423	0.345	0.232

4.4.3 Phenolic compounds

Kaur *et al* (2014) showed a wide variation in total phenols content in brinjal genotypes, ranging from 22.62 to 234.46 mg GAE/100g FW (244.28 to 2990.64 mg GAE/100g DW) having nearly 15 fold variations. In present study, the phenols content varied in the range of 32.85 to 122.90 mg/100g DW (Table). This showed that the treatment done with PGPR7 led to high content of total phenols which have high free radical scavenging activity (Lutharia and Mukhopadhay 2006). The profile of phenolic compounds is given in table 7. The ODHs varied from 11.40 to 70.78 mg/100g having highest content with the treatment done with PGPR3 (70.78±0.52 mg/100g). Highest content of flavonols in brinjal was found when treated with PGPR7 (148.85±0.26 mg/100g). The distribution of flavonols is shown in table 7. Helmja *et al* (2007) reported that brinjal has highest amount of flavonols (660mg/100g) among Solanaceae family.

Brinjal phenolics have been recommended as a choice diet for the management of type 2 diabetes (Pinto *et al* 2009; Nickavar and Yousefian 2009). Phenolic compounds embrace a wide range of plant substances which possess common aromatic ring bearing one or more hydroxyl substituents. They tend to be water soluble since they most frequently occur combined with sugar as glycosides and are usually located in the cell wall vacuole. Phenolic compounds are plant-derived antioxidants that possess metal-chelating capabilities and radical-scavenging properties (Bors and Saran 1987; Lopes *et al* 1999). Phenols display a vast variety of structures which can be divided into three main classes, which are flavonoids, tannins and phenolic acids (Strube *et al* 1993). Phenolic compounds known to possess high antioixidant activity are commonly found in fruits, vegetables and herbs. Somawathi *et al* (2014) reported that *S. melongena* was found to have varying levels of total phenolic content, ranging from 48.67±0.27 to 61.11±0.26 mg GAE/100g on fresh weight basis. Nisha *et al* (2009) found the total phenolic content of brinjal to be in the range of 49.02±1.3-106.98±2.2 mg gallic acid equivalents/100g. Eggplant has a high antioxidant capacity (Cao *et al* 1996), which is of great interest for improving the marketing of this crop (Picha 2006; Sun-Waterhouse 2011). The main phenolics in the eggplant flesh are chlorogenic acid and its conjugates, which normally account for up to 95% of the total phenolics in the fruit flesh (Whitaker and Stommel 2003). Chlorogenic acid and AA have similar antioxidant activities (Kim et al 2002; Triantis *et al* 2005), and the chlorogenic acid values are much higher (on average 39 times higher), which confirms that phenolic acids account for most of the antioxidant activity of eggplant (Akanitapichat *et al* 2010; Luthria 2012). Here, the values we have obtained are similar to those found in other studies (Prohens *et al* 2007; Raigon *et al* 2008, 2010; Concellon *et al* 2012).

Table 7: Content of phenolic compounds in response to different PGPR cultures

Treatments	Total Phenols (mg/100g)	ODH Content (mg/100g)	Flavonols (mg/100g)
Control	39.45±0.41	31.34±0.08	14.06±0.10
T1	70.24±0.09	52.38±0.09	62.61±0.13
T2	83.77±0.09	11.40±0.14	77.06±0.07
T3	100.40±0.13	70.78±0.52	54.55±0.24
T4	32.85±0.16	58.83±0.15	25.21±0.04
T5	36.80±0.10	29.80±0.13	71.36±0.09
T6	98.75±0.10	62.79±0.20	82.98±0.19
T7	129.90±0.10	39.73±0.09	148.64±0.26
T8	129.59±0.26	26.82±0.17	100.02±0.07
T9	111.77±0.11	45.94±0.06	133.27±0.09
T10	92.03±0.11	15.32±0.08	88.94±0.06
CD (5%)	0.292	0.411	14.01

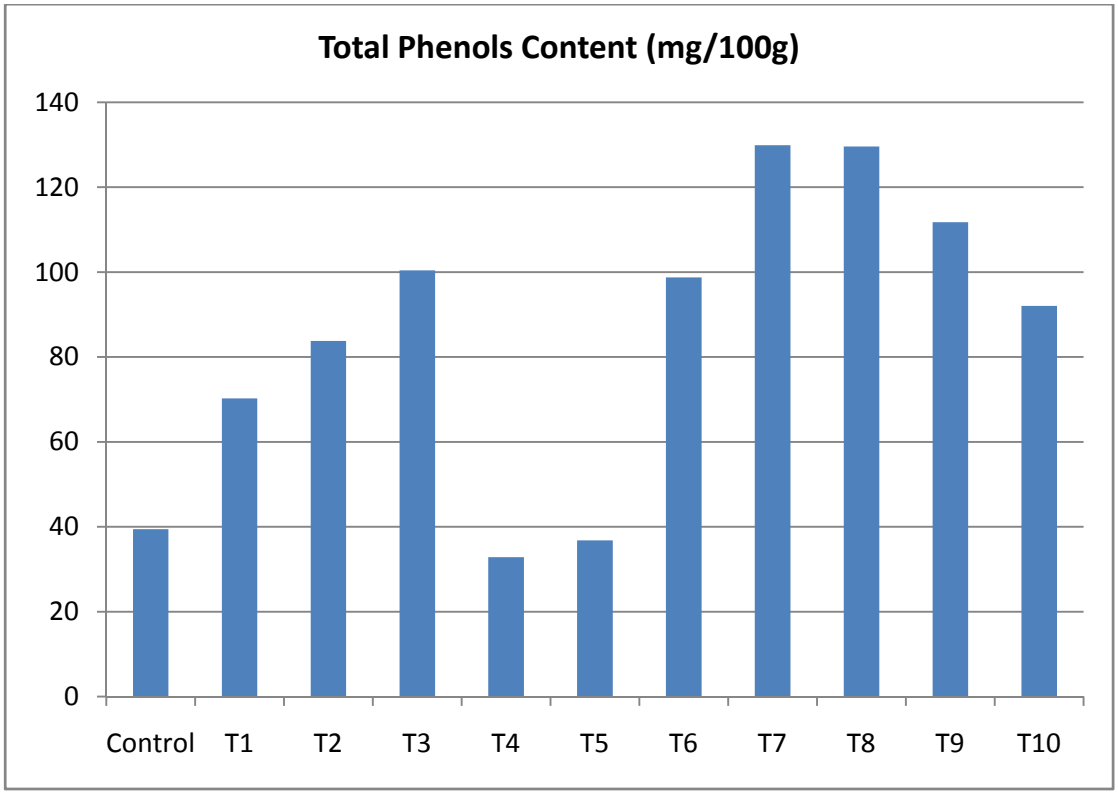

Fig 10: Total phenolic content in the fruits of brinjal in response to different PGPRs

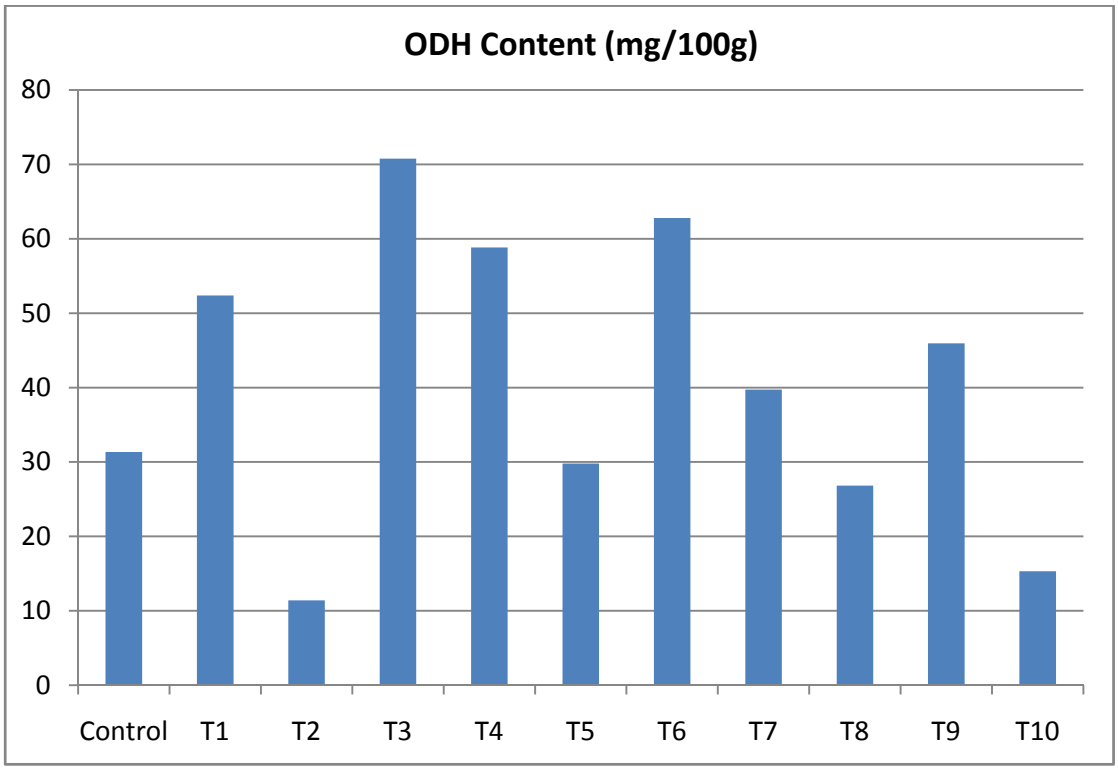

Fig 11: ODHs in the fruits of brinjal in response to different PGPR cultures

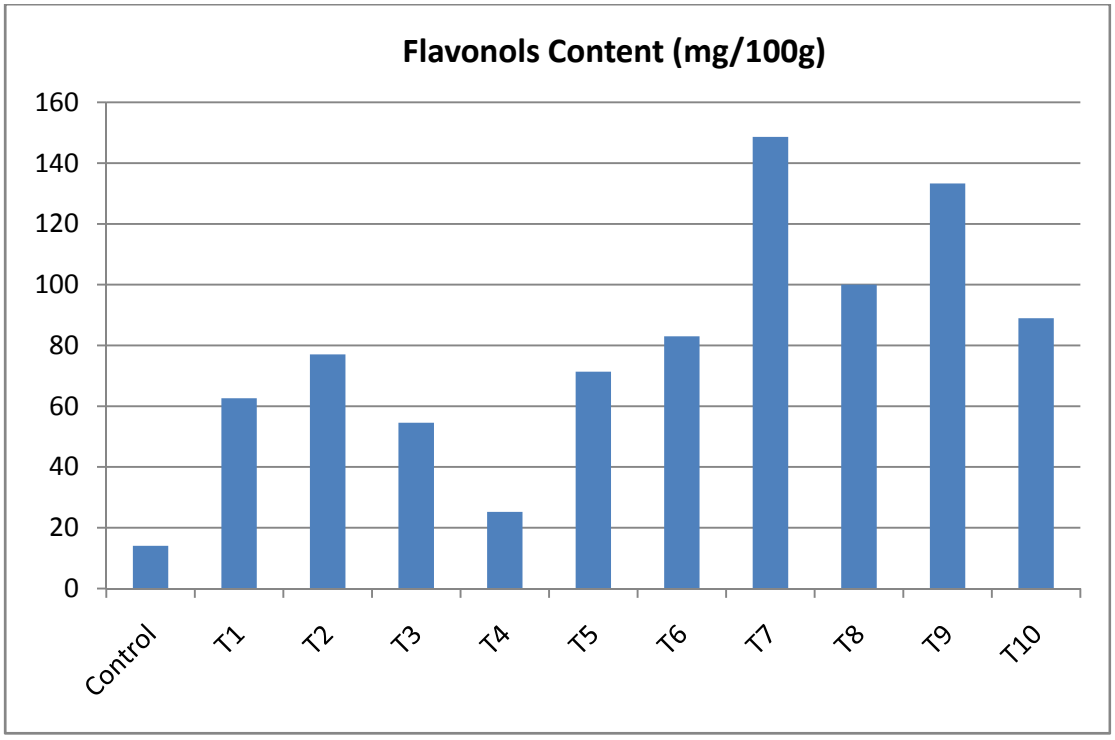

Fig 12: Flavonols content in the fruits of brinjal in response to different PGPR cultures

4.4.4 Activities of sucrose metabolizing enzymes

Sucrose synthase and invertase activities were assayed in the fruits of brinjal. In the present study, maximum activity of sucrose synthase was shown with the treatment PGPR9 (2.46±0.10 µg sucrose/min/mg FW). Maximum invertase activity was shown with the treatment PGPR7 (29.85± 0.32 µmole glucose/h/g FW) as presented in table 8.

Tomlinson *et al* (1991) assayed Invertase and sucrose synthase activities in the vascular bundles isolated from grapefruit (*Citrus paradisi*) during periods of rapid sucrose translocation into fruit. Sucrose synthase activity per unit protein was greater in the isolated dorsal vascular bundles than in any other fruit tissue assayed. Total invertase activity was five-to 10-fold less than that of sucrose synthase in all tissues examined.

A study on sucrose metabolism was done in maize seedlings. The sucrose synthase activity was detected in the cell elongation zone of the seminal root and root apices of lateral roots. These results suggested that sucrose is transported to the root elongation zone and the surrounding tissue of the lateral root primordial, and is cleaved into glucose, fructose, and UDP-glucose by invertase or sucrose synthase. This suggested that sucrose contributes to root formation by serving as the energy source, the carbon source for cell wall synthesis, and as a compatible solute for cell elongation. Invertase activity was detected as the modulated cell wall signal and the weak intercellular signal in the epidermis, cortex, and central cylinder (Kim *et al* 2000). Albrecht and Mustroph (2003) showed that increased sucrose synthase

activity was observed in the tip region and stele of root in wheat subjected to the O_2 deficit condition.

Invertase activity has been negatively correlated with sucrose accumulation in organs that store sucrose. Immature sugarbeat root (Giaquinta 1979) and immature citrus fruit (Kate and Kubota 1978) exhibited high invertase activity that disappeared prior to sucrose accumulation. Moreover, high invertase activity was correlated with rapid growth of stem tissue in sugar cane, but was barely detectable as sucrose accumulated (Hatch *et al* 1963).The most common sugars are fructose and glucose, while sucrose content is low, suggesting that there is a considerable invertase activity in the eggplant fruit (Boo *et al* 2010).

Table 8: Effect of different plant growth promoting rhizobacteria on the activities of sucrose metabolizing enzymes in brinjal at different stages of growth

Treatments	Invertase (µmole glucose/h/g FW)	Sucrose synthase (µg sucrose/min/mg FW)
Control	6.85±4.12	1.7±0.14
T1	1.65±0.11	0.64±0.06
T2	3.4±0.29	2.07±0.10
T3	8.70±0.12	1.94±0.05
T4	1.55±0.23	1.57±0.11
T5	8.62±0.10	0.81±0.14
T6	10.38±0.12	1.82±0.12
T7	9.85±0.32	2.16±0.06
T8	8.94±0.15	1.66±0.10
T9	19.23±0.28	2.46±0.10
T10	1.59±0.35	2.33±0.06
CD (5%)	2.78	0.21

4.4.5 Dry matter, iron and zinc content

In the present study, the dry matter in the fruits of brinjal was determined in response to different PGPR cultures. The treatment with PGPR10 showed the maximum dry matter content (10.01±0.01%) as presented in the table 9. Iron and Zinc contents were also measured in the fruits. It was observed that the treatment with PGPR6 had maximum Zinc content (0.32±0.02%) and the treatment given with PGPR7 showed the maximum Iron content

(0.76±0.76%) as presented in table 9. A similar analysis of dry matter, protein and phenolics content was done in 31 varieties of eggplant. Considerable differences in the composition among varieties were detected in all the traits studied with relative differences between the lowest and highest values of 42.9% for dry matter. Positive mean heterosis was detected for dry matter content (8.4%). Contents of dry matter and protein were positively correlated (Raigon *et al* 2008).

Nitrogen and calcium are the two elements among the main (N, P, K, Ca, Mg) nutrients, that are responsible for proper nutrition state of the plants. Within intensive horticulture the proper nutrition of the plants forms the main factor shaping the volume and quality of vegetable crops (Kader 2008, Fallovo *et al* 2009).

Table 9: Dry matter and mineral content (Fe, Zn) in the fruits of brinjal in response to different PGPR cultures

Treatments	Dry matter (%)	IRON (mg/100g)	ZINC (mg/100g)
Control	8.60±0.16	0.25±0.04	0.17±0.03
T1	8.54±0.06	0.05±0.05	0.18±0.03
T2	9.04±0.18	0.28±0.28	0.19±0.05
T3	8.86±0.05	0.20±0.19	0.05±0.02
T4	7.52±0.09	0.33±0.33	0.15±0.03
T5	8.85±0.30	0.37±0.37	0.11±0.01
T6	9.26±0.19	0.24±0.24	0.32±0.02
T7	9.50±0.20	0.76±0.76	0.24±0.02
T8	7.95±0.05	0.22±0.22	0.16±0.03
T9	10.01±0.05	0.46±0.46	0.25±0.02
T10	9.68±0.43	0.21±0.21	0.13±0.02
CD (5%)	0.41	0.74	0.59

4.5 Effect of different plant growth promoting rhizobacteria on the fruit quality of brinjal (plant height, average fruit weight and number of fruits per plant)

Maximum plant height was observed to be (71.21±0.30 cm); maximum average fruit weight was observed to be (86.56±0.91g) and the maximum number of fruits was observed to be 35.33±0.47 with PGPR1 as presented in Table 10. These agronomic characteristics have been shown in plate 2.

Plate 1: DPPH radical scavenging activity of *Solanum melongena* L.

Table 10: Effect of PGPR cultures on the fruit quality and agronomic characters in the fruits of brinjal

Treatments	Plant height (cm)	Average fruit weight (g) at final harvest	Number of fruits per plant at final harvest
Control	50.57±0.31	59.89±0.09	19.33±0.47
T1	60.61±0.60	65.58±2.21	35.67±0.94
T2	55.15±0.30	60.19±0.26	10.33±0.47
T3	45.58±1.08	78.60±0.35	19.67±0.47
T4	63.59±0.75	74.83±2.55	28.33±0.47
T5	58.04±0.14	76.44±2.79	24.33±1.70
T6	53.58±0.85	70.51±0.57	24.67±0.47
T7	70.23±0.74	86.85±0.91	20±0
T8	58.3±0.73	62.77±0.63	20.67±0.94
T9	67.58±0.32	78.93±1.42	25.67±0.47
T10	56.15±0.60	64.56±0.90	21.67±0.47
CD (5%)	1.34	3.05	1.56

PGPRs promote plant growth by secreting phytohormones. One such phytohormone is gibberellin. An experiment was carried out during kharif season 2013 to study the efficacy of growth regulators on growth characters and yield attributes in brinjal. It was found that Gibberellin can promote the activity of xyloglucan endotransglycosylase (XET) which loosens the cell wall and increase cell permeability (Saptari and Dewi 2013). Similar result was found in brinjal by Meena and Dhaka (2003). Exogenous supply of growth regulators may enhance the accumulation of photosynthates and efficient utilization of food reserves for the development of fruit. These results were supported by the findings of Mehta *et al* (1989) in chilli. Plants exhibited improvement relevant to growth, sustainable health and enhancement in flowering and fruit parameters in a coordinated manner for collective contribution towards the higher weight of fruit. These results are in confirmation with reported findings of Sumiati (1987), and Edison (1991) in tomato and Pampapathy and Rao (1975) in brinjal.

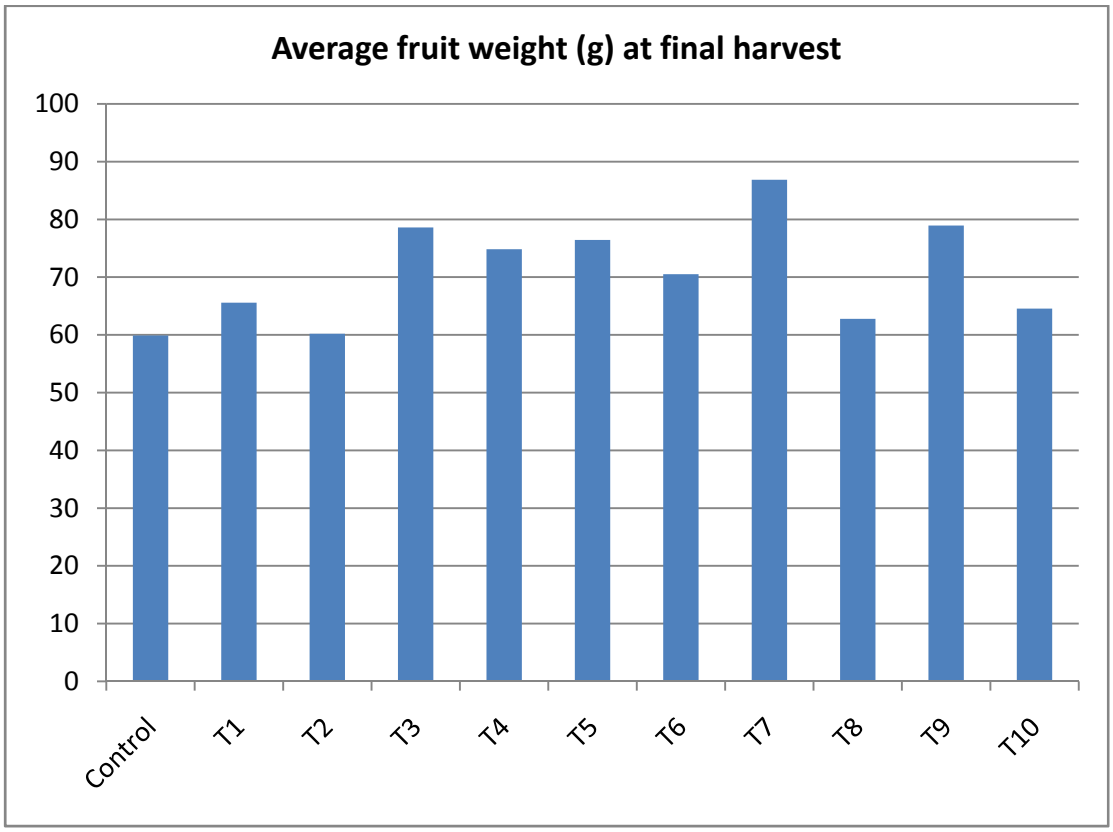

Fig 13: Average fruit weight of brinjal in response to different PGPR cultures

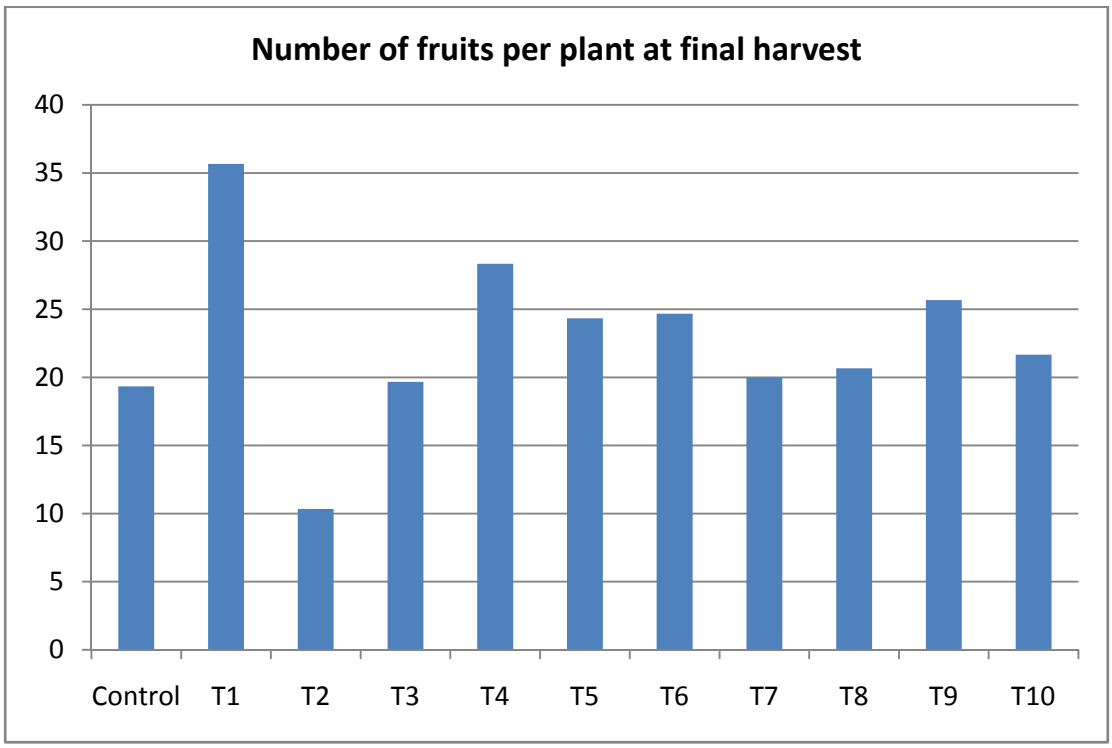

Fig 14: Effect of different PGPR on the number of fruits of brinjal at the final harvest

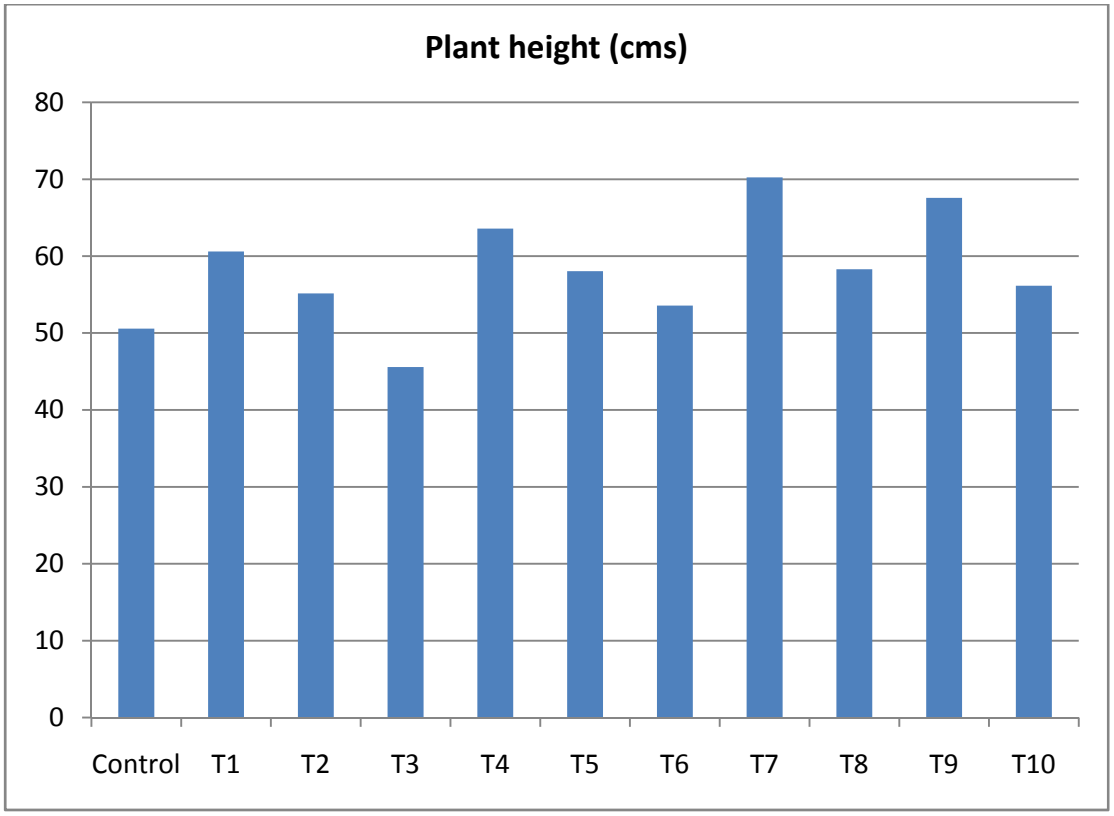

Fig 15: Effect of different PGPR cultures on the plant height in brinjal

CHAPTER-V

SUMMARY

Brinjal (*Solanum melongena* L.) is a tropical and subtropical crop of warm areas. Brinjal belongs to Solanaceae family which also includes crops such as potato, sweet pepper and tomato. The name Brinjal is popular in Indian subcontinents and is derived from Arabic and Sanskrit whereas the name eggplant has been derived from the shape of the fruit of some varieties, which are white and resemble in shape to chicken eggs. It is also called Aubergine in Europe.

The fruits of brinjal are an excellent remedy for liver problems. The green brinjal is good for diabetic patients (Shukla and Naik 1993). Brinjal is a warm season crop, therefore susceptible to severe frost. Low temperature during cool season causes deformation of fruits. A long and warm growing season is desirable for successful Brinjal production. Cool nights and short summers are unsuited to satisfactory yield. A daily mean temperature of 13 degree centigrade to 21 degree centigrade is favorable for optimum growth and yield (Kalloo *et al* 1990, Kumar *et al* 2000, Mohanty and Prusty 2000, Thapa 2002).

The present investigation was conducted to study the effect of plant growth promoting rhizobacteria on germination, antioxidant enzymes and enzymes of starch degradation in the laboratory experiment; and also their effect on various agronomic characters, fruit quality, antioxidant capacity, various biochemical plant growth parameters and yield of brinjal after harvesting the crop at maturity.

Various PGPR cultures cause significant increase in germination percentage of brinjal seeds. PGPR7 significantly enhanced the seed germination to 90% and also increased the shoot length in brinjal to 46.37 cm as presented in table 2. Maximum root length (51.07cm) was observed in seedlings treated with PGPR7. Treatment with PGPR7 showed maximum SOD and Catalase activities i.e. 94.83 units/min/mg FW and 62.56 µmole/min/mg FW respectively.

PGPR9 induced peroxidase activity (7.80 change in absorbance min/g FW) in comparison with the control group (5.49 change in absorbance min/g FW). Antioxidant capacity in the seedlings treated with PGPR7 showed a higher content of 94.80% over the untreated control.

Maximum amylase activity has been shown with the treatment given with PGPR7 (0.42±0.42 mg maltose/mg protein/h) followed by PGPR9.

In the present study, the anthocyanin content was found in the range 86.56mg/100g to 104.50mg/100g. Treatment with PGPR7 gave the maximum anthocyanin content (104.50mg/100g).

In the present study, the reducing sugars content was found to be maximum with the treatment done with PGPR7 (8.36%) whereas the treatment done with PGPR4 showed the minimum content of reducing sugars (1.17%) as compared to the untreated control which showed 2.43% of reducing sugars. The content of non-reducing sugars was also observed to be maximum with the treatment given with PGPR7 (8.78±0.17%). The ODHs varied from 11.40 to 70.78 mg/100g having highest content with the treatment done with PGPR3 (70.78±0.52 mg/100g). Highest content of flavonols content in brinjal was found when treated with PGPR7 (148.85±0.26 mg/100g). In the present study, maximum activity of sucrose synthase was shown with the treatment PGPR9 (2.46±0.10 µg sucrose/min/mg FW). PGPR9 showed maximum invertase activity (19.23 µmole glucose/h/g FW), maximum peroxidase activity (7.80 change in absorbance/min/g FW), maximum nitrogen content (2.85±0.09%), maximum starch content (4.22±0.22%) and maximum activity of sucrose synthase (2.46±0.10 µg sucrose/min/mg FW). It was observed that the treatment with PGPR6 had maximum zinc content (0.32±0.02%) and the treatment given with PGPR7 showed the maximum iron content (0.76±0.76%) and maximum invertase activity in fruits (29.85±0.32 µmole glucose/h/g FW). The highest plant height, average fruit weight and number of fruits in brinjal were observed with the treatments given with PGPR2, PGPR7 and PGPR7 respectively. Maximum plant height was observed to be (71.21±0.30 cm); maximum average fruit weight was observed to be (86.56±0.91g) and the maximum number of fruits was observed to be 35.33±0.47. The treatment with PGPR10 showed more dry matter content (10.01±0.01%). Treatment with PGPR7 gave highest protein content (3.64±0.07%).

From this it was concluded that the treatment given with PGPR7 led to better germination, higher activities of antioxidant enzymes at different stages of growth and other plant growth parameters, antioxidant capacity and fruit quality in brinjal (PBHL 51) under study.

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