# Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against Bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)

**THESIS**

***Submitted in partial fulfilment of the requirements for the award of degree***

***Of***

***DOCTOR OF PHILOSOPHY***

***IN***

***BOTANY***

***BY***

**MEHJABEEN AFAQUE**

**DEPARTMENT OF BIOLOGICAL SCIENCES**

**SAM HIGGINBOTTOM UNIVERSITY OF AGRICULTURE TECHNOLOGY AND SCIENCES**

**ALLAHABAD-211007**

**2018**

**ID. NO. 12PHBOTA106**


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## Certificate of Original Work

This is to certify that the thesis entitled, "**Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against bacterial wilt of Tomato (*Lycopersicon esculentum* Mill.)**" submitted for the degree of Doctor of Philosophy in Botany, Department of Biological Sciences, Faculty of Science, Sam Higginbottom University of Agriculture, Technology and Sciences, is a bonafide research carried out by Ms. **Mehjabeen Afaque**, ID number **12PHBOTA106**, under my supervision and guidance.

The experimental findings presented in this thesis are genuine and original to the best of my knowledge, the script of the thesis has been written by the candidate herself and no part of this thesis has been submitted for any other degree or diploma in any other university. The assistance and help received during the course of this investigation has been duly acknowledged.

The thesis is being forwarded for acceptance in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy in Botany** of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P.)

Place: Allahabad
Date: May 20, 2017

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## CERTIFICATE OF RECOMMENDATION BY STUDENT ADVISORY COMMITTEE

This thesis entitled '**Efficacy of plant growth promoting Rhizobacteria (PGPR) against bacterial wilt of Tomato (*Lycopersicon esculentum* Mill.)**' has been prepared and submitted by Ms. **Mehjabeen Afaque** ID no. **12PHBOTA106** for the award of degree of DOCTOR OF PHILOSOPHY in Botany of the faculty of Science of Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad.

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# DECLARATION

I hereby declare that the thesis **"Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against Bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)"** being submitted as partial fulfilment for the degree of Ph. D. in botany, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad (U.P) is an original piece of research work done by me under the supervision of Dr. Suchit. A. John, Associate Professor. To the best of my knowledge, no part or whole of the thesis has not been submitted elsewhere for the award of any other degree or any other qualification of any university or examining body.

Place: Allahabad

Date:

(Mehjabeen Afaque)

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*Place: Allahabad*

*Date:* *(MehjabeenAfaque)*

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| % | : | Per cent |
| & | : | And |
| / | : | Per |
| @ | : | At the rate of |
| $^{0}$B | : | Degree Brix |
| $^{0}$C | : | Degree Celsius |
| ANOVA | : | Analysis of variance |
| C. D. | : | Critical difference |
| cm | : | Centimeter |
| CRD | : | Completely Randomized Design |
| CV | : | Coefficient of variation |
| d.f. | : | Degree of freedom |
| DAT | : | Days after transplanting |
| DAI | : | Days after inoculation |
| DMSO | : | Dimethyl sulphoxide |
| ESS | : | Error sum squares |
| *et al.* | : | And other |
| F (cal.) | : | F calculated |
| g | : | Gram |
| ha | : | Hectare |
| *i.e.* | : | That is |
| kg | : | Kilogram |

| | | |
|---|---|---|
| Max. | : | Maximum |
| Min. | : | Minimum |
| ml | : | milliliter |
| mm | : | Millimeter |
| mg | : | milligram |
| µl | : | micro liter |
| nm | : | nanometre |
| No. | : | Number |
| R | : | Replication |
| S | : | Significant |
| S.E(m) | : | Standard error of mean |
| S.E(d) | : | Standard error of Deviation |
| t | : | Tonnes |
| *viz.* | : | Namely |

# Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against Bacterial Wilt of Tomato (*Lycopersicon esculentum Mill.*)


## Abstract

Tomato (*Lycopersicon esculentum Mill.*) is one of the most consumed and widely grown vegetable crops in the world which is an important source of vitamins and minerals in human diet. They contain the carotene, lycopene, one of the most powerful natural antioxidants. Tomato suffers from many diseases caused by fungi, bacteria, viruses and nematodes. Over 200 diseases have been reported to affect the tomato plants in the world. Among the bacterial diseases, bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most destructive and widespread disease of bacterial origin affecting all solanaceous crop causing plant death and significant yield losses. The management of Bacterial wilt of tomato is difficult but managed by chemical. However chemicals have many ill effects on the environment, farmers and consumers and also affect the non-target organisms. Keeping in mind, losses of yield and there harmful effect we selected bio agents i.e. PGPR for the management of disease. In view of this, the aim of the study was to screen the potential PGPR against the bacterial wilt pathogen *in-vitro* via well-agar diffusion method and disc diffusion method against the pathogen *Ralstonia solanacearum*. The Selected five potential PGPRs PR3, PR9, PR17, PR25 and 3NAA8 were evaluated against the bacterial wilt pathogen on three different varieties of tomato in *in-situ* condition. The varieties are Pusa-120, Navodaya and Golden green. Among all, the reduction in disease incidence percentage was recorded in plants treated with PR17 and 3NAA8 among all. In addition, the plant growth and yield was also improved by PR17 and 3NAA8 out of all five potential PGPR. Therefore, these results suggest that out of five antagonistic strains PR17 (*Enterobacter* spp.) and 3NAA8 (*Bacillus* spp.) support good antagonistic activity and could be applied as biocontrol agents against Bacterial wilt of tomato and for their potential to promote tomato plant growth.



**Keywords**: Biocontrol; PGPR (Plant growth promoting rhizobacteria); Bacterial wilt; *Ralstonia solanacearum*;

# Chapter- 1

# Introduction

# INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated vegetable crops which is grown worldwide **(Balemi *et al.*, 2005)**. Tomato is a prominent member of Solanaceae family **(Harlan, 1992; Ali *et al.*, 2012).** Tomatoes are valuable and key food crop throughout the tropics and subtropics for low-income farmers. Tomato is warm season annual plant that grows with the average optimum temperature range of 25°C to 29°C **(Ejaz *et al.*, 2011)**.In India, tomato occupies 4180ha with annual production of 6.4 million tonnes **(Anon, 1988).**Tomatoes contain essential nutrients including vitamin A, C and E **(Beecher, 1998)**, providing approximately 20 mg of vitamin C per 100 grams **(Wilcox *et al.*, 2003)**.One medium size tomato (~145 grams) can provide up to 20% of vitamin A and 40% of the vitamin C of Recommended Daily Allowance of vitamin A and C **(Kelly and Boyhan, 2010)**. Tomatoes hold a significant position based on nutritional view point as its 100 g encompasses virtually 48 mg calcium, 27 mg ascorbic acid, 20 mg phosphorus, 3.6 g carbohydrates, 0.9 g proteins, 0.8 g fibre, 0.4 mg iron, 0.2 g fats and 20 K calories of energy. Besides these nutrients it also contains $\beta$-carotene and Lycopene pigments, also calcium, water, and niacin, which are essential for metabolism **(Olaniyi *et al.*, 2010).** The red colour of tomato is due to lycopene is extremely vital. Tomatoes prevent scurvy and also keep the blood vessels healthy **(Ejaz *et al.*, 2011).** Tomatoes are very nutritious and are low in calories. Besides being eaten fresh, the versatile tomato can be baked, stewed, fried, juiced, or pickled and can be used in soups, salads, and sauces. Tomatoes can be grown in different soil types, but a deep, loamy, well-drained soil is ideal. Tomatoes grow best in a slightly acid soil with a pH of 6.2-6.8.

Bacterial wilt of tomato incited by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.,* (Syn. *Pseudomonas solanacearum* E.F. Smith) is one of the devastating diseases which affect the vascular bundles of plants. Bacterial wilt also known as solanaceous wilt southern bacterial wilt, and southern blight firstly was described in 1896 by E.F. Smith in tomato, potato and eggplant. The disease is known to spread very quickly through furrow irrigation as well as rain **(Taylor *et al.*, 2011)**. The disease affects a wide range of crops especially members of Solanaceae family such as pepper, brinjals, and potatoes as well as their weed relatives and ornamental crops **(Champoiseau and Momol, 2009; Elphinstone, 2005).**

It is considered as one of the major diseases of tomato and other solanaceous plants. It occurs in the wet tropics, sub-tropics and temperate regions of the world. The management of disease is difficult because of its wide host range, the exceptional ability of the pathogen to survive in the roots of non-host plants and in the soil **(Kumar and Sood, 2001)**.

Symptom expression is favoured by high temperatures (85-95°F / 29-35°C) and symptoms of the disease may progress rapidly after infection. At the early stages of disease, the first visible symptoms of bacterial wilt are usually occurring on the foliage of plants. These symptoms consist of wilting of the youngest leaves at the ends of the branches during the hottest part of the day. At this stage, only one or half a leaflet may wilt, and plants may appear to recover at night, when the temperatures are cooler. When disease develops under favourable conditions, the whole plant may wilt quickly and desiccate although dried leaves remain green, leading to general wilting and yellowing of foliage and eventually plant death **(Kelman 1953; Smith 1920)**. The other common symptom which can be associated with bacterial wilt in the field is the stunting of plants. These symptoms can appear at any stage of plant growth, although in the field it is common for healthy-appearing plants to suddenly wilt when fruits are rapidly expanding. In young tomato stems, infected vascular bundles may become visible as long, narrow, dark brown streaks **(Kelman 1953)**. In young, succulent plants of highly susceptible varieties, collapse of the stem may also be observed.

*Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum* and *Burkholderia solanacearum* is the causal agent of bacterial wilt of tomato. *R. solanacearum* is anaerobic non-sporing, Gram negative plant pathogenic bacterium **(Denny 2006)**. It is soil borne and motile with a polar flagellar tuft and sometimes 1 to 4 polar flagella. It colonises the xylem, causing wilt in a very wide range of potential host plants **(Martin *et al.*, 1978)**. The organism grows most favourably in temperatures of 35 - 37ºC, but can survive in tomato plants across a temperature range of 10 - 41$^{O}$C **(Hayward, 1991)**.*Ralstonia solanacearum* can survive during winters in plant debris or diseased plants, wild hosts, seeds or vegetative propagative organs **(Elphinstone *et al.*, 2005)**. *R. solanacearum* can also survive in cool weather in a viable stage but not culturable **(Countinho, 2005)**. The large number of *R. solanacearum* can shed from roots of infected plants and dispersed on plant surfaces, can enter the surrounding soil or water or may be acquired by insect vectors **(Haywards, 1991)**. *Ralstonia solanacearum* affect several crops like potato, eggplant, tomato, pepper, tobacco etc. **(Hayward, 1991)**. *Ralstonia solanacearum* was classified into five races on the basis of differences host range **(Buddenhagen *et al.*, 1962; He *et al.*, 1983; Pegg and Moffet, 1971)**, and six biovars

according to ability to oxidase three hexose alcohols and three disaccharides **(Hayward, 1964 and 1991; Hayward and Hartman, 1994; He *et al.*, 1983)**. Unlike other phytopathogenic bacteria, race systems of *R. solanacearum* are not based on gene-for-gene interactions instead these are determined based on the pathogenicity of each isolate in 2 different kinds of host plants. Although the biovar and race systems are widely accepted for the classification of *R. solanacearum,* there is no definite correlation between biovar and race.

Bacteria that colonize roots of plants and enhance the plant growth are termed as plant growth-promoting rhizobacteria (PGPR) **(Kloepper *et al.*, 1980)**. Independent of the mechanisms of plant growth promotion, PGPRs colonize the rhizosphere, the rhizoplane, i.e. root surface, or the root itself i.e. within radicular tissues **(Gray and Smith, 2005)**. In the Rhizosphere only 1 to 2% of bacteria promote growth in plants **(Antoun and Kloepper, 2001)**. PGPR affect in two different ways for plant growth i.e. indirectly or directly. The direct enhancement of plant growth by PGPR results either by providing the plant with a compound like phytohormones that is synthesized by the bacterium, or promote the uptake of some nutrients from the environment **(Glick, 1995)**. The indirect promotion of plant growth takes place when PGPR suppress the deleterious effects of harmful phytopathogenic organisms. This can occur by producing antagonistic substances that inhibits pathogen growth or by inducing resistance to pathogens **(Glick, 1995)**. A particular PGPR may affect plant growth and development by using any these mechanisms. PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as production of phytohormone for e.g. Auxin **(Patten and Glick, 2002)** cytokinins **(Timmusk *et al.*, 1999)**, gibberellins **(Gutie´rrez-Man˜ero *et al.*, 2001)**, decrease of plant ethylene levels **(Glick *et al.*, 2007)** or nitrogen fixing associated with roots **(Dobereiner, 1992)** solubilisation of inorganic phosphate and mineralization of organic phosphate or other nutrient **(De Freitas *et al.*, 1997; Jeon *et al.*, 2003)** antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds and competition with detrimental microorganisms **(Dobbelaere *et al.*, 2002; Dey *et al.*, 2004; Lucy *et al.*, 2004)**.

Bacterial wilt control is challenging as use of chemical products including antibiotics, fertilizers and fungicides are not effective in managing the disease **(Hartman and Elphinstone, 1994).** Approaches for disease control such as field sanitation, crop rotation, and application of resistant varieties, have shown limited success **(Guo *et al.*, 2004)**.Biological control offer an eco-friendly and cost-effective alternative as an important component of an integrated disease

management program **(Li *et al.,* 2007)**.Several potential biocontrol agents against bacterial wilt of tomato have been reported **(Guo *et al.,* 2004; Lemessa and Zeller, 2007; Xue *et al.,* 2009)**. Application of biological controls products for soil-borne pathogen has gained popularity in recent years due to environmental concerns raised on the use of chemical products in disease control **(Haas and De'fago, 2005).** Biological control methods have been widely accepted and advocated for as key practice in sustainable agriculture with the biggest potential of the biological control being microorganisms, arbuscular mycorrhizal fungi (AMF) **(Sharma and John, 2002; Tahat *et al.,* 2010)** and some naturally occurring antagonistic rhizobacteria such as *Bacillus* sp., *Pseudomonas* sp. **(Guo *et al.,* 2004).**

## JUSTIFICATION

*Ralstonia solanacearum* being soil-borne poses serious challenges in its management and control especially in already infected fields leading to reduced incomes to small scale growers **(Taylor *et al.,* 2011).** A sustainable, affordable and effective control method needs to be introduced to prevent further crop loss, also which do not harm the environment too. The strategy to control bacterial wilt of tomato must guarantee continuous and increased production of tomato. Tomato production being a key income earner to some families, it will ensure increased incomes for the farmer and fair prices to the consumers **(Taylor *et al.,* 2011).**

Biological control not only increases the crop yield and suppresses disease but also avoids environmental pollution. It has been estimated that more than 100 million tonnes of nitrogen, potash and phosphate-chemical fertilizers have been used annually in order to increase plant yield. **(Glick *et al.*,1999).**Keeping in view the extent of damages caused by the pathogen *Ralstonia solanacearum* to the tomato crop and the ill effects of chemical treatments on the host, the present study entitled "**Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against Bacterial Wilt of Tomato (*Lycopersicon esculentum* Mill.)**" is conducted on three different varieties Pusa 120, Navodaya and Golden Green which is an effort to improve tomato crop through the use of PGPR by reducing the disease effects.

## OBJECTIVES

The study entitled, "**Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)**" was carried out with the following objectives:

1) To screen potential PGPR against *Ralstonia solanacearum.*
2) To compare the efficacy of selected PGPR on growth of tomato.
3) To evaluate selected potential PGPR for the management of bacterial wilt disease on tomato.

# Chapter-2

# Review of Literature

# REVIEW OF LITERATURE

## 2.1 Tomato- the crop

Tomato (*Lycopersicon esculentum* Mill.) is second most **(Dorais *et al.*, 2008)** important vegetables worldwide **(Balemi *et al.*, 2005)**. It is the world's largest vegetable crop after potato and sweet potato but it tops the list of canned vegetables **(Olaniyi *et al.*, 2010)**. Tomato belongs to genus *Lycopersicon* under the family *Solanaceae* having chromosome number 2n=24. This family also includes other well-known species, such as potato, tobacco, peppers and eggplant. Common names for the tomato are: tomate (Spain, France), tomat (Indonesia), faan ke'e (China), tomati (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili). Tomato is an annual plant, which can reach a height of over two metres. Tomato requires a moderately cool, dry climate for high yield and premium quality. However, it is adapted to a wide range of climatic conditions from temperate to hot and humid tropical. The required optimum temperature for most varieties lies between 21 and 24 °C. The plants can survive a range of temperatures, but the plant tissues are damaged below 10 °C and above 38 °C **(Balemi *et al.*, 2005)**. It is an important condiment in most diets and a very reliable source of vitamins. It also contains a large quantity of water (%), calcium (%) and Niacin all of which are of great importance in the metabolic activities of man. The world produced 163.29 million tons of tomatoes in the year 2014-15 with the highest production of 51 million tonnes in China followed by India 18.73 million tons and America 13.8 million tons **(Singh *et al.*,2016).**

## 2.2 The pathogen-*Ralstonia solanacearum*

*R. solanacearum* (Smith 1896) **(Yabuuchi *et al.*, 1995)** is a soil-born bacterial plant pathogen which comprises a "species complex" provided its high heterogeneity **(Fegan and Prior, 2005).** *R. solanacearum* is distributed in many habitats all over the world and has an unusually broad host range **(Denny, 2006)**. It can infect over 200 plant species representing over 50 botanical families **(Hayward, 1991)**. *R. solanacearum* has turned into a model system to study plant-microbe interactions, pathogenicity determinants and pathogen ecological activities due to its global distribution, adaptive potential and large host range. Given the fact that *R. solanacearum* is a soil-borne pathogen and that resistance of the host is limited, Bacterial wilt is very difficult to control **(Saddler, 2005)**.

*R. solanacearum* was not the first name of this species. On the contrary, it has been classified in numerous genera since it was first described **(Smith, 1896)**. Although T.J. Burrill was the first to isolate the bacterium in 1890, E.F. Smith was the first to publish a scientific description and classify it in the genus *Bacillus* as *B. solanacearum* in 1896 **(Smith, 1908; Kelman, 1953)**. However, afterwards it was moved to the genus *Bacterium*, and to the genus *Pseudomonas* with the name of *Pseudomonas solanacearum*, temporary reclassified in the genera *Phytomonas* and *Xanthomonas* and eventually transferred back to the genus *Pseudomonas* in 1948. In 1992 it was placed in the genus *Burkholderia* **(Yabuuchi *et al.*,1992)** but, more recent phylogenetic and polyphasic phenotypic analyses pointed out that it would rather be accommodated in the new established genus of *Ralstonia*, in 1995 **(Yabuuchi *et al.*,1995)**. Since then, the bacterium is named *R. solanacearum* and belongs to the family *Ralstoniaceae* included in the β-subdivision of the Proteobacteria **(Stackebrandt *et al.*, 1988)**.

*Ralstonia solanacearum* on the basis of differences host range was classified into five races **(Buddenhagen *et al.*, 1962; He *et al.*, 2003),** and six biovars according to ability to oxidase three disaccharides and three hexose alcohols **(Hayward 1991; Hayward and Hartman, 1994; He *et al.*, 1983)**.*Ralstonia solanacearum* has broad host range throughout the world including more than 450 host species **(Wicker *et al.*, 2007).**

*R. solanacearum* single cell is a small rod shaped with rounded ends **(Smith, 1896; Kelman, 1953)**, with 0.5 to 0.7 by 1.5 to 2.5 μm of average size and are non encapsulated **(Deny and Hayward, 2001)**. It is a Gram-negative bacteria, and polar flagella when present. The bacterium has an oxidative metabolism and is generally considered a strict aerobe. However, under some circumstances, it is able to limited, slow growth when cells are not in direct contact with the air **(Kelman, 1951)**. *R. solanacearum* is catalase positive, oxidase positive, and reduces nitrates. *R. solanacearum* produces poly-β-hydroxybutyrate granules as cell energetic reserve. *R. solanacearum* strains from tropical areas all over the world have a high temperature optimum (35°C), whereas that of strains taking place at higher altitudes in the tropics and in subtropical and temperate areas is lower (27°C); but at 40°C no growth has been observed **(EPPO, 2004)**.Regarding pH requirements, in general *R.solanacearum* growth is inhibited in acid media but favoured in alkaline conditions (**Kelman 1953**). *R. solanacearum* can grow in 1% NaCl liquid media but, little or none in 2% NaCl. The bacterium can produce one to several polar flagella **(Clough *et al.*, 1997)** which provide it swimming motility **(Tans-Kersten *et al.*, 2001)**. *R. solanacearum* possesses diverse genes involved in colonization and wilting of host plants, such as those coding for lytic enzymes

and EPS, hypersensitive reaction and pathogenicity (*hrp*) genes, structural genes encoding effector proteins injected by a type III secretion system (T3SS) from the bacterium into the plant cell, genes coding for factors occupied in cell adherence, and others **(Schell 2000, Genin and Boucher 2004)**. In *R. solanacearum*, the *hrp* genes control induction of both, disease development and the hypersensitive reaction (HR) **(Boucher and Genin, 2001; Boucher *et al.*, 1992).**

This soil-borne bacterium belongs to the betaproteobacteria is responsible for bacterial wilt in important crops such as potato, tomato, eggplant, pepper, tobacco and banana. In fact, bacterial wilt is considered the single most destructive bacterial plant disease because of its extreme ferociousness, unusually broad host range and wide geographic distribution **(Prior *et al.*, 1998)**.

*R. solanacearum* is well thought-out a species complex-a heterogeneous group of related but genetically distinctive strains **(Allen *et al.*, 2005; Prior and Fegan 2005)**.*R. solanacearum* inhabits the vascular tissue of its hosts. The bacterium normally enters plant roots from the soil through natural openings or wounds where secondary roots emerge **(Araud-Razou *et al.*, 1998)**, colonizes the intercellular space of the root cortex and vascular parenchyma, and in due course enters the xylem vessels and spreads to the stem and leaves, where the pathogen cell density commonly surpasses $10^9$ CFU/g of host tissue **(Vasse *et al.*, 1995)**. After *R. solanacearum* has colonized the xylem, large numbers of bacterial cells are shed from roots, providing a pathway for bacteria to return to the soil and set off new infections **(Hayward, 1991)**. Affected plants usually die rapidly after suffering from chlorosis, stunting, wilting. The survival of *Ralstonia solanacearum* was strongly dependent on temperature irrespective of inoculums density and physiological state, with maximum survival occurring at $12^0$C, $20^0$C and $28^0$C and it can persist in sediment saturated with drainage water but died out when the sediment was died **(Elsas *et al.*, 2001)**. *Ralstonia solanacearum* causes symptoms in both above and below ground organs of the potato plants **(Weller *et al.*, 2000).**

**2.3 Taxonomic position of *Ralstonia solanacearum*:**

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Beta Proteobacteria

Order: Burkholderiales

Family: *Ralstoniaceae* **(Smith *et al.*, 1896)**

Bacteria, Gracilicutes, *Proteobacteria* β subdivision. It belongs to rRNA homology group II (nonfluorescent) within the *Pseudomonads*. *R. Pickettii* (saprophyte or human facultative pathogen), *Pseudomonas syzygii* (causing Sumatra disease of cloves), and the so-called Blood Disease Bacterium (BDB, causing blood disease of banana in Indonesia) are closely related to *R. solanacearum* and maycross-react in serological and DNA-based detection methods. Subclassification of *R. solanacearum* based on RFLP and other genetic fingerprinting studies **(Hayward, 2000)** is into Division I (biovars 3, 4 and 5 originating in Asia) and II (biovars 1, 2Aand 2T, originating in S. America). Further taxonomic division mainly based on nucleic sequence analysis into phyllovars and sequevars has been proposed **(Poussier *et al.*, 1999; Taghavi *et al.*, 1996)**.

### 2.4 Biochemical Tests of *Ralstonia solanacearum*

*Ralstonia solanacearum* isolates are gram's negative, the elastic thread or viscid material raised the loop from the bacterial solution by toothpick a few centimetres from glass slides as the indication of gram negative bacteria on Potassium hydroxide solubility test, isolates able to developed deep blue colour with oxidase reagent within few second which indicated the tested isolates were gram negative and the test was positive on Kovac's oxidse test **(Hossain, 2006)**. A number of methods have been developed to detect *Ralstonia solanacearum* where it exists as a latent pathogen, particularly in potato. These include indirect immuno fluorescence (IIF) microscopy followed by isolations on semi- selective media, fatty acid analysis and pathogenecity tests on indicator plants such as tomato **(Janse, 2008)**. The KOH technique is easier and more rapid than the traditional gram-strain in which dyes are engaged. The only disadvantage is that one dose not view the morphology of the bacteria in the course of the test. Place a generous loopful of bacterial culture (approximately 24 hours growth) on a microscope slide. Add a loopful of 3% KOH and stir for 5 to 10 seconds. Raise the loop from the bacterial solution and look for an elastic thread, DNA, I to 2 cm long that will stretch from the slide to the loop. Stringing forms with gram- negative bacterial cultures. The lack of stringing is indicative of gram-positive organisms **(Suslow *et al.*, 1982).**

**Hayward (1964)** devised Sucrose Peptone Agar (SPA) medium for characterizing difference in *Pseudomonas solanacearum.* It is non-selective medium that is useful for general purpose; however, it is not recommended for culturing *Agrobacterium*. It is particularly useful when looking for levan producing *pseudomonades*. Tetrazolium Medium (TTC) is used to test for pathogenic strains of the vascular bacterial pathogen, *Ralstonia solanacearum* **(Kelman**

**1954)**. Two colony types appear on this medium' the non-pathogenic or weakly pathogenic colonies are round, butyrous and deep red with narrow bluish borders. The highly pathogenic colonies are irregularly-round, fluidal, white colonies or white colonies with pink centres.

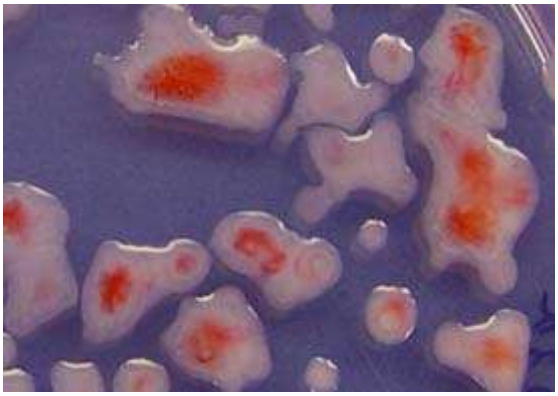


**Plate.2.4 Culture of virulent *Ralstonia solanacearum* on TZC agar.** (Photo courtsey of T. Momol and S.M. Olson, University of Florida, source: Google)

## 2.5 The disease- Bacterial wilt of tomato

Bacterial wilt is one of the major destructive diseases of tomato and other solanaceous plants. The disease is known to occur in the wet tropics, sub-tropics and some temperate regions of the world. The disease is difficult to manage because of its wide host range, the exceptional ability of the pathogen to survive in the roots of non-host plants and in the soil **(Kumar and Sood, 2001)**.In India, a yield loss study with one cultivar of tomato showed 10-100% mortality of plants and 0-91% yield loss **(Elphinstone, 2005).**

The symptoms include wilting of the youngest leaves which may appear to recover at night. Under favourable conditions, the entire plant may wilt quickly and desiccate, although dried leaves remain green, leading to general wilting and yellowing of foliage and eventually death of the plant. In young stems, infected vascular bundles may become observable as long, narrow, dark brown streaks. The bacterium can survive for days to years in water, wet soils or deep soil layers (>75 cm), depending on temperature conditions. In aquatic habitats, factors such as pH, salt level, surfaces provided by particulate matter, and the presence of competing, antagonistic or parasitic organisms can affect bacterial survival. Soil moisture content, soil type and plant material content in soil also can play a critical role in its survival in this habitat. At low temperatures (<4°C), bacterial population densities fall rapidly but the bacteria still can endure, often in a physiological latent state **(Patrice 2008).**

The browning and necrosis of the vascular tissues of the bacterial wilt infected potato tuber from which extensive secondary rotting can develop. Bacterial wilt causes desiccation and rapid death of the plant under warm environment **(Elphinstone, 2005)**. Cross-section of bacterial wilt infected potato tubers may reveal a grey-brown discoloration of vascular tissues, also called a vascular ring. The discoloration continues into the pith or cortex of the tuber. The presence of bacteria cells indicated by the oozing of milky-white sticky exudates (ooze), might also be observed in freshly-cut sections of infected tubers. Bacterial ooze may also be visible to eyes or at the point where the stolon attaches to the tuber **(Swanson *et al.*,2005)**. Tomato bacterial wilt is caused by *Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum.* The drooping of leaf followed by complete wilting of plant within a few days, vascular bundle becomes discoloured. The pathogen has different races attacking different crop plants. Tomato bacterial wilt is caused by race 1 strains, which has a wide host range and can survive for long time in soil **(Wang and Lin, 2005)**.

The weeds can be responsible for transmission of the pathogen through successive seasons **(Lopez and Biosca, 2005)**. The stunting of plant infected by bacterial wilt in the field can take place at any stage of plant growth (**Allen *et al.*, 2001).**The bacterial wilt caused by *Ralstonia solanacearum* is a major constraint to the production of economically important agricultural crops. This is widely distributed in wet tropics, sub-tropics and some warm temperate regions of the world. The scale to determine disease severity 1= No symptom, 2 = Top young leaves wilted, 3 = Two leaves wilted, 4 = Four or more leaves wilted and 5 = Plant dies **(Grey and Steck, 2001).** The bacterial wilt persists in the soil all year round. In general, race 3 of the bacterium survives better under conditions of high soil moisture and low temperature. Race 3 will usually decreases in virulence when temperatures exceed 35°C. The disease is most severe at 24-35°C and is seldom found in temperate climates where the mean temperature for any winter month falls below 10°C **(Stansbury *et al.*, 2001)**. The survival of *R. solanacearum* strongly dependent on temperature irrespective of inoculums density and physiological state, with maximum survival occurring at $12^0$C, $20^0$C and $28^0$C and it can persist in sediment saturated with drainage water **(Elsas *et al.*, 2001).** The bacterial wilt caused by *R. solanacearum* is particularly important disease during Kharif in India **(Singh and Pandey, 1988)**. The late planting reduced the incidence of *P. solanacearum* and advised that late planting could be a good practice to minimize the disease **(Ahmed and Talukdar 1978)**.

## 2.6 Management of bacterial wilt of tomato

Since *R. solanacearum* is a soil-borne pathogen and host resistance is limited, bacterial wilt is difficult to control. Some highly aggressive strains have been reported to cause severe symptoms, even to tomato varieties classified as resistant **(Denny *et al.,* 2006)**. Crop resistance has also been observed to be overcome due to high genetic diversity of the bacteria **(Wang *et al.,* 1988)**. Other methods like intercropping and crop rotation are often hampered due to a wide range of pathogens **(Nguyen *et al.,* 2010)**. Chemical control is nearly impossible to apply though use of antibiotics to control bacterial wilt started as early as 1952. Soil fumigants showed either slight or no effects **(Takahashi, 1984)**. Antibiotics such as streptomycin, ampicillin, tetracycline and penicillin showed hardly any effect **(Farag *et al.,* 1982)**. This is mainly because *R. solanacearum* is a soil-borne pathogen and is systemic in its action **(Sangoyomi *et al.,* 2011)**. The perception of biological control of plant diseases involves reduction or decrease in inoculums potential of a pathogen brought about directly or indirectly by other biological agencies **(Johnson *et al.,* 1972)**. Plant growth promoting rhizobacteria are known to exhibit bio-control of parasitic pathogens. Recent studies indicate that biological control of bacterial wilt disease could be achieved using antagonistic bacteria **(Ciampi-Panno *et al.,* 1989)**. Among PGPRs, *Pseudomonas fluorescens* have been reported to be effective against a broad spectrum of plant pathogens **(Loon *et al.,* 1998)**. Similarly the sporulating Gram positive bacteria like Bacillus spp. have also been used successfully for the control of plant diseases **(Kloepper *et al.,* 2004)**. Amongst fungi, Trichoderma spp. is known to be effective biological means to control soil borne diseases **(Howell, 2003)**. *Bacillus subtilis* has been reported to be effective in the management of bacterial wilt disease in tomato **(Sinha *et al.,* 2012)**. *P. aeruginosa* KUCd1, a cadmium tolerant strain reported to have PGP effect shows antagonistic effect towards several plant pathogens **(Guha *et al.,* 2007, and Sarkar *et al.,* 2013)** 79–81 though its effectiveness in controlling bacterial wilt has not been reported. Trichoderma spp. has proved to be helpful in the control of phytopathogens affecting different crops **(Benitez *et al.,* 2004 and Soytong *et al.,* 2005).**

## 2.7 The disease symptoms

Disease distribution may range from a few scattered plants or loci of infection in fields where low or erratic natural infestations occur to larger areas of wilting and dead plants in a field **(Kelman and Sequeira, 1965)**. Under natural conditions, the initial symptom in mature plants is wilting of upper leaves during hot days followed by recovery throughout the evening and early hours of the morning. The wilted leaves maintain their green colour as the disease progresses. Under hot and humid conditions, complete wilting occurs and eventually the plant dies. Occasionally, one-sided wilting is noticed whereby only some branches/shoots in a plant are seen to exhibit wilting **(Agrios, 2005)**. Massive invasion of the cortex might result in the appearance of water-soaked lesions on the external surface of infected stems. If an infected stem is cut crosswise, tiny drops of dirty white or yellowish viscous ooze exude from several vascular bundles **(Champoiseau *et al.*, 2009)**. There may also be discoloration of the vascular system, expressed in form of pale yellow to dark brown colour **(Gota, 1992)**.

**Plate.2.7 (a) Bacterial streaming from an Infected tomato stem.**

**(b)Progression of symptoms of bacterial wilt of tomato.**

## 2.8 Plant growth promoting rhizobacteria (PGPR)

The rhizosphere is a narrow zone of soil which contains diverse range of microorganisms such as the fungi, algae, nematodes, actinomycetes, protozoa and bacteria. The group of bacteria is subdivided into three subgroups (neutral, negative or positive) according to their effects on the plant physiology. Bacteria (rhizobacteria) are the most known (95%) and the most plentiful because of their high growth rate and capability to use different carbon and nitrogen sources among the microbial community of rhizosphere **(Glick, 2012).**These rhizobacteria can affect the plants physiology through different ways. Thus, the interactions and activities between rhizobacteria and plant can be advantageous, destructive or neutral **(Ordookhani and Zare, 2011).**

Plant-associated bacteria can be classified into beneficial, deleterious and neutral groups on the basis of their effects on plant growth **(Dobbelaere *et al.*, 2003**). Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria (PGPR**) (Kloepper *et al.*, 1989).**Thus, PGPR is a group of bacteria capable to actively colonize the plant root system and improve their growth and yield **(Wu *et al.*, 2005)**. For the first time by **Kloepper *et al.*, (1980)** proposed the term PGPR and was used specifically for the fluorescent *Pseudomonas* involved in the biological control of pathogens and enhancing plant growth. Later **Kapulnik (1981)** extended this term to the rhizobacteria capable to promote directly plant growth. Today, the term PGPR is used to refer to all rhizospheric bacteria capable to improve the plant growth by one or more mechanisms **(Haghighi *et al.*, 2011)**. **Bashan and Holguin (1998)** proposed the division of PGPR into two classes: biocontrol-PGPB (plant growth promoting bacteria) and PGPB.

A large variety of species belonging to the genus *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* was reported as PGPR (**Saharan and Nehra, 2011).** The PGPR effects depend on ecological and soil factors, plant species, plant age, development phase and soil type **(Werner, 2001).** For example, a bacterium certainly not produce beneficial effects to the plant when the soil receives chemical fertilizers, which promotes plant growth through nitrogen fixation or phosphorus solubilisation (compounds often present at low dose in many soils),. Also, the mutant bacterium *Pseudomonas fluorescens* BSP53a, hyper producing indole acetic acid (IAA) and stimulating root development of blackcurrant (*Ribes nigrum* L.) inhibits root development of Cherry (*Prunus avium* L.) (**Dubeikovsky *et al.*, 1993).**

Independent of the mechanisms of vegetal growth promotion, PGPRs colonize the rhizosphere, the rhizoplane (root surface), or the root itself (within radicular tissues**) (Gray and Smith, 2005)**. It is well established that only 1 to 2% of bacteria promote plant growth in the rhizosphere **(Antoun and Kloepper, 2001).** Bacteria of various genera have been acknowledged as PGPR, of which *Bacillus* and *Pseudomonas* spp. are the leading **(Podile and Kishore, 2006)**.

PGPR are classified based on their functional activities (Somers *et al.*, 2004) as (i) bio pesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites), (ii) rhizoremediators (degrading organic pollutants) (plant growth promotion, generally through phytohormones), (iii) phytostimulators and (iv) biofertilizers (increasing the availability of nutrients to plant) **(Antoun and Pre´vost, 2005).**

PGPR affect plant growth indirectly or directly. The direct promotion of plant growth by PGPR entails either providing the plant with a compound that is synthesized by the bacterium, for example phytohormones, or facilitating the uptake of certain nutrients from the environment (**Glick, 1995).** The indirect promotion of plant growth takes place when the ruinous effects of one or more phytopathogenic organisms inhibited by PGPR. This can happen by producing antagonistic substances or by inducing resistance to pathogens (**Glick, 1995).** PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion**, (Patten and Glick, 2002).**


**Plate.2.8 Mechanism of plant growth promotion by rhizobacteria (Nandal and Hooda, 2013)**

## 2.9 Mechanisms of plant growth promotion

In general, PGPR promote plant growth directly by either facilitating resource acquisition which may be nitrogen, phosphorus and essential minerals or modulating plant hormone levels, or indirectly by diminishing the inhibitory effects of various pathogens on plant growth and improvement in the forms of biocontrol agents **(Glick, 2012)**.

### 2.9.1 Direct mechanisms of action

#### 2.9.1.1 Nitrogen fixation

Nitrogen (N) is the most important nutrient for plant growth and production. Although, there is about 80% N2 in the atmosphere, but it is unavailable to the growing plants as they are not in the form in which plants need. The N2 which is present in atmospheric is converted into plant-utilizable forms by biological N2 fixation (BNF) which converts nitrogen to ammonia by nitrogen fixing microorganisms by the use of complex enzyme system known as nitrogenase **(Kim and Rees, 1994)**.

Biological nitrogen fixation takes place, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature **(Raymond *et al.*, 2004)**. Furthermore, BNF represents an economically beneficial and environmentally sound alternative to chemical fertilizers**(Ladha *et al.*,1997)**.The atmospheric nitrogen fixed by Nitrogen-fixing (diazotrophic) bacteria by means of the enzyme nitrogenase, a two constituent metallo enzyme composed of (a) dinitrogenase reductase,a dimer of two identical subunits that contains the sites for Mg ATP Binding and hydrolysis, and supplies the reducing power to the dinitrogenase ,and (b) the dinitrogenase component that contains a metal cofactor **(Dean and Jacobson 1992).** The nodulation process consists of steps like (a) Interaction of rhizobial rhicadhesin with host lectins and rhizobial attachment with root cells. (b) Excretion of nod factors by rhizobia causes root hair curling. (c) Rhizobia penetrate root hair and form an infection thread through which they penetrate the cortical cells and form bacteroid state thereby nodules are formed. Nitrogen fixing organisms are generally categorized as (a) symbiotic N2 fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. rhizobia) **(Zahran, 2001)** and non-leguminous trees (e.g. Frankia) and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as *cyanobacteria (Anabaena, Nostoc), Azospirillum,Azotobacter, Gluconoacetobacter diazotrophicus* and *Azocarus* etc. **(Bhattacharyya and Jha, 2012)**. However, non-symbiotic nitrogen fixing bacteria provide

only a small amount of the fixed nitrogen that the bacterially-associated host plant requires **(Glick, 2012)**. Symbiotic nitrogen fixing rhizobia within the rhizobiaceae family (α-proteobacteria) infect and establish symbiotic relationship with the roots of leguminous plants. The establishment of the symbiosis involves a complex interplay between host and symbiont **(Giordano and Hirsch, 2004)** resulting in the formation of the nodules wherein the rhizobia colonize as intracellular symbionts.

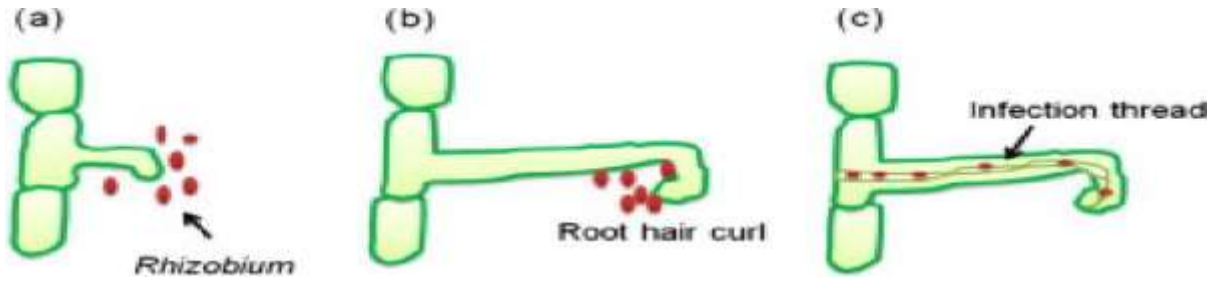



**Plate.2.9.1 (a) Nitrogen fixation by PGPR (Nandal and Hooda, 2013)**

### 2.9.1.2 Phosphate Solubilisation

Since phosphate is an essential macronutrient for plant growth and has only limited bioavailability, it is considered to be one of the elements that limit plant growth **(Feng *et al.*, 2004)**. Phosphate in soil is present in two insoluble forms: mineral forms such as apatite, hydroxyapatite, and oxyapatite, and organic forms including in phosphomonoesters, phosphodiesters, and phosphotriesters **(Khan *et al.*, 2007)**. Solubilisation and mineralization of P by Phosphate-Solubilising Bacteria (PSB) is one of the most important bacterial physiological traits in soil biogeochemical cycles **(Jeffries *et al.*,2003)**, as well as in plant growth **(Richardson 2001)**. Bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (**Bhattacharyya and Jha, 2012).** The solubilisation of inorganic phosphorus occurs as a outcome of the action of low molecular weight organic acids which are synthesized by a range of soil bacteria **(Zaidi *et al.*, 2009)**. Conversely, the mineralization of organic phosphorus occurs through the synthesis of a variety of different phosphatases, catalyzing the hydrolysis of phosphoric esters **(Glick, 2012)**. Importantly, phosphate solubilisation and mineralization can coexist in the same bacterial strain **(Tao *et al.*, 2008)**. Besides providing P to the plants, the PS bacteria also augment the growth of plants by stimulating the efficiency

of BNF, enhancing the availability of other trace elements (Such as iron, zinc) and by synthesizing important plant growth promoting substances **(Ponmurugan and Gopi, 2006; Mittal *et al.*, 2008).** To make this form of Phosphorus accessible by plants for plant nutrition, it must be hydrolyzed to inorganic Phosphorus by means of acid and alkaline phosphatase enzymes. Because the pH of most soils ranges from acidic to neutral values acid phosphatases should play the major role in this process **(Rodríguez and Fraga, 1999)**. The possibility of enhancing P uptake of crops by artificial inoculation with P-solubilising strains of rhizobacteria presents an immense interest to agricultural microbiologists.

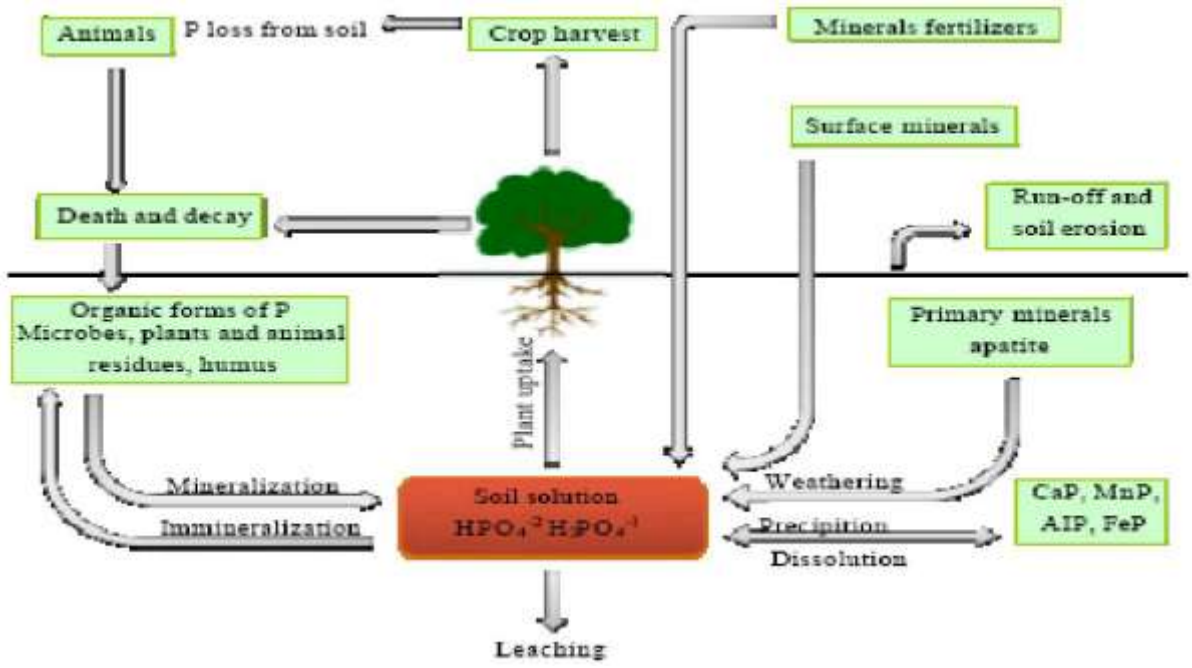



**Plate.2.9.1 (b) Solubilisation and mineralization of Phosphorus (Nandal and Hooda, 2013)**

### 2.9.1.3 Siderophore production

Iron is a fundamental nutrient for more or less all forms of life. All microorganisms known hitherto, with the exception of certain lactobacilli, essentially require iron **(Neilands, 1995)**. In the aerobic environment, iron occurs principally as Fe3+ and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to both plants and microorganisms **(Rajkumar *et al.*, 2010)**. Commonly, bacteria acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores which have high association constants for complexing iron. Most of the siderophores are water-soluble and can be divided

into extracellular siderophores and intracellular siderophores. Generally, rhizobacteria differs regarding the siderophore cross-utilizing ability; some are proficient in using siderophores of the same genus while others could utilize those produced by other rhizobacteria of different genera **(Khan *et al.*, 2009)**. In both Gram-negative and Gram-positive rhizobacteria, iron (Fe3+) in Fe3+-siderophore complex on bacterial membrane is reduced to Fe2+ which is further freed into the cell from the siderophore by mechanism involving the inner and outer membranes. During this reduction process, the siderophore may be destroyed/recycled **(Rajkumar *et al.*, 2010; Neilands, 1995)**. Thus, siderophores perform as solubilising agents for iron from minerals or organic compounds under circumstances of iron limitation **(Indiragandhi *et al.*, 2008)**. Not only iron, siderophores also form stable complexes with other heavy metals that are of environmental concern, such as Al, Cd, Cu, Ga, In, Pb and Zn, as well as with radio nuclides including U and Np **(Neubauer *et al.*, 2000)**. The soluble metal concentration increases by Binding of the siderophore to a metal **(Rajkumar *et al.*, 2010)**. Hence, bacterial siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals.

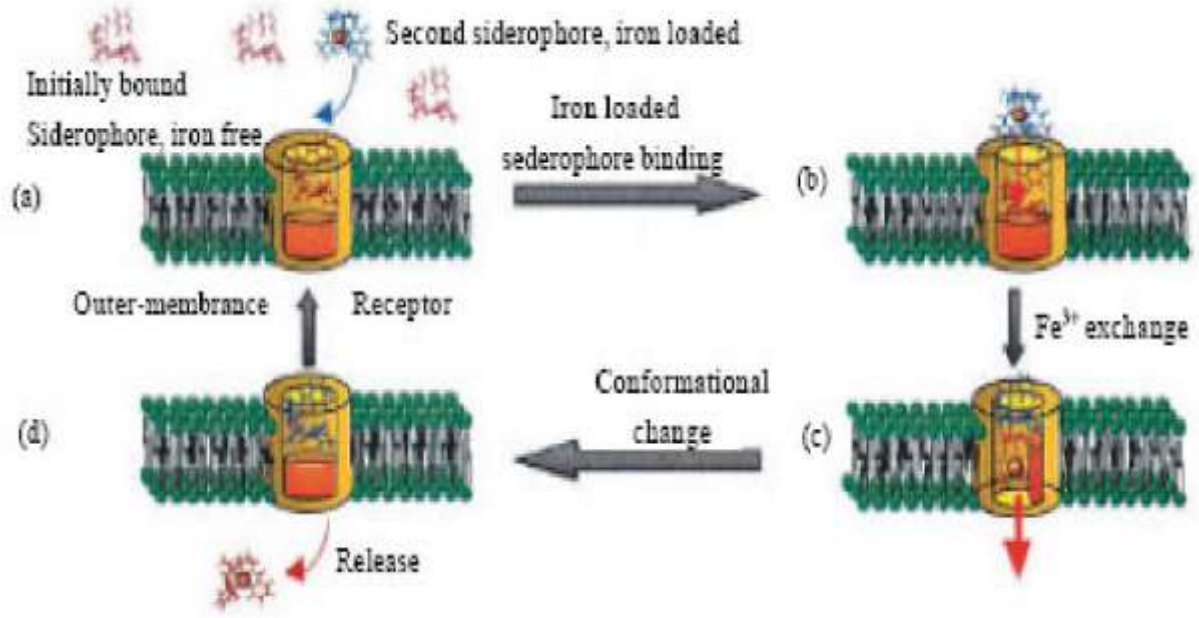



**Plate.2.9.1(c) The siderophores shuttle iron delivery mechanism (Nandal and Hooda, 2013.**

#### 2.9.1.4 Synthesis of Plant Hormones

Phytohormones are signal molecules which act as chemical messengers and play an important role as growth and development regulators in the plants. Phytohormones are extremely low concentration organic compounds that influence physiological, morphological and biochemical processes in plants, and their synthesis is finely regulated **(Fuentes-Ramírez and Caballero-Mellado, 2006)**. PGPR can increase root surface and length and promote in this way plant development with the production of different phytohormones like indole-3-acetic acid (IAA), gibberellic acid and cytokinins **(Kloepper *et al.*, 2007).** IAA (auxin) is the most important phytohormone which is produced by PGPR, and treatment with auxin-producing rhizobacteria increased the plant growth **(Vessey, 2003)**. Production of other phytohormones by biofertilizing-PGPR has been identified, but not nearly to the same extent as bacteria which produce IAA **(Vessey, 2003)**. A few PGPR strains were reported to produce cytokinins and gibberellins (gibberellic acid**) (Gutiérrez-Mañero *et al.*, 2001; Vessey, 2003).** Bacteria produce cytokinins and gibberellins, in addition to IAA like *Azospirillum* and spp. Many PGPR are capable to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme which cleaves ACC, the immediate precursor of ethylene in the biosynthetic pathway for ethylene in plants **(Glick *et al.*, 1998).**

### 2.9.2 In Direct mechanisms

The application of PGPR to control several diseases, which is a form of biological control, is an environment-friendly approach **(Lugtenberg and Kamilova, 2009)**. The major indirect mechanism of plant growth promotion in rhizobacteria is through acting as biocontrol agents **(Glick, 2012)**.

#### 2.9.2.1 Antibiotics

Antibiosis is highly effective and attractive mode of action of rhizobacteria for suppression of soil borne infections in crops **(Handelsman and Stab, 1996)**. Most biocontrol strains of PGPR have the capability to produce one or several groups of antibiotics, which inhibit fungal pathogens **(Haas and Defago, 2005)**. These biocontrol PGPR produce antibiotics which reduce or suppress soil-borne infections of cereal crops including wheat, rice, maize, chickpea, and barley **(Raaijmakers *et al.*, 2002)**.Some of these antibiotics cause membrane damage to pathogens such as *Pythium* spp. and inhibit zoospores formation **(De Souza *et al.*, 2003)**. Others such as the phenazines inhibit electron transport in disease causing organisms

and also act by damaging lipids and other macromolecules **(Haas and Defago, 2005)**.Genetic analysis of many biocontrol strains of *Pseudomonas* showed that there is a correlation between antibiotic production and disease suppression **(Vincent *et al.*, 1991)**. It was confirmed that with increase in the populations of *Pseudomonas* spp., which produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG), there was a rapid decline in disease in wheat caused by the fungus *Gaeumanomyces graminis* var. *tritici* **(Raaijmakers and Weller 1998; De Souza *et al.*, 2003)**. Bacterization of wheat seeds with *P. fluorescens* strains 2–79 producing the antibiotic PCA resulted in significant suppression of take-all in about 60% of field trials (**Weller 2007)**. There is a growing list of reports of *Bacillus* spp. as biocontrol agents in various crops. **Kim *et al.*, (1997**), for instance, isolated and discovered a potential biocontrol strain, *Bacillus* sp. L324-92, with a broad spectrum inhibitory activity against take all, root rot caused by *Rhizoctonia. solani and Pythium ultimum*. In other experiment **(El-Meleigi *et al.*, 2007),** treatment of spring wheat seeds with antibiotic producing strains of *Bacillus* spp. has been reported as a powerful tool to control root rot causing fungal pathogens in dry land fields. According to this work, application of *Paenibacillus polymyxa* to wheat seeds suppressed infection by root rot pathogens *Fusarium graminearum* and *Cochliobolus sativum*. The potential uses of antibiotic producing PGPR as biocontrol agents have been reported in many other cereals including maize, sorghum, rice, and chickpea. In maize for instance, *Fusarium verticilloides*, causing root rot and yield loss, has been significantly suppressed by the application of *Bacillus amyloliquifaciens* as seed treatment **(Pereira *et al.*, 2009)**. **Von der Weid *et al.*, (2005)** recently described *Paenibacillus brasilensis* PB177, a new strain isolated from the rhizosphere of maize in Brazil that produces antimicrobial substances suggesting that it could be a potential biocontrol agent in the rhizosphere of maize. In another biocontrol experiments, **Idris *et al.*, (2007, 2008)** confirmed the bio-control of *F. oxysporum* and *Pythium ultimum* on sorghum with *Bacillus* spp. It was verified that the bacterial strains generate antimicrobial metabolites, possibly antibiotics, which suppressed the growth of the fungal pathogens in vitro.

#### 2.9.2.2 Siderophores

Biocontrol PGPRs also exert their antagonistic activities which are not in favour of plant pathogens by means of secretion of siderophores. These low molecular weight compounds (400–1, 500 Da) preferentially chelate iron (Fe3+) and transport it into the cell across the cell membrane **(Neilands, 1995; Wandersman and Delepelaire, 2004)**. The siderophores attach

most of the Fe+3 in the rhizosphere and effectively avert the proliferation of fungal pathogens by depriving them of available iron **(Kloepper *et al.*, 1980; O' Sulivan and O' Gara 1992).** Inhibition of the pathogens arises because iron deficiency causes growth inhibition, decrease in nucleic acid synthesis, inhibition of sporulation, and causes changes in cell morphology **(Mathiyazhagan *et al.*, 2004).**

Among the biocontrol rhizobacteria, the *fluorescent Pseudomonas* spp. sourceful competitors for iron ($Fe^{+3}$) in the rhizosphere of various crops producing two major forms of siderophores: the fluorescent pigmented pyoverdins (pseudobactins) **(Lemanceau *et al.*, 1993)** and the non-fluorescent siderophore which is called as pyochelins **(Leeman *et al.*, 1996)**. Siderophores formed by certain strains of the *P. fluorescens-putida* group are responsible for improved plant growth and biocontrol and are most often associated with fungal suppression in the rhizosphere of several crops **(Battu and Reddy 2009)**. According to these workers, siderophore mediated the suppression of rice fungal pathogens *R. solani* and *Pyricularia oryze* in an in-vitro assay on Kings-B medium. Earlier**, Becker and Cook (1988)** reported the role of siderophores produced by *Pseudomonas* strain B324 in the suppression of *Pythium* root rot of wheat. Mutants lacking in pyoverdins production are less effectual than parental strains in suppression of fungal pathogens **(Loper and Henkels 1999)**. It is thus assumed that siderophore production is another important mechanism by which some strains of bacteria defend plants against root pathogens.

### 2.9.2.3 Cell Wall Degrading Enzymes

One of the major mechanisms used by biocontrol agents to control soilborne pathogens involves the production of cell wall degrading enzymes **(Chet *et al.*, 1990; Kobayashi *et al.*, 2002)**. Cell wall degrading enzymes such as b-1, 3-glucanase, chitinase, cellulase, and protease secreted by biocontrol strains of PGPR exert a direct inhibitory effect on the hyphal growth of fungal pathogens. Chitinase and b-1,3-glucanase degrade chitin, an insoluble linear polymer of b-1,4-N-acetylglucoseamine, which is the major component of the fungal cell wall. The b-1, 3-glucanase synthesized by strains of *Paenibacillus* and *Streptomyces* spp. lyse fungal cell walls of pathogenic *F. oxysporum*. In a similar manner, *Bacillus cepacia* synthesizes b-1,3-glucanase, which destroys the cell walls of the soilborne pathogens *R. solani, P. ultimum, and S. rolfsi* **(Compant *et al.*, 2005)**. Potential biocontrol agents with chitinolytic activities include *B. licheniformis, B. cereus, B. circulans, and B. thuringiensis* **(Sadfi *et al.*, 2001)**. Among the Gram-negative bacteria, *Serratia marcescens, Enterobacter*

*agglomerans, Pseudomonas aeruginosa,* and *P. fluorescens* have been found to have chitinolytic activities **(Nelson and Sorenson, 1999)**. Cell wall degrading enzymes of rhizobacteria affect the structural integrity of the walls of the target pathogen **(Budi *et al.,* 2000)**. **Someya *et al.,* (2000)** studied the chitinolytic and antifungal activities of a potent biocontrol strain of *S. Marcescens* B2 against the soilborne pathogens *R. solani* and *F. oxysporum.* The mycelia of the fungal pathogens coinoculated with this strain showed various abnormalities such as partial swelling in the hyphae and at the tip, hyphal curling or bursting of the hyphal tip. Examples of protection from phytopathogenic infection as a result of the activity of cell wall degrading enzymes include control of *Sclerotium rolfsii* and *F. oxysporum* on beans **(Felse and Panda, 1999)**.

### 2.9.2.4 Hydrogen cyanide and volatile compounds production

The antagonistic activity of PGPR also results in the production of volatile compounds. The best known compound is hydrogen cyanide (HCN). **Devi *et al.,* (2007)** reported the excretion of HCN by rhizospheric strains. *Pseudomonas* strains which produce HCN are used against bacterial canker of tomato as biological control **(Lanteigne *et al.,*2012)**. *P. corrugata* showed antagonistic activity against *Alternaria alternata* and *Fusarium oxysporum* pathogen microorganisms of several cultures such as maize **(Trivedi *et al.,*2008)**. This antagonism has been linked with the production of volatile compounds, although *P. corrugata* also produced some hydrolytic enzymes. *Bacillus subtilis* strains isolated from tea, producing volatile antifungal compounds induced structural defects on six pathogenic fungi under *in vitro* culture conditions **(Chaurasia *et al.,*2005)**. *B. megaterium* inhibits the growth of two plant pathogens *A. alternate* and *F. oxysporum* through the production of volatile compounds **(Trivedi and Pandey, 2008)**.

### 2.9.2.1 Induction of Systemic Resistance

Induced systemic resistance (ISR) is the condition of defensive capacity developed by the plant when stimulated by diverse agents together with rhizobacteria **(Loon *et al.,*1998)**. Once resistance is induced in plants, it will result in nonspecific protection against pathogenic fungi, bacteria, and viruses **(Silva *et al.,* 2004)**. The mode of action of disease suppression by non-pathogenic rhizosphere bacteria should be distinguished from pathogen induced systemic acquired resistance (SAR) **(Bakker *et et al.,* 2003)**. Colonization of the plant root system by rhizobacteria can indirectly lead to reduced pathogen attack through induction of systemic

resistance **(Kloepper and Beauchamp 1992)**. PGPR elicit ISR in plants by increasing the physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reactions of the host. This result in the synthesis of defence chemicals such as chitinase, peroxidase, and pathogenesis-related proteins **(Ramamoorthy *et al.*, 2001; Nandakumar *et al.*, 2001; Silva *et al.*, 2004)**.In rice, *P. fluorescens* strains showed inhibitory effect on the mycelial growth of *R. solani* by inducing resistance in the plant **(Radjacommare *et al.*, 2004)**. The bacteria induced resistance against the sheath blight fungus by activating chitinasegenes in rice **(Nandakumar *et al.*, 2001)**. Another biocontrol PGPR, *S. Marcescens* strain B2, which inhibits several soil borne pathogens including under greenhouse conditions, could not inhibit the same pathogens in a dual culture assay indicating that this is due to the induction of systemic resistance **(Someya *et al.*, 2002)**.

In beans, *P. aeroginosa* ISR against infection by *Collehotricum lindemuthianu* **(Bigirimana and Hofte, 2002.** Pea roots inoculated with *P. fluorescens* strain 63–28produced more chitinase at the site of penetration by *F. oxysporum* f. sp. pisi. Several strains of *Bacillus* spp. also have the capacity to induce systemic resistancein various crops against a wide range of pathogens in *Pisum sativum* **(Benhamou *et al.*, 1996)**. *Bacillus subtilis* AF1 isolated from soils suppressive to pigeon pea (Cajanus cajan) wilt caused by *Fusarium* sp. caused lysis of *Aspergillus niger* by stimulating the production of phenylalanine ammonia lyase and peroxidase by the plant thereby eliciting induction of systemic resistance **(Kloepper *et al.*, 2004)**. Root Colonization and Rhizosphere Competence Root colonization is an important prerequisite for bacteria to be considered as true PGPRs, and it is commonly believed that a biocontrol agent should colonize the rhizosphere and the surface of the plant it protects **(Silva *et al.*, 2003; Handelsman and Stab 1996; Benizri *et al.*, 2001)**. Therefore, any given PGPR is often in effective as a biocontrol agent against root disease if it does not colonize the roots efficiently **(Montealegre *et al.*, 2003)**.

*Pseudomonas* and *Bacillus* spp. is the most significant root colonizing PGPR in various crops. Several members of this group have widespread distribution in the soil, are efficient colonizers of the rhizosphere, and produce various types of metabolites inhibitory to a wide range of pathogens in plants **(Rangarajan *et al.*, 2003)**. Many other root colonizing strains of PGPR have also been found to have antifungal properties toward a number of pathogens in soil. However, for many of the potential biocontrol strains including *Pseudomonas* and *Bacillus* spp., biological control of soil borne diseases is often inconsistent. One of the major factors associated with this inconsistency is insufficient root colonization by introduced bacteria **(Bloemberg and Lugtenberg, 2001)**. Correlation of poor biocontrol performance of

a biocontrol agent with inefficient root colonization has been confirmed by means of mutants of *Pseudomonas* strains, which had lost their biocontrol activity. In this regard, it is essential to understand the bacterial traits that contribute to root colonization.

**2.10 Latest Advances and Future Prospects of PGPR as Biocontrol**

Agents in Plants with the advancement and innovations of current biotechnological research overthe past ten years, there is now vastly improved knowledge on the beneficial effects of both biocontrol and growth enhancing PGPR. Several strategies have so far been exploited to increase the efficacy of biocontrol strains to develop them for wide spread use in agriculture. Because of their metabolic versatility, excellent root colonization capability, and their capacity to produce a wide range of antifungal metabolites, intense biotechnological research is being done on the soil borne *P.fluorescens* **(Walsh *et al.*, 2001)**. For example, the antifungal metabolite2,4-diacetylphloroglucinol (2,4 DAPG) is an important metabolite produced by these biocontrol strains. In this regard, the development of sensitive *in situ* detection methods of 2,4-DAPG helped to understand the relationship between effective BCA *Pseudomonads* and suppressive soils in the suppression of take-all disease caused by *Gaeumanomyces graminis* var. *tritici* **(Raaijmakers *et al.*, 1999; Walsh *et al.*, 2001)**.

Improving the biocontrol efficacy of potential rhizobacteria by means of genetic modifications involves, for instance, the construction of strains that produce increased levels of antimicrobial and growth enhancing metabolites **(Walsh *et al.*, 2001)**. By transforming *P. fluorescens* CHAO with the gene coding for 1-aminocyclopropane-1-carboxylic acid deaminase, for instance, the plant growth promotion and biocontrol capacity of this strain have been increased **(Wang *et al.*, 2000)**.Novel perspectives are emerging regarding biocontrol and optimizing the application of biocontrol strains for future use. The identification of *P. fluorescens* genes associated with root colonization and that are specifically expressed in the rhizosphere (rhi genes) by means of in-vivo expression technology (IVET) is another important innovation **(Bloemberg andLugtenberg, 2001)**. Many such root colonizing genes and traits from *P. fluorescens* have been identified and used to improve root colonization patterns of wild type *Pseudomonas* strains **(Lugtenberg and Dekkers, 1999)**. In some biocontrol PGPR, efficient root colonization is linked to a site-specific recombinase gene, and transfer of this gene from a rhizosphere competent *P. fluorescens* strain to a non-competing strain improved its root colonization ability **(Compant *et al.*, 2005)**.

## 2.11 Biological control of Bacterial wilt

Methods for enhanced growth of economically important plants are constantly evolving. Widespread use of nitrogen fertilizer has increased crop yield, but has also caused deleterious effects on ecosystems, i.e., nitrate (NO3) pollution of ground and surface waters, soil acidification, and production of the greenhouse gas nitrous oxide (N2O) through denitrification **(Biswas *et al.*, 2000)**. In order to reduce negative environmental effects associated with food and fibre production, there has been a recent resurgence of interest in environmentally safe, sustainable, and organic agricultural practices **(Lind *et al.*, 2004)**.‘‘Organic agriculture’’ is a production system, which avoids or minimizes the use of synthetic fertilizers, pesticides, and growth regulators, relying instead on bio-fertilization, crop rotation, crop residues, mechanical cultivation, and biological pest control to maintain soil productivity. Reduced yield in organic production systems is an important problem (**Lind *et al.*, 2004)**.

**Guo *et al.*, (2004)** evaluated three strains of plant growth-promoting rhizobacteria (PGPR), *Serratia* sp. J2, *Pseudomonas fluorescens* J3, and Bacillus sp. BB11 for biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum*, and concluded that Yield increases with these bacteria and also PGPR products provides stable formulations that retain biological control and plant growth-promoting activities.**Doan and Nguyen (2005)** concluded that two strains of *P. fluorescens* (B16 and VK58) and one strain of *B. subtilis* (B16) showed promising results for biological control of bacterial wilt in the greenhouse and in the field. In the field trial conducted on tomato and groundnut with B16. B.16 and VK58 in Hanoi, Bac Giang and Bac Ninh provinces the tomato yield increased from 3.3 to 4.1 tons/ha and the groundnut yield from 0.7 to 0.94 tons/ha. B.16 has a higher effect in disease control, a optimistic effect on growth and yield of groundnut and can replace 20% NPK fertiliser without major changes in crop yield. **Yang *et al.*, (2012)** screened 420 bacterial strains isolated from rhizosphere soil and the plant surface as well as the stem, leaf, and root tissue of ginger out of which *Bacillus subtilis* strain 1JN2, *Myroides odoratimimus* 3YW8, *Bacillus amyloliquefaciens* 5YN8, and *Stenotrophomonas maltophilia* 2J showed biocontrol efficacies greater than 50%. A bacterial strain, J12, isolated from the rhizosphere soil of tomato plants strongly inhibited the growth of phytopathogenic bacteria *Ralstonia solanacearum*. Strain J12 was identified as *Pseudomonas brassicacearum* based on its 16S rRNA gene sequence. **(Zhou *et al.*, 2012)**

*Bacillus strains* are effective biocontrol agents of the cucumber wilt caused by *Fusarium oxysporum f*. sp. *Cucumerinum* **(Li *et al.,* 2012).** Chitosan and two *Paenibacillus* strains, in particular *Paenibacillus polymyxa* MB02-1007, were found to have strong *in vitro* antibacterial activities against *R. solanacearum (***Algam *et al.,* 2010***). B. subtilis* PFMRI, *P. Macerans* BS-DFS, and PF9, and *P. fluorescens*PF20 have shown the highest degree of inhibition in vitro **(Aliye *et al.,* 2008)**. The mechanism involved during *in-vitro* inhibition could be due to antibiosis, siderophore production or both **(Adesina *et al.,* 2007)**.

Isolates of Pseudomonads which were produced by Diacetylphloroglucinol (DAPG) were screened against bacterial wilt in brinjal caused by *R. solanacearum.* The highest percentage of wilt disease reduction was observed in seedlings treated with *P. plecoglossicida* BA11D1 (95.8%), *P. putida* CK24E (62.5%) and *P. plecoglossicida* BA3D1 (41.7%) (**Arulmani *et al.,* 2013**).

*Bacillus amyloliquifaciens* and *Serratia marcescens* were effective for disease suppression of bacterial wilt of ginger and growth promotion both in green house and field **(Bini *et al.,* 2011).** Seed treatment + seedling treatment + soil application of *P. flourescens* along with vermi compost as substrate was most effective on bacterial wilt disease reduction in bell pepper **(Bora and Bora, 2009).** Inhibitory activity of *P. fluorescens* was tested against *R. solanacearum* following dual culture method. The increase in yields might also be due to the ability of the antagonist to decrease disease incidence and increase plant growth **(Mavrodi *et al.,* 2003).**

*P. fluorescens* Pf11, *P. fluorescens* Pf16, *P. putida* Pp17, *Paenibacillus* spp. Pb28 and *Enterobacter* sp. En38, *P. fluorescens* Pp23 and Serratia sp. Se40. Strains Pf11, Pf16, Pp17 and Pb28 significantly inhibited the growth of the pathogen *R. solanacearum* and controlled Bacterial wilt of potato **(Kheirandish and Harighi, 2015).** *Pseudomonas putida* strain WCS358 and *P. fluorescens* strains WCS374 and WCS417, and their rifampin-resistant derivatives all inhibited growth of *R. solanacearum* in vitro. *P. fluorescens* WCS417r significantly suppressed bacterial wilt in Eucalyptus urophylla **(Ran *et al.,* 2005).** APF1 *P. fluorescens* and B2G *B. subtilis* strains significantly reduced disease incidence and increased weight of tomato plants. Inoculation with APF1 and B2G strains reduced area under disease progress curves (AUDPC) by 60% and 56% in plants respectively. Plant dry weight increase in plants inoculated with APF1 *P. fluorescens*and B2G *B. subtilis* strains was 96% and 75%, respectively. APF1 was found to be the most beneficial strain in disease suppression and also growth promotion resulting in 63% dry weight increase compared to untreated control **(Lemessa and Zeller, 2007).**

The galloping growth of world population estimated around 7 billion people and may reach 8 billion by 2020 **(Glick, 2012)**, generates several struggle including food insecurity and famine. So it is urgent to double the agricultural manufacture in order to decrease the risk of malnutrition and increased poverty **(Soulé *et al.*, 2008)**. In return to this, new seeds varieties of high-yield were introduced into agricultural production systems in several countries. The utilize of these new varieties is accompanied by a growing and excessive use of chemical fertilizers and pesticides. Although the use of these chemical products has many advantages such as the ease to handle and convincing results, they produce the environmental and public health problems. Among these problems, (i) groundwater and crop products contamination by heavy metals from the use of these agricultural inputs, (ii) physical and chemical deterioration of agricultural soils, can be mentioned (iii) destruction of the soil biological communities, and (iv)interruption of the natural ecological cycle of nutrients. Indeed, the prolonged use of mineral fertilizers without addition of organic matter leads to the poor soils inorganic matter, more sensitive to wind and rain erosion **(Alalaoui, 2007).** Heavy metals contamination of groundwater and crop products is one of the major causes of the cancer occurrence **(Koo *et al.*, 2009)**.

## 2.12 Yield enhancement by PGPR

Different bacterial groups are essential components of soils. They make soil dynamic for nutrient turn over and sustainable for crop production are involved by involving in various biotic activities of the soil ecosystem **(Ahemad *et al.*, 2009; Chandler *et al.*, 2008).** In addition to providing the mechanical support and facilitating water and nutrient uptake, plant roots also synthesize, accumulate, and secrete a diverse array of compounds called as root exudates **(Walker *et al.*, 2003).**These exudates also promote the plant advantageous symbiotic interactions and inhibit the growth of the hostile plant species **(Nardi *et al.*, 2000)**. Generally, PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or

indirectly by decreasing the inhibitory effects of different pathogens on plant growth and

improvement in the forms of biocontrol agents **(Glick, 2012)**. The most common direct mechanism for plant growth promotion by bacteria is the production of plant growth substances such as auxins, gibberellins **(Holl *et al.*, 1988; Chanway, 2002).**

Significant increases in root and shoot fresh weight, root dry weight and total root length in tomatoes was shown by *Bacillus cereus* (KFP9-F). Possible mechanisms like Indole acetic acid production, phosphate solubilisation and siderophore secretion may be the reason for plant growth enhancement by bacterial isolate KFP9-F **(Hassen and Labuschagne, 2010)**. In apricot, shot-hole disease was decreased by *Bacillus* OSU-142, also yield, growth and PNE contents of leaves has increased by floral and foliar application of *Bacillus* OSU-142 **(Esitken *et al.*, 2002, 2003)**. Under organic growing conditions, *Bacillus* M3 alone or in combination with Bacillus OSU-142 have the potential to increase the yield, growth and enhance nutrition of raspberry plant **(Orhan, 2006).**

The *Pseudomonas fluorescens* are a group of PGPR reported to improve the overall growth of various crops **(Dey *et al.*, 2004; Vikram *et al.*, 2007)**. The auxins and cytokinins plays important role in enhancing plant cell division and root development **(Arshad and Frankenberger, 1993)**. Root exudates which are the chemicals secreted into the soil by roots, are known to manipulate the activity of microbial communities in the rhizosphere. Thus, root border cells can inhibit the growth of pathogenic organisms and encourage the growth of beneficial microorganisms **(Humphris *et al.*, 2005).** IAA is involved in root initiation, cell division, and cell enlargement **(Gray and Smith, 2005),** and increases root surface area and consequent access to soil nutrients by enhanced formation of lateral and adventitious roots. Cell division, cell enlargement, and tissue expansion in certain plant parts are promoted by Cytokinins **(Dey *et al.*, 2004; Gray and Smith, 2005).** The quantity and quality of exudates produced by border cells depend on the plant genotype. The microbial communities present in the rhizosphere are certainly influenced by the ability of the microorganisms to respond to and utilize particular compounds released from border cells **(Hawes *et al.*, 2003)**. Significant increases in shoot length shoot weight, number of leaf, number of node, and root dry weight was showed by *P. fluorescens* and *Bradyrhizobium* sp. in comparison to control and plants treated with other PGPR. Essential oil yield was also significantly without alteration of oil composition. *P. fluorescens* has clear commercial possible for economic cultivation of O. Majorana **(Banchio *et al.*, 2008)**. *Bacillus cereuses* (KFP9-F) resulted in significant increases in roots and shoots fresh weight, root dry weight and total root length in tomatoes. Indole acetic acid production, phosphate solubilisation and siderophore secretion are possible mechanisms by which the bacterial isolate KFP9-F enhanced plant growth **(Hassen and Labuschagne, 2010)**. **Harthmann et al. (2009)** found that the yield of onion bulb was promoted significantly by PGPR *Bacillus cereus* (UFV40).

Different species of PGPR differ in the type of benefits they grant on growth and development of plants **(Ekanayake *et al.*, 1994)**. Inoculation could improve a plant development and compensate for nutrient deficiency through production of plant growth regulators by microbes at the root interface, which stimulated root development of plants and resulted in better absorption of water and nutrients from the soil **(Kloepper and Beauchamp, 1992; Zimmer *et al.*, 1995; Wu *et al.*, 2005)**.Strains of Pseudomonas have increased root and shoot elongation in canola, lettuce and tomato **(Glick *et al.*, 1997)**. **Nielson and Sorensen (1999)** result confirmed that *P. fluorescens* have antagonistic potential against *R. solani* and *Pythium ultimum,* and produced lytic enzymes.

# Chapter -3

# Materials and Methods

# MATERIALS AND METHODS

The experiment entitled, "Efficacy of plant growth promoting rhizobacteria against bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)" was carried out under the agro-climatic conditions of Allahabad. The materials used and methods adopted during the course of investigation are presented with a brief description of site of experiment, soil properties, cropping history, sampling techniques and statistical analysis in this chapter.

## 3.1 Geographical location of the experimental site

The experiment was conducted in Department of Biological Sciences, SHUATS (Formerly Allahabad Agricultural Institute), Allahabad (U.P.) which is situated at $25^0$ 24' 42" N latitude, $87^0$ 50 56"' E longitude and 98 m altitude from the sea level. This is situated on the right side of the river *Yamuna*. All the facilities required for crop cultivation are available.

## 3.2 Soil analysis

The mechanical and chemical analysis of soil was done before the start of the experiment to assess the initial fertility gradient. Aggregate soil samples were drawn from experiment field (0-30cm depth) prior to application of farm yard manure to assess the initial physical and chemical status of soil. The result of analysis is presented in Table 3.3.

**Table 3.2 Physical and chemical properties of the soil:**

| Sr. No. | Particulars | Value (%) | Method followed |
|---|---|---|---|
| **Physical properties** | | | |
| 1 | Sand | 48.15 | Boyounce Hydrometer **(Piper, 1966)** |
| 2 | Silt | 21.34 | |
| 3 | Clay | 30.51 | |
| 4 | Textural class | Sandy loam | |
| **Chemical properties** | | | |
| 1 | Soil pH | 7.47 | Potentiometery**(Jackson, 1973)** |
| 2 | Organic carbon (%) | 0.41 | Walkely and Black's wet digestion **(Jackson, 1973)** |
| 3 | Available nitrogen (kg/ha) | 114.56 | Alkaline permanganate method **(Subbaiah and Asija, 1956)** |
| 4 | Available phosphorus (kg/ha) | 17.14 | Bray's method **(Jackson, 1973)** |
| 5 | Available potash (kg/ha) | 157.2 | Photometery**(Jackson, 1973)** |

## 3.3 Experimental Details

### 3.3.1 Experimental design

The experiment was conducted in pot laid out in CRD design consist of seven treatments with seven replications.

**Table 3.3.1 Treatments Detail.**

| S.NO | TREATMENTS | TREATMENT NAME |
|---|---|---|
| **1.** | **T1** | Control( uninoculated) |
| **2.** | **T2** | Control (inoculated) |
| **3.** | **T3** | PR3 |
| **4.** | **T4** | PR9 |
| **5.** | **T5** | PR17 |
| **6.** | **T6** | PR25 |
| **7.** | **T7** | 3NAA8 |

### 3.3.2 EXPERIMENT DESCRIPTION:

Crop : Tomato (*Lycopersicon esculentum* Mill.)

Varieties : 3 **(Pusa 120, NAVODAYA, GOLDEN GREEN)**

Design of Experiment : Completely Randomized Design (CRD)

Total trials : 2 (2014, 2015)

No. of replications : 7

No. of treatments : 7

**3.3.3 Source of pathogen:**

The causal organism of bacterial wilt of tomato, *R. solanacearum* was collected from Division of Plant Pathology, ICAR-IARI, New Delhi.

**3.3.4 Source of antagonistic organism:**

The 250 bacterial isolates were collected from the Department of Biological sciences, SHUATS, Allahabad.

**3.3.5 Source of planting material:**

The seeds of Pusa-120 were collected from IARI, New Delhi, while the seeds of Navodaya and Golden green were collected from local market of Allahabad.

## 3.4 Preliminary screening for antagonistic activity *in-vitro*:

This test was performed to screen antagonistic activity of obtained isolates against *R. solanacearum* according to the method described by **Li *et al.*, (2008)**. Cultures of *R. solanacearum* was grown overnight in nutrient broth. 0.5 ml of the liquid culture was mixed with 15 ml lukewarm melted nutrient agar in sterile plates and allowed to solidify. Afterwards, 250 isolates were spotted on the surface of agar plates and incubated them at 28°C for 72 h. Consequently, those bacteria that displayed positive inhibition activity were considered as antagonistic isolates and selected for further investigations.

## 3.5 Laboratory *in vitro* assay for studying antagonism

To further study the antagonism, systematically, two techniques were applied:

1) Agar-well techniques

2) Disc-diffusion techniques

For agar well, 100 µl of *R. solanacearum* suspension was spread on NA plates and two holes of 9 mm diameter were punched into the agar. In these holes, one hole was filled with water was treated as control and in other hole 30 µl suspension of each test antagonist was added and the plates incubated at 28 ºC for 48 h. Inhibition of *R. solanacearum* growth was assessed by measuring the radius of inhibition zone (mm) after incubation for 48 h at 28ºC **(Lemessa *et al.*, 2001).**

Paper disc, the nutrient glucose agar was seeded with *R. solanacearum* by evenly spreading 0.1 ml of the suspension.10 mm disc was immersed in each test antagonist and was spotted at the centre of the pathogen-inoculated-plate. Paper disc immersed in distilled sterile water and

spotted at the centre of the plates with the pathogen was used as control. Completely randomized design (CRD) with four replications was used for both techniques. Average diameter of the inhibition zones after 72 h of incubation at 30ºC was used as a measure of antagonism **(Dhingra *et al.*, 1995)**. The study was repeated twice. The potential bacterial antagonists that showed strong (11–20 mm inhibition diameter) and very strong (over 20 mm inhibition diameter) degree of inhibition were labelled and maintained separately. The most effective in vitro inhibitors (>11 mm inhibition diameter) were further evaluated for in vivo.

## 3.6 CULTURAL PRACTICES

### 3.6.1 Nursery preparation

Tomato seeds were surface sterilised by immersion in 70% ethanol for 1 minute and then washed 3 to 4 times with distilled water. To raise seed bed of 2m length, 1m width and 5cm height with fine tilt were prepared without use of organic manure and fertilizers. The furrow were made at a distance 3cm across the length of bed, the seeds of all the three varieties of tomato PUSA 120, Navodaya, Golden green were mulched. The seedbed was watered when needed. The healthy seedlings were transplanted after 30 days to the experimental pots.

### 3.6.2 Transplantation

In pots, 5kg of soil mixed with FYM was filled in each 6kg pot and 30 days old seedlings of same height were transplanted in each pot. Every pot has two set of plants.

### 3.6.3 Irrigation:

Irrigation was given immediately after transplanting and second light irrigation was given on third day after transplanting. Thereafter, regular irrigation was given when the crop required.

### 3.6.4 Weeding:

Weeding was carried out generally at an interval of 20 – 30 days interval to avoid competition for moisture and nutrient in the soil. The experimental pots were kept weed free for best growth and development of plants. Hand weeding was followed to remove the weeds.

### 3.6.5 Harvesting:

The fruits were harvested at the maturity and harvesting was done at an interval of 5-6 days and it was continued up to last fruiting in each treatment, it was done by hand picking.

## 3.7 COLLECTION OF DATA

Plants were selected randomly from each pot for data collection. Data on the following parameters were recorded from the sample plants during the course of experiment.

### 3.7.1 SELECTION OF ANTAGONISTIC PGPR (*In- vitro*)

The antagonistic PGPR were selected on the basis of maximum inhibition zone formed against *R. solanacearum* in Agar well diffusion and Disk diffusion method which were applied to observe zone of inhibition.

### 3.7.2 DISEASE INCIDENCE

Tomato seedlings were transplanted in pots filled with sterilized soil (sterilized by acid sterilization technique). Two set of controls were used, one set of control were grown without inoculation (neutral) and one set inoculated with *R.solanacearum.* The antagonist potential was tested individually for PR3, PR9, PR17, PR25, 3NAA8. Soil pre-treatment with antagonist was done a week prior to the pathogen inoculation soil drenching method. 20ml of suspension of pathogen was inoculated into the soil through soil drenching method after seven days of application of the antagonists. The disease incidence was calculated over a period of 42 days, beginning at 7 days after inoculation (**Sarkar and Chaudhri, 2013**).

The percentage incidence of wilt disease was estimated as follows:

$$\textbf{DISEASE INCIDENCE } \% = \frac{\textbf{NUMBER OF INFECTED PLANTS}}{\textbf{TOTAL NUMBER OF PLANTS}} \times \mathbf{100}$$

### 3.7.3 GROWTH PARAMETERS

1) **Number of branches per plant**

   Numbers of branches arising on the main shoot were counted at period of 30, 60 and 90 Days after transplanting (DAT). The number of branches per plant of each replication was recorded and subjected to statistical analysis.

2) **Number of leaves per plant**

   Number of leaves per plant was counted at 30, 60 and 90 days after transplanting (DAT). The average number of leaves per plant of each replication was recorded and subjected to statistical analysis.

3) **Number of flowers per plant**

   Number of flowers was counted from each replication of all treatments at 60 days after transplanting (DAT). The average number of flowers per plant of each replication was recorded and subjected for statistical analysis

4) **Number of fruits per plant**

   The number of fruits was counted for each plant after 90 days of transplanting (DAT). The average recorded data was subjected for statistical analysis.

5) **Fresh weight and dry weight of plant**

   The fresh weight of plant were taken after full harvesting, as well as fresh weight of shoot and root and dry weight of shoot and root data were also recorded.

6) **Fresh weight of fruit**

   The fresh weights of fruits were taken after harvesting of the plant. The average data of weight was subjected for statistical analysis.

7) **Total yield per plant**

   The total yield of tomato per plant was taken after the harvesting was done. The average data of yield was then subjected for statistical analysis.

### 3.7.4 GERMINATION OF TOMATO SEEDS

Uniform sized tomato seeds were surface sterilized with 0.1% mercuric chloride for 5 min, rinsed with sterilized distilled water (SDW). The bacterial suspension of were prepared and tested for their efficacy on seed germination and seedling growth, which was carried out by the roll towel method **(Abdul Baki and Anderson, 1973)**. Sterilized seeds were soaked in bacterial suspension for 24h and sterile blank nutrient broth served as control. On 3$^{rd}$ day the germination percentage was calculated. After 5, 7 and 11 days ten seedlings were taken randomly from each replication and length of root and shoot measured separately and data were recorded. The experiment was repeated three times.

## 3.8 BIOCHEMICAL PARAMETERS

### 3.8.1 Estimation of chlorophyll (mg/g leaf fresh weight) by DMSO method (dimethyl sulphoxide):

The estimation of chlorophyll was done using dimethyl sulphoxide (DMSO) extraction procedure. Plant samples were collected at random and leaves were chopped into fine pieces. 50mg sample from these chopped leaves were added in replicated tubes each containing 10 ml dimethyl sulphoxide (DMSO).

The tubes containing leaf pieces and DMSO were incubated at 65ºC for 3 hours in oven. After complete extraction, leaf supernatants were used for measuring the absorbance with the help of spectrophotometer against DMSO blank. The absorbance at 663nm and 645nm are read in a spectrophotometer. Using the absorption coefficients, the amount chlorophyll a and chlorophyll b were calculated according to the formula given below on mg g$^{-1}$ fresh weight of leaf tissue basis**.**

Chlorophyll a (mg/g fresh weight)

$$= (12.7 \times A_{663} - 2.63 \times A_{645}) \times (V/1000 \times W)$$

Chlorophyll b (mg/g fresh weight)

$$= (22.9 \times A_{645} - 4.48.63 \times A_{663}) \times (V/1000 \times W)$$

Where,

A= absorbance of specific wave length

V= volume

W= fresh weight of sample

### 3.8.2 T.S.S. (Total soluble solids) $^0$Brix:

With the use Erma Japan hand refractrometer the T. S. S of 3 fruits of each treatment were recorded as the percentage. A drop of juice was used to record the TSS and values were expressed as degree ºBrix.

### 3.8.3 Ascorbic acid (mg/100 g fruit pulp):

**Ascorbic acid** content was determined by visual titration method and expressed in mg/100g as prescribed by A.O.A.C, 1990.

The juice was filtered through muslin cloth 10 ml (w) of the juice was taken with the help of a pipette in 100 ml volumetric flask and the volume ($V_1$) was made up with 1.0% oxalic acid solution. The flask was shaken well. The juice was filtered, and known quantity ($V_2$) of solution was titrated against standard dye (V) solution till a faint pink colour appeared and persisted for 15 second. The amount of ascorbic acid was calculated by the formula (**A.O.A.C, 1990**).

$1/1 \times V \times V_1 / V_2 \times 100 / W$ = Vitamin C mg/100 mg of fruit.

Where,

V = ml of dye indicator used in titration

$V_1$ = volume to which the juice is diluted

T = titrate volume of dye with standard solution of vitamin C

$V_2$ = volume of filtrate taken for titration.

W = volume of the juice initially taken for the determination.

### 3.8.4 Lycopene

Lycopene extraction and determination was based on the method of **Fish *et al.*, (2002*)***. In brief, tomato fruit were finely ground for 1 min to puree using blender. Ground tissues were kept on ice and out of light after preparation and until assayed. An approximate 1 g of the puree (without seeds) was put in 50 ml aluminium wrapped test tubes while they were on ice. Lycopene extraction solution (39 ml) consisting of hexane, 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol in a ratio of 1:1:1 was added to the tubes and shaken for 10 min at 180 rpm using table top shaker while they were on ice. To each tube

6 ml of cold double distilled water was added and agitated for an additional 5 min for better separation of polar and non-polar compounds. Tubes were then left for 15 min in room temperature for separation into polar and non-polar layers. The absorbance of supernatant (hexane layer) containing Lycopene was read three times using spectrophotometer at the wavelength of 503 nm. Absolute hexane was used as blank. The amounts of Lycopene in tissues were estimated by the following formula:

$$\textbf{Lycopene (mg/100g)} = (x/y) \times A_{503} \times 3.12$$

Where x is the amount of hexanes (ml), *y* the weight of fruit tissue (g), $A_{503}$ the absorbance at 503 nm and 3.12 is the extinction coefficient.

### 3.8.5 Relative water content (RWC)

For the determination of RWC, fully expanded leaves of the same physiological age as were collected and weighed immediately to record fresh weight (FW). The leaves were then floated in distilled water inside a closed glass petridish and weighed periodically after gently wiping water from the leaf surface with tissue paper to get turgid weight (TW). The leaves were then dried at 70 ºC for 48 h to get oven-dried weight (ODW). The RWC was determined using the equation as described by **Kaya and Higgs (2003)**.

**RWC (%) = [(FW-ODW)/ (TW-ODW] × 100**

## 3.9 Skeleton of ANOVA Table.

## Table 3.9 Anova table skeleton.

| Source of variation | d.f | S.S | M.S.S. | F.Cal. | F Tab. At 5% |
|---|---|---|---|---|---|
| **Due to replication** | r-1 | RSS | $\frac{RSS}{r-1}$ | $\frac{RMSS}{EMSS}$ | 3.00 |
| **Due to treatments** | t-1 | TSS | $\frac{TSS}{t-1}$ | $\frac{TSS}{MESS}$ | |
| **Due to error** | (r-1)(t-1) | ESS | $\frac{MESS}{(r-1)\,(t-1)}$ | | |

Where,

r = Number of replications

t = Number of treatments

Ssr = Sum of squares due to replications

Sst = Sum of squares due to treatment

Sse = Sum of squares due to error

Mssr = Mean Sum of squares due to replications

Msst = Mean Sum of squares due to treatment

Tss = Total sum of square

$$SE = \sqrt{\frac{2 \times MES}{r}}$$

CD = S.Ed + t (5%) on error d.f.

**Table 3.10 Calendar of operation during the experimentation.**

| Operations | Date | | Method used |
|---|---|---|---|
| | 2014 | 2015 | |
| **For nursery bed** | | | |
| 1) Seed bed preparation | 3rd October | 3rd October | Manual labour |
| 2) Nursery preparation | 5th October | 5th October | Manual labour |
| **For pots** | | | |
| 3) Transplanting | 8th November | 7th November | Manual labour |
| 4) Irrigation | Alternate days | Alternate days | Manual labour |
| 5) Soil treatment with PGPR | 16th November | 15th November | Soil drenching method |
| 6) Irrigation | Alternate days | Alternate days | Manual labour |
| 7) Pathogen inoculation in soil | 24th November | 24th November | Soil drenching method |
| 8) Irrigation | Alternate days | Alternate days | Manual labour |
| 9) Weeding | Alternate weeks | Alternate weeks | Manual labour |
| 10) Harvesting | 10th May | 13th May | Manual labour |

# Chapter -4

# Results

# RESULTS

The current investigation was focused on the control of bacterial wilt of tomato caused by pathogen *Ralstonia solanacearum* by the use of antagonistic PGPRs and their effects on the yield and growth of tomato plant. In this chapter, the results of the experiments have been portrayed in the form of tables, figures and plates.

**4.1 Screening and selection of efficient PGPR against *Ralstonia solanacearum*:**

Out of 250 rhizobacterial isolates, after preliminary screening 20 isolates of PGPR showed effective activity against the pathogen i.e. *Ralstonia solanacearum.* These 20 isolates **2CBA2, 2CBA4, 2CBA18, 2CBA42, 2AAB27, 2BAB1, 1NAB15, 2NAB20, 3NAA1, 3NAA7, 3NAA8, 3NAA12, 3NAA17, PR2, PR3, PR9, PR17, PR25, PR26, and PR27** were selected for the *in-vitro* laboratory assay to measure their inhibition zone against the pathogen.

**4.2 The 250 isolates of PGPR screened against *Ralstonia solanacearum.***

**4.2.1 *Pseudomonas:***

1CBA1, 1CBA2, 1CBA7, 1CBA8, 1CBA11, 1CBA17, 1CBA18, 1CBA19, 1CBB3, 1CBB5, 1CBB10, 1CBB12, 1CBB13, 1CBB15, 1CBB16, 1CBB19, 2CBA1, **2CBA2,** 2CBA3, **2CBA4,** 2CBA6, 2CBA6, 2CBA16, 2CBA17, **2CBA18,** 2CBA41, **2CBA42,** 2CBA43, 3CBA2, 3CBA3, 3CBA4, 3CBA5, 3CBA8, 3CBB1, 3CBB3.

**4.2.2 *Azotobactor***:

1AAB1, 1AAB3, 1AAB5, 1AAB6, 2AAB1, 2AAB13,2AAB15, 2AAB16, 2AAB17, 2AAB18, 2AAB22, 2AAB23, 2AAB24, **2AAB27,** 2AAB28, 2AAB29, 2AAB30, 2AAB31, 2AAB35, 2AAB36, 2AAB37, 2AAB38,2AAB39, **2BAB1,** 2BAB10, 2BAB14, 2BAB16, 2BAB17, 2BAB18, 2BAB24, 2BAB26, 2BAB27, 2BAB28, 3AAB2, 3AAB3, 3AAB5, 3AAB6, 3AAB8, 3BAB12, 3BAB13, 3BAB15, 3BAB16, 1BAB8, 1BAB13, 1BAB15.

**4.2.3 *Rhizobium***:

1RBA1, 1RBA2, 1RBA4, 1RBA5, 1RBA8, 1RBA9, 1RBA10, 1RBA13, 1RBA14, 1RBA18, 1RBA19, 1RBA20, 1RBB1, 1RBB2, 1RBB4, 1RBB9, 1RBB13, 1RBB14, 1RBB16, 1RBB17, 1RBB18, 1RBB19, 1RBB20, 2RBA2, 2RBA6, 2RBA8, 2RBA11, 2RBA16,

2RBA18, 2RBA23, 2RBA24, 2RBA27, 2RBA28, 2RBA36, 2RBA38, 2RBA39, 2RBA40, 2RBB1, 2RBB6, 2RBB7,2RBB8, 2RBB12, 2RBB14, 2RBB16, 2RBB17, 2RBB19, 2RBB21, 3RBA1, 3RBA2, 3ARB1, 3ARB2, 3ARB5, 3ARB6, 3BRB3, 3BRB4, 3BRB7.

**4.2.4 *Bacillus***:

1NAA1, 1NAA2, 1NAA3, 1NAA5, 1NAA7, 1NAA8, 1NAA9, 1NAA10, 1NAA11, 1NAA12, 1NAA15, 1NAA17, 1NAA19, 1NAA20, 1NAA24, 1NAB3, 1NAB6, 1NAB7, 1NAB10, 1NAB11, 1NAB14, **1NAB15,** 1NAB16, 1NAB23, 1NAB24, 1NAB28, 1NAB29, 1NAB35,1NAB36, 1NAB38, 1NAB42, 1NAB43, 1NAB45, NAB46, 2NAA3, 2NAA6, 2NAA7, 2NAA10, 2NAA11, 2NAA14**,** 2NAA15, 2NAA16, 2NAA23, 2NAA24, 2NAA28, 2NAA29, 2NAA35, 2NAA36, 2NAA38, 2NAA42, 2NAA43, 2NAA45, 2NAA46, 2NAB1, 2NAB4, 2NAB5, 2NAB7, 2NAB8, 2NAB9, 2NAB10, 2NAB11, 2NAB12, 2NAB13, 2NAB14, 2NAB15, 2NAB18, 2NAB19, **2NAB20,** 2NAB21, 2NAB22, 2NAB26, 2NAB27, 2NAB28, 2NAB29, 2NAB30 , 2NAB31, 2NAB32 , 2NAB34 , 2NAB35, 2NAB36, 2NAB37, 2NAB38 , **3NAA1**, 3NAA2, 3NAA3, 3NAA4, 3NAA5, 3NAA6, **3NAA7**, **3NAA8**, 3NAA9, 3NAA10, 3NAA11, **3NAA12**, 3NAA14, 3NAA15, 3NAA16, **3NAA17**, 3NAA19, 3NAA20 , 3NAA21 , 3NAA22, 3NAA23, 3NAA24, 3NAA25.

**4.2.5 PR31**:

PR1 , **PR2** , **PR3** , PR4, PR5, PR6, PR7, PR8, **PR9** , PR10, PR11 , PR12 , PR13 , PR14 , PR15, PR16, **PR17**, PR18, PR19 , PR20, PR21 , PR22 , PR23 , PR24, **PR25**, **PR26**, **PR27**, PR28 , PR29, PR30, PR31 .

**4.3. Laboratory *in-vitro* assay**

The selected 20 potential PGPR isolates were further screened by two techniques. In both agar-well diffusion and disc-diffusion method, the five strains that showed the highest inhibition zones are **PR3, PR9, PR17, PR25 (*Enterobactor* sp.), 3NAA8 (*Bacillus* sp.).** Among these five strains the maximum inhibition zone was shown by PR17 as 33.50mm, 25.30mm and lowest zone by PR25 as 27.60mm, 19.30mm. PR17 also found significant from all other isolates while 3NAA8 was found non-significant from PR17 but significant from other isolates of PGPR.

**Table 4.3 *In-vitro* antagonistic effect of selected 20 PGPR against *R. solanacearum*, (inhibition zone (mm)).**

| Treatments | Agar well diffusion (diameter area in mm) | Disk diffusion (diameter area in mm) |
|---|---|---|
| PR3 | 29.30 | 19.30 |
| PR2 | 14.00 | 15.60 |
| PR9 | 29.60 | 20.30 |
| PR17 | 33.50 | 25.30 |
| PR25 | 27.60 | 19.30 |
| PR26 | 27.00 | 16.60 |
| PR27 | 20.40 | 17.30 |
| 3NAA1 | 24.30 | 12.30 |
| 3NAA7 | 8.60 | 7.60 |
| 3NAA8 | 30.60 | 20.30 |
| 3NAA12 | 19.30 | 10.30 |
| 3NAA17 | 17.30 | 18.00 |
| 1NAB15 | 10.00 | 8.30 |
| 1NAB20 | 21.30 | 10.30 |
| 2CBA2 | 18.60 | 8.30 |
| 2CBA4 | 16.30 | 16.30 |
| 2CBA18 | 12.30 | 10.50 |
| 2CBA42 | 9.00 | 8.30 |
| 2BAB1 | 7.00 | 8.60 |
| 2AAB27 | 7.30 | 7.60 |
| **F- test** | S | S |
| **S. Ed. (±)** | 0.406 | 0.389 |
| ***C. D. (P = 0.05)*** | *0.861* | *0.826* |

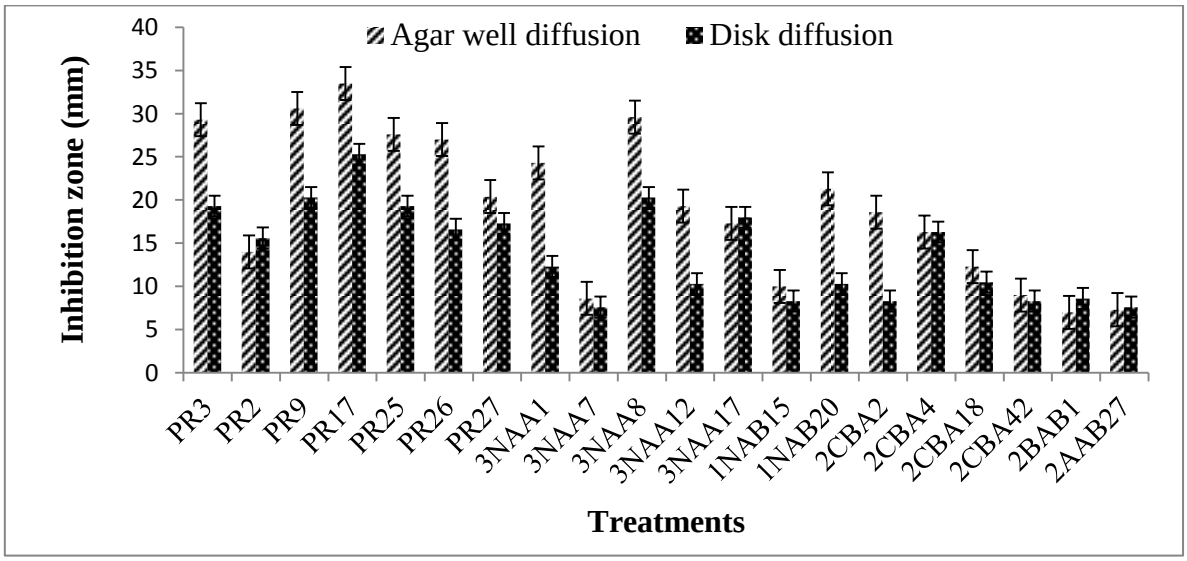



**Fig.4.3.The inhibition effect expressed by inhibition diameter area (mm) of twenty antagonistic bacterial strains on *Ralstonia solanacearum* growth in agar-well diffusion and disc diffusion method.**

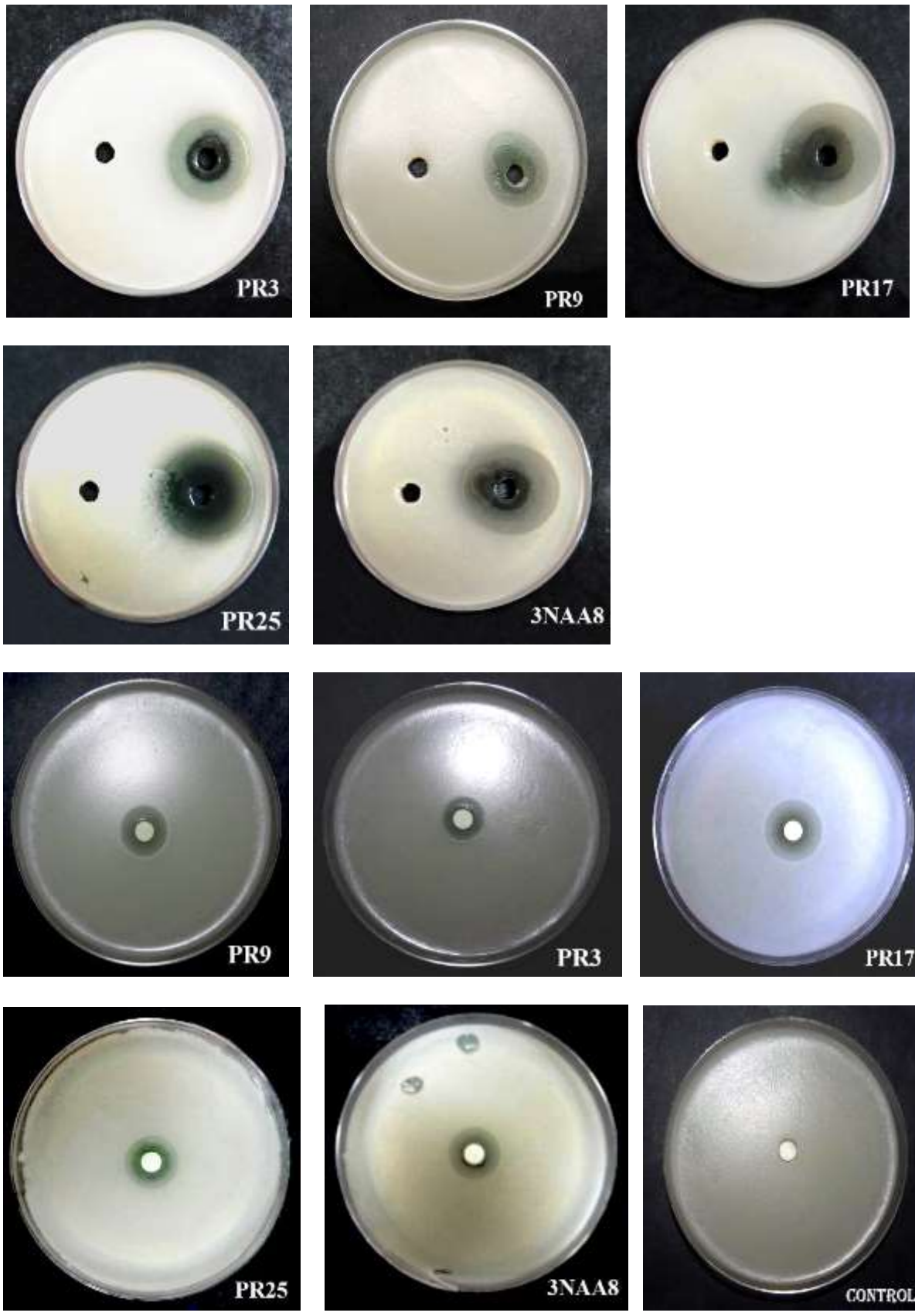



**Plate.4.3 The inhibition zone showed by the five potential PGPR PR3, PR9, PR17, PR25 and 3NAA8 against *Ralstonia solanacearum* in agar well diffusion and disk diffusion technique.**

## 4.4. DISEASE INCIDENCE

In the experiment, the antagonistic bacterial strains showed significant difference for their abilities to reduce tomato bacterial wilt incidence which are presented in the **tables (4.4.a-c)**. In all the three varieties PR17 and 3NAA8 found highest significant, also the other PGPR treatments were significant from each other.

In variety Pusa 120, the lowest disease incidence 60% was found with PR17 following 3NAA8 showing disease incidence of 67.86% in comparison to control(with pathogen) which showed the highest disease incidence of 99.78% after 42 days from day of inoculation. The control without any pathogen and treatment have 0% disease incidence (**Table 4.4.(a))**.

In variety Navodaya, PR17 showed the lowest disease incidence of 50.02% following 3NAA8 which was found to have disease incidence of 64.28% in comparison to control (with pathogen) which showed the highest disease incidence of 99.91% after 42 days from day of inoculation. The control without any pathogen and treatment have 0% disease incidence (**Table 4.4.(b)).**

Whereas in variety Golden Green, PR17 showing the lowest disease incidence 53.49% following 3NAA8 found with 67.86% of disease incidence in comparison to control which showed the disease incidence of 99.50% after 42 days from days of inoculation. The control without inoculation of pathogen and treatments showed no disease incidence (**Table 4.4.(c)).**

In all the three varieties, PR17 and 3NAA8 showed the lowest value of disease incidence percentage against *R. solanacearum.*

**Plate.4.4 The preparation of antagonistic PGPRs for the inoculation in tomato plants to observe their effects against *Ralstonia solanacearum*.**

**Table 4.4.(a) Disease incidence values showed by five antagonistic PGPR after inoculation against Bacterial wilt of tomato in Pusa 120 during different stages of days after inoculation (DAI).**

| Treatments | Disease incidence (%) | | | | | |
|---|---|---|---|---|---|---|
| | 7 DAI | 14 DAI | 21 DAI | 28DAI | 35DAI | 42DAI |
| Control( uninoculated) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Control (inoculated) | 10.73 | 25.08 | 42.72 | 61.00 | 92.25 | 99.78 |
| PR3 | 0.00 | 17.93 | 42.81 | 64.31 | 78.60 | 85.72 |
| PR9 | 0.00 | 10.86 | 28.61 | 57.13 | 71.45 | 82.15 |
| PR17 | 0.00 | 0.00 | 21.50 | 39.29 | 57.17 | 60.98 |
| PR25 | 0.00 | 14.28 | 32.14 | 53.54 | 64.28 | 75.00 |
| 3NAA8 | 0.00 | 14.28 | 32.14 | 46.32 | 53.57 | 67.86 |
| C.D. (5%) | 0.01 | 0.12 | 0.14 | 0.27 | 0.26 | 0.25 |
| S. Ed. | 0.00 | 0.06 | 0.07 | 0.13 | 0.28 | 0.12 |

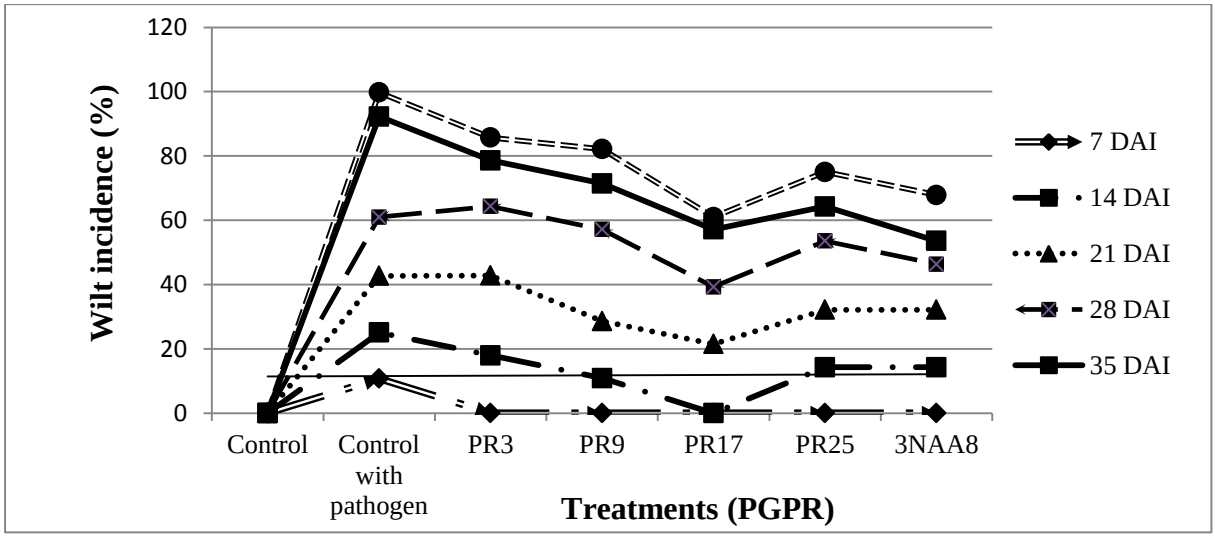



**Fig.4.4.(a) Disease incidence values on tomato plants of variety Pusa 120 when treated by five potential antagonists recorded different DAI (days after inoculation) against bacterial wilt of tomato. Control (uninoculated) was free of symptoms.**


**(A)** **(B)** **(C)**


**(D)** **(E)** **(F)**

**Plate.4.4(a) Infected plants of Pusa 120 showing the disease incidence of bacterial wilt by different given PGPR treatments T1(Control (inoculated)),T2(PR3), T3(PR9), T4(PR17), T5(PR25),T6(3NAA8).**

**Table 4.4.(b) Disease incidence values showed by five antagonistic PGPR after inoculation against Bacterial wilt of tomato in Navodaya during different stages of days after inoculation (DAI).**

| Treatments | Disease incidence (%) | | | | | |
|---|---|---|---|---|---|---|
| | 7 DAI | 14 DAI | 21 DAI | 28 DAI | 35 DAI | 42 DAI |
| Control ( uninoculated) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Control (inoculated) | 17.86 | 35.66 | 57.15 | 82.14 | 99.78 | 99.91 |
| PR3 | 0.00 | 17.85 | 39.30 | 57.06 | 71.42 | 78.57 |
| PR9 | 0.00 | 17.86 | 32.14 | 46.43 | 67.86 | 78.57 |
| PR17 | 0.00 | 0.00 | 14.29 | 32.15 | 42.77 | 50.02 |
| PR25 | 0.00 | 18.88 | 39.05 | 53.57 | 67.86 | 78.57 |
| 3NAA8 | 0.00 | 14.29 | 32.31 | 46.43 | 60.72 | 64.28 |
| C.D. (5%) | 0.01 | 0.11 | 0.08 | 0.25 | 0.13 | 0.08 |
| S. Ed. | 0.00 | 0.55 | 0.12 | 0.04 | 0.07 | 0.04 |

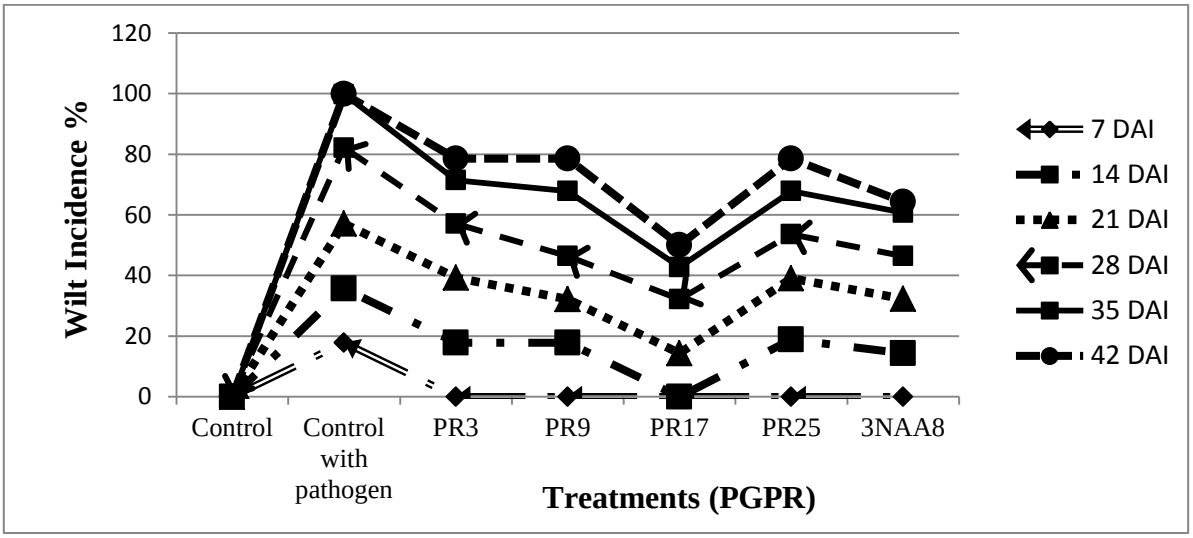



**Fig.4.4.(b).Disease incidence values on tomato plants of variety Navodaya when treated by five potential antagonists recorded at 7, 14, 21, 28, 35, 42 days after inoculation (DAI) against bacterial wilt of tomato. Control (uninoculated) was free of symptoms.**


**(A)** **(B)** **(C)**


**(D)** **(E)** **(F)**

**Plate.4.4.(b) Infected plants of Navodaya showing the disease incidence of bacterial wilt by different given PGPR treatments T1(Control (inoculated)),T2(PR3), T3(PR9), T4(PR17), T5(PR25),T6(3NAA8).**

**Table 4.4.(c). Disease incidence values showed by five antagonistic PGPR after inoculation against Bacterial wilt of tomato in Golden Green during different stages of days after inoculation (DAI).**

| Treatments | Disease incidence (%) | | | | | |
|---|---|---|---|---|---|---|
| | 7 DAI | 14 DAI | 21 DAI | 28 DAI | 35 DAI | 42 DAI |
| Control ( uninoculated) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Control (inoculated) | 17.85 | 39.30 | 67.86 | 89.30 | 97.01 | 99.50 |
| PR3 | 0.00 | 14.29 | 32.15 | 49.77 | 60.72 | 71.44 |
| PR9 | 0.00 | 17.86 | 39.32 | 60.72 | 75.00 | 82.15 |
| PR17 | 0.00 | 0.00 | 17.86 | 32.15 | 46.36 | 53.49 |
| PR25 | 0.00 | 7.16 | 21.44 | 42.79 | 60.72 | 67.86 |
| 3NAA8 | 0.00 | 7.14 | 21.44 | 39.28 | 49.90 | 60.72 |
| C.D. (5%) | 0.00 | 0.01 | 0.08 | 0.18 | 0.28 | 0.27 |
| S. Ed. | 0.00 | 0.01 | 0.02 | 0.09 | 0.28 | 0.14 |

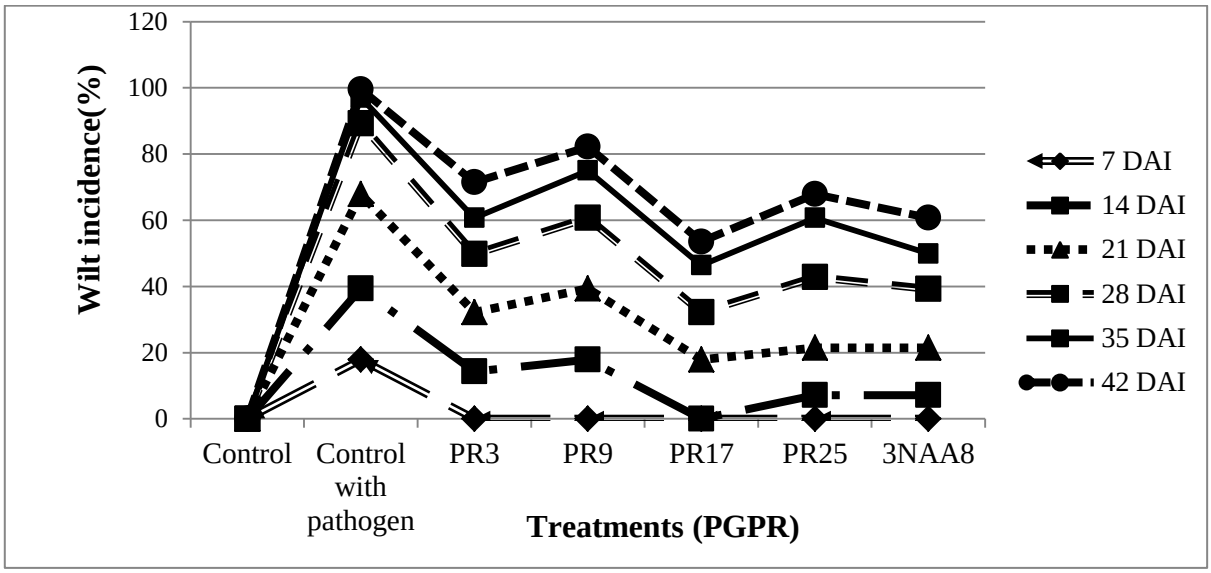
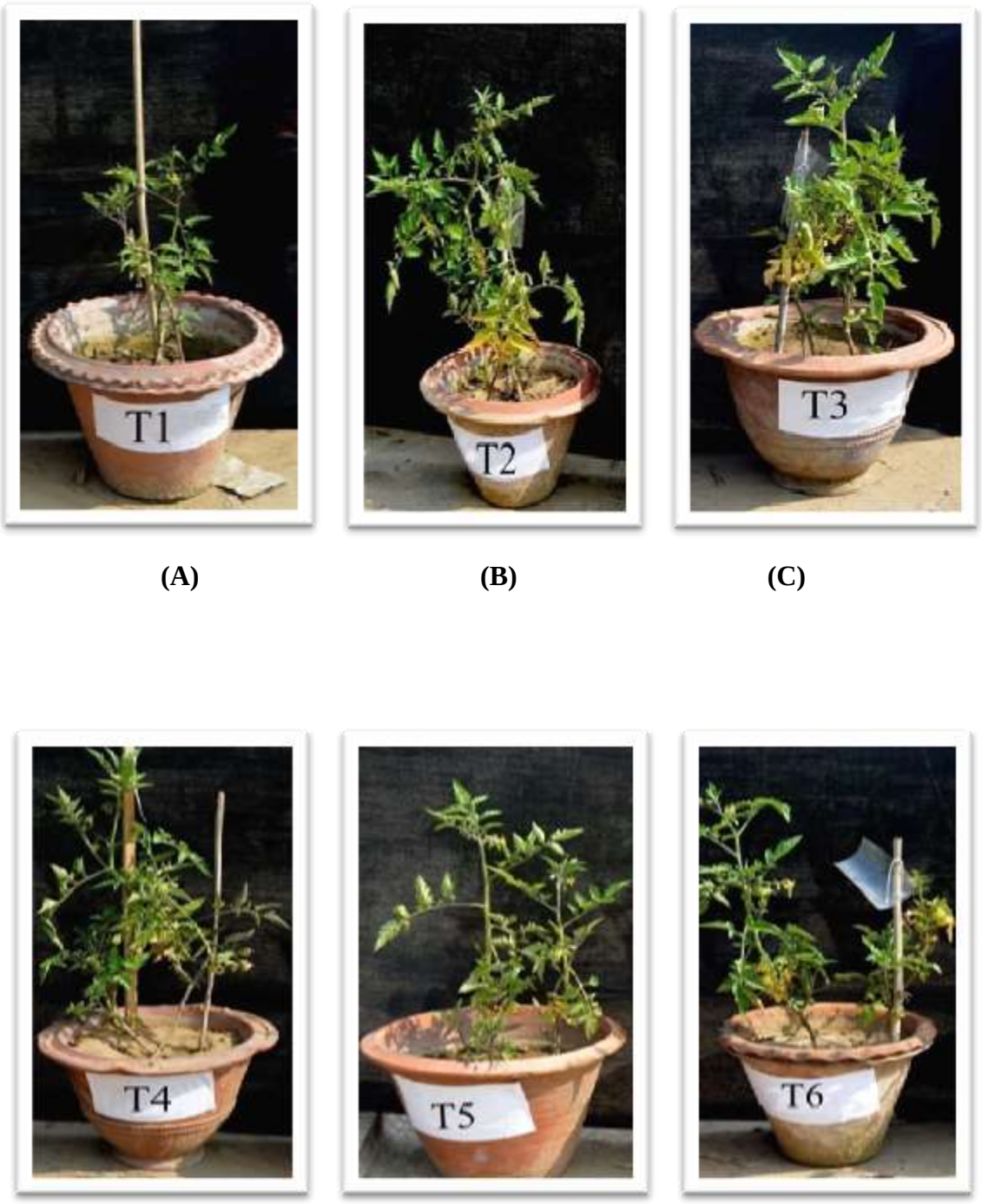



**Fig.4.4.(c) Disease incidence values on tomato plants of variety Golden Green when treated by five potential antagonists recorded at 7, 14, 21, 28, 35, 42 days after inoculation (DAI) against bacterial wilt of tomato.**




**(A)** **(B)** **(C)**

**(D)** **(E)** **(F)**

**Plate.4.4.(c) Infected plants of Golden Green showing the disease incidence of bacterial wilt by different given PGPR treatments T1(Control(inoculated)),T2(PR3), T3(PR9), T4(PR17), T5(PR25),T6(3NAA8).**

## 4.5. SEED GERMINATION PARAMETERS:

### 4.5.1. Germination percentage:

The growth promoting activity by five selected PGPR strains was tested for the seed germination by treating tomato seeds with these strains in roll towel. It is evident that there is significant differences in germination percentage due to different treatments in presented in Table 4.5.1 below.

In Pusa 120, the strain 3NAA8 was found with highest germination percentage 96.67 % followed by PR17 which showed 93.33% of germination percentage among all the potential PGPR strains. The lowest germination percentage was observed in PR3 and PR 9 with 88.33%. The control showed the least germination percentage with 43.33%.

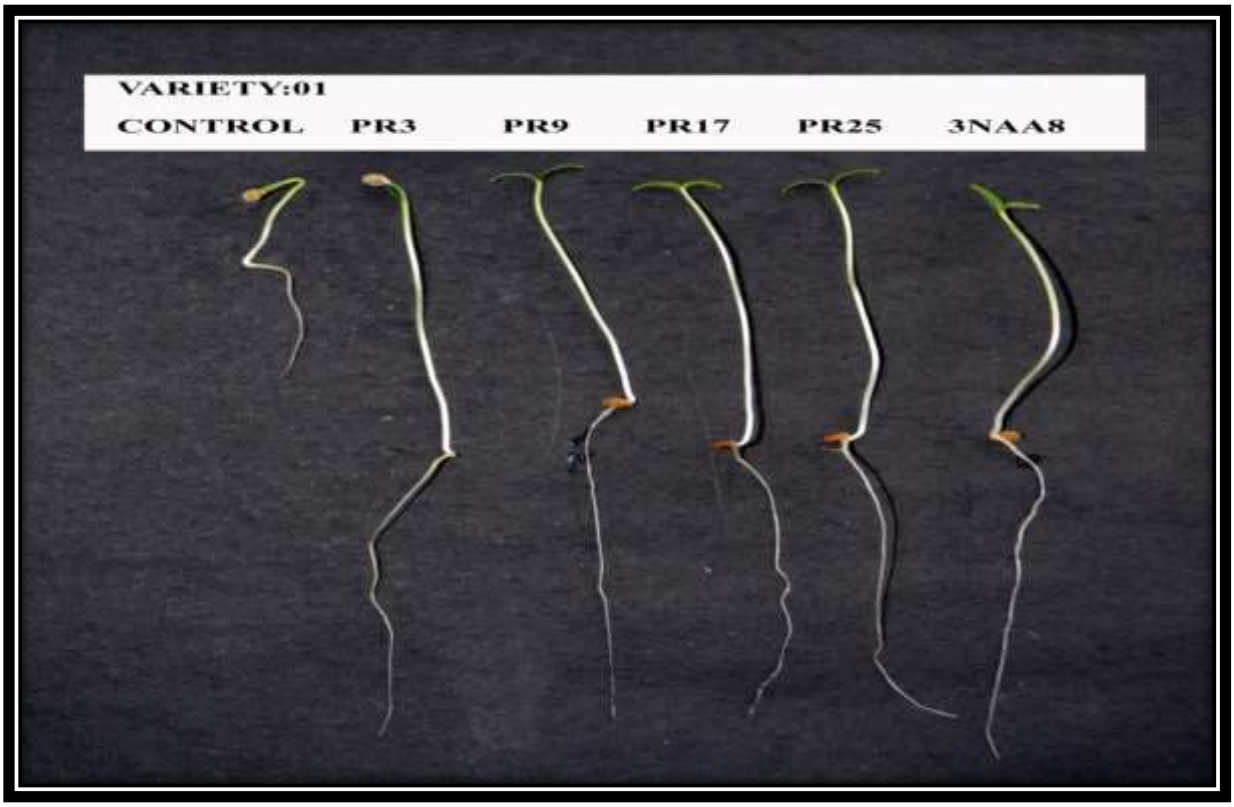
In Navodaya also the strain 3NAA8 showed the highest germination percentage of 95% followed by PR17 found with 91.67% of germination percentage. The lowest percentage of germination was observed in PR3 with 81.67%.

In Golden Green, the strains PR17 and 3NAA8 both showed the same highest percentage of germination 95%. The lowest germination percentage was observed in PR3 and PR9 with same 83.33% respectively.

In all the three varieties PR17 and 3NAA8 showed the best germination percentage among the five potential strains. However 3NAA8 was found non-significant from PR17 and PR25 whereas PR17 and PR25 were found significant from PR9, PR3 and control but they are non-significant from each other.

**Table 4.5.1 Germination % of tomato seeds in variety Pusa 120, Navodaya and Golden Green by different five PGPR strains PR3, PR9, PR17, PR25, 3NAA8 (Roll towel method).**

| Treatments | Germination % | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 43.33 | 43.33 | 50 |
| PR3 | 88.33 | 81.67 | 83.33 |
| PR9 | 88.33 | 86.67 | 83.33 |
| PR17 | 93.33 | 91.67 | 95 |
| PR25 | 90.00 | 86.67 | 85 |
| 3NAA8 | 96.67 | 95 | 95 |
| C.D. (5%) | 7.93 | 7.03 | 7.03 |
| S. Em. | 2.55 | 2.26 | 2.26 |
| S. Ed. | 3.60 | 3.19 | 3.19 |
| C.V. | 5.29 | 4.84 | 4.77 |

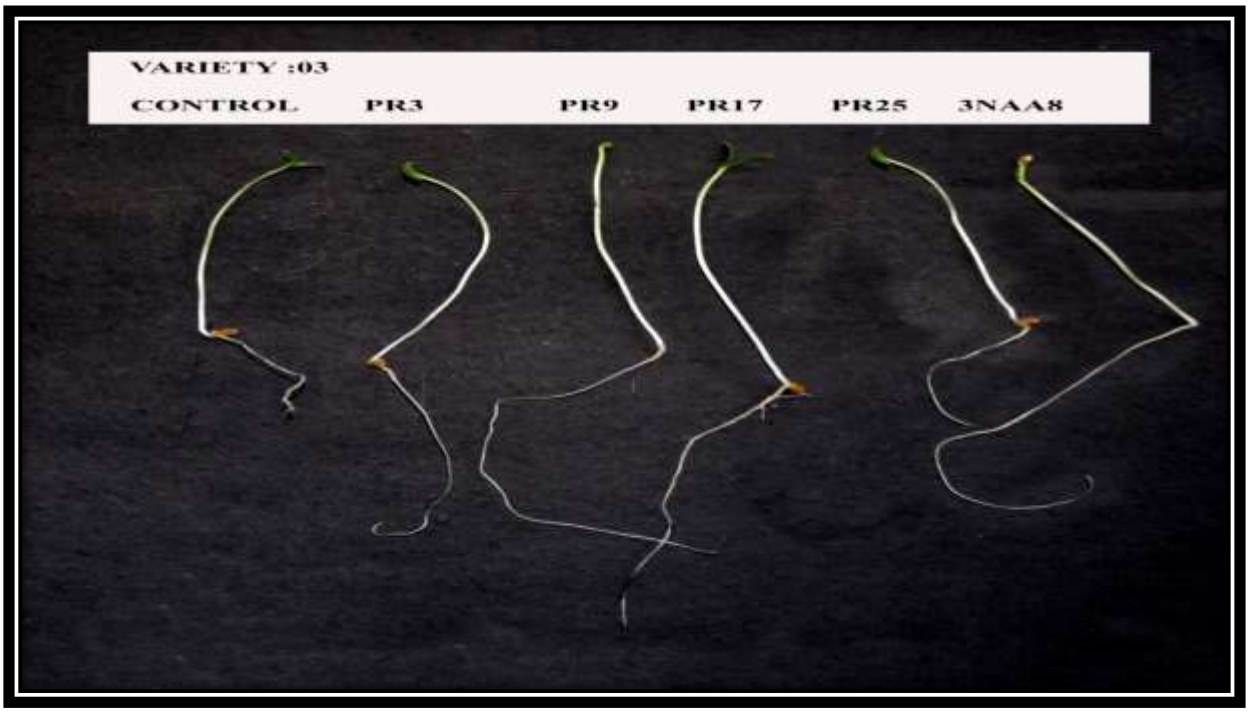



**(A)**

**Plate.4.5.1 Effect of five potential PGPR on seed germination of Pusa 120.**


**(B)**

**Plate.4.5.1 Effect of five potential PGPR on seed germination of Navodaya.**




**(C)**

**Plate.4.5.1 Effect of five potential PGPR on seed germination of Golden Green.**

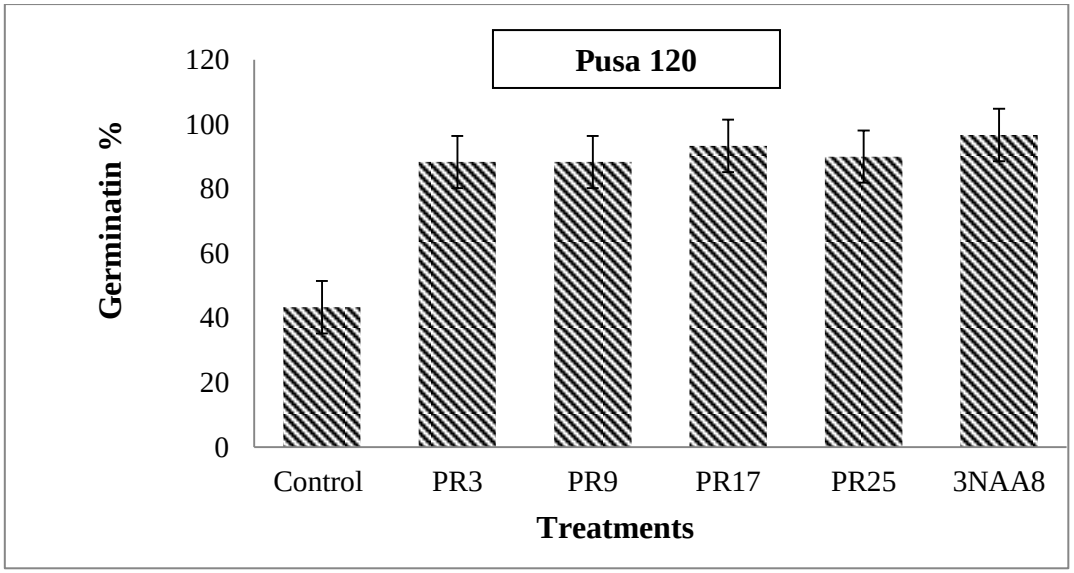



**Fig.4.5.1(a) Germination % of tomato seeds of variety Pusa 120 treated with five selected PGPR strains.**

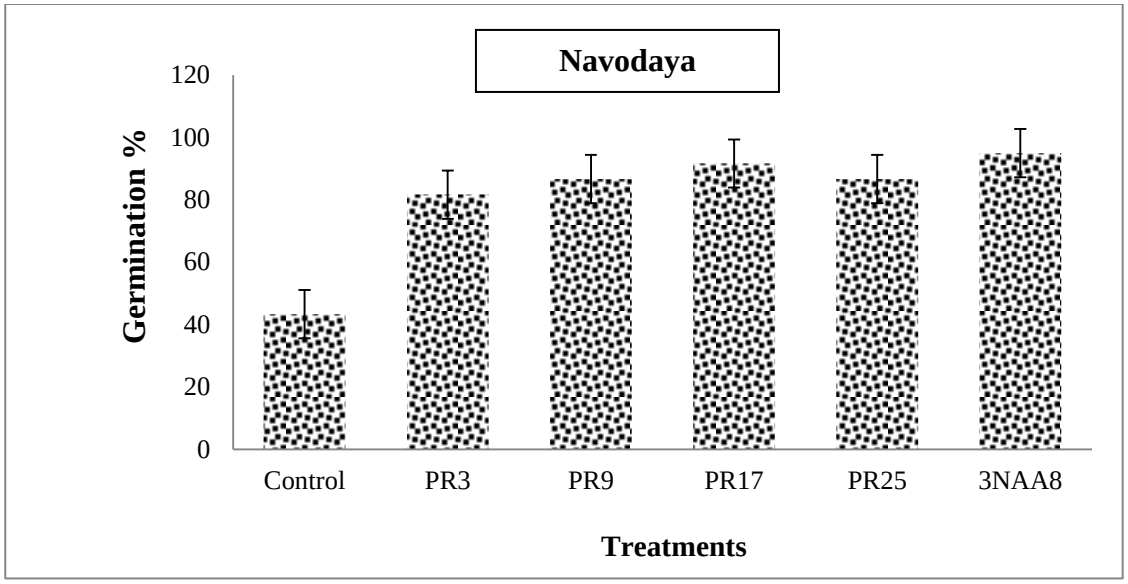



**Fig.4.5.1(b) Germination % of tomato seeds of variety Navodaya treated with five selected PGPR strains.**

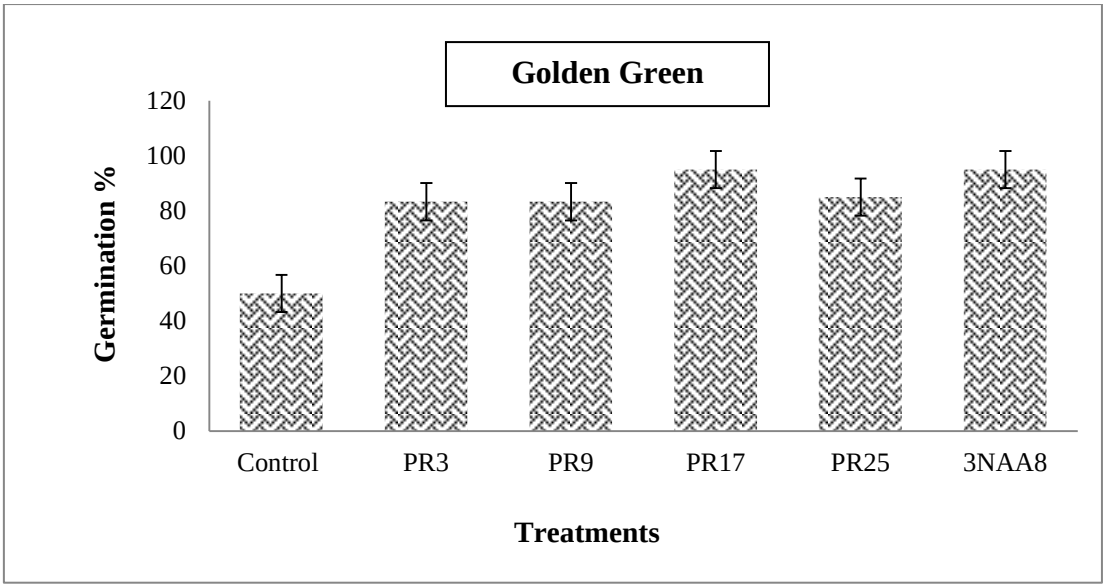



**Fig.4.5.1(c) Germination % of tomato seeds of variety Golden Green treated with five selected PGPR strains.**

**4.5.2 Root length (cm):**

The tables below show that there is a significant difference in root length among the varieties after the treatment with antagonistic PGPR strains. 3NAA8 and PR17 show the maximum increase in root length in all the three varieties, but 3NAA8 was found non-significant from PR17 but significant from other PGPR treatment while PR17 was found significant from all.

In Pusa 120, The maximum root length at 5 DAI(3.77)cm,7 DAI(5.90)cm,9 DAI(8.00)cm,11 DAI (9.17)cm was observed in PR17 followed by 3NAA8 at 5 DAI(4.00)cm,7 DAI(5.63)cm,9 DAI(8.03)cm,11 DAI (8.97)cm. The lowest root length was found in PR9 at 5 DAI(3.00)cm,7 DAI(4.60)cm,9 DAI(7.33)cm,11 DAI (8.40)cm(Table 4.5.2(a)).

In Navodaya, The maximum root length at 5 DAI(4.07)cm,7 DAI(5.00)cm,9 DAI(6.00)cm,11 DAI (7.03)cm was observed in PR17 followed by 3NAA8 at 5 DAI(4.03)cm,7 DAI(4.93)cm,9 DAI(5.90)cm,11 DAI (6.90)cm. The lowest root length was found in PR9 at 5 DAI(3.49)cm,7 DAI(4.37)cm,9 DAI(5.27)cm,11 DAI (6.27)cm(Table 4.5.2(b))..

In Golden Green, The maximum root length at 5 DAI(3.90)cm,7 DAI(5.00)cm,9 DAI(6.57)cm,11 DAI (8.73)cm was observed in 3NAA8 followed by PR17 at 5 DAI(3.93)cm,7 DAI(5.03)cm,9 DAI(6.47)cm,11 DAI (8.63)cm. The lowest root length was found in PR9 at 5 DAI(3.47)cm,7 DAI(4.50)cm,9 DAI(5.73)cm,11 DAI (7.87)cm(Table 4.5.2(a)).

**Table 4.5.2(a) Effect of PGPR strains on Root length (cm) of tomato seedlings in Pusa 120 at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Root length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.97 | 2.67 | 4.10 | 4.67 |
| PR3 | 3.73 | 5.07 | 7.70 | 8.60 |
| PR9 | 3.00 | 4.60 | 7.33 | 8.40 |
| PR17 | 3.77 | 5.90 | 8.00 | 9.17 |
| PR25 | 4.00 | 5.47 | 7.57 | 8.37 |
| 3NAA8 | 4.00 | 5.63 | 8.03 | 8.97 |
| C.D. (5%) | 0.30 | 0.42 | 0.82 | 0.60 |
| S. Em. | 0.10 | 0.13 | 0.26 | 0.19 |
| S. Ed. | 0.14 | 0.19 | 0.37 | 0.27 |
| C.V. | 4.94 | 4.75 | 6.41 | 4.17 |

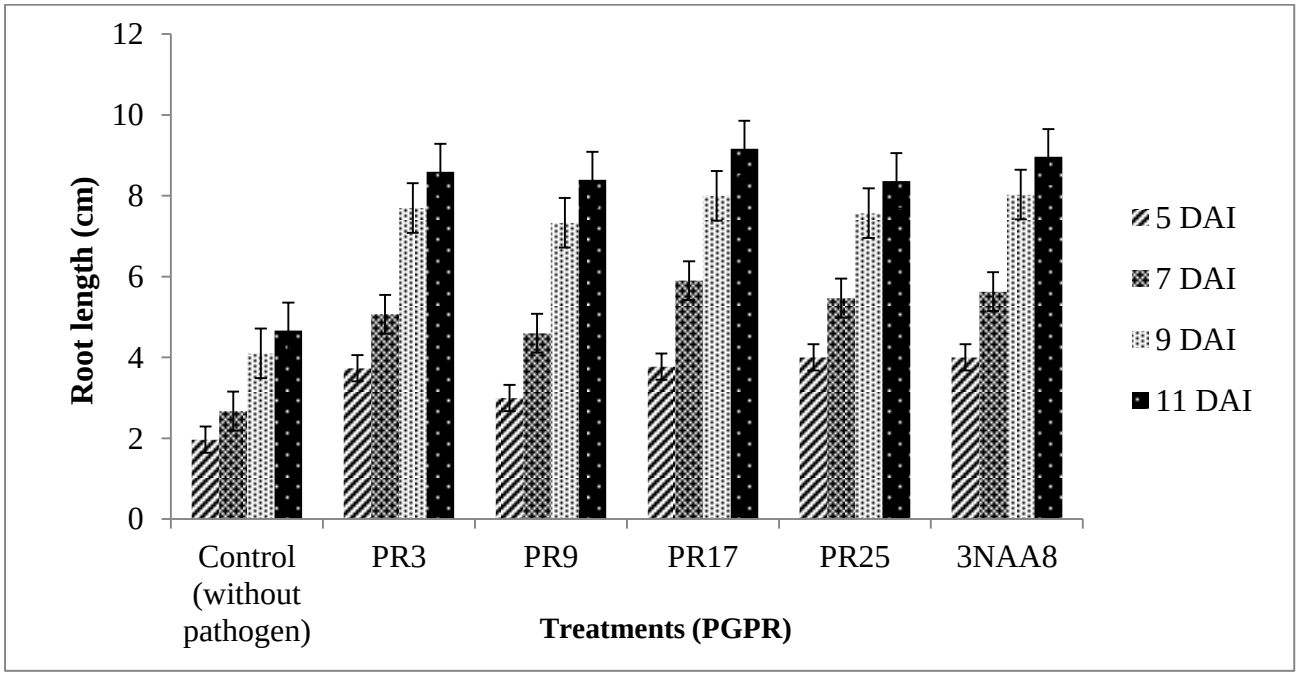



**Fig.4.5.2(a) Effect of PGPR strains on Root length (cm) of tomato seedlings in variety Pusa 120 at different interval of days after inoculation (DAI) of PGPR strains.**

**Table 4.5.2(b) Effect of PGPR strains on Root length (cm) of tomato seedlings in Navodaya at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Root length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.97 | 2.47 | 3.80 | 4.77 |
| PR3 | 3.67 | 4.60 | 5.37 | 6.57 |
| PR9 | 3.49 | 4.37 | 5.27 | 6.27 |
| PR17 | 4.07 | 5.00 | 6.00 | 7.03 |
| PR25 | 3.53 | 4.33 | 5.23 | 6.23 |
| 3NAA8 | 4.03 | 4.93 | 5.90 | 6.90 |
| C.D. (5%) | 1.32 | 0.37 | 0.31 | 0.26 |
| S. Em. | 0.42 | 0.12 | 0.10 | 0.08 |
| S. Ed. | 0.60 | 0.17 | 0.14 | 0.12 |
| C.V. | 2.27 | 4.80 | 3.29 | 2.31 |

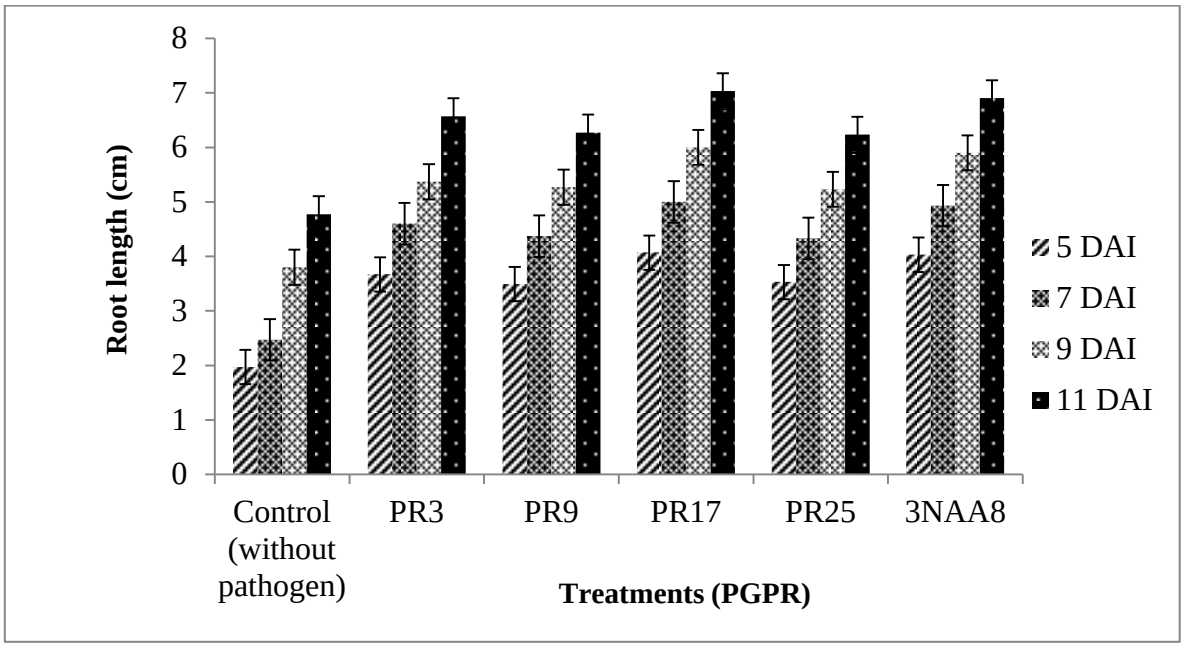



**Fig.4.5.2(b) Effect of PGPR strains on Root length (cm) of tomato seedlings in variety Navodaya at different interval of days after inoculation (DAI) of PGPR strains.**

**Table 4.5.2(c) Effect of PGPR strains on Root length (cm) of tomato seedlings in Golden Green at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Root length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.90 | 2.60 | 3.70 | 4.90 |
| PR3 | 3.60 | 4.63 | 5.87 | 7.97 |
| PR9 | 3.47 | 4.50 | 5.73 | 7.87 |
| PR17 | 3.93 | 5.03 | 6.47 | 8.63 |
| PR25 | 3.67 | 4.67 | 5.93 | 8.07 |
| 3NAA8 | 3.90 | 5.00 | 6.57 | 8.73 |
| C.D. (5%) | 0.18 | 0.16 | 0.33 | 0.65 |
| SE(m) | 0.06 | 0.05 | 0.11 | 0.21 |
| SE(d) | 0.08 | 0.08 | 0.15 | 0.30 |
| C.V. | 2.93 | 2.07 | 3.25 | 4.71 |

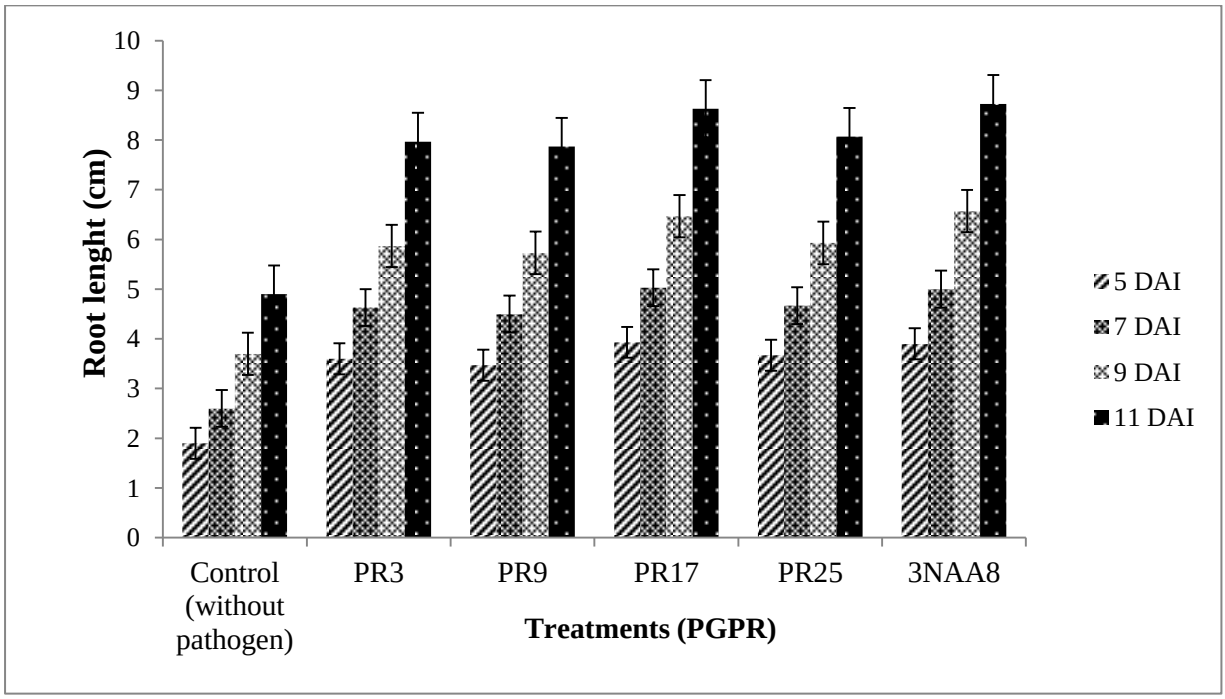



**Fig.4.5.2(c) Effect of PGPR strains on Root length (cm) of tomato seedlings in variety Golden Green at different interval of days after inoculation (DAI) of PGPR strains.**

**4.5.3 Shoot length (cm):**

The table 4.5.3(a-c) shows that there is a significant difference in shoot length among the varieties after the treatment with antagonistic PGPR strains.

In Pusa 120, The maximum shoot length at 5 DAI(2.83)cm,7 DAI(4.43)cm,9 DAI(6.87)cm,11 DAI (7.77)cm was observed in 3NAA8 followed by PR17 at 5 DAI(2.73)cm,7 DAI(4.60)cm,9 DAI(6.47)cm,11 DAI (7.43)cm. The lowest shoot length was found in PR9 at 5 DAI(2.33)cm,7 DAI(3.83)cm,9 DAI(5.80)cm,11 DAI (6.60)cm (Table 4.5.3(a)). 3NAA8 was found non-significant from PR17 and PR9 but significant from PR25 and PR9 while PR17 was found significant to all but non-significant from PR3.

In Navodaya, The maximum shoot length at 5 DAI(3.20)cm,7 DAI(3.97)cm,9 DAI(4.80)cm,11 DAI (5.90)cm was observed inPR17 followed by3NAA8 at 5 DAI(3.07)cm,7 DAI(3.90)cm,9 DAI(4.70)cm,11 DAI (5.87)cm. The lowest shoot length was found in PR9 at 5 DAI(2.63)cm,7 DAI(3.30)cm,9 DAI(4.07)cm,11 DAI (5.23)cm(Table 4.5.3(b)). Whereas 3NAA8 was found significant from all other PGPR treatments, while PR17 was found non-significant from 3NAA8 but significant from other PGPRs.

In Golden Green, The maximum shoot length at 5 DAI(3.93)cm,7 DAI(4.00)cm,9 DAI(5.00)cm,11 DAI (7.37)cm was observed in PR17 followed by 3NAA8 at 5 DAI(3.90)cm,7 DAI(3.97)cm,9 DAI(5.33)cm,11 DAI (7.17)cm. The lowest shoot length was found in PR3 at 5 DAI(3.60)cm,7 DAI(3.47)cm,9 DAI(4.37)cm,11 DAI (6.37)cm(Table 4.5.3(a)). PR17 and 3NAA8 found significant from PR3 but non-significant from other PGPR treatments.

In all the three varieties 3NAA8 and PR17 showed the maximum increase in the length of shoot under treatment of potential antagonistic.

**Table 4.5.3(a) Effect of PGPR strains on Shoot length (cm) of tomato seedlings in Pusa 120 at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Shoot length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.23 | 2.00 | 2.93 | 3.53 |
| PR3 | 2.77 | 4.10 | 6.03 | 7.07 |
| PR9 | 2.33 | 3.83 | 5.80 | 6.60 |
| PR17 | 2.73 | 4.60 | 6.47 | 7.43 |
| PR25 | 2.90 | 4.53 | 6.13 | 6.70 |
| 3NAA8 | 2.83 | 4.43 | 6.87 | 7.77 |
| C.D. (5%) | 0.25 | 0.41 | 0.65 | 0.76 |
| S. Em. | 0.08 | 0.13 | 0.21 | 0.24 |
| S. Ed. | 0.11 | 0.19 | 0.30 | 0.35 |
| C.V. | 5.65 | 5.80 | 6.35 | 6.49 |

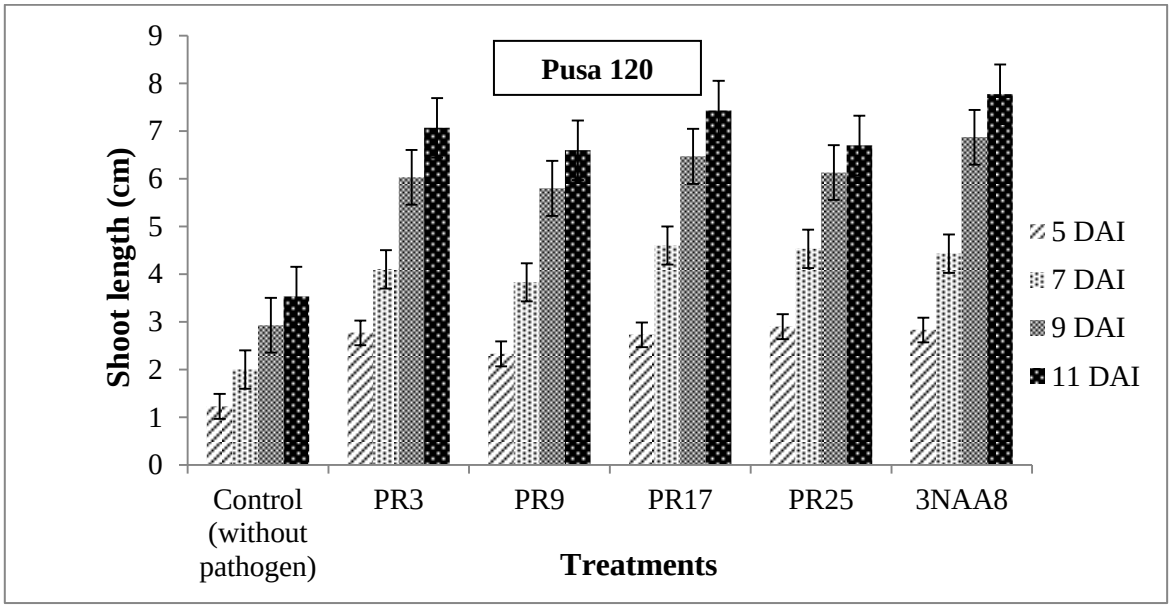



**Fig.4.5.3(a) Effect of five PGPR strains on Shoot length (cm) of tomato seedlings in Pusa 120 at different interval of days after inoculation (DAI) of PGPR strains.**

**Table 4.5.3(b) Effect of PGPR strains on Shoot length (cm) of tomato seedlings in Navodaya at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Shoot length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.03 | 1.87 | 2.70 | 3.73 |
| PR3 | 2.83 | 3.57 | 4.30 | 5.43 |
| PR9 | 2.63 | 3.30 | 4.07 | 5.23 |
| PR17 | 3.20 | 3.97 | 4.80 | 5.90 |
| PR25 | 2.73 | 3.37 | 4.07 | 5.20 |
| 3NAA8 | 3.07 | 3.90 | 4.70 | 5.87 |
| C.D. (5%) | 0.28 | 0.30 | 0.35 | 0.36 |
| S. Em. | 0.11 | 0.09 | 0.12 | 0.10 |
| S. Ed. | 0.16 | 0.13 | 0.16 | 0.14 |
| C.V. | 7.52 | 4.65 | 4.84 | 3.16 |

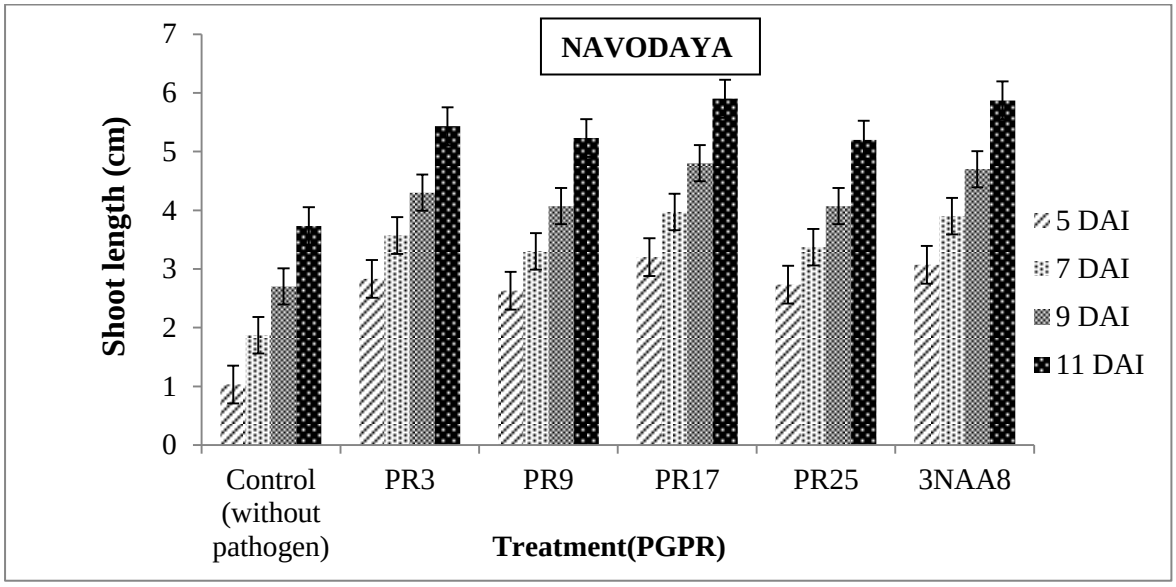



**Fig.4.5.3(b) Effect of five PGPR strains on Shoot length (cm) of tomato seedlings in Navodaya at different interval of days after inoculation (DAI) of PGPR strains.**

**Table 4.5.3(c) Effect of PGPR strains on Shoot length (cm) of tomato seedlings in GOLDEN GREEN at different interval of days after inoculation (DAI) of PGPR strains.**

| Treatments | Shoot length (cm) | | | |
|---|---|---|---|---|
| | 5 DAI | 7 DAI | 9 DAI | 11 DAI |
| Control (uninoculated) | 1.90 | 1.57 | 2.67 | 3.63 |
| PR3 | 3.60 | 3.47 | 4.37 | 6.37 |
| PR9 | 3.47 | 3.37 | 4.50 | 6.53 |
| PR17 | 3.93 | 4.00 | 5.00 | 7.37 |
| PR25 | 3.67 | 3.63 | 4.63 | 6.77 |
| 3NAA8 | 3.90 | 3.97 | 5.33 | 7.17 |
| C.D. (5%) | 0.18 | 0.20 | 0.43 | 0.71 |
| S. Em. | 0.06 | 0.07 | 0.14 | 0.23 |
| S. Ed. | 0.08 | 0.09 | 0.20 | 0.32 |
| C.V. | 2.93 | 3.39 | 5.42 | 6.24 |

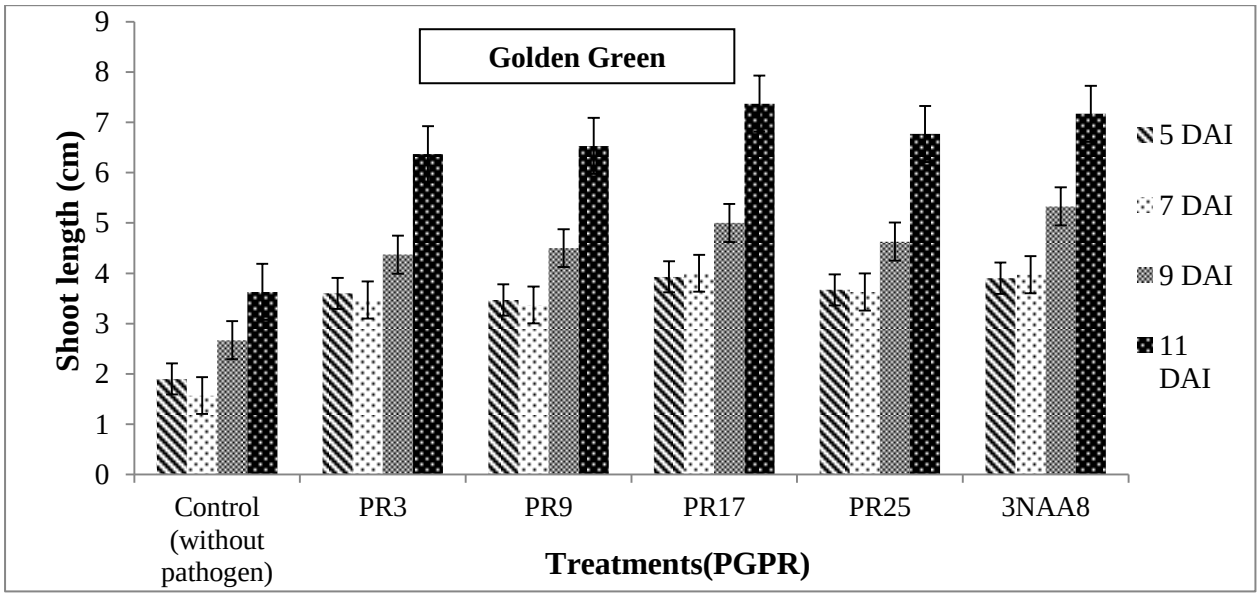



**Fig.4.5.3(c) Effect of five PGPR strains on Shoot length (cm) of tomato seedlings in GOLDEN GREEN at different interval of days after inoculation (DAI) of PGPR strains.**

## 4.6 GROWTH PARAMETERS

### 4.6.1 Number of leaves per plant

The data presented in the 4.6.1(a-c) below shows at 30DAT, 60DAT and 90DAT, the effect of selected PGPR at different growth stage of tomato crop. Statistical analysis of number of leaves showed the significant growth difference due to the treatments of selected PGPR.

In variety-1 i.e. Pusa 120, the maximum increase in number of leaves was observed in PR17 at 30DAT (48.86), 60 DAT (57.18) and 90 DAT (95.57) followed by 3NAA8at 30 DAT (48.79), 60 DAT (57.32) and 90 DAT (95.25)out of the five potential strains. The minimum number of leaves was shown by PR3 at 30 DAT (40.29), 60 DAT (47.57) and 90 DAT (74.89) respectively (Table 4.6.1(a)). 3NAA8 found significant from all other PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant from PR25, PR9, PR3 and control.

Where as in variety-2 i.e. Navodaya, it is found that the maximum number of leaves at all stages of growth was 3NAA8 at 30 DAT (49.21), 60 DAT (58.32) and 90 DAT (95.21) followed by PR17 at 30 DAT (48.39), 60 DAT (57.79) and 90 DAT (93.39). The lowest number of leaves was found in PR3 at 30 DAT (41.04), 60 DAT (47.75) and 90 DAT (78.04) (Table 4.6.1(a)). PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from rest of PGPR treatments.

In variety-3 i.e. Golden Green, 3NAA8 was found to show the maximum number of leaves at 30 DAT (48.50), 60 DAT (57.36) and 90 DAT (92.93) followed by PR17 at 30 DAT (48.29), 60 DAT (56.43) and 90 DAT (92.46) among all the PGPRs. The minimum number of leaves was observed in PR3 at 30 DAT (40.21), 60 DAT (47.29) and 90 DAT (76.39) (Table 4.6.1(c)). PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9, PR3 and control.

Therefore, in all the three varieties the maximum increase in number of leaves were observed in PR17 and 3NAA8 at different growing stages of plant and the lowest was found to be PR9 among the applied potential PGPR strains.

**Table 4.6.1(a) Effect of Five selected PGPR on number of leaves at 30, 60 and 90 DAT (Days after transplanting) on Pusa 120.**

| Treatments | Number of leaves per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 26.93 | 31.54 | 58.36 |
| Control (inoculated) | 25.79 | 30.32 | 55.18 |
| PR3 | 40.29 | 47.57 | 74.89 |
| PR9 | 41.61 | 49.29 | 78.96 |
| PR17 | 48.86 | 57.18 | 95.57 |
| PR25 | 41.18 | 49.07 | 79.36 |
| 3NAA8 | 48.79 | 57.32 | 95.25 |
| C.D. (5%) | 1.20 | 1.35 | 3.06 |
| S. Em. | 0.42 | 0.47 | 1.07 |
| S. Ed. | 0.59 | 0.67 | 1.51 |
| C.V. | 2.84 | 2.71 | 3.68 |

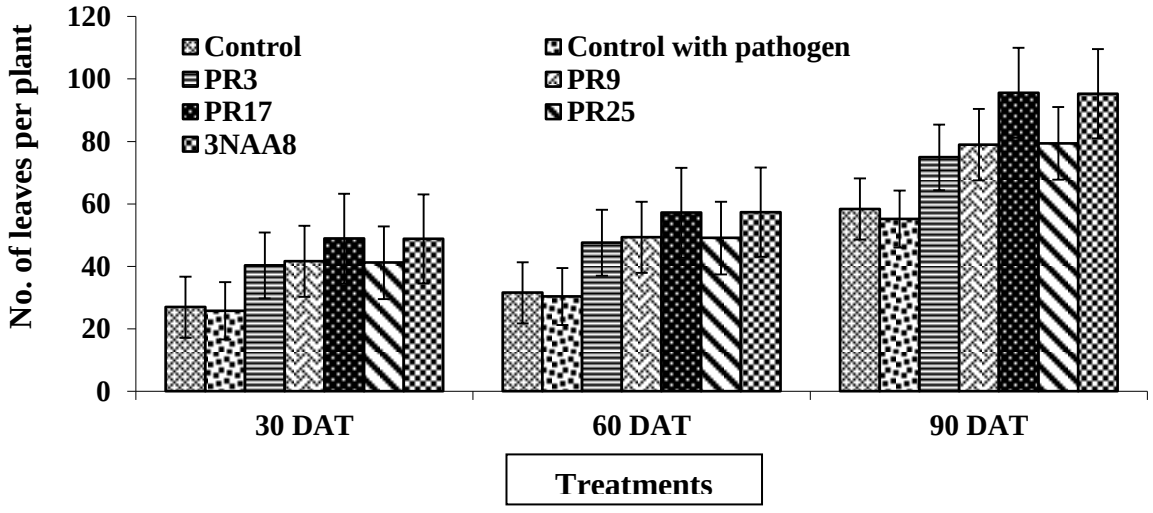



**Fig. 4.6.1(a) Influence of Five selected PGPR on number of leaves in variety Pusa 120 of tomato at different days after transplanting (DAT).**

**Table 4.6.1(b) Effect of Five selected PGPR on number of leaves at different days after transplanting (DAT) on Navodaya.**

| Treatments | Number of leaves per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 27.75 | 32.46 | 55.93 |
| Control (inoculated) | 25.89 | 30.36 | 50.75 |
| PR3 | 41.04 | 47.75 | 78.04 |
| PR9 | 40.93 | 48.57 | 78.54 |
| PR17 | 48.39 | 57.79 | 93.39 |
| PR25 | 41.93 | 48.93 | 77.93 |
| 3NAA8 | 49.21 | 58.32 | 95.21 |
| C.D. (5%) | 1.54 | 1.28 | 2.27 |
| S. Em. | 0.54 | 0.45 | 0.79 |
| S. Ed. | 0.76 | 0.63 | 1.12 |
| C.V. | 3.61 | 2.54 | 2.78 |

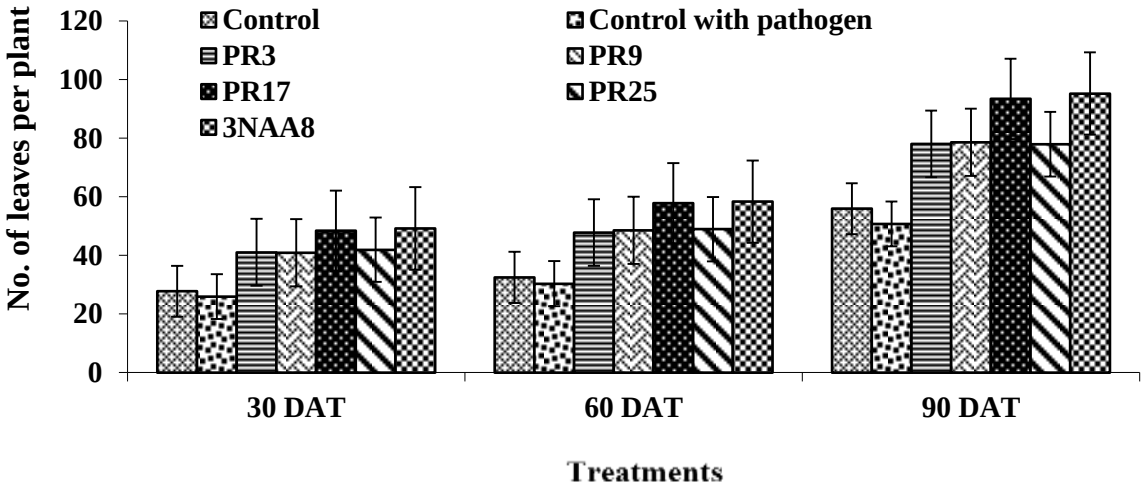



**Fig. 4.6.1(b) Influence of Five selected PGPR on number of leaves in variety Navodaya of tomato at different days after transplanting (DAT).**

**Table 4.6.1(c) Effect of Five selected PGPR on number of leaves at different days after transplanting (DAT) on Golden Green.**

| Treatments | Number of leaves per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 27.79 | 33.07 | 54.50 |
| Control (inoculated) | 25.61 | 31.00 | 49.71 |
| PR3 | 40.21 | 47.29 | 76.39 |
| PR9 | 40.39 | 47.64 | 78.71 |
| PR17 | 48.29 | 56.43 | 92.46 |
| PR25 | 41.32 | 48.57 | 79.16 |
| 3NAA8 | 48.50 | 57.36 | 92.93 |
| C.D. (5%) | 1.62 | 1.84 | 2.39 |
| S. Em. | 0.56 | 0.64 | 0.84 |
| S. Ed. | 0.80 | 0.91 | 1.18 |
| C.V. | 3.84 | 3.70 | 2.95 |

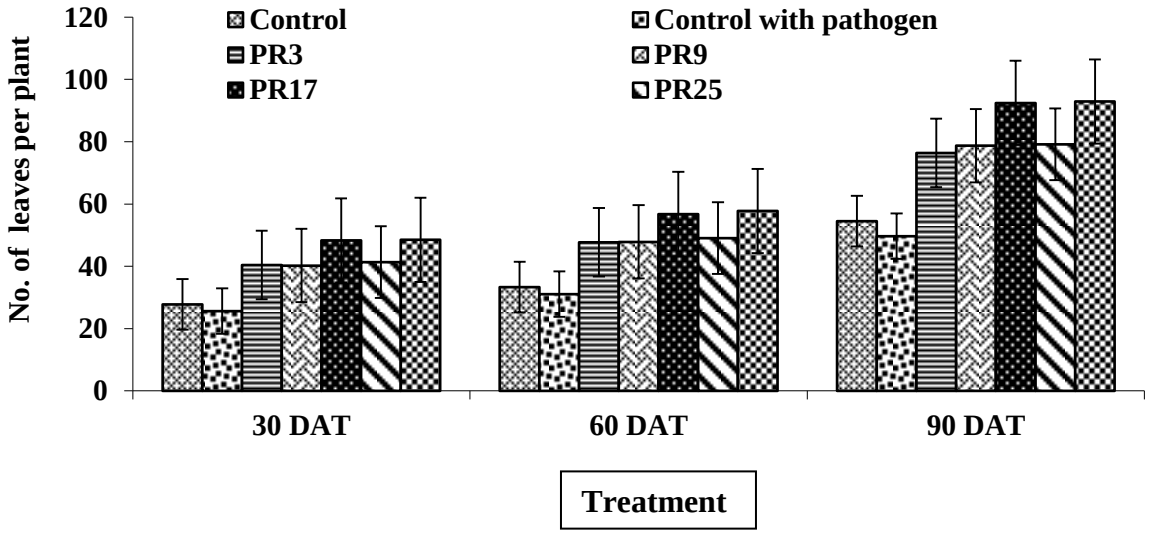



**Fig.4.6.1(c) Influence of Five selected PGPR on number of leaves in variety Golden Green of tomato at different days after transplanting (DAT).**

### 4.6.2 Number of branches per plant:

The data presented in the 4.6.2(a-c) below at 30 DAT, 60 DAT and 90 DAT shows the effect of selected PGPR at different growth stage of tomato crop. Statistical analysis of number of leaves showed the significant growth difference due to the treatments of selected PGPR.

In variety-1 which Pusa 120, the maximum increase in number of leaves was found in 3NAA8at 30 DAT (8.29), 60 DAT (10.57) and 90 DAT (14.25) followed by PR17 at 30 DAT (8.43), 60 DAT (10.39) and 90 DAT (14.04) out of the five potential strains. The minimum number of leaves was shown by PR3 at 30 DAT (7.75), 60 DAT (9.71) and 90 DAT (12.86) respectively (Table 4.6.2(a)). PR17 was found significant from all PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9, PR3 and control.

And in variety-2 i.e. Navodaya, it was observed that the maximum number of leaves at all stages of growth was shown by PR17 at 30 DAT (8.39), 60 DAT (10.79) and 90 DAT (13.55) followed by 3NAA8 at 30 DAT (8.50), 60 DAT (10.89) and 90 DAT (13.46). The lowest number of leaves was shown in PR3 at 30 DAT (7.71), 60 DAT (9.71) and 90 DAT (12.32) (Table 4.6.2(b)). PR17 and 3NAA8 was found non-significant from each other and all other PGPR treatments were also found non-significant from each other.

In variety-3 i.e. Golden Green, it was found that3NAA8 showed the maximum number of leaves at 30 DAT (8.61), 60 DAT (10.86) and 90 DAT (14.57) followed by PR17 at 30 DAT (8.32), 60 DAT (10.75) and 90 DAT (13.82) among all the PGPRs. The minimum number of leaves was observed in PR3 at 30 DAT (7.75), 60 DAT (9.93) and 90 DAT (12.29) (Table 4.6.2(c)). 3NAA8 was found significant from all the PGPR treatments whereas PR17 was found non-significant from PR25 but significant from PR9, PR3 and control.

Therefore, among the applied potential PGPR strains in all the three varieties, the maximum increase in number of leaves were observed in PR17 and 3NAA8 at different growing stages of plant and the lowest was found to be PR9.

**Table 4.6.2(a) Effect of Five selected PGPR on number of branches at different days after transplanting (DAT) on Pusa 120.**

| Treatments | Number of branches per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 6.00 | 7.86 | 10.64 |
| Control (inoculated) | 5.46 | 7.43 | 9.86 |
| PR3 | 7.75 | 9.71 | 12.86 |
| PR9 | 7.93 | 9.86 | 13.07 |
| PR17 | 8.43 | 10.39 | 14.04 |
| PR25 | 7.57 | 9.54 | 13.11 |
| 3NAA8 | 8.29 | 10.57 | 14.25 |
| C.D. (5%) | 0.53 | 0.52 | 0.61 |
| S. Em. | 0.18 | 0.18 | 0.21 |
| S. Ed. | 0.26 | 0.26 | 0.30 |
| C.V. | 6.60 | 5.12 | 4.50 |

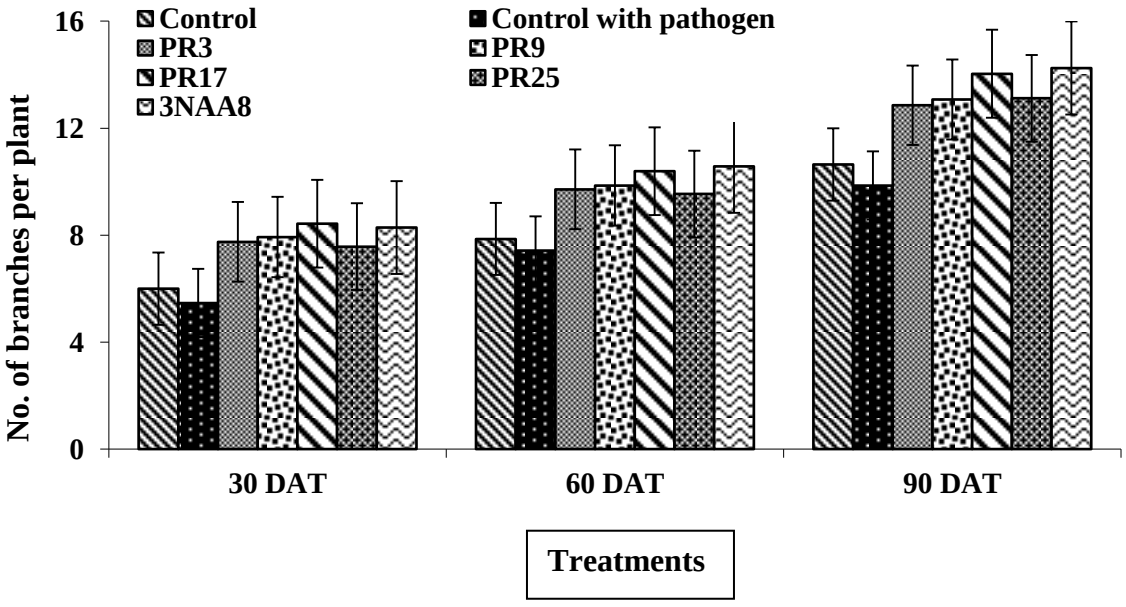



**Fig.4.6.2(a) Influence of different PGPR on number of branches in variety Pusa 120 of tomato different days after transplanting (DAT).**

**Table 4.6.2(b) Effect of Five selected PGPR on number of branches at different days after transplanting (DAT) on Navodaya.**

| Treatments | Number of branches per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 6.25 | 8.21 | 10.54 |
| Control (inoculated) | 6.00 | 8.04 | 10.07 |
| PR3 | 7.71 | 9.71 | 12.32 |
| PR9 | 7.75 | 9.86 | 13.32 |
| PR17 | 8.39 | 10.79 | 13.55 |
| PR25 | 7.75 | 10.04 | 13.29 |
| 3NAA8 | 8.50 | 10.89 | 13.46 |
| C.D. (5%) | 0.52 | 0.44 | 0.55 |
| S. Em. | 0.18 | 0.15 | 0.19 |
| S. Ed. | 0.26 | 0.22 | 0.27 |
| C.V. | 6.41 | 4.23 | 4.08 |

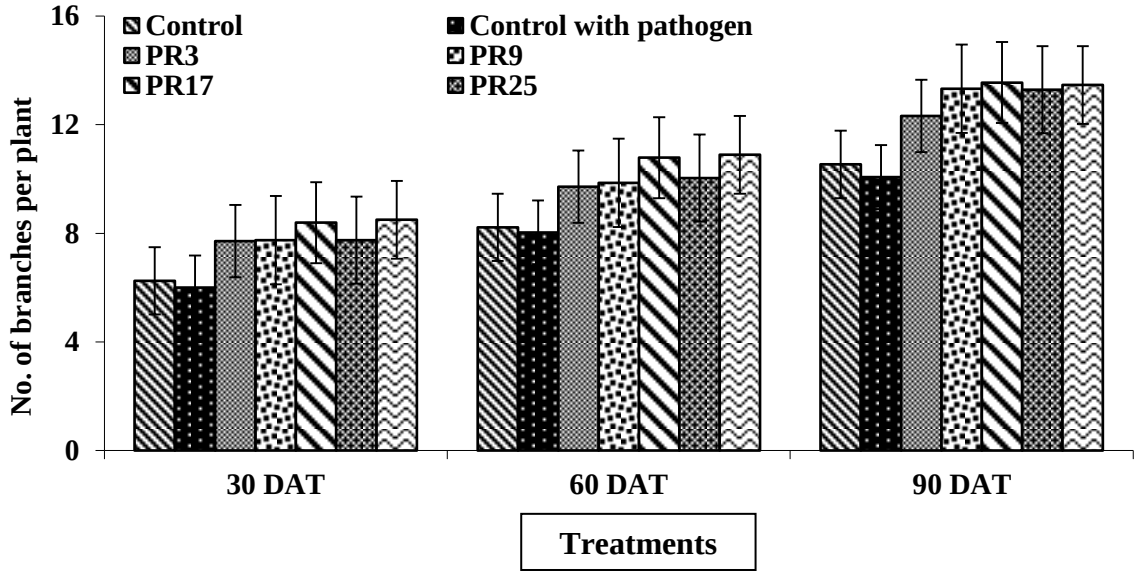



**Fig. 4.6.2(b) Influence of different PGPR on number of branches in variety Navodaya of tomato at different days after transplanting (DAT).**

**Table 4.6.2(c) Effect of Five selected PGPR on number of branches at different days after transplanting (DAT) on Golden Green.**

| Treatments | Number of branches per plant | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| Control (uninoculated) | 6.14 | 8.29 | 10.46 |
| Control (inoculated) | 5.61 | 7.75 | 9.79 |
| PR3 | 7.75 | 9.93 | 12.29 |
| PR9 | 7.87 | 10.29 | 13.14 |
| PR17 | 8.32 | 10.75 | 13.82 |
| PR25 | 7.96 | 10.21 | 13.57 |
| 3NAA8 | 8.61 | 10.86 | 14.57 |
| C.D. (5%) | 0.44 | 0.49 | 0.56 |
| S. Em. | 0.15 | 0.17 | 0.19 |
| S. Ed. | 0.22 | 0.24 | 0.27 |
| C.V. | 5.40 | 4.62 | 4.09 |

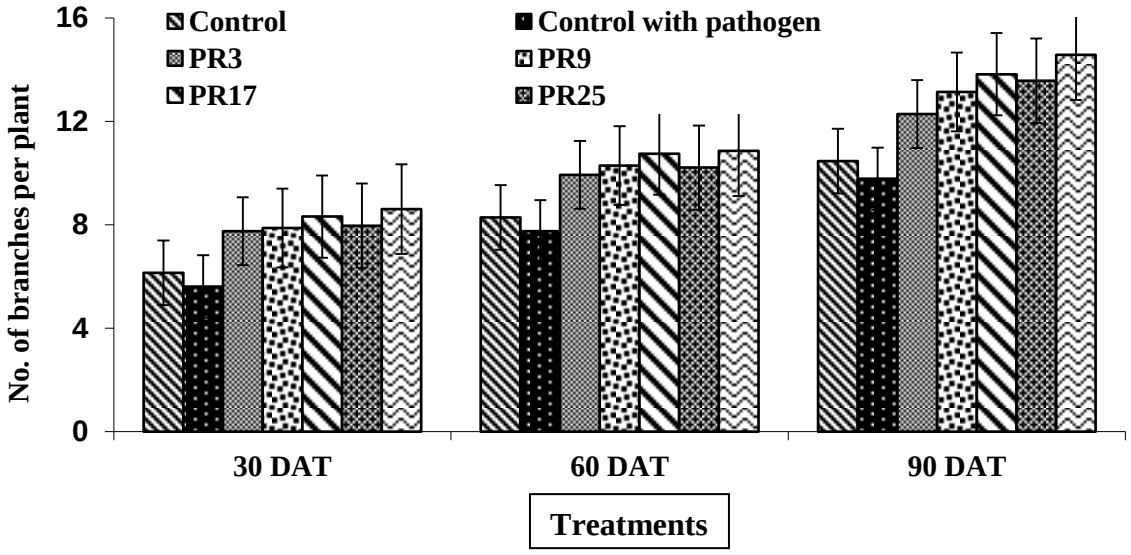



**Fig.4.6.2(c) Influence of different PGPR on number of branches in variety Golden Green of tomato at different days after transplanting (DAT).**

### 4.6.3 Number of flowers per plant:

The data presented in Table 4.6.3 below shows the significant increase in the number of flowers at 60 DAT (days after transplanting) in all the three varieties of tomato by five different effective PGPR strains.

In variety-1 which Pusa 120, the maximum increase in number of flowers was found in 3NAA8 at 60 DAT (22.57) followed by PR17 at 60 DAT (21.54) out of the five potential strains. The minimum number of flowers was shown by PR3 at 60 DAT (18.96) respectively. 3NAA8 and PR17 both were found significant from all other PGPR treatments whereas PR25, PR9 and PR3 were found non-significant from each other.

And in variety-2 i.e. Navodaya, it was observed that the maximum number of flowers at all stages of growth was shown by 3NAA8 at 60 DAT (22.25) followed by PR17 at 60 DAT (22.00). The lowest number of flowers was shown in PR3 at 60 DAT (19.18). PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from rest of the PGPR treatment.

In variety-3 i.e. Golden Green, it was found that 3NAA8 showed the maximum number of flowers at 60 DAT (22.36) followed by PR17 at 60 DAT (22.21) among all the PGPRs. The minimum number of flowers was observed in PR3 at 60 DAT (20.00). PR17 was found significant from all other treatments whereas 3NAA8 was found non-significant to PR17 but significant to other PGPR treatments.

**Table 4.6.3 Effect of five selected PGPR on number of flowers per Plant at 60 DAT (Days after transplanting) on Pusa 120, Navodaya and Golden Green.**

| Treatments | No. of Flowers per Plant (60 DAT) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 11.07 | 10.89 | 11.00 |
| Control (inoculated) | 10.46 | 9.71 | 9.89 |
| PR3 | 18.96 | 19.18 | 20.00 |
| PR9 | 20.71 | 19.86 | 20.14 |
| PR17 | 21.54 | 22.00 | 22.21 |
| PR25 | 20.39 | 20.25 | 20.64 |
| 3NAA8 | 22.57 | 22.25 | 22.36 |
| C.D. (5%) | 0.73 | 0.59 | 0.61 |
| S. Em. | 0.25 | 0.21 | 0.21 |
| S. Ed. | 0.36 | 0.29 | 0.30 |
| C.V. | 3.75 | 3.07 | 3.10 |

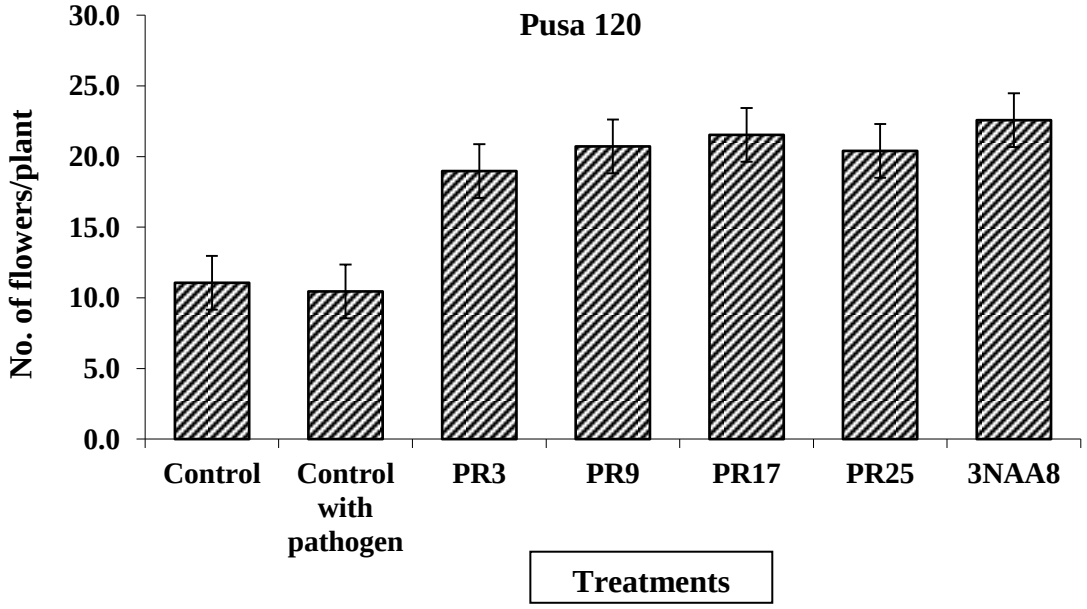



**Fig. 4.6.3(a) Effect of different PGPR on number of flowers per plant in variety Pusa 120 of tomato at 60 DAT (days after transplanting).**

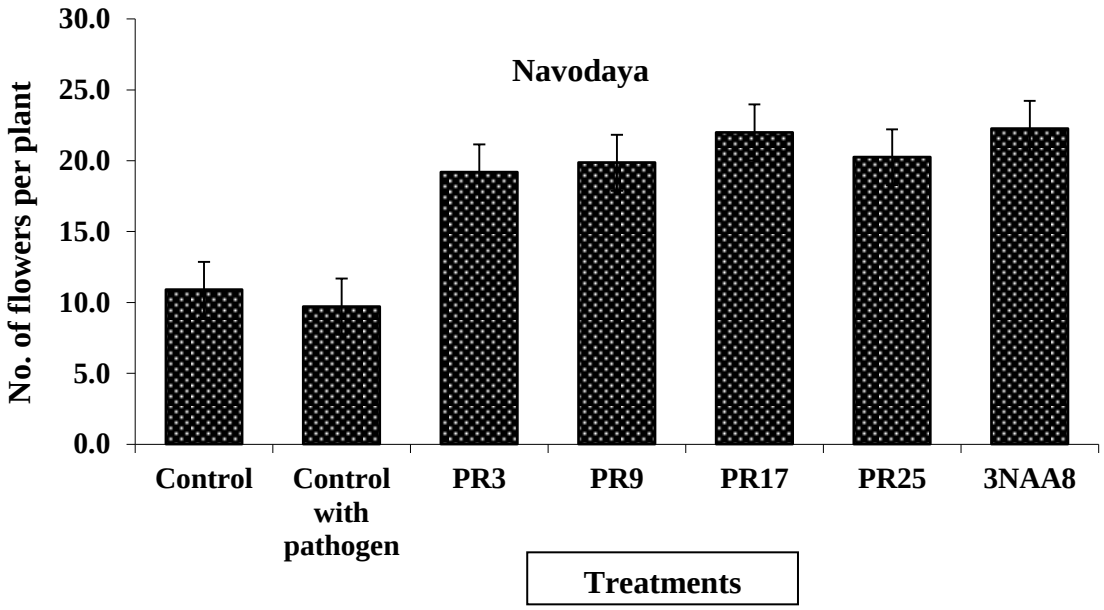



**Fig. 4.6.3(b) Effect of different PGPR on number of flowers/plant in variety Navodaya of tomato at 60 DAT (days after transplanting).**

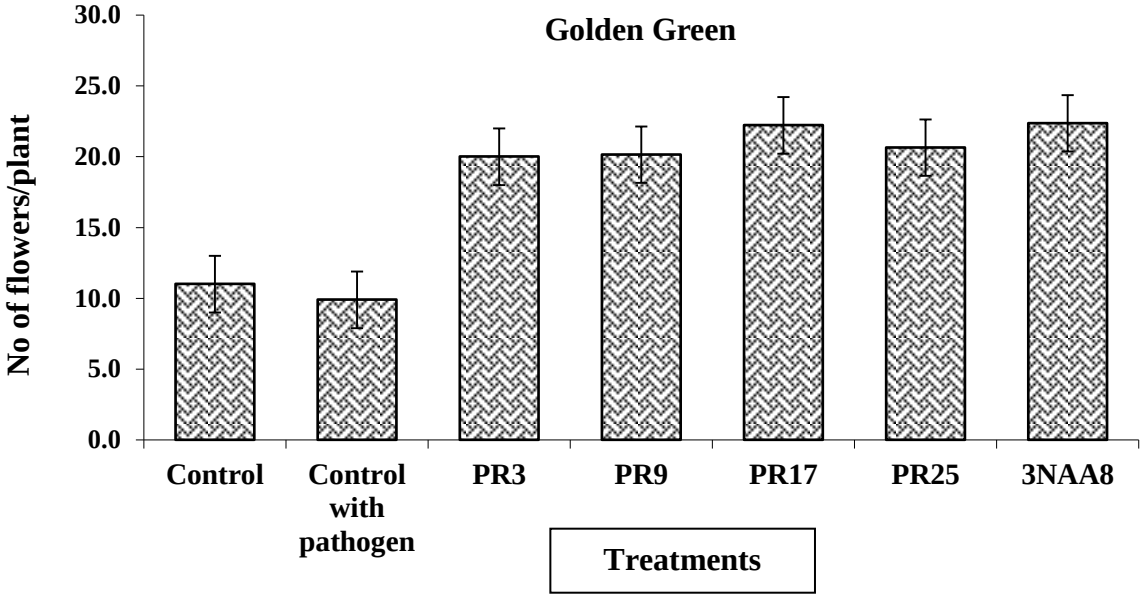



**Fig. 4.6.3(c) Effect of different PGPR on number of flowers/plant in variety Golden Green of tomato at 60 DAT (days after transplanting).**

## 4.7 YIELD PARAMETERS

### 4.7.1 Number of fruits per plant:

Data presented below in Table 4.7.1 at 60DAT (days after transplant) shows the effect of PGPR on number of fruits produced. Statistical analysis showed the significant difference due to treatment of PGPR.

In variety-1 which Pusa 120, the maximum increase in number of fruits was observed in 3NAA8 at 60 DAT (12.93) followed by PR17 at 60 DAT (12.89) out of the five potential PGPRs. The minimum number of leaves was shown by PR3 at 60 DAT (10.64) respectively. PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 and significant from PR25, PR9, PR3 and control but they are non-significant from each other.

And in variety-2 i.e. Navodaya, it was found that the maximum number of fruits at all stages of growth was shown by PR17 at 60 DAT (13.68) followed by 3NAA8 at 60 DAT (13.28). The lowest number of leaves was shown in PR3 at 60 DAT (11.54). 3NAA8 was found significant from all other PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant from rest of the PGPR treatments. Also they are significant from each other.

In variety-3 i.e. Golden Green, it was found that 3NAA8 showed the maximum number of fruits at 60 DAT (13.29) followed by PR17 at 60 DAT (13.18) among all the PGPRs. The minimum number of leaves was observed in PR3 at 60 DAT (11.75). PR17 was found significant from all PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3.

**Table4.7.1 Influence of Potential PGPR strains on number of fruits/plant in varieties Pusa 120, Navodaya and Golden Green of tomato on 60 DAT (Days after transplant).**

| Treatments | No. of Fruits per Plant | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 6.79 | 6.89 | 6.71 |
| Control (inoculated) | 6.43 | 6.29 | 6.21 |
| PR3 | 10.64 | 11.54 | 11.75 |
| PR9 | 11.04 | 11.57 | 12.29 |
| PR17 | 12.89 | 13.68 | 13.18 |
| PR25 | 11.79 | 12.32 | 12.00 |
| 3NAA8 | 12.93 | 13.28 | 13.29 |
| C.D. (5%) | 0.58 | 0.58 | 0.61 |
| S. Em. | 0.20 | 0.20 | 0.21 |
| S. Ed. | 0.29 | 0.29 | 0.30 |
| C.V. | 5.17 | 4.98 | 5.24 |

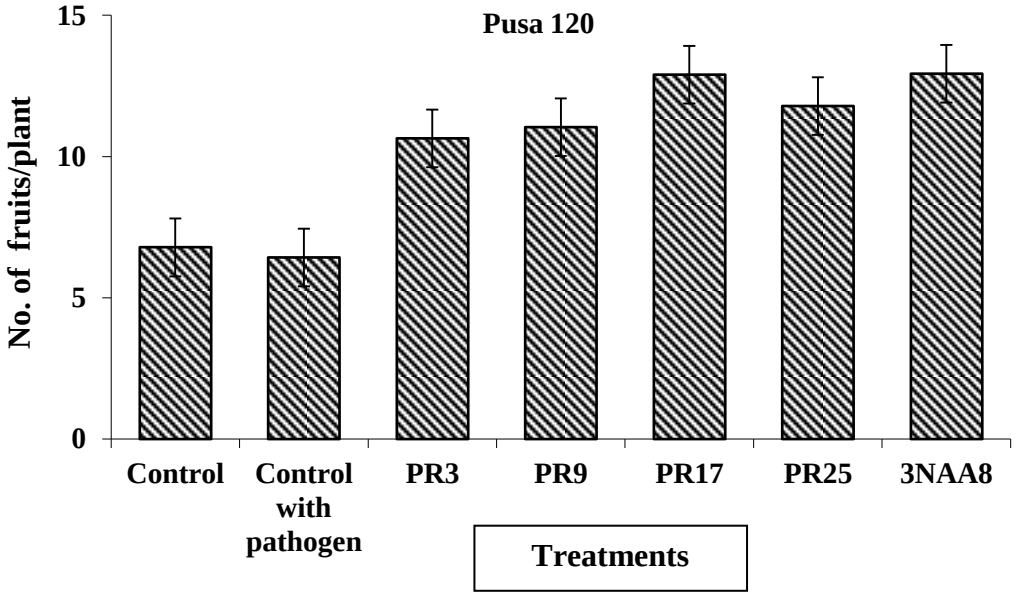



**Fig. 4.7.1(a) Influence Effect of different PGPR on number of fruits/plant in variety Pusa 120 of tomato.**

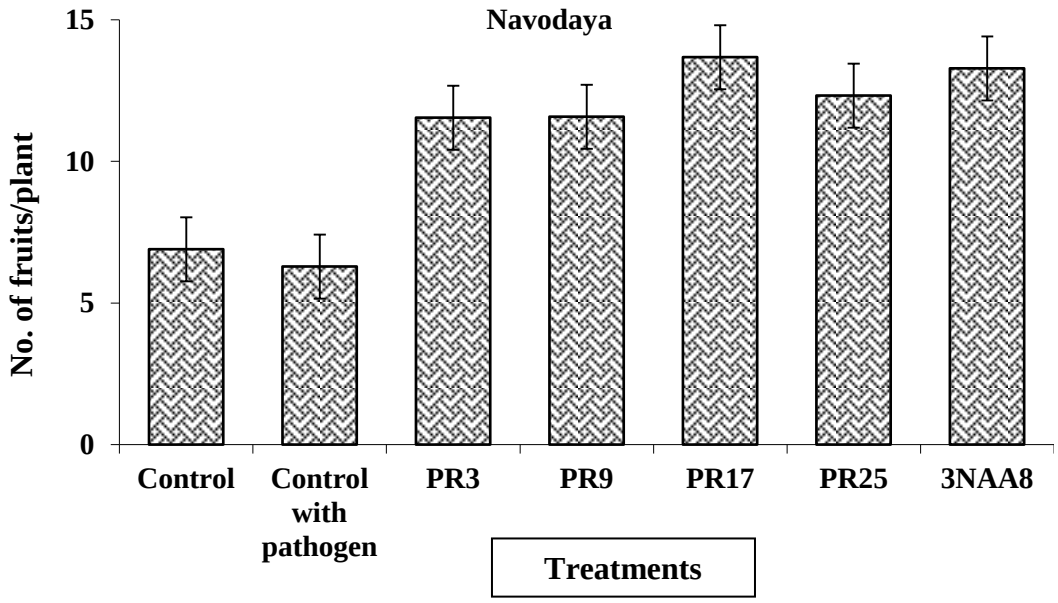



**Fig.4.7.1(b) Influence Effect of different PGPR on number of fruits/plant in variety Navodaya of tomato.**

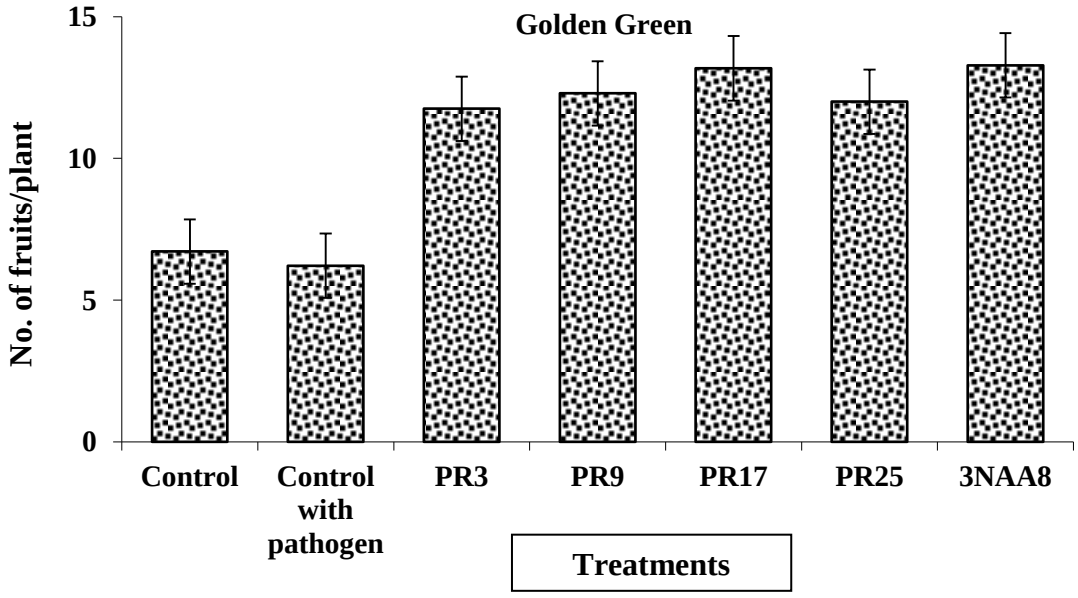



**Fig.4.7.1(c) Influence Effect of different PGPR on number of fruits/plant in variety Golden Green of tomato.**

### 4.7.2 Total fresh weight of plant (g):

The data presented in Table 4.7.2 and figures below showed that there was significant difference in plant fresh weight among the varieties. PR17 was found significant from all other PGPR treatments whereas 3NAA8 was non-significant from but was found significant from PR25, PR9 and PR3 in all the three varieties.

In Pusa 120, the highest fresh of plant was found in 3NAA8 (60.90)g followed by PR17(57.56)g whereas the lowest fresh weight of plant was recorded in PR3(29.48)g among all the treatments given.

In Navodaya also the highest fresh weight of plant was shown by 3NAA8 (62.41)g followed by PR17 (61.29)g. The lowest fresh weight of plant was observed in PR3 (32.82)g among all the potential PGPRs.

In Golden Green, the highest fresh weight was found in 3NAA8 (61.13)g followed by PR17 (60.29)g. The lowest fresh weight was recorded in PR3 (39.08)g among all the treatments.

**Table 4.7.2 Effect of potential PGPR on total fresh weight (g) of tomato plant in varieties Pusa 120, Navodaya and Golden Green.**

| Treatments | Total Fresh weight of Plant (g) | | |
|---|---|---|---|
| | **Pusa 120** | **Navodaya** | **Golden Green** |
| Control (uninoculated) | 15.91 | 16.88 | 16.54 |
| Control (inoculated) | 14.49 | 15.53 | 15.11 |
| PR3 | 29.48 | 32.82 | 39.08 |
| PR9 | 37.89 | 40.02 | 42.67 |
| PR17 | 57.56 | 61.29 | 60.29 |
| PR25 | 54.12 | 58.47 | 57.56 |
| 3NAA8 | 60.90 | 62.41 | 61.13 |
| C.D. (5%) | 1.22 | 1.45 | 2.09 |
| S. Em. | 0.43 | 0.51 | 0.73 |
| S. Ed. | 0.60 | 0.72 | 1.03 |
| C.V. | 2.91 | 3.27 | 4.62 |

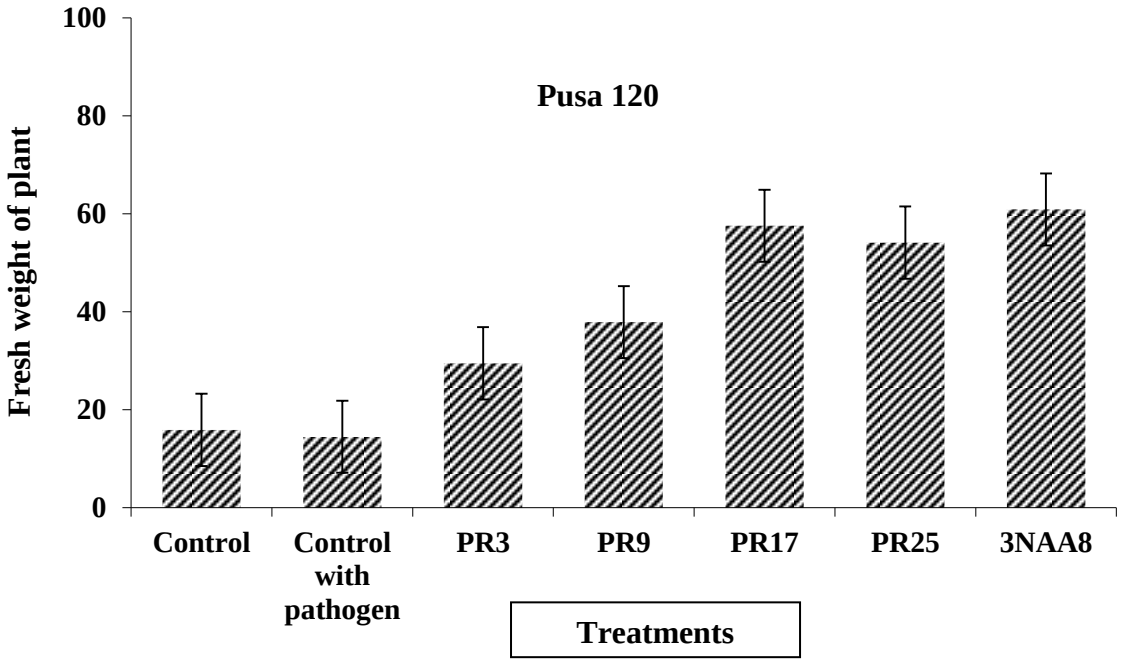



**Fig.4.7.2(a) Effect of PGPR on total fresh weight (g) of tomato plant on variety Pusa 120.**

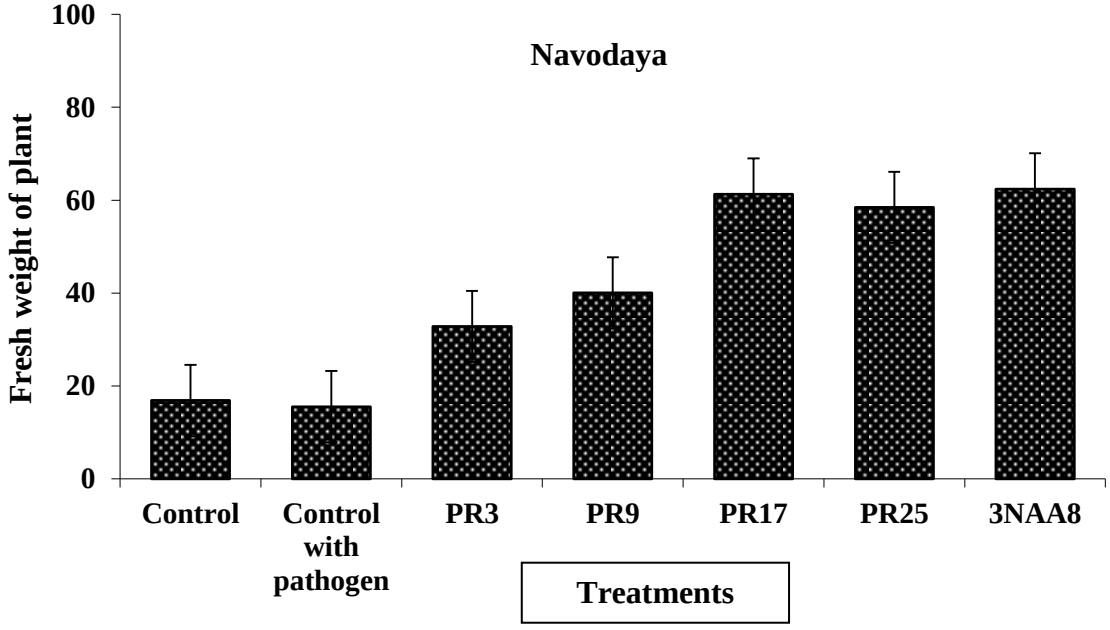



**Fig.4.7.2(b)Effect of PGPR on total fresh weight (g) of tomato plant on variety Navodaya.**

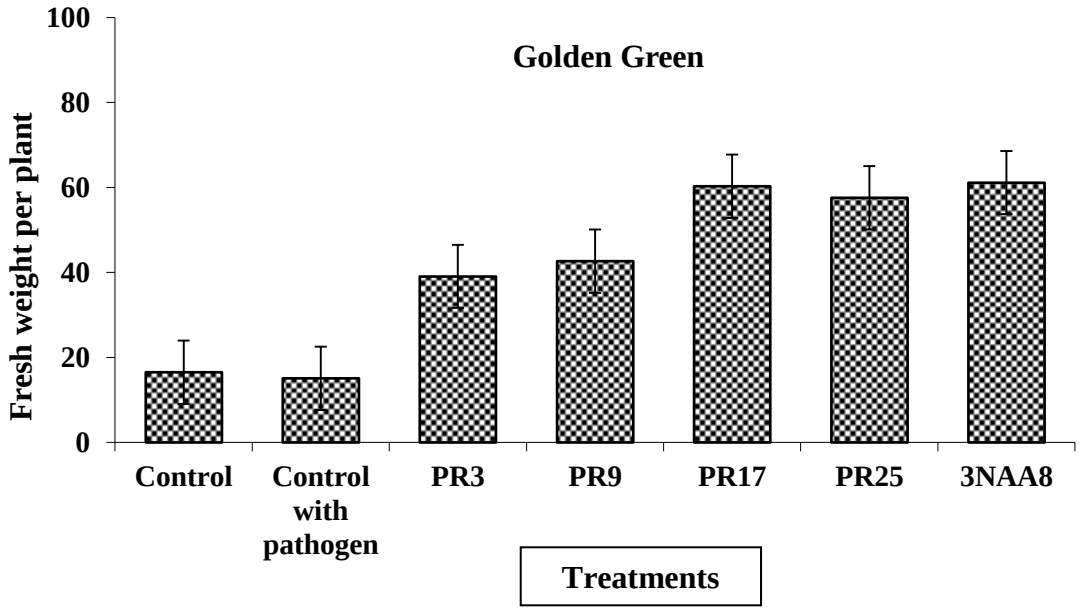



**Fig. 4.7.2(c) Effect of PGPR on total fresh weight (g) of tomato plant on variety Golden Green.**

### 4.7.3 Fresh weight of shoot (g):

The data presented in Table 4.7.3below showed that there was significant difference in plant fresh weight of shoot among the three varieties of tomato. All the PGPR treatments were found significant from each other except in variety Navodaya where 3NAA8 was found non-significant from PR17.

In Pusa 120, the highest fresh weight of shoot was found in 3NAA8 (53.65)g followed by PR17(50.57)g whereas the lowest fresh weight of shoot was recorded in PR3(24.77)g among all the treatments given.

In Navodaya also the highest fresh weight of shoot was shown by PR17 (54.97)g followed by 3NAA8 (54.70)g. The lowest fresh weight of shoot was observed in PR3 (25.87)g among all the potential PGPRs.

In Golden Green, the highest fresh weight of shoot was showed in 3NAA8 (54.62)g followed by PR17 (54.49)g. The fresh weight of shoot was recorded in PR3 (28.32)g among all the treatments.

**Table.4.7.3 Effect of PGPR on fresh weight (g) of shoot on varieties Pusa 120, Navodaya and Golden Green of tomato.**

| Treatments | Fresh weight of shoot (g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 11.85 | 11.83 | 11.88 |
| Control (inoculated) | 10.90 | 10.79 | 10.28 |
| PR3 | 24.77 | 25.87 | 28.32 |
| PR9 | 32.08 | 33.96 | 34.94 |
| PR17 | 50.57 | 54.97 | 54.49 |
| PR25 | 45.69 | 52.09 | 49.39 |
| 3NAA8 | 53.65 | 54.70 | 54.62 |
| C.D. (5%) | 0.62 | 0.75 | 0.75 |
| S. Em. | 0.26 | 0.22 | 0.26 |
| S. Ed. | 0.37 | 0.30 | 0.37 |
| C.V. | 2.11 | 1.63 | 1.99 |

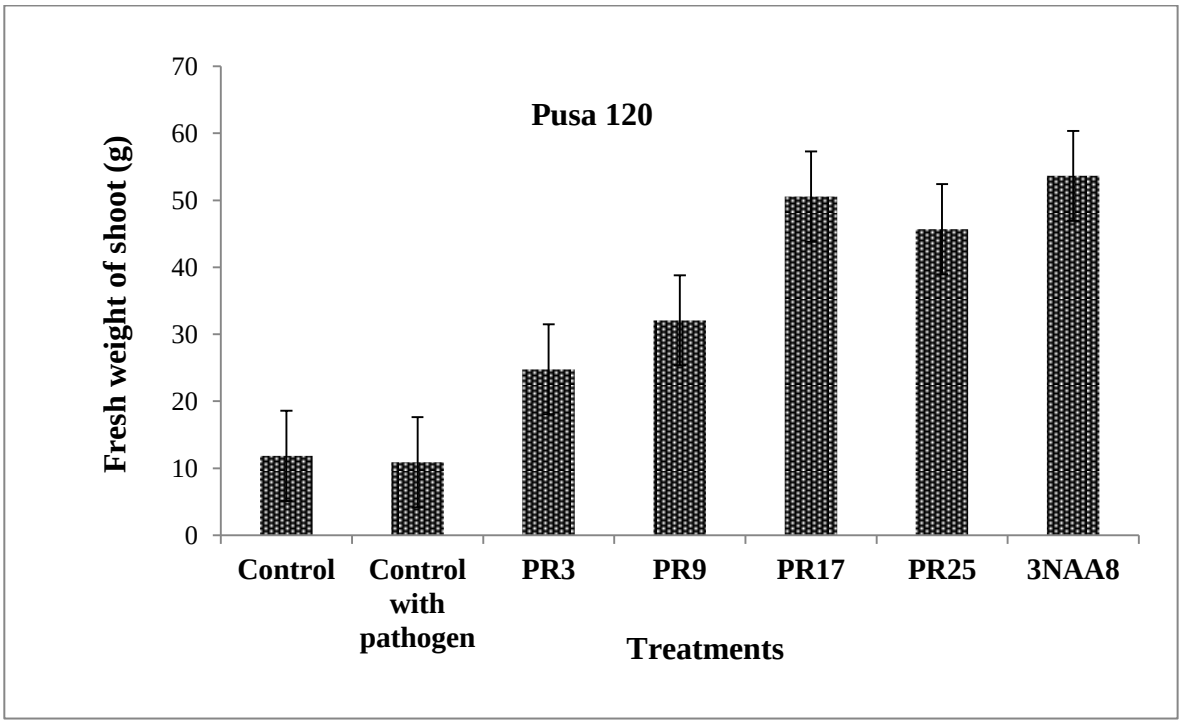



**Fig.4.7.3(a) Effect of PGPR on fresh weight (g) of shoot on variety Pusa 120 of tomato.**

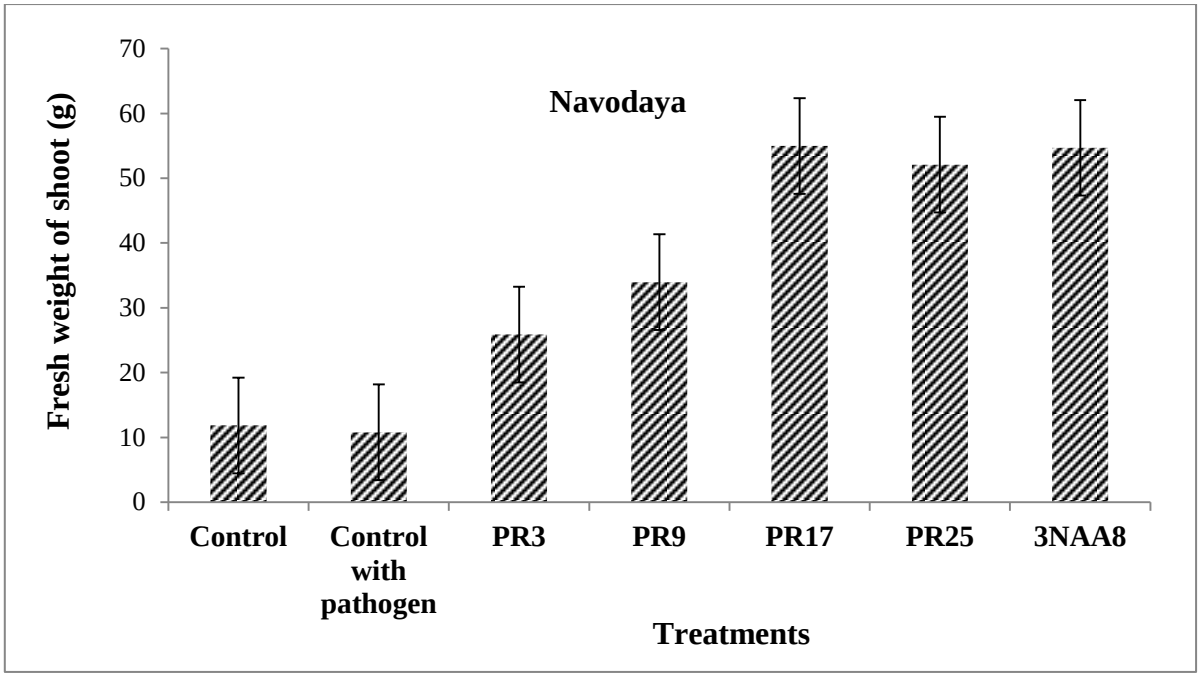



**Fig.4.7.3(b) Effect of PGPR on fresh weight (g) of shoot on variety Navodaya of tomato.**

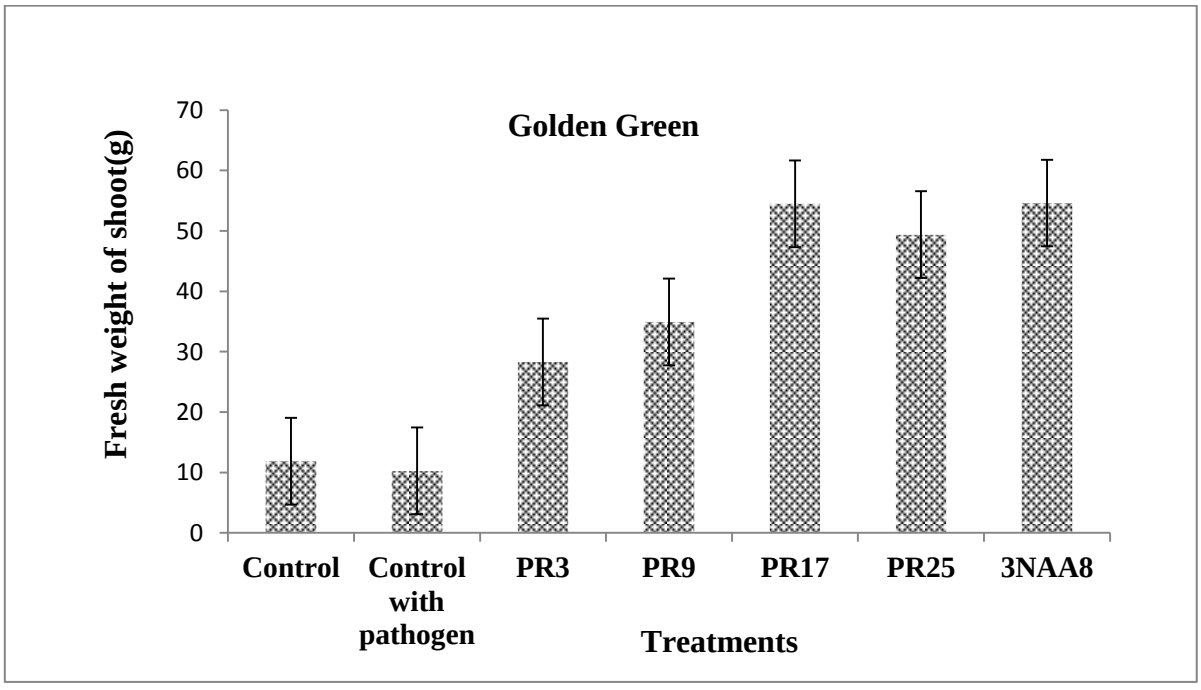



**Fig.4.7.3(c) Effect of PGPR on fresh weight (g) of shoot on variety Golden Green of tomato.**

### 4.7.4 Fresh weight of root per plant (g):

Data presented (in Table 4.7.4) shows the effect of five potential selected PGPR on fresh weight of root in three varieties of tomato.

In Pusa 120, the highest fresh weight of root was found in PR17 (7.04)g followed by 3NAA8 (7.03)g whereas the lowest dry of root was recorded in PR3(3.41)g among all the treatments given. 3NAA8 was found significant from all the PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant to PR25, PR9, PR3 and control.

In Navodaya also the highest fresh weight of root was shown by PR17 (6.91)g followed by 3NAA8 (6.85)g. The lowest fresh weight of root was observed in PR3 (3.63)g among all the potential PGPRs. 3NAA8 was found significant from all PGPR treatments whereas PR17 was non-significant from 3NAA8 but significant from other PGPR treatments.

In Golden Green, the highest fresh weight of root was showed in 3NAA8 (6.94)g followed by PR17 (6.88)g. The lowest fresh weight of root was recorded in PR3 (4.35)g among all the treatments. PR17 was found significant from all the PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from other PGPR treatments.

**Table 4.7.4 Effect of PGPR on fresh weight (g) of root on varieties Pusa 120, Navodaya and Golden Green of tomato.**

| Treatments | Fresh weight of root (g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 2.46 | 2.61 | 2.60 |
| Control (inoculated) | 1.96 | 2.23 | 2.24 |
| PR3 | 3.41 | 3.63 | 4.35 |
| PR9 | 5.85 | 5.18 | 5.95 |
| PR17 | 7.04 | 6.91 | 6.88 |
| PR25 | 6.22 | 5.86 | 5.93 |
| 3NAA8 | 7.03 | 6.85 | 6.94 |
| C.D. (5%) | 0.16 | 0.15 | 0.15 |
| S. Em. | 0.06 | 0.05 | 0.05 |
| S. Ed. | 0.08 | 0.07 | 0.07 |
| C.V. | 3.06 | 2.86 | 2.72 |

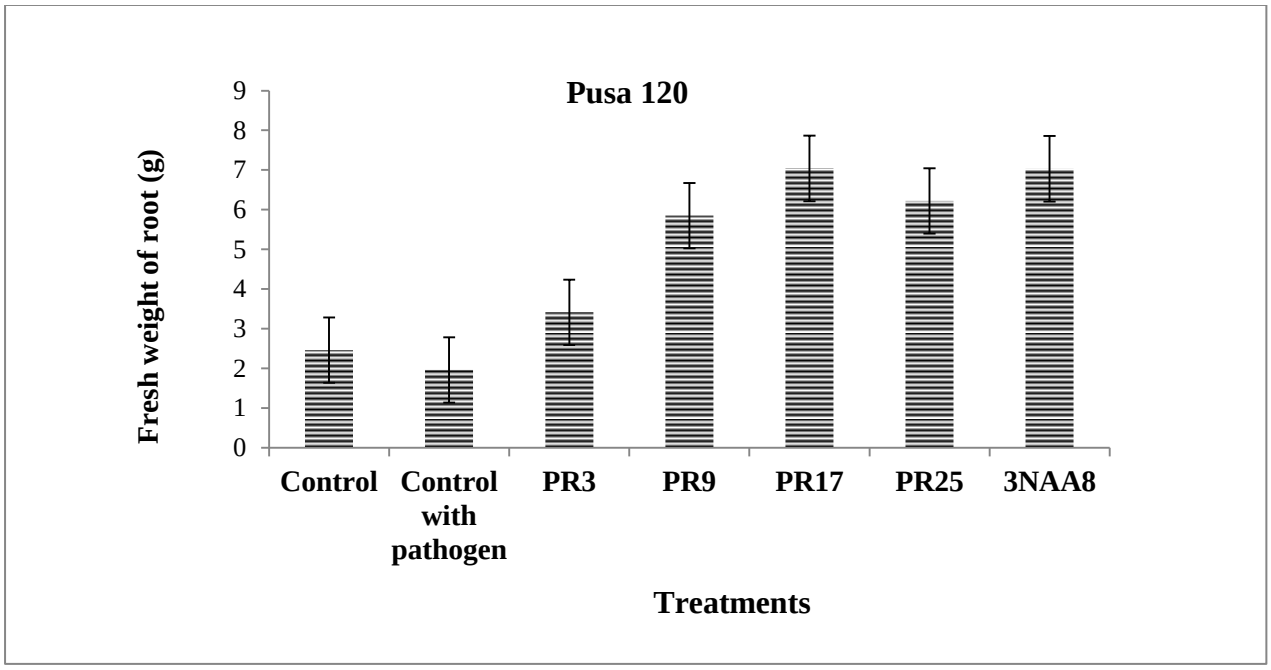



**Fig.4.7.4(a) Effect of PGPR on fresh weight (g) of root on variety Pusa 120 of tomato.**

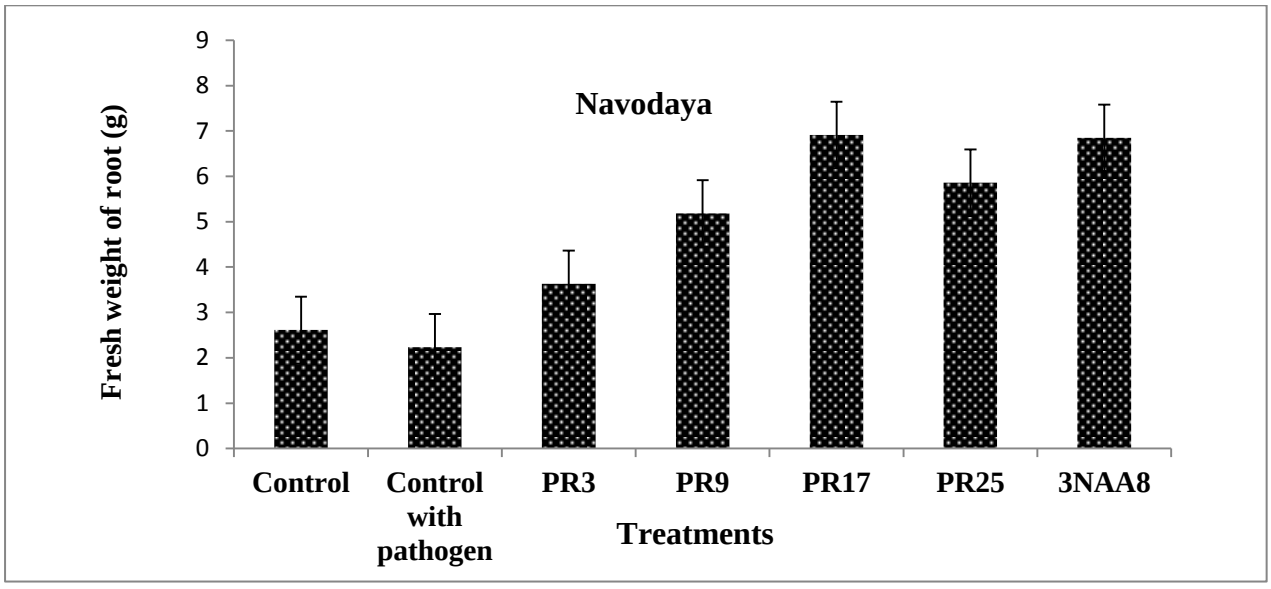



**Fig.4.7.4(b) Effect of PGPR on fresh weight (g) of root on variety Navodaya of tomato.**

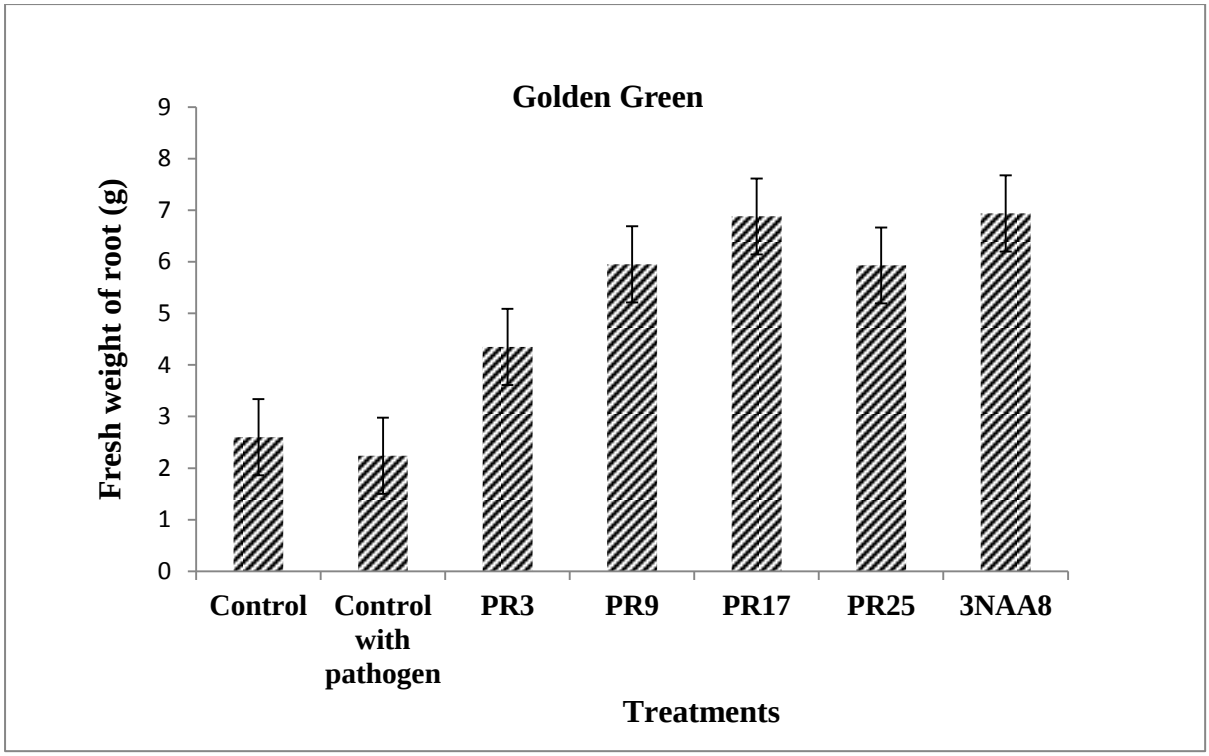
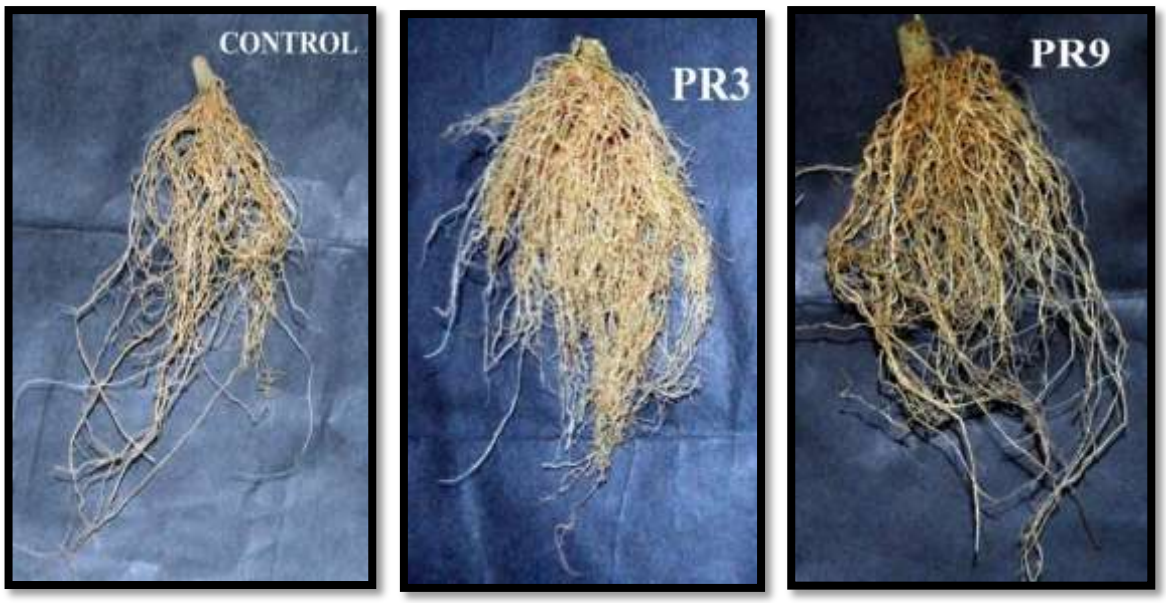


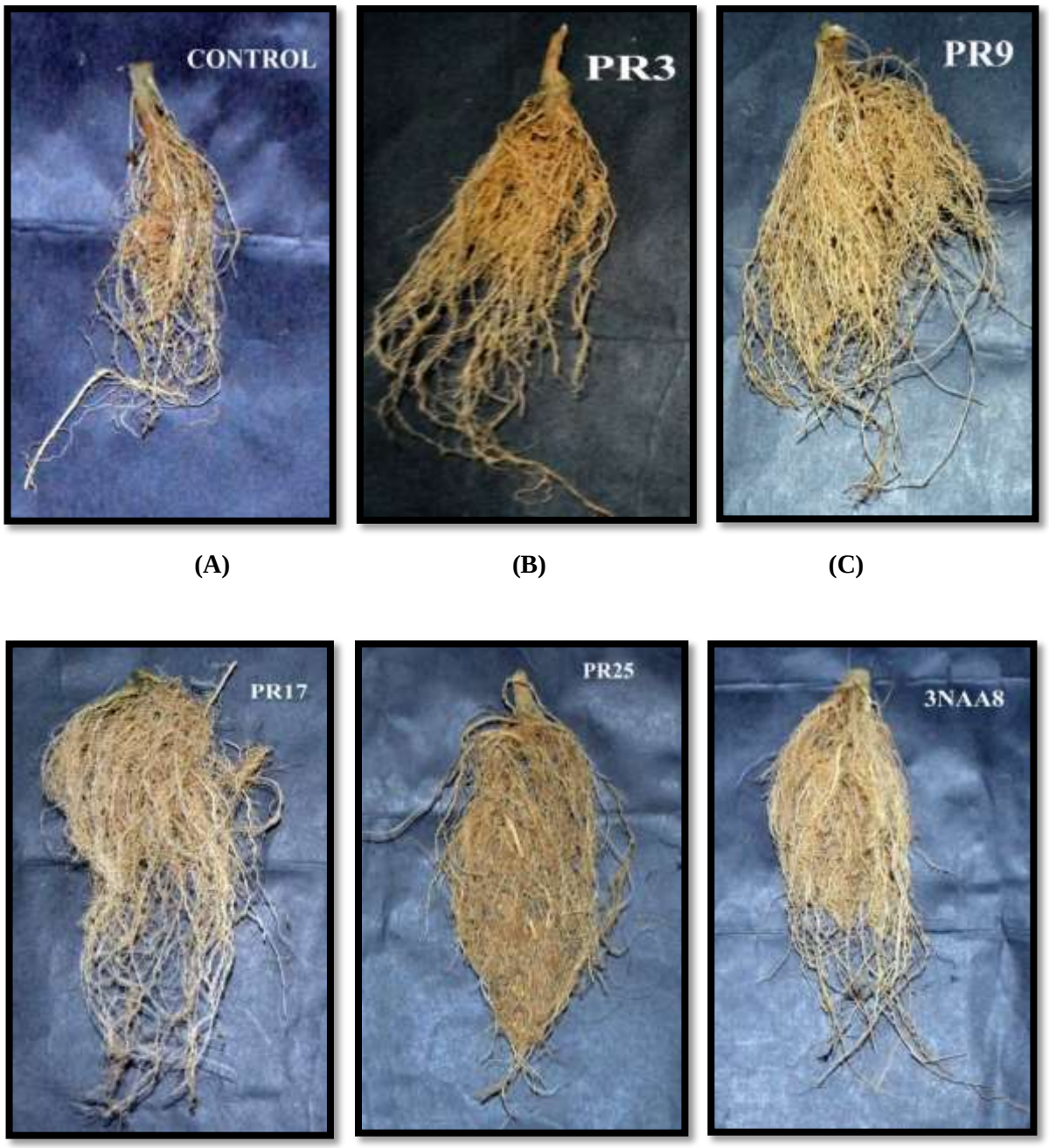

**Fig.4.7.4(c) Effect of PGPR on fresh weight (g) of root on variety Golden Green of tomato.**




**(A)** **(B)** **(C)**


**(D)** **(E)** **(F)**

**Plate.4.7.4 (a) Effect of PGPR on the fresh weight (g) of tomato root on variety Pusa 120.**




**(A)** **(B)** **(C)**

**(D)** **(E)** **(F)**

**Plate.4.7.4 (b) Effect of PGPR on the fresh weight (g) of tomato root on variety Navodaya.**


**(A)** **(B)** **(C)**

**(D)** **(E)** **(F)**

**Plate.4.7.4 (c) Effect of PGPR on the fresh weight (g) of tomato root on variety Golden Green.**

### 4.7.5 Dry weight of shoot (g):

Statistically significant results were observed regarding dry weight of shoot which was shown in Table 4.7.5. All the treatments were found significant from each other except PR17 in variety Navodaya where it is found non-significant from 3NAA8 only.

In Pusa 120, the highest dry of shoot was found in 3NAA8 (18.12)g followed by PR17(17.59)g whereas the lowest dry of shoot was recorded in PR3(15.36)g among all the treatments given.

In Navodaya also the highest dry of shoot was shown by 3NAA8 (18.12)g followed by PR17 (17.87)g. The lowest dry of shoot was observed in PR3 (15.77)g among all the potential PGPRs.

In Golden Green, the highest dry of shoot was showed in 3NAA8 (17.96)g followed by PR17 (17.74)g. The dry of shoot was recorded in PR3(15.79)g among all the treatments.

**Table 4.7.5 Effect of PGPR on dry weight (g) of shoot on varieties Pusa 120, Navodaya and Golden Green of tomato.**

| Treatments | Dry weight of Shoot (g) | | |
|---|---|---|---|
| | **Pusa 120** | **Navodaya** | **Golden Green** |
| Control (uninoculated) | 12.47 | 12.59 | 12.59 |
| Control (inoculated) | 11.44 | 11.75 | 11.74 |
| PR3 | 15.36 | 15.77 | 15.79 |
| PR9 | 16.46 | 16.80 | 16.78 |
| PR17 | 17.59 | 17.87 | 17.74 |
| PR25 | 16.89 | 17.53 | 17.55 |
| 3NAA8 | 18.12 | 18.12 | 17.96 |
| C.D. (5%) | 0.29 | 0.19 | 0.16 |
| S. Em. | 0.10 | 0.07 | 0.06 |
| S. Ed. | 0.14 | 0.09 | 0.08 |
| C.V. | 1.72 | 1.10 | 0.94 |

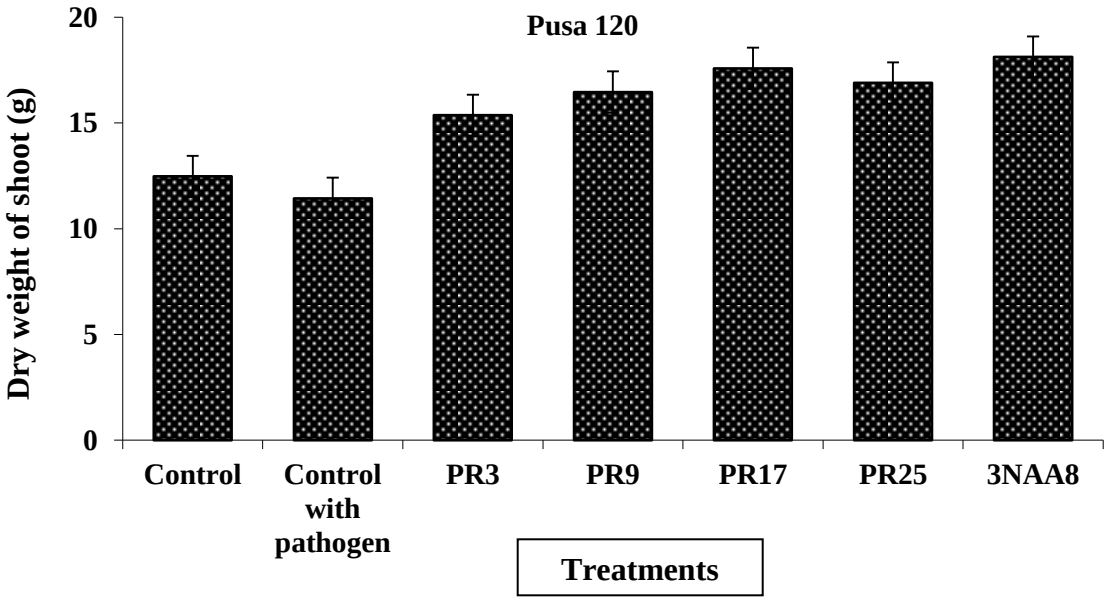



**Fig. 4.7.5(a) Effect of PGPR on dry weight (g) of shoots on PUSA 120 variety of tomato.**

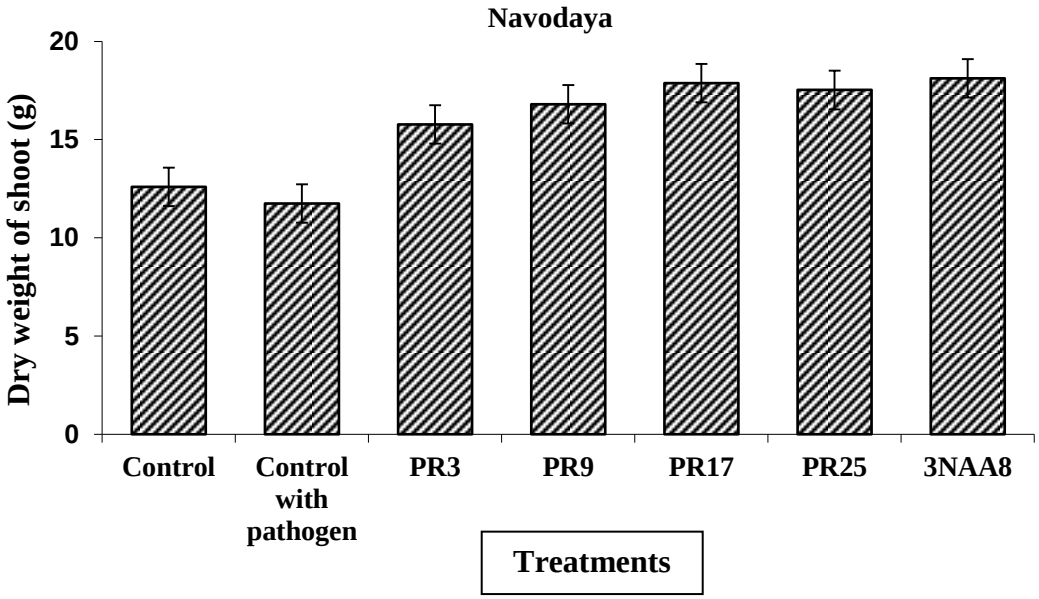



**Fig. 4.7.5(b) Effect of PGPR on dry weight (g) of shoot on Navodaya variety of tomato.**

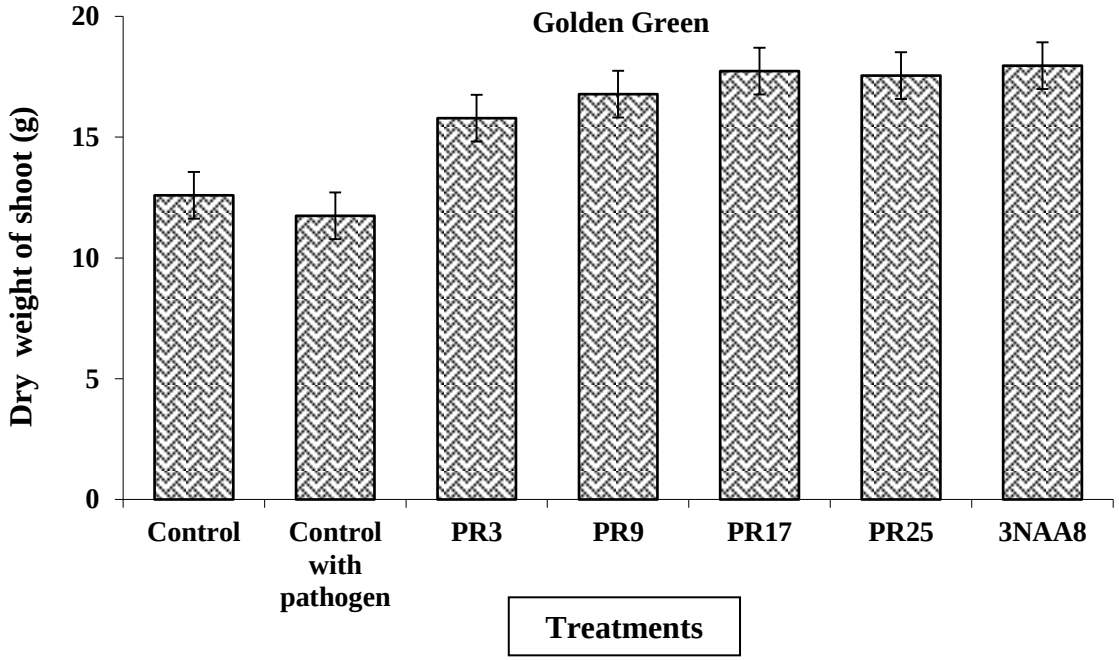



**Fig. 4.7.5(c) Effect of PGPR on dry weight (g) of shoot on Golden Green variety of tomato.**

## 4.7.6 Dry weight of root (g):

In Table 4.7.6 the significant increase in dry weight of root was observed. All the potential PGPR treatments were found significant from each other except 3NAA8 of variety Golden Green where it is found non-significant from PR17 but significant to other PGPR treatments.

In Pusa 120, the highest dry of root was found in 3NAA8 (5.56)g followed by PR17(5.01)g whereas the lowest dry of root was recorded in PR3(3.11)g among all the treatments given.

In Navodaya also the highest dry of shoot was shown by 3NAA8 (5.45)g followed by PR17 (5.05)g. The lowest dry of root was observed in PR3 (2.94)g among all the potential PGPRs.

In Golden Green, the highest dry of root was showed in 3NAA8 (5.29)g followed by PR17 (5.21)g. The minimum dry of root was recorded in PR3 (2.88)g among all the treatments.

**Table 4.7.6 Effect of PGPR on dry weight (g) of root on Pusa 120, Navodaya and Golden Green varieties of tomato.**

| Treatments | Dry weight of root (g) | | |
|---|---|---|---|
| | **Pusa 120** | **Navodaya** | **Golden Green** |
| Control (uninoculated) | 0.69 | 0.81 | 0.77 |
| Control (inoculated) | 0.33 | 0.37 | 0.36 |
| PR3 | 3.11 | 2.94 | 2.88 |
| PR9 | 3.90 | 3.80 | 3.68 |
| **PR17** | **5.01** | **5.05** | **5.21** |
| PR25 | 4.53 | 4.32 | 4.11 |
| **3NAA8** | **5.56** | **5.45** | **5.29** |
| C.D. (5%) | 0.31 | 0.18 | 0.16 |
| S. Em. | 0.11 | 0.06 | 0.06 |
| S. Ed. | 0.15 | 0.09 | 0.08 |
| C.V. | 8.69 | 5.16 | 4.77 |

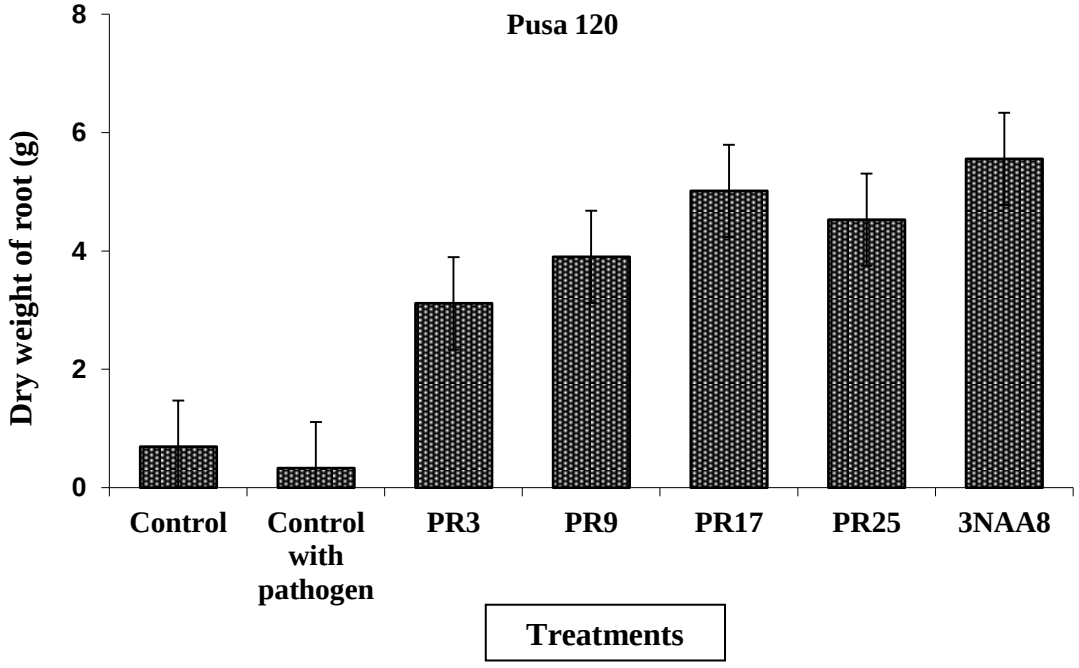



**Fig. 4.7.6(a) Effect of PGPR on dry weight (g) of root on Pusa 120 variety of tomato**

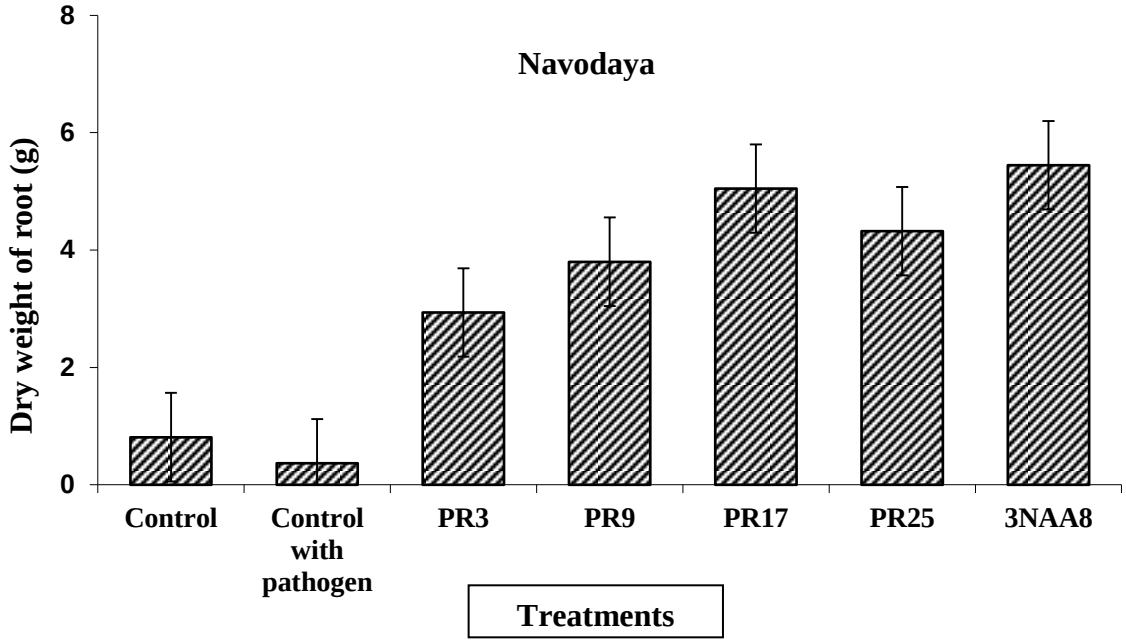



**Fig. 4.7.6(b) Effect of PGPR on dry weight (g) of root on Navodaya variety of tomato.**

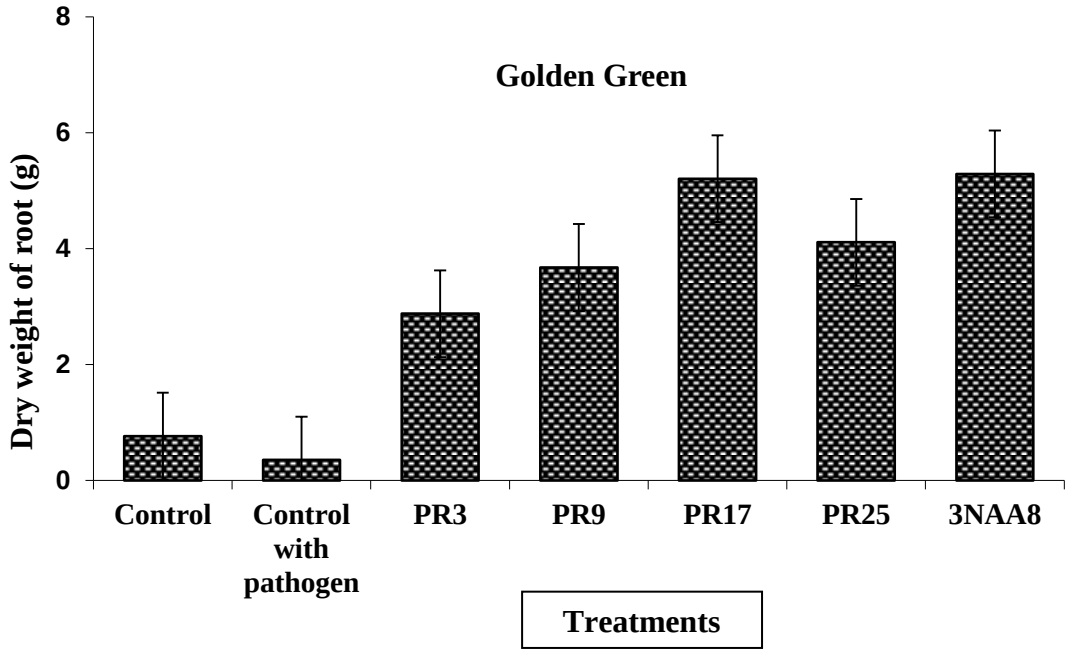



**Fig. 4.7.6(c) Effect of PGPR on dry weight (g) of root on Golden Green variety of tomato.**

## 4.7.7 Fresh weight of tomato per plant

Statistically significant results were observed regarding fresh fruit weight which was shown in Table 4.7.7. In Pusa 120 and Golden Green PR17 was found significant from all other PGPR treatments and 3NAA8 was found non-significant from PR17 but significant from PR3, PR9 and PR 25 also they are found non-significant from each other whereas in Navodaya 3NAA8 was found significant and PR17 was found to be non-significant from 3NAA8 but significant from rest of the PGPR treatments.

In Pusa 120, the maximum average fresh weight of tomato per plant was observed in 3NAA8 (66.96)g followed by PR17(65.29)g whereas the average fresh weight of tomato per plant was recorded in PR25 (57.69)g among all the treatments given.

In Navodaya also maximum average fresh weight of tomato per plant was shown by PR17 (63.61)g followed by 3NAA8 (63.06)g. The lowest average fresh weight of tomato per plant was observed in PR3 (55.19)g among all the potential PGPRs.

In Golden Green, the highest average fresh weight of tomato per plant was showed in 3NAA8 (65.30)g followed by PR17 (63.29)g. The lowest average fresh weight of tomato per plant was recorded in PR3 (53.22)g among all the treatments.

**Table 4.7.7 Effect of PGPR on fresh fruit weight (g) of Tomato of Pusa 120, Navodaya, and Golden Green.**

| Treatments | Fresh fruit weight (g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 36.78 | 36.51 | 36.46 |
| Control (inoculated) | 27.09 | 25.54 | 26.41 |
| PR3 | 58.50 | 57.34 | 53.22 |
| PR9 | 58.18 | 57.83 | 54.54 |
| PR17 | 65.29 | 63.61 | 63.29 |
| PR25 | 57.69 | 55.19 | 56.68 |
| 3NAA8 | 66.96 | 63.06 | 65.30 |
| C.D. (5%) | 2.74 | 1.77 | 1.58 |
| S. Em. | 0.96 | 0.62 | 0.55 |
| S. Ed. | 1.35 | 0.87 | 0.78 |
| C.V. | 4.78 | 3.18 | 2.87 |

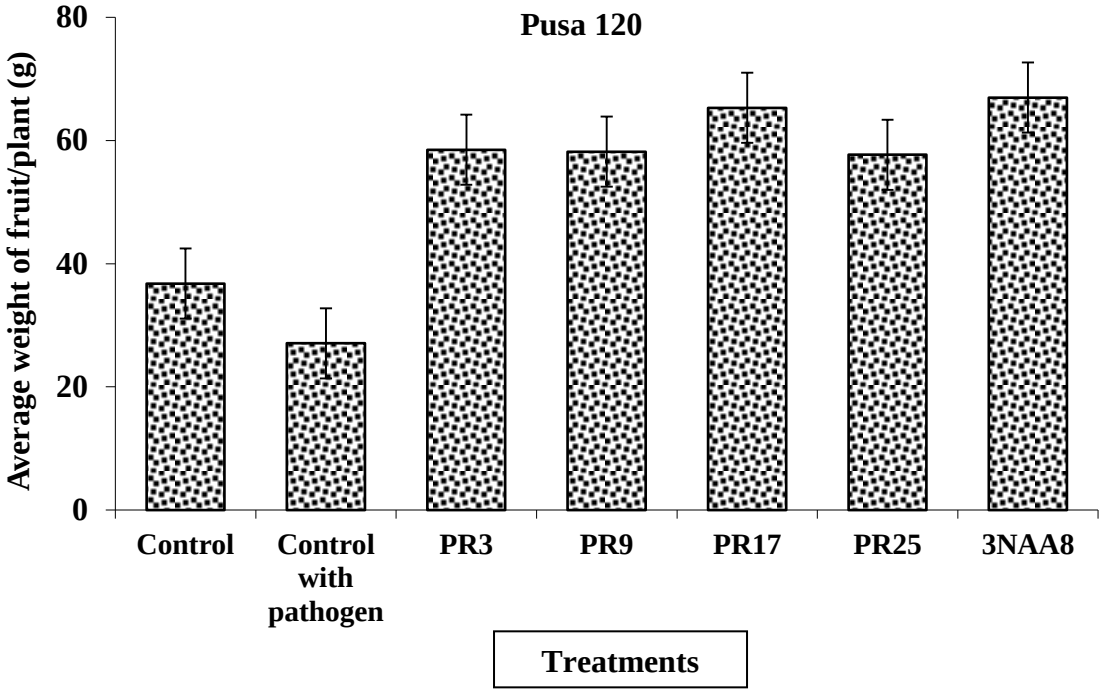



**Fig.4.7.7(a) Effect of PGPR on average weight of fruit/plant on Pusa 120 variety of tomato.**

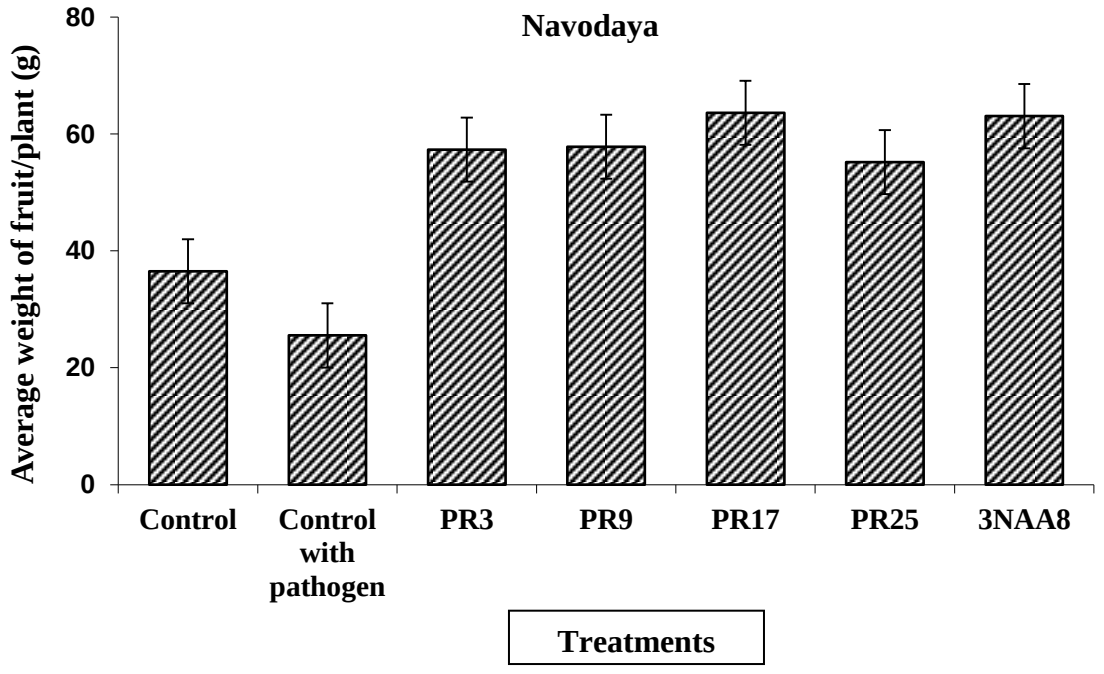



**Fig.4.7.7(b) Effect of PGPR on average weight of fruit/plant on Navodaya variety of tomato.**

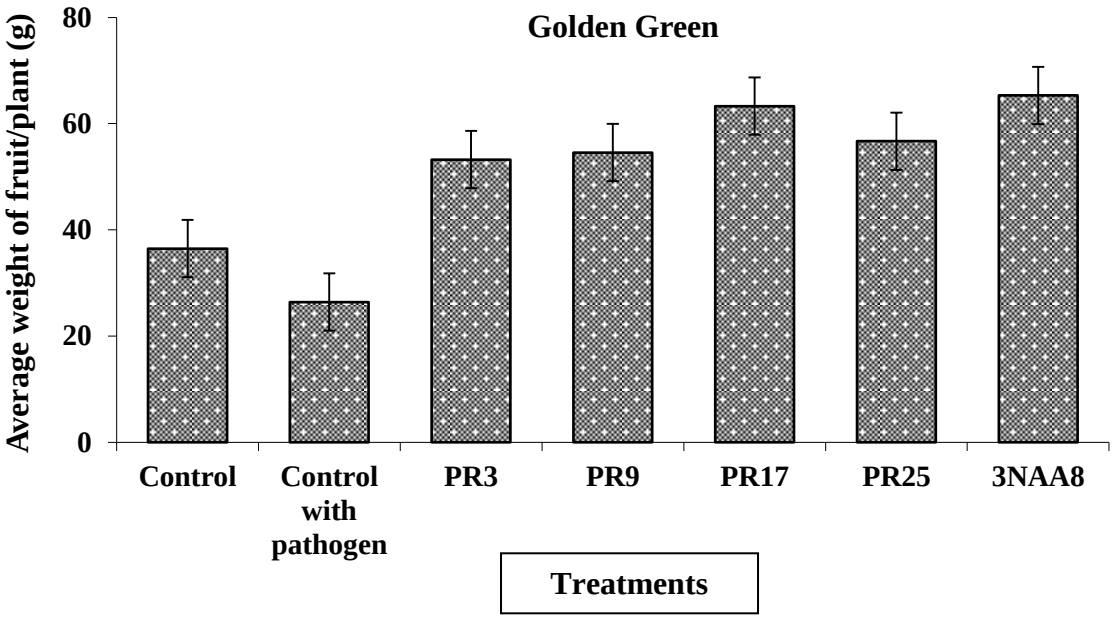



**Fig.4.7.7(c) Effect of PGPR on average weight of fruit/plant on Golden Green variety of tomato.**

## 4.7.8 Yield per plant

There was significant variation in fruit yield per plant in respect of different PGPRs treatment which was shown in table 4.8. In Pusa 120 and Golden Green 3NAA8 was found significant from all other PGPR treatments but in Navodaya PR17 was found to be significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3, also they are found significant from each other.

In Pusa 120, the plants treated with PR17 showed the maximum fruits yield (664.91g) per plant followed by the treatment 3NAA8 of (660.34g), the minimum fruits yield per plant (571.86g) was obtained from PR3.

In Navodaya, the maximum fruits yield per plant was showed by 3NAA8 (659.62)g followed by PR17 (655.48)g. the minimum fruits yield per plant was observed in PR3 (544.89)g among all the potential PGPRs.

In Golden Green, the maximum fruits yield per plant showed in PR17 (655.21g followed by 3NAA8 (653.39)g. The minimum fruits yield per plant was recorded in PR25 (551.26)g among all the treatments.

**Table 4.7.8 Effect of PGPRs on Fruit Yield /plant (g) of Tomato in varieties Pusa 120, Navodaya, and Golden Green.**

| Treatments | Fruit Yield /plant (g) | | |
|---|---|---|---|
| | **Pusa 120** | **Navodaya** | **Golden Green** |
| Control (uninoculated) | 273.92 | 241.48 | 256.02 |
| Control (inoculated) | 170.05 | 136.31 | 158.68 |
| PR3 | 571.86 | 544.89 | 561.06 |
| PR9 | 587.19 | 549.58 | 562.14 |
| PR17 | 664.91 | 655.48 | 655.21 |
| PR25 | 569.61 | 556.84 | 551.26 |
| 3NAA8 | 660.34 | 659.62 | 653.39 |
| C.D. (5%) | 9.92 | 5.50 | 5.52 |
| S. Em. | 3.46 | 1.92 | 1.93 |
| S. Ed. | 4.90 | 2.72 | 2.73 |
| C.V. | 1.83 | 1.06 | 1.05 |

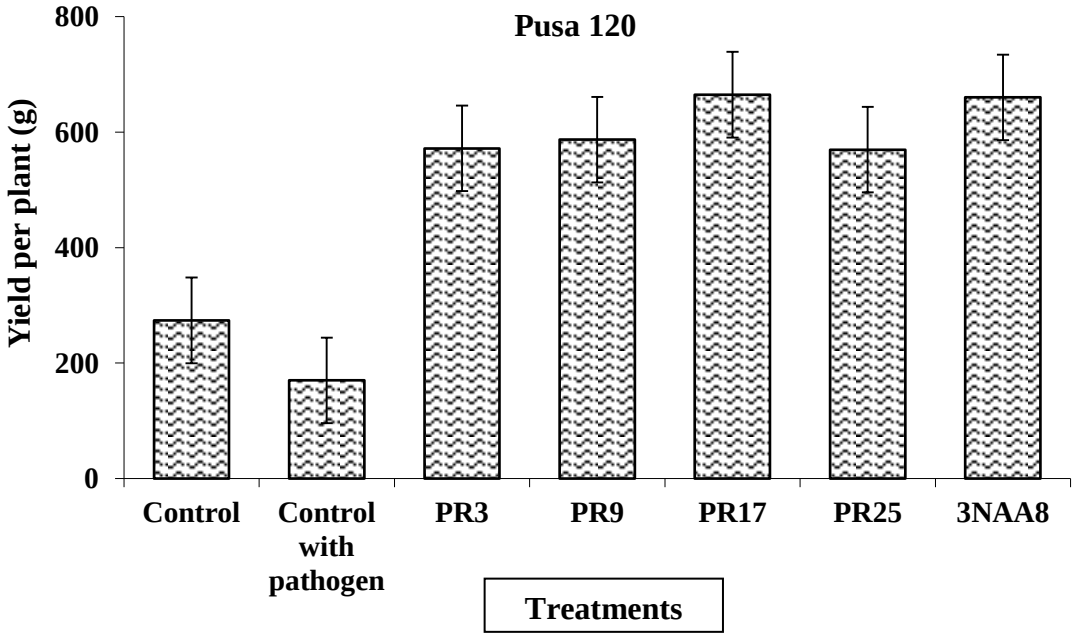



**Fig. 4.7.8(a) Effect of PGPR on Yield per plant on Pusa 120 variety of tomato.**

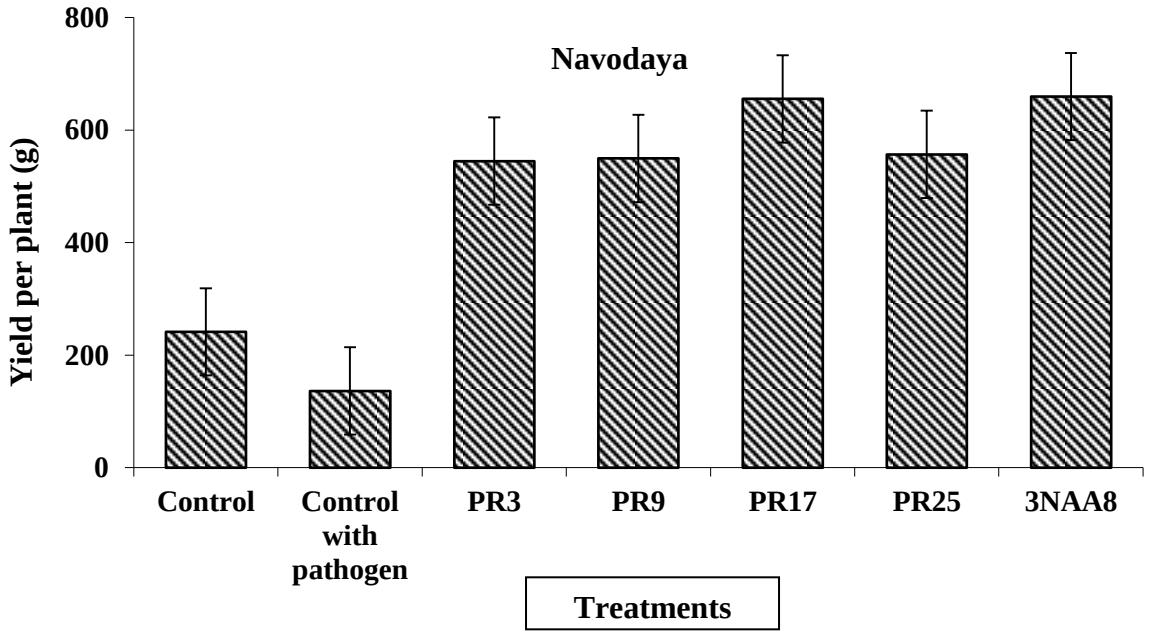



**Fig. 4.7.8(b) Effect of PGPR on Yield per plant on Navodaya variety of tomato.**

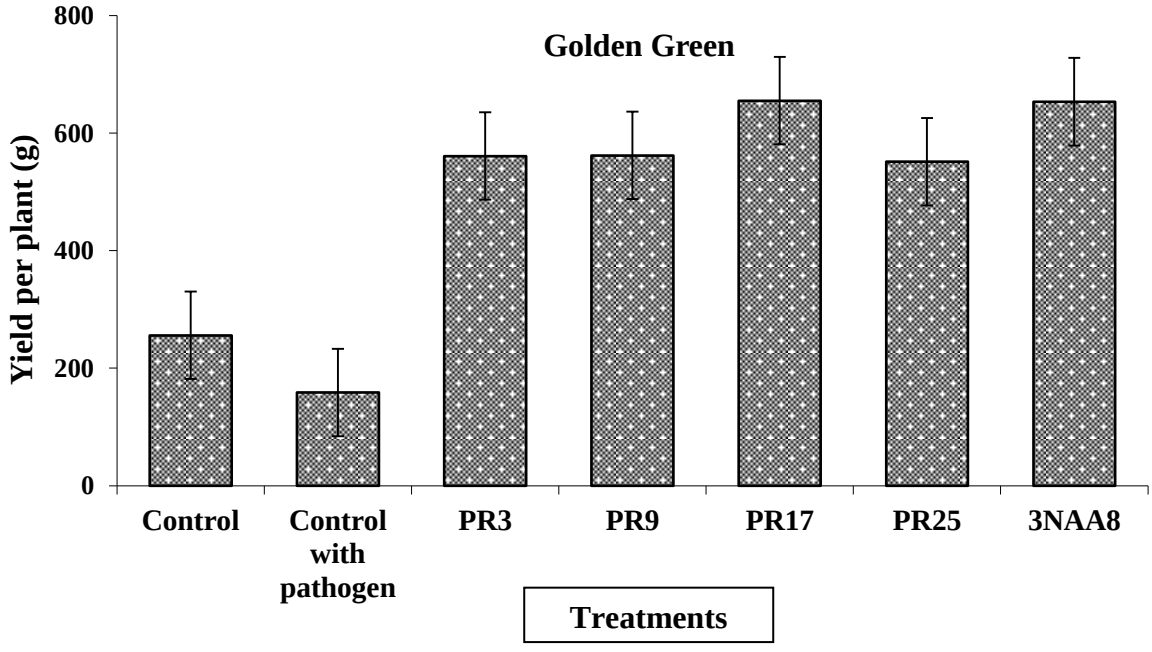



**Fig. 4.7.8(c) Effect of PGPR on Yield per plant on Golden Green variety of tomato.**

## 4.8. BIOCHEMICAL PARAMETERS

### 4.8.1 Total Soluble Solid (°Brix):

Total Soluble Solid of tomato juice was determined from fruits of each treatment at the time of Harvest. The data recorded was put under analysis reveals that TSS of tomato of each variety was significantly influenced by different potential PGPRs.

In Pusa 120, the highest T.S.S. of tomato was observed in 3NAA8 (6.91) followed by PR17 (6.63) whereas the lowest T.S.S. of tomato was recorded in PR3 (5.44) among all the treatments given. All the PGPR treatments were found significant from each other.

In Navodaya the highest T.S.S. of tomato was shown by PR17 (6.82) followed by 3NAA8 (6.78). The lowest T.S.S. of tomato was observed in PR9 (5.53) among all the potential PGPRs. 3NAA8 was found to be significant from all other PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant from PR3, PR9 and PR25. Also they are found significant from each other.

In Golden Green, the highest T.S.S. of tomato was showed in 3NAA8 (6.77) followed by PR17 (6.50). The lowest T.S.S. of tomato was recorded in PR3 (5.56) among all the treatments. PR17 was found to be significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR3, PR9 and PR25. Also they are found significant from each other.

**Table 4.8.1 Effect of five potential PGPR on Total soluble solids (°Brix) in varieties of tomato Pusa 120, Navodaya and Golden Green.**

| Treatments | Total soluble solids (°Brix) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 4.20 | 4.00 | 4.21 |
| Control (inoculated) | 3.71 | 3.74 | 3.61 |
| PR3 | 5.44 | 5.61 | 5.56 |
| PR9 | 5.64 | 5.53 | 5.68 |
| PR17 | 6.63 | 6.82 | 6.50 |
| PR25 | 6.01 | 5.69 | 5.85 |
| 3NAA8 | 6.91 | 6.78 | 6.77 |
| C.D. (5%) | 0.25 | 0.32 | 0.31 |
| S. Em. | 0.09 | 0.11 | 0.11 |
| S. Ed. | 0.12 | 0.16 | 0.15 |
| C.V. | 4.15 | 5.35 | 5.23 |

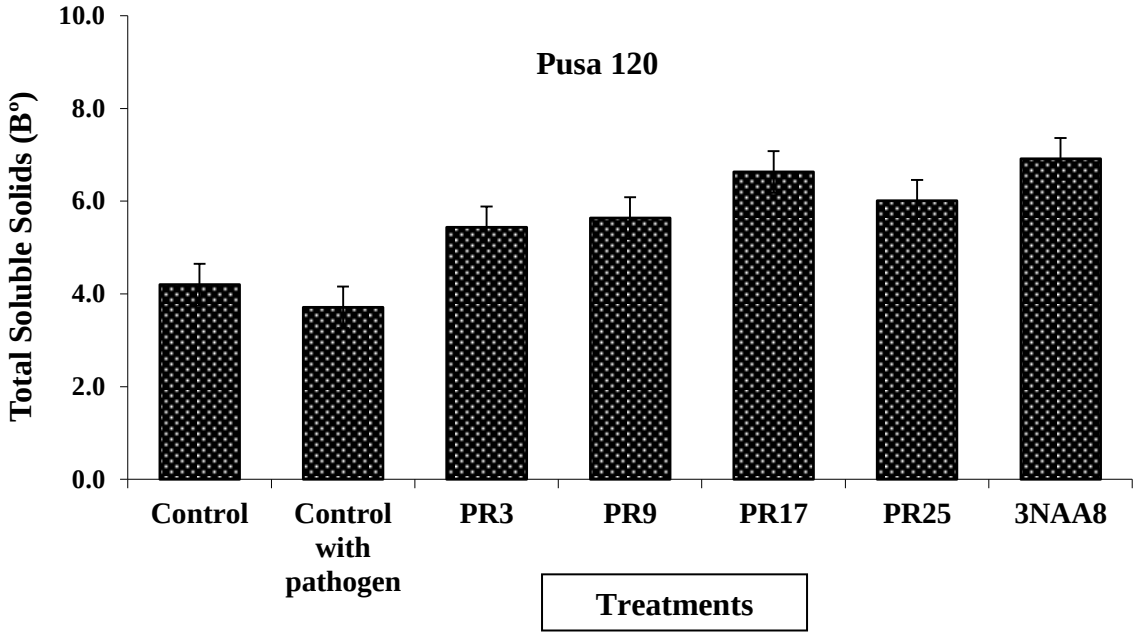



**Fig.4.8.1(a) Effect of PGPR on T.S.S. (ºBrix) value on Pusa 120 variety of tomato.**

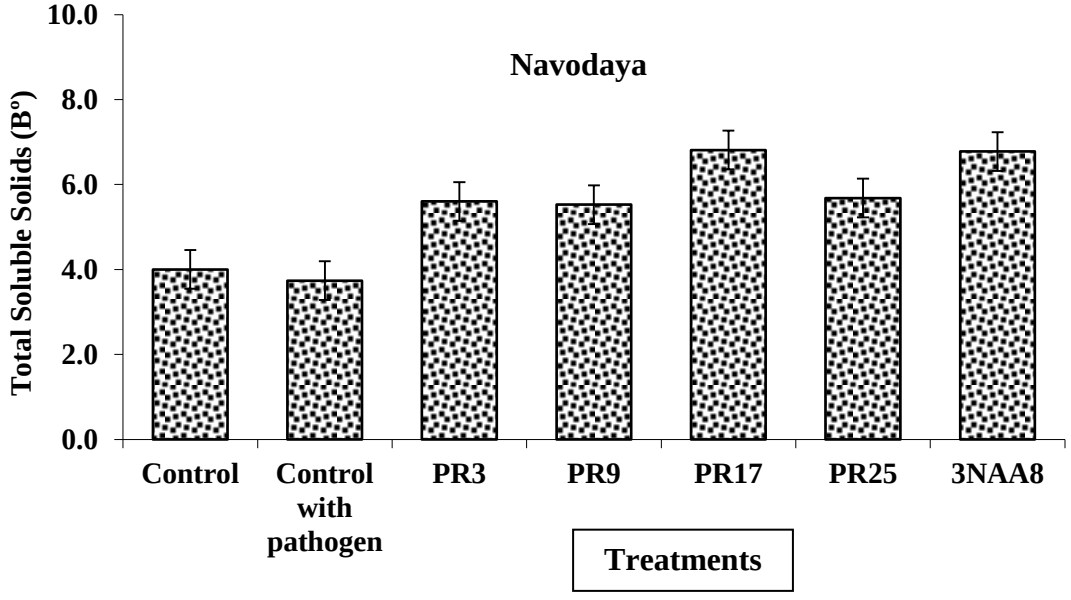



**Fig.4.8.1(b) Effect of PGPR on T.S.S. (Bº) value on Navodaya variety of tomato.**

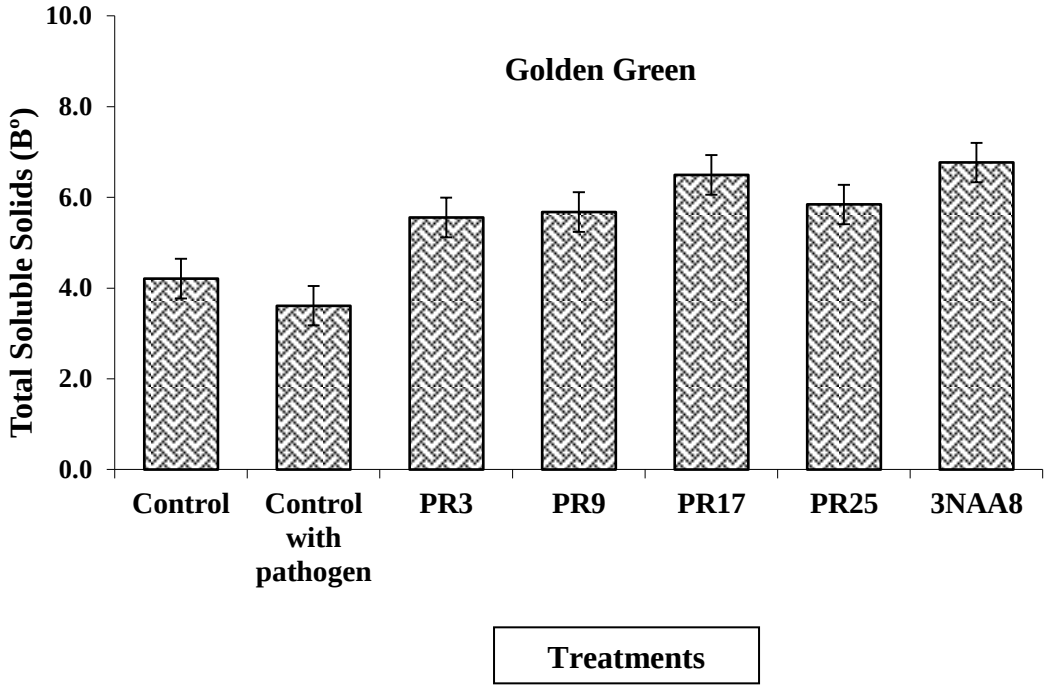



**Fig.4.8.1(c) Effect of PGPR on T.S.S. (Bº) value on Golden Green variety of tomato.**

### 4.8.2 LYCOPENE

The data shown below in the Table 4.8.2 clearly indicates that there is significant difference in Lycopene (mg/100g) due to the treatment. In all the three varieties 3NAA8 was found to be significant from all other PGPR treatments given whereas PR17 was found non-significant from 3NAA8 and significant to PR25, PR9 and PR3 but they are non-significant to each other.

In Pusa 120, the highest Lycopene content of tomato was observed in PR17 (3.19) followed by 3NAA8 (3.19) whereas the lowest Lycopene content of tomato was recorded in PR3 (2.76) among all the treatments given.

In Navodaya, the highest Lycopene content of tomato was shown by PR17 (3.41) followed by 3NAA8 (3.38). The lowest Lycopene content of tomato was observed in PR9 (2.87) among all the potential PGPRs.

In Golden Green, the highest Lycopene content of tomato was showed in PR17 (3.19) followed by 3NAA8 (3.17). The lowest Lycopene content of tomato was recorded in PR25 (2.86) among all the treatments.

**Table 4.8.2 Effect of five potential PGPR on Lycopene (mg/100g) in varieties of tomato Pusa 120, Navodaya and Golden Green.**

| Treatments | Lycopene (mg/100g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 2.64 | 2.50 | 2.56 |
| Control (inoculated) | 2.27 | 2.16 | 2.23 |
| PR3 | 2.76 | 2.87 | 2.87 |
| PR9 | 2.90 | 2.82 | 2.83 |
| PR17 | 3.19 | 3.41 | 3.19 |
| PR25 | 2.83 | 2.92 | 2.86 |
| 3NAA8 | 3.14 | 3.38 | 3.17 |
| C.D. (5%) | 0.20 | 0.17 | 0.15 |
| S. Em. | 0.07 | 0.06 | 0.05 |
| S. Ed. | 0.10 | 0.08 | 0.07 |
| C.V. | 6.70 | 5.43 | 4.86 |

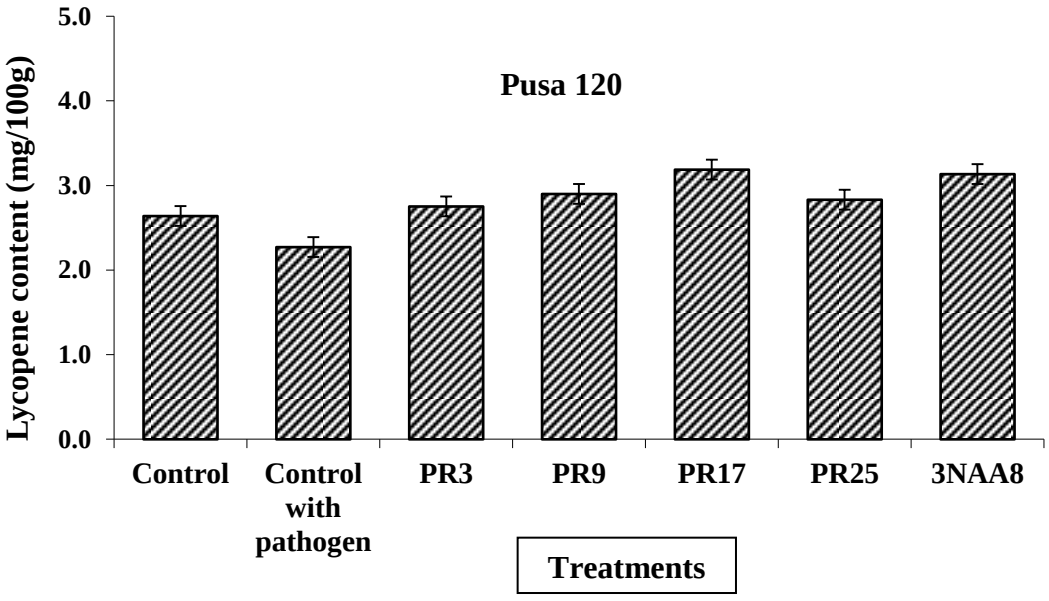



**Fig.4.8.2(a) Effect of PGPR on Lycopene content (mg/100g) value on Pusa 120 variety of tomato.**

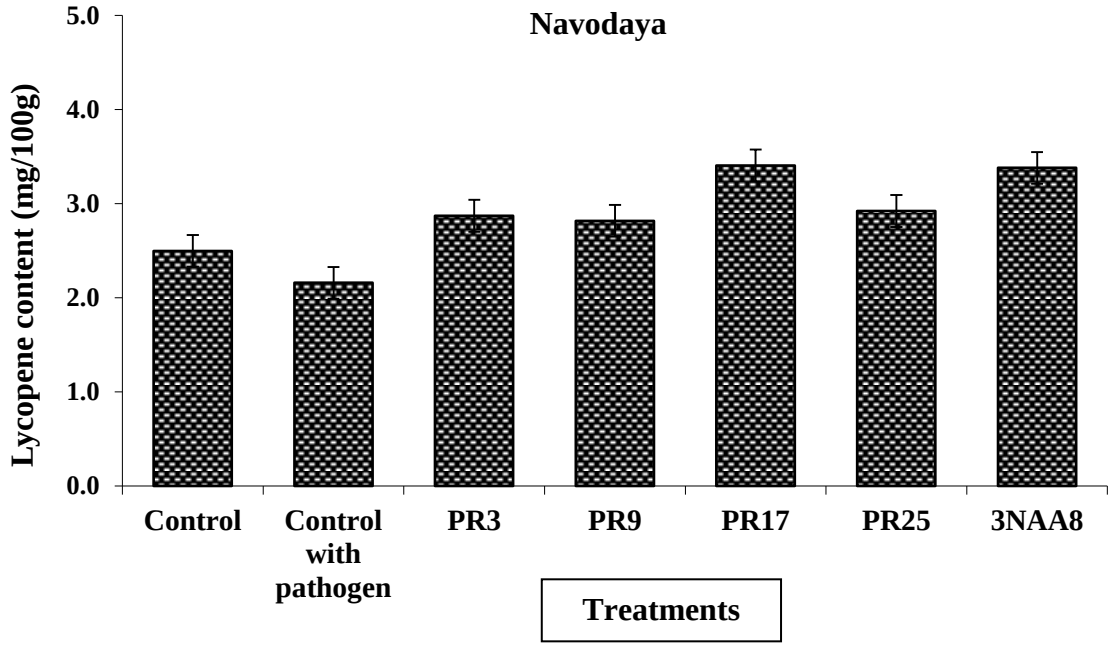



**Fig.4.8.2(b) Effect of PGPR on Lycopene content (mg/100g) value on Navodaya variety of tomato.**

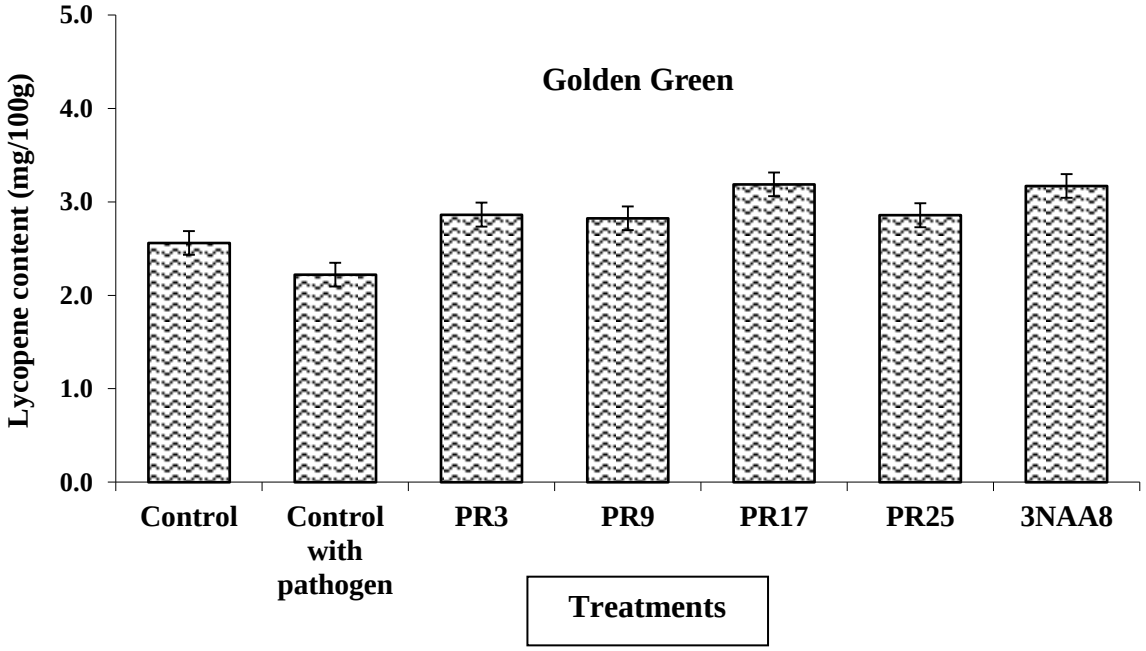



**Fig.4.8.2(c) Effect of PGPR on Lycopene content (mg/100g) value Golden Green variety of tomato.**

### 4.8.3. ASCORBIC ACID (mg/100g):

There was significant variation in ascorbic acid in respect of different PGPRs treatment which was shown in table 4.8.3. In all the three varieties PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found to be non-significant from PR17 and significant to PR25, PR9 and PR3 but they are non-significant from each other.

In Pusa 120, the plants treated with PR17 showed the highest of ascorbic acid (32.97mg/100g) per plant followed by the treatment 3NAA8 of (33.77mg/100g), the lowest of ascorbic acid (24.05mg/100g) was obtained from PR9.

In Navodaya, the maximum of ascorbic acid was showed by 3NAA8 (34.57mg/100g) followed by PR17 (34.19mg/100g). The minimum of ascorbic acid was observed in PR25 (25.25mg/100g) among all the potential PGPRs.

In Golden Green, the maximum of ascorbic acid showed in PR17 (34.71mg/100g) followed by 3NAA8 (35.02mg/100g). The minimum of ascorbic acid was recorded in PR25 (25.28mg/100g) among all the treatments.

**Table 4.8.3 Effect of five potential PGPR on Ascorbic acid (mg/100g) in varieties of tomato Pusa 120, Navodaya, and Golden Green.**

| Treatments | Ascorbic acid (mg/100g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 20.52 | 20.64 | 20.44 |
| Control (inoculated) | 20.14 | 20.12 | 19.60 |
| PR3 | 24.69 | 25.51 | 24.95 |
| PR9 | 24.05 | 24.93 | 24.82 |
| PR17 | 32.97 | 34.19 | 34.71 |
| PR25 | 24.31 | 25.25 | 25.28 |
| 3NAA8 | 33.77 | 34.57 | 35.02 |
| C.D. (5%) | 0.97 | 0.78 | 0.50 |
| S. Em. | 0.34 | 0.27 | 0.17 |
| S. Ed. | 0.48 | 0.39 | 0.24 |
| C.V. | 3.49 | 2.72 | 1.73 |

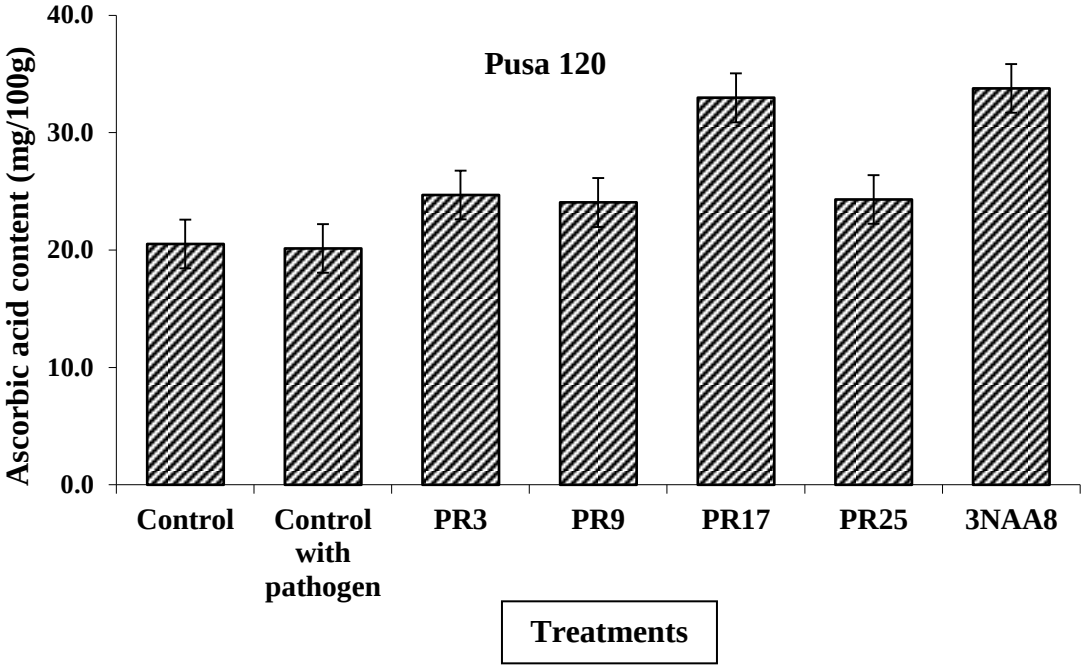



**Fig.4.8.3(a) Effect of PGPR on ascorbic acid content (mg/100g) value Pusa 120 variety of tomato.**

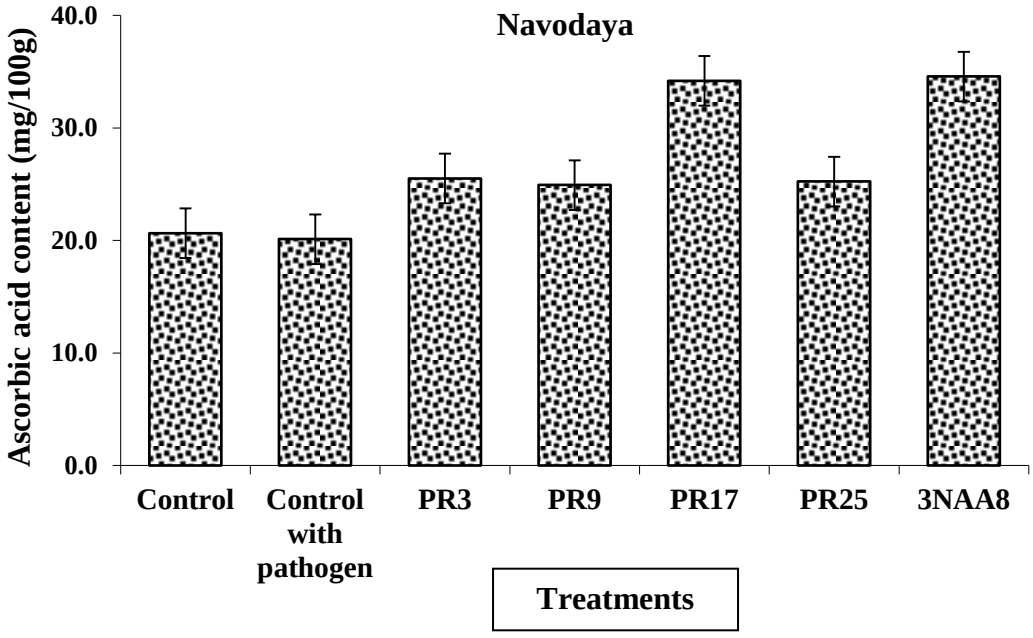



**Fig.4.8.3(b) Effect of PGPR on ascorbic acid content (mg/100g) value on Navodaya variety of tomato.**

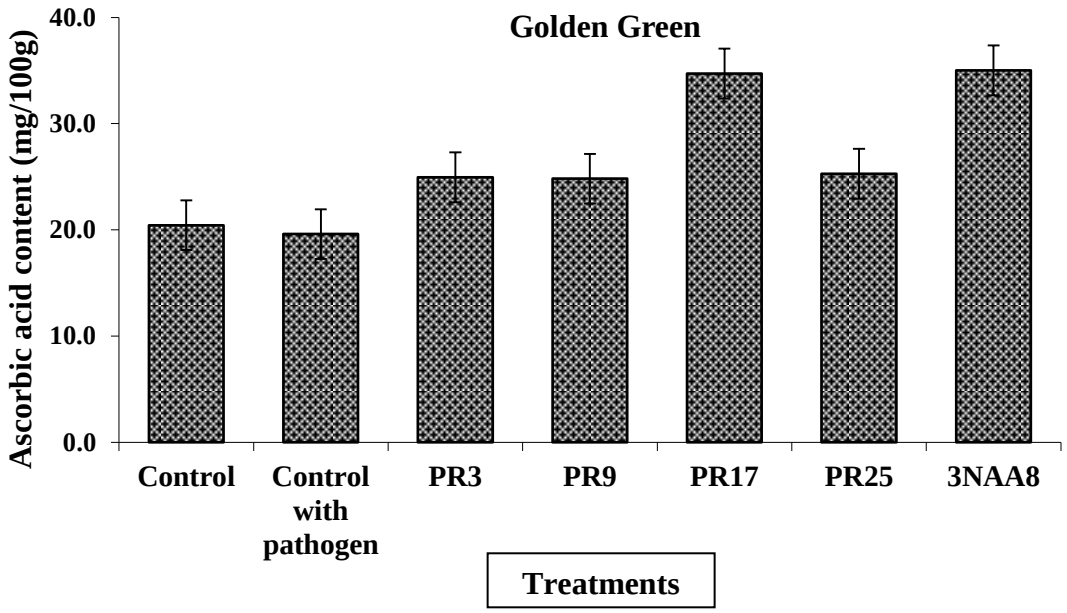



**Fig.4.8.3(b) Effect of PGPR on ascorbic acid content(mg/100g) value on Golden Green variety of tomato.**

### 4.8.4 CHLOROPHYLL (mg/g fresh weight):

#### 4.8.4(A) Chlorophyll a (mg/g leaf fresh weight) at flowering stage:

The data shown below in the Table 4.8.4(A) clearly indicates that there is significant difference in Chlorophyll a (mg/g) due to the treatments given. In PUSA 120 and Navodaya 3NAA8 was found significant from all other PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant from PR9, PR25 and PR3 while they are found non-significant from each other. In Golden Green PR17 was found significant from all whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3 while they are non-significant from each other.

In Pusa 120, the plants treated with PR17 showed the highest chlorophyll a (2.04mg/g) per plant followed by the treatment 3NAA8 of (1.99mg/g), the lowest of chlorophyll a (1.44mg/g) was obtained from PR3.

In Navodaya, the maximum of chlorophyll a was showed by PR17 (1.97mg/g) followed by3NAA8 (1.95mg/g). The minimum of chlorophyll a was observed in PR3 (1.38mg/g) among all the potential PGPRs.

In Golden Green, the maximum of chlorophyll a showed in 3NAA8 (1.94mg/g) followed by PR17 (1.92mg/g). The minimum of chlorophyll a was recorded in PR25 (1.51mg/g) among all the treatments.

**Table 4.8.4(A) Effect of five potential PGPR on chlorophyll a mg/g fresh weight in varieties of tomato Pusa 120, Navodaya, and Golden Green.**

| Treatments | Chlorophyll a (mg/g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 0.97 | 0.96 | 0.95 |
| Control (inoculated) | 0.84 | 0.81 | 0.80 |
| PR3 | 1.44 | 1.38 | 1.51 |
| PR9 | 1.52 | 1.46 | 1.57 |
| PR17 | 2.04 | 1.97 | 1.92 |
| PR25 | 1.48 | 1.44 | 1.61 |
| 3NAA8 | 1.99 | 1.95 | 1.94 |
| C.D. (5%) | 0.14 | 0.09 | 0.09 |
| S. Em. | 0.05 | 0.03 | 0.03 |
| S. Ed. | 0.07 | 0.04 | 0.04 |
| C.V. | 8.98 | 5.74 | 5.43 |

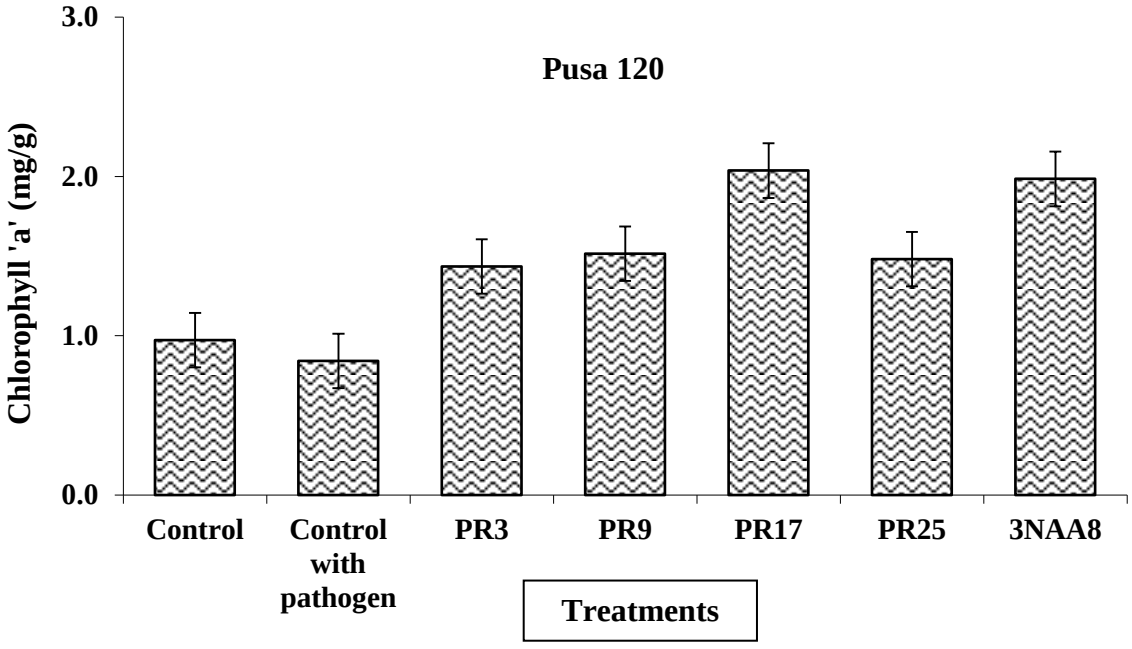


**Fig.4.8.4(A)(a) Effect of PGPR on Chlorophyll a(mg/g) value on Pusa 120 variety of tomato.**

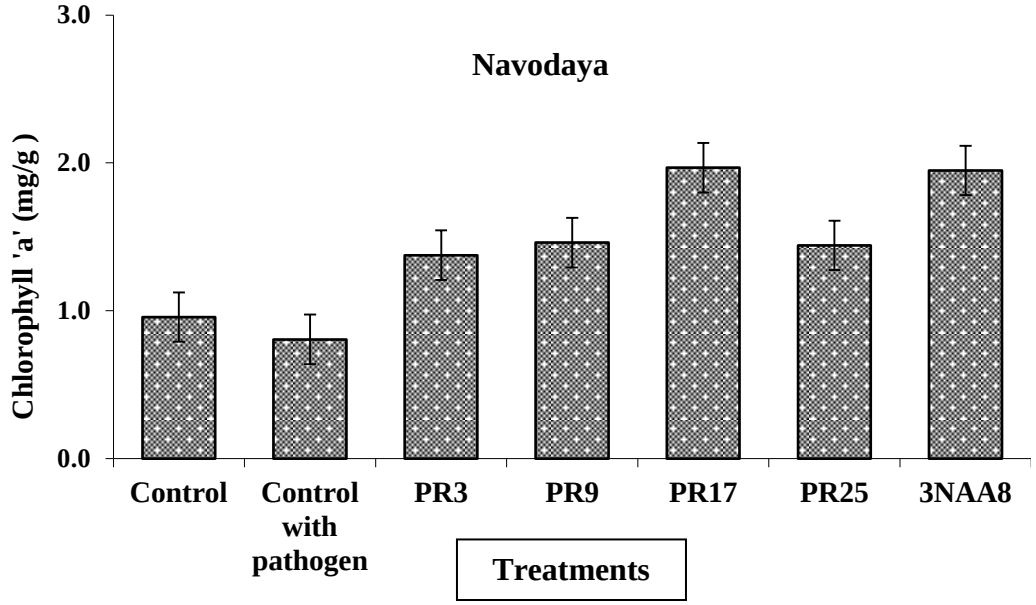



**Fig.4.8.4(A)(b) Effect of PGPR on Chlorophyll a(mg/g) value on Navodaya variety of tomato.**

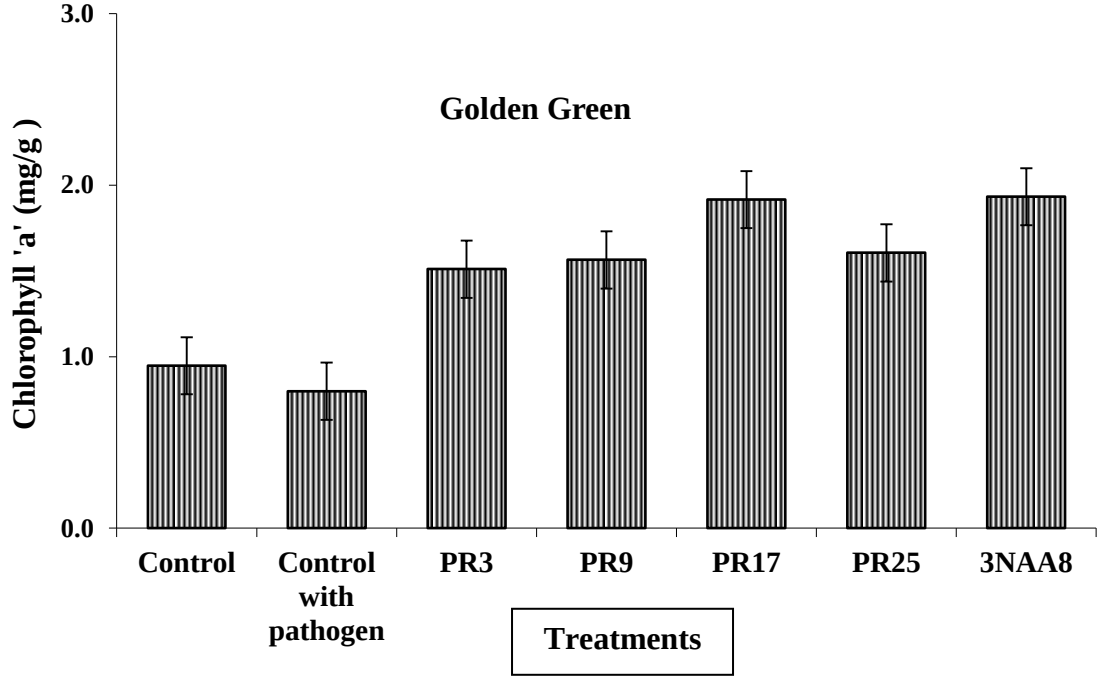



**Fig.4.8.4(A)(c) Effect of PGPR on Chlorophyll a(mg/g) value on Golden Green variety of tomato.**

**4.8.4(B) Chlorophyll b (mg/g leaf fresh weight) at flowering stage:**

The data shown below in the Table 4.8.4(B) clearly indicates that there is significant difference in Chlorophyll b (mg/g) due to the treatment.

In Pusa 120, the plants treated with PR17 showed the highest chlorophyll b (1.89mg/g) per plant followed by the treatment 3NAA8 of (1.86mg/g), the lowest of chlorophyll b (1.29mg/g) was obtained from PR3. 3NAA8 was found significant from all other PGPR treatments whereas PR17 was found non-significant from 3NAA8 but significant from PR25, PR9 and PR3 while they are found to be non-significant from each other.

In Navodaya, the maximum of chlorophyll b was showed by 3NAA8 (1.83mg/g) followed by PR17 (1.79mg/g). The minimum of chlorophyll b was observed in PR3 (1.30mg/g) among all the potential PGPRs.PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3 while they are found to be non-significant from each other.

In Golden Green, the maximum of chlorophyll b showed in 3NAA8 (1.85mg/g) followed by PR17 (1.83mg/g). The minimum of chlorophyll b was recorded in PR3 (1.43mg/g) among all the treatments. PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3 while they are found to be non-significant from each other.

**Table 4.8.4(B) Effect of five potential PGPR on chlorophyll b mg/g fresh weight in varieties of tomato Pusa 120, Navodaya and Golden Green.**

| Treatments | Chlorophyll b (mg/g) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 0.95 | 0.89 | 0.87 |
| Control (inoculated) | 0.80 | 0.76 | 0.75 |
| PR3 | 1.29 | 1.30 | 1.43 |
| PR9 | 1.42 | 1.40 | 1.50 |
| PR17 | 1.89 | 1.79 | 1.83 |
| PR25 | 1.38 | 1.36 | 1.53 |
| 3NAA8 | 1.86 | 1.83 | 1.85 |
| C.D. (5%) | 0.12 | 0.09 | 0.09 |
| S. Em. | 0.04 | 0.03 | 0.03 |
| S. Ed. | 0.06 | 0.05 | 0.04 |
| C.V. | 7.95 | 6.45 | 5.72 |

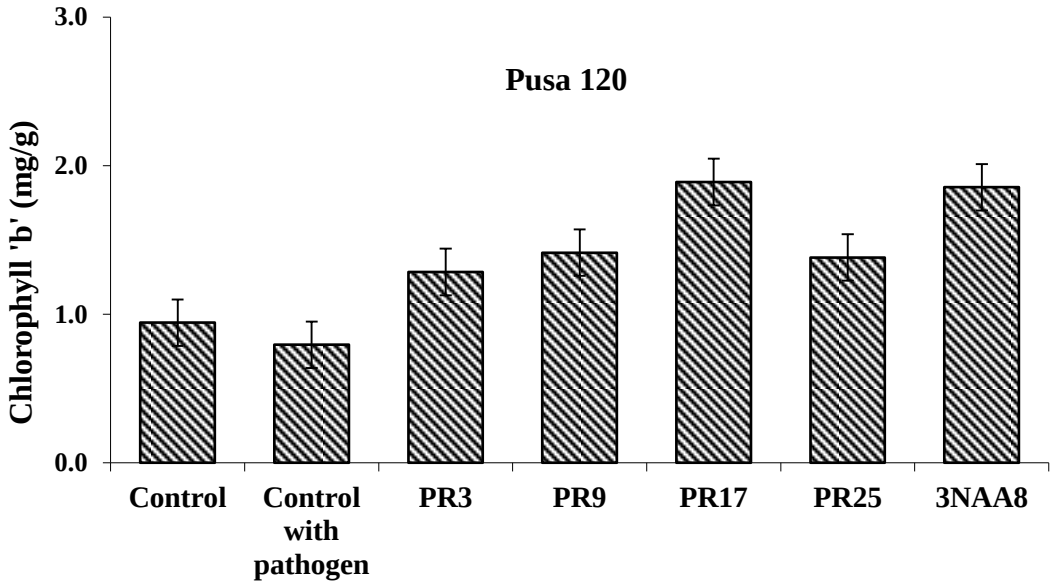



**Fig.4.8.4(B)(a) Effect of PGPR on Chlorophyll b (mg/g) value on Pusa 120 variety of tomato.**

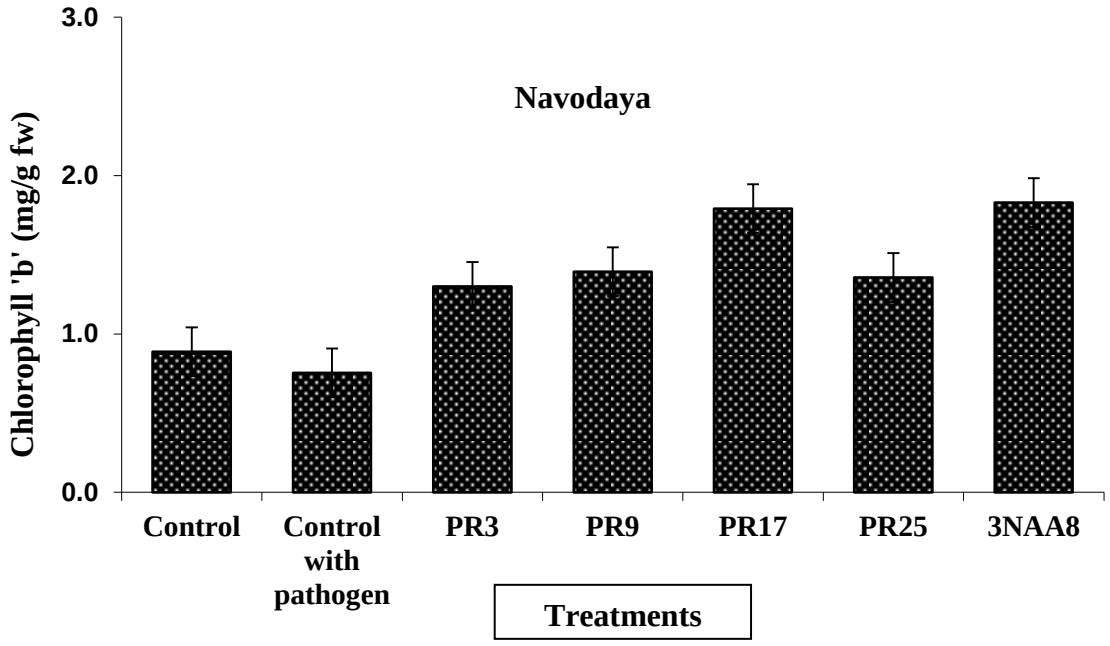



**Fig.4.8.4(B) (b) Effect of PGPR on Chlorophyll b (mg/g) value on Navodaya variety of tomato.**

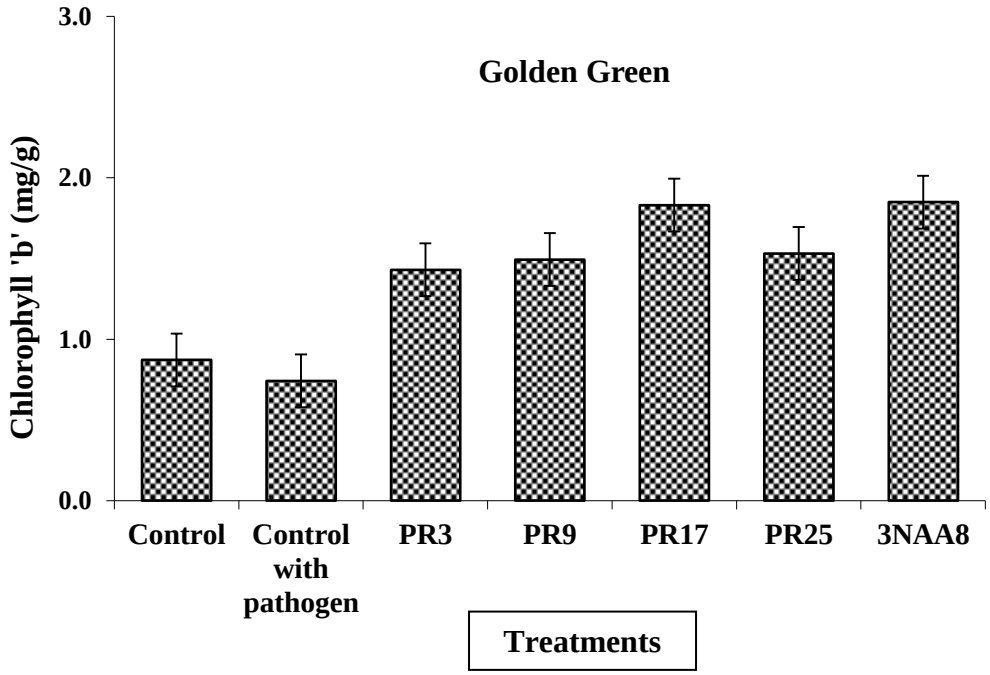



**Fig.4.8.4(B) (c) Effect of PGPR on Chlorophyll b (mg/g) value on Golden Green variety of tomato.**

### 4.8.5 RWC (Relative water content):

The data shown below in the table 4.8.5 clearly indicates that there is significant difference in relative water content due to the treatments. In all the three varieties PR17 was found significant from all other PGPR treatments whereas 3NAA8 was found non-significant from PR17 but significant from PR25, PR9 and PR3 but they are found non-significant from each other.

In Pusa 120, the highest relative water content (%) of tomato was observed in 3NAA8 (66.47) followed by PR17 (66.34) whereas the lowest relative water content (%)of tomato was recorded in PR3 (60.54) among all the treatments given.

In Navodaya, the highest relative water content (%) of tomato was shown by 3NAA8 (66.88) followed by PR17 (66.82). The lowest relative water content (%) of tomato was observed in PR3 and PR25 (61.12) among all the potential PGPRs.

In Golden Green, the highest relative water content (%) of tomato was showed in 3NAA8 (66.58) followed by PR17 (66.37). The lowest relative water content (%) of tomato was recorded in PR25 (62.40) among all the treatments.

**Table 4.8.5 Effect of five potential PGPR on Relative water content (%) in varieties of tomato Pusa 120, Navodaya, and Golden Green.**

| Treatments | Relative water content (%) | | |
|---|---|---|---|
| | Pusa 120 | Navodaya | Golden Green |
| Control (uninoculated) | 53.24 | 51.86 | 51.90 |
| Control (inoculated) | 46.11 | 47.51 | 48.39 |
| PR3 | 60.54 | 61.12 | 62.55 |
| PR9 | 64.04 | 62.10 | 64.13 |
| PR17 | 66.34 | 66.82 | 66.37 |
| PR25 | 61.59 | 61.12 | 62.40 |
| 3NAA8 | 66.47 | 66.88 | 66.58 |
| C.D. (5%) | 2.17 | 1.39 | 1.05 |
| S. Em. | 0.76 | 0.49 | 0.37 |
| S. Ed. | 1.07 | 0.69 | 0.52 |
| C.V. | 3.36 | 2.16 | 1.61 |

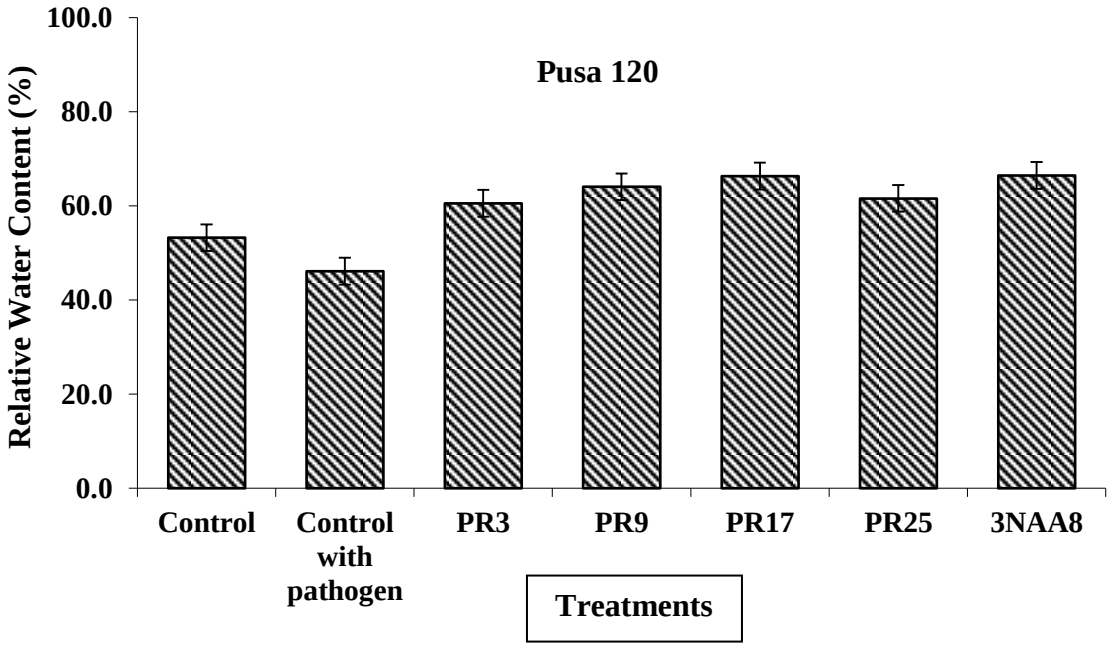



**Fig.4.8.5(a) Effect of PGPR on Relative water content (%) value on Pusa 120 variety of tomato.**

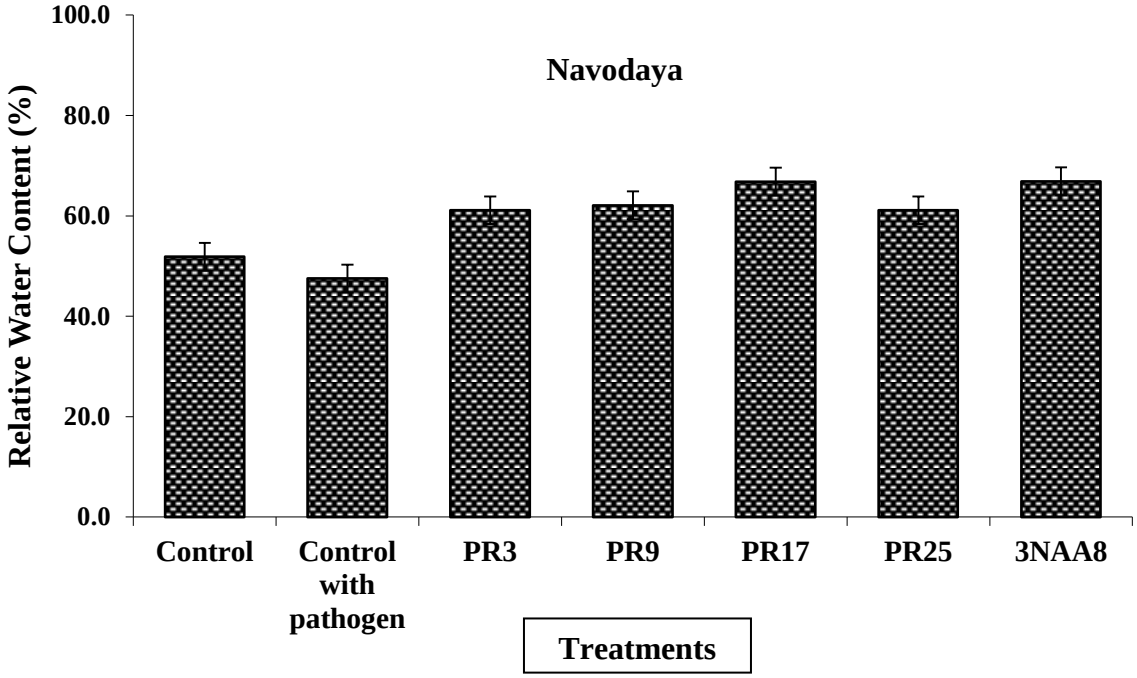



**Fig.4.8.5(b) Effect of PGPR on Relative water content (%)value on Navodaya variety of tomato.**

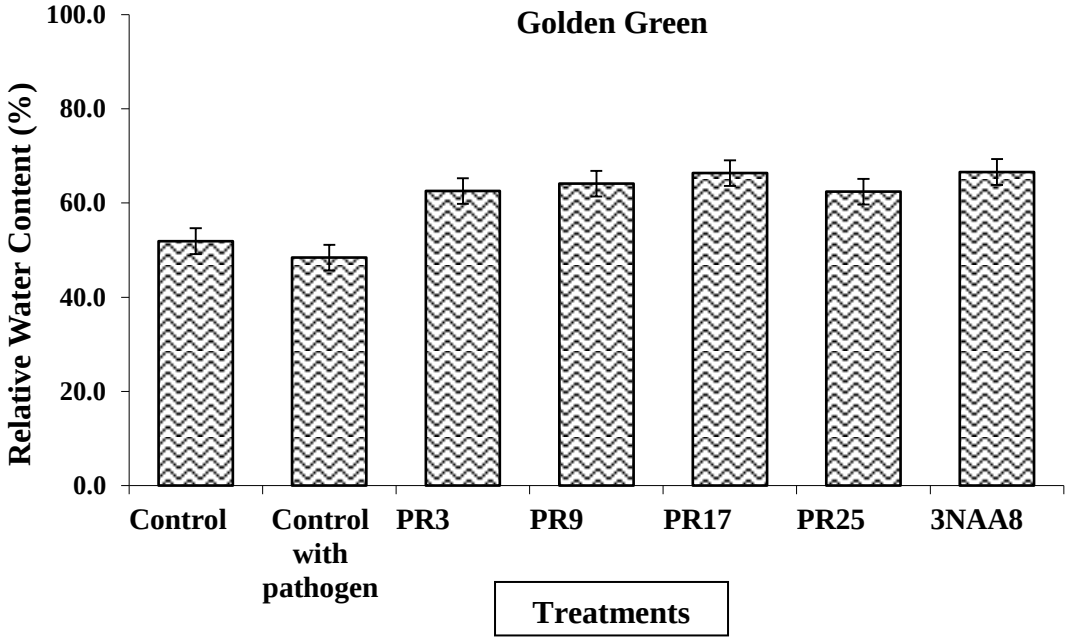



**Fig.4.8.5(c) Effect of PGPR on Relative water content (%) value on Golden Green variety of tomato.**

# Chapter -5

# Discussion

# DISCUSSION

Root associated bacteria are an important functional group of beneficial bacteria used for control of soil borne pathogens and plant growth promotion **(Gamalero *et al.*, 2003; Rajkumar *et al.*, 2005)**. Several tomato diseases and disorders cause wilting of plant, leaf spots and fruit rots etc. Usually, these diseases do not kill the plants, but they can lead to significant yield and quality losses. Many disease-causing microorganisms can survive in plant debris, on seed, or in the soil. Thus there is a need of controlling the adverse effects of diseases and their causing pathogens. Keeping this fact in mind, an experiment was conducted with three different varieties of tomato namely Pusa 120, Navodaya, Golden green to study the effects of five potential PGPR PR3, PR9, PR17, PR25, 3NAA8 against the pathogen, bacterial wilt disease and growth and yield enhancement of plant.

PGPRs are a group of bacteria capable to actively colonize the plants root system and improve their growth and yield **(Wu *et al.*, 2005).** They colonize all ecological niches of root to all stages of plant development, even in the presence of a competing micro flora. 2 to 5% of total rhizospheric bacteria represented by PGPR **(Antoun and Kloepper, 2001).** The term PGPR was proposed by **Kloepper *et al.*, (1980)** and has been implied for a long time, especially for fluorescent *Pseudomonas* involved in the biological control of pathogens and enhancing plant growth. Later, **Kapulnik *et al.*, (1981)** used this term to the rhizobacteria which have the ability to directly promote plant growth. Today PGPR refer to all bacteria existing in the rhizosphere and improve plant growth through one or more mechanisms. A broad range of species belonging to the genus *Pseudomonas*, *Enterobacter*, *Alcaligenes*, *ArthrobacterAzospirillum*, *Azotobacter*, *Klebsiella*, , *Burkholderia*, *Bacillus* and *Serratia*was reported as PGPR **(Saharan and Nehra, 2011).**

Two years of trials (2014 and 2015) under organic growing conditions showed that PGPR treatments including PR3, PR9, PR17, PR25, 3NAA8 applications significantly affected all parameters tested in this study.

## 5.1 Screening and selection of efficient PGPR against *Ralstonia solanacearum.*

20 isolates of PGPR out of 250 rhizobacterial isolates, in preliminary screening which showed activity against the pathogen i.e. *Ralstonia solanacearum* was selected on the basis of their slight zone creation in plates. These 20 isolates were 2CBA2, 2CBA4, 2CBA18, 2CBA42, 2AAB27, 2BAB1, 1NAB15, 2NAB20, 3NAA1, 3NAA7, 3NAA8, 3NAA12, 3NAA17, PR2, PR3, PR9, PR17, PR25, PR26, and PR27.

## 5.2 *In-vitro* assay

The selected potential 20 PGPR isolates which were found positive was further screened by both agar-well diffusion and disc-diffusion method, the five strains that showed the highest inhibition zones are **PR3, PR9, PR17, PR25, 3NAA8.** Among these five strains the maximum inhibition zone was shown by PR17 as 33.50mm, 25.30mm following 3NAA8 as 30.60mm, 20.30mm, PR9 as 29.60mm, 20.30mm, PR3 as 29.30mm, 19.30mm and lowest zone by PR25 as 27.60mm, 19.30mm. These selected efficient PGPR were used for further studies. PGPR strains could promote the growth in earthen pots culture. Variability in the nature and magnitude of growth promotion due to bacterial inoculation is not uncommon and presents a significant barrier to the evaluation of bacterial inoculants **(Schroth and Weinhold, 1986).** Among the five efficient PGPR, the PR3, PR9, PR17, PR25 belongs to *Enterobactor* species and 3NAA8 belongs to *Bacillus* species.

## 5.3 Disease Incidence

Bacterial wilt is one of the most destructive diseases of tomato crops worldwide among all the diseases. *R. Solanacearum* is the causal agent which affect a wide range of other crops such as tobacco (*Nicotiana tabacum* L.), banana (*Musa paradisiacal* L.), peanut (*Arachis hypogaea* L.), etc. in almost every region in the warm temperate, semitropical, tropical and cool climates of the world **(Hayward, 1994).**

In the experiment, the wilt incidence was reduced markedly by treating the tomato plants with five potential selected PGPR. The disease incidence was remarkably reduced by PR17 and 3NAA8 after 42 days from the day of inoculation in all three varieties. The Pusa 120 had disease incidence of PR17 (60%) and 3NAA8 (67.86%) while Navodaya had PR17 (50.02%)

and 3NAA8 (64.28%) and Golden green had calculated disease incidence of PR17 (53.49%) and 3NAA8 (67.86%) respectively.

Recent studies indicated that biological control of bacterial wilt disease could be achieved using antagonistic bacteria. Toyota and Kimura have reported the suppressive effect of some antagonistic bacteria on *R.Solanacearum* (**Toyota *et al.*, 2000).** Bacteria had biocontrol efficacy on bacterial wilt of tomato plants under greenhouse conditions which belong to genera *Bacillus* **(Chen *et al.*,2013; Tan *et al.*,2013)**.Some naturally occurring antagonistic rhizobacteria such a as *Bacillus* spp., *Pseudomonas* spp. have been known to control this disease. The PGPR increase root development either directly by producing phytohormones, or indirectly by inhibiting pathogens through the production of different compounds. In vitro experiment showed inhibitory activity of *Paenibacillus* sp. Pb28 against bacterial wilt of tomato which could be due to production of protease **(Kheirandish and Harighi 2015).** Strain *P. fluorescens* CHA0 is able to produce several antibiotics, including 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, and HCN **(Duffy and De´fago, 1999; Maurhofer *et al.*,1994)**.

In our study, a comparative analysis of the antagonistic potential of five Plant Growth Promoting Rhizobacteria PR3, PR9, PR17, PR25 and 3NAA8 was done to identify stable antagonists against bacterial wilt of tomato. To make a confirmative analysis and to compare the antagonistic behaviour of these organisms on both cultural and field conditions, in-vitro and in-vivo assays were designed. Among the strains used in experiment, 3NAA8 which belongs to *Bacillus* spp. and PR17 which belongs to *Enterobactor* spp. have been reported to be most effective PGPRs against bacterial wilt disease in tomato in comparison to other treatments and control. This may be due to the Antibiotics production, Lytic enzymes production, Hydrogen cyanide production, Induction of systemic resistance or Competition for Iron, nutrient and space.

The main functions of PGPR to control pathogens are Antibiotics production by **(Ongena *et al.*, 2005),** Lytic enzymes production **(Joshi *et al.*, 2012),** Hydrogen cyanide production **(Lanteigne *et al.*, 2012),** Volatile compounds production **(Trivedi *et al.*, 2008),** Induction of systemic resistance **(Doornbos *et al.*, 2012),** Competition for Iron, nutrient and space **(Innerebner *et al.*, 2011).**

The use of plant growth-promoting rhizobacteria (PGPR) represents a potentially attractive alternative disease management approach since PGPR have been reported to increase yield and protect crops simultaneously **(Ramamoorthy *et al.*, 2002; Raupach, 1998).** PGPR have been reported to stimulate plant growth and improve stand under stress conditions **(van Loon *et al.*, 1998).** Three strains of PGPR, *Bacillus subtilis* MBI600 (Microbio, Bolder, CO) and GBO3 (Gustafson, Inc., Plano, TX), and *Bacillus amyloliquefaciens* IN937 (Auburn University, Auburn, AL), have been reported to act as biological control agents against various plant pathogens in numerous field and vegetable crops **(Martinez-Ochoa, 2000; Zehnder *et al.*,2000; Raupach, 1998; Ryu *et al.*,2000; EPA, 2004).** Plant disease control using PGPR has been variable across locations and crops; several factors may influence the ability of PGPR to affect plant growth parameters and disease suppression such as colonization **(Bloemberg and Lugtenberg 2001; Benizri *et al.*,2001)**, soil moisture **(Meikle *et al.*,1995)**, competition **(Young *et al*. 1995),** nutrients, inorganic compounds and plant-derived factors **(Milner *et al.*,1995; Rodriguez and Pfender, 1997).**A number of studies have confirmed that *Pseudomonas* strains was most successful in controlling the plant root pathogens including *F. oxysporum* and *R. solani* by the production of siderophores, other secondary metabolites and lytic enzymes **(O'Sullivan and O'Gara, 1992; Nagrajkumar *et al.*, 2004).**

## 5.4 SEED GERMINATION PARAMETERS

Seed treatment with plant growth promoting rhizobacterial strains for measuring the germination enhancement in tomato is reported in the present study. On treatment with five potential PGPR PR3, PR9, PR17, PR25 and 3NAA8, a gradual increase in the germination of tomato seeds was observed. The maximum germination percentage 96.67 % was shown by 3NAA8 followed by PR17 which showed 93.33% of germination percentage among all the potential PGPR strains and the lowest was by PR9 (88.30%) in Pusa 120. In Navodaya maximum germination percentage was found by 3NAA8 by 95% followed by PR17 found with 91.67% of germination percentage and lowest by PR3 as 81.67% while in Golden green highest germination percentage was shown by PR17 and 3NAA8 by 95% and the least shown by PR3 and PR9 as 83.33%.

The maximum root length in Pusa 120 at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in PR17 followed by 3NAA8 and the lowest root length was found in PR9. In Navodaya, The

Maximum root length at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in PR17 followed by 3NAA8 and the lowest root length was found in PR9. While In Golden green, maximum root length at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in 3NAA8 followed by PR17 and the lowest root length was found in PR9 among all the potential PGPRs.

In Pusa 120, the maximum shoot length at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in 3NAA8 followed by PR17. The lowest shoot length was found in PR9. In Navodaya, The maximum shoot length at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in PR17 followed by 3NAA8. The lowest shoot length was found in PR9.

In GOLDEN GREEN, The maximum shoot length at 5 DAI, 7 DAI, 9 DAI and 11 DAI was observed in PR17 followed by 3NAA8. The lowest shoot length was found in PR3. Similar studies were conducted by **Nezarat and Gholami (2009)** and it was reported that seed inoculation with PGPR enhanced seed germination and transplant vigour of maize. Similar findings were reported by **Murphy *et al.*, (2000).**

Growth promotion may be attributed to other mechanisms such as production of plant growth promoting All the isolates showed increase in seed germination over control in roll towel method, similar findings in cereals such as sorghum enhancement of seed germination parameters by rhizobacteria has been reported **(Raju *et al.*, 1999)** and pearl millet **(Niranjan *et al.*, 2004, 2003).** The improvement in seed germination by PGPR was also found in work with wheat and sunflower **(Shaukat *et al.*, 2006a, 2006b),** where it was found that some PGPR induced seeds results in increase in seed emergence, in some cases achieving increases up to 100% greater than control. These findings may be due to the increased synthesis of hormones like gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, such as amylase, which have brought an increase in availability of starch assimilation.

It has been reported that as compared to non rhizospheric soil, the higher concentrations of phosphate-solubilising bacteria are commonly found in rhizosphere soil **(Reyes and Valduz, 2006).** The capability of bacteria to generate IAA in the rhizosphere depends on the accessibility of precursors and uptake of microbial IAA by plant. Improved IAA production was found in *B. subtilis* strain BCA-6. **Jagnow, (1987)** recorded production of IAA by *Bacillus* and *Pseudomonas*. Bacterial strain *Pseudomonas putida* BHUPSB04 showed highest significant concentration of IAA followed by *P. Aeruginosa* BHUPSB02, *B. subtilis* BHUPSB13, *P. polymyxa* BHUPSB17 and *B .boronophillus* **(Yadav *et al.*, 2010).** Pseudomonas produced higher level of IAA was recorded by **Xie *et al.*, (1996).**

## 5.5 GROWTH AND YIELD PARAMETERS

The Five potential PGPR PR3, PR9, PR17, PR25 and 3NAA8 enhanced the growth parameters of tomato including number of leaves (30, 60, 90 DAT), number of branches (30, 60, 90 DAT), number of flowers per plant (60 DAT) and yield parameters of tomato including total fresh weight of plant, fresh weight of shoot and root, dry weight of root and shoot, number of fruit per plant and fruit yield per plant. Among the five PGPR the highest values were shown by 3NAA8 and PR17 in comparison to both the controls.

Our results are in agreement with the work done by **Nandakumar *et al.*, (2001)** and **Raupauch *et al*., (1996),** who recorded that various PGPR strains improved area and shoot length of cucumber plants. Also with **Almaghrabi *et al.*, (2013)** who illustrated the increase in tomato plant growth, shoot dry weight of plant (g), plant height (cm), number of fruits/plants and weight of yield/plant using six isolates of PGPR *P. putida, P. fluorescence, S. mar- cescens, B. amyloliquefaciens, B. subtilis* and *B. cereus*. Similarly **Kandan (2000)** found improved leaf area and shoot length in tomato plants treated with *P. fluorescence* strain CHAO, Co-T-. Seed bacterization of chickpea and soybean with *fluorescent Pseudomonads*, RBT13, increased seed germination, shoot length and root growth **(Kumar and Dube, 1992)**. Similar studies for a number of species have been done which showed increased growth and development in plants inoculated with PGPR **(Vessey, 2003; Gray and Smith, 2005; Van Loon, 2007).** It was reported by **Walia et al. 2013** that the strain of *B. subtilis* improved seed germination (35.08%), shoot dry weight (63.50%) and root dry weight (54.08%), shoot length (5.22%), root length (21.12%)of tomato transplants compared to the control**.**

Similarly, the efficiency of PGPR formulations on yield attributes of a variety of crops was reported by **Weller and Cook (1983)**. **Nandakumar *et al*. (2001)** reported that application of *P. fluorescens* strains increased the yield in rice. The seed treatment with PGPR resulted in increased plant growth and yield in potato under field conditions **(Kloepper *et al.*, 1980).**The improvement and enhancement of tomato growth and yield parameters evaluated in this study may be attributed to growth-promoting substances that take action on plant metabolic processes. For any particular PGPR strain, growth enhancement of the host plant is typically due to a combination of accessible mechanisms **(Lucy *et al.*, 2004; Van Loon, 2007).**

The possible reason might be related with the preliminary increase in root growth by the application of PGPR strains, which would have helped in promoting enhanced absorption of

vital nutrients that are responsible for highly active photosynthesis as well as protein synthesis. Better root growth may be responsible for better synthesis of hormones like auxin and cytokine in which would have helped in better partitioning efficiency which later resulted in increased economic yield. PGPR synthesize phytohormones that promote plant growth at various stages **(Kloepper *et al.*, 1986)**.The plant growth may be stimulated by several mechanisms activated by Rhizosphere bacteria which includes mechanisms such, production of plant growth regulators, nitrogen fixation, suppression of disease. The production of plant growth substances such as auxins and gibberellins is one of the most reported direct plant growth promotion mechanism by bacteria **(Holl *et al.*, 1988; Chanway, 2002)**. It has also been recognized that rhizobacteria possess an enzyme ACC-deaminase which hydrolyses ACC into ammonia and a-ketobutyrate **(Mayak et al. 1999).** The root elongation, nodulation can be facilitated and yield of plant can be improved with PGPR containing ACC-deaminase which could suppress accelerated endogenous ethylene synthesis **(Zafar-ul-Hye 2008)**.The plant bio mass was significantly increased by strains of *fluorescent pseudomonads*. This could be due to induction of systemic resistance, competition advantage to root colonization by antagonists and production of siderophore **(Aspiras and Cruz 1986, Henok *et al.*, 2007; Lemessa and Zeller 2007).** Some *Bacillus* spp. have been found to produce secondary metabolites mainly with inhabitant pathogen activity such as lipopeptides, surfactins, bacillomycin D, and fengycins **(Chen *et al.*,2006)**.

## 5.6 BIOCHEMICAL PARAMETERS

The most five potential PGPR were used studying their effect on the biochemical parameters of tomato in three different varieties. The biochemical parameters including Chlorophyll a, b, Lycopene**,** Ascorbic acid**,** TSS, Relative water content were improved by the inoculation of PGPR PR3, PR9, PR17, PR25 and 3NAA8.

Based on the results, in all the three varieties PUSA 120, N Navodaya and Golden green the most effective PGPR were 3NAA8 and PR 17that improved Chlorophyll a, b, Lycopene**,** Ascorbic acid**,** TSS, Relative water content. Similar studies of TSS and Lycopene and for ascorbic acid were observed in **Ordookhani *et al.*, (2011)** and **Kumari, (2012).** Also the PGPR enhanced the ascorbic acid and chlorophyll content in all the three varieties of tomato. Similar studies were done for chlorophyll and ascorbic acid and were observed that PGPR inoculation increased the chlorophyll content (**Singh and Singh 2004 and Salam *et al.*, 2011).** In the study done by **Ekinci *et al.*, (2014)** showed that bacterial inoculation of the

strains of *Bacillus megaterium* TV-3D, *B. megaterium* TV-91C, *Pantoea agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10 increased plant growth parameters such as chlorophyll contents, fresh shoot weight, dry shoot weight, root diameter, root length, fresh root weight, dry root weight of cauliflower. Similar findings were reported by **Orhan *et al.*, (2006)** where he suggested that *Bacillus* M3 alone or in combination with *Bacillus* OSU-142 have the potential to increase the yield, growth and nutrition (TSS) of raspberry plant under organic growing conditions. The enhance growth may be due to rising nutrient uptake providing plant growth hormones, improving chlorophyll content and organic acids with bacterial applications. Seeds inoculated with *Pseudomonas, Azotobacter and Azospirillum* were found to enhance the fruit lycopene content. Similarly, antioxidant activity increased significantly following the same treatment **(Ordookhani *et al.*, 2010).**

In present study we have found that the relative water content was also increased by the inoculation of PGPR treatments in comparison to control. RWC is a useful measure of the physiological water status of plants **(Gonzalez and Gonzalez-Vilar, 2001)** .the similar findings were reported for the Increased Relative water content (RWC) by PGPR for radish **(Yildirim *et al.*, 2008)**, for lettuce **(Yildirim *et al.*, 2011),** and for strawberry **(Karlidag *et al.*, 2011)**. **Mayak *et al.*, (2004)** reported that the growth of root and growth of plant can be facilitated by the use of PGPR under salt stress by improving the water use efficiency.

**Ordookhani *et al.*, (2010)** showed that PGPR can increase tomato fruit quality. It may be related to increasing of minerals by plant inoculated. Increased nutrient uptake by plants inoculated with plant-growth promoting bacteria has been attributed to the production of plant growth regulators at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil **(Kloepper *et al.*, 1992; Zimmer *et al.*, 1995; Hoflich *et al.*, 1996).**

# Chapter- 6

# Summary and Conclusion

# SUMMARY

The present investigation entitles "**Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)**" .The experiment was laid out in a customised randomized design and replicated seven times. The study has carried out during 2014-2016 at Department of Biological Sciences, SHUATS, Allahabad (U.P.), Sam Higginbottom University of Agriculture, Technology and Science, Allahabad.

The salient finding of the experiment is summarized and conclusion is drawn. The general result of the investigation together with conclusion has been summarized below.

As we used the three varieties of tomato viz PUSA-120, Navodaya and Golden green (V1, V2, V3), all the treatments improved the quality of crop. From all varieties we cannot say particularly one variety showed best result but over all Golden green showed the maximum rate of improvement.

1. In all the three varieties namely PUSA-120, Navodaya and Golden green, among the given PGPR 3NAA8 and PR17 reduced the disease incidence successively at stages of 7, 14, 21, 28, 35 and 42 DAI. There was significant difference between the different treatments at their successive growth stages. And the minimum where recorded in control (inoculated with pathogen).
2. In seed germination, all the PGPR which were given to observe the efficacy on seeds of tomato of three different varieties showed a remarkable improvement in germination percentage, also in shoot length and root length. The treatment which showed the highest rate of effect in germination is 3NAA8 and PR17.
3. There was significant difference in the Number of leaves and maximum Number of leaves were recorded in treatment 3NAA8 and PR17 following PR25, PR9 and PR3 at 30, 60 and 90 DAT. And the minimum where recorded in treatment is control.
4. The significant variation observed in Number of branches under five treatment at 30, 60 and 90 DAS, the maximum Number of branches recorded with treatment 3NAA8 and PR17 and the minimum where recorded in treatment is control.
5. The number of flowers per plant was found to be statistically significant under various treatments. The maximum numbers of flowers per plant were recorded with treatment 3NAA8 and PR17 and the minimum where recorded in is control.

6. The numbers of fruits per plant were found maximum in treatment 3NAA8 and PR17 and the minimum where recorded in treatment cT1 that is control.
7. The average fruit weight per plant was improved significantly by 3NAA8 and PR17 among all the five potential PGPR.
8. The yield per plant was enhanced in all the three varieties by PGPR 3NAA8 and PR17 among all the PGPRs inoculated in plant.
9. The total fresh of plant was increased significantly with the inoculation of selected PGPR. The fresh weight of shoot and root, and dry weight of shoot and root were also increased by the inoculation of potential PGPR. The maximum Fresh weight of plant was recorded with PGPR 3NAA8 and PR17 and the minimum where recorded in that is control.
10. In the case of Chlorophyll a, b, we observe that there was significant difference between Chlorophyll a, b. The maximum Chlorophyll a, b recorded in the treatment 3NAA8 and PR17 and minimum was observed control.
11. There was a significant difference between the treatment combination in case of Ascorbic acid in soil the maximum moisture contents (%) was observed in treatment 3NAA8 and PR17 and the minimum was recorded in control.

15. In the case of TSS, we observed that there was significant difference between TSS. The maximum TSS was recorded in the treatment 3NAA8 and PR17 and minimum was observed in control.

17. The significant variation where observed in case of Lycopene. The maximum lycopene was recorded in the treatment combination 3NAA8 and PR17 and the minimum was observed in control.

# CONCLUSION

The present study entitled "**Efficacy of Plant Growth Promoting Rhizobacteria (PGPR) against bacterial wilt of tomato (*Lycopersicon esculentum* Mill.)**" revealed beneficial effects of PGPR among the potential five PGPR which were selected among 250 isolate. PGPR **PR17 and 3NAA8** have proved to be potential biocontrol agents against the pathogen *R. solanacearum*, in addition to their capability to improve tomato growth under the Allahabad environment. Though all the PGPR showed the effective improvement in the germination of seed and elongation of root and shoot length. These PGPR also increase the growth parameters including number of leaves, branches, flowers, fruits and enhanced the yield per plant etc. Also PR17 and 3NAA8 significantly increased the chlorophyll content, Lycopene content, T.S.S, Ascorbic acid and RWC. However the other remaining PGPR also decrease the effect of disease and also improve the nutrition value and quality of crop as well. It may be concluded that PGPR 17 and 3NAA8 therefore can be used for the management of bacterial wilt of tomato. It is therefore clear that the objectives of chemical fertilizers and pesticides use can be reached with PGPR use. Further studies are, however, needed to investigate the mode of action of these strains in terms of inducing systemic resistance and enhancing their antibiosis activity against plant pathogens.

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**Plate.4.8 Preparation of nursery bed for different varieties**

**Plate.4.9 Soil sterilisation by acid sterilisation method.**

**Plate.4.10 Pots with transplanted seedling from nursery bed.**

**Plate.4.11 Estimation of TSS $^{0}$Brix of tomato fruit juice using Hand Refractrometer.**

**Plate.4.12 Yield of tomato**

# Appendix

# APPENDICES

**Effect of PGPR on Fresh weight of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 15,943.11 | 2,657.18 | 2,106.44 | S |
| Error | 42 | 52.981 | 1.261 | | S |
| Total | 48 | 15,996.09 | | | |

**Effect of PGPR on Fresh weight of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 17,313.54 | 2,885.59 | 1,598.36 | S |
| Error | 42 | 75.825 | 1.805 | | S |
| Total | 48 | 17,389.37 | | | |

**Effect of PGPR on Fresh weight of tomato plant of variety Golden Green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 16,259.89 | 2,709.98 | 727.891 | S |
| Error | 42 | 156.368 | 3.723 | | S |
| Total | 48 | 16,416.26 | | | |

**Effect of PGPR on Number of leaves per plant 30 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 3,685.01 | 614.169 | 499.145 | S |
| Error | 42 | 51.679 | 1.23 | | S |
| Total | 48 | 3,736.69 | | | |

**Effect of PGPR on Number of leaves per plant 30 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 3,547.01 | 591.169 | 293.778 | S |
| Error | 42 | 84.517 | 2.012 | | S |
| Total | 48 | 3,631.53 | | | |

**Effect of PGPR on Number of leaves per plant 30 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 3,432.05 | 572.009 | 257.043 | S |
| Error | 42 | 93.464 | 2.225 | | S |
| Total | 48 | 3,525.52 | | | |

**Effect of PGPR on Number of leaves per plant 60 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 5,134.27 | 855.711 | 346.766 | S |
| Error | 42 | 103.643 | 2.468 | | S |
| Total | 48 | 5,237.91 | | | |

**Effect of PGPR on Number of leaves per plant 60 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 4,838.99 | 806.498 | 250.911 | S |
| Error | 42 | 135 | 3.214 | | S |
| Total | 48 | 4,973.99 | | | |

**Effect of PGPR on Number of leaves per plant 60 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 4,850.41 | 808.402 | 363.884 | S |
| Error | 42 | 93.307 | 2.222 | | S |
| Total | 48 | 4,943.72 | | | |

**Effect of PGPR on Number of leaves per plant 90 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 12,097.03 | 2,016.17 | 271.408 | S |
| Error | 42 | 312 | 7.429 | | S |
| Total | 48 | 12,409.03 | | | |

**Effect of PGPR on Number of leaves per plant 90 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 12,105.91 | 2,017.65 | 280.866 | S |
| Error | 42 | 301.714 | 7.184 | | S |
| Total | 48 | 12,407.62 | | | |

**Effect of PGPR on Number of leaves per plant 90 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 10,615.86 | 1,769.31 | 249.004 | S |
| Error | 42 | 298.434 | 7.106 | | S |
| Total | 48 | 10,914.30 | | | |

**Effect of PGPR on Number of branches per plant 30 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 55.727 | 9.288 | 39.503 | S |
| Error | 42 | 9.875 | 0.235 | | S |
| Total | 48 | 65.602 | | | |

**Effect of PGPR on Number of branches per plant 30 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 40.444 | 6.741 | 29.305 | S |
| Error | 42 | 9.661 | 0.23 | | S |
| Total | 48 | 50.105 | | | |

**Effect of PGPR on Number of branches per plant 30 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 53.947 | 8.991 | 55.33 | S |
| Error | 42 | 6.825 | 0.163 | | S |
| Total | 48 | 60.772 | | | |

**Effect of PGPR on Number of branches per plant 60 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 62.462 | 10.410 | 45.511 | S |
| Error | 42 | 9.607 | 0.229 | | S |
| Total | 48 | 72.069 | | | |

**Effect of PGPR on Number of branches per plant 60 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 53.883 | 8.981 | 54.02 | S |
| Error | 42 | 6.982 | 0.166 | | S |
| Total | 48 | 60.865 | | | |

**Effect of PGPR on Number of branches per plant 60 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 62.298 | 10.383 | 51.413 | S |
| Error | 42 | 8.482 | 0.202 | | S |
| Total | 48 | 70.781 | | | |

**Effect of PGPR on Number of branches per plant 90 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 116.695 | 19.449 | 61.13 | S |
| Error | 42 | 13.363 | 0.318 | | S |
| Total | 48 | 130.057 | | | |

**Effect of PGPR on Number of branches per plant 90 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 90.887 | 15.148 | 59.411 | S |
| Error | 42 | 10.709 | 0.255 | | S |
| Total | 48 | 101.596 | | | |

**Effect of PGPR on Number of branches per plant 90 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 134.069 | 22.345 | 85.04 | S |
| Error | 42 | 11.036 | 0.263 | | S |
| Total | 48 | 145.105 | | | |

**Effect of PGPR on Number of Flowers per plant 60 DAT of tomato plant of variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,065.42 | 177.57 | 392.523 | S |
| Error | 42 | 19 | 0.452 | | S |
| Total | 48 | 1,084.42 | | | |

**Effect of PGPR on Number of Flowers per plant 60 DAT of tomato plant of variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,138.44 | 189.741 | 638.441 | S |
| Error | 42 | 12.482 | 0.297 | | S |
| Total | 48 | 1,150.93 | | | |

**Effect of PGPR on Number of Flowers per plant 60 DAT of tomato plant of variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,169.27 | 194.878 | 624.459 | S |
| Error | 42 | 13.107 | 0.312 | | S |
| Total | 48 | 1,182.38 | | | |

**Effect of PGPRs on Chlorophyll a of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 8.621 | 1.437 | 82.673 | S |
| Error | 42 | 0.73 | 0.017 | | S |
| Total | 48 | 9.351 | | | |

**Effect of PGPRs on Chlorophyll a of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 8.219 | 1.37 | 204.682 | S |
| Error | 42 | 0.281 | 0.007 | | S |
| Total | 48 | 8.5 | | | |

**Effect of PGPRs on Chlorophyll a of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 8.171 | 1.362 | 213.287 | S |
| Error | 42 | 0.268 | 0.006 | | S |
| Total | 48 | 8.439 | | | |

**Effect of PGPRs on Chlorophyll b of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 7.167 | 1.195 | 100.985 | S |
| Error | 42 | 0.497 | 0.012 | | S |
| Total | 48 | 7.664 | | | |

**Effect of PGPRs on Chlorophyll b of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 6.96 | 1.160 | 157.142 | S |
| Error | 42 | 0.31 | 0.007 | | S |
| Total | 48 | 7.27 | | | |

**Effect of PGPRs on Chlorophyll b of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 7.886 | 1.314 | 205.897 | S |
| Error | 42 | 0.268 | 0.006 | | S |
| Total | 48 | 8.154 | | | |

**Effect of PGPRs on Lycopene of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 4.049 | 0.675 | 18.937 | S |
| Error | 42 | 1.497 | 0.036 | | S |
| Total | 48 | 5.545 | | | |

**Effect of PGPRs on Lycopene of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 8.359 | 1.393 | 57.577 | S |
| Error | 42 | 1.016 | 0.024 | | S |
| Total | 48 | 9.375 | | | |

**Effect of PGPRs on Lycopene of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 4.802 | 0.800 | 42.811 | S |
| Error | 42 | 0.785 | 0.019 | | S |
| Total | 48 | 5.587 | | | |

**Effect of PGPRs on Ascorbic acid of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,269.84 | 211.639 | 261.325 | S |
| Error | 42 | 34.015 | 0.81 | | S |
| Total | 48 | 1,303.85 | | | |

**Effect of PGPRs on Ascorbic acid of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,429.75 | 238.291 | 459.429 | S |
| Error | 42 | 21.784 | 0.519 | | S |
| Total | 48 | 1,451.53 | | | |

**Effect of PGPRs on Ascorbic acid of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 1,617.42 | 269.57 | 1,290.74 | S |
| Error | 42 | 8.772 | 0.209 | | S |
| Total | 48 | 1,626.19 | | | |

**Effect of PGPRs on TSS of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 59.13 | 9.855 | 188.79 | S |
| Error | 42 | 2.192 | 0.052 | | S |
| Total | 48 | 61.323 | | | |

**Effect of PGPRs on TSS of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 57.226 | 9.538 | 104.352 | S |
| Error | 42 | 3.839 | 0.091 | | S |
| Total | 48 | 61.065 | | | |

**Effect of PGPRs on TSS of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 55.744 | 9.291 | 114.278 | S |
| Error | 42 | 3.415 | 0.081 | | S |
| Total | 48 | 59.159 | | | |

**Effect of PGPRs on Related water content of tomato in variety PUSA 120.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 2,376.82 | 396.136 | 98.207 | S |
| Error | 42 | 169.415 | 4.034 | | S |
| Total | 48 | 2,546.23 | | | |

**Effect of PGPRs on Related water content of tomato in variety Navodaya.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 2,254.82 | 375.803 | 227.052 | S |
| Error | 42 | 69.516 | 1.655 | | S |
| Total | 48 | 2,324.34 | | | |

**Effect of PGPRs on Related water content of tomato in variety Golden green.**

| Source | D.F. | SS | MSS | F Cal. | Result |
|---|---|---|---|---|---|
| Treatment | 6 | 2,190.09 | 365.015 | 387.319 | S |
| Error | 42 | 39.581 | 0.942 | | S |
| Total | 48 | 2,229.67 | | | |