MANAGEMENT OF EARLY BLIGHT DISEASE OF TOMATO (*Solanum lycopersicum* L.) UNDER PROTECTED CULTIVATION

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(2016-21-007)

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THESIS

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DECLARATION

I, Sumbula V. (2016-21-007) hereby declare that this thesis entitled **"Management of early blight disease of tomato (*Solanum lycopersicum* L.) under protected cultivation"** is a bonafide record of research work done by me during the course of research and that the thesis has not been previously formed for the award of any degree, diploma, fellowship or other similar title, of any other university or society.

28/11/2020

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CERTIFICATE

Certified that this thesis entitled **"Management of early blight disease of tomato (*Solanum lycopersicum* L.) under protected cultivation"** is a record of research work done independently by **Mrs. Sumbula V.** (2016-21-007) under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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INTRODUCTION

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most remunerative and widely grown vegetables in the world. In fact, it is fifth important cultivated crop after rice, wheat, maize and potato. Globally, the crop occupies an area of 4.8 million hectares with a production of 125.4 million tonnes. After China, India is the second largest producer of tomato accounting eleven per cent share of world production.It occupies an area of 0.797 million hectare, with a production of 20708 metric tons and productivity of 20.7 metric tons/ha in the year 2017 (FAO, 2017). In India, it is grown in a wide range of climate across states of Andhra Pradesh, Odisha, Karnataka, Maharashtra, Tamil Nadu, West Bengal, Bihar, Gujarat, Uttar Pradesh, Madhya Pradesh and Chhattisgarh. In Kerala, its commercial cultivation is limited to Chittoor tracts of Palghat district, even though it has good demand throughout the state. Recently, an increase in tomato cultivation was observed in the state with the advance of protected cultivation techniques. With the coordinated efforts of central and state Governments, protected cultivation is gaining popularity in Kerala. Production of vegetable crops under protected structures during off-season helps to fetch better market prices for the produce.

Cultivation of tomato is constrained by various fungal, bacterial and viral diseases. Among the fungal diseases, early blight caused by *Alternaria solani* is the most common and destructive disease widespread in tropical, subtropical and temperate regions of the world. It is increasingly becoming a limiting factor for successful cultivation of tomato and causes yield lose in the range of 15-100 per cent (Sahu *et al.*, 2013). Under favourable conditions more than 80 per cent disease severity of early blight has been recorded in tomato crop (Kumar and Srivastava, 2013). The disease can occur over a wide range of climatic conditions, but is most prominent in areas with heavy dew, rainfall and high relative humidity (Abada *et al.*, 2011). Moreover, prevalence of microclimate which is congenial for multiplication and spread of the pathogens, high density cropping and mono-cropping of high yielding genotypes make the plants under polyhouse predisposed to pathogens.

Primary methods of controlling the disease include preventing long periods of wetness on the leaf surface, cultural scouting, sanitation, and development of disease resistant varieties. Moreover, the ultimate control of this disease can be achieved through cultivation of resistant varieties, but it remains limited by the evolution of new strains of the pathogens. Hence, farmers mainly rely up on fungicides for the control of pathogens *viz. A. solani*, the casual organism of early blight. However, unplanned or overdosed and wide indiscriminate use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. Hence, application of fungicides at proper dose and time interval is mandatory. Moreover, innovative and safe methods like use of biocontrol agents need to be identified and evaluated to reduce the dependence on harmful chemicals. So, the fungicides and bioagents are commonly used to manage plant pathogens in many parts of the world. But little is known about their effects on the non-target microbial communities that inhabit inside and outside the plant. These treatments may lead to destruction of valuable beneficial microbes which in turn lead to increased vulnerability of crops. Hence, it has become necessary to consider the effect of different foliar treatments on target and non-target microbial communities while formulating disease management strategies.

Effect of fungicides and bioagents on culturable microorganisms can be assessed by serial dilution plating and their effect on non-culturable microorganism can be studied only by metagenomic analysis. Metagenomics is a recent technique used for studying microbial communities from their natural environment, without prior culturing. Since metagenomic analysis reveals the diversity of total microbiota in plant the loss of valuable microbial diversity can be assessed by the technique. Understanding the changes in properties such as structure, diversity, richness, and dynamics of indigenous microbial communities due to the application of fungicides and bioagents are essential for formulating sustainable, economical and environment friendly management strategies against plant diseases. In this context, metagenomics is the most suitable technique that

provides insightful predictions about micro inhabitants of plants and underlying processes that govern the organization of those systems.

Considering the importance of high value crop tomato, it has become necessary to formulate effective chemical and biological treatments against early blight disease of tomato under protected cultivation with emphasis on their effects on non-target microbial communities. Hence, the present study was undertaken giving much emphasis on the following aspects.

- Symptomatology of the disease
- Cultural and morphological characters of the pathogen
- *In vitro* evaluation of fungicides and bioagents against the pathogen
- Management of early blight disease of tomato under protected cultivation
- Enumeration of non-target culturable microflora
- Survival of the bio control agents on the phylloplane of tomato
- Metagenomic analysis to assess the impact of foliar treatments on non-target microflora

REVIEW OF LITERATURE

2. Review of literature

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop next to potato. Varied climatic adaptability and high nutritive value are made the tomato cultivation more popular all over the world. Tomato is the richest source of vitamin A and C and supplies a sufficient amount of the antioxidant lycopene pigment, this helps to protect the body against cancer and heart disease (Bohm *et al.* 2016). Because of its wide use and nutritional values, there is a high demand for both fresh and processed tomatoes. Increasing the production of tomato is therefore required to fulfill the ever-increasing demand.

The productivity of tomato is greatly hampered by environmental and biotic factors. The environmental factors such as radiation intensity, temperature, concentration of carbon dioxide (CO2) in the atmosphere, availability of nutrients (Krumbein *et al*., 2006) and biotic factors *viz.* fungal, bacterial and viral infestation (Fakhro *et al*., 2010) affect the growth and productivity of the crop, and quality of tomato fruits. Among the fungal diseases, early blight caused by *Alternaria solani* is the most common, destructive and widespread in all the tomato growing tracts and cause yield losses up to 79 and 67 per cent under field and protected cultivation respectively (Basu, 2011).

2.1. Protected cultivation of tomato

The open field production of various vegetables is subjected to both direct biotic and abiotic factors such as heavy rain, thunderstorms, excessive solar radiation, temperatures and humidity levels above plant growth optima (Kleinhenz *et al*. 2006; Nguyen *et al*. 2009) the high insect pest infestation pressure (Fuchs *et al* 2006; Max *et al*., 2012) and fungal diseases (Heine *et al*., 2012). Hence, protected cultivation can provide sustainable solutions for these problems. Furthermore, production of vegetable crops under protected structures during off-season helps to fetch better market prices for the produce.

Protected cultivation is a technique wherein the microclimate in the surrounding area of the plant is controlled partially or fully or modified to protect the crop from weather especially very low or high temperatures, hail storms and heavy rains. This technology is useful for protecting the plants from birds, insects etc. and conserving the soil moisture simultaneously (Ummyiah *et al.*, 2017). Protected conditions for vegetable cultivation are created by using different types of structures, which are season- and location-specific. These structures are designed as per climate modification requirement of the area. Temperature, humidity, wind velocity, soil conditions, etc. also play a major role in the design of protected structures for growing vegetable crops (Sirohi *et al.*, 2017). The demand of vegetables and drastically shrinking land holding, protected cultivation of vegetable crops is becoming popular among the farmers and best alternative for using land and other resources more efficient (Sindhu and Chatterjee, 2020)

Tomato being a high value vegetable crop, has been more popular for cultivation in protected structures. Generally indeterminate tomato varieties are suitable for polyhouse cultivation (Chandra *et al*., 2000). Cheema *et al.* (2004) studied the production of off-season tomato crop under net house conditions at Ludhiana (India). The results revealed that net house cultivation has extended the fruit availability of tomato from last week of January to first week of June. These studies have offered the possibility of raising off-seasonal crop of tomato and enhancing the fruit availability period by using non-chemical methods of pest control.

Parvej *et al.* (2010) studied phenology behavior in tomato under greenhouse condition and concluded that fruit maturity in polyhouse was advanced 17 by 5 days compared to crop raised in open field condition. The plant in polyhouse had higher number of flower clusters/plant, fruits/plant, fruit length, fruit diameter, individual fruit weight, fruit weight/plant and fruit yield over open field condition.

Kaddi *et al.* (2014) reported that the vine length, number of leaves, fruit weight, fruit length and fruit width were significantly higher under naturally ventilated polyhouse and insect-proof net house compared to open field conditions. The seed yield/fruit and seed

yield/1000m^2 were significantly higher in kharif season compared to summer. However, studies revealed that incidence and severity of bacterial wilt and early blight was more in polyhouse condition (Amar and Banyal, 2011). Protected structures have led to changes in the microclimate of protected crops. Restricted air exchange results in the atmospheric humidity being much higher inside insulated greenhouses than conventional ones which encourage several plant diseases and cause physiological disorders. This microclimate change could alter stages and rates of development of the pathogen, modify host resistance, and result in changes in the physiology of host - pathogen interactions (El-Mougy *et al.*, 2011).

2.2. Early blight disease of tomato

Early blight of tomato caused by *A. solani* is an important and widely distributed disease throughout the world. The disease was first described by Ellis and Martin (1882) from U.S.A. on potato and the causal organism was identified as *Macrosporium solani*. Later Jones and Grout (1897) transferred the fungus to the genus *Alternaria* on the basis of formation of spores in catenulate (in chains) in culture. The name early blight was ascribed to the disease by Jones (1991) because of the sever attacks on early maturing cultivars than medium or late maturing cultivars. It is the most catastrophic diseases incurring loss both at pre and post-harvest stages causing 35 to 78 per cent reduction in yield (Jones, 2000). Every one per cent increase in intensity can reduce yield by 1.36 per cent and complete crop failure can occur when the disease is more severe. Under favorable conditions more than 80 per cent disease severity of early blight has been recorded in tomato crops (Kumar and Srivastava, 2013).

The disease can occur over a wide range of climatic conditions, but it is most prominent in areas where received heavy dew deposition, heavy rainfall precipitation and high relative humidity. In severe rainfall, high humidity and fairly high temperatures 24-30^{0}C are more favourable for disease development (Peralta *et al.* 2005). Recently, a survey on field diseases of tomato in four districts of West Bengal, revealed that amongst the fungal diseases, blight caused by *Alternaria* species was the most predominant one with

the crop loss in field ranging from 70 to 100% (Kanjilal *et al.*, 2016). It occurs to some extent every year wherever tomatoes are grown. In spite of its name, the disease may occur at any time during the growing season (Vloutoglou and Kalogerakis, 2016).

Early blight is a three-phase disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is the most common and destructive part of the disease responsible for significant economic losses sustained by tomato producer each year. Infection accompanied by the production of toxins by *A. solani*, including some non-host specific toxins called alternaric acid, zinniol, altersolanol and macrosporin. The toxins act on the host protoplast to disturb physiological processes that sustain plant health (Koley *et al*., 2017).

2.3. Pathogen

The genus *Alternaria* is large and consists of several economically-important plant pathogens including *A. solani*, *A. alternata* and *A. brassicicola*. This genus was first recognized by Berkeley (1836) and he identified the causal fungus on plants belonging to family Brassicaceae as *Macrosporium brassicae* which was later renamed as *A. brassicae* (Berk.) Sacc. Butler (1903) reported *A. solani* for first time in India on *S. tuberosum* from Farukhabad district of Uttar Pradesh. Many species of *Alternaria* are significant causes of necrotrophic diseases of crops. For most species including *A. solani*, no sexual stages have been reported. *Alternaria* produce unique club-shaped conidia, often beaked with horizontal and often vertical septa that may be produced either individually or in a chain, depending on the species. Hyphal cells are darkly pigmented with melanin, which guard's hyphae and spores against environmental stress and allows spores to survive in soil for a long periods of time (Rotem, 1994).

The classification of *Alternaria solani* belongs to the phylum *Ascomycota*, subdivision *Pezizomycotina*, class *Dothediomycetes*, order *Pleosporales*, family *Pleosporaceae* genus *Alternaria* and species *Alternaria solani* (Simmons, 2007). *A. solani* reproduces asexually; a sexual stage of this fungus is unknown. The fungus overwinters in soil, plant debris, seed and alternate hosts in the form of either conidia or

mycelia, which may serve as primary sources of inoculum. The thick cell wall of conidia enables the fungus to adapt to adverse climatic conditions (Foolad *et al*., 2008). Infection occurs during warm and humid conditions. Conidia germinates at temperature of 8–32 °C in cool and humid conditions in the presence of moisture to form germ tubes (Jones, 1991). Germ tubes penetrate host tissue directly or enter through stomata or through wounds, thereby causing infection. Lesions appear after 2–3 days of infection depending on environmental conditions, leaf age and cultivar susceptibility, and spores are produced 3–5 days after the appearance of lesions (Agrios, 2005).

Generally, a long period of wetness is needed for spore production, but spores are also produced during alternate wet and dry conditions. First, conidiophores are developed during wet nights, which then produce spores or conidia in another wet night after the period of day light and dryness. In the next step, conidia are rapidly dispersed through wind and rain splash and continue the disease cycle in other healthy parts of the same plant or different plants. Early blight has the potential of causing polycyclic infection because of its short disease cycle. *Alternaria solani* has ability to survive for a long time on the diseased plant deberies in soil in the absence of main host (Basu, 2005). Rotem (2006) reported that *Alternaria solani* may survive for more than ten years in the soil on plant debris and seeds at optimum temperatures. *Alternaria solani* survived on also other solanaceous cultivated crops such as potato, pepper, egg plant and weed host.

2.4. Symptomatology

Symptoms of early blight disease first appears as small brown-to-black lesions on older foliage. The tissue surrounding the primary lesions may become bright yellow, and when lesions are numerous, entire leaves may become chlorotic. As the lesions enlarge, they often develop concentric rings giving them a bull's-eye or target-spot appearance (Walker, 1952). Ramakrishnan *et al*. (1971) reported that the spots were oval or angular in shape ranging from 0.3 to 0.4 cm diameter in size with usually narrow chlorotic zone around the spot. Symptoms were progress from lower leaves to upper leaves. Leaf spots

begin as small brown areas on lower leaves. As the spots matured, concentric rings of raised and depressed brown tissue were evident. Heavily infected plants often become defoliated.

Datar and Mayee (1981) observed cankerous spots on tomato stems of seedling causing by *A. solani*. They were especially injurious when they occurred at the juncture of the stem and side branches. Tomato fruit, both green and ripe, may also become infected with the fungus. Infection generally begins at the end of calyx region. Brown leathery areas were formed at infection sites and a mass of black spores may be evident on fruit lesions when ideal weather conditions exist. Sherf and MacNab (1986) described the first symptoms of early blight as small, dark, necrotic lesions that usually appear on the older leaves which subsequently spread upward as the plants become older. In severe epidemics *A. solani* can cause premature defoliation, which weakens the plants and exposes the fruit to injury from sunscald.

Chaerani *et al.* (2006) noticed symptoms on tomato due to early blight as collar rot on stem of seedlings, lesions on stem of adult plant and rotting on fruits. Foolad *et al.* (2008) described early blight symptoms on potato and tomato foliage as small, dark, circular lesions becoming distinctly zonate as they develop. Stem lesions was occurred on diseased tomato plants as roughly circular, sunken, dark and zonate. He also found that the pathogen can attack on green and ripe fruits at the stem end and cause cracks and other wounds. Blancard *et al*. (2012) observed the appearance of rot spots on green and ripe fruit of tomato.

Peralta *et al.* (2005) reported that early blight is the major disease symptom caused by the fungus *A. solani*. This disease can lead to complete defoliation in severe case in regions where received heavy rainfall, high humidity and fairly high temperatures (24°–29°C). When conditions are favorable for disease development, lesions can become numerous and plants defoliate, reducing both fruit quantity and quality. Fruit can become infected either in the green or ripe stage through the stem attachment. Lesions can become quite large, involve the whole fruit, and have characteristic concentric rings. Infected fruit often drops, and losses of immature fruit may occur. Fruit on defoliated plants are also

subject to sunscald. Stems and petioles affected by early blight have elliptical concentric lesions, which severely weaken the plant. Lesions at the base of emerging seedlings can cause collar rot. If this arises consecutively on many seedlings, it may indicate contamination of tomato seeds or soil used for planting (Fulya and Sally, 2010). Early blight starts appearing from the seedling stage and persists till the last harvest of fruits. The disease appears on leaves, stem and fruits, causing defoliation, drying of twigs and premature fruit drop depending upon the severity (Verma and Verma, 2010).

2.5. Pathogenicity

Barksdale (1968) and Dhiman *et al*. (1980) confirmed the pathogenicity of early blight of tomato caused by *A. solani* by spore suspension ($2x10^{4/ml}$). Further, they atomized the culture suspension on three leaf stage seedlings at the rate of 30 ml per seedling for successful inoculation. Coffey *et al*. (1975) observed that the early blight severity was gradually increased on young tomato plants with increased as conidial concentration from 5×10^3 to 8×10^4 conidia/ ml. A positive relationship between inoculum concentration and symptom development has also been demonstrated for other *Alternaria* species by Vloutoglou and Kalogerakis (2000).

Vloutoglou *et al.* (2001) studied the effects of inoculum concentration, wetness duration and plant age on development of early blight and on shedding of leaves in tomato plants. They reported the main effect of early blight was premature defoliation which was linearly related to the percentage of leaf area showing symptoms. They also observed that the 4-6 hrs of wetness are sufficient to initiate the disease on plants and as wetness duration increased up to 24 h, there was an increase in the percentage leaf area showing symptoms and in the percentage of defoliation. Arunakumara (2006) conducted pathogenicity test by inoculating of spore suspension and homogenized mycelium bits (2×10^4 spore ml-1) of *A. solani* on foliage of 30 days old tomato seedlings. Tippeswamy *et al*. (2010) confirmed the pathogenicity of early blight of tomato by spraying $1x10^4$conidial suspension of *A. solani* on 30 days old seedlings, before flowering (60 days) and after flowering (90 days).

Andrus *et al.* (1945) confirmed the pathogenicity on tomato by using mycelial fragments of *A. solani* as inoculum. Locke (1949) used blended mycelial fragments of *A. solani* for puncture inoculation. Brock (1950), Henning and Alexander (1959) used the suspension of mycelial fragments of *A. solani* to inoculate the leaves of field or green house grown plants. Barksdale (1968) and Dhiman *et al*. (1980) used suspension containing $2x10^4$ spores/ml for proving pathogenicity of early blight of tomato caused by *A. solani.* Further, they atomized the culture suspension on three leaf stage seedlings at the rate of 30 ml per seedling for successful inoculation. A positive relationship between inoculum concentration and symptom development has also been demonstrated for other *Alternaria* species (Vloutoglou *et al.*, 2001). Pathogenicity test was carried out by inoculating with spore suspension and homogenized mycelial bits (2×10^4 spores/ml) of *A. solani* on foliage of 30 days old tomato seedlings (Arunakumara, 2006). Tippeswamy *et al*. (2010) confirmed the pathogenicity of early blight of tomato by spraying 10^4 conidial suspension of *A. solani* to one-month seedlings (30 days), before flowering (60 days) and after flowering (90 days).

2.6 Characterization of pathogen

2.6.1 Cultural characters

The taxonomy of *Alternaria*, particularly as it relates to early blight, is undergoing revision. In 2000, Simmons recognized new species among the *A. solani*-like isolates from Solanaceae hosts. Based on cultural and morphological differences, Simmons proposed *A. tomatophila* as "the common and widely distributed causal agent of early blight of tomato" (Simmons, 2007). More recently, Woudenberg *et al*. (2014) grouped several large-spored isolates including those from Solanaceae, Cucurbitaceae and Scrophulariaceae into a new species designated *A. linearia*e . Large spore species were distinguished from small spore forms including *A. alternata* and *A. arborescens* (*A. alternata* fsp *lycopersici*), which have also been isolated from solanaceous plants and have been reported to cause early blight-like symptoms in some situations (Bessadat *et al*., 2017). To differentiate these closely-

related large-spored *Alternaria* species, several morphological, molecular and chemotaxonomic approaches have been applied.

Kaul and Saxena (1988) noted the cultural variability of *A. solani* isolates on PDA and classified into 4 distinct cultural groups based on types of growth, colony colour, colour of the substrate and growth rate. *A. solani* culture on PDA medium produced hyphae, which were grey white or grey brown in colour and even yellow pigment was secreted by some isolates. Moreover, Perez and Martinez (1995) reported that variability in 4 isolates of *A. solani* with respect to cultural characters like colony growth, colony diameter, mycelial colour, colony texture and pigmentation on medium differed between isolates and it was concluded that *A. solani* exhibited variability. The pathogenicity varied significantly among isolates (Yunhui *et al.*, 1994).

Babu *et al*. (2000) described the variability in cultural characteristics for isolates of *A. solani*, the incitant of early blight of tomato collected from 20 locations in Madurai and Dindigul districts of Tamil Nadu. The pigmentation varied from yellow, brown, black, brownish to greenish black in isolates of *A. solani* on potato dextrose agar medium. In general, radial growth of all isolates ranged between 14.9 mm and 32.2 mm on PDA and 24.3 mm to 53.7 mm on three selective media i.e., ASM, V-8 juice agar and V-8 juice agar (synthetic) on the fourth day. Most of the isolates showed smooth mycelial growth with circular and irregular margin and with concentric zonation (Kumar *et al.*, 2013*)*.

Nikam *et al.* (2015) studied the pathogenic variability in tomato cultivar Pusa Ruby with the eight isolates of *A. solani*. The test isolates could grow better on the basic culture medium potato dextrose agar; however, highest mycelial growth was recorded on the isolate AsLt (88.50 mm), followed by AsBd (82.36mm) and AsHl (78.40 mm), with excellent sporulation. All the eight test isolates exhibited a wide range of variability in respect of their mycelial and conidial dimensions and septation.

2.6.2 Morphological characters

The morphology of an individual is the ultimate expression of its growth processes and final display of all its complex relationships with its normal habitat. Joly (1959) studied on morphological variations of *Alternaria* species and later during 1964 divided in three sections and proposed a simple key for identification at species level. Further, Ellis and Gibson (1975) noticed the conidia of *Alternaria solani* are muriform and beaked.

Neergaard (1945) reported that *Alternaria solani* as large spores producing group of fungus and characterized by separate conidia borne singly on simple conidiophores within the genus *Alternaria.* The mycelium consisted of septate, branched, light brown hyphae, which turned darker with age. The conidiophores were short, 50 to 90 μm and dark coloured. Conidia were 120-296 x 12-20 μm in size, beaked, muriform dark coloured and borne singly (Bose and Som, 1986). However, in culture they formed short chains. According to Singh (1987) the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

According to morphological characters and physiologic analysis, *A. solani* belongs to large, long beaked and concatenated spores (Simmons, 2007). The mycelium consisted of septate, branched, light brown hyphae, which turned darker with age. The conidiophores were short, 50 to 90 μm and dark coloured. Conidia were 120-296 x 12-20 μm in size, beaked, muriform dark coloured and borne singly. However, in culture they formed short chains. According to Singh (1987) the conidia contained 5-10 transverse septa and 1-5 longitudinal septa.

2.7 Disease management

Early blight is one of the most destructive diseases of tomato, causing considerable loss to quality and quantity of fruits. Therefore, effective, safe and economical strategies should formulate for the better management of early blight disease. The goal of plant disease management is to reduce the economic and aesthetic damage caused by plant diseases. Successful disease control requires thorough knowledge of the causal agent and

the disease cycle and host-pathogen interactions in relation to environmental factors, and cost.

2.7.1 Chemical control

It is well known that using the fungicides is considered as the shortest way to obtain efficient results of disease management. Several research workers have reported that timely application of fungicides is the best method to control early blight (Lodha and Prasad, 1973; Bartlett *et al.*, 2002; Kapsa and Osowski, 2003; Singh and Singh, 2006). Lodha and Prasad (1973) reported that copper-based fungicides gave good control of *A. solani in vitro*. Mallikarjun (1996) evaluated eight fungicides against *A. alternata* causing leaf blight of turmeric *in vitro* condition. Propiconazole (Tilt) was found superior in inhibiting the growth of the fungus. Dubey *et al*. (2000) investigated the effect of combination of different fungicides to inhibit the growth of *A. alternata* which is responsible for *Alternaria* blight of broad bean. He found that Hexaconazole (Contaf) inhibited total growth of *A. alternata* under *in vitro* conditions.

According to Dahmen and Staub (1992) difenoconazole (Score 250) was found to be very effective against early blight of potato because of its protectant, curative, eradicant mode of action and especially its long-lasting protective activity up to 3 weeks. Vloutoglou *et al.* (2001) tested the efficacy of Mancozeb (0.14% a.i.), Iprodione (0.075% a.i.), Prochloraz (0.025% a.i.), Chlorothalonil (0.15% a.i.) and Azoxystrobin (0.025% a.i.) in tomato plants with spore suspension of *Alternaria solani* as a protective and curative. They were found effective when the fungicides were applied one day prior to inoculation with reduction in disease severity by 91-100% and defoliation by 100% as compared to untreated control.

Prasad and Naik (2003) tested the efficacy of non-systemic fungicides (Iprodione, Mancozeb, Copper Oxychloride and Saaf), systemic fungicides (Thiophanate methyl, Triademefon, Benomyl and Carbendazim) in controlling early blight of tomato. Mancozeb gave the highest cost benefit ratio of 1: 1.14 in addition to reducing the disease incidence.

Abhinandan *et al*., (2004) confirmed the efficacy of commercial fungicides (Dithane M-45), (Mancozeb) at 0.25%, Kavach @ 0.25%, Rovral (Iprodione) @ 0.20%, Copper oxychloride (Blitox) @ 0.25%, Antracol (Propineb) @ 0.15%, Propiconazole @ 0.05%, Penconazole @ 0.05% in controlling early blight disease.

Miles *et al*. (2005) evaluated efficacy of strobilurins azoxystrobin, trifloxystrobin, pyraclostrobin and copper hydroxide, mancozeb, captan, iprodione and chlorothalonil in the field for control of brown spot caused by *A. alternata* on Citrus. They observed in all experiments, that strobilurins incorporated with copper, mancozeb and iprodione were better than the stand-alone application. Ashour (2009) noticed efficacy of mancozeb in early blight disease management and resulted in producing the highest fruit yield compared with antioxidants as well as the alternation between them.

Gaur (2009) evaluated nine fungicides against *A. alternata* under *in vitro* condition. Among the tested fungicides Prochloraz (95.3%) found most effective in inhibiting mycelial growth followed by Propineb (65.8%), Saaf (60.5%) and Mancozeb (57.8%). Patel and Choudhary (2010) screened the efficacy of different systemic and contact fungicides against early blight of tomato. Among systemic fungicides, Difenconazole inhibited maximum growth of *A. solani* and in contact fungicides, Mancozeb gave highest per cent inhibition.

Horsfield *et al*., (2010) conducted series of experiments to evaluate fungicide use strategies for the control of early blight, the most significant foliar disease of potatoes in Australia. The protective and curative activity of fungicides was evaluated in glasshouse and field studies. Boscalid, azoxystrobin, iprodione and propineb were highly effective in the control of early blight when applied up to three days before or three days after inoculation. Tofoli *et al*. (2010) reported the efficacy of different fungicides *viz.* difenconazole, tebuconazole, chlorothalonil, mancozeb, propineb, copper hydroxide and iprodione for the management of Alternaria leaf blight of tomato.

Sahu *et al.* (2013) opined that, the fungicides like Pristine, Maccani, Boscalid, Pyraclostrobin and mancozeb can be used not only to manage early blight but to increase the yield of tomato as well. The field evaluation of different fungicides indicated that fenamidone 10% + mancozeb 50% (0.2%) was most effective followed by Propiconazole 25% EC, Dimethomorph 9% + Mancozeb 60%, Cymoxanil 8% + Mancozeb 64% (0.2%) and Mancozeb (0.25%) and also economical in reducing severity of the early blight and increasing yield over control (Kumar *et al.*, 2018).

Unplanned, overdosed and wide use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. Hence, application of fungicides at proper dose and time interval is mandatory.

2.7.2 Biological control

The worldwide trend towards environmentally safe methods of plant disease control in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. Hence, recent efforts have focused on developing environmentally safe, long-lasting, and effective biocontrol methods for the management of plant diseases.

Trichoderma spp. are versatile fungi with diverse genetic variability, ubiquitousness, synthesize variety of secondary metabolites, colonize substrate effectively and grow under extremes of climatic regimes. This fungus show rapid growth, establish chemical communication with other soil microbiota and plants and posses ability to serve as plant's safeguard and growth promoter (Contreras-Cornejo *et al*., 2016; Kashyap *et al*., 2017). The principle biocontrol attribute of *Trichoderma* is believed to involve mechanism of mycoparasitism, antibiosis and niche exclusion, however, other indirect mechanisms of induced systemic resistance and growth promotion are also acknowledged to play a crucial role in biocontrol as well as in amelioration of abiotic stresses; enhancing plant growth and yield (Bae *et al*., 2016; Youssef *et al*., 2016).

Martinez and Solano (1995) were studied the antagonism of *Trichoderma* strains against *A. solani* on tomato. Strains L12 and LI7 showing 3 types of antagonism gave 45.7

and 38.77 per cent control of *A. solani* respectively. Kota (2003) reported that *Trichoderma harzianum* and *T. virens* highly inhibited the growth of *A. alternata* under *in vitro* condition. Later, inhibition of *A. solani* by *T. viride*, *Aspergillus niger*, *T. harzianum*, *T. koningii* and *T. hamatum* with inhibition per cent 51.7%, 50.6%, 48.3%, 42.5% and 42.5% respectively was reported by Mishra and Gupta (2008). However, Panchal and Patil (2009) reported a higher per cent inhibition with *T. viride* (65%) and *T. harzianum* (54%).

Plant growth-promoting microorganism (PGPM) is yet another group which play a vital role in the suppression of plant diseases. PGPRs such as *P. fluorescens* induce resistance in plants and suppress plant pathogens causing fungal, bacterial and viral diseases (Kloepper and Schroth 1978). Moreover, *Pseudomonads* are well known to show the growth promotion activity in plants and simultaneously activating the control of diseases (Weller, 1988). Casida and Lukezie (1992) were reported that *Pseudomonas strain* 679-2, was able to reduce the severity of the leafspot disease caused by *A. solani*. Babu *et al*. (2000) observed that spraying tomato cultivar PKM-1 with suspension of *P. fluorescens* strains 48 hours after inoculation with *A. solani* reduced leaf blight disease by 15-38 per cent compared to control.

Biocontrol methods such as seedling dip and foliar applications of *T. harzianum*, *T. viride* and *P. fluorescens* were found to decrease the early blight incidence up to 62 per cent and increased tomato yield up to 37 per cent (Ramanujam *et al.*, 2015). Lal *et al.* (2016) observed the effectiveness of seed treatment + foliar spray of *T. harzianum* and *P. fluorescens* in reducing the disease intensity of early blight disease of potato and also increasing tuber yield.

Several members of the genus *Bacillus*, including *Brevibacillus* and *Paenibacillus* species, produce various antimicrobial substances, e.g. antibiotics. Being capable of producing more than 70 different antibiotics, *B. subtilis* is one of the major producers of these substances in the genus (Katz and Demain, 1977). *Brevibacillus brevis* is also characterized by its capability to produce antibiotics. In addition, a wide range of antimicrobial substances are produced by *B. licheniformis*, *B. pumilus*, *B. circulans*, *B.*

cereus, Brevibacillus laterosporus, *Paenibacillus polymyxa* and other species (Foldes, *et al.*, 2000 and Peralta *et al.*, 2005). Furthermore, *Bacillus*-formulated bio-pesticides i.e. *B. thuringiensis, B. subtilis,* and *B. sphaericus* have a great potential for systematic plant resistance by inhibition of pathogen growth, development, feeding, and reproduction (Mnif and Ghribi, 2015). Additionally, several *Bacillus* spp. including *Bacillus subtilis* are globally dispersed bacteria that exhibit various inhibitory effects against phytopathogenic fungi and can synthesize many biologically active compounds including antibiotics, siderophores, lipopeptides, enzymes and exopolysaccharides (Rahman *et al.*, 2018).

Abdalla *et al.* (2014) conducted an *in vitro* screening of 45 *Bacillus* isolates against *A. alternata* and 27 *Bacillus* isolates showed antagonistic properties. Koley *et al.* (2017) studied efficacy of six bio-control agents and among them *B. subtilis*, showed the highest growth inhibition (52.77%) of the fungus, *A. solani* over the control. This was followed by two isolates of *P. fluorescens* with 47.22% and 45.55% of growth inhibition, respectively. *T. harzianum* and *T. viride* resulted less than 40 % growth inhibition. Moreover, earlier studies also confirmed the foliar application of *B. subtilis* managed early blight disease and improved growth in greenhouse and field conditions (Sundaramoorthy, 2013; Pane and Zaccardelli, 2015; Basamma and Kulkarni, 2016). Similarly, another study revealed that foliar application of *B. subtilis* alone and in combination with the plant nutrients managed EB disease significantly by 67–83%, while improved plant growth attributes by 20–77%. *B. subtilis* and plant nutrients helped the tomato plant to fight off the plant hacker by up-regulating the production of total phenolic contents and defensive enzymes (Awan and Shoaib, 2019).

2.8. Phylloplane microflora

Microbial communities on or around plants play a major role in plant functioning and vigour. The phyllosphere is an ecologically and economically important ecosystem that hosts a large and diverse microbial community. The phyllosphere, which consists of the aerial parts of plants, and therefore primarily, of the set of photosynthetic leaves, is one of the most prevalent microbial habitats on earth. The leaf surface habitat is vast: vegetation

modelling gives an estimated global leaf area of 508,630,100 km^2, which corresponds to 1,017,260,200 km^2 of upper and lower leaf surface, an area approximately twice as great as the land surface (Vorholt, 2012). The phylloplane – that is, plant foliage as a microbial habitat – is considered a hostile environment for survival and colonization by microorganisms due to the rapid fluctuation in solar radiation, temperature, humidity and heterogeneous availability of nutrients (Lindow and Brandl, 2003; Bulgarelli *et al*., 2013). Nonetheless, the phylloplane is populated by a large and diverse microbiota of bacteria, fungi, yeast, archaea and other microorganisms that have commensal, pathogenic and mutualistic interactions with the plant host. These communities can improve plant growth and health status through different microbe host interactions (Rastogi *et al*., 2013).

Bacteria are estimated to be the most abundant colonists of leaf surfaces with densities reaching as high as 10^8 cells per cm^2 (Leveau, 2006). The total size of the fungal population of the phylloplane has not yet been estimated but is expected to be lower. Beneficial bacteria predominate in this biotype, but some phytopathogens also take advantage of the conditions they find there and use it for the first stage of infection. Bacterial communities are generally dominated by the phylum *Proteobacteria*, including *Methylobacterium*, *Sphingomonas*, *Xanthomonas*, *Pantoea*, and *Pseudomonas* (Delmotte *et al.*, 2009; Vorholt, 2012; Rastogi *et al*., 2013), although other bacterial phyla such as *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* have also been identified at the core of the phyllosphere community (Vorholt, 2012 and Bulgarelli *et al.*, 2013). Most of them colonize the plant surfaces as large and heterogeneous aggregates, which allows them to withstand the surrounding conditions (Vorholt, 2012 and Rastogi *et al*., 2013). These aggregate structures confer on them the advantage of maintaining a hydrated surface through production of extracellular polymeric substances (Lindow and Brandl, 2003; Whipps *et al.*, 2008: Vogel *et al.*, 2016). This production is playing an important role (adhesion) in the epiphytic style of some of the pathogenic bacteria that colonize the plant surface.

Foliar surfaces also support a diverse community of fungi; however, their population sizes are typically lower than their bacterial counterparts. Phyllosphere fungal communities are known to impact the fitness of their host plant (Herre *et al.*, 2007; Sunshine *et al*., 2009). Compared with bacterial communities, relatively little is known about fungal community structure and function on leaf surfaces or how fungi impact bacterial community composition. Suda *et al.* (2009) demonstrated a larger population size, greater functional diversity, and increased species richness in bacterial communities in the phyllosphere of powdery mildew fungus-infected leaves.

The most abundant of the fungi on the surfaces of leaves are the members of phylum Ascomycota, Basidiomycota. Jumpponen & Jones (2009) investigated the fungal communities in the phyllosphere of oak trees (Quercus macrocarpa) and found that, *Devriesia*, *Mycosphaerella*, *Ramularia*, *Stenella*, *Dioszegia*, *Paraphaeosphaeria*, *Phaeosphaeria* and *Sphaceloma* were dominant fungal genus. Larran *et al.* (2010) identified *Alternaria, Stemphilum, Penicllium, Epicoccum, Ulocladium, Nigrospora, Torula, Septonema, Aspergillus* and *Phaeoseptoria* spp. as species predominantly colonizing wheat leaves. Luo *et al.* (2019) reported the occurance of *Sporobolomyces, Davidiella, Phoma, Alternaria, Aureobasidium, Pleosporales* on cucumber phyllosphere.

For enumerating the phylloplane microflora, microorganisms are usually washed off the leaf surface in a wash buffer (Lindow *et al.* 1978 and Hirano *et al.* 1982), which is then inoculated onto artificial growing media and incubated. Using buffers instead of water to wash the microorganisms from the leaf surfaces ensures the stability of the osmotic pressure and maintains the pH at a predetermined level that allows survival of the microorganisms under investigation (Morris *et al.* 1997). Dilution plating and culturing, the traditional culture-based analysis of can be effectively used to evaluate the heterotrophic portion of the microbial population (Kirk *et al.*, 2004), while 99 percent of the population lies beyond the limit of being grown on a culture media. An advantage of this method is that many leaves can be combined into one sample, while dilution ranges can be used to control the density of microorganisms on the plates and to recalculate the

number of colony forming units (cfu) recovered from the leaves. The bulking of samples allows large numbers of samples to be processed in a relatively short period (Bakker, 2004).

2.8.1 Impact of disease management practices on non-target culturable phylloplane microflora

Different factors contribute to the shaping of microbial communities in the phyllosphere, including environmental cues, agricultural practices, microbial interactions, plant genotypes and phenotypes (Lindow and Brandl, 2003). However, microbial populations on foliage in agricultural settings are influenced by management practices such as organic vs. conventional farming (Waipara *et al.,* 2002), application of pesticides (Zhang *et al.*, 2009) fungicides (Newton *et al.*,2010) and antibiotics (Balint and Stapleton, 2011) and as well as nitrogen fertilization (Ikeda *et al*., 2011).

The use of foliar pesticides to control diseases can cause major disruption of phylloplane microorganism populations, often reducing the number and diversity of organisms. This can have a negative effect on naturally-occurring biological control, which in some cases, makes the plants more susceptible to other disorders (Hislop 1976; Bosshard *et al.* 1987*).* For example, when pathogenic microorganisms on the leaf surface are less sensitive to pesticides than their antagonists are, this may result in increased disease severity (Fenn *et al.* 1989). In an experiment by Dix and Webster (1995), the use of non-selective fungicides, such as zineb and mancozeb, increased disease severity due to the removal of antagonists from the leaf surface, while the more selective fungicides benomyl and maneb initially reduced pathogen as well as epiphytic populations, but antagonist populations recovered and disease severity was reduced. Pandey and Kumar (1988) found that population of *Alternaria alternata* on soybean leaves were reduced by thiram, and that captan, ziram, mancozeb, zineb and carbendazim caused decline in species richness, whilst populations of *Aspergillus nidulans* and *Penicillium citrinum* increased. Evidence suggests that increased infection of apricot by *Eutypa armeniacae* (die back) when copper fungicides are used is due to a fungicidal reduction of the antagonistic leaf saprophyte *Fusarium lateritium* (Stirling *et al*., 1999).

The occurrence of fungicide-insensitive nontarget pathogens or of fungicide-resistant strains of target pathogens after fungicide application may be the result of a disturbance of the balance among pathogens, between the pathogen and antagonistic saprophytes, or both. Elmholt (2013) found that propiconazole had severe effects on mycorrhiza in wheat and on non-target soil fungi. John and Charles (2014) reported 10 to 1000-fold reduction in microbial populations on fungicide treated apple leaves. Raj (2016) observed a drastic reduction of cucumber phylloplane microbes after spraying fungicides like mancozeb, cymoxanil + mancozeb and potassium phosphonate + hexacoazole. Pawar (2017) reported more than 50% reduction of phylloplane microbial population after spraying fungicides *viz.* mancozeb, tebuconazole and difenoconazole on capsicum. The negative effects of these fungicides last long 6 months noticed that iprodione and difenoconazole + propiconazole had severe effects on non-target fungal populations of citrus leaves. He also observed that the negative effects of propineb and metiram disappeared two months after application, then the fungal population started to increase again.

2.9. Endophytic microflora

Endophytes are bacterial or fungal or actinomycetes microorganisms that colonize healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease (Wilson, 1995). They are ubiquitous, colonize in all plants, and have been isolated from stem, roots, petioles, leaf segments, inflorescences of weeds, fruit, buds, seeds and also dead and hollow hyaline cells of plants (Hata and Sone, 2008; Specian *et al*., 2012; Stępniewska and Kuzniar, 2013). The population of endophytes in a plant species is highly variable and depends on various components, such as host species, host developmental stage, inoculum density and environmental condition (Dudeja and Giri, 2014).

Endophytes association can be obligate or facultative and causes no harm to the host plants. They exhibit complex interactions with their hosts which involves mutualism

and antagonism. Plants strictly limit the growth of endophytes, and these endophytes use many mechanisms to gradually adapt to their living environments (Dudeja *et al*., 2012). These microorganisms induce plant growth using several mechanistic approaches such as biological nitrogen fixation (Rayan, 2008) phytohormone production (Hurek and Hurek, 2011), phosphate solubilization (Brader *et al*., 2014), inhibition of ethylene biosynthesis (Hamilton *et al*., 2012). The microorganism also play a major role in tolerance to abiotic stresses by inducing resistance in plant to counteract against pathogenic attacks (Raaijmakers and Mazzola, 2012) or by the release of secondary metabolites such as enzymes, siderophore, and antibiotics (Christina *et al*., 2013).

There are endophytic bacteria, fungi, and/or actinomycetes whose isolation from the plant tissues has been a challenge since the studies on endophytes started. Several researchers have reviewed extensively different methods of the isolation of bacterial endophytes (Hallmann *et al.*, 1997 and Hurek and Hurek, 1998). Endophytes are isolated by initial surface sterilization followed by culturing from ground tissue extract (Rai *et al.*, 2007) or by direct culturing of plant tissues (Hata and Sone, 2008) on media suitable for bacteria or fungi or actinomycetes. Previous studies showed significant difference in endophytic colonisation and the type of endophytes between root, stem and leaf tissues (Lodge *et* al., 1996). Mostly, roots have the high population of endophytes when compared to stem and leaves (Rosenblueth and Martinez, 2004). Yang *et al.* (2011) recorded endophytic bacteria from leaves and found that, the isolation efficiency of bacteria from stems was higher than from leaves. Patel *et al*. (2012) isolated 18 bacterial endophytes from root and stem of tomato plants collected from different regions of Gujarat. Philip *et al.* (2020) reported the use of antagonistic endophytic bacteria against the control of rubber disease.

Endophytes are associated with plants in various forms, including bacteria or fungi or actinomycetes that have been colonized inside the plant tissues. Jacobs *et al.* (1985) reported seven bacterial genera such as *B. subtilis, Erwinia herbicola, P. fluresence, Corynebacterium* sp., *Lactobacillus* sp. and *Xanthomonas* sp. from healthy sugar beet root

tissue. Filho *et al.* (2010) observed the presence of *Paenibacillus macerans* and *Bacillus pumilus* as endophytic bacteria in tomato and reported its antagonistic activity against early blight pathogen *A.solani.* James and Mathew (2015) isolated 79 endophytic bacteria from tomato root and stem and among these 12 bacteria were found highly antagonistic to *R. solanacearum*. More than 200 genera from 16 phyla of bacterial species have been reported to be associated with endophytes and among them, most of the species belong to the phyla Actinobacteria, Proteobacteria, and Firmicutes (Golinska *et al*., 2015). The diversity of endophytic bacteria ranges from gram-positive to gram-negative bacteria, such as *Achromobacter, Acinetobacter, Agrobacterium, Bacillus, Brevibacterium, Microbacterium, Pseudomonas, Xanthomonas* etc. (Sun *et al*., 2015).

Actinomycetes are prokaryotic microorganisms that belong to the phylum Actinobacteria and possess mycelium like fungus and forms spores (Chaudhary *et al*., 2013; Barka *et al*., 2016). Castillo *et al.* (2002) isolated endophytic actinomycetes from stem and root of tomato, banana and wheat. Sreeja (2011) isolated five endophytic actinomycetes from tomato which belonged to genus *Streptomyces*. Endophytic actinomycetes are known to produce various chemical entities with unique structures of considerable medicinal importance (Gayathri and Muralikrishnan, 2013; Singh and Dubey, 2015).

Fungi are a heterotrophic group of organisms with various life cycles that include symbiotic relationships with a wide variety of autotrophic organisms (Dayle *et al*., 2001). Endophytic fungi have been classified into two broad groups based on their phylogeny and life history traits. These include the clavicipitaceous, which infect some grasses confined to cool regions and the non-clavicipitaceous endophytes, which are from asymptomatic tissues of non-vascular plants, ferns and allies, conifers and angiosperms and are limited to the Ascomycota or Basidiomycota group (Jalgaonwala *et al*., 2011; Bhardwaj and Agrawal, 2014). The foliage isolates usually correspond among the Ascomycota to the Sordariomycetes, Dothidiomycetes, Pezizomycetes, Leotiomycetes and Eurotiomycetes, while basidiomycetous isolates belonging to Agaricomycotina, Pucciniomycotina and Ustilaginomycotina have been less frequently isolated from foliage than from woody

tissues. This could be, however, underestimated because of the low recovering on agar cultures (Arnold *et al.,* 2007). Kim *et al.* (2007) isolated different endophytic fungus from tomato leaves among which *F. oxysporum* EF119 showed the most potent *in vivo* anti-oomycete activity against tomato late blight. Kurian (2011) obtained *Penicillium minioluteum* as promising endophytic fungus from cacao against *Phytophthora* pod rot. Andrade-Linares *et al.* (2011) reported 51 fungal isolates from tomato roots, of which, 20 isolates belonged to *Fusarium* spp. and only three isolates showed antagonistic property. Xia *et al*. (2019) studied endophytic fungal communities of corn, tomato, pepper, and watermelon and found that *Pichia guilliermondi*, *Trichoderma spirale*, *Trichoderma atroviride*, *Chaetomium globosum*, *Fusarium* sp., *Periconia macrospinosa* and *Rhinocladiella* sp. were abundant in all tested crops.

2.9.1. Impact of disease management practices on non-target culturable endophytic microflora

The fungicides used to control crop diseases can have adverse effects on endophytic microorganisms, with possible consequences for plant health and productivity (Rachel *et al*., 2014). Reduction of *Lolium* an endophyte in ryegrass plants and seeds was observed with the application of fungicides *viz.* prochloraz, imazalil and propiconazole at 50 ppm concentration (Harvey *et al.*, 1982). Elmholt (2013) found that propiconazole had severe effects on mycorrhiza in wheat and on non-target soil fungi. John and Charles (2014) reported 10 to 1000-fold reduction in microbial populations on fungicide treated apple leaf endophytes.

Applications of the systemic fungicide Benlate resulted in increased populations of endophytic deleterious fluorescent pseudomonads and total culturable bacteria (Klopper *et al*., 2012). With citrus and leatherleaf fern, benlate treatment also resulted in higher percentages of virulent endophytic bacteria as shown in the hypersensitive reaction in tobacco and the production of pectinolytic enzymes in the potato slice assay (Klopper *et al*., 2013). Collectively the results and the emerging microbial ecology model indicate that

the development of systemically active agrichemicals should include assessment of effects on endophytic microbes.

Prior *et al.* (2017) studied the impact of copper, sulfur, and azoxystrobin based fungicides on fungal endophytic communities of common bean and broad bean. Treatments with systemic fungicide azoxystrobin showed the strongest effect on endophytic communities followed by copper and sulphur respectively and endophytes took five weeks to recover after treatment with azoxystrobin.

2.10. Metagenomic analysis- A culture independent approach

The term 'metagenomics' was introduced to provide an insight on the unculturable microflora that remain unidentified and unexplored and the ones that has not yet been catalogued using 16S rDNA sequences (Handelsman, 2004). In the area of microbial ecology, the term 'metagenomics' is now synonymous with the culture-independent application of genomics techniques to the study of microbial communities in their natural environments (Chen and Pachter, 2005). Current estimates indicate that only 1% microorganism present in most habitats are culturable and rest 99% are non-culturable and therefore they cannot be studied and understood in a way that microbial ecologists have become accustomed to over the past century. Metagenomics exploits the fact that while some microorganisms are culturable and others are not, all of them are life-forms based on DNA as a carrier of genetic information. The metagenomic toolbox allows accessing, storing, and analysing this DNA and thus can provide an otherwise hard-to-attain insight into the biology and evolution of environmental microorganisms, independent of their culturable status (Leveau, 2007).

Microbial communities on or around plants play a major role in plant functioning and vigour. Many of them have beneficial effects on plants as they may provide nutrients, antagonize pathogens and reduce plant stress symptoms. Plants are usually colonized by complex endophytic and phyllosphere communities, which to a large extent are not easily to cultivate. Different techniques have applied so-called 'first-generation' molecular

techniques [e.g. clone library Sanger sequencing, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism] to describe variation in community structure in the context of plant genotype, plant phenotype, and geographical location (Hunter *et al.*, 2010; Vokou *et al*., 2012; Izhaki *et al*., 2013). However, these techniques are low throughput and relatively expensive and allow only a superficial comparison of microbial communities (Rastogi & Sani, 2011). The advent of next-generation DNA sequencing significantly reduced costs and permitted multiplexing of hundreds of samples in a single sequencing run. This is dramatically changing the landscape of microbial ecology and offering new windows of 'omic exploration'. The 454-pyrosequencing platform was one of the 'first' to be widely implemented in microbial community analysis through 16SrRNA or ITS amplicon sequencing, shotgun metagenomics, wholegenome sequencing, and transcriptional profiling (Delmotte *et al*., 2009; Rastogi *et al*., 2013).

'Second' next-generation sequencing technology such as the Illumina platform (Degnan and Ochman, 2012) allows ultra-high-throughput sequencing of microbial communities and yields amounts of sequence data that are several orders of magnitude higher. Convergence of metagenomic with metaproteomic analysis, popularly known as proteogenomics (Rastogi *et al*., 2013), represents another important technical advancement. Combined, these technological innovations are greatly facilitating comparative ecological analyses and provide new insights into the structure, function, and variability of microbiota in the phyllosphere and other environments.

Application of next-generation molecular technologies in the area of phyllosphere and endophytic microbiology is rapidly increasing and so is the number of new studies providing further insights into community variation, drivers, functions, and interactions with biotic and abiotic components (Rastogi *et al*., 2013; Romero *et al.*, 2014; Aragón *et al*., 2017). The importance of microbial diversity on and in the plants has an emerging field of research enabling to studying the various beneficial properties of phyllosphere and endophytic microbial community. Jumpponen and Jones (2009) investigated the fungal communities in the phyllosphere of oak trees (*Quercus macrocarpa*) under rural or urban

management practices. High-throughput pyrosequencing of fungal internal transcribed spacer 1 (ITS1) exposed differences in the foliar fungal community composition and identified specific taxa that were responsive to these two management practices. *Devriesia*, *Mycosphaerella, Ramularia*, *Stenella, Dioszegia, Paraphaeosphaeria, Phaeosphaeria*, and *Sphaceloma* were more common in the oak trees in the rural environments, while *Aureobasidium, Davidiella, Didymella*, and *Microsphaeropsis* occurred more frequently in urban environments.

Bodenhausen *et al.* (2013) used 454 pyrosequencing to characterize the bacterial communities associated with the roots and the leaves of wild *Arabidopsis thaliana* and observed that Proteobacteria, Actinobacteria, and Bacteroidetes were the most abundant phyla in both leaf and root samples. Romero *et al.* (2014) analysed endophytic bacterial communities of tomato leaves by 16SrRNA gene pyrosequencing and found that leaf endophytes mainly comprised five phyla, among which Proteobacteria was the most represented, followed by Actinobacteria, Planctomycetes, Verrucomicrobia, and Acidobacteria.

Karlsson *et al.* (2014) described the major phyllosphere fungal community of wheat as 'pink' yeasts (*Sporobolomyces* and *Rhodotorula*),'white' yeasts (*Cryptococcus*) and ascomycete saprotrophs such *Cladosporium* and *Alternaria* by amplicon metagenomic analysis. Akinsanya *et al.* (2015) applied the high throughput techniques of NGS to the metagenomics study of endophytic bacteria in Aloevera plant, by assessing its PCR amplicon of 16S rDNA sequences (V3-V4 regions) with the Illumina metagenomics technique revealed Proteobacteria, Firmicutes, Actinobacteria and Bacteriodetes as the predominant phyla. Similarly, Luo *et al.* (2019) examined the microbial community structure and diversity of cucumber leaves by Illumina MiSeq sequencing and the cucumber phyllosphere microbiota was observed to be dominated by bacterial populations from Proteobacteria, Actinobacteria, and Firmicutes, as well as fungal species from Ascomycota and Basidiomycota.

2.10.1. Impact of disease management practices on non-target microflora by metagenomic analysis

Over the last two decades, there have been several concerns over the short term and long-term effects of the fungicides and bioagents application on crop plants. Such concerns relate to aspects such as effects on biodiversity, environmental and agricultural sustainability, survival and diversity of beneficial non-target microflora, food web and/or food chain, gene pool, gene transfer (Zhang *et al*., 2009). Next generation sequencing (NGS), together with the evolution of bioinformatic tools, and the emergence of metagenomic approaches have made it easier to comprehensively analyse microbial communities on or in any type of matrix, including plant tissues (Newton *et al*., 2010 and Alves *et al*., 2018).

Perazzolli *et al.* (2014) investigated the effect of penconazole fungicide on the leaf microbiota of the grapevine by pyrosequencing analysis. While comparing with untreated leaves, an increased abundance of bacterial family *Alcaligenaceae* and reduced abundance of *Microbacteriaceae* and *Methylobacteriaceae* was observed in penconazole treated leaves. He also reported the relative abundances of grapevine leaf bacteria (*Haemophilus*, *Swaminathania*, *Paracoccus*, *Roseomonas*, *Kineosporia*, and *Porphyromonas*) and fungi (*Epicoccum*, *Teratosphaeria*, *Exophiala*, *Claviceps*, and *Chalastospora*) on fungicide treated leaves was negatively correlated with downy mildew severity, suggesting that some strains of these genera could have a role as biocontrol agents. Karlsson *et al.* (2014) studied non target effect of fungicides *viz.* azoxystrobin, bixafen, cyprodinil, difenoconazole, fenpropimorph, metrafenone, picoxystrobin, prochloraz, propiconazole, prothioconazole and pyraclostrobin on wheat phyllosphere microflora by metagenomics approach. The microbial community composition was significantly different for fungicide-treated and untreated samples and fungicide treatments affected community evenness negatively. The proportion of Leucosporidiales and Dothideales was lower in fungicide-treated samples and relative abundance of Dioszegia, Aureobasidium pullulans and Leucosporidium golubevii was lower in fungicide-treated leaves than control leaves.

Similarly, fungicides like enostroburin and metalaxyl cause significant changes in the bacterial communities of the wheat and pepper phyllosphere (Gu *et al.*, 2010 and Moulas *et al*., 2013). The reduction of phyllospheric microbiota of strawberry plants treated with fungicides like cyprodinil, fludioxonil, pyraclostrobin, boscalid and fenexamid has been revealed by metagenomic analysis and suggested that overall higher fungal diversity detected in leaves and flowers, relative to fruits may have reflected different responses to the applied chemicals. (Abdelfattah *et al.,* 2016).

Chemical applications have been reported to have a significant impact on non-target organisms and reduce overall genetic diversity (Pinto *et al.*, 2014 and Singh *et al.*, 2015). Several hypotheses can be put forward to explain the cause of these negative or positive correlations. First, a difference in fungicide sensitivity can cause some taxa to decrease relative to others in the community (Cadez, *et al.,* 2010). Secondly, a specific taxon may be affected by the fungicides indirectly through changes in the abundance of investigate the impact of conventional chemical treatments on fungal diversity and community structure (Worthington, 2012). Moreover, the impacts of chemical and biological treatments on the phyllosphere microbiota are dependent on the dosage and frequency of applications and the mechanisms of action of the products tested, as well as the types of indigenous microorganisms and the weather conditions (Imfeld and Vuilleumier, 2012).

The use of biological control agents offers a promising alternative or supplement to chemical fungicides for the control of crop diseases. In terms of safety, biocontrol agents should not have any effects on nontarget organism (Cook *et al.*, 1996). However, several workers were reported the significant correlation between application of biocontrol agents (BCAs) and changes in the relative abundance of certain non-target microflora (Sylla *et al.*, 2013; Scheepmaker and Kassteele, 2011).

Johansen & Olsson (2008) reported transient effects of the biocontrol agent *Pseudomonas fluorescens* DR54 on the barley rhizosphere microbiota, whereas *P. fluorescens* CHA0 did not change the diversity of culturable fungi in the cucumber rhizosphere significantly (Girlanda *et al*., 2009). Zhang *et al.* (2010) assessed the effect of

Bacillus thuringiensis (Bt) on the microbial communities within the pepper plant phyllosphere using culture-independent methodologies. Their analysis suggested that the bacterial and fungal biomass were not significantly affected following Bt application. However, Bt did change the phyllosphere microbial community structure significantly ie Bt application enhances abundance of Gammaproteobacteria.

The effect of a biocontrol agents (BCA) can also depend on its interaction with other factors: metagenomic amplicon sequencing analysis revealed significant modifications of the bacterial community composition of lettuce rhizosphere following application of the BCA *P. jessenii* (Schreiter *et al*., 2014). The impact of the pathogen *R. solani* and/or of the BCA strain FZB42 on phyllosphere and rhizosphere bacterial communities of lettuce was also studied more in depth by amplicon sequencing (Erlacher *et al*., 2014). The plant microbiota shifted as a consequence of pathogen attack, but these effects were offset by FZB42.These results suggest a novel mode of action for the BCA, ie. selective compensation of the impact of a pathogen on the plant-associated microbiota by the phyllosphere and rhizosphere.

The interaction between *P. nicotianae-Trichoderma* strains and rhizosphere of fortified compost treatment showed *Bacteroidetes* enrichment and in particular the more relative abundance of *Pedomicrobium, Hyphomicrobium, Bacillus, Bdellovibrio* and *Gammaproteobacteria* compared to non-fortified compost treatment, indicating that they may be involved in disease suppression of *P.nicotianae* (Winding *et al.*, 2010). Similarly, *T. harzianum* fortified compost showed a different fungal diversity, by increasing *Ascomycota* and by decreasing *Basidiomycota*, to the normal compost treatments (Boland and Brimner, 2011).

Two recent publications compared the results obtained using NGS and low-throughput approaches (Schmidt *et al.*, 2014; Sylla *et al.*, 2013). They both showed that classical techniques failed to detect alterations in microbial communities found when using NGS technologies. These examples show that NGS technologies provide a more holistic and in-depth analysis of the microbiome, and could therefore be much more appropriate to

detect alterations in microbial community diversity and abundance after BCA treatment. As a consequence, the results of some publications using low-throughput technologies and underlining the absence of effect of a biocontrol agents should be taken with care or would need further confirmation from NGS tools.

Thus, it is crucial to understand the community structure and diversity of phyllosphere and endophytic bacteria on plant leaves. It could further contribute to understanding the significant roles of plant microbiota in supporting their multiple bioactivities. Moreover, it will help to develop a more effective and less environmentally damaging strategies for improving plant health and plant protection.

MATERIALS AND METHODS

3. Material and methods

The present study on "Management of early blight disease of tomato (*Solanum lycopercium* L.) under protected cultivation" was conducted at the department of Plant Pathology, during the period 2016-2020. Isolation of metagenomic DNA was carried out in the high-tech seed testing laboratory and Department of Agricultural Microbiology College of Horticulture, Vellanikkara.

3.1. Isolation of the pathogen and pathogenicity test

The pathogen was isolated from infected tomato leaves collected from the research plot of Vegetable Science Department, College of Horticulture. showing typical symptom. The leaves were cut into small bits of 5 mm size and surface sterilized with one per cent sodium hypochlorite solution for one minute and washed in three changes of sterile water. The bits were then transferred aseptically to sterile Petri dishes containing potato dextrose agar (Appendix I). Inoculated dishes were incubated under room temperature (27 ± 1^0C) for 2-5 days and observed for the fungal growth arising from the plant tissue. Fungal growth on the medium was subcultured and purified by hyphal tip method. Pure cultures of the fungus were maintained on potato dextrose agar slants for the subsequent use. Pathogenicity test was carried out on the three-month old tomato seedlings (Akshya variety) under *in vivo* condition adopting spore inoculation method. Spore suspension (10^6 ml^{-1}) of water was prepared and drop of Tween-20 was added as spreading agent. The seedlings were sprayed with 10 ml of spore suspension of pathogen on leave surface with and without giving five pin pricks. Seedlings inoculated with sterile water served as control. The inoculated plants were covered with moistened polythene bags to provide humidity and observed daily for the symptom appearance when typical blight symptoms were observed on the leaves, the pathogen was reisolated and compared with the original cultures.

3.2. Symptomatology

Symptomatology of the disease on various plant parts such as leaves, shoots and fruits were studied under both natural and artificial conditions. For artificial inoculation, spore suspension was used as mentioned under 3.1.

3.3 Characterisation of the pathogen

The cultural and morphological characters of the isolated pathogen *viz.* colour, growth pattern and rate, conidia and conidiophore characters were studied on potato dextrose agar (PDA). For studying cultural characters, 8 mm sized culture disc from five-day old culture of the pathogen was placed at the center of the mediated plates and incubated at 27 ± 1^0C and three replications were kept. Morphological characters were studied by slide culture technique and microphotographs and measurements were taken using ultrascope.

3.4. *In vitro* evaluation of fungicides and bioagents against the pathogen

Efficacy of the selected contact and systemic fungicides and bioagents were tested against the pathogen under *in vitro* condition. Details of fungicides and bioagents used for the *in vitro* evaluation are given in the Table 3.1 and 3.2.

3.4.1. *In vitro* evaluation of fungicides

Efficacy of seven fungicides were studied by poisoned food technique (Zentmyer, 1955). Potato dextrose agar, 100 ml was taken in 250 ml conical flask and sterilized at 1.05 kg/cm^2 pressure for 20 min. The fungicides were mixed separately with medium in suitable proportion to get the desired concentrations (Table 3.1) and poured to sterilized Petri dishes @ 20 ml/plate. Eight mm sized disc from five-day old culture of the pathogen was placed at the center of each plate containing poisoned medium. Plates without the fungicides served as control. Three replications were maintained for each fungicide. Observations were recorded till the pathogen attained full growth in control. The per cent inhibition of pathogen was calculated using the formula suggested by Vincent (1927).

$$\text{Per cent inhibition of pathogen} = \frac{C - T}{C} X\ 100$$

C = Growth or colony diameter of the pathogen in control

T = Growth or colony diameter of the pathogen in treatment

3.4.2. *In vitro* evaluation of antagonists

Endophytic *Bacillus subtilis* isolated from cocoa leaves, three reference cultures *viz. Trichoderma viride* (KAU), *Pseudomonas fluorescens* (KAU) and *B. subtilis* (KAU) and one plant growth promoting microbial consortium (PGPM mix of KAU) and its individual microorganisms *viz. T. viride*, *T. harzianum*, *P. fluorescens* and *B. megatherium* were screened for their antagonistic activity against the pathogen by employing dual culture technique (Johnson and Curl, 1972).

3.4.2.1. *In vitro* evaluation of fungal antagonists

Antagonistic activities of fungal antagonists were tested against the pathogen by adopting differed antagonism method. Sterilized Petri dishes containing PDA medium were inoculated with the 8 mm mycelial disc of five-day old cultures of the pathogen at 2 cm from the periphery. After 48 h of incubation, 8 mm disc of five-day old culture of antagonist was placed at the opposite end in the same plate, at 2 cm distance from the periphery. Three replications were kept for each antagonist and observations were recorded daily till the pathogen grew and fully covered the plates in control.

3.4.2.2 *In vitro* evaluation of bacterial antagonists

The bacterial antagonists were evaluated for antagonistic activity against the pathogen by simultaneous antagonism method. Eight mm mycelial disc of five-day old cultures of the pathogen was inoculated at the centre of the PDA mediated Perti dish and the bacterial antagonists were streaked on either side of the pathogen at 2 cm from the periphery of the dish. The pathogen grown as monoculture served as control. The inoculated plates in triplicates were incubated at room temperature and observations were recorded daily till pathogen attained full growth in control. The per cent inhibition of growth of pathogen was calculated by formula suggested by Vincent (1927).

3.5. Management of early blight disease of tomato under protected cultivation

Field experiments were conducted under rain shelter of size 200 m^2 and polyhouse of size 300 m^2 both having gable type roof, constructed in the North-South direction in the Department of Plant Pathology, College of Horticulture, Vellanikkara.

The experiments were carried out during July to January, 2017-2018 to find out the efficacy of selected fungicides and antagonists on the management of early blight disease of tomato under protected condition. The experiment details are given below.

Design : RBD

No.of treatments : 11

Replication : 3

No.of plants/replication : 10

Variety : Akshaya

Plot size : 3.0 x 1.0 m^2

Spacing : 0.6 x 0.6 m^2

Season : July to January

Table 3.1 Details of the fungicides used for *in vitro* evaluation

Treatment	Chemical name	Trade name	Concentration (%)
T_1	Propineb 70% WP	Anthracol	0.1, 0.2, 0.3
T_2	Copper hydroxide 77% WP	Kocide	0.15, 0.2, 0.25
T_3	Pyraclostrobin 20% EC	Insignia	0.025, 0.05, 0.075
T_4	Azoxystrobin 23% SC	Amistar	0.1, 0.15, 0.2
T_5	Hexaconazole 5% EC	Contaf	0.05, 0.1, 0.15
T_6	Difenoconazole 25% EC	Score	0.025, 0.05, 0.075
T_7	Iprodione 25% + Carbendazim 25% WP	Quintal	0.1, 0.2, 0.3
T_8	Trifloxystrobin 25% + Tebuconazole 55%	Nativo	0.025, 0.05, 0.075

Table 3.2 List of bioagents used for *in vitro* evaluation

Treatment	Bioagents
T_1	*Trichoderma viride* (KAU)
T_2	PGPM mix (KAU) [*T. viride*, *T. harzianum*, *Bacillus megatherium*, *Pseudomonas fluorescens*]
T_3	*Pseudomonas fluorescens* (KAU)
T_4	*Bacillus subtilis* (KAU)
T_5	*Bacillus subtilis* (Endophyte from cocoa)
T_6	*T. viride* (PGPM mix)
T_7	*T. harzianum* (PGPM mix)
T_8	*Pseudomonas fluorescens* (PGPM mix)
T_9	*Bacillus megatherium* (PGPM mix)

Treatments were applied as seed treatment and foliar spray. Seeds of tomato were treated with the fungicides and antagonist. Total of 150 seeds were used for each treatment. Seeds were soaked in respective fungicides and antagonist (10^6 ml^{-1}) for 30

min, air dried and sown in pro trays containing sterilized potting mixture. Foliar application was given at the onset of disease. Subsequent sprays were given at 15 days interval. Systemic fungicides were sprayed twice and contact fungicides and biocontrol agents were applied three times. Plants in each plot were separated using plastic screen during foliar spray. Artificial inoculation was given by spraying spore suspension as mentioned in 3.1.3. The details of treatments are given in Table 33.

3.5.1 Preparation of nursery

Nursery was raised during July –September 2017, in pro tray using soil less medium consisting, consisting of coir pith, perlite and vermiculate in 3:1:1 proportion by weight. Tomato seeds were treated as per the technical programme and sown one seed per cavity. The pro trays were kept in net house and irrigated regularly. Observations on germination percentage, root and shoot length, girth at collar region and number of leaves were recorded 30 days after sowing. Vigour index was calculated using the formula, suggested by Elliot and Lynch (1984).

Vigour index = (Root length + shoot length) x germination percentage

3.5.2 Soil solarisation

Beds of 3 x 1 m^2 size and 10 cm height were taken inside the polyhouse and rain shelter; irrigated using rose can and the beds were perfectly levelled. Transparent polythene sheet of 150-gauge thickness was stretched and spread over the beds so that it was placed touching the surface and there were no air pockets in between. The sides of the polythene sheet were sealed by putting soil. Soil solarisation was carried out during April- June for a period of 90 days in protected structures.

Table 3.3 List of treatments used for field experiment

Treatment No.	Treatments
T_1	Foliar spray with propineb 70% WP (0.1%)
T_2	Foliar spray with propineb 70% WP (0.2%)
T_3	Foliar spray with hexaconazole 5% EC (0.05%)
T_4	Foliar spray with hexaconazole 5% EC (0.1%)
T_5	Foliar spray with difenoconazole 25% EC (0.05%)
T_6	Foliar spray with iprodione 25% + carbendazim 25% WP (0.1%)
T_7	Foliar spray with iprodione 25% + carbendazim 25% WP (0.2%)
T_8	Foliar spray with *Trichoderma viride* (KAU)
T_9	Foliar spray with PGPM mix (KAU)
T_{10}	*Bacillus subtilis (*Endophyte from cocoa)
T_{11}	Untreated control

3.5.3 Filed preparation

Filed experiments were conducted in polyhouse and rain shelter simultaneously. Land inside the structures were ploughed and thoroughly prepared into bed size 3.0x 1.0 m^2. All beds were subjected to soil solarisation for 90 days, then polythene sheet was removed. Thirty-day old seedlings were transplanted at a spacing of 0.6x 0.6 m and 3-4 cm depth. Agronomic practices were adopted as per the package of practices recommendations of crops (KAU, 2011). Fertigation and irrigation were carried out using drip irrigation system. Details of treatments applied are mentioned in Table 3.3.

3.5.4 Observations recorded

3.5.4.1 Disease incidence

Observations were recorded one week after the challenge inoculation and per cent disease incidence was calculated using the formula suggested by Wheeler (1969).

$$\text{Per cent disease incidence (PDI)} = \frac{\text{Number of plants infected X 100}}{\text{Total number of plants observed}}$$

3.5.4.2 Disease severity

Observations were recorded before spray and after 10 days of each spray. Disease was scored using 0-5 scale suggested by Bora *et al.* (2014).

Per cent disease severity (PDS) was calculated using the formula suggested by Wheeler (1969).

$$\text{PDS} = \frac{\text{Sum of all numerical ratings X 100}}{\text{Total number of leaves assessed X maximum disease grade}}$$

Score chart used for early blight of tomato

Score	Description of the symptom
0	Leaves free from infection
1	Small irregular spots covering < 5% leaf area
2	Small irregular brown spots with concentric rings covering 5.1-10% leaf area
3	Lesions enlarging, irregular brown with concentric rings covering 10.1-25% leaf area
4	Lesions coalesce to form irregular and appears as a typical blight symptom covering 25.1- 50% leaf area
5	Lesions coalesce to form irregular and appears as a typical blight symptom covering >50% leaf area

3.5.4.3 Biometric observations

Observations on plant height, girth at collar region, number of leaves per plant, number of flowers per plant, number of fruits per plant, average fruit weight and yield per plot were recorded.

3.5.4.4 Meteorological parameters

Temperature and relative humidity inside the polyhouse and rain shelter were recorded at 7.30 am and 2.30 pm daily during the experiment using temperature and moisture meter which are permanently installed inside the structures. Correlation analysis was performed between major meteorological factors and disease severity using SPSS v16.0 data editor.

3.6. Residue analysis

Residue analysis was carried out to find fungicide residue level in harvested tomato fruits from plants treated with different fungicides in polyhouse and rain shelter. The fruit samples were collected from the plant at one, three, five and seven days after spray and sent to Pesticide Residue Research and Analytical Laboratory, College of Agriculture, Vellayani.

3.7 Enumeration of non-target culturable microflora

The phylloplane and endophytic microflora (fungi, bacteria and actinomycetes) of the crop was enumerated before and after treatment application using serial dilution plating to know the changes due to the treatments.

3.7.1 Enumeration of phylloplane microflora

The phylloplane microflora (fungi, bacteria and actinomycetes) of the crop was enumerated using serial dilution plating of leaf washings. The methodology adopted by Elad and Kirshner (1993) was used for studying the enumeration of phylloplane microflora. Tomato leaves were collected before the treatment application and ten days after treatment application. Area of the leaf used for the study was measured by graph paper method. Then the leaf was cut into small pieces and added to 100 ml sterile water, agitated well for one minute and serially diluted up to 10^{-5} dilution and fungi, bacteria and actinomycetes were plated on suitable media. Based on the number of colonies developed, dilutions 10^{-2}, 10^{-4} and 10^{-1} were used for isolation of fungi, bacteria and actinomycetes respectively. The media used were Martin's Rose Bengal Agar, Nutrient Agar and Ken Knights Agar (Appendix I) for fungi, bacteria and actinomycetes respectively.

Microbial suspension (1ml) of the respective dilution was pippeted into sterile Petri dish and 15 ml of molten cooled medium was added. Three replications were kept for each sample. The plates were then incubated at room temperature. After incubation fungal, bacterial and actinomycetes colonies were counted at 24 h, 48 h and 7 days after inoculation respectively. Population of the phylloplane microflora was expressed as number of colonies forming units per unit area of leaf (cfu cm^{-2}).

3.7.2 Enumeration of endophytic microflora

The endophytic microflora (fungi, bacteria and actinomycetes) present in leaf, stem and root of the crop was isolated by destructive sampling and enumerated using serial dilution plate technique. One plant each from each plot were uprooted, brought to the laboratory and washed under running tap water to remove the soil particle adhering to the plant. The root portion 5 cm below the soil line and stem portion 20 cm above the soil line were taken for the isolation. The skin of the stem was peeled off and the root skin scraped off to remove external contaminants and these were cut into bits of 1 cm length. The leaves samples were collected randomly from each treatment before and after treatment application.

Isolation of endophytes from leaves, stem and root samples were carried as suggested by Haiyan *et al.* (2005). One gram each of leaf, stem and root samples were surface sterilized separately by sequentially treating with 0.5 per cent sodium hypochlorite and 70 per cent ethanol for 2 min and rinsed with two changes of sterile water followed by two changes of sterile 0.02 M tris phosphate buffer. An aliquot of 1 ml of the final buffer wash was transferred to sterile Petri plate to which respective media (Martin's Rose Bengal Agar for fungi, Nutrient Agar for bacteria and Ken Knights Agar for actinomycetes) was added and it served as sterility check. Each sample was then triturated in 9 ml of final buffer wash using a sterile pestle and mortar and dilutions were prepared up to 10^{-5} from this triturate. For isolation of fungi, bacteria and actinomycetes 10^{-3}, 10^{-5} and 10^{-1} dilutions were used respectively. One ml from each dilution pipetted into sterile Petri plates and 15 ml each of molten and cooled medium was poured separately. The plates were incubated at room temperature. Fungal, bacterial and actinomycetes colonies were counted at 24 h, 48 h and 7 days after inoculation respectively.

Microbial population in each dilution were recorded and number of colonies in each sample was calculated using the following equation.

$$\text{Number of cfu/g sample} = \frac{\text{Number of colonies on the plate x dilution factor}}{\text{Volume plated (ml)}}$$

3.8 Survival of biocontrol agents on the phylloplane of tomato

The population of biocontrol agents sprayed on tomato leaves was estimated at periodical intervals of 5, 10,15 days after spraying using serial dilution plating of leaf washings as described in 3.8.1. The media used for the isolation of *Trichoderma* sp. and *P. fluorescens* were *Trichoderma* selective medium (TSM) and King's B agar (KBA) respectively. Leaves were collected from tomato plants in rain shelter and polyhouse before and after treatment application at an interval of 5 days. For isolation of *Trichoderma* sp. and *P. fluorescens,* 10^{-3} and 10^{-5} dilutions were used respectively. The respective dilutions were plated as per 3.8.1. Population of biocontrol agents was expressed as colony forming units per unit area of leaf (cfu cm^{-2}).

3.9 Statistical analysis

Analysis of variance was performed on the data collected in various experiments using the statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatment means were done using DMRT.

3.10 Metagenomic analysis to assess the impact of foliar spray on non-target microflora

3.10.1 Collection of samples

Tomato leaves were collected from all the treatments after second foliar application by random sampling technique. Collected samples were kept in sterile plastic bags in a thermally insulated. Collected samples were kept in sterile plastic bags in a thermally insulated container. All samples were immediately placed on ice and transported to the high-tech seed testing laboratory attached to the Department of vegetable Science, College of Horticulture and stored at −80 °C prior to processing.

3.10.2 Metagenomic DNA extraction

The extraction of metagenomic DNA was done with an objective to construct metagenomic libraries of the samples. Collected samples were pooled and aseptically

ground to a fine powder in liquid nitrogen using sterilised pestle and mortar. Ground tissue powder (100 mg) were then stored at −80 °C. Metagenomic DNA extraction was done by using the DNeasy Plant Mini kit (QIAGEN, Dusseldorf, Germany). The standard protocol provided in the user manual was followed to obtain the best result. Samples were first mechanically disrupted and then chemically lysed. RNA is removed by RNAse digestion during lysis. Cell debries, precipitated proteins, and polysaccharides are removed and the sample was homogenized by centrifugation through a QIAshredder spin column. Buffering conditions were adjusted and the lysate was loaded onto the DNEasy Plant Mini spin column. During a brief spin, DNA selectively binds to the silica membrane while contaminants pass through. Remaining contaminants and enzyme inhibitors were removed in one or two efficient wash steps using washing buffer. The DNA was finally eluted using elution buffer and metagenomic DNA dissolved in 100 µl of elution buffer was obtained from each sample.

3.10.3 Agarose Gel Electrophoresis

The quality of the isolated metagenomic DNA was analyzed by agarose gel electrophoresis. The metagenomic DNA was run in 0.8 per cent agarose gel for the qualitative analysis. About 250 ml 1X TAE buffer was made from a stock solution of 50X TAE buffer (pH 8.0). The agarose gel was prepared by dissolving 0.8 g agarose in 100 ml 1X TAE buffer. The solution was heated for proper mixing and after cooling, ethidium bromide was added to it at a concentration of 0.5 µg ml^{-1} prepared from a stock solution of 10 mg ml^{-1}. The molten agarose suspension was then poured into a casting tray and a comb was placed carefully after wiping both the casting tray and comb with alcohol. The agarose was allowed to solidify for 30.0 minutes. The comb was gently removed from the solidified gel to obtain wells and the gel was then placed in the buffer tank filled with 1X TAE buffer with the side with the wells facing the cathode. About 5 µl of DNA was mixed with 2 µl gel loading dye and the mixture was carefully loaded into the respective wells using a micro-pipette. The molecular weight marker used was λDNA/*Eco*R1 + *Hind* III double digest (Sisco research laboratory; Biolit, Mumbai). The electrodes were connected to the power pack and a constant

electric potential of 80 V was applied till the tracking dye reached almost 3 cm away from the end.

3.10.4 Gel documentation

The gel documentation was done using GeNei UVITEC Cambridge gel documentation system. The agarose gel was visualized in the presence of UV light to illuminate the DNA bands and images of the gel was captured using the software system attached to it and the band size was compared with the ladder.

3.10.5 Quantitative analysis of metagenomic DNA

The quantity and purity of the DNA samples were analyzed using NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies., USA). The instrument was cleaned using distilled water and the blank value was recorded using sterile distilled water. The absorbance was measured at 260 nm and 280 nm. The 260/280 ratio was calculated to understand the purity of the DNA samples. The quantity of DNA was also measured.

3.10.6 Metagenomic DNA sequencing

Metagenomic DNA from all the treatments were extracted and analysed for the qualitative and quantitative standards. Out of the 22 metagenomic DNA samples collected from plants which received various treatments, four were selected for sequencing. The selected samples were DNA from plants sprayed with 1) the best performing systemic fungicide, 2) best performing contact fungicide, 3) best among the biocontrol agents and 4) from the plants in control. The samples were sequenced at a private scientific facility Xcelris Labs Limited, Ahmedabad.

3.10.6.1. 16S RNA gene and ITS amplicon library sequencing using Next Generation Illumina Miseq™

The amplicon library was prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S and ITS metagenomic sequencing library preparation protocol. Primers for the amplification of the V3-V4 hyper-variable region (Table 4) of 16S rDNA gene

of bacteria and archaea and ITS2 region (Table 5) were designed in Xcelris NGS Bioinformatics Lab and synthesized in Xcelris PrimeX facility. The amplicon with the Illumina adaptors were amplified using i5 and i7 primers that add multiplexing index sequences as well as common adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The 16S RNA gene and ITS amplicon libraries were purified by 1X AMpureXP beads, checked on Agilent DNA1000 chip on Bioanalyzer 2100 and quantified by Qubit Fluorometer 2.0 using Qubit dsDNA HS Assay kit (Life Technologies).

3.10.6.2 Cluster Generation and Sequencing

Library was loaded onto Illumina platform at appropriate concentration (10-20pM) for cluster generation and sequencing after obtaining the Qubit concentration for the library and the mean peak size from Bioanalyser profile. Paired-End sequencing allowed the template fragments to be sequenced in both the forward and reverse directions on Illumina platform. The samples were bound to complementary adapter oligos on paired-end flow cell using the regents supplied with the kit. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

3.11. Bioinformatics analysis and data processing

Sequence analysis was carried out using the quantitative insights into microbial ecology (QIIME) pipeline (Caporaso *et al.*, 2010). For sequence filtering, reads shorter than 200 bases or longer than 1,000 bases were discarded, sequences with homopolymer runs longer than six bases or more than six ambiguous bases were also discarded, whereas one barcode correction and two primer mismatches were accepted. Chimeras were removed using the UCHIME program (Edgar, 2010) according to the USEARCH pipeline. Operational taxonomic units (OTU) were determined using the UCLUST algorithm (Edgar, 2010) at 97% sequence similarity.

Based on the sequence data bacteria present in the samples were identified and classified employing MG RAST server, an open source system into which raw sequence

data in fasta format were uploaded (Meyer e*t al.,* 2008). The raw sequences were automatically processed and the taxonomic distribution data were generated by the MG RAST pipeline by comparing the sequences against the RDP database at 97 per cent identity and an e-value of five. The taxonomy assignment of the fungal OTU was carried out using one codex server, which uses exact k-mer alignment to classify sequences against a reference database of ~40 000 complete microbial genomes including bacteria, viruses, fungi, protists, and archaea (Minot *et al.*, 2015). Singleton OTU were removed for faciliating statistical analysis. For downstream analysis, the OTU table was rarefied at an even depth to reduce bias in sequencing depth. Alpha diversity was calculated using observed species, Shannon, Good's coverage and Chao1 estimates. The Illumina sequence data was then submitted to Sequence Read Archive (SRA) (http://www.ncbi.nlm. nih.gov/sra) of GenBank database and the accession number was obtained.

Table-3.4. Primers used for 16S r DNA in the present study

Sl.No.	Oligo Name	Oligo Sequence (5' to 3')	Length of primer	Product size (Approx.)
1.	Prokaryote V3-Forward	CCTACGGGNBGCASCAG	17	~ 460 bps
2.	Prokaryote V4-Reverse	GACTACNVGGGTATCTAATCC	21	

Table-3.5. Primers used for ITS region of fungal DNA in the present study

Sl.No.	Oligo Name	Oligo Sequence (5' to 3')	Length of primer	Product size (Approx.)
1.	ITS2-Forward	GCATCGATGAAGAACGCAGC	20	~ 350 bps
2.	ITS2-Reverse	TCCTCCGCTTATTGATATGC	20	

RESULTS

4. Results

The studies on "Management of early blight disease of tomato (*Solanum lycopersicum* L.) under protected cultivation" was conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara during 2016-2020. The study consisted of isolation and characterisation of pathogen causing early blight disease of tomato, experiments to evaluate different treatments against the disease under protected structures *viz.* polyhouse and rain shelter, enumeration of phylloplane and endophytic microflora, metagenomic analysis to assess the impact of foliar spray on non-target microflora. The results obtained are presented below.

4.1 Isolation of the pathogen and pathogenicity test

Tomato (variety Akshaya) leaves showing typical early blight symptoms of dark brown spots with concentric rings surrounded by discoloured tissue were collected from infected field. A fungal growth showing black mycelia surrounded by white young tip of hyphae was observed from the infected tissue by the standard tissue isolation technique. A pure culture of pathogen was obtained from colony showed greenish brown mycelia and surrounded by white young tip of hyphae at initial stage turning later to ash brown and finally grey colour in the potato dextrose agar medium (Plate 4.1).

Pure culture of pathogen was used for pathogenecity test following Koch's postulates. The pathogenicity test was carried out as described in materials and methods (3.1) by spray inoculation with spore suspension of *A. solani* on foliage of 30 days old tomato seedlings. Symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue (Plate 4.2). Infection and initial symptoms were noticed in 8-9 days after inoculation (DAI) when spore suspension was sprayed after giving injury. In the case of intact leaves ie without pin pricks, infection was noticed in 10-12 DAI only. Reisolation of the fungus from infected part and confirmed the identity of the pathogen culturally and morphologically and thus fulfilled Koch postulates.

a. Early blight symptom

b. Isolated pathogen on PDA

Plate 4.1. Isolation of pathogen from infected tomato leaves

a. Tomato plants kept for incubation

b. Symptoms on tomato leaves

Plate 4.2. Pathogenicity test

4.2. Symptomatology

Symptoms of the disease on tomato was studied under both natural and artificial conditions. Symptoms observed on artificial inoculation were similar to those produced under natural condition. The first symptom of the early blight disease was observed on older tomato leaves as light-yellow discolored spots mostly near the leaf margin. Later the spot become a small angular oval brown water-soaked lesion, one to four mm in diameter. A narrow chlorotic halo also developed around the spot. Later, the spots enlarged with characteristic concentric rings in the center to produce a target board effect and the colour of the spots changed from brown to dark brown. Finally, the adjacent spots eventually coalesced to form large irregular spots leading to drying and defoliation (Plate 4.3).

Older leaves got infection first and later it progressed upward. When plants were 60-90 days old, symptoms also appeared on stem and petioles as brown to dark brown elongated cankerous target board type spots (Plate 4.4). These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepals and fruits resulting in pre-mature dropping of fruits. The symptoms on fruits appeared first at stem end as black or brown sunken spots both on green and ripe fruits which enlarged within eight days involving most of the fruits, finally the fruits were rotted (Plate 4.5).

4.3 Characterisation of the pathogen

Cultural and morphological characteristics of the pathogen were studied on potato dextrose agar.

4.3.1. Cultural characters

Cultural characters like colony colour/ pigmentation, Margin colour, Margin growth pattern, Topography and Zonation observed (Table 4.1). Pathogen produced profuse mycelial growth on PDA. Initially, the mycelium was greenish brown surrounded by white young tip of hyphae and later turned to ash brown and finally turned to grey colour

(Plate 4.6). It produced septate mycelia with aerial topography and irregular rough growth patterns with concentric zonation. Sporulation was observed six days after incubation.

4.3.2. Morphological characters

Morphological characters such as type of conidiophore, conidium, size, shape of conidium and number of septa in conidium were observed under microscope (Table 4.2). The conidiophores were straight or flexuous, sometimes geniculate brown to olivaceous brown, slightly swollen at apex having terminal scars indicating the point of attachment of conidia. The conidia were solitary straight or muriform or oblong tapering to beak, pale or olivaceous brown, length 40-110 μm and 7-15 μm thick in the broadest part with 2-8 transverse and 0-3 longitudinal septa. The beaks were flexuous, pale and sometimes branched (Plate 4.7).

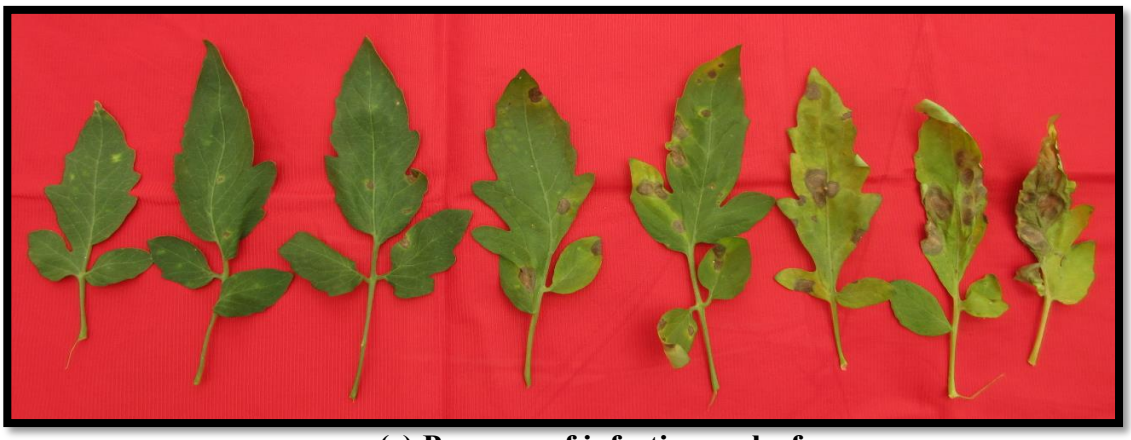
The cultural and morphological characters of the pathogen were completely fitted into the description of *Alternaria solani* described by Alexopoulos *et al.* (1996). Hence, it is confirmed that the symptom observed on tomato leaves are those of early blight disease caused by *A. solani.*

Table 4.1. Cultural characters of the pathogen

Cultural characters of the pathogen				
Colony colour	**Margin growth pattern**	**Margin colour**	**Topography**	**Zonation**
Greenish brown	Irregular rough	Whitish brown	Aerial	Concentric zonation

(a) Initial symptom on leaf

(b) Targeted spots with concentric zonation

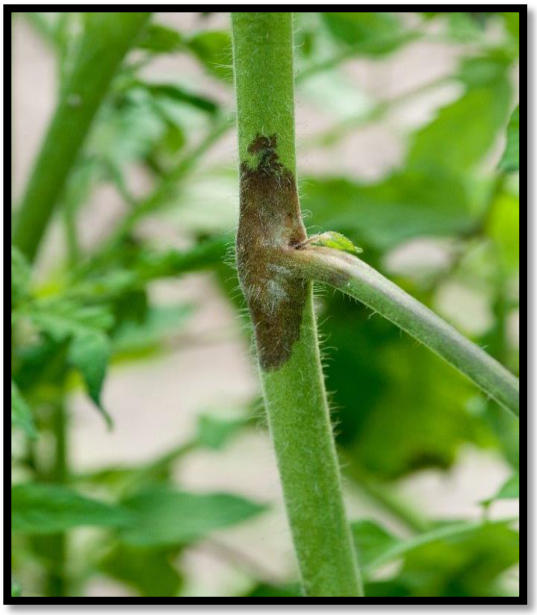


(c) Progress of infection on leaf

(d) Adjacent spots coalesced to form large irregular spots

(e) Complete drying of leaves

Plate 4.3. Symptoms on tomato leaves



(a) Brown elongated spot

(b) Brown spot near flower buds

(c) Lesion spread to petioles and leaves

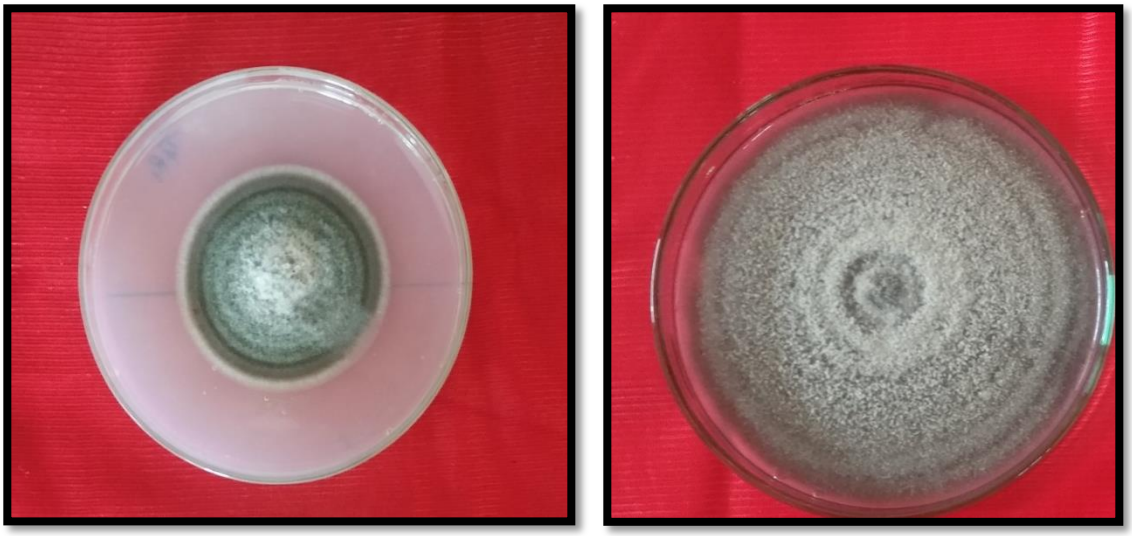
(d) Spots enlarged

Plate 4.4. Symptoms on tomato stem and petioles

(a) Symptoms on fruits under natural condition

(b) Symptoms on fruits under artificial condition

Plate 4.5. Symptoms on tomato fruits



(a) *Alternaria solani* 4 DAI **(b) *Alternaria solani* 7 DAI**

Plate 4.6. Cultural characters of the pathogen on potato dextrose agar

(a) Conidia formation (400X) **(b) Conidia maturation (400X)**

(c) Matured conidia (400X)

Plate 4.7. Morphological characters of the pathogen on potato dextrose agar medium

Table 4.2. Morphological characters of the pathogen

Conidiophore	Conidia	Size of conidia	No.of septa in conidia
Brown in colour Straight /flexuous	Slightly flexuous oblong or muriform	Length- 25-44µm Width-7-15µm	Horizontal septa-2-8 Vertical septa-1-3

4.4 *In vitro* evaluation of fungicides and bioagents against the pathogen

Eight fungicides (with three concentration) and eleven bioagents were screened for their inhibitory effect against the pathogen *A. solani* under *in vitro* condition.

4.4.1 *In vitro* evaluation of fungicides against the pathogen

The inhibitory effect of different fungicides on *A. solani* was studied by poisoned food technique. The efficacy of eight fungicides against *A. solani* at different concentrations is given in Table 4.3.

Table 4.3. *In vitro* evaluation of fungicides against *Alternaria solani*

Sl. No.	Fungicide	Conc (%)	Per cent inhibition over control
1.	Propineb 70% WP (Anthracol)	0.1	100(10)[a]
		0.2	100(10)[a]
		0.3	100 (10)[a]
2.	Copper hydroxide 77% WP (Kocide)	0.15	68.33(8.26)[f]
		0.2	68.32(8.26)[f]
		0.25	81.11(9)[d]
3.	Pyraclostrobin 20% EC	0.025	79.44(8.91)[d]
		0.05	81.11(9)[d]
		0.075	85.55(9.24)[c]
4.	Azoxystrobin 23% SC (Amistar)	0.1	41.66(6.45)[j]
		0.15	46.10(6.78)[h]
		0.2	46.66(6.83)[h]
5.	Hexaconazole 5% EC (Contaf)	0.05	100(10)[a]
		0.1	100(10)[a]
		0.15	100(10)[a]
6.	Difenoconazole 25% EC (Score)	0.025	94.99(9.7)[b]
		0.05	97.21(9.8) [b]
		0.075	100(10) [a]
7.	Iprodione 25% + Carbendazim 25% WP (Quintal)	0.1	100(10)[a]
		0.2	100(10)[a]
		0.3	100(10)[a]
8.	Trifloxystrobin 25% + Tebuconazole 55% (Nativo)	0.025	44.44(6.66)[i]
		0.05	62.22(7.88)[j]
		0.075	73.33(8.56)[e]
	CD (0.05)		0.121

*Mean of the three replications
√x+0.5 transformed values are given in parentheses

All the fungicides tested were found to be effective against the pathogen; however, the efficiency varied with the chemical (Plate 4.8). There was a positive correlation between the concentration and per cent inhibition of growth of mycelium except propineb, hexaconazole iprodione 25% + carbendazim. All the three concentrations of propineb (0.1%, 0.2% & 0.3%), hexaconazole (0.05%, 0.1% & 0.15%) and iprodione 25% + carbendazim (0.1%, 0.2% & 0.3%) recorded cent per cent inhibition of the pathogen. Hence, it revealed that, even the lower concentration of these fungicides was effective against the pathogen.

All the three concentration of difenoconazole was significantly superior over copper hydroxide, pyraclostrobin, azoxystrobin and trifloxystrobin 25% + tebuconazole in inhibiting mycelial growth and recorded 94.99-100 per cent inhibition. Similarly, lower and higher concentration of pyraclostrobin was more effective than copper hydroxide, azoxystrobin and trifloxystrobin 25% + tebuconazole and showed 79-85 per cent mycelial growth inhibition. The contact fungicide copper hydroxide recorded 68-81 per cent reduction over control and the combination fungicide, trifloxystrobin 25% + tebuconazole, was the least effective and recorded only 73.33 per cent inhibition even at the concentration of 0.075 per cent.

Based on *in vitro* screening, effective fungicides at their effective concentration *viz.* two lower concentrations of propineb (0.1% & 0.2%), hexaconazole (0.05% & 0.1%) and iprodione 25% + carbendazim (0.1% & 0.2%) and second lower concentration of difenoconazole (0.05%) were selected for field experiment.

4.4.2 *In vitro* evaluation of bioagents against the pathogen

Endophytic *B. subtilis* isolated from cocoa leaves, three reference cultures *viz. Trichoderma viride* (KAU), *Pseudomonas fluorescens* (KAU) and *B. subtilis* (KAU) and one plant growth promoting microbial consortium (PGPM mix of KAU) and its individual microorganisms *viz. T. viride*, *T. harzianum, P.fluorescens* and *B. megatherium* were

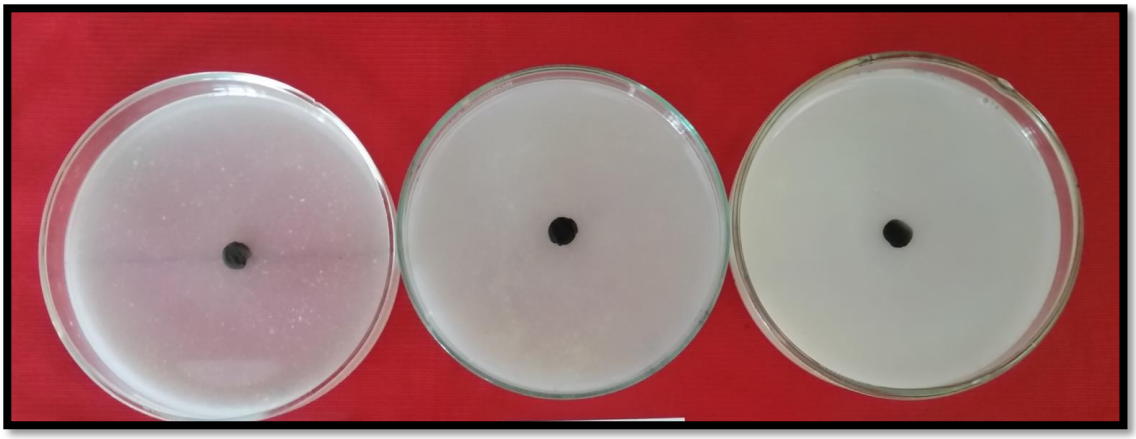
screened for their antagonistic activity against the pathogen and the findings are presented in Table 4.4.

Table 4.4. *In vitro* evaluation of bioagents against *Alternaria solani*

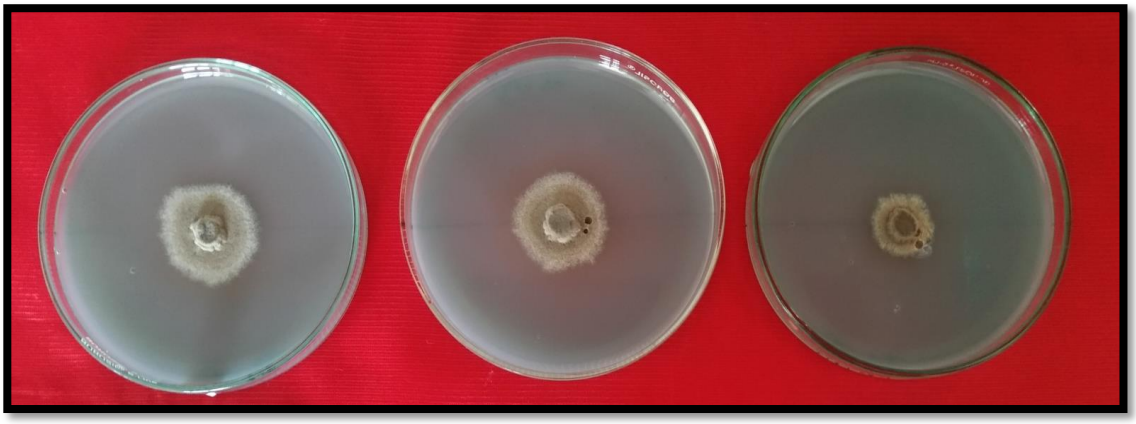
Sl.No.	Bioagents	Per cent inhibition over control
1.	*Trichoderma viride* (KAU)	100(10)[a]
2.	PGPM mix (KAU) [*T. viride*, *T. harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*]	100(10)[a]
3.	*Pseudomonas fluorescens* (KAU)	36.66(6.04)[d]
4.	*Bacillus subtilis* (KAU)	46.11(6.78)[bc]
5.	*Bacillus subtilis* (Endophyte from cocoa)	51.66(6.78)[bc]
6.	*T. viride* (PGPM mix)	100(10)[a]
7.	*T. harzianum* (PGPM mix)	100(10)[a]
8.	*Pseudomonas fluorescens* (PGPM mix)	34.44(5.85)[d]
9.	*Bacillus megatherium* (PGPM mix)	43.32 (6.57)[c]
	CD (0.05)	0.511

*Mean of the three replications
√x+0.5 transformed values are given in parentheses

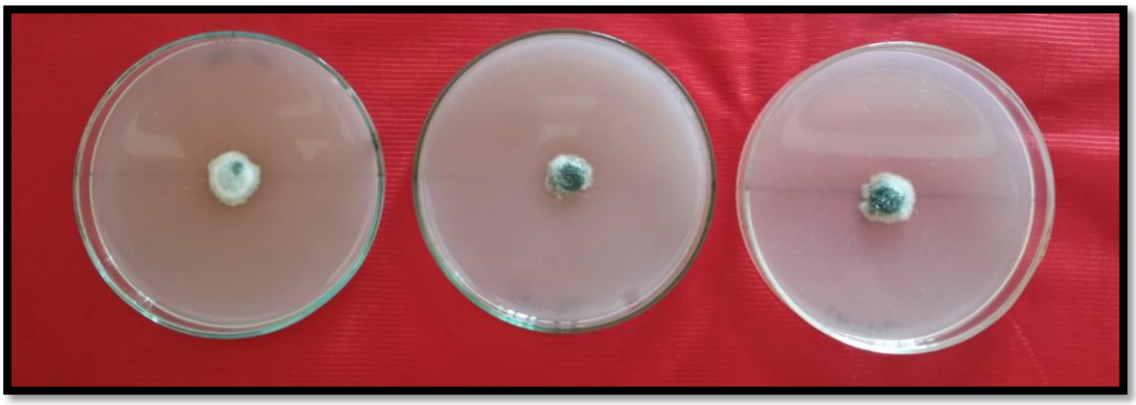
All the nine antagonists showed some antagonistic activity against the pathogen. However, *T. viride* (KAU), *T. viride* (PGPM mix), *T. harzianum* (PGPM mix) and plant growth promoting microbial consortium (PGPM mix of KAU) showed cent per cent inhibition of pathogen by the overgrowth mechanism of antagonism, causing complete disintegration of the pathogen. However, less than 50 per cent inhibition was observed for



(a) T_1-Propineb70%WP (0.1%, 0.2%, 0.3%)

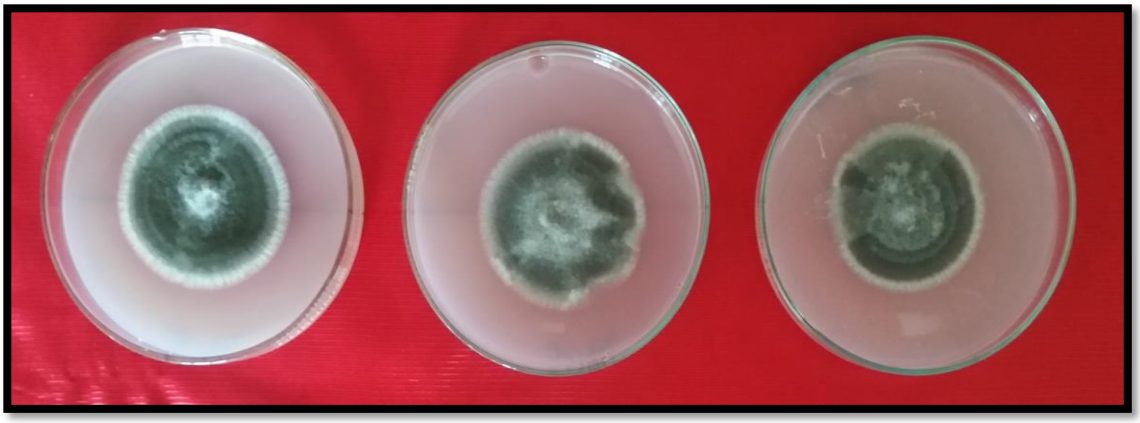


(b) T_2-Copper hydroxide 77% WP (0.15%, 0.2%, 0.25%)

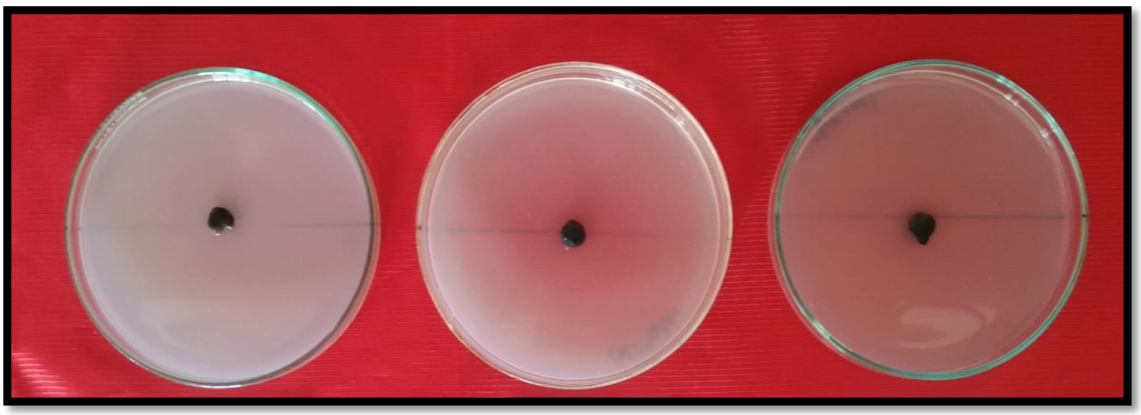


(c)T_3-Pyraclostrobin 20% EC (0.025%, 0.05%, 0.075%)

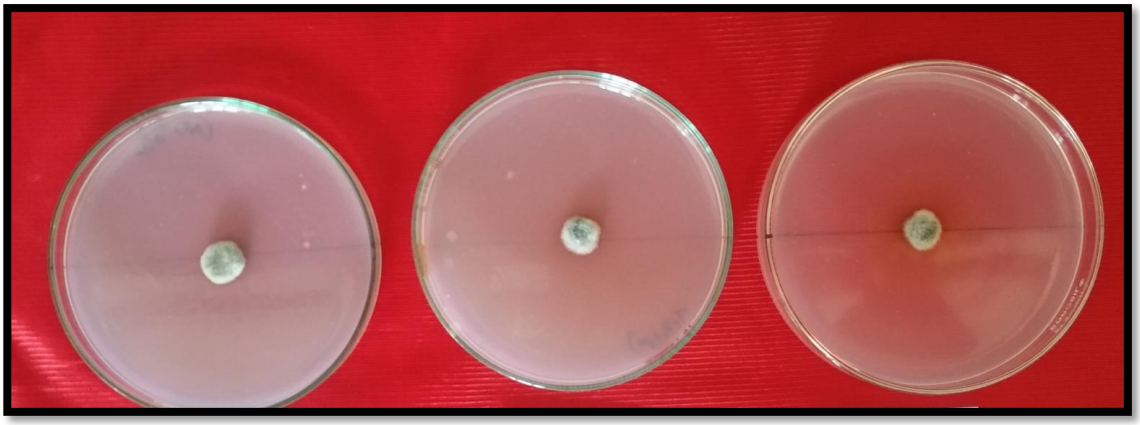
Plate 4.8.1. *In vitro* evaluation of fungicides against the pathogen



(d) T_4-Azoxystrobin 23% SC (0.1%, 0.15%, 0.2%)

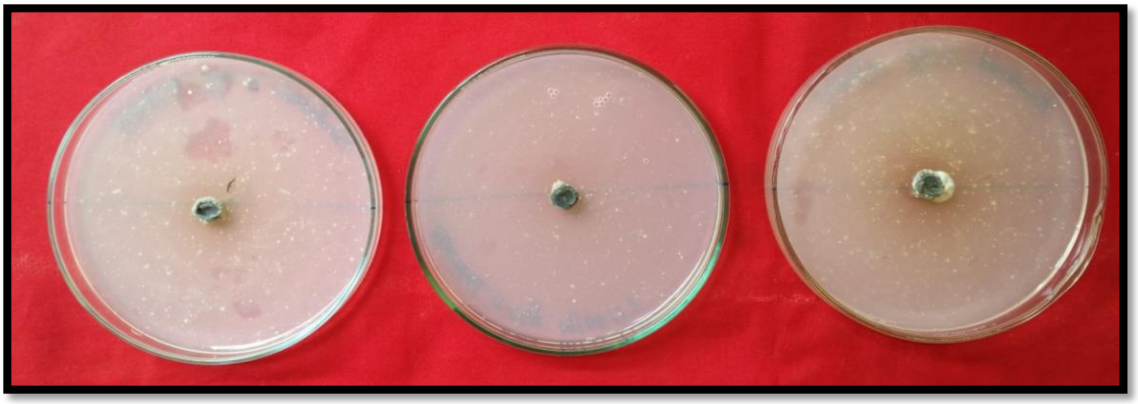


(e) T_5-Hexaconazole 5% EC (0.05%, 0.1%, 0.15%)

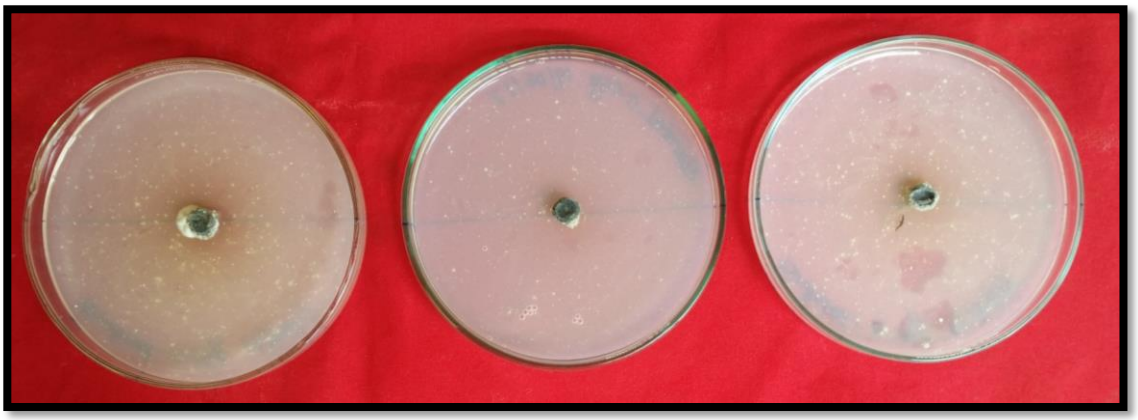


(f) T_6-Difenoconazole 25% EC (0.025%, 0.05%, 0.075%)

Plate 4.8.2. *In vitro* evaluation of fungicides against the pathogen

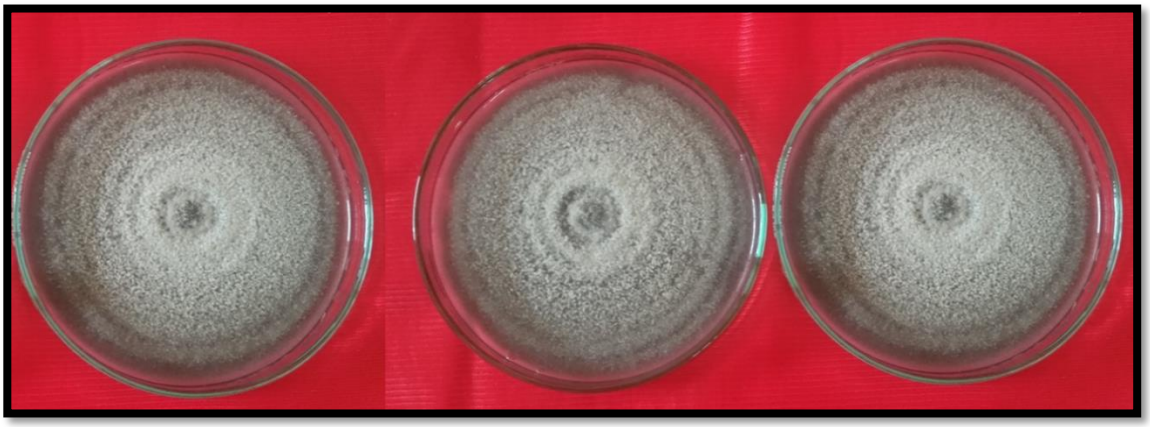


(g) T_9. Iprodione 25% + Carbendazim 25% WP (0.1%, 0.2%,0.3%)



(h) T_{10}. Trifloxystrobin 25% + Tebuconazole 55% WG

(0.025%, 0.05%, 0.075%)



(i) Control

Plate 4.8.2. *In vitro* evaluation of fungicides against the pathogen

all the bacterial antagonists, except *Bacillus subtilis* (Endophyte from cocoa) which showed 51.66 per cent. Among different bacterial antagonists *P. fluorescens*, showed the lowest growth inhibition (34.44%) of the fungus *A. solani* over the control. It was evident from the results that among individual microorganism of PGPM mix of KAU, fungal counterpart *viz. T. viride* (PGPM mix), *T. harzianum* (PGPM mix) showed maximum inhibition than the bacterial counterpart (Plate 4.9).

Among the nine antagonists, three efficient antagonists one each from reference culture [*T. viride* (KAU)], endophytes [*Bacillus subtilis*] and plant growth promoting microbial consortium [PGPM mix of KAU] were selected for field screening.

4.5. Management of early blight disease of tomato under protected cultivation

Field experiments were conducted simultaneously in polyhouse ($300m^2$) and rain shelter ($200m^2$) during July to January, 2017-2018 to evaluate selected fungicidal and bioagents treatments for the management of *A. solani* causing early blight disease of tomato under protected condition. The structures are facing North South direction and with a gable type roof. Soil solarization was carried out before transplantation of tomato seedling for a period of 90 days in polyhouse and rain shelter (Plate 4. 10). Treatments were applied as seed treatment and foliar spray. Observation on biometric characters during nursery and field and disease incidence and severity were recorded.

4.5.1 Effect of seed treatment on seed germination

Seeds treatment was carried out with selected fungicides and bioagents before sowing to study the effect of seed treatment on seed germination and seedling vigour (Plate 4. 11). The results of seed germination were furnished in Table 4.5.

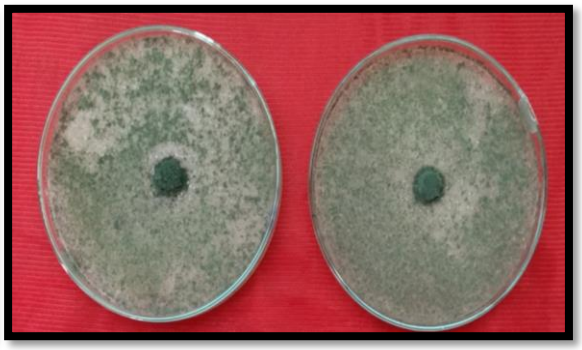
The results show that, there was a significant difference among treatments in number of seed germinated and germination per cent at five days after sowing. The maximum per cent germination (38.66%) was recorded for T_9 (PGPM mix of KAU) and which was on par with T_8 (*T. viride* of KAU) and T_{10} (*Bacillus subtilis* 1) and minimum (20.66%) was in T_4

and this was on par with T_7 and T_6 respectively. However, ten days after sowing, treatments were found non-significant on per cent seed germination and almost all seeds were germinated. Hence, results revealed that, even though treatments were non-significant on total per cent seed gemination, there was a difference among treatments in earliness of seed germination.

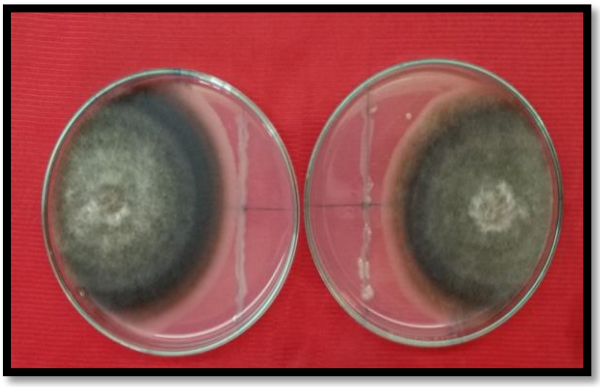
4.5.1.2 Effect of seed treatment on seedling vigour

Biometric characters including shoot length, root length, girth and number of leaves were recorded for each treatment (Table. 4.6). All parameters showed significant difference between different treatments (Plate 4.12). With respect to shoot length, the maximum shoot length of 15.11 cm was noticed in PGPM mix (T_9) treatment which was followed by T_8 (*T. viride* of KAU) and T_{10} (*B. subtilis* 1) respectively and minimum was in control (T_{11}) with 11.6 cm which was on par with T7 (Iprodione 25% + carbendazim - 0.2%) and T4 (Hexaconazole- 0.1%). Similarly, the maximum root length was observed in T_9 (9.43 cm) and minimum was in T_4 (5.99 cm) and which was on par with T_7 (6.25 cm) and T_{11} (6.74 cm) respectively. Likewise, girth was found maximum in T_9 (2.02 cm) which was on par with T_8 (1.93 cm) and minimum was in T_7 (1.34 cm). while in case of number of leaves, most of the treatments was on par which each other.

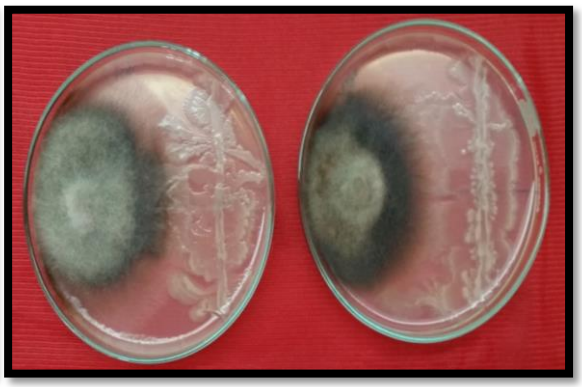
(a) T_{1-} *Trichoderma viride* (KAU)



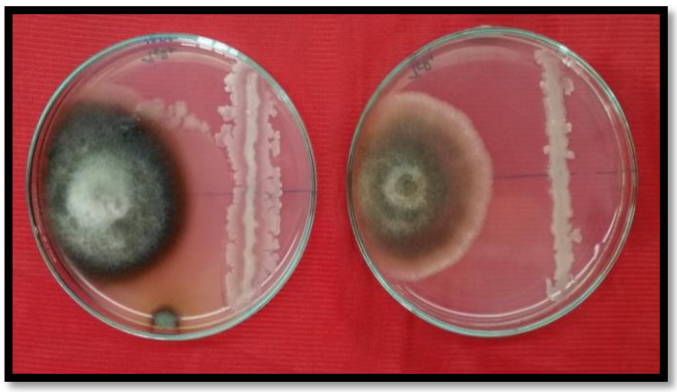
(b) T_2-PGPM mix (KAU)



(c) T_3-*Pseudomonas fluorescens* (KAU)

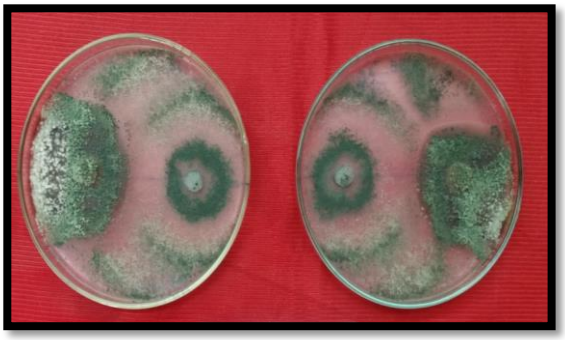


(d) T_4-*Bacillus subtilis* (KAU)

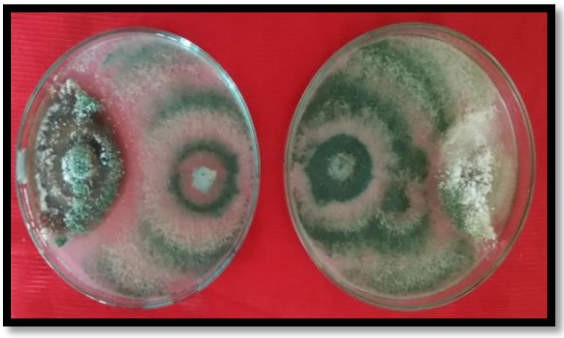


(e) T_5-*Bacillus subtilis* (Endophyte from cocoa)

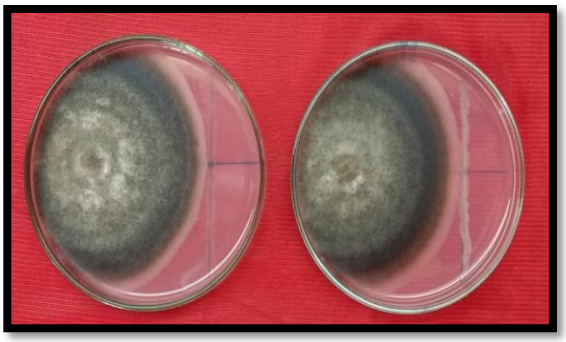
Plate 4.9.1. *In vitro* evaluation of bioagents against the pathogen



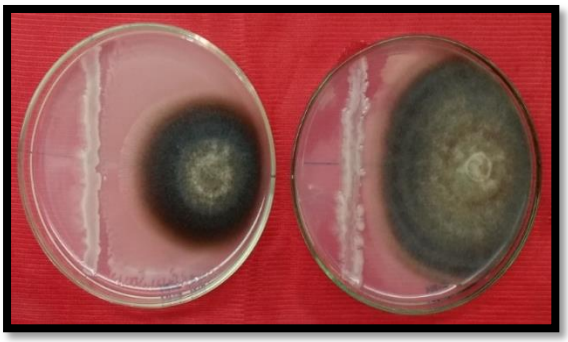
(f) T_6-*T. viride (*PGPM mix)



(g)T_7-*T. harzianum*(PGPM



(h)T_8-*Pseudomonas fluorescens* (PGPM



(i)T_9-*Bacillus megatherium* (PGPM mix)

(j) Control

Plate 4.9.2. *In vitro* evaluation of bioagents against the pathogen

(a) Polyhouse **(b)Rain shelter**

Plate 4.10. Soil solarization inside polyhouse and rain shelter

Plate 4.11. Seed treatments with selected fungicides and bioagents

Table 4.5. Effect of seed treatments on seed germination

Treatment	5 DAS		10 DAS	
	No. of seed germinated	Per cent Germination	No. of seed germinated	Per cent Germination
T_1- Propineb 70% WP (0.1%)	14.66^{bcd}	29.33^{bcd}	50	100
T_2-Propineb 70% WP (0.2%)	14.33^{cd}	28.66^{cd}	48	96
T_3- Hexaconazole 5% EC (0.05%)	13.66^{de}	27.33^{de}	50	100
T_4- Hexaconazole 5% EC (0.1%)	10.33^{f}	20.66^{f}	48.33	96.66
T_5- Difenoconazole 25% EC (0.05%)	17.66^{ab}	35.33^{ab}	49.33	98.66
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	12.33^{def}	24.66^{def}	49	98
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	10.66^{ef}	21.33^{ef}	49.33	98.66
T_8- *Trichoderma viride* (KAU)	18^{a}	36.66^{a}	49.33	98.66
T_9- PGPM mix (KAU)	19.33^{a}	38.66^{a}	50	100
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	18.33^{a}	36.66^{a}	50	100
T_{11}- Untreated control	17^{abc}	34.00^{abc}	48.66	97.33

Table 4.6. Effect of treatments on seedling vigour (24 DAS)

Treatments	*Shoot length (cm)	*Root length (cm)	*Girth (cm)	*No. of leaves	Vigour index
T_1- Propineb 70% WP (0.1%)	13.52^{bc}	8.38^{b}	1.84^{bc}	3.00^{c}	2057.99^{cd}
T_2-Propineb 70% WP (0.2%)	12.72^{cd}	7.75^{bc}	1.74^{cd}	3.66^{ab}	1956.09^{de}
T_3- Hexaconazole 5% EC (0.05%)	12.00^{de}	6.95^{cde}	1.92^{ab}	4.00^{a}	1955.33^{de}
T_4- Hexaconazole 5% EC (0.1%)	11.63^{e}	5.99^{f}	1.80^{bc}	4.00^{a}	1775.88^{f}
T_5- Difenoconazole 25% EC (0.05%)	13.75^{b}	8.00^{b}	1.34^{f}	3.66^{ab}	2081.98^{bcd}
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	11.26^{e}	6.74^{def}	1.64^{de}	3.00^{c}	1920.64^{e}
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	11.60^{e}	6.25^{ef}	1.55^{e}	4.00^{a}	1775.96^{f}
T_8- *Trichoderma viride* (KAU)	14.27^{ab}	8.24^{b}	1.93^{ab}	4.00^{a}	2202.66^{b}
T_9- PGPM mix (KAU)	15.11^{a}	9.43^{a}	2.02^{a}	4.00^{a}	2454.66^{a}
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	13.79^{b}	8.22^{b}	1.72^{cd}	3.33^{bc}	2150.78^{bc}
T_{11}- Untreated control	13.57^{bc}	7.62^{bcd}	1.61^{de}	4.00^{a}	1997.65^{de}

DAS -days after sowing
*Mean of the three replications

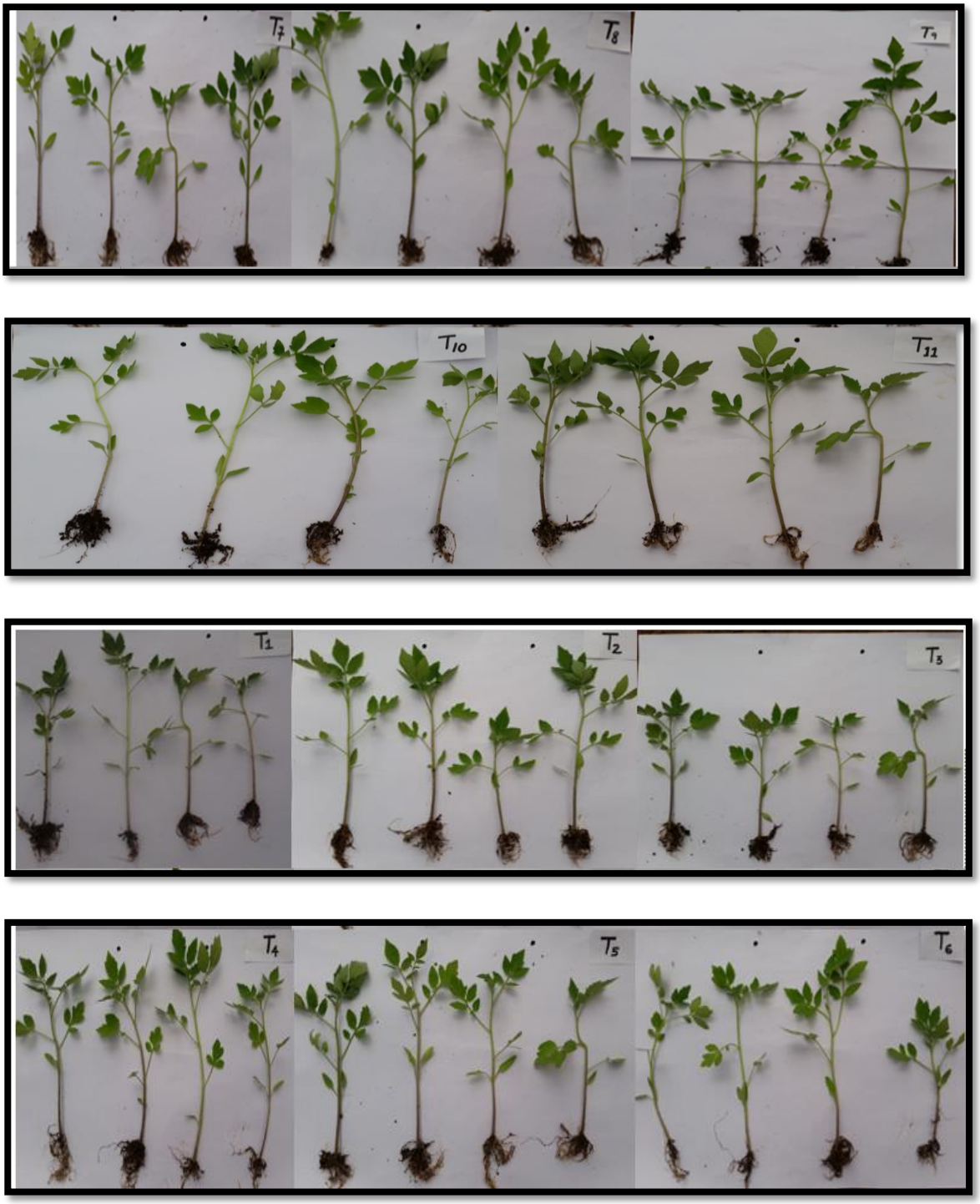


Plate 4.12. Effect of treatments on seedling vigour

It was evident from the data that, even though there is a significant difference between the treatments, difference between fungicidal treatments *viz.* T_7 and T_4 with control was almost on par. However, bioagents treatments showed clear difference from all fungicidal and control treatments.

Vigour index of tomato seedlings was determined using the formula given in 3.6.1 and showed in Table 10. Significant difference in vigour index was noticed between treatments. The treatment T_9 recorded highest vigour index of 2454.66 and which was followed by T_8 and T_{10} with vigour index of 2202.66 and 2150.78 respectively. The lowest vigour index was recorded in T_4 (1775.88) and T_7 (1775.96). the results indicated that, seed treatments with selected bioagents had a good effect in enhancing the seedling vigour index compared to fungicidal treatments and control. Moreover, it was found that, seed treatments with fungicides *viz.* propineb (0.1% & 0.2%) and difenoconazole (0.05%) was superior over control for enhancing seedling vigour index (Plate 4.13 & 4.14).

4.5.3. Effects of treatments on per cent disease incidence under polyhouse and rain shelter

Disease incidence were recorded one week after the challenge inoculation of the pathogen. Spore suspension spray was used for the artificial inoculation as mentioned in 3.1. The per cent disease incidence was found out using the formula as described in 3.5.4.1 (Table 4.7).

As artificial inoculation was given, early blight incidence was noticed in most of the plants in all the treatments recording 83 to 94 per cent incidence. Hence, no significant difference in per cent disease incidence was found between different treatments under polyhouse and rain shelter conditions.

Table 4.7. Effect of treatments on per cent disease incidence

Treatments	Per cent disease incidence	
	Rain shelter	Polyhouse
T_1- Propineb 70% WP (0.1%)	83.55	85.99
T_2-Propineb 70% WP (0.2%)	85.03	87.99
T_3- Hexaconazole 5% EC (0.05%)	86.44	89.22
T_4- Hexaconazole 5% EC (0.1%)	86.22	93.99
T_5- Difenoconazole 25% EC (0.05%)	89.77	89.66
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	84.75	91.55
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	91.99	91.03
T_8- *Trichoderma viride* (KAU)	83.99	89.33
T_9- PGPM mix (KAU)	84.36	89.22
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	88.88	94.06
T_{11}- Untreated control	91.91	92.44
CD (0.01)	NS	NS

Plate 13. Various stages of crop in rain shelter

Plate 4.14. Various stages of crop in polyhouse

However, while comparing early blight incidence in polyhouse and rain shelter, per cent incidence was more in polyhouse ranging from 85-95 per cent with respect to 83-91per cent in rain shelter. Similarly, days taken for disease incidence was more in rain shelter (12 DAI) compared to polyhouse (9 DAI) condition.

4.5.4. Effect of treatments on per cent disease severity

The effectiveness of selected 7 fungicides and 3 bioagents were tested against the early blight disease of tomato under polyhouse and rain shelter. Foliar application of treatments was given at the onset of early blight disease. Early blight disease severity was recorded at the time of treatment applications and 10 days after first, second and third spraying of treatments application simultaneously in polyhouse and rain shelter condition (Plate 4.15). Six plants per replication per treatments were selected randomly and observation on severity of the disease on the foliage was recorded. The per cent disease severity was calculated as described in 3.5.4.2.

4.5.4.1. Effect of treatments on per cent disease severity under polyhouse

Results of the field experiment for management of early blight disease of tomato caused by *A. solani* in polyhouse are presented in Table 4.8.

It was observed from the data that, all treatments were superior to control and significant difference was noticed among the treatments at all intervals of observations. Moreover, it was noticed that, rate of disease progress was very less in all treatments compared to control at different intervals of observation which indicate the positive effect of treatments on the spread of infection.

Table 4.8. Effect of treatments on per cent disease severity of tomato early blight under polyhouse condition

Treatments	Per cent disease severity				Per cent reduction over control
	Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T_1- Propineb 70% WP (0.1%)	21.9 (4.67)	23.46 (4.84)^{e}	25.73 (5.07)^{ef}	26.13 (5.11)^{fg}	70.83
T_2-Propineb 70% WP (0.2%)	22.24 (4.71)	22.66 (4.75)^{ef}	24.80 (4.97)^{fg}	25.20 (5.01)^{fg}	71.87
T_3- Hexaconazole 5% EC (0.05%)	21.52 (4.63)	27.46 (5.24)^{cd}	28.00 (5.29)^{de}	28.53 (5.34)^{de}	68.18
T_4- Hexaconazole 5% EC (0.1%)	20.58 (4.52)	24.80 (4.97)^{de}	26.40 (5.13)^{ef}	27.20 (5.21)^{ef}	69.64
T_5- Difenoconazole 25% EC (0.05%)	22.67 (4.75)	27.46 (5.24)^{cd}	29.33 (5.41)^{d}	29.86 (5.46)^{d}	66.67
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	20.93 (4.57)	22.13 (4.70)^{ef}	24.53 (5.03)^{fg}	25.33 (5.03)^{fg}	71.72
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	19.40 (4.40)	20.26 (4.49)^{f}	23.46 (4.89)^{g}	24.00 (4.89)^{g}	73.21
T_8- *Trichoderma viride* (KAU)	22.37 (4.72)	36.00 (5.99)^{b}	38.40 (6.32)^{b}	40.06 (6.32)^{b}	55.29
T_9- PGPM mix (KAU)	22.21 (4.71)	30.40 (5.51)^{c}	32.80 (5.88)^{c}	34.66 (5.88)^{c}	61.31
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	22.96 (4.79)	38.40 (6.19)^{b}	39.20 (6.45)^{b}	41.73 (6.45)^{b}	53.42
T_{11}- Untreated control	24.81 (4.98)	82.40 (9.07)^{a}	87.73 (9.46)^{a}	89.60 (9.47)^{a}	-

Figures in parenthesis are transformed values

0 -no infection **1 -below 5% infection** **2 -5.1-10% infection**

3 -10.1-25% infection **4 -25.1-50% infection** **5 -above 50% infection**

Plate 4.15. Score chart for early blight of tomato

Since artificial inoculation of pathogen was given uniformly, there was no significant difference in disease severity among treatments before foliar application. Among the eleven treatments, spraying of iprodione 25% + carbendazim 25% WP (0.2%) (T_7) showed the lowest disease severity at all spray intervals recording 20.26, 23.46 and 24.0 per cent with 73.21 per cent reduction over control. This was statistically on par with spraying of iprodione 25% + carbendazim 25% WP (0.1%) (T_6), propineb 70% WP- 0.1% (T_1) and propineb 70% WP- 0.2% (T_2) with 25.33, 26.13 and 25.20 per cent disease severity after third spray and 71.71, 70.83 and 71.87 per cent reduction over control respectively.

The plants treated with hexaconazole (T3 and T4) showed low disease severity (28.53% and 27.20%) compared to difenoconazole treated plants (T5) which recorded 29.86 per cent severity after third spray. Hence, it was evident that, among fungicidal treatments difenoconazole was least efficient in the management of early blight disease in polyhouse.

All bioagents recordedmore than 50 per cent reduction of early blight disease over control. Among the three bioagent treatments, foliar spray with PGPM mix (T_9) gave best result in the reduction of early blight severity recording 34.66 per cent with 61.31 per cent reduction over control. This was followed by T_8 (*T. viride*) where 40.06 per cent severity and 55.29 per cent reduction was recorded and it was statistically on par with T_{10} (*B. subtilis*) which accounted 41.73 per disease severity and 53.42 per cent reduction of early blight disease over control.

4.5.4.2. Effect of treatments on per cent disease severity under rain shelter

Results of field experiment on management of early blight disease of tomato under rain shelter are furnished in Table 4.9.

Table 4.9. Effect of treatments on per cent disease severity of tomato early blight under rain shelter

Treatments	Per cent disease severity				Per cent reduction over control
	Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T1- Propineb 70% WP (0.1%)	20.16 (4.49)	22.40 (4.73)^{ef}	24.33 (4.93)^{efg}	25.33 (5.03)^{ef}	71.79
T2-Propineb 70% WP (0.2%)	21.06 (4.58)	21.20 (4.59)^{fg}	22.53 (4.71)^{gh}	24.00 (4.89)^{fg}	73.27
T3- Hexaconazole 5% EC (0.05%)	20.46 (4.52)	24.53 (4.95)^{de}	26.13 (5.11)^{de}	27.33 (5.22)^{e}	70.31
T4- Hexaconazole 5% EC (0.1%)	19.66 (4.43)	25.33 (5.03)^{d}	26.00 (5.09)^{ef}	26.66 (5.16)^{e}	69.56
T5- Difenoconazole 25% EC (0.05%)	20.60 (4.53)	26.66 (5.16)^{d}	28.26 (5.31) ^{d}	29.60 (5.44)^{d}	67.04
T6- Iprodione 25% + carbendazim 25% WP (0.1%)	18.70 (4.31)	20.53 (4.52)^{fg}	23.86 (4.88)^{fgh}	24.26 (4.92)^{fg}	72.98
T7- Iprodione 25% + carbendazim 25% WP (0.2%)	18.48 (4.29)	19.93 (4.35)^{g}	21.86 (4.67)^{h}	22.93 (4.78)^{g}	74.46
T8- Trichoderma viride (KAU)	20.46 (4.52)	34.66 (5.88)^{b}	37.60 (6.13)^{b}	38.73 (6.22)^{b}	56.87
T9- PGPM mix (KAU)	20.80 (4.55)	30.40 (5.51)^{c}	32.00 (5.65)^{c}	34.13 (5.84)^{c}	62.00
T10- Bacillus subtilis (Endophyte from cocoa)	21.12 (4.59)	36.80 (6.06)^{b}	38.66 (6.21)^{b}	39.40 (6.27)^{b}	56.12
T11- Untreated control		79.73 (8.92)^{a}	86.93 (9.32)^{a}	89.80 (9.47)^{a}	-

Figures in parenthesis are transformed values

Results revealed that, all treatments were effective for the management of early blight disease with 56-74 per cent reduction of disease over control and significant difference was noticed among the treatments at all intervals of observations. It was noticed that, even though there was a slight increase in disease per cent severity after each spray, all treatments at all stages of observations were found superior to control in reducing the rate of disease progress. Moreover, disease severity trends among different treatments were same after first, second and third spray.

Data presented in the Table 4.9 showed that, plants treated with iprodione 25% + carbendazim 25% WP (0.2%) (T_7) was best among all treatments recorded lowest disease severity (18.48, 19.93 and 21.86 per cent) at all intervals of observation and highest disease reduction (74.46 per cent). This was statistically on par with T_2 (propineb 70% WP - 0.2%) and T_6 (iprodione 25% + carbendazim 25% WP -0.1%) and which showed 21.20 and 20.53 per cent severity after first spray, 22.53 and 23.86 per cent after second spray and 24.0 and 24.26 per cent after third spray respectively. These were followed by T_1, T_4 and T_3 recording 25.33, 26.0 and 26.66 per cent disease severity respectively after third spray. Plants treated difenoconazole (T5) showed higher per cent of severity (29.60%) and lowest disease reduction (67.04%) among different fungicidal treatment.

All the bioagents evaluated against early blight disease of tomato under rain shelter gave more than fifty-five per cent reduction disease over control plants. Among the three bioagents treatment tested, plants sprayed with PGPM mix (T_9) recorded highest disease reduction (62 per cent) and lowest disease severity (34.13 per cent). This was followed by T_8 and T_9 and found statistically on par with each other with per cent disease severity of 38.73 and 39.40 respectively after third spray.

Summing up the results of effect of the experiment on the management of early blight disease of tomato under polyhouse and rain shelter, it is clearly evident that, fungicides *viz.* iprodione 25% + carbendazim 25% WP (0.1% and 0.2%) and propineb (0.2%) was more effective and PGPM mix application was most efficient treatments among different bioagents. Moreover, efficacy of selected fungicides and bioagents showed same

trends both under polyhouse and rain shelter conditions. However, disease severity was comparatively more in polyhouse than rain shelter at different intervals of observation. Hence, per cent disease reduction was observed more for plants cultivated in the rain shelter.

4.5.5. Effect of treatments on biometric characters of tomato

Biometric characters such as plant height, collar girth, days to flowering, number of flowers per plant, number of fruits per plant, average fruit weight and yield per plot were recorded during field experiment under polyhouse and rain shelter and presented in Table 4.10, 4.11, 4.12, 4.13, and 4.14.

4.5.5.1. Effect of treatments on plant hight

It is observed from the data presented in Table 4.10 that, significant difference was noticed among different treatments with respect to plant hight both under polyhouse and rain shelter condition.

In polyhouse, the maximum plant height was observed in T_9 (308.80 cm) and it was followed by T_{10} and T_8 with 294.33 cm and 290.66 cm respectively. Plants in control recorded 283.59 cm which was higher than all the fungicidal treated plant's height except, difenoconazole -0.05% (T_5) which was statistically on par with control (284.13 cm). Hexaconazole - 0.1% (T_4) treated plants showed the lowest plant height (259.99 cm).

In rain shelter, plants treated with PGPM mix (T_9) showed maximum plant height (280.78 cm). This was followed by T_{10} (268.10 cm) and it was on par with T_8 recording 263.16 cm. Here also difenoconazole -0.05% (T_5) treated plants showed comparatively better response on plant height than other fungicides however, this was statistically on par with control (T_{11}), propineb -0.1% and 0.2% (T_1 and T_2) and iprodione 25% + carbendazim - 0.1% (T_6). The minimum plant height of 241.14 cm was recorded in T_4.

Summing up the results, the effect of different treatments on plant height shows same almost same trend in polyhouse and rain shelter. However, in polyhouse treatments,

plant growth rate was more compared to rain shelter. In both cases, plants treated with bioagents showed the highest plant height than fungicide treated plants and control. In both cases the plants applied with PGPM mix (T_9) recorded maximum plant and minimum in hexaconazole - 0.1% (T_4) treated plants. Similarly, among different fungicides, difenoconazole showed a better response with respect to plant height.

4.5.5.2. Effect of treatment on collar girth

It is evident from the Table 4.11 that, in polyhouse condition, effects of selected treatments on collar girth were significantly different and maximum (3.66 cm) was recorded for plants sprayed with PGPM mix (T_9) and this was followed by foliar application of *Bacillus subtilis* (T_{10}) and *Trichoderma viride* (T_8) with a value of 3.47 cm and 3.33 cm respectively. Among the selected fungicidal treatments, plants sprayed with difenoconazole -0.05% (T_5) showed highest collar girth and lowest was recorded for hexaconazole –0.1% (T_4) and iprodione 25% + carbendazim - 0.2% (T_7) treated plants.

In rain shelter, significant difference among treatment on collar girth was not observed during one month after transplanting. Even though bioagents applications showed slightly better performance than fungicides and control treatments. However, two months after transplanting, a slight significant difference was observed and maximum collar girth was observed for T_9 (3.63 cm) which was on par with T_{10}, T_8, T_1, T_5 and T_2 respectively and hexaconazole - 0.05% and 0.1% (T_3 and T_4) recorded minimum collar girth.

Table. 4.10 Effect of treatment on plant height

Treatments	Plant height (cm)			
	Rain shelter		Polyhouse	
	*1 MAT	*2 MAT	*1 MAT	*2 MAT
T_1- Propineb 70% WP (0.1%)	68.13^{cd}	257.63^{cd}	83.13^{c}	274.73^{d}
T_2-Propineb 70% WP (0.2%)	64.20^{de}	254.86^{cd}	74.60^{d}	274.50^{d}
T_3- Hexaconazole 5% EC (0.05%)	60.76^{ef}	244.53^{ef}	74.4^{d}	270.33^{de}
T_4- Hexaconazole 5% EC (0.1%)	50.33^{g}	241.14^{f}	59.93^{f}	259.99^{f}
T_5- Difenoconazole 25% EC (0.05%)	71.26^{c}	261.1^{bcd}	82.10^{c}	284.13^{c}
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	61.91^{ef}	254.57^{cd}	70.86^{d}	270.93^{de}
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	56.06^{fg}	252.04^{de}	66.46^{e}	266.33^{e}
T_8- *Trichoderma viride* (KAU)	73.13^{bc}	263.16^{bc}	84.13^{c}	290.66^{b}
T_9- PGPM mix (KAU)	81.88^{a}	280.78^{a}	93.24^{a}	308.80^{a}
T_{10}- *Bacillus subtilis* 1 (Endophyte from cocoa)	78.13^{ab}	268.10^{b}	88.53^{b}	294.33^{b}
T_{11}- Untreated control	64.4^{de}	258.16^{cd}	74.73^{d}	283.59^{c}

***MAT- month after transplanting**

Table 4.11. Effect of treatment on girth at collar

Treatments	Girth at collar (cm)			
	Rain shelter		Polyhouse	
	*1 MAT	*2 MAT	*1 MAT	*2 MAT
T_1- Propineb 70% WP (0.1%)	3.04	3.56^{ab}	2.72^{cde}	3.18^{cde}
T_2-Propineb 70% WP (0.2%)	2.98	3.50^{ab}	2.56^{def}	3.12^{def}
T_3- Hexaconazole 5% EC (0.05%)	2.81	3.08^{c}	2.47^{fg}	3.03^{ef}
T_4- Hexaconazole 5% EC (0.1%)	2.92	3.08^{c}	2.3^{g}	2.98^{f}
T_5- Difenoconazole 25% EC (0.05%)	3.08	3.56^{ab}	2.78^{bcd}	3.28^{cd}
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	2.96	3.46^{ab}	2.44^{fg}	3.12^{def}
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	2.62	3.26^{bc}	2.3^{g}	2.96^{f}
T_8- *Trichoderma viride* (KAU)	3.09	3.56^{ab}	2.82^{bc}	3.33^{bc}
T_9- PGPM mix (KAU)	3.17	3.63^{a}	3.31^{a}	3.66^{a}
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	3.06	3.57^{ab}	2.97^{b}	3.47^{b}
T_{11}- Untreated control	2.68	3.42^{abc}	2.52^{efg}	3.13^{def}

***MAT- month after transplanting**

4.5.5.3. Effect of treatments on days to flowering and number of flowers

Effect of treatments on days to flowering and number of flowers under polyhouse and rain shelter was studied and found significant difference among treatments in case of days to flowering while treatments were more or less uniform with respect to number of flowers (Table 4.12). In polyhouse, first flowering took place within 34 -42 days after transplanting while it was 36-43 in rain shelter. In both cases, all the three bioagent treatments (T_8, T_9, T_{10}) recorded slight earliness in flowering compared to fungicidal treatments as well as control. It also observed that, the days required for first flowering was almost same for all fungicidal treatments. Two months after transplanting, the number of flowers per plant were 27 -30 and 21-28 in polyhouse and rain shelter respectively. Hence, from the above results it was noticed that, earliness in flowering and number of flowers per plant were more under polyhouse condition than rain shelter.

4.5.5.4. Effect of treatments on yield

Significant difference was noticed among the treatments with respect to all the observed yield parameters such as average number of fruits per plant, fruit weight and yield per plot (Table 4.13). Moreover, all treatments under polyhouse and rain shelter were significantly superior to control in all the observed yield attributes (Plate 16 & 17).

In case of polyhouse treatments, average number of fruits per plant was more in plants sprayed with iprodione 25% + carbendazim 25% WP -0.2% (T_7) with 9 fruits per plant and this was statistically on par with T_6 (iprodione 25% + carbendazim 25% WP - 0.1%) obtained 8 fruits per plant. It was followed by T_1, T_2 and T_4 with 7 fruits per plant. Least average number of fruits per plant was recorded in control (2 per plant). While in case of average weight of fruits, T_9 (PGPM mix application) recorded maximum (38.12g) and it was on par with T_8 (36.21g) and T_{10} (36.07g) and remaining treatments were almost on par which each other. Regarding average yield per plot, all treatments were significantly superior to control and maximum was observed in T_7 and it was on par with T_6 and T_2 with 1.13 kg, 1.08 kg and 1.0 kg respectively against 0.72 kg in control.

Table 4.12 Effect of treatment on flowering

Treatments	Polyhouse			Rain shelter		
	Days to flowering	Number of flowers		Days to flowering	Number of flowers	
		*1 MAT	*2 MAT		*1 MAT	*2 MAT
T_1- Propineb 70% WP (0.1%)	38.66[c]	5.00	30.66	40[b]	2.33	24.26
T_2-Propineb 70% WP (0.2%)	40.66 [abc]	4.33	30.33	43[a]	2.66	24.26
T_3- Hexaconazole 5% EC (0.05%)	39.66 [abc]	4.33	29.00	43[a]	3.33	21.20
T_4- Hexaconazole 5% EC (0.1%)	41.66 [ab]	4.66	27.33	43.66[a]	3.66	21.20
T_5- Difenoconazole 25% EC (0.05%)	40.33 [abc]	3.33	29.00	43.33[a]	4.00	15.00
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	40.66[abc]	6.00	30.66	43.33[a]	3.30	24.46
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	42.33[a]	5.00	30.00	43.33[a]	5.00	22.06
T_8- *Trichoderma viride* (KAU)	39.33[d]	7.00	30.66	37[cd]	5.00	27.06
T_9- PGPM mix (KAU)	34.33[d]	8.00	33.00	36.66[d]	7.00	28.26
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	35.33[d]	7.00	30.00	38.33[c]	6.00	24.80
T_{11}- Untreated control	39.33[bc]	4.33	29.33	41[b]	3.33	21.86
CD (0.05)	2.708	NS	NS	1.58	NS	NS

Table 4.13. Effect of treatment on yield

Treatments	Rain shelter			Polyhouse		
	Average number of fruits/plant	Average weight of fruits (g)	Average Yield/plot (kg)	Average number of fruits/plant	Average weight of fruits (g)	Average Yield/plot (kg)
T_1- Propineb 70% WP (0.1%)	16.00^{cd}	39.10^{cd}	5.42^{cd}	6.66^{b}	33.00^{bc}	0.89^{cde}
T_2-Propineb 70% WP (0.2%)	19.00^{ab}	43.36^{bc}	5.68^{c}	7.00^{b}	29.73^{c}	1.00^{abc}
T_3- Hexaconazole 5% EC (0.05%)	15.00^{de}	40.74^{cd}	4.84^{ef}	4.33^{def}	28.73^{c}	0.84^{def}
T_4- Hexaconazole 5% EC (0.1%)	17.33^{bc}	41.46^{bcd}	5.51^{c}	6.66^{b}	30.74^{bc}	0.97^{bcd}
T_5- Difenoconazole 25% EC (0.05%)	15.00^{de}	38.21^{d}	4.51^{fg}	6.00^{bc}	28.21^{c}	0.79^{ef}
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	18.00^{b}	41.84^{bcd}	6.17^{b}	8.00^{a}	30.25^{bc}	1.08^{ab}
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	20.00^{a}	41.92^{bcd}	7.26^{a}	9.00^{a}	31.92^{bc}	1.13^{a}
T_8- *Trichoderma viride* (KAU)	14.00^{ef}	46.21^{ab}	4.74^{f}	4.00^{ef}	36.21^{ab}	0.83^{ef}
T_9- PGPM mix (KAU)	15.33^{de}	48.52^{a}	5.13^{de}	5.33^{cd}	38.12^{a}	0.84^{def}
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	13.00^{ef}	46.07^{ab}	4.25^{g}	3.66^{f}	36.07^{ab}	0.79^{ef}
T_{11}- Untreated control	11.00^{g}	37.87^{d}	3.77^{h}	1.66^{g}	27.87^{c}	0.72^{f}

(a) T2 (Propineb - 0.2%) **(b)T6 (Iprodione + carbendazim- 0.1%)** **(c)T7 (Iprodione + carbendazim - 0.2%)**

(d) T9 (PGPM mix (KAU) **(e)T11 (Untreated control)**

Plate 4.16. Effect of treatments on yield under rain shelter condition

(a) T2 (Propineb - 0.2%) **(b)T6 (Iprodione + carbendazim 0.1%)** **(c)T7 (Iprodione + carbendazim - 0.2%)**

(d)T9 (PGPM mix -KAU) **(e)T11 (Untreated control)**

Plate 4.17. Effect of treatments on yield under polyhouse condition

Yield parameters under rain shelter experiments were also found significantly superior to control. The treatment iprodione + carbendazim - 0.2% (T_7) recorded significantly higher number of fruits of 20 per plant and it was followed by T_2 (19/ plant) and T_6 (18/ plant) respectively and lower number of fruits was observed in control with 11 per plant. However, average weight of tomato fruits was recorded higher for treatment T_9 (48.52g) followed by T_8 (46.21g) and T_9 (46.07 g). Even though the remaining treatments were almost on par with each other, average fruit weight was lowest in control plants recording 37.87g. With respect to average yield per plot, all treatments were significantly superior to control and maximum was observed in T_7 followed by T_6 and $T_2$with 7.26 kg, 6.17 kg and 5.68 kg respectively and minimum was in control with 3.77 kg.

Summing up the findings of effects of treatments on yield, it is observed that, all the treatments under polyhouse and rain shelter were superior to control and increased the yield. While comparing yield obtained from polyhouse and rain shelter, yield was more in rain shelter and it was very low in polyhouse. However, trends of selected treatments were more or less same under both conditions. In both cases, the average number of fruits and yield per plot was maximum in T_7 and average fruit weight was recorded maximum for bioagent treatments compared to fungicidal treatment.

Since maximum tomato yield was obtained from rain shelter, an additional crop was laid in rain shelter and the eleven treatments were repeated to conform the efficacy of the selected treatments against early blight disease of tomato (Plate 18). The results on effect of treatments on per cent disease severity are expressed in Table 4.14. Results revealed that, all treatments showed similar trend as that of first crop in rain shelter and were effective for the management of early blight disease with 53 -73 per cent of disease control and significant difference was noticed among the treatments at all intervals of observations. Like the first crop experiment results, here also iprodione 25% + carbendazim 25% WP (0.2%) (T_7) was best among all treatments recorded lowest disease severity (24 per cent) and this was statistically on par with T_6 (iprodione 25% + carbendazim 25% WP -0.1%), T_2 (propineb 70% WP - 0.2%) and T_1 (propineb 70% WP - 0.1%) and difenoconazole (T5) showed higher per cent of severity (29.86 per cent) and among different fungicidal treatment.

Table.4.14 Effect of treatments on per cent disease severity – Rain shelter II

Treatments	Per cent disease severity				Per cent reduction over control
	Before spray	After 1st spray	After 2nd spray	After 3rd spray	
T_1- Propineb 70% WP (0.1%)	20.34 (4.50)	23.46 (4.84)^{e}	25.73 (5.07)^{ef}	26.13 (5.11)^{fg}	70.83
T_2-Propineb 70% WP (0.2%)	18.52 (4.29)	22.66 (4.75)^{ef}	24.80 (4.97)^{fg}	25.20 (5.01)^{fg}	71.87
T_3- Hexaconazole 5% EC (0.05%)	18.90 (4.34)	27.46 (5.24)^{cd}	28.00 (5.29)^{de}	28.53 (5.34)^{de}	68.18
T_4- Hexaconazole 5% EC (0.1%)	17.66 (4.19)	24.80 (4.97)^{de}	26.40 (5.13)^{ef}	27.20 (5.21)^{ef}	69.64
T_5- Difenoconazole 25% EC (0.05%)	23.49 (4.82)	27.46 (5.24)^{cd}	29.33 (5.41)^{d}	29.86 (5.46)^{d}	66.67
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	18.84 (4.35)	22.13 (4.70)^{ef}	24.53 (5.03)^{fg}	25.33 (5.03)^{fg}	71.72
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	20.04 (4.47)	20.26 (4.49)^{f}	23.46 (4.89)^{g}	24.00 (4.89)^{g}	73.21
T_8- *Trichoderma viride* (KAU)	19.05 (4.36)	36.00 (5.99)^{b}	38.40 (6.32)^{b}	40.06 (6.32)^{b}	55.29
T_9- PGPM mix (KAU)	20.21 (4.49)	30.40 (5.51)^{c}	32.80 (5.88)^{c}	34.66 (5.88)^{c}	61.31
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	20.00 (4.46)	38.40 (6.19)^{b}	39.20 (6.45)^{b}	41.73 (6.45)^{b}	53.42
T_{11}- Untreated control	19.36 (4.39)	82.40 (9.07)^{a}	87.73 (9.46)^{a}	89.60 (9.47)^{a}	-

Figures in parenthesis are transformed values

Table.4.15 Effect of treatments on yield – Rain shelter II

Treatments	Rain shelter			
	Average number of fruits/plants	**Average weight of fruits (g)**	**Average Yield/plot (kg)**	**B:C ratio**
T_1- Propineb 70% WP (0.1%)	17.33^{cd}	41.43^{cd}	5.89^{cd}	1.51
T_2-Propineb 70% WP (0.2%)	20.00^{b}	42.13^{bcd}	6.82^{b}	1.58
T_3- Hexaconazole 5% EC (0.05%)	16.66^{def}	39.73^{cd}	5.78^{cd}	1.43
T_4- Hexaconazole 5% EC (0.1%)	18.00^{cd}	42.28^{bcd}	6.02^{c}	1.46
T_5- Difenoconazole 25% EC (0.05%)	16.00^{ef}	38.91^{d}	5.32^{de}	1.40
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	19.66^{bc}	42.65^{bcd}	6.12^{c}	1.48
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	22.00^{a}	44.05^{bcd}	7.66^{a}	1.53
T_8- *Trichoderma viride* (KAU)	15.33^{fg}	46.76^{ab}	5.08^{e}	1.34
T_9- PGPM mix (KAU)	16.66^{def}	49.19^{a}	5.27^{de}	1.38
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	14.91^{fg}	46.82^{ab}	4.93^{e}	1.32
T_{11}- Untreated control	12.66^{h}	38.53^{d}	4.06^{f}	1.17
CD (0.05)	1.69	4.85	0.66	

Similarly, among bioagents treatments also same trend was obtained as that of previous experiment in rain shelter and plants sprayed with PGPM mix (T_9) recorded highest disease reduction (61.31 per cent) and lowest disease severity (34.66 per cent). Hence, these results conformed the effect of selected treatments on the management of early blight disease of tomato.

Yield and yield parameters for the additional crop also recorded and it is presented in Table 4.15 The results showed significant difference among treatments and recorded a slight overall increase in number of fruits and yield per plot compared to first crop. But, the trend of different treatments on yield and yield parameters was same as that of first crop. Here also, the maximum yield (7.66 Kg) and number of fruits per plant (22) was observed for plant treated with iprodione 25% + carbendazim 25% WP (0.2%) (T_7) and maximum average fruit weight was recorded for the fruits which are collected from plant sprayed with PGPM mix (T_9). Control plants recorded poor yield and yield parameters compared to other treatments.

The benefit to cost ratio (B:C ratio) was calculated for all the treatments and is given in Table 4.15. At the market price of Rs. 24/Kg of fruit, the highest (1.58) benefit to cost ratio was obtained in the treatment T_2 (Propineb 0.2%) followed by T_7 (1.53) and T_1 (1.51) respectively. Whereas the lowest (1.17) B:C ratio was observed in untreated control plants.

4.5.6 Meteorological parameters

Temperature and relative humidity inside the poly house and rain shelter was recorded at 7.30 am and 2.30 pm daily during the experiment (Table 4.16). From the readings it was found that temperature and relative humidity were higher in polyhouse compared to rain shelter. In polyhouse temperature varied from 20.46 – 29.40 ^{0}C at7.30 am and 32.48 – 39.92 at 2.30 pm while in rain shelter it was recorded from 19.80 to 23.90 at 7.30 am and 19.80 – 36.30 at 2.30 pm. Similarly, relative humidity in polyhouse was within the range of 48-97 per cent at 7.30 am and 49-88 per cent at 2.30 pm whereas, it was 37-88 per cent at 7.30 am and 43-75 per cent at 2.30 pm inside the rain shelter.

Plate 4.18. Various stages of crop in rain shelter-II

Table 4.16 Meteorological data during the period of experiment in polyhouse and rain shelter

Std. week	Meteorological parameters							
	Polyhouse				Rain shelter			
	7.30 am		2.30 pm		7.30 am		2.30 pm	
	Temp.(^{0}C)	R.H (%)	Temp.(^{0}C)	R.H (%)	Temp.(^{0}C)	R.H (%)	Temp.(^{0}C)	R.H (%)
29	26.50	96	34.20	88	22.10	86	30.90	70
30	27.30	94	35.91	85	23.00	81	31.60	71
31	27.55	94	34.95	86	23.90	85	31.20	71
32	26.75	95	33.40	84	23.60	84	29.90	70
33	27.20	96	34.95	88	23.40	88	31.00	71
34	26.60	97	34.97	86	23.10	86	30.01	70
35	26.25	95	32.48	83	23.10	88	29.40	71
36	27.65	94	34.04	81	23.60	86	31.70	71
37	27.70	96	35.50	85	23.00	87	32.40	70
38	26.40	95	34.48	83	22.20	83	30.60	71
39	27.05	94	34.48	83	22.90	82	31.20	71
40	27.10	95	35.95	82	22.80	84	31.40	71
41	27.10	93	34.95	80	22.80	84	31.40	75
42	26.55	95	35.94	83	22.20	87	30.90	70
43	26.75	91	35.97	83	21.50	78	32.00	71
44	28.10	83	37.14	75	22.70	70	33.50	72

45	26.55	85	36.94	77	21.90	73	32.30	67
46	26.80	93	36.91	80	20.80	75	32.80	66
47	20.46	92	35.30	80	21.60	73	33.80	64
48	27.25	82	36.78	78	22.50	71	32.00	70
49	26.90	89	37.16	80	21.00	73	32.80	71
50	27.05	87	37.54	78	21.40	72	32.70	67
51	26.55	67	38.16	64	21.50	54	32.30	63
52	26.55	68	38.16	65	20.30	52	32.80	64
1	26.50	75	38.32	56	19.80	58	33.20	61
2	27.25	73	37.40	67	21.80	56	32.70	61
3	27.25	63	36.40	54	20.70	48	33.80	52
4	27.75	68	39.48	63	21.40	53	34.10	62
5	27.40	48	38.13	49	20.50	37	34.30	48
6	28.80	77	39.92	62	22.30	59	35.30	49
7	29.40	66	38.28	61	22.50	51	36.30	43

4.5.6.1. Correlation analysis of severity of early blight disease of tomato with major meteorological parameters

Correlation analysis was performed by using the data collected during the filed experiments (Table 4.17). A significant positive correlation was found between severity of early blight disease and temperature in polyhouse. Where as in rain shelter, the disease severity was showed significant positive correlation with relative humidity.

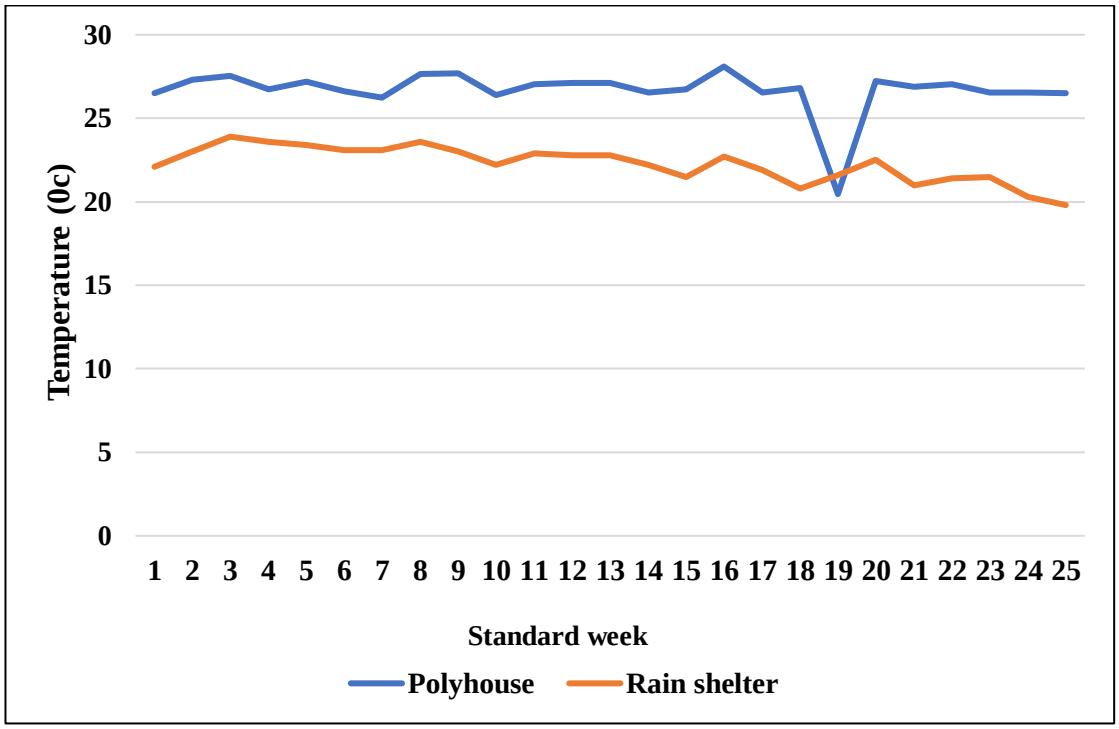


Fig. 4.1 Comparison of the temperature inside polyhouse and rain shelter

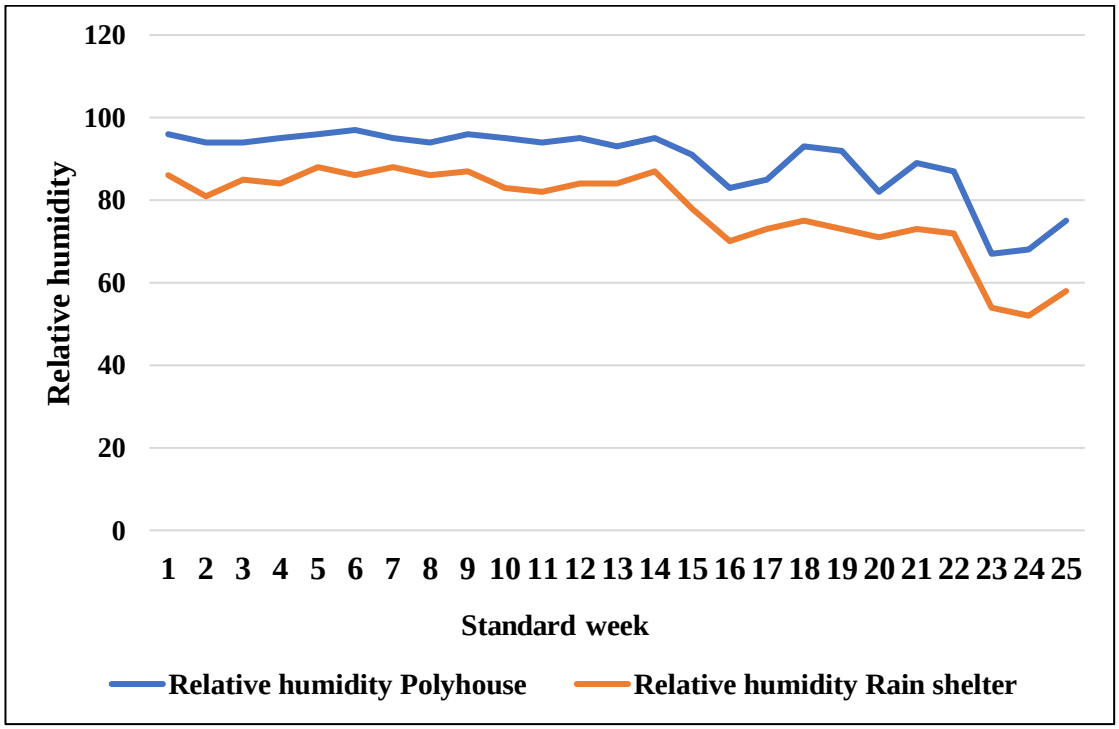


Fig. 4.2 Comparison of relative humidity inside polyhouse and rain shelter

Table 4.17 Correlation analysis of severity of early blight disease with temperature and relative humidity

Meteorological parameter	Correlation coefficient	
	Polyhouse	Rain shelter
	PDS	PDS
RH	-0.089	0.83**
Temperature	0.699*	-0.275

PDS- Per cent disease severity **RH-** Relative humidity

***Correlation is significant at 5% level ** Correlation is significant at 1% level

4.6. Residue analysis

The results of residue analysis are presented in Table 22 and 23. Since the Pesticide Residue Research and Analytical Laboratory has no facility to analyse propineb and iprodione, residue analysis was carried out for fungicides *viz*. hexaconazole (T_4), difenoconazole (T_5) and carbendazim (T_7). In ployhouse fruiting was very less hence, residue analysis was performed at first DAS and fifth DAS while in rain shelter it was performed at one, three, five and seven DAS. From the Table 4.18 and 4.19 it was clear that in both cases maximum residue was obtained from tomatoes which are picked from carbendazim treated plant. At fifth day after spraying its level was 0.25 ppm and 0.43 ppm in tomatoes collected from polyhouse and rain shelter respectively. The results also showed that even at seven days after spray carbendazim residue was at detectable level. Hexaconazole residue level was found below detectable at 5 DAS and one DAS in polyhouse and rain shelter respectively while difenoconazole residue was attained below datable level in tomato fruits at 5 DAS in polyhouse and at 7 DAS in rain shelter. Hence, these results indicate that degradation rate of fungicides was more under polyhouse condition than rain shelter.

Table 4.18 Residue analysis of tomato fruits obtained from polyhouse

Treatment	Residues in ppm (DAS)	
	1	**5**
T4-Hexaconzole	0.055	BDL
T5- Difenaconazole	0.44	BDL
T7- Carbendazim	0.86	0.25

Table 4.19 Residue analysis of tomato fruits obtained from rain shelter

Treatment	Residues in ppm (DAS)			
	1	3	5	7
T4-Hexaconzole	BDL	BDL	BDL	BDL
T5- Difenaconazole	0.6	0.14	0.08	BDL
T7- Carbendazim	0.65	0.44	0.43	0.29

DAS- Days After Spray

4.7. Enumeration of non-target culturable microflora under protected condition

The culturable phylloplane and endophytic microflora such as fungi, bacteria and actinomycetes of the tomato crop was enumerated before and after treatment application using serial dilution plating to know the changes due to the treatments (Plate 19).

4.7.1 Enumeration of phylloplane microflora

Culturable phylloplane microflora *viz.* fungi, bacteria and actinomycetes on the tomato leaves were enumerated using serial dilution plating of leaf washings to study the effects of different treatments on phylloplane microbes in polyhouse and rain shelter and the results are furnished in Tables 4.20, 4.21, 4.22, 4.23, 4.24 & 4.25.

4.7.1.1 Population of phylloplane fungi in polyhouse

Effects of selected treatments on phylloplane fungal population in polyhouse are presented in Table 4.20. Before the treatment application, more or less uniform population of phylloplane fungi was observed in all the treatment which ranged from 1.52 x 10^2 cfu cm^{-2} to 1.82 x 10^2 cfu cm^{-2}. However, after the first spray significant difference in phylloplane fungal population among the treatments were observed. A drastic reduction in the fungal population was found in all the fungicidal treatments while increased population was observed in control as well as in bioagents treatments except in plants treated with *B. subtilis* 1(T_{10}).

The highest reduction was noticed in T_7 and it was on par with T_6 and T_2 with a population of 0.73 x 10^2 cfu cm^{-2}, 0.96 x 10^2 cfu cm^{-2} and 1.07 x 10^2 cfu cm^{-2} respectively. Among different fungicidal treatments, difenoconazole -0.05% (T_5) treated plants showed comparatively less reduction of phylloplane fungi. Hence, in case of fungicidal treatments the per cent reduction of fungal population on leaves ranged from 32.18 to 64.10 per cent.

In bioagents treatment such as T_8 and T_9, where *Trichoderma* spp. was sprayed on leaves there was a drastic increase in the population of phylloplane fungal flora and the population was 3.10 x 10^2 cfu cm^{-2} and 3.72 x 10^2 cfu cm^{-2} respectively after first spray. However, in T10 where *B. subtilis* 1 was sprayed there was reduction in fungal flora, but the reduction was lesser compared to chemical treatments. Control plants recorded an increased fungal population of 3.08 x 10^2 cfu cm^{-2} after first spray. Moreover, the trends observed in the population of phylloplane fungal flora after second spray was same as that of first spray. Hence, after second spray also there was reduction in phylloplane fungal population in case of all fungicidal treatments and bioagent treatment T_{10} and population increase in control and bioagents treatments *viz.* T_8 and T_9.

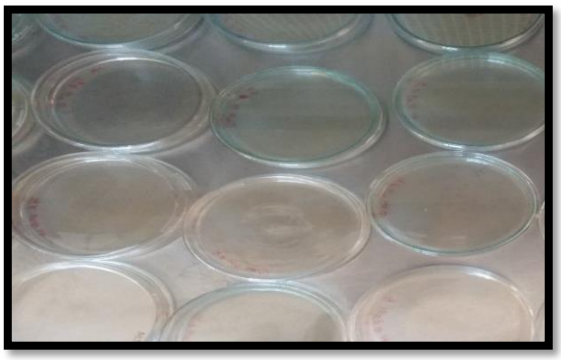
(a) Media used for isolation

(b) Extraction of phylloplane microflora in sterile water

(c) Extraction of endophytic microflora in 0.02 M tris phosphate buffer

(d) Serial dilution

(e) Incubation of plates (Martin's Rose Bengal Agar)



(f) Incubation of plates (Nutrient Agar)

Plate 4.19. Isolation of phylloplane and endophytic microflora

Table 4.20 Effect of treatments on phylloplane fungi of tomato in polyhouse

Treatments	Fungi (x 10^2 cfu cm^{-2})			
	Pre treatment	**After 1st spray**	**After 2nd spray**	**Per cent reduction**
T_1- Propineb 70% WP (0.1%)	1.64	1.29^{de}	1.07^{de}	34.75
T_2-Propineb 70% WP (0.2%)	1.72	1.07^{def}	0.87^{e}	49.41
T_3- Hexaconazole 5% EC (0.05%)	1.60	1.41^{d}	1.06^{de}	33.75
T_4- Hexaconazole 5% EC (0.1%)	1.82	1.3^{de}	1.07^{de}	41.20
T_5- Difenoconazole 25% EC (0.05%)	1.74	1.16^{de}	1.18^{d}	32.18
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	1.72	0.96^{ef}	0.8^{ef}	53.48
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.56	0.73^{f}	0.56^{f}	64.10
T_8- *Trichoderma viride* (KAU)	1.52	3.10^{b}	3.36^{b}	-121.05
T_9- PGPM mix (KAU)	1.58	3.72^{a}	4.02^{a}	-154.43
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.68	1.56^{c}	1.34^{c}	20.23
T_{11}- Untreated control	1.56	3.08^{b}	3.22^{b}	-106.41

4.7.1.2 Population of phylloplane bacteria in polyhouse

In Table 4.21 changes in phylloplane bacterial population due to foliar treatments are furnished. The results showed that, before the treatment application the population was more or less uniform on the leaves which ranges from 1.28 x 10^4 cfu cm^{-2} to 1.78 x 10^4 cfu cm^{-2}. But after the foliar application significant difference in bacterial population among the treatments was observed and population reduction was recorded in all chemical treatments as well as treatment with *T. viride* (T_8). However, population increase was observed in control and bioagent treatments *viz.* T_9 and T_{10}.

After the first spray, the treatment with iprodione 25% + carbendazim 25% W - 0.2% (T_7), propineb 70% WP- 0.2% (T_2) and hexaconazole 5% EC- 0.1% (T_4) recorded highest reduction of phylloplane bacteria with a population of 0. 87 x 10^4 cfu cm^{-2}, 0.81 x 10^4 cfu cm^{-2} and 0.78 x 10^4 cfu cm^{-2} respectively. This was followed by T_6 and T_3 recording 1.05 x 10^4 cfu cm^{-2} and 1.12 x 10^4 cfu cm^{-2} respectively. Among fungicidal treatments, minimum per cent reduction 38.28 per cent was noticed in plants treated with difenoconazole 25% EC (0.05%) (T_5) against 61.48 per cent in fungicidal treatment T_7.

Among the bioagents treatments, T_8 (*T. viride*) showed reduction in phylloplane bacterial population recorded 1.48 x 10^4 cfu cm^{-2} while an increased bacterial population of 2.62 x 10^4 cfu cm^{-2} and 2.34 x 10^4 cfu cm^{-2} was recorded in T_9 and T_{10} respectively after first spray. In control plants also there was an increase in bacterial population but the rate of increase was lesser compared to T_9 and T_{10}. The same pattern of reduction and increase was noticed for all treatments even after the second foliar application.

Table 4.21 Effect of treatments on phylloplane bacteria of tomato in polyhouse

Treatments	Bacteria (x 10^4 cfu cm^{-2})			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction
T_1- Propineb 70% WP (0.1%)	1.45	1.24^{de}	0.86^{def}	40.66
T_2-Propineb 70% WP (0.2%)	1.38	0.81^{g}	0.72^{ef}	47.82
T_3- Hexaconazole 5% EC (0.05%)	1.78	1.12^{ef}	1.05^{de}	41.01
T_4- Hexaconazole 5% EC (0.1%)	1.53	0.78^{g}	0.74^{ef}	51.63
T_5- Difenoconazole 25% EC (0.05%)	1.75	1.36^{cd}	1.08^{de}	38.28
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	1.72	1.05^{f}	0.72^{ef}	58.13
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.48	0.87^{g}	0.57^{f}	61.48
T_8- *Trichoderma viride* (KAU)	1.56	1.48^{c}	1.28^{d}	17.94
T_9- PGPM mix (KAU)	1.30	2.62^{a}	3.23^{a}	-14.84
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.28	2.34^{b}	2.56^{b}	-10.00
T_{11}- Untreated control	1.42	1.46^{c}	1.75^{c}	-02.30

4.7.1.3 Population of phylloplane actinomycetes in polyhouse

Data on population of phylloplane actinomycetes in polyhouse presented in Table 4.22. The results indicate that the effects of different treatments on actinomycetes population on the tomato leaves were more or less uniform among treatments in each intervals of observation. However, an overall reduction in population was noticed after the foliar application except in T_{10} (*B. subtilis* 1) where actinomycetes population was increased from 0.22 x 10 cfu cm^{-2} to 0.30 x 10 cfu cm^{-2}. The maximum per cent reduction was observed in T_7 (59.25 per cent) followed by T_2 and T_4 recording 58.82 per cent and 56.66 per cent respectively. In bioagents treatments such as T_8 and T_9 reduction rate was less compared to fungicidal treatments while it was more compared to control in which per cent reduction was only 8.69.

4.7.1.4. Population of phylloplane fungi in rain shelter

The results of selected treatment application's impact on phylloplane fungi in rain shelter are presented in the Table 4.23. Before the treatment application, there was no significant difference among treatments with regard to phylloplane fungal population and it was more or less uniform ranged from 1.99 x 10^2 cfu cm^{-2} to 2.50 x 10^2 cfu cm^{-2}. But, after the foliar application significant difference in fungal population among the treatments was observed and population reduction was recorded in all chemical treatments as well as treatment with *B. subtilis* (T_{10}). However, a drastic increase in the population was noticed in control and bioagent treatments *viz.* T_8 and T_9.

Table 4.22. Effect of treatments on phylloplane actinomycetes of tomato in polyhouse

Treatments	Actinomycetes (x 10 cfu cm^{-2})			
	Pre treatment	**After 1st spray**	**After 2nd spray**	**Per cent reduction over control**
T_1- Propineb 70% WP (0.1%)	0.29^{abc}	0.22^{bc}	0.13^{bc}	55.17
T_2-Propineb 70% WP (0.2%)	0.34^{a}	0.18^{c}	0.14^{bc}	58.82
T_3- Hexaconazole 5% EC (0.05%)	0.29^{abc}	0.21^{bc}	0.14^{bc}	51.72
T_4- Hexaconazole 5% EC (0.1%)	0.30^{ab}	0.18^{c}	0.13^{bc}	56.66
T_5- Difenoconazole 25% EC (0.05%)	0.24^{bcd}	0.21^{bc}	0.14^{bc}	41.66
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	0.28^{abc}	0.21^{bc}	0.13^{bc}	53.57
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	0.27^{abc}	0.18^{c}	0.11^{c}	59.25
T_8- *Trichoderma viride* (KAU)	0.28^{abc}	0.28^{abc}	0.21^{abc}	25.00
T_9- PGPM mix (KAU)	0.27^{abc}	0.37^{a}	0.23^{ab}	14.81
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	0.22^{d}	0.28^{abc}	0.30^{a}	-36.36
T_{11}- Untreated control	0.23^{cd}	0.31^{ab}	0.21^{abc}	8.69

Table 4.23. Effect of treatments on phylloplane fungi of tomato in rain shelter

Treatments	Fungi (x 10^{-2} cfu cm^{-2})			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	2.45	1.38^{def}	1.17^{def}	52.24
T_2-Propineb 70% WP (0.2%)	2.48	1.22^{ef}	0.99^{efg}	60.08
T_3- Hexaconazole 5% EC (0.05%)	2.37	1.48^{de}	1.23^{de}	48.10
T_4- Hexaconazole 5% EC (0.1%)	2.05	1.23^{ef}	1.03^{defg}	49.75
T_5- Difenoconazole 25% EC (0.05%)	2.50	1.68^{d}	1.49^{d}	40.40
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	2.18	0.99^{fg}	0.83^{fg}	61.92
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.99	0.79^{g}	0.67^{g}	73.20
T_8- *Trichoderma viride* (KAU)	2.36	3.62^{a}	3.96^{ab}	-67.79
T_9- PGPM mix (KAU)	2.05	4.18^{a}	4.27^{a}	-108.20
T_{10}- *Bacillus subtilis* 1 (Endophyte from cocoa)	2.45	2.12^{c}	2.07^{c}	15.51
T_{11}- Untreated control	2.41	3.54^{b}	3.58^{b}	-48.54

After the first spray, the maximum reduction of phylloplane fungi was observed in T_7 (0.79 x 10^2 cfu cm^{-2}) which was statistically on par with T_6 (0.99 x 10^2 cfu cm^{-2}) and this was followed by T_4 (1.23 x 10^2 cfu cm^{-2}) and T_2 (1.22 x 10^2 cfu cm^{-2}). Difenoconazole -0.05% (T_5) treated plants showed comparatively less reduction among different fungicidal treatments recording 1.68 x 10^2 cfu cm^{-2} after first spray. The same trend was observed among treatments even after the second spray and the per cent reduction of fungal flora on the tomato leaves were varied from 40.40 to 73.20 for chemical treatments.

Among the three bioagents treatments, only T_{10} (*B. subtilis*) treated plants showed reduction in fungal flora and recorded 2.12 x 10^2 cfu cm^{-2} fungal population after first spray. While bioagents treatment such as T_8 and T_9, where *Trichoderma* spp. was sprayed on leaves there was a drastic increase in the fungal flora and the population was 4.18 x 10^2 cfu cm^{-2} and 3.62 x 10^2 cfu cm^{-2} respectively after first spray. Control plants recorded an increased fungal population of 3.54 x 10^2 cfu cm^{-2} after first spray. After the second spray also, the same trend was repeated among bioagents treatments as well as control i.e. population increase in T_8, T_9 and control and decrease in T_{10}.

4.7.1.5. Population of phylloplane bacteria in rain shelter

Effects of foliar application different treatments on phylloplane bacterial population in rain shelter is documented in the Table 4.24. Before the treatment application, more or less uniform bacterial population was observed in all the treatment which ranged from 1.74 x 10^4 cfu cm^{-2} to 2.2 x 10^4 cfu cm^{-2}. However, after the foliar application, significant difference among the treatments was observed and drastic reduction in bacterial population was found in all the fungicidal treatments, bioagent treatment T_8 (*T. viride*) and control treatment while increased population was noticed in bioagents treatments such as T_9 and T_{10}.

Table 4.24. Effect of treatments on phylloplane bacteria of tomato in rain shelter

Treatments	Bacteria (x 10^{-4}cfu cm^{-2})			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	1.93	1.12^{ef}	1.01^{def}	47.66
T_2-Propineb 70% WP (0.2%)	1.83	1.05^{f}	0.82^{ef}	55.19
T_3- Hexaconazole 5% EC (0.05%)	1.93	1.24^{de}	1.03^{def}	46.63
T_4- Hexaconazole 5% EC (0.1%)	1.83	0.81^{g}	0.81^{f}	55.73
T_5- Difenoconazole 25% EC (0.05%)	2.07	1.36^{cd}	1.29^{cde}	37.68
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	1.74	0.87^{g}	0.75^{f}	56.89
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.78	0.78^{g}	0.63^{f}	64.60
T_8- *Trichoderma viride* (KAU)	1.83	1.46^{c}	1.31^{cd}	28.41
T_9- PGPM mix (KAU)	2.16	2.62^{a}	3.88^{a}	-64.35
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	2.22	2.34^{b}	3.31^{b}	-49.0
T_{11}- Untreated control	2.22	1.48^{c}	1.74^{c}	21.62

The highest reduction was noticed in T_7 and it was on par with T_4 and T_6 with a population of 0.78 x 10^4 cfu cm^{-2}, 0.81 x 10^4 cfu cm^{-2} and 0.87 x 10^4 cfu cm^{-2} respectively after the first spray. Among different chemical treatments, difenoconazole -0.05% (T_5) treated plants showed comparatively less reduction of phylloplane bacteria recording 1.36 x 10^4 cfu cm^{-2} population after the first spray. After the second spray also, the same trend was observed among treatments and the per cent reduction of phylloplane bacterial population among chemical treatments were ranges from 37.68 to 64.60 per cent.

In bioagents treatment such as T_9 and T_{10}, where *P. fluorescens* and *B. subtilis* were given as foliar spray, there was a drastic increase in phylloplane bacterial count and the population was 2.62 x 10^4 cfu cm^{-2} and 2.34 x 10^4 cfu cm^{-2} respectively after first spray. However, in T_8 where *T. viride* was sprayed there was reduction in bacterial population, but the reduction was lesser compared to chemical treatments. Control plants also recorded reduction in phylloplane bacterial population, but per cent reduction was less than chemical treatments and *T. viride* (T_8) treatment. Moreover, the trends observed in the bacterial population among chemical and bioagents treatments after second spray were the same as that of first spray. Hence, after second spray also there was reduction in phylloplane bacterial population in case of all fungicidal treatments, bioagent treatment T_8, and control and population increase in bioagents treatments *viz.* T_9 and T_{10}.

4.7.1.5. Population of phylloplane actinomycetes in rain shelter

Results of phylloplane actinomycetes population revealed that changes due to foliar application of selected treatments are given in the Table 4.25. The results indicate that the effects of different treatments on actinomycetes population on the tomato leaves were more or less uniform among treatments in each intervals of observations. Before the treatment application actinomycetes count was within range of 0.17- 0.33 x 10 cfu cm^{-2}.

Table 4.25. Effect of treatments on phylloplane actinomycetes of tomato in rain shelter

Treatments	Actinomycetes (x 10^{-1} cfu cm^{-2})			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	0.30	0.21^{bc}	0.22	26.66
T_2-Propineb 70% WP (0.2%)	0.25	0.18^{c}	0.17	32
T_3- Hexaconazole 5% EC (0.05%)	0.21	0.21^{bc}	0.16	23.8
T_4- Hexaconazole 5% EC (0.1%)	0.23	0.18^{c}	0.17	26.08
T_5- Difenoconazole 25% EC (0.05%)	0.17	0.21^{bc}	0.13	23.52
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	0.33	0.21^{bc}	0.17	33.33
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	0.26	0.18^{c}	0.14	33.33
T_8- *Trichoderma viride* (KAU)	0.20	0.28^{abc}	0.17	15.0
T_9- PGPM mix (KAU)	0.21	0.31^{ab}	0.17	19.04
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	0.22	0.28^{abc}	0.25	-13.63
T_{11}- Untreated control	0.26	0.37^{a}	0.25	3.84

An overall reduction in population was noticed after the foliar application except in T_{10} (*B. subtilis*) where actinomycetes population was increased from 0.22 x 10 cfu cm^{-2} to 0.28 x 10 cfu cm^{-2} after the first spray and then decrease to 0.25 x 10 cfu cm^{-2}. Similarly, in bioagent treatments such as T_8 and T_9 and control treatments actinomycetes population was increased after the first spray and reduced to a count lesser than its initial count after second spray. The maximum per cent reduction was observed in T_7 and T_6 (33.33 per cent). In bioagents treatments such as T_8 and T_9 reduction rate was less compared to fungicidal treatments while it was more compared to control in which per cent reduction was only 3.84.

Summing up the above study on effects of selected treatments on tomato phylloplane culturable microbial population under protected cultivation, it was found that application of fungicides on tomato plant drastically reduced the tomato phylloplane microflora while bioagent applications response was varied depending up on the type of microflora (Fig. 4.1-4.6) (Plate 20, 21 & 22). In general, fungal population was increased with the application of bioagent treatment T8 and T9, bacterial population was increased with T9 and T10 and population of actinomycetes increased in T10. Even though reduction of phylloplane microflora was observed with the application of bioagents treatments, the rate of reduction was very less compared to chemical treatments. Among fungicidal treatments T7 recorded maximum and T5 recorded minimum phylloplane microbial reduction both under polyhouse and rain shelter condition. The results also showed that, natural population of phylloplane fungi and bacteria was comparatively more in rain shelter tomato than in polyhouse whereas phylloplane actinomycetes population was more in polyhouse tomato plants.

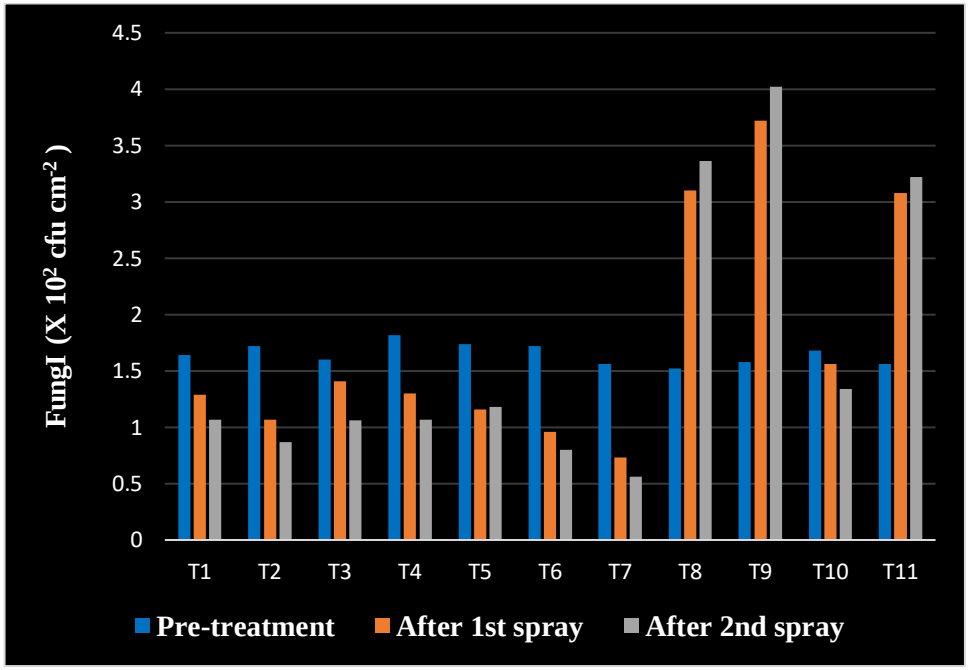


Fig.4.3. Effect of treatments on phylloplane fungi of tomato in polyhouse

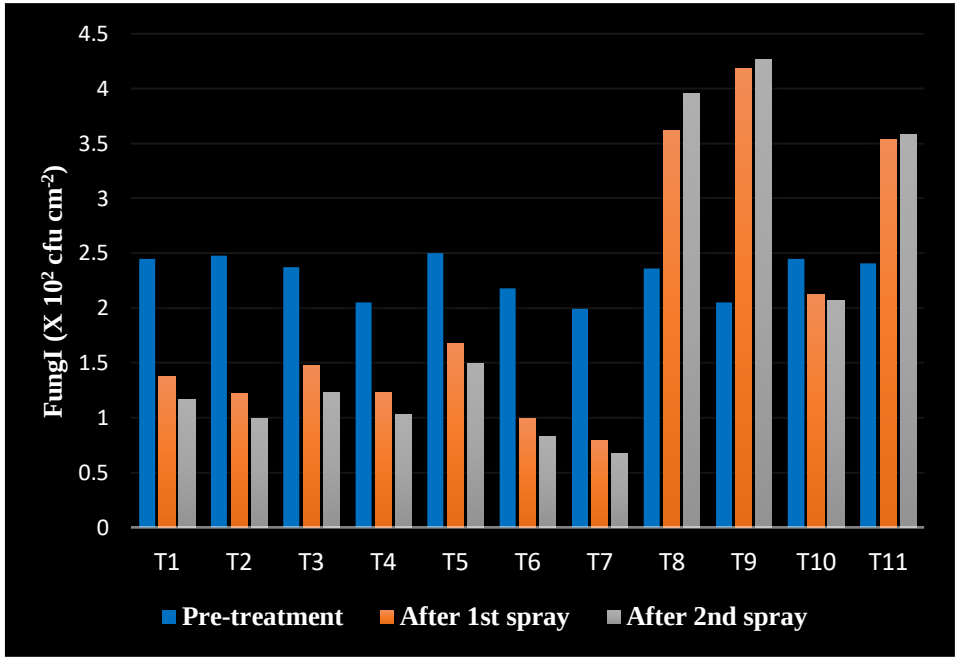


Fig.4.4 Effect of treatments on phylloplane fungi of tomato in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

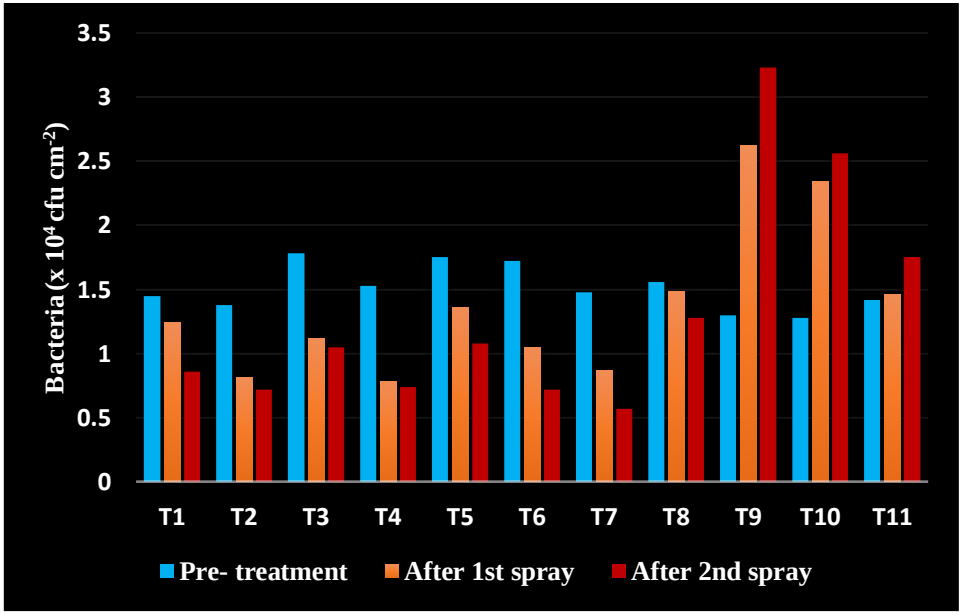


Fig.4.5. Effect of treatments on phylloplane bacteria of tomato in polyhouse

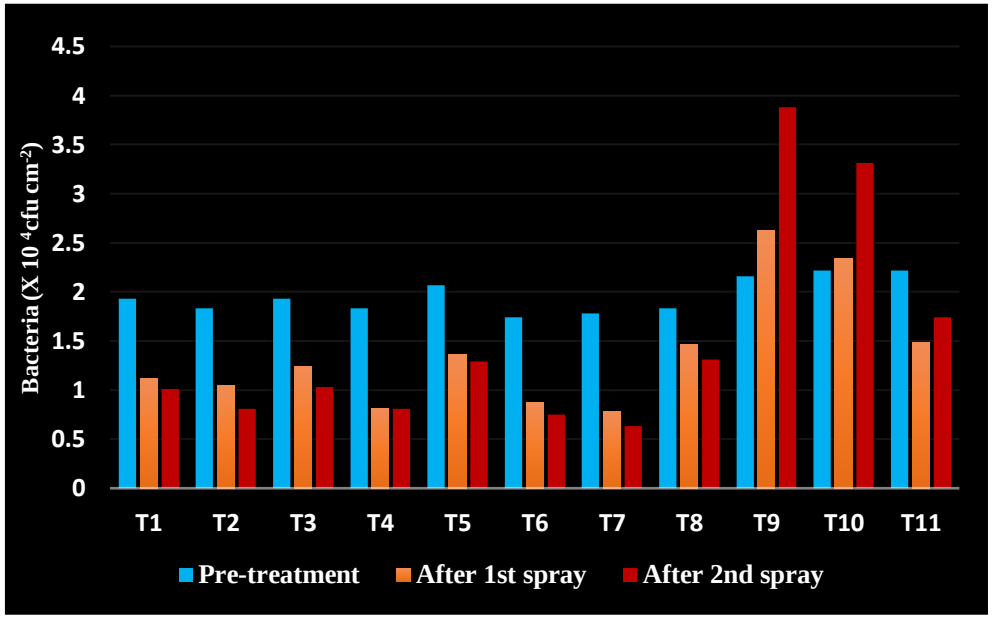


Fig.4.6. Effect of treatments on phylloplane bacteria of tomato in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

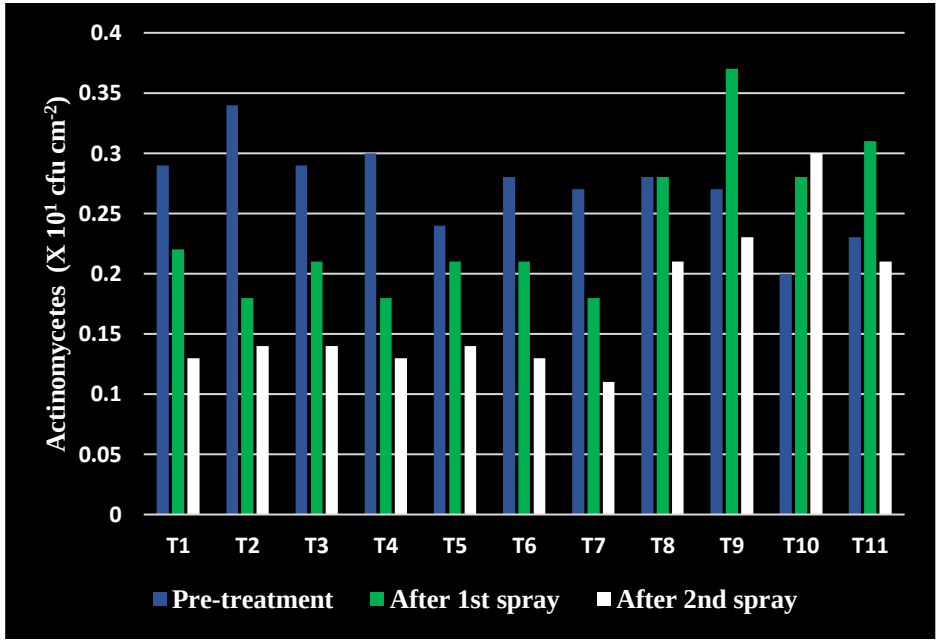


Fig.4.7. Effect of treatments on phylloplane actinomycetes of tomato in polyhouse

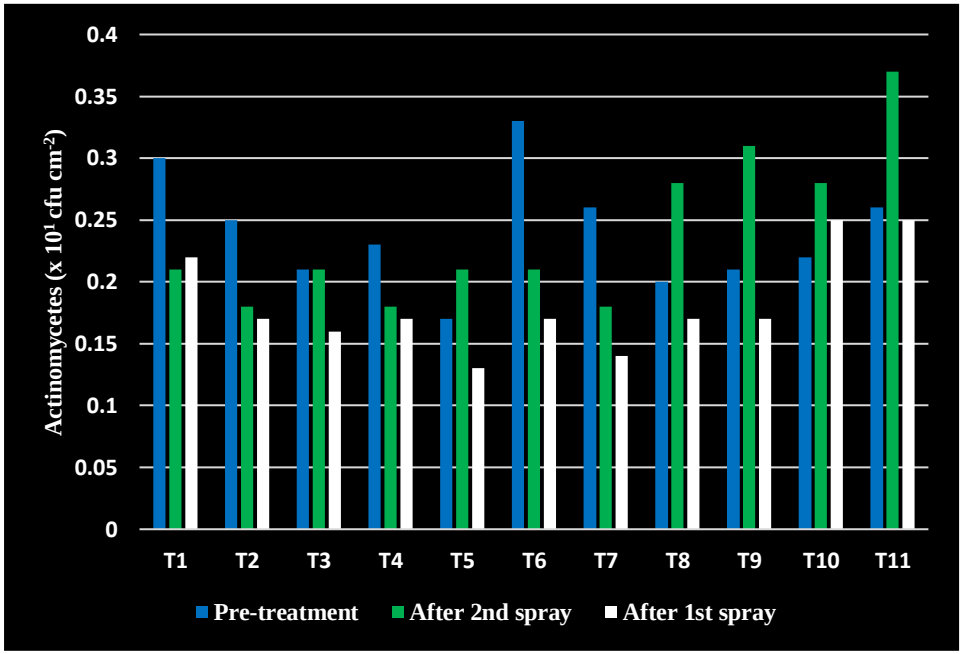


Fig.4.8. Effect of treatments on phylloplane actinomycetes of tomato in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

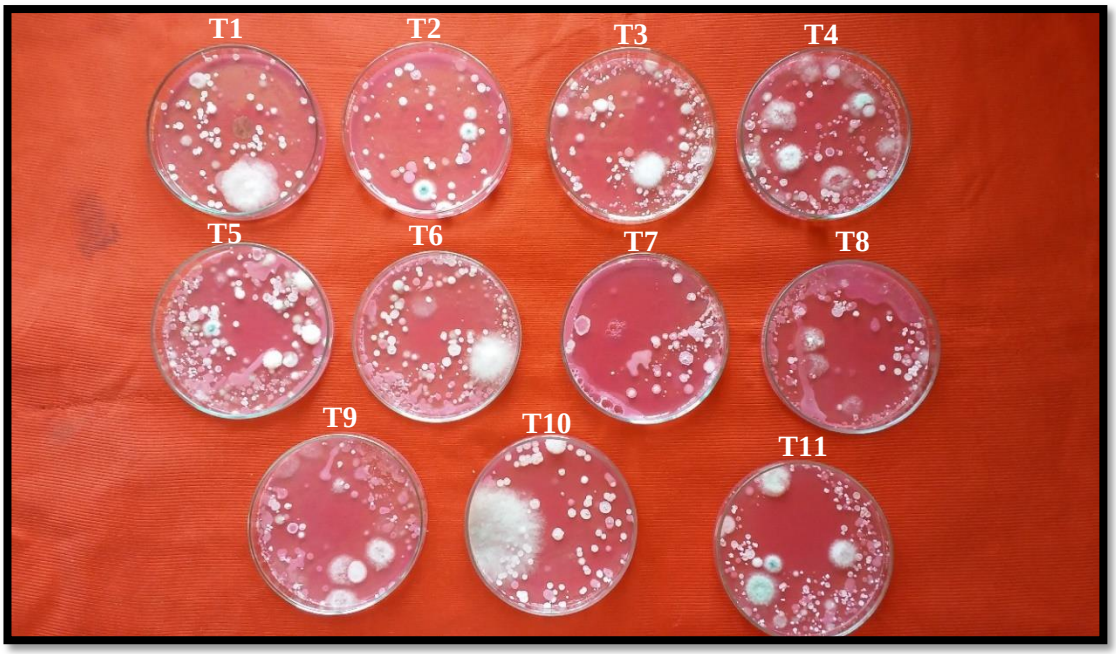
T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

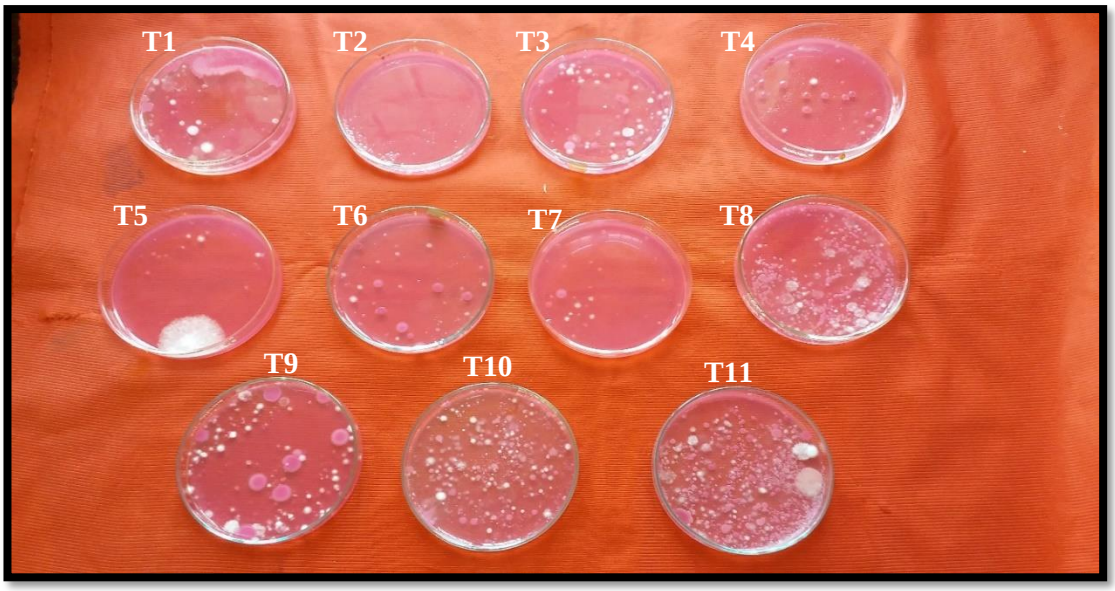
T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

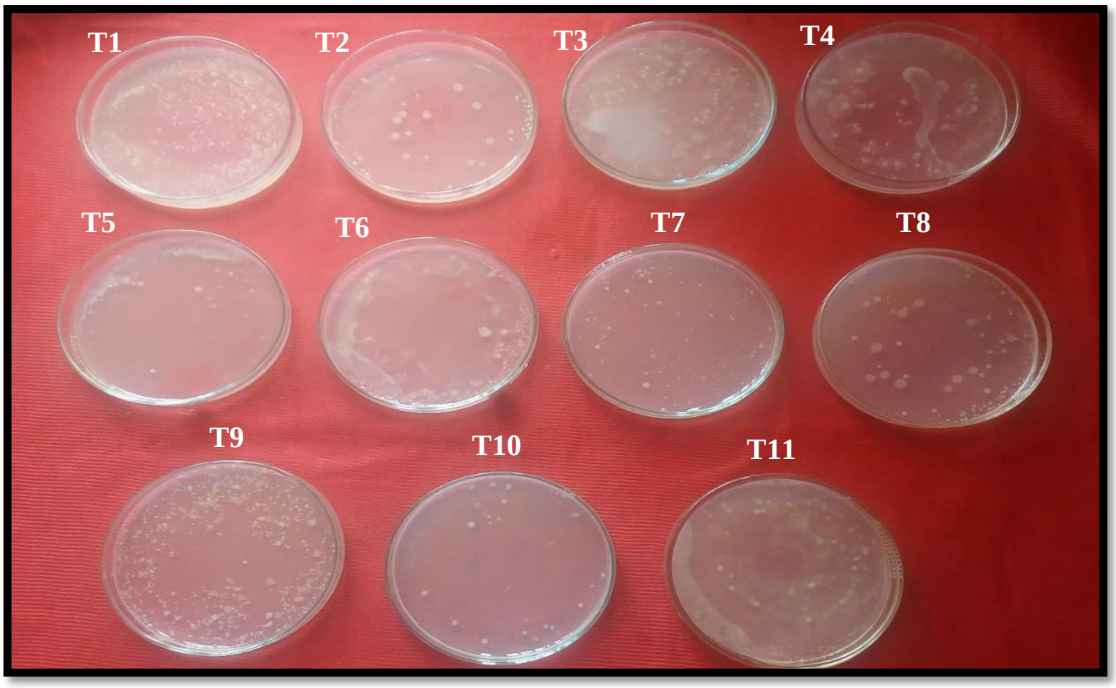


(a) Phylloplane fungi before treatment application

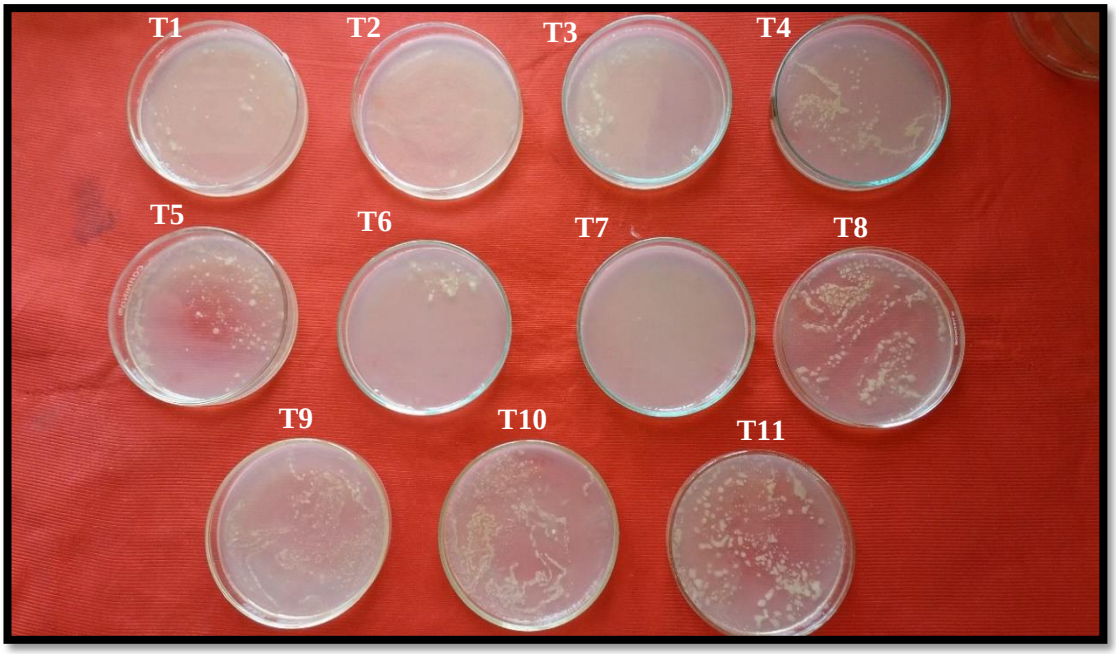


(b) Phylloplane fungi after treatment application

Plate 4.20. Effects of treatments on phylloplane fungi

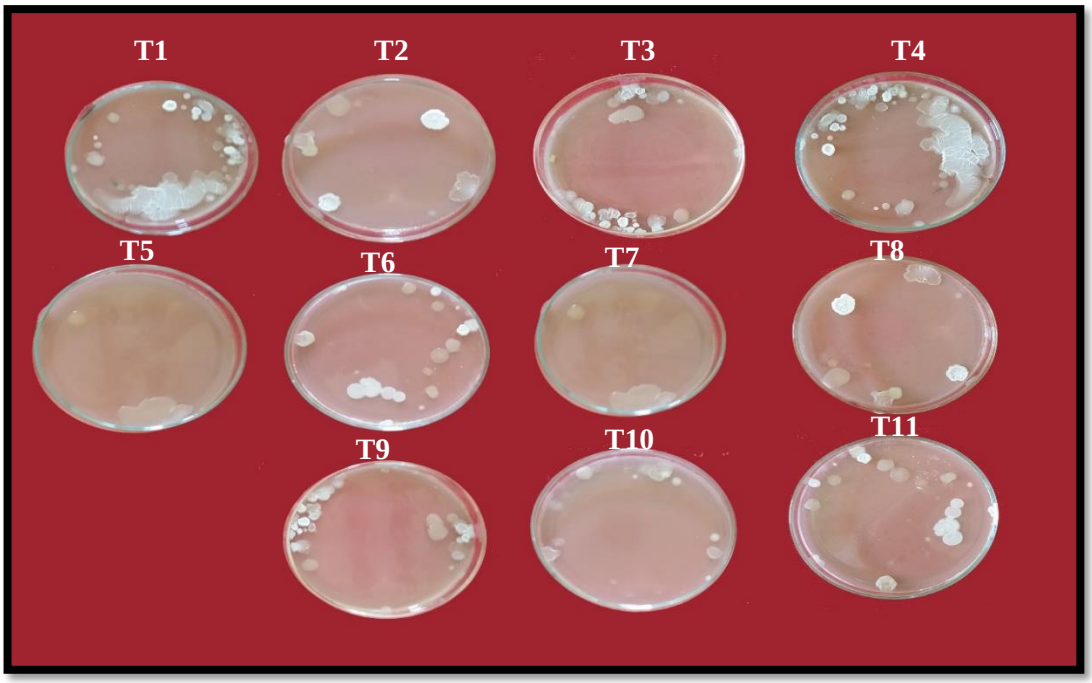


(a) Phylloplane bacteria before treatment application

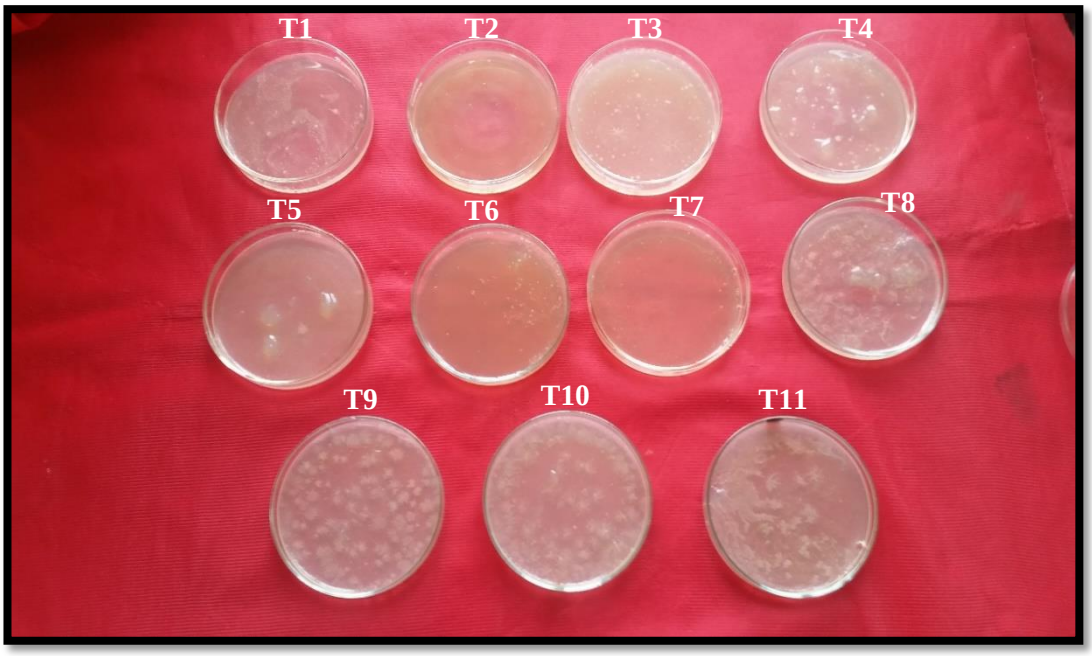


(b) Phylloplane bacteria after treatment application

Plate 4.21. Effects of treatments on phylloplane bacteria



(a) Phylloplane actinomycetes before treatment application



(b) Phylloplane actinomycetes after treatment application

Plate 4.22. Effects of treatments on phylloplane actinomycetes

4.7.2 Enumeration of endophytic microflora

Culturable endophytic microorganisms *viz.* fungi, bacteria and actinomycetes were isolated from leaf, stem and root of tomato plants before and after treatment application and enumerated using serial dilution plating to study the effects of different treatments on endophytic microbes in polyhouse and rain shelter and the results are presented in Tables 4.26 to 4.43.

4.7.2.1 Population of endophytic fungi in polyhouse

Endophytic fungi from leaves, stem and root were isolated before and after foliar spray and the results of quantitative estimation of fungal endophytes are given in Tables 4.26, 4.27 & 4.28.

4.7.2.1.1 Population of leaf fungal endophytes in polyhouse

Data presented in Table 4.26 revealed that, before the treatment application, there was no significant difference among treatments with respect to population of leaf endophytic fungi and it was ranged from 25.66 to 31.66 x 10^3 cfu g^{-1}. However, after the foliar application, significant difference among the treatments was observed and reduction in fungal population was found in all the fungicidal treatments and bioagent treatment T_{10} (*B. subtilis*) while increased population was noticed in control plants as well as bioagents treatments such as T_8 and T_9. After the first spray, the maximum reduction of fungi was observed in T_7 (15 x 10^3 cfu g^{-1}) which was statistically on par with T_6 (15.66 x 10^3 cfu g^{-1}) and T_2 () and T_2 (1.22 x 10^2 cfu cm^{-2}). Difenoconazole -0.05% (T_5) treated plants showed comparatively less reduction among different fungicidal treatments recording 22 x 10^3 cfu g^{-1} after first spray. The same trend was observed among treatments even after the second spray and the per cent reduction of fungal flora on the tomato leaves were varied from 29.61 to 57.59 for chemical treatments.

Table 4.26. Effect of treatments on endophytic fungi of tomato in polyhouse

Treatments	Fungi (x 10^3 cfu g^{-1}) leaf			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	30.33	21.0^{ef}	17.33^{de}	42.86
T_2-Propineb 70% WP (0.2%)	31.66	18.33^{fg}	15.0^{ef}	52.62
T_3- Hexaconazole 5% EC (0.05%)	27.0	21.66^{def}	18.66^{d}	30.88
T_4- Hexaconazole 5% EC (0.1%)	30.0	20.66^{ef}	16.66^{def}	44.46
T_5- Difenoconazole 25% EC (0.05%)	25.66	22.0^{de}	18.06^{d}	29.61
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	27.0	15.66^{g}	14.0^{ef}	48.14
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	30.66	15.0^{g}	13.3^{f}	57.59
T_8- *Trichoderma viride* (KAU)	29.33	41.66^{b}	46.33^{b}	-57.96
T_9- PGPM mix (KAU)	27.33	46.0^{a}	51.66^{a}	-89.02
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	28.33	25.0^{d}	24.0^{c}	15.28
T_{11}- Untreated control	31.66	37.33^{c}	43.0^{b}	-35.81

Similarly, among the three bioagents treatments, only T_{10} (*B. subtilis*) treated plants showed reduction in fungal flora and recorded 25 x 10^3 cfu g^{-1} fungal population after first spray. While bioagents treatment such as T_8 and T_9, where *Trichoderma* spp. was sprayed on leaves there was a drastic increase in the fungal flora and the population was 46 x 10^3 cfu g^{-1} and 46.33 x 10^3 cfu g^{-1} respectively after first spray. Control plants recorded an increased fungal population of 37.33 x 10^3 cfu g^{-1} after first spray. The same trends were observed among treatments even after the second spray.

4.7.2.1.2 Population of fungal stem endophytes in polyhouse

Data presented in Table 4.27 showed that, before treatment application endophytic fungal population from the stem was more or less uniform varied from 17.66 x 10^3 cfu g^{-1} to 20 x 10^3 cfu g^{-1}. But, after the foliar application significant difference was observed and population reduction was recorded in all chemical treatments as well as treatment with *B. subtilis* (T_{10}) while population increase was noticed in control and bioagent treatments *viz.* T_8 and T_9. However, among different chemical treatments applied, population of tomato stem fungal endophytes were more or less same which ranged from 9.66 x 10^3 cfu g^{-1} to 17 x 10^3 cfu g^{-1} after first spray and 9.0 x 10^3 cfu g^{-1} - 16.33 x 10^3 cfu g^{-1} after second spary and the per cent reduction of stem fungal endophytes among chemical treatments were ranges from 18.35 to 50.9 per cent.

Among bioagents treatments T_8 and T_9, where *Trichoderma were* given as foliar spray, there was a drastic increase in fungal endophytes from stem and the population was 27.66 x 10^3 cfu g^{-1} and 27 x 10^3 cfu g^{-1} respectively after first spray. However, in T_{10} where *B. subtilis* was sprayed there was reduction in endophytic fungal population, but the reduction was lesser compared to chemical treatments. Control plants also recorded an increase in fungal population, but it was less than biological treatments *viz.* T_8 and T_9.

Table 4.27. Effect of treatments on endophytic fungi of tomato in polyhouse

Treatments	Fungi (x 10^3 cfu g^{-1}) stem			
	Pre treatment	**After 1st spray**	**After 2nd spray**	**Per cent reduction over control**
T_1- Propineb 70% WP (0.1%)	20.0	17.0^{d}	16.33^{c}	18.35
T_2-Propineb 70% WP (0.2%)	17.66	12.66^{d}	12.33^{d}	30.18
T_3- Hexaconazole 5% EC (0.05%)	18.33	12.33^{d}	11.33^{d}	38.18
T_4- Hexaconazole 5% EC (0.1%)	20.0	11.33^{d}	10.66^{d}	46.7
T_5- Difenoconazole 25% EC (0.05%)	18.66	12.0^{d}	10.66^{d}	42.87
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	20.0	11.66^{d}	10.66^{d}	46.7
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	18.33	9.66^{d}	9.0^{d}	50.9
T_8- *Trichoderma viride* (KAU)	18.66	27.0^{a}	28.33^{ab}	-51.82
T_9- PGPM mix (KAU)	18.33	27.66^{a}	29.33^{a}	-60.01
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	20.0	17.66^{c}	17.0^{c}	15.0
T_{11}- Untreated control	18.66	23.0^{b}	25.33^{b}	-35.75

4.7.2.1.3 Population of fungal root endophytes in polyhouse

The results of different treatment's impact on fungal root endophytes in rain shelter are presented in the Table 4.28. From the results it was found that, before the treatment application, there was no significant difference among treatments with regard to fungal root endophytes population. During this time population count was within the range of 17 x 10^3 cfu g^{-1}- 22.33 x 10^3 cfu g^{-1}. However, after the treatment application, significant difference in endophytic fungal population were observed and reduction in the fungal population was found in all the fungicidal treatments while increased population was observed in control as well as in bioagents treatments except in plants treated with *B. subtilis* 1(T_{10}). After the first spray, the maximum reduction was observed in T_7 (11.66 x 10^3 cfu g^{-1}) which was statistically on par with T_4 (15 x 10^3 cfu g^{-1}) and T_4 (15.33 x 10^3 cfu g^{-1}) and this was followed by T_5 (15.66 x 10^3 cfu g^{-1}). Propineb -0.1% (T_1) treated plants showed comparatively less reduction among different fungicidal treatments recording 20 x 10^3 cfu g^{-1} after first spray. The same trend was observed among treatments even after the second spray and the per cent reduction of endophytic root fungal flora were varied from 7.88 to 51.57 for chemical treatments.

Similarly, among the three bioagents treatments, only T_{10} (*B. subtilis*) showed reduction in fungal flora and it recorded 25 x 10^3 cfu g^{-1} fungal population after first spray while bioagents treatment such as T_8 and T_9, where *Trichoderma* spp. was sprayed on leaves there was a gradual increase in the fungal flora and the population was 27.66 x 10^3 cfu g^{-1} and 30 x 10^3 cfu g^{-1} respectively after first spray. Control plants also recorded an increased fungal population of 25.33 x 10^3 cfu g^{-1} after first spray. The same trends were observed among treatments even after the second spray.

Summing up the above studies on effects of selected treatments on endophytic fungal (leaf, stem and root) population, it is clearly evident that, all chemical as well as *B. subtilis* treated plants exhibited a reduction in endophytic fungal population whereas in plants treated with biocontrol agents such as *Trichoderma viride* and PGPM mix exhibited

Table 4.28. Effect of treatments on root endophytic fungi of tomato in polyhouse

Treatments	Fungi (x 10 cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	22.0	20.0^{cd}	19.33^{c}	7.88
T_2-Propineb 70% WP (0.2%)	17.0	16.66^{cde}	15.66^{cd}	12.13
T_3- Hexaconazole 5% EC (0.05%)	20.0	16.66^{cde}	15.0^{d}	21.7
T_4- Hexaconazole 5% EC (0.1%)	22.33	15.0^{ef}	13.0^{de}	41.78
T_5- Difenoconazole 25% EC (0.05%)	18.66	15.66^{def}	14.0^{de}	24.97
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	21.33	15.33^{ef}	13.0^{de}	38.09
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	21.33	11.66^{f}	10.33^{e}	51.57
T_8- *Trichoderma viride* (KAU)	19.33	27.66^{ab}	30.66^{a}	-58.61
T_9- PGPM mix (KAU)	18.0	30.0^{a}	31.33^{a}	-74.05
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	21.66	20.33^{c}	19.33^{c}	10.75
T_{11}- Untreated control	21.0	25.33^{b}	26.33^{b}	-25.38

an increase in fungal endophytes. In all the cases the maximum reduction in endophytic fungal population was recorded by T_7 and maximum increase was notice in T_9. Moreover, among different fungicidal treatments, systemic fungicide difenoconazole (0.05) showed the minimum fungal population reduction in case of leaf fungal endophytes while in case of stem and root fungal endophytes, the contact fungicide propineb recorded minimum per cent reduction of fungal population. Similarly, due to the different treatment application, the changes in fungal population was more in leaf fungal compared to stem and root fungal endophytes. The results also revealed that, population of tomato fungal endophytes was more in leaves compared to roots and stem.

4.7.2.2 Population of endophytic bacteria in polyhouse

Bacterial endophytes were isolated from tomato leaves, stem and root before and after treatment application and the results of quantitative estimation of bacterial endophytes are given in Tables 4.29, 4.30 & 4.31.

4.7.2.2.1 Population of bacterial leaf endophytes in polyhouse

It is observed from the data presented in Table 4.29 that, before the foliar spray, there was no significant difference in bacterial colony count between different treatments and bacterial population was more or less uniform ranged from 33.66 to 39.66 x 10^5 cfu g^{-1}. But, after the foliar application, significant difference was observed and reduction in bacterial population was found in all the fungicidal treatments and bioagent treatment T_8 (*T. viride*) while increased population was noticed in control plants as well as bioagents treatments such as T_9 and T_{10}. After the first spray, the maximum reduction of bacteria was observed in T_7 (19.33 x 10^5 cfu g^{-1}) followed by T_4 (27.33 x 10^5 cfu g^{-1}) which was statistically on par with all the remaining chemical treatments *viz.* T_2, T_3, $T_1$and T_5. The same trend was observed among chemical treatments even after the second spray and the per cent reduction of leaf endophytic bacterial population were varied from 26.05 to 46.52 for chemical treatments.

Among the three bioagents treatments, treatment such as T_9 and T_{10}, where *P. fluorescens* and *B. subtilis* was sprayed on leaves showed drastic increase in bacterial count and the population was 68 x 10^5 cfu g^{-1} and 63.66 x 10^5 cfu g^{-1} respectively after first spray while T_8 (*T. viride*) showed a slight increase in bacterial count (36.33 x 10^5 cfu g^{-1}) after first spray and then bacterial count was decreased (33 x 10^5 cfu g^{-1}) after second spray. But the rate of decrease was

less compared to chemical treatments. Control plants also recorded an increased bacterial population of 41.66 x 10^5 cfu g^{-1} after first spray. The same trends were observed among treatments even after the second spray.

4.7.2.2.1 Population of stem endophytic bacteria in polyhouse

Effects of foliar application different treatments on stem endophytic bacterial population in polyhouse is documented in the Table 4.30. Before the treatment application, more or less uniform bacterial population was observed in all the treatment which ranged from 23 x 10^5 cfu g^{-1} to 28.33 x 10^5 cfu g^{-1}. However, after the foliar application, significant difference among the treatments was observed and a sudden reduction in bacterial population was found in all the fungicidal treatments while increased population was noticed in bioagents treatments such as T_9 and T_{10}. The highest reduction was noticed in T_7 and it was on par with T_6 and T_4 with a population of 17.33 x 10^5 cfu g^{-1}, 18.66 x 10^5 cfu g^{-1} and 19.66 x 10^5 cfu g^{-1} respectively after the first spray. Among different chemical treatments, propineb - 0.1% (T_1) treated plants showed comparatively less reduction of endophytic bacteria recording 21.66 x 10^5 cfu g^{-1} population after the first spray. After the second spray also, the same trend was observed among treatments and the per cent reduction of endophytic bacterial population among chemical treatments were ranges from 9.97 to 46.15 per cent.

In bioagents treatment such as T_9 and T_{10}, where *P. fluorescens* and *B. subtilis* were given as foliar spray, there was a drastic increase in endophytic bacterial count and the population was 38.33 x 10^5 cfu g^{-1} and 35.66 x 10^5 cfu g^{-1} respectively after first spray. However, in T_8 where *T. viride* was sprayed there was slight increase in bacterial population after first spray and then reduced to initial count after second spray. Control plants also recorded an increase in stem endophytic bacteria, but rate of increase was less than bioagent treatments *viz*. T_9 and T_{10}.

Table 4.29. Effect of treatments on leaf endophytic bacteria of tomato in polyhouse

Treatments	Bacteria (x 10^5 cfu g^{-1}) leaf			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	36.33	29.33^{cd}	26.66^{d}	26.61
T_2-Propineb 70% WP (0.2%)	33.66	27.66^{d}	24.33^{d}	27.71
T_3- Hexaconazole 5% EC (0.05%)	35.33	29.0^{d}	27.33^{cd}	22.64
T_4- Hexaconazole 5% EC (0.1%)	37.0	27.33^{d}	27.0^{cd}	27.02
T_5- Difenoconazole 25% EC (0.05%)	39.66	32.33^{cd}	29.33^{cd}	26.04
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	38.66	26.0^{d}	24.33^{d}	37.06
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	33.66	19.33^{e}	18.0^{e}	46.52
T_8- *Trichoderma viride* (KAU)	35.33	36.33^{bc}	33.0^{c}	6.59
T_9- PGPM mix (KAU)	34.33	68.0^{a}	69.0^{a}	-100.9
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	39.66	63.66^{a}	65.0^{b}	-63.89
T_{11}- Untreated control	42.33	41.66^{b}	45.0^{b}	-6.30

Table 4.30. Effect of treatments on stem endophytic bacteria of tomato in polyhouse

Treatments	Bacteria (x 10^5 cfu g^{-1}) stem			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	26.66	21.66^{c}	24.0^{cd}	9.97
T_2-Propineb 70% WP (0.2%)	25.66	21.00^{cd}	21.66^{d}	15.58
T_3- Hexaconazole 5% EC (0.05%)	27.66	20.33^{cd}	18.0^{e}	34.92
T_4- Hexaconazole 5% EC (0.1%)	27.33	19.66^{cde}	16.33^{ef}	40.24
T_5- Difenoconazole 25% EC (0.05%)	27.33	20.66^{cd}	16.66^{ef}	39.04
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	28.33	18.66^{de}	17.0^{ef}	39.99
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	26.0	17.33^{e}	14.0^{f}	46.15
T_8- *Trichoderma viride* (KAU)	25.33	26.0^{b}	25.33^{c}	0
T_9- PGPM mix (KAU)	28.66	38.33^{a}	40.66^{a}	-41.81
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	28.33	35.66^{a}	39.33^{b}	-38.82
T_{11}- Untreated control	23.0	26.0^{b}	25.33^{c}	-10.13

4.7.2.2.1 Population of root endophytic bacteria in polyhouse

From the results presented in Table 4.31. It was found that, before the treatment application, there was no significant difference among treatments with regard to bacterial root endophytes population and at this time population count was within the range of 34.33 x 10^5 cfu g^{-1} - 47.33 x 10^5 cfu g^{-1}. However, after the treatment application, reduction in endophytic bacterial population was found in all the fungicidal treatments as well as in control. But reduction was less in control compared to chemical treatment while increased population was observed in all bioagents treatments except in T_8 in which a minute population reduction was recorded. After the first spray, the maximum reduction was observed in T_7 (27.33x 10^5 cfu g^{-1}) which was statistically on par with all the remaining chemical treatments. Even though, propineb -0.1% (T_1) treated plants showed comparatively less per cent reduction of bacterial population (7.75 per cent) among different chemical treatments. The same trend was observed among treatments even after the second spray and the per cent reduction of endophytic root fungal flora were varied from 7.88 to 51.57 for chemical treatments. In T_9 and T_{10}, where bacterial biocontrol agents were sprayed there was a gradual increase in the bacterial population and count was 55 x 10^5 cfu g^{-1} and 53 x 10^5 cfu g^{-1} respectively after first spray. The same trend was observed among all the treatments even after the second spray.

Summing up the findings on effects of treatments applications on endophytic bacterial population in polyhouse, it is observed that, all chemical treated plants exhibited a reduction in endophytic bacterial population whereas in plants treated with biocontrol agents such as PGPM mix and *B. subtilis* exhibited an increased bacterial count while *T. viride* treated plants has no or little effects on endophytic bacterial population. However, the per cent reduction as well as increase of endophytic bacterial population was less compared to phylloplane bacteria. In leaves, stem and root the maximum reduction in endophytic bacterial population was recorded by T_7 and maximum increase was notice in T_9. Moreover, among different fungicidal treatments, systemic fungicides recorded the maximum endophytic bacterial reduction compared to contact fungicide propineb. Similarly, due to the different treatment application, the changes in bacterial population was more in leaf compared to stem and root bacterial endophytes. The results also revealed that, population of tomato bacterial endophytes was more in roots compared to leaves and stem.

Table 4.31. Effect of treatments on root endophytic bacteria of tomato in polyhouse

Treatments	Bacteria (x 10^5cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	38.66	40.33^{cd}	35.66^{de}	7.75
T_2-Propineb 70% WP (0.2%)	41.33	36.66^{cde}	33.66^{def}	18.55
T_3- Hexaconazole 5% EC (0.05%)	47.33	35.33^{cde}	32.33^{ef}	31.69
T_4- Hexaconazole 5% EC (0.1%)	47.0	34.66^{de}	31.0^{ef}	34.04
T_5- Difenoconazole 25% EC (0.05%)	44.66	36.33^{de}	32.66^{ef}	27.13
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	42.33	32.66^{de}	29.0^{ef}	31.49
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	34.33	27.33^{e}	25.66^{f}	45.40
T_8- *Trichoderma viride* (KAU)	42.33	41.66^{cd}	42.0^{cd}	0.77
T_9- PGPM mix (KAU)	41.33	55.0^{a}	55.66^{a}	-34.67
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	42.0	53.0^{ab}	54.0^{ab}	-28.57
T_{11}- Untreated control	47.0	44.66^{bc}	48.33^{bc}	10.63

4.7.2.3 Population of endophytic actinomycetes in polyhouse

Endophytic actinomycetes were isolated from tomato leaves, stem and root before and after foliar application and the results of population of endophytic actinomycetes are given in Tables 4.32, 4.33 & 4.34.

4.7.2.3.1 Population of leaf endophytic actinomycetes in polyhouse

Data on population of endophytic actinomycetes isolated from tomato leaves in polyhouse is presented in Table 4.32. Before and after treatments application, significant difference was not observed among the treatment with regard to endophytic actinomycetes. However, an increase in endophytic actinomycetes count was found in all treatments after first and second spray. Before the treatment application, actinomycetes population was with the range of 0.66 x 10 cfu g^{-1} to 2.33 x 10 cfu g^{-1} while after the treatment it varied from 1.66 x 10 cfu g^{-1} to 3.33 x 10 cfu g^{-1}. The maximum increase in endophytic actinomycetes count was found in T_9 (3.33 x 10 cfu g^{-1}) and minimum was recorded in T_3(1.66 x 10 cfu g^{-1}) after the second spray. In control plants also there was an increase in actinomycetes population from 1.66 x 10 cfu g^{-1} to 2.3 x 10 cfu g^{-1} during different intervals of observations.

4.7.2.3.2 Population of stem endophytic actinomycetes in polyhouse

Data on endophytic actinomycetes population from tomato stem are given in the Table 4.33. The data reveled that, with respect to endophytic actinomycetes population, there was no significant difference among treatment before and after foliar application. However, after the foliar spray, endophytic actinomycetes population was increased in all treatments except T_1 and T_5 in which population count remains same even after the treatment application. Before treatment application actinomycetes population was within the range of 1.33 x 10 cfu g^{-1} to 2.66 x 10 cfu g^{-1} and after the treatment application it increased to the range of 2.0 x 10 cfu g^{-1} to 3.66 x 10 cfu g^{-1}. Control plants also recorded increased endophytic actinomycetes count ranges from 2 x 10 cfu g^{-1} to 3 x 10 cfu g^{-1}.

Table 4.32. Effect of treatments on leaf endophytic actinomycets of tomato in polyhouse

Treatments	Actinomycetes (x 10 cfu g^{-1}) leaf			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	1.33	1.66	2.0	-50.37
T_2-Propineb 70% WP (0.2%)	1.66	1.66	2.33	-40.36
T_3- Hexaconazole 5% EC (0.05%)	0.33	2.0	1.66	-24.81
T_4- Hexaconazole 5% EC (0.1%)	1.0	1.33	1.33	-33
T_5- Difenoconazole 25% EC (0.05%)	2.33	1.0	3.0	-28.75
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	0.66	3.0	2.33	-40.36
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.66	2.33	2.33	-40.36
T_8- *Trichoderma viride* (KAU)	1.33	1.66	2.0	-50.37
T_9- PGPM mix (KAU)	1.33	1.0	3.33	-150.37
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.66	1.33	2.66	-60.24
T_{11}- Untreated control	1.66	1.66	2.3	-38.55

Table 4.33. Effect of treatments on stem endophytic actinomycets of tomato in polyhouse

Treatments	Actinomycetes (x 10 cfu g^{-1}) stem			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	2.66	2.66	2.66	0
T_2-Propineb 70% WP (0.2%)	2.0	2.66	2.66	-33
T_3- Hexaconazole 5% EC (0.05%)	1.33	1.66	2.0	-50.37
T_4- Hexaconazole 5% EC (0.1%)	2.0	3.0	3.33	-66.5
T_5- Difenoconazole 25% EC (0.05%)	1.66	1.66	1.66	0
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	1.66	1.66	2.0	-20.48
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	2.0	2.33	2.33	-16.5
T_8- *Trichoderma viride* (KAU)	2.66	2.33	3.66	-37.5
T_9- PGPM mix (KAU)	2.66	2.66	3.33	-25.0
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.33	1.66	2.33	-75.18
T_{11}- Untreated control	2.0	2.0	3.0	-50.0

4.7.2.3.3 Population of root endophytic actinomycetes in polyhouse

In Table 4.34, endophytic actinomycetes population from tomato roots before and after treatments application in polyhouse are furnished. It was found that, before and after treatment application significant difference among treatments were absent and it was ranged from 1.66 x 10 cfu g^{-1} to 3 x 10 cfu g^{-1} and 2.33 x 10 cfu g^{-1} to 3.66 x 10 cfu g^{-1} respectively. The data also showed that, after foliar spray endophytic actinomycetes population was increased in all treatments except T_6 and T_7 in which population count remains same even after the treatment application. In control plants endophytic actinomycetes count was increased from 3 x 10 cfu g^{-1} to 3.66 x 10 cfu g^{-1} after the second spray.

Thus, the results on effects of different treatments on tomato endophytic actinomycetes population revealed that, even though population was increased in all treatments after foliar application, significant difference among treatments was not observed. Since, increased population of endophytic actinomycetes was also observed in untreated control plants, we can conclude that the selected treatments have little or no effects on endophytic actinomycetes population of tomato plants. The results also revealed that, among various parts of tomato plants, root occupied maximum endophytic actinomycetes population count.

4.7.2.4 Population of endophytic fungi in rain shelter

Endophytic fungi from leaves, stem and root were isolated before and after foliar spray from rain shelter experiment and the results of quantitative estimation of fungal endophytes are given in Tables 4.35, 4.36 & 4.37.

4.7.2.4.1 Population of leaf endophytic fungi in rain shelter

It is observed from the data presented in Table 4.35 that, before the foliar spray, there was no significant difference in fungal colony count between different treatments and fungal population was more or less uniform ranged from 31.33 x 10 cfu $g^{-1}$to 37.66. But, after the foliar application, significant difference was observed and reduction in endophytic fungal population was found in all the fungicidal treatments and bioagent treatment T_{10} (*B. subtilis*) while increased population was noticed in control plants as well as bioagents treatments such as T_8 and T_9. After the first spray, the maximum reduction of fungi was

Table 4.34. Effect of treatments on root endophytic actinomycets of tomato in polyhouse

Treatments	Actinomycetes (x 10 cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	2.33	3.33	2.66	-14.16
T_2-Propineb 70% WP (0.2%)	3.0	2.33	3.33	-10.0
T_3- Hexaconazole 5% EC (0.05%)	1.66	2.66	2.0	-20.48
T_4- Hexaconazole 5% EC (0.1%)	1.66	4.0	3.0	-80.72
T_5- Difenoconazole 25% EC (0.05%)	2.66	3.33	3.33	-25.18
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	2.33	2.33	2.33	0
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	2.33	2.0	2.33	0
T_8- *Trichoderma viride* (KAU)	2.66	3.33	3.33	-25.18
T_9- PGPM mix (KAU)	2.66	3.0	3.66	-37.59
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.66	1.66	2.33	-40.36
T_{11}- Untreated control	3.0	3.33	3.66	-22.0

Table 4.35. Effect of treatments on leaf endophytic fungi of tomato in rain shelter

Treatments	Fungi (x 10^3 cfu g^{-1}) leaf			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	37.66	21.33^{de}	18.33^{de}	51.32
T_2-Propineb 70% WP (0.2%)	36.33	20.33^{de}	17.33^{de}	52.29
T_3- Hexaconazole 5% EC (0.05%)	35.0	22.66^{de}	21.33^{de}	39.05
T_4- Hexaconazole 5% EC (0.1%)	34.0	21.33^{de}	19.33^{de}	43.14
T_5- Difenoconazole 25% EC (0.05%)	33.0	24.33^{d}	22.0^{d}	33.33
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	31.66	21.0^{de}	17.66^{de}	44.21
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	31.33	18.66^{e}	17.0^{e}	45.73
T_8- *Trichoderma viride* (KAU)	36.66	44.66^{ab}	52.33^{a}	-42.74
T_9- PGPM mix (KAU)	34.0	47.33^{a}	53.33^{a}	-56.85
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	31.66	38.33^{c}	31.33^{c}	1.04
T_{11}- Untreated control	36.33	39.33^{bc}	42.66^{b}	-17.42

observed in T_7 (18.66 x 10^3 cfu g^{-1}) and which was statistically on par with all the remaining chemical treatments except T_5. The same trend was observed among chemical treatments even after the second spray and the per cent reduction of leaf endophytic fungal population were varied from 39.05 to 45.73 for chemical treatments.

Among the three bioagents treatments, treatment such as T_8 and T_9, where *Trichoderma* was sprayed on leaves showed drastic increase in fungal colony count and the population was 44.66 x 10^3 cfu g^{-1} and 47.33 x 10^3 cfu g^{-1} respectively after first spray while T_{10} showed a slight increase in fungal colony count (38.33 x 10^3 cfu g^{-1}) after first spray and then population was decreased (31.33 x 10^3 cfu g^{-1}) after second spray. But the rate of decrease was less compared to chemical treatments. Control plants also recorded an increased fungal population of 39.33 x 10^5 cfu g^{-1} after first spray. The same trends were observed among treatments even after the second spray.

4.7.2.4.2 Population of stem endophytic fungi in rain shelter

Effects of foliar application different treatments on stem endophytic fungal population in rain shelter is documented in the Table 4.36. Before the treatment application, more or less uniform fungal population was observed in all the treatment which ranged from 17.33 x 10^3 cfu g^{-1} to 24.66 x 10^3 cfu g^{-1}. However, after the foliar application, significant difference among the treatments was observed and a sudden reduction in fungal population was found in all the fungicidal treatments and T_{10} while increased population was noticed in control plants as well as bioagents treatments such as T_8 and T_9. The highest reduction was noticed in T_7 and it was on par with T_6 with a population of 10.66 x 10^3 cfu g^{-1} and 11.66 x 10^3cfu g^{-1} respectively after the first spray. Among different chemical treatments, propineb - 0.1% (T_1) treated plants showed comparatively less reduction of endophytic fungi recording 17 x 10^3 cfu g^{-1} population after the first spray. After the second spray also, the same trend was observed among treatments and the per cent reduction of endophytic fungal population among chemical treatments were ranges from 23.80 to 67.33 per cent.

In bioagents treatment such as T_8 and T_9, where *Trichoderma* were given as foliar spray, there was a drastic increase in endophytic fungal colony count and the population was 28 x 10^3 cfu g^{-1} and 30.33 x 10^3 cfu g^{-1} respectively after first spray.

Table 4.36. Effect of treatments on stem endophytic fungi of tomato in rain shelter

Treatments	Fungi (x 10^{-3} cfu g^{-1}) stem			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	21.00	17.00^{b}	16.0^{c}	23.80
T_2-Propineb 70% WP (0.2%)	17.66	16.33^{b}	12.66^{cde}	28.31
T_3- Hexaconazole 5% EC (0.05%)	19.66	13.33^{bc}	9.0^{de}	54.22
T_4- Hexaconazole 5% EC (0.1%)	22.66	13.0^{bc}	9.0^{de}	60.28
T_5- Difenoconazole 25% EC (0.05%)	20.33	14.0^{bc}	12.0^{cde}	40.97
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	21.66	11.66^{c}	8.0^{e}	63.06
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	17.33	10.66^{c}	5.66^{e}	67.33
T_8- *Trichoderma viride* (KAU)	24.66	28.0^{a}	33.0^{ab}	-33.81
T_9- PGPM mix (KAU)	24.66	30.33^{a}	34.33^{a}	-39.21
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	20.33	17.33^{b}	16.33^{c}	19.67
T_{11}- Untreated control	22.00	26.33^{a}	27.0^{b}	-22.7

However, in T_{10} where *B. subtilis* was sprayed there was decrease in fungal population from 20.33 x 10^3 cfu g^{-1} to 16.33 x 10^3 cfu g^{-1} after the foliar application. Control plants recorded an increase in stem endophytic fungi, but rate of increase was less than bioagent treatments *viz*. T_8 and T_9.

4.7.2.4.3 Population of root endophytic fungi in rain shelter

From the results presented in Table 4.37 it was found that, before the treatment application, there was no significant difference among treatments with regard to fungal root endophytic population and at this time population count was within the range of 20.66 x 10^3 cfu g^{-1} – 28.66 x 10^3 cfu g^{-1}. However, after the treatment application, reduction in fungal endophytes was found in all the fungicidal treatments as well as in bioagents treatment T_{10} while increased population was observed in control plants and bioagents treatments T_8 and T_9. After the first spray, the maximum reduction was observed in T_7 (14.66 x 10^3 cfu g^{-1}) which was statistically on par with all the remaining chemical treatments except in propineb treated plants (T_1 and T_2). The per cent reduction of fungal population was less in propineb treated plants (8.33-8.66 per cent) compared to other fungicidal treatments. The same trend was observed among treatments even after the second spray and the per cent reduction of endophytic root fungal flora were varied from 7.88 to 51.57 for chemical treatments.

In T_8 and T_9, where fungal biocontrol agents were sprayed, there was a gradual increase in the fungal population and count was 31.33 x 10^3 cfu g^{-1} and 33 x 10^3 cfu g^{-1} respectively after first spray. Control plants also recorded an increased root endophytic fungus (27.33 x 10^3 cfu g^{-1}) after first spray. The same trend was observed among all the treatments even after the second spray.

Table 4.37. Effect of treatments on root endophytic fungi of tomato in rain shelter

Treatments	Fungus (x 10^3 cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	24.0	21.01^{bc}	22^{b}	8.33
T_2-Propineb 70% WP (0.2%)	27.0	27.33^{ab}	24.66^{b}	8.66
T_3- Hexaconazole 5% EC (0.05%)	28.66	20.33^{cd}	16.0^{cd}	38.17
T_4- Hexaconazole 5% EC (0.1%)	25.33	20.0^{cd}	15.66^{cd}	29.39
T_5- Difenoconazole 25% EC (0.05%)	22.66	21.33^{bcd}	19.33^{bc}	14.69
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	20.66	19.67^{cd}	15.0^{cd}	27.39
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	22.0	14.66^{d}	11.33^{d}	48.5
T_8- *Trichoderma viride* (KAU)	20.66	31.33^{a}	37.0^{a}	-79.0
T_9- PGPM mix (KAU)	24.33	33.0^{a}	38.33^{a}	-57.4
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	22.0	24.0^{bc}	20.33^{bc}	8.35
T_{11}- Untreated control	24.0	27.33^{ab}	34.0^{a}	-41.66

Summing up the above studies on effects of selected treatments on endophytic fungal (leaf, stem and root) population in rain shelter, it is clearly evident that, all chemical as well as *B. subtilis* treated plants exhibited a reduction in endophytic fungal population whereas in plants treated with biocontrol agents such as *T. viride and* PGPM mix exhibited an increase in fungal endophytes. In all the cases the maximum reduction in endophytic fungal population was recorded by T_7 and maximum increase was notice in T_9. Moreover, among different fungicidal treatments, systemic fungicide difenoconazole (0.05) showed the minimum fungal population reduction in leaf fungal endophytes while in case of stem and root fungal endophytes, the contact fungicide propineb recorded minimum per cent reduction of fungal population. The results also revealed that, population of tomato fungal endophytes was more in leaves compared to roots and stem. While comparing these results with polyhouse, it was found that natural population of leaf and stem endophytic fungi and its reduction per cent due to different treatment application was more in plants cultivated under rain shelter. However, more or less similar population was observed in case of root endophytic fungi. Moreover, in T_8 and T_9 the rate of increase in fungal population was recorded more under rain shelter condition (Fig. 4.6-4.12).

4.7.2.5 Population of endophytic bacteria in rain shelter

Bacterial endophytes were isolated from tomato leaves, stem and root before and after treatment application in rain shelter and the results of quantitative estimation of bacterial endophytes are given in Tables 4.38, 4.39 & 4.40.

4.7.2.5.1 Population of bacterial leaf endophytes in rain shelter

Data presented in Table 4.38 revealed that, before the treatment application, there was no significant difference among treatments with respect to population of leaf endophytic bacteria and it was ranged from 38 x 10^5 cfu g^{-1} to 46.66 x 10^5 cfu g^{-1}. However, after the foliar application endophytic population varied significantly with different treatment and reduction in bacterial population was found in all the fungicidal treatments,

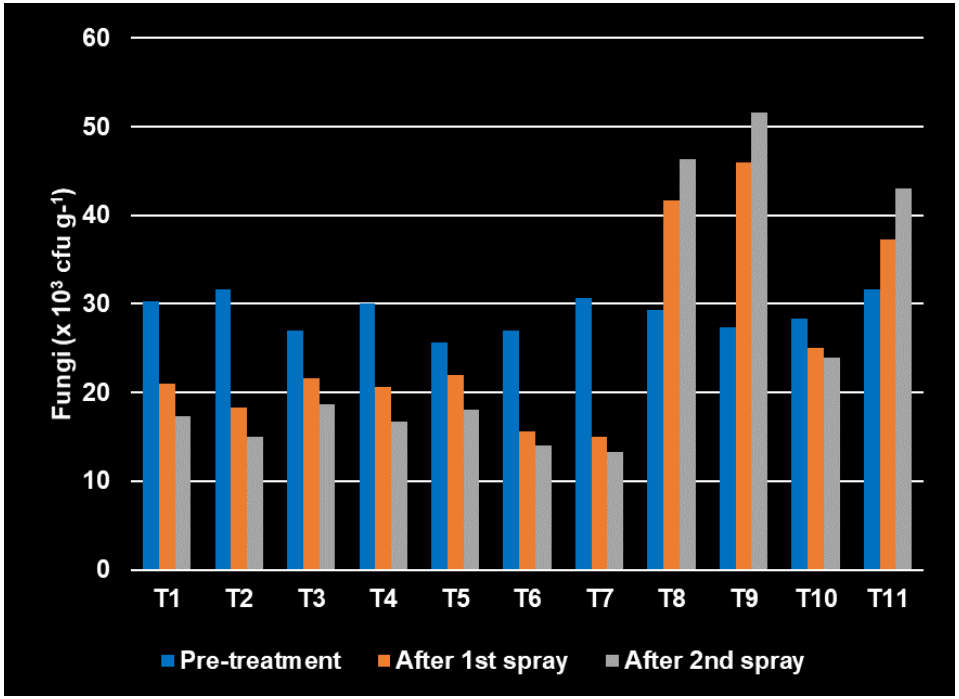


Fig. 4.9. Effect of treatments on endophytic fungi of tomato leaves in polyhouse

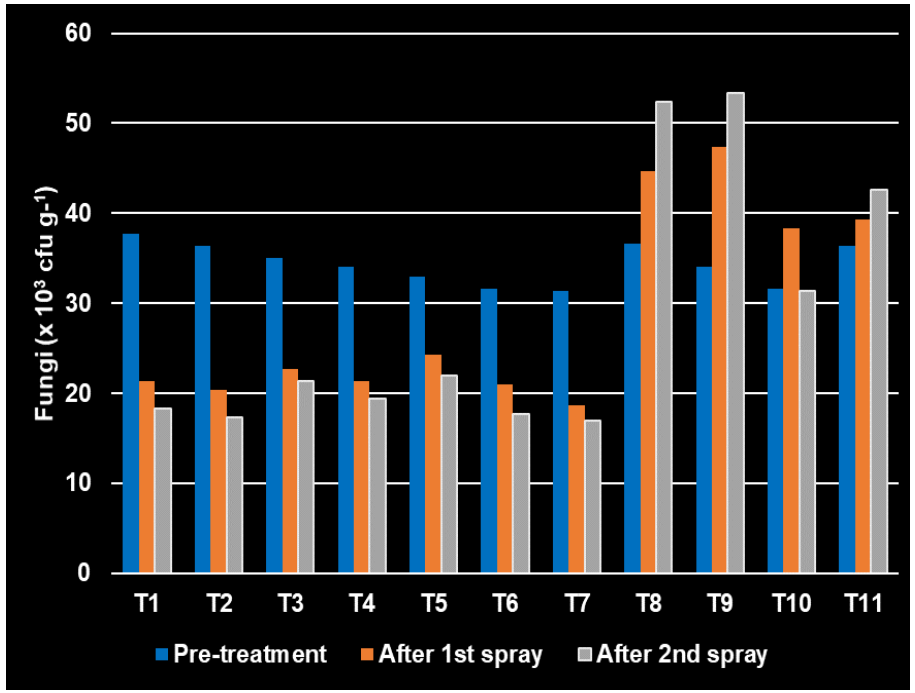


Fig. 4.10. Effect of treatments on endophytic fungi of tomato leaves in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

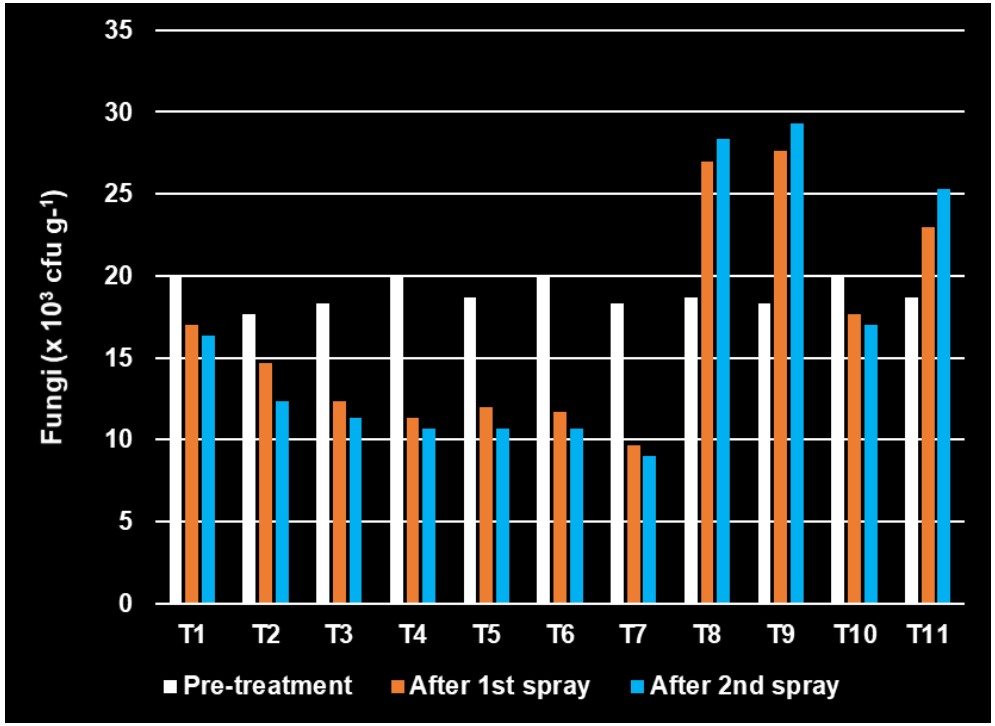


Fig. 4.11. Effect of treatments on endophytic fungi of tomato stem in polyhouse

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

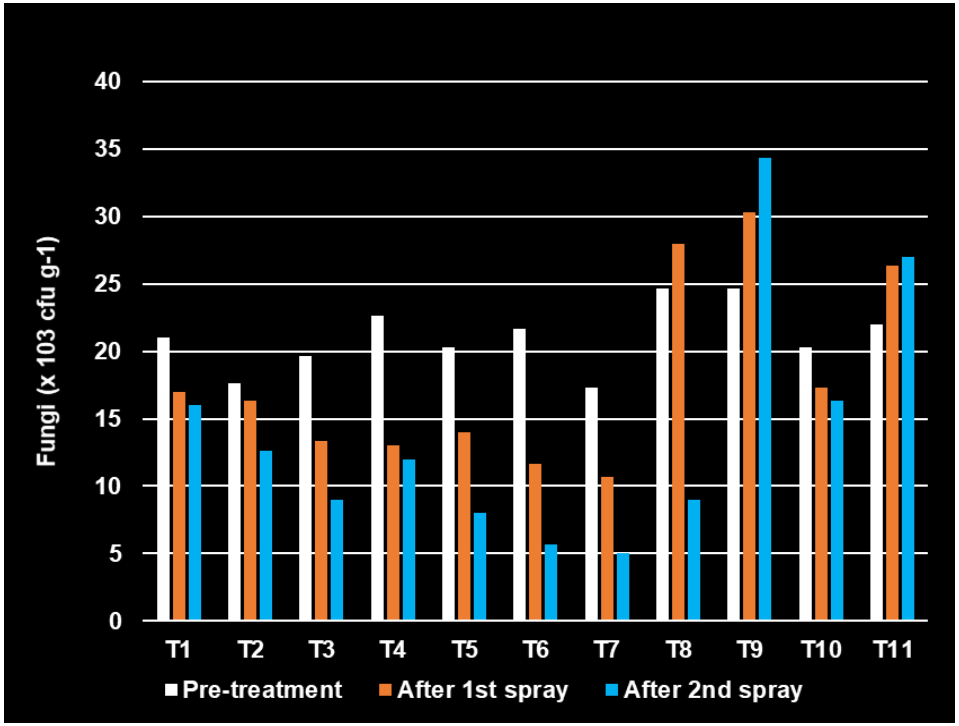


Fig. 4.12. Effect of treatments on endophytic fungi of tomato stem in rain shelter

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

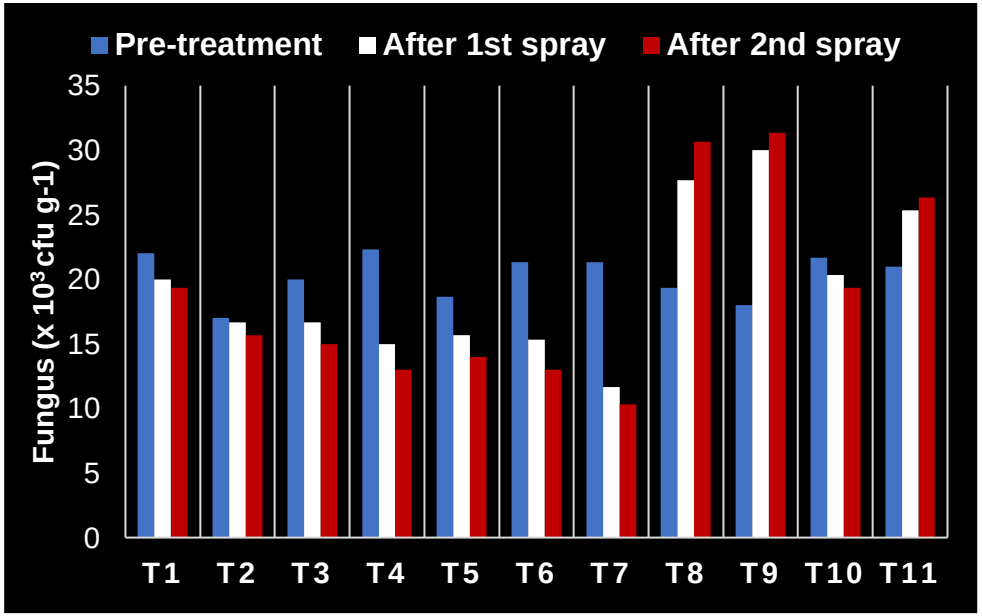


Fig.4.13. Effect of treatments on endophytic fungi of tomato root in polyhouse

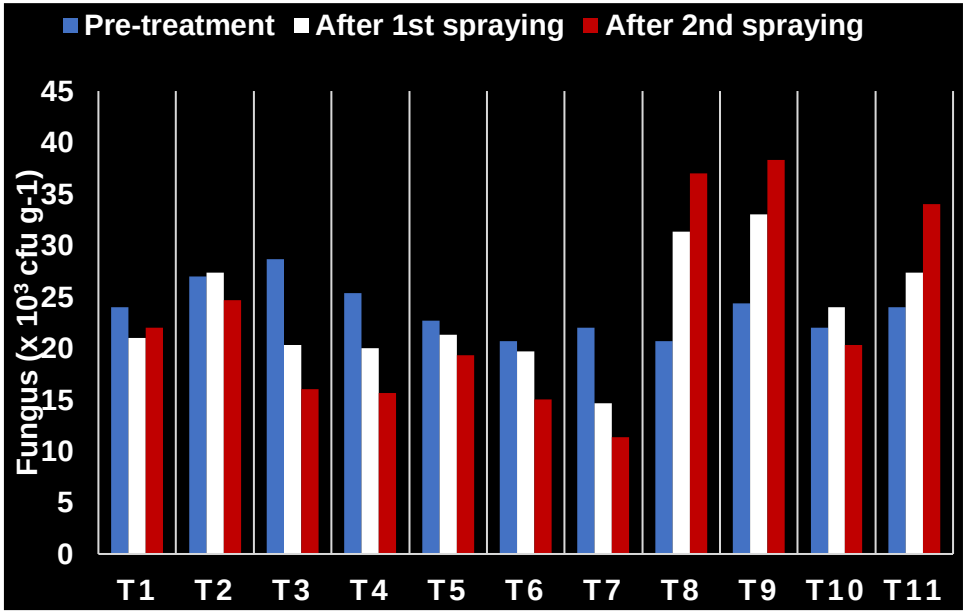


Fig.4.14. Effect of treatments on endophytic fungi of tomato root in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

Table 4.38. Effect of treatments on leaf endophytic bacteria of tomato in rain shelter

Treatments	Bacteria (x 10^5 cfu g^{-1}) leaf			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	39.0	37.33^{cde}	35.33^{cd}	9.41
T_2-Propineb 70% WP (0.2%)	45.66	34.33^{cdefg}	31.0^{cde}	32.10
T_3- Hexaconazole 5% EC (0.05%)	42.66	36.66^{cdef}	29.66^{def}	30.47
T_4- Hexaconazole 5% EC (0.1%)	42.33	28.66^{fg}	22.66^{f}	29.12
T_5- Difenoconazole 25% EC (0.05%)	38.00	31.66^{defg}	30.0^{def}	22.62
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	42.00	29.33^{efg}	25.0^{ef}	40.47
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	46.66	26.66^{g}	25.66^{ef}	45.0
T_8- *Trichoderma viride* (KAU)	38.66	40.0^{c}	36.33^{cd}	6.02
T_9- PGPM mix (KAU)	39.33	62.0^{a}	56.0^{b}	-42.38
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	42.00	52.33^{b}	65.66^{a}	-56.33
T_{11}- Untreated control	41.00	39.66^{cd}	38.66^{c}	5.70

control and in bioagent treatment T_8 (*T. viride*) while increased population was noticed in bioagents treatments such as T_9 and T_{10}. After the first spray, the maximum reduction of fungi was observed in T_7 (26.66 x 10^5 cfu g^{-1}) which was statistically on par with T_6 (29.33 x 10^5 cfu g^{-1}) and T_4 (28.66). Propineb -0.1% (T_1) treated plants showed comparatively less reduction among different fungicidal treatments recording 37.33 x 10^5 cfu g^{-1} after first spray. The same trend was observed among treatments even after the second spray and the per cent reduction of fungal flora on the tomato leaves were varied from 9.41 to 45 for chemical treatments.

Similarly, among the three bioagents treatments, only T_8 (*B. subtilis*) showed reduction in bacterial count and recorded 40 x 10^5 cfu g^{-1} fungal population after first spray. While bioagents treatment such as T_9 and T_{10}, where bacterial biocontrol agents were sprayed on leaves there was a drastic increase in the bacterial colony and the population was 62 x 10^5 cfu g^{-1} and 52.33 x 10^5 cfu g^{-1} respectively after first spray. In control plants bacterial population was reduced from 41 x 10^5 cfu g^{-1} to 38.66 x 10^5 cfu g^{-1} recorded during different intervals of observations. The same pattern of reduction and increase was recorded in all the treatments even after the second spray.

4.7.2.5.2 Population of stem endophytic bacteria in rain shelter

Data furnished in Table 4.39 showed that, before treatment application, isolation of endophytic bacteria from tomato stem yielded more or less uniform population between different treatments and it was varied from 32.33 x 10^5 cfu g^{-1} to 37.33 x 10^5 cfu g^{-1}. But, after the foliar application significant difference was observed and population reduction was recorded in all fungicidal treatments and control treatment whereas population increase was noticed in all bioagent treatments. Among different chemical treatments applied, population of tomato stem bacterial endophytes in T_7, T_6, T_4, T_5 and T_3 were more or less same which ranged from 27.66 x 10^5 cfu g^{-1} to 31.33 x 10^5 cfu g^{-1} after first spray and 26.33 x 10^3 cfu g^{-1} – 28.66 x 10^3 cfu g^{-1} after second spray. Among fungicidal treatments, T_1 and T_2 showed comparatively less reduction recording 35.33 x 10^5 cfu g^{-1} and 32 x 10^5 cfu g^{-1} respectively after first spray. The per cent reduction of stem bacterial endophytes among chemical treatments were ranges from 15.08 to 28.58 per cent.

Among the three bioagents treatments, T_9 recorded maximum increase in endophytic bacterial population (54.33 x 10^5 cfu g^{-1}) followed by T_{10} and T_8 recording 45.33 x 10^5 cfu g^{-1}

and 40 x 10^5 cfu g^{-1} respectively. Control plants also recorded reduction in bacterial count, but the reduction was lesser compared to chemical treatments.

4.7.2.5.3 Population of root endophytic bacteria in rain shelter

On evaluation of selected treatments on root endophytic bacterial population in rain shelter, it is noted from Table 4.40 that, before foliar spray bacterial population was almost equal in all the treatments and it was ranged from 59.33 x 10^5 cfu g^{-1} to 70.66 x 10^5 cfu g^{-1}. But, after the foliar spray significant difference was noticed between chemical treatments and biological treatments. Endophytic bacterial population reduction was recorded in all fungicidal treatments as well as in bioagent treatment T_8 while population increase was noticed in control and bioagent treatments *viz.* T_9 and T_{10}. However, significant difference was not found between different chemical treatments and population ranges from 52 x 10^5 cfu g^{-1} to 63.66 x 10^5 cfu g^{-1} after first spray and 49 x 10^5 cfu g^{-1} to 63.33 x 10^5 cfu g^{-1} after second spray. Even though the maximum per cent reduction in bacterial population (22.22 per cent) was recorded in T_4 and minimum in T_1 among chemical treatments. Among bioagents treatments, maximum increase in endophytic bacterial count was found in T_9 (92.66 x 10^5 cfu g^{-1}) and which was statistically on par with T_{10} (80.66 x 10^5 cfu g^{-1}). Control plants also recorded an increase in bacterial count, but the rate was very less compared bioagents treatments T_9 and to T_{10}. Hence, while examining the results of changes in endophytic bacterial population isolated from leaves, stem and root of different treatment applied tomato plant in rain shelter, we can find that, all chemical treated plants exhibited a reduction in endophytic bacterial population whereas in plants treated with biocontrol agents such as PGPM mix and *B. subtilis* exhibited an increased bacterial count. However, *T. viride* treated plants showed reduction in leaf and root endophytic bacterial population while it recorded an increase in case of stem bacterial endophytes.

Table 4.39. Effect of treatments on stem endophytic bacteria of tomato in rain shelter

Treatments	Bacteria (x 10^5 cfu g^{-1}) stem			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	35.33	35.33^{de}	30.0^{de}	15.08
T_2-Propineb 70% WP (0.2%)	32.33	32.0^{ef}	26.66^{e}	17.53
T_3- Hexaconazole 5% EC (0.05%)	35.33	31.33^{efg}	26.66^{e}	20.0
T_4- Hexaconazole 5% EC (0.1%)	37.0	29.0^{fg}	26.33^{e}	28.58
T_5- Difenoconazole 25% EC (0.05%)	35.33	29.0^{fg}	28.66^{e}	21.91
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	37.33	28.33^{fg}	28.0^{e}	22.92
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	35.0	27.66^{g}	27.33^{e}	24.99
T_8- *Trichoderma viride* (KAU)	36.33	40.0^{c}	40.33^{b}	-11.01
T_9- PGPM mix (KAU)	34.66	54.33^{a}	56.0^{a}	-61.56
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	35.0	45.33^{b}	51.66^{a}	-47.6
T_{11}- Untreated control	37.33	36.33^{cd}	35.66^{bc}	4.47

Table 4.40 Effect of treatments on root endophytic bacteria of tomato in rain shelter

Treatments	Bacteria (x 10^5 cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	59.33	55.33^{cd}	54^{bcd}	8.98
T_2-Propineb 70% WP (0.2%)	70.00	63.66^{cd}	63.33^{bc}	9.52
T_3- Hexaconazole 5% EC (0.05%)	61.0	54.33^{cd}	50.33^{cd}	17.49
T_4- Hexaconazole 5% EC (0.1%)	63.0	52.0^{d}	49.0^{d}	22.22
T_5- Difenoconazole 25% EC (0.05%)	59.33	55.33^{cd}	54.0^{bcd}	11.62
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	70.66	59.66^{cd}	57.66^{bcd}	18.57
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	62.33	56.0^{cd}	51.66^{bcd}	17.11
T_8- *Trichoderma viride* (KAU)	65.0	65.6^{bc}	63.66^{bc}	2.06
T_9- PGPM mix (KAU)	68.33	92.66^{a}	93.33^{a}	-36.58
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	66.0	80.66^{ab}	82.0^{a}	-24.24
T_{11}- Untreated control	61.00	65.33^{cd}	64.66^{b}	-6.0

Similarly, control plants also recorded an increase in root endophytic bacterial population and reduction in leaf and stem bacterial endophytes. In all cases, there was little or no significant difference among different chemical treatments except in the treatment with contact fungicide propineb which recorded minimum per cent reduction of endophytic bacteria. Moreover, the changes due to different treatment application was more in leaf compared to stem and root bacterial endophytes. The results also revealed that, the maximum population of tomato endophytic bacteria was in root followed by leaves and stem respectively. Comparing the results on enumeration of tomato endophytic bacteria from rain shelter and polyhouse, it was noticed that natural population was more in rain shelter while the per cent population reduction due to different treatments were more in polyhouse condition. Moreover, the rate of increase in bacterial population in biocontrol treatments such T_9 and T_{10} was higher in polyhouse cultivated plant (Fig 4.13-4.18).

4.7.2.6 Population of endophytic actinomycetes in rain shelter

Endophytic actinomycetes were isolated from tomato leaves, stem and root before and after foliar application and the results is shown in Tables 4.41, 4.42 & 4.43.

4.7.2.6.1 Population of leaf endophytic actinomycetes in rain shelter

In Table 4.41, endophytic actinomycetes population from tomato roots before and after treatments application in polyhouse are furnished. It was found that, before and after treatment application significant difference among treatments were absent and it was ranged from 1.0 x 10 cfu g^{-1} to 2.3 x 10 cfu g^{-1} and 1.0 x 10 cfu g^{-1} to 2.0 x 10 cfu g^{-1} respectively. The data also showed that, after foliar spray endophytic actinomycetes population was decreased in all treatments except T_3 and T_4 in which population count remains same even after the treatment application. In control plants endophytic actinomycetes count was decreased from 2 x 10 cfu g^{-1} to 1.3 x 10 cfu g^{-1} after the second spray.

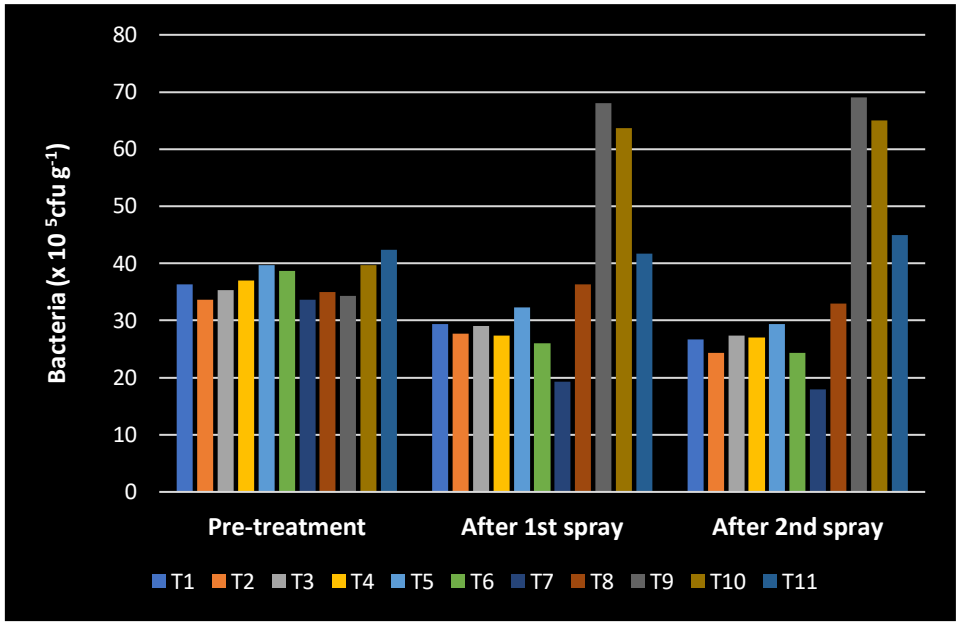


Fig.4.15. Effect of treatments on endophytic bacteria of tomato leaves in polyhouse

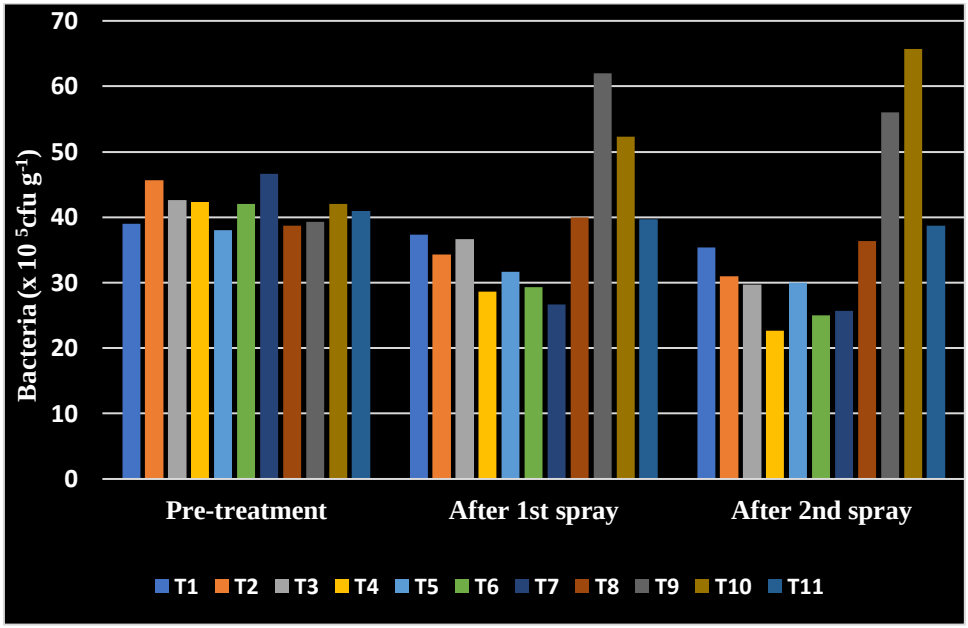


Fig.4.16. Effect of treatments on endophytic bacteria of tomato leaves in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

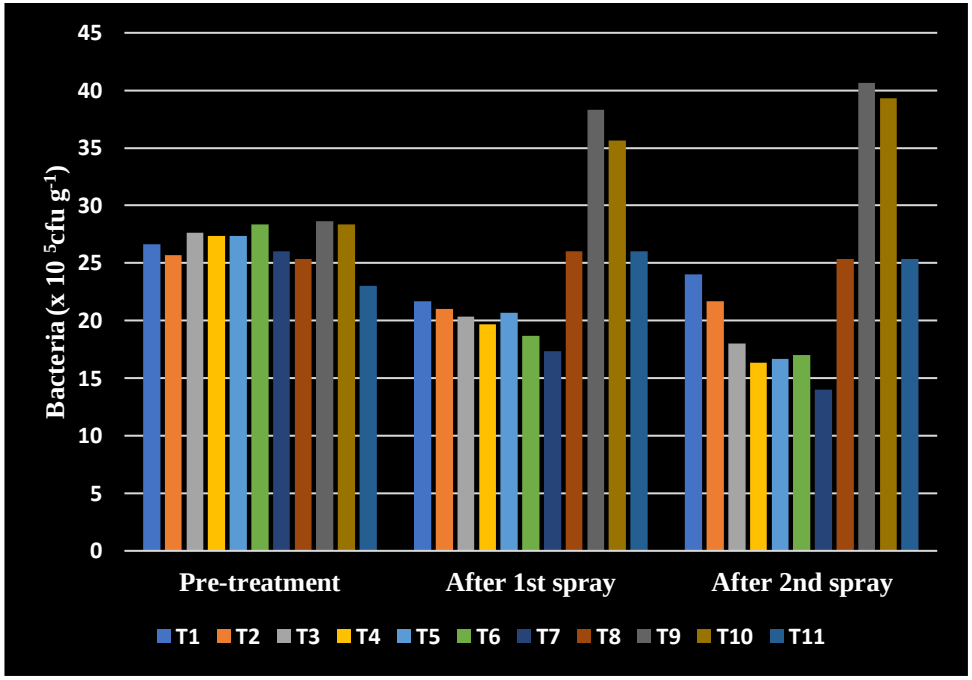


Fig.4.17. Effect of treatments on endophytic bacteria of tomato stem in polyhouse

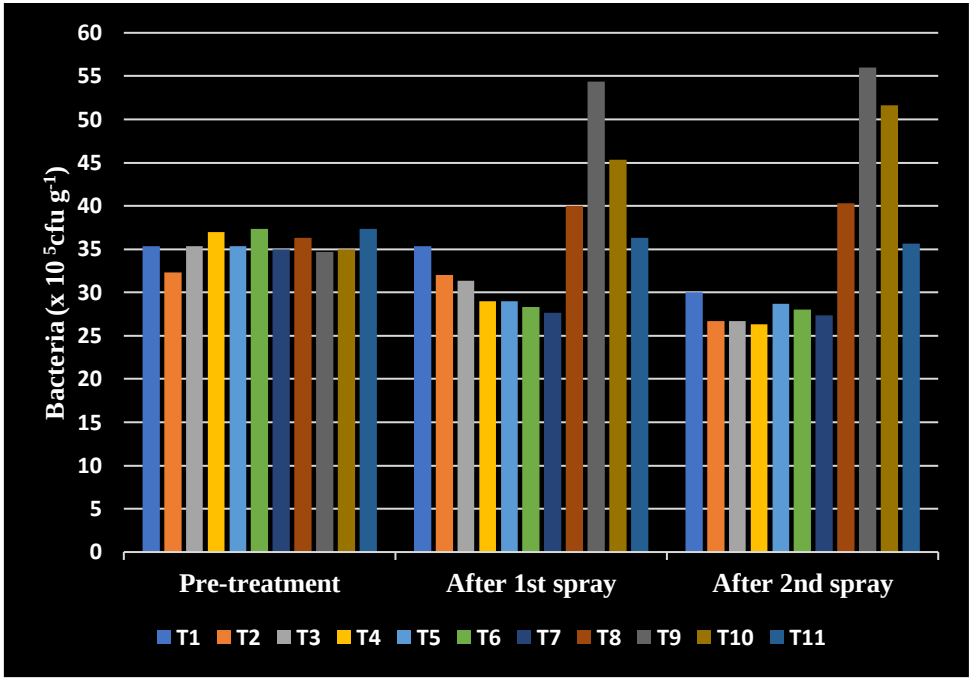


Fig.4.18. Effect of treatments on endophytic bacteria of tomato stem in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

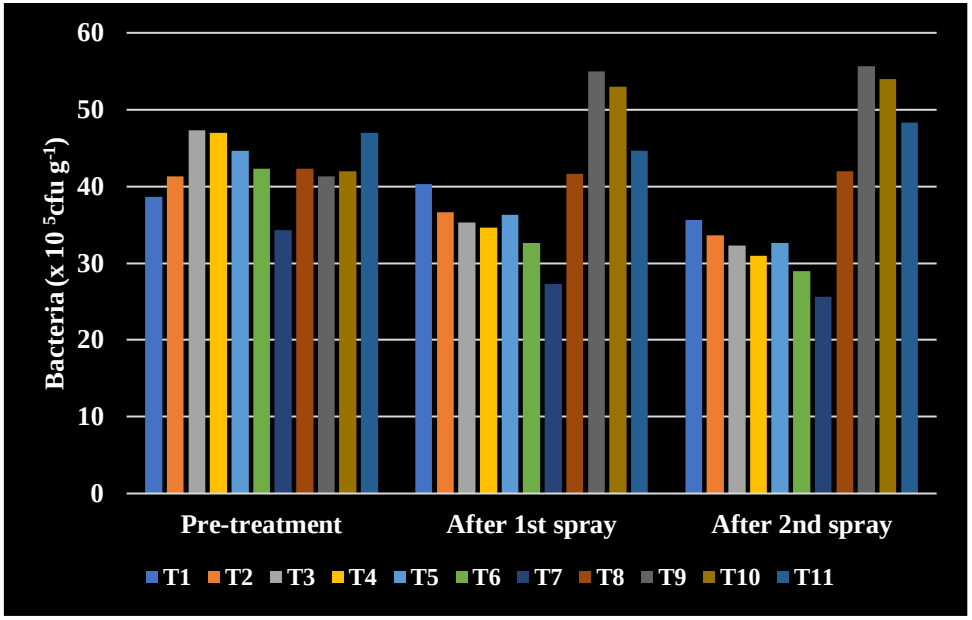


Fig.4.19. Effect of treatments on endophytic bacteria of tomato root in polyhouse

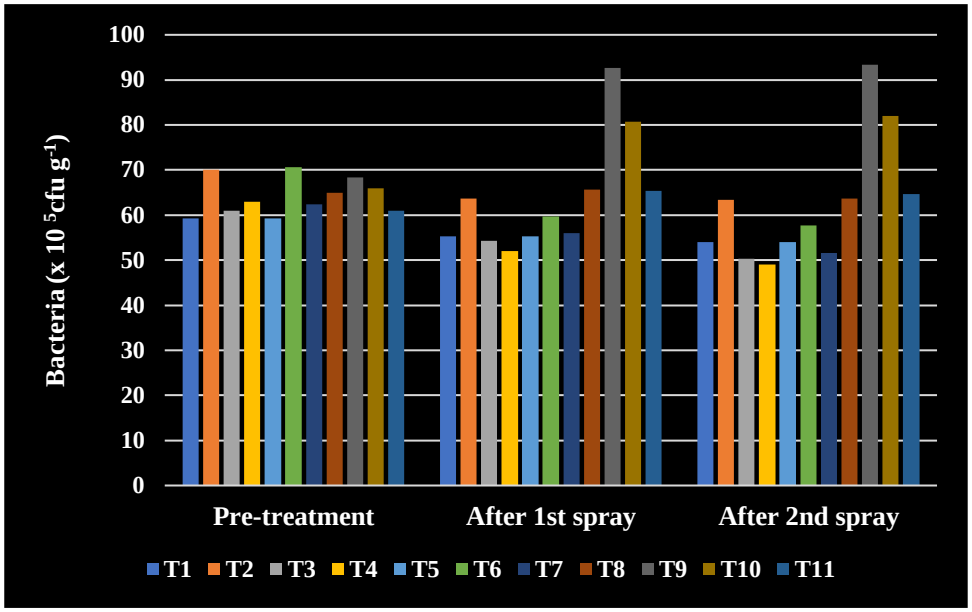


Fig.4.20. Effect of treatments on endophytic bacteria of tomato root in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

Table 4.41 Effect of treatments on leaf endophytic actinomycets of tomato in rain shelter

Treatments	Actinomycetes (x 10 cfu g^{-1}) leaf			
	Pre treatment	**After 1st spray**	**After 2nd spray**	**Per cent reduction over control**
T_1- Propineb 70% WP (0.1%)	1.06	1.66	1.33	19.87
T_2-Propineb 70% WP (0.2%)	2.0	2.0	1.66	17
T_3- Hexaconazole 5% EC (0.05%)	1.0	1.0	1.0	0
T_4- Hexaconazole 5% EC (0.1%)	2.33	2.0	2.0	14.16
T_5- Difenoconazole 25% EC (0.05%)	1.66	1.66	1.66	0
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	2.33	1.66	2.0	14.16
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	2.33	1.33	1.33	42.91
T_8- *Trichoderma viride* (KAU)	1.66	1.33	1.33	19.87
T_9- PGPM mix (KAU)	1.66	1.66	1.33	19.87
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	2.33	2.33	2.0	14.16
T_{11}- Untreated control	2.0	1.66	1.33	9.57
CD (0.05)	NS	NS	NS	-

4.7.2.6.2 Population of stem endophytic actinomycetes in rain shelter

Results on endophytic actinomycetes population from tomato stem are given in the Table 4.42. The data reveled that, with respect to endophytic actinomycetes population, there was no significant difference among treatment before and after foliar application. However, an overall increase in endophytic actinomycetes population was observed in all treatments except T_3 in which population count remains same even after the treatment application. Before treatment application actinomycetes population was within the range of 0.66 x 10 cfu g^{-1} to 1.33 x 10 cfu g^{-1} and after the treatment application it increased to the range of 1.0 x 10 cfu g^{-1} to 2.33 x 10 cfu g^{-1}. Control plants also recorded increased endophytic actinomycetes count ranges from 1.33 x 10 cfu g^{-1} to 1.66 x 10 cfu g^{-1}.

4.7.2.6.3 Population of root endophytic actinomycetes in rain shelter

Data on population of endophytic actinomycetes isolated from tomato leaves in rain shelter is presented in Table 4.43. It was evident from the results that, before and after treatments application, significant difference was not observed among the treatment with regard to endophytic actinomycetes. However, after the treatment application, an overall decrease in leaf endophytic actinomycetes population was found in all treatments excepts T_5 and T_{10} in which population remain constant even after treatment application. Before the treatment application, actinomycetes population was with the range of 1.66 x 10 cfu g^{-1} to 3 x 10 cfu g^{-1} while after the treatment it varied from 1.06 x 10 cfu g^{-1} to 2.33 x 10 cfu g^{-1}. Similarly, the per cent reduction of leaf endophytic actinomycetes due to different treatment application was ranged from 0 to 36.14.

Thus, the results on effects of different treatments on tomato endophytic actinomycetes population revealed that, there was no significant difference of endophytic actinomycetes population among different treatments. However, an overall slight increase in stem endophytic actinomycetes and reduction in leaf and root endophytic actinomycetes population was observed after treatment application. Hence, we can conclude that, the selected treatments have little or no effects on endophytic actinomycetes population of tomato plants since, population increase and decrease was also noticed in untreated control plants.

Table 4.42 Effect of treatments on stem endophytic actinomycets of tomato in rain shelter

Treatments	Actinomycetes (x 10 cfu g^{-1}) stem			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	0.66	1.0	1.0	-51.51
T_2-Propineb 70% WP (0.2%)	1.33	1.66	1.66	-24.81
T_3- Hexaconazole 5% EC (0.05%)	1.33	0.66	1.33	0
T_4- Hexaconazole 5% EC (0.1%)	0.66	1.0	1.0	-51.5
T_5- Difenoconazole 25% EC (0.05%)	1.0	1.33	2.33	-33
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	1.33	1.66	1.66	-24.81
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	0.66	1.0	1.0	-51.51
T_8- *Trichoderma viride* (KAU)	1.0	0.66	2.0	-100
T_9- PGPM mix (KAU)	1.33	0.66	1.66	-24.81
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.33	1.66	2.0	-50.37
T_{11}- Untreated control	1.33	1.66	1.66	-24.81
CD (0.05)	NS	NS	NS	-

Table 4.43. Effect of treatments on root endophytic actinomycets of tomato in rain shelter

Treatments	Actinomycetes (x 10 cfu g^{-1}) root			
	Pre treatment	After 1st spray	After 2nd spray	Per cent reduction over control
T_1- Propineb 70% WP (0.1%)	1.66	1.66	1.06	36.14
T_2-Propineb 70% WP (0.2%)	2.33	1.66	1.66	28.75
T_3- Hexaconazole 5% EC (0.05%)	2.0	2.0	1.66	17
T_4- Hexaconazole 5% EC (0.1%)	3	2.33	2.0	33.33
T_5- Difenoconazole 25% EC (0.05%)	2.33	1.0	2.33	0
T_6- Iprodione 25% + carbendazim 25% WP (0.1%)	2.66	2.33	2.0	14.16
T_7- Iprodione 25% + carbendazim 25% WP (0.2%)	1.33	1.0	1.0	24.81
T_8- *Trichoderma viride* (KAU)	2.0	1.66	1.66	17
T_9- PGPM mix (KAU)	1.66	1.66	1.33	19.87
T_{10}- *Bacillus subtilis* (Endophyte from cocoa)	1.66	1.33	1.66	0
T_{11}- Untreated control	2.0	1.66	1.66	17

The results also revealed that, among various parts of tomato plants, root occupied maximum endophytic actinomycetes population count followed by leaf and stem respectively. While comparing the results of endophytic actinomycetes population from polyhouse and rain shelter it was found that natural population of leaf endophytic actinomycetes was more in rain shelter while stem endophytic actinomycetes was more in polyhouse. But root actinomycetes population was found almost same in both conditions. Even though there was no significant difference in endophytic actinomycetes population among different treatments, a general population increase was observed in polyhouse treatments whereas population decrease was found in leaf and root endophytes under rain shelter condition (Fig. 4.9-4.12).

4.8 Survival of the biocontrol agents on the phylloplane of tomato under protected cultivation

Survival of the biocontrol agents sprayed on leaves in treatments such as T_8 and T_9 was assessed by re-isolation and enumeration using serial dilution plating on suitable selective media at periodical intervals 5, 10, 15 days of first, second and third spray.

4.8.1 Survival of *Trichoderma* on the phylloplane of tomato under protected cultivation

Inside the polyhouse, before treatment application the natural population of *Trichoderma* in T_8 and T_9 was 1.03 x 10^{-2} cfu cm^{-2} and 0.84 x 10^{-2} cfu cm^{-2} respectively (Table 4.44) whereas five days after first spraying, there was more than threefold increase in the population in T_8 and T_9. Thereafter, it gradually decreased from 3.46 x 10^{-2} cfu cm^{-2} at 5 DAS to 2.14 x 10^{-2} cfu cm^{-2} at 15 DAS and from 3.82 x 10^{-2} cfu cm^{-2} to 2.73 x 10^{-2} cfu cm^{-2} respectively in T_8 and T_9. Consequent to second and third spraying these treatments showed same trends as in first spraying (Plate 23).

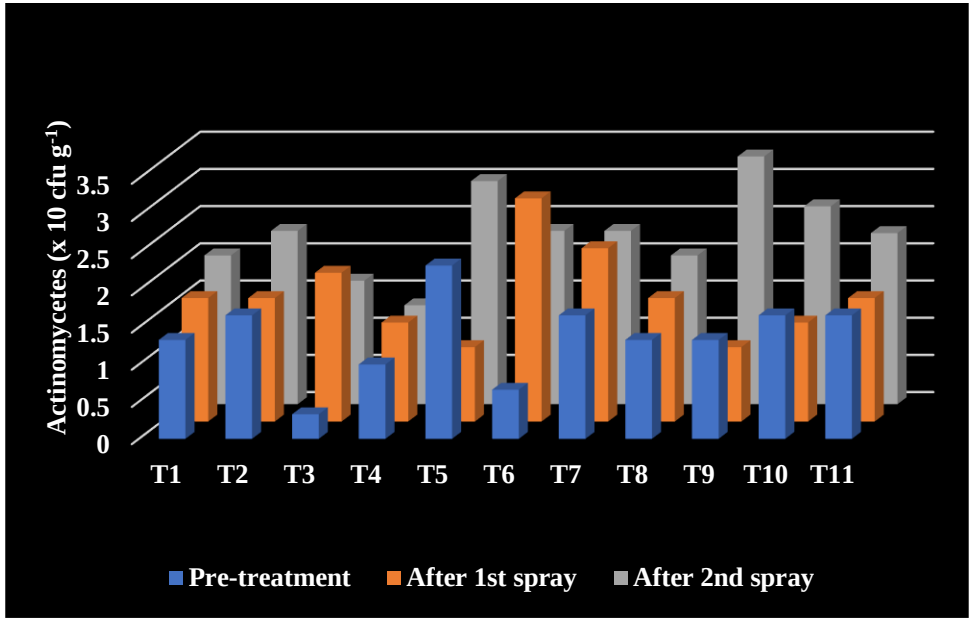


Fig.4.21. Effect of treatments on endophytic actinomycetes of tomato leaves in polyhouse

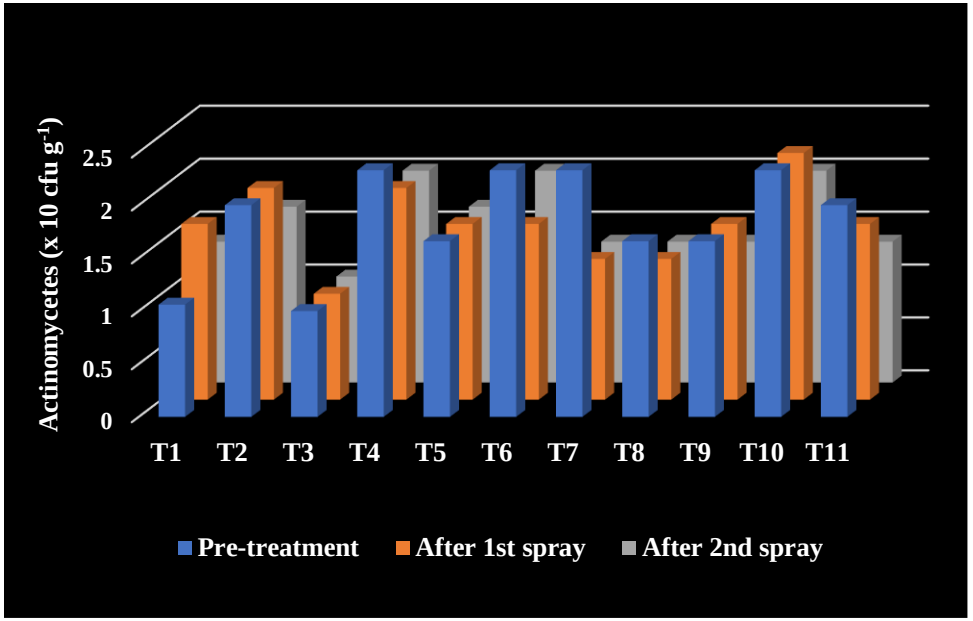


Fig.4.22. Effect of treatments on endophytic actinomycetes of tomato leaves in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

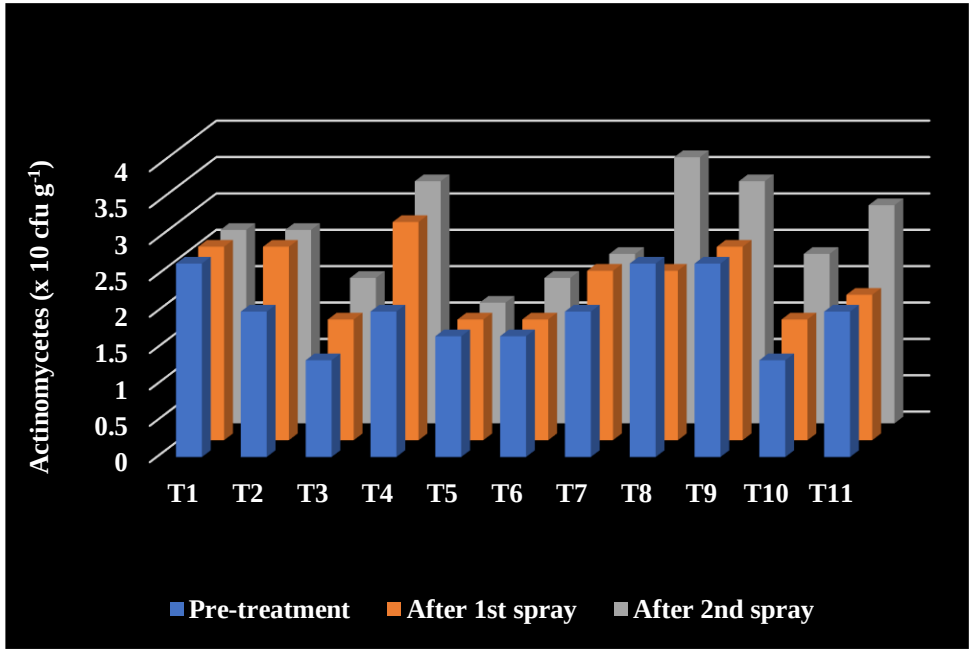


Fig.4.23. Effect of treatments on endophytic actinomycetes of tomato stem in polyhouse

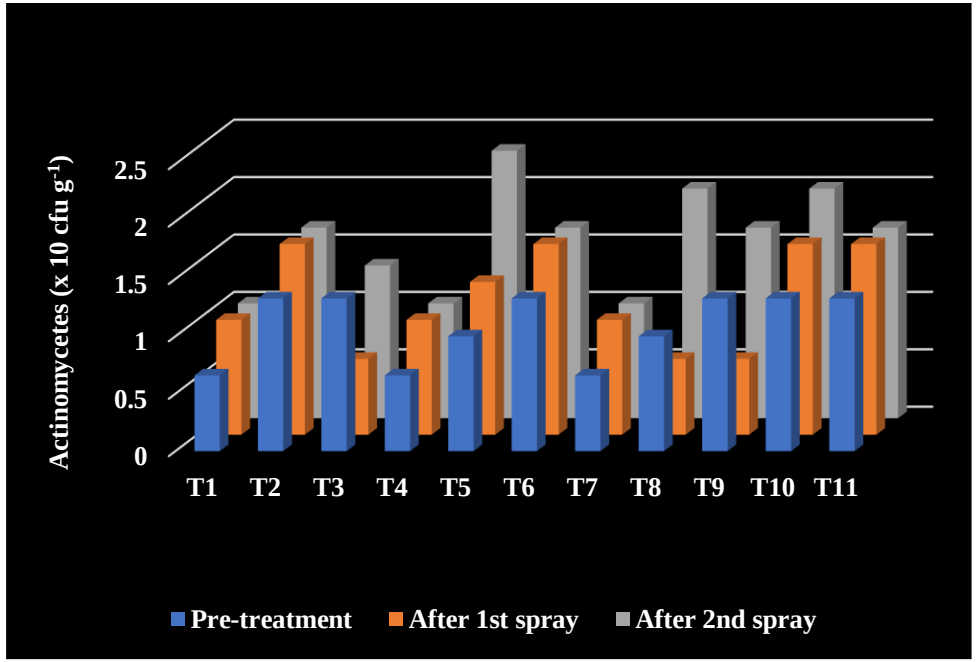


Fig.4.24. Effect of treatments on endophytic actinomycetes of tomato stem in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

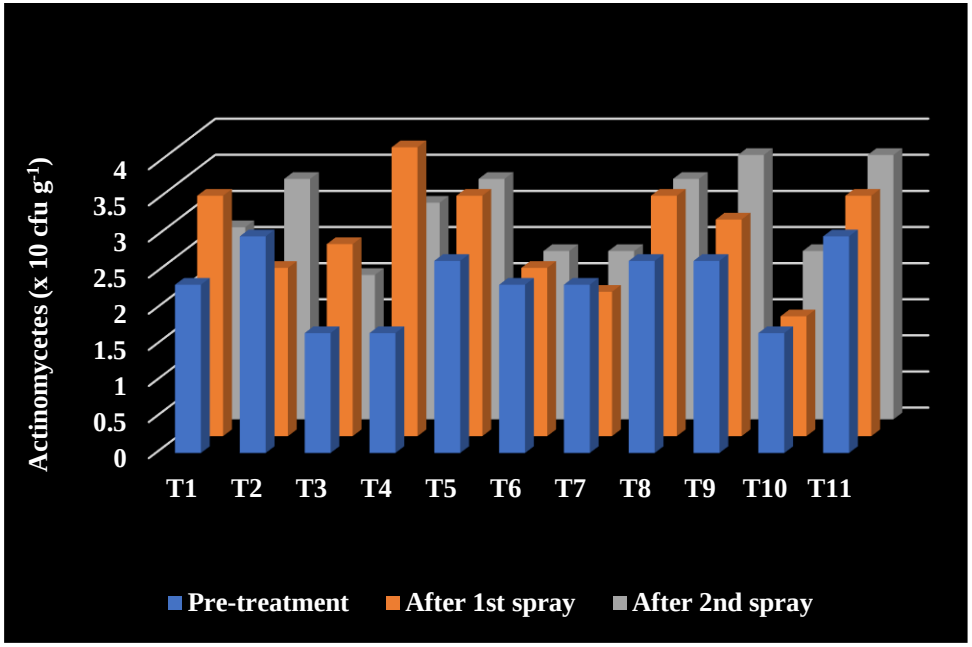


Fig.4.25. Effect of treatments on endophytic actinomycetes of tomato root in polyhouse

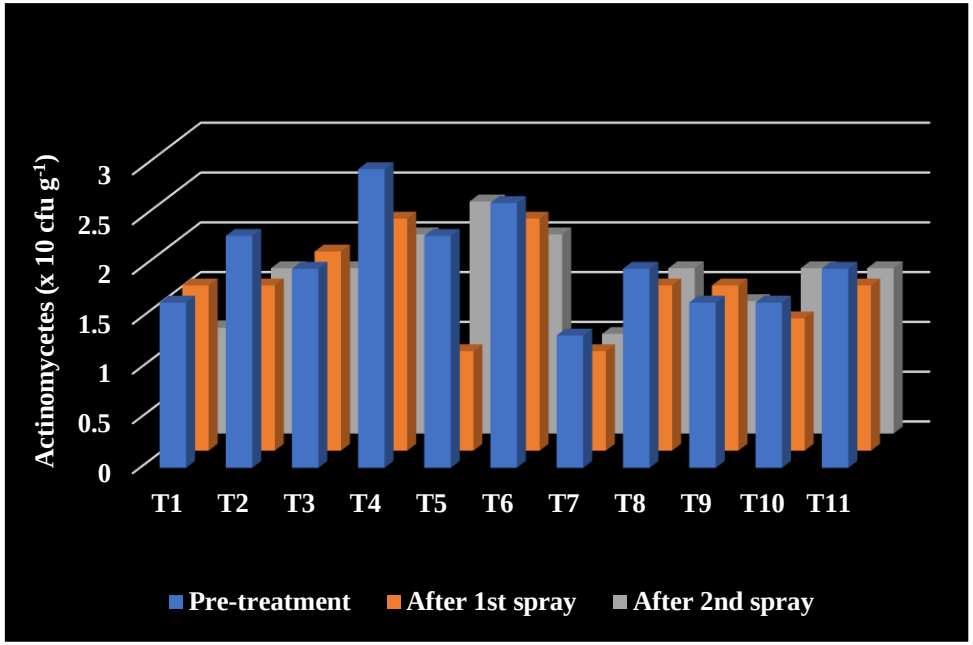


Fig.4.26. Effect of treatments on endophytic actinomycetes of tomato root in rain shelter

T_1 – FS - Propineb 70% WP (0.1%)

T_2 – FS- Propineb 70% WP (0.2%)

T_3 – FS- Hexaconazole 5% EC (0.05%)

T_4- FS- Hexaconazole 5% EC (0.1%)

T_5- FS- Difenoconazole 25% EC (0.05%)

T_6- FS- Iprodione 25% + carbendazim 25% WP (0.1%)

T_7- FS- Iprodione 25% + carbendazim 25% WP (0.2%)

T_8 -FS- *Trichoderma viride* (KAU)

T_9 –FS- PGPM mix (KAU)

T_{10}-FS- *Bacillus subtilis* (Endophyte from cocoa)

T_{11}-FS- Untreated control

FS- Foliar spray

In rain shelter also, the population of *Trichoderma* followed the same pattern as in polyhouse, but compared to polyhouse, the natural population was more in rain shelter condition and it was 1.26 x 10^{-2} cfu cm^{-2} and 1.32 x 10^{-2} cfu cm^{-2} respectively in T_8 and T_9 (Table 4.45). Here also, three-fold increase in *Trichoderma* population was recorded five days after spraying. Then in T_8 the population was gradually decreased to 2.58 x 10^{-2} cfu cm^{-2} and 2.83 x 10^{-2} cfu cm^{-2} in T_9 after fifteen days and similar pattern was observed during second and third spray.

Table 4.44. Survival of *Trichoderma* on the phylloplane of tomato in polyhouse

Treatment	*Trichoderma* (x 10 cfu cm^{-2})								
	Pre-treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_8. Foliar spray with *T. viride* (KAU)	1.03	3.46	2.96	2.14	4.05	3.47	2.89	4.52	4.17	3.61
T_9. Foliar spray with PGPM mix (KAU)	0.84	3.82	3.05	2.73	4.46	3.83	3.15	4.92	4.28	3.83

Table 4.45. Survival of *Trichoderma* on the phylloplane of tomato in rain shelter

Treatment	*Trichoderma* (x 10 cfu cm^{-2})								
	Pre-treatment	First spraying			Second spraying			Third spraying	
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_8. Foliar spray with *T. viride* (KAU)	1.26	3.65	3.10	2.58	4.37	3.84	3.14	5.03	4.47	3.75
T_9. Foliar spray with PGPM mix (KAU)	1.32	3.91	3.25	2.83	4.68	4.03	3.36	5.25	4.61	3.96

4.8.2. Survival of *Pseudomonas fluorescens* on the phylloplane of tomato under protected cultivation

Since *P. fluorescens* was one of the plant growths promoting microorganism in PGPM mix, its survival on the tomato phylloplane was checked in T_9 treated plants (Table 4.46 and 4.47). It was noticed that, before treatment application natural population of *P. fluorescens* in polyhouse (0.67×10^{-4} cfu cm^{-2}) was less compared to rain shelter (1.23×10^{-4} cfu cm^{-2}). However, in both cases, there was an increase in the *P. fluorescens* population in T_9 at 5 DAS but, it gradually decreased from 3.14×10^{-4} cfu cm^{-2} to 1.64×10^{-4} cfu cm^{-2} and from 3.94×10^{-4} cfu cm^{-2} to 2.43×10^{-4} cfu cm^{-2} in polyhouse and rain shelter respectively. Similar trend was observed after second and third spray under polyhouse and rain shelter condition (Plate 4.24).

Table 4.46. Survival of *Pseudomonas fluorescens* on the phylloplane of tomato in polyhouse

Treatment	*Pseudomonas fluorescens* (x 10^2 cfu cm^{-2})									
	Pre-treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_9. Foliar spray with PGPM mix (KAU)	0.67	3.14	2.92	1.64	4.32	3.81	3.01	4.16	3.79	2.14

Table 4.47. Survival of *Pseudomonas fluorescens* on the phylloplane of tomato in rain shelter

Treatment	*Pseudomonas fluorescens* (x 10^2 cfu cm^{-2})									
	Pre-treatment	First spraying			Second spraying			Third spraying		
		5DAS	10DAS	15DAS	5DAS	10DAS	15DAS	5DAS	10DAS	15DAS
T_9. Foliar spray with PGPM mix (KAU)	1.23	3.94	3.16	2.43	4.87	4.15	3.53	4.96	4.21	3.3

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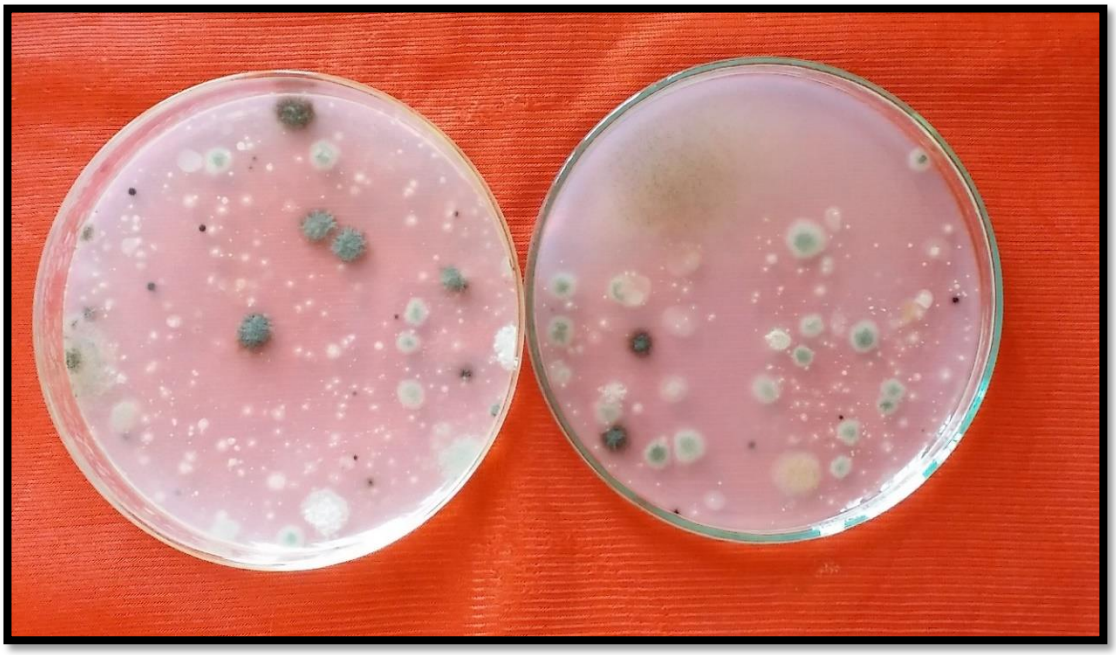


Plate 23. Survival of *Trichoderma viride* on tomato phylloplane

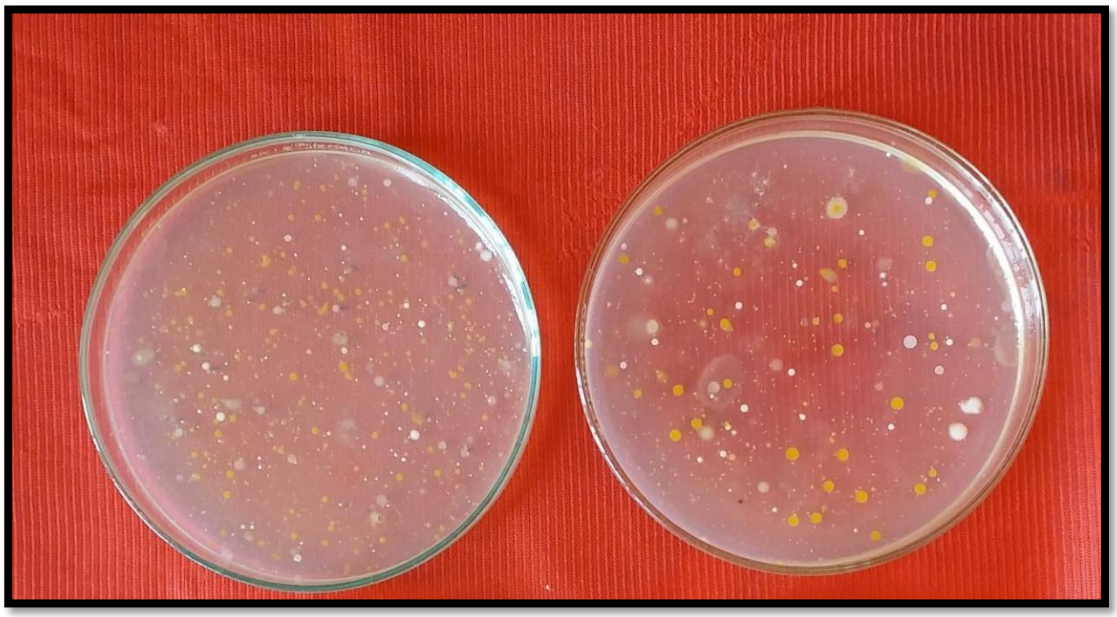


Plate 24. Survival of *Pseuodomonas fluorescens* on tomato phylloplane

4.9. Metagenomic analysis to assess the impact of foliar spray on non-target microflora

Metagenomics DNA was extracted from twenty-two samples representing eleven treatments both from polyhouse and rain shelter experiment as described in 3.9.2.

4.9.1. Quality and quantity of metagenomic DNA

The quality of the metagenomic DNA isolated from 22 leaves samples using DNeasy Plant Mini kit was analysed using agarose gel electrophoresis. Electrophoresis on 0.8 per cent agarose gel revealed a single intact band corresponding to the 21226 bp band in the marker (Plate 25). The quantitative analysis of the metagenomic DNA was done by spectrophotometry using NanoDrop. The concentration of DNA and the ratio of absorbance between 260/280 nm were estimated and provided in Table 4.48. Since all the samples expressed good quality and quantity of genomic DNA, these samples were further checked for PCR amplification in the 16S rDNA region and the ribosomal internal transcribed spacer (ITS) region for the presence of bacteria and fungi respectively in the samples and confirmed the presence of bacteria and fungi in all the samples (Plate 26 & 27). Among the total 22 DNA samples, four samples which is collected each one from best contact (T_2) and systemic (T_7) fungicides and biocontrol agent (T_9) treated plants and from control plants (T_{11}) from rain shelter experiments were selected for further downstream processing.

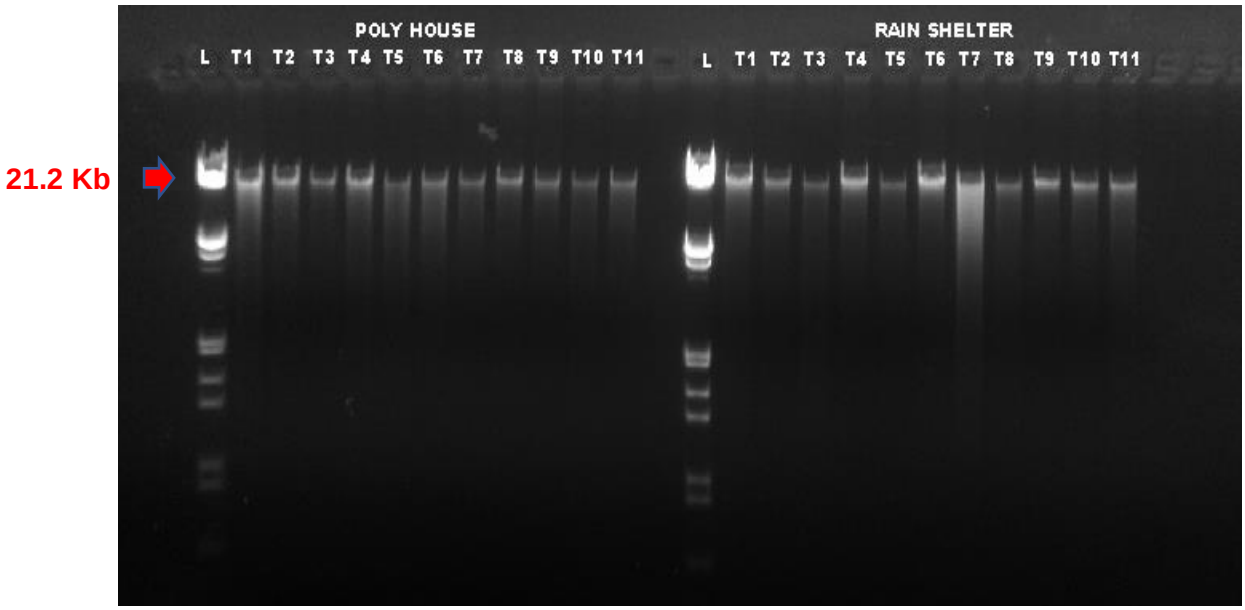


Plate 4.25. Metagenomic DNA from tomato leaves collected from polyhouse and rain shelter on 0.8% agarose gel
L : Ladder (λ*Eco*R1+ *Hind*III double digest)

Table 4.48. Qualitative and quantitative parameters of the isolated metagenomic DNA

Treatment	Rain shelter		Poly house	
	Concentration of DNA (ng/µl)	A260/280	Concentration of DNA (ng/µl)	A260/280
T1- Propineb 70% WP (0.1%)	61.40	1.78	58.70	1.82
T2-Propineb 70% WP (0.2%)	39.50	1.78	59.30	2.20
T3- Hexaconazole 5% EC (0.05%)	34.50	1.79	32.90	1.80
T4- Hexaconazole 5% EC (0.1%)	56.00	1.80	59.20	1.80
T5- Difenoconazole 25% EC (0.05%)	22.80	1.81	38.30	1.82
T6- Iprodione 25% + carbendazim 25% WP (0.1%)	67.30	1.83	45.20	1.79
T7- Iprodione 25% + carbendazim 25% WP (0.2%)	61.10	1.81	29.00	1.78
T8- Trichoderma viride (KAU)	25.10	1.81	38.40	1.79
T9- PGPM mix (KAU)	31.10	1.83	32.50	1.83
T10- Bacillus subtilis (Endophyte from cocoa)	34.90	1.78	25.10	1.86
T11- Untreated control	37.60	1.79	29.10	1.78

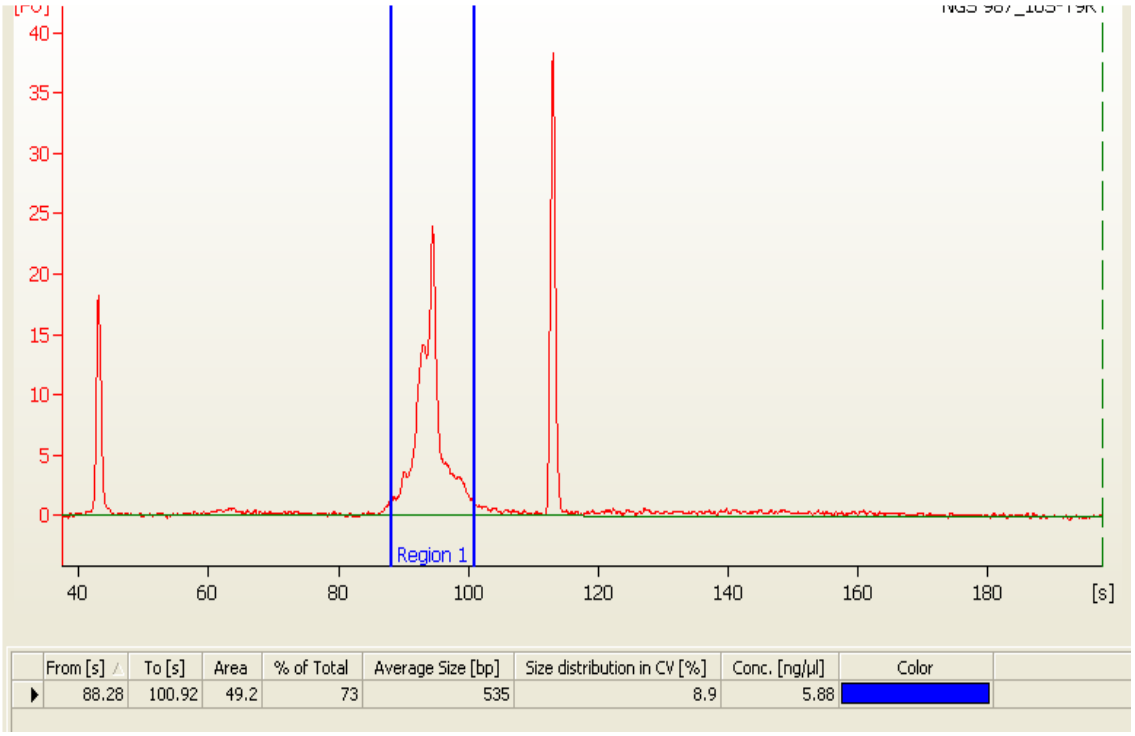
4.9.2. 16S RNA gene and ITS amplicon library sequencing using Next Generation Illumina Miseq™

4.9.2.1 Metagenomic library preparation

The amplicon libraries were prepared from given gDNA sample after amplifying V3-V4 region of 16S segment for bacteria and ITS2 region of ITS segment for fungi. The amplicon libraries were purified by 1X AMpureXP beads, checked on Agilent DNA1000 chip on Bioanalyzer 2100 (Fig.4.25-4.32). It was found that, the 16S library mean size and ITS library mean size was maximum in sample T_9R recording 613bp and 594bp respectively and minimum was in T_7R (515bp for 16S and 551 for ITS library). The mean 16S and ITS library size for the sample T_2R were 535bp and 582bp respectively and which was 537bp and 584bp for the sample from control plants ($T_{11}R$). Then these libraries were sequenced using the Illumina 2 x 250 bp sequencing chemistry to generate ~150Mb of data per library.

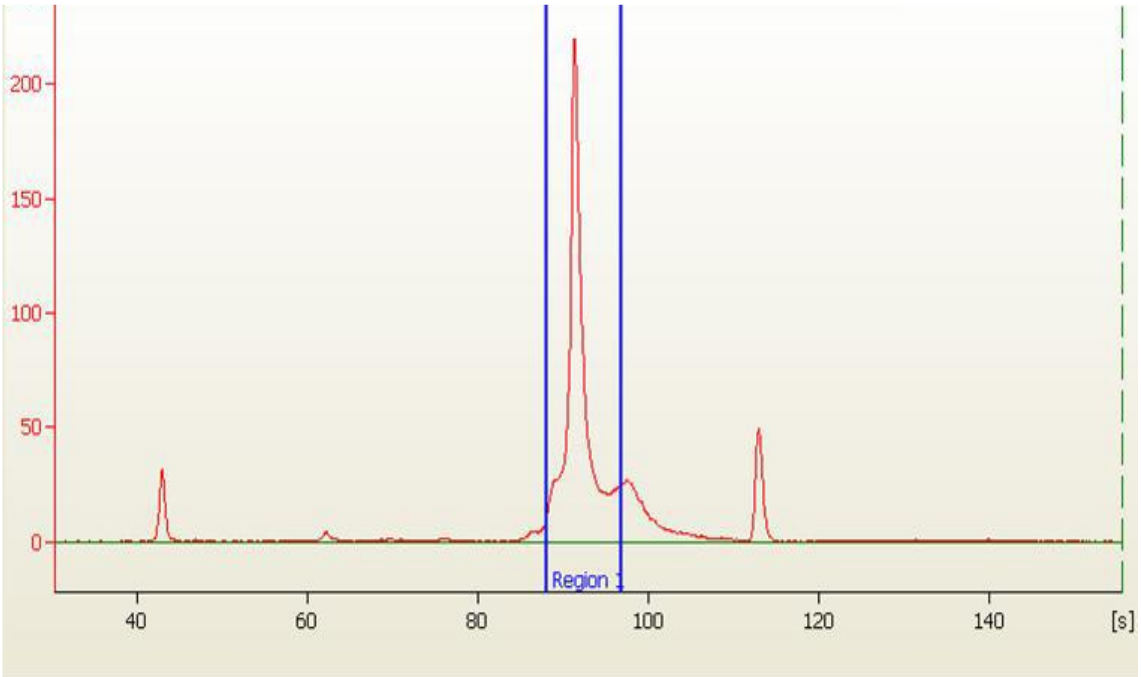
4.9.2.2. Next generation sequencing and sequence assembly

The next generation sequencing was performed using 2x250 PE chemistry on the Illumina platform. Paired end sequence assembly was carried out for data generated using FLASH (Parameter Minimum overlap of 10 bases) assembler. FLASH (Fast Length Adjustment of Short reads) is a very fast and accurate software tool to merge paired-end reads from next-generation sequencing experiments**.** Hence, the consensus reads were obtained after separating them from the paired-end sequences followed by trimming of unwanted sequences (Table 4.49 and 4.50). After the sequence assembly 348771, 310249, 361198 and 458124 reads of bacteria and 542721, 382832, 377122 and 503544 reads of fungi were obtained for the samples T_2R, T_7R, T_9R and $T_{11}R$ respectively.



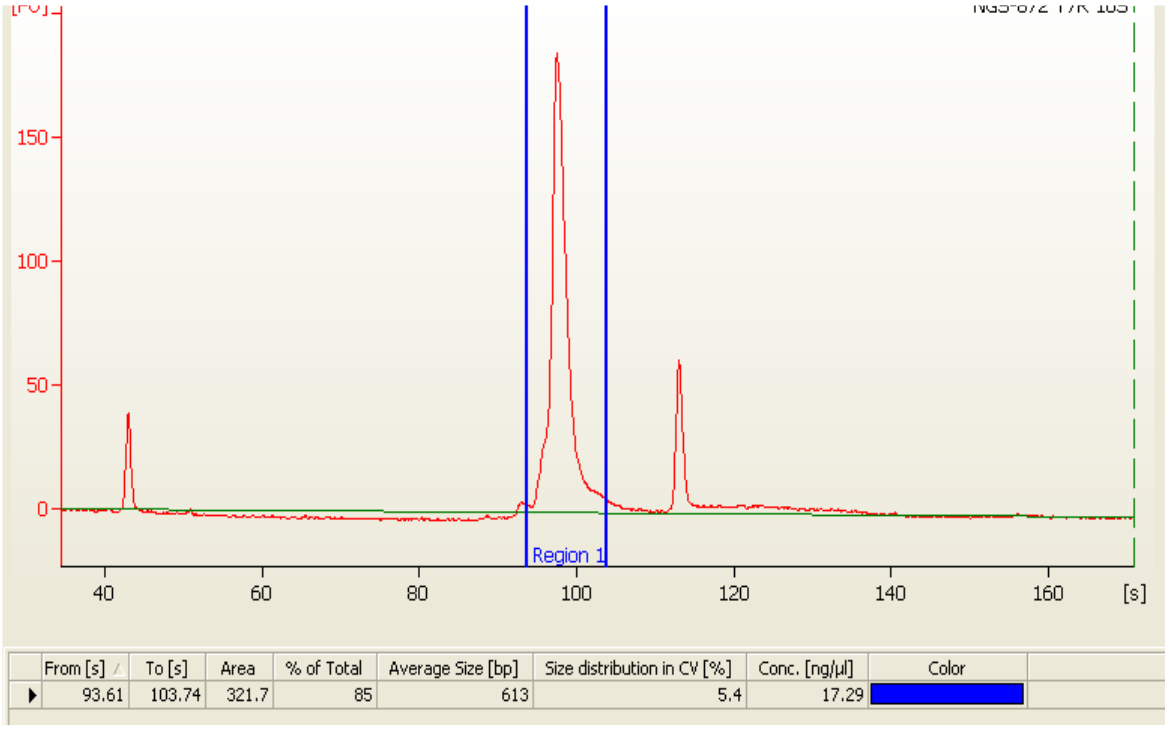
From [s]	To [s]	Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [ng/µl]	Color
88.28	100.92	49.2	73	535	8.9	5.88	

Figure 4.27: 16S Library profile of propineb treated tomato leaves



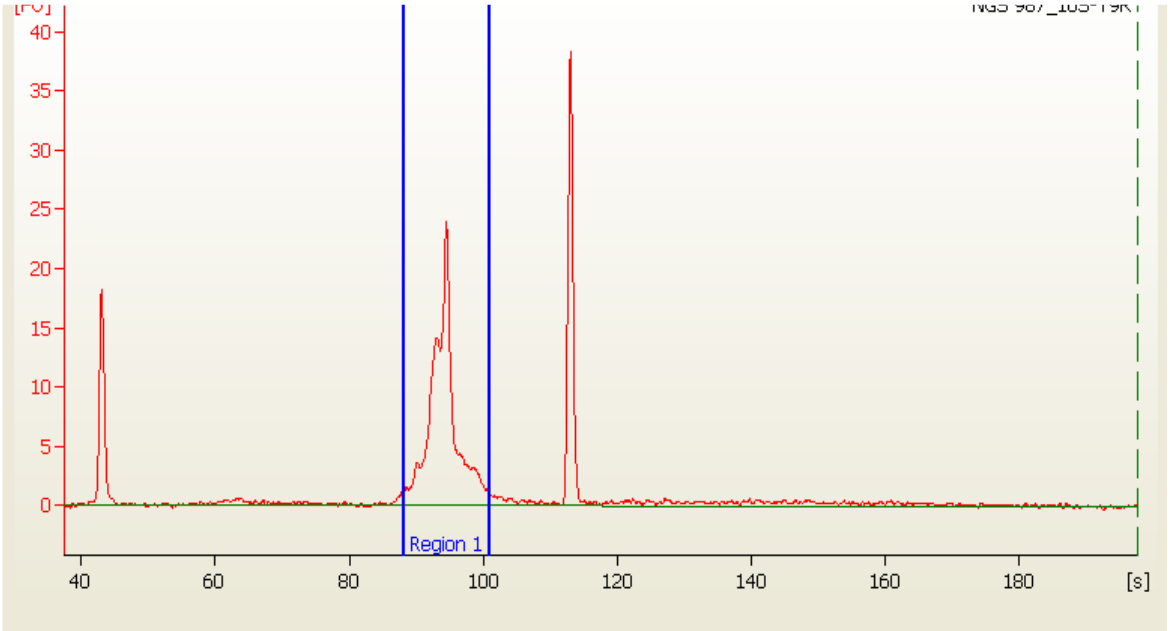
From [s]	To [s]	Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [ng/µl]	Color
88.03	96.89	362.4	74	515	6.5	23.98	

Figure 4.28: 16S Library profile of iprodione + carbendazim treated tomato leaves



From [s]	To [s]	Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [ng/µl]	Color
93.61	103.74	321.7	85	613	5.4	17.29	

Figure 4.29: 16S Library profile of PGPM mix treated tomato leaves



From [s]	To [s]	Area	% of Total	Average Size [bp]	Size distribution in CV [%]	Conc. [ng/µl]	Color
88.28	100.92	49.2	73	535	8.9	5.88	

Figure 4.30: 16S Library profile of untreated tomato leaves

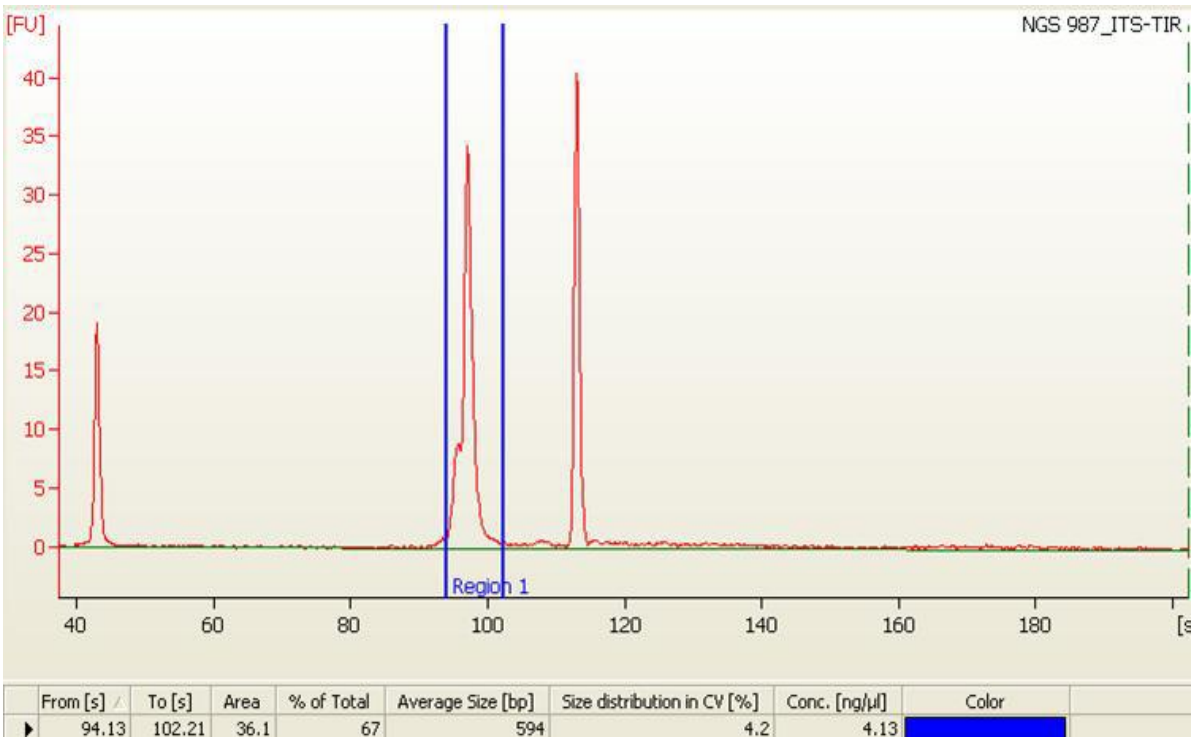


Figure 4.31: ITS Library profile of propineb treated tomato leaves

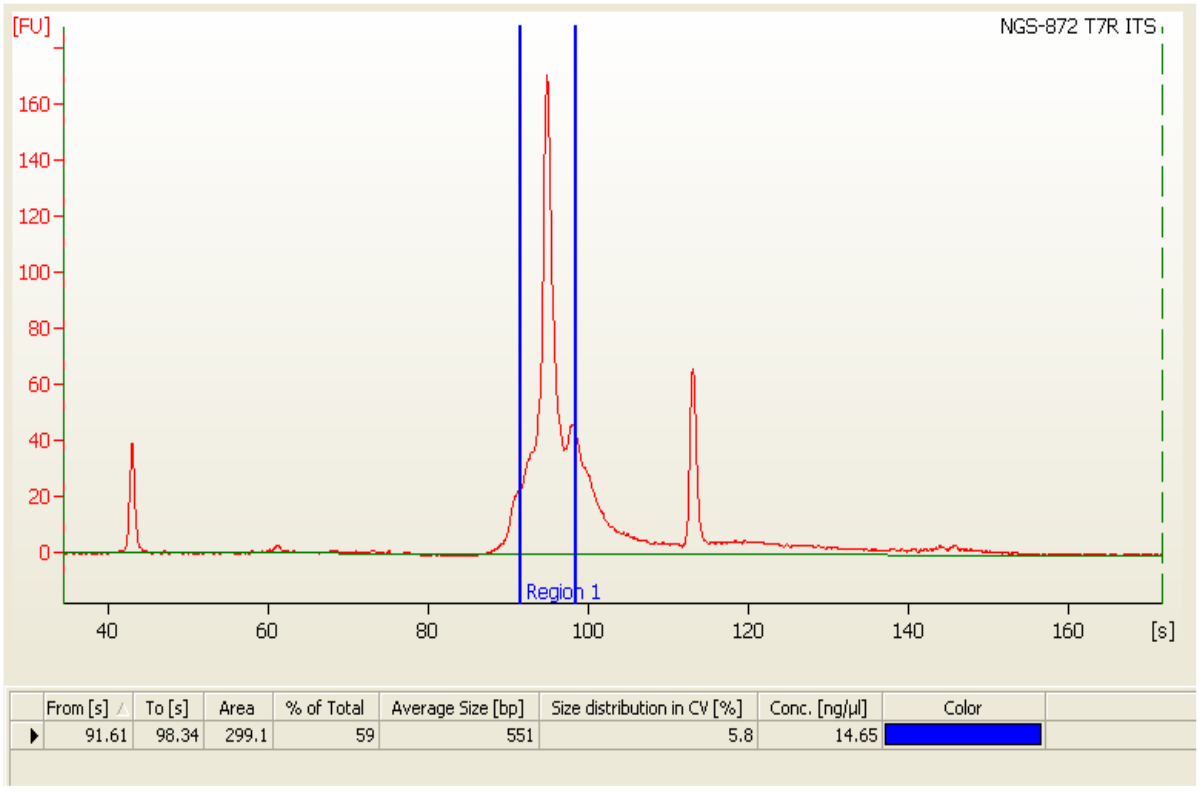


Figure 4.32: ITS Library profile of iprodione + carbendazim treated tomato leaves

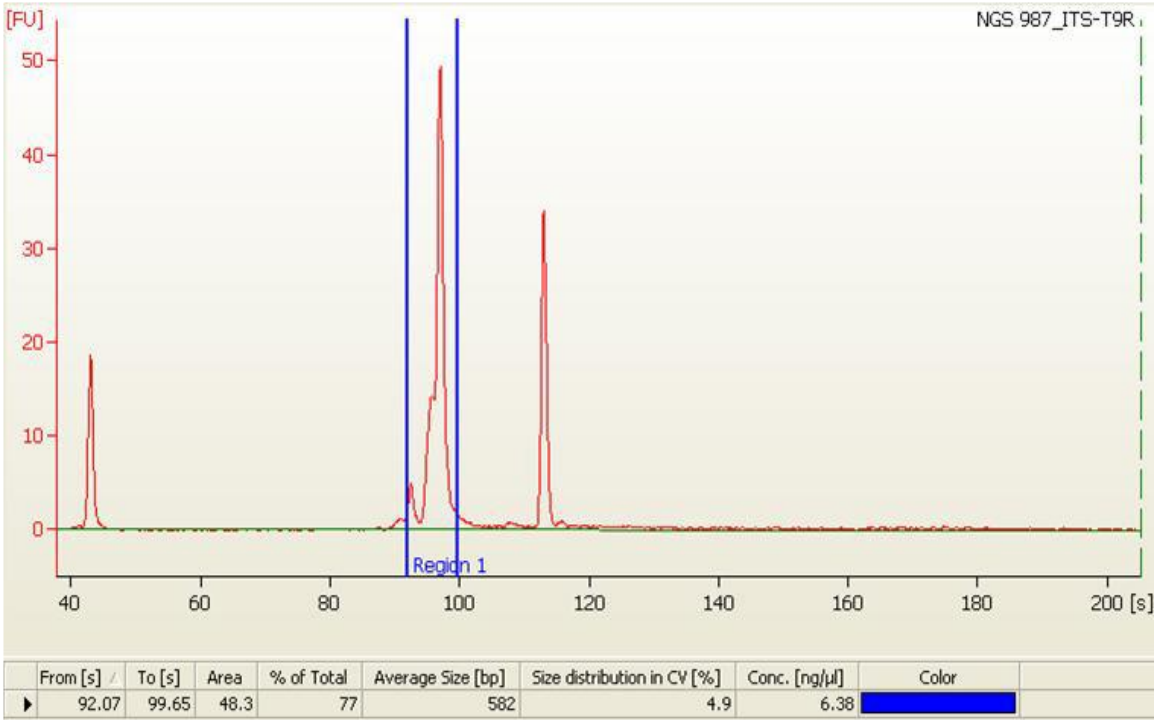


Figure 4.33: ITS Library profile of PGPM mix treated tomato leaves

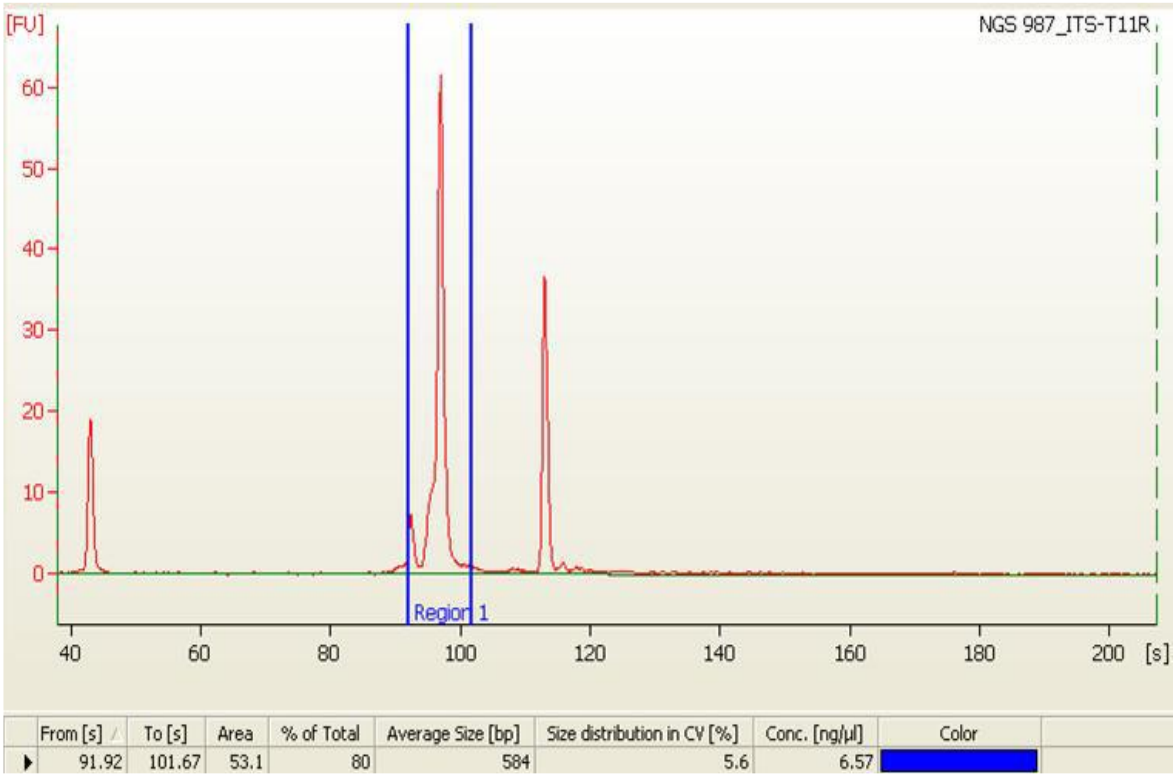


Figure 4.34: ITS Library profile of untreated tomato leaves

Table 4.49: Read statistics of 16S amplicon

Sample No.	PE Reads	Total Reads (R1+R2)	Total Bases (R1+R2)	Data in Mb	Flash Reads
T2R	354041	708082	106212300	106	348771
T7R	315659	631318	946977001	94	310249
T9R	400784	801568	152627000	195	361198
T11R	464519	929038	139355700	139	458124

Table 4.50: Read statistics of ITS amplicon

Sample No.	PE Reads	Total Reads (R1+R2)	Total Bases (R1+R2)	Data in Mb	Flash Reads
T2R	578320	1156640	276347966	276	542721
T7R	400274	800548	180027531	197	382832
T9R	403685	807370	180303762	180	377122
T11R	542608	1085216	234337414	234	503544

4.9.3. Chimera filter

Chimeras are DNA sequences composed of DNA from two or more microbial species. Hence, the chimeric sequences were removed from the consensus reads to obtain the pre-processed reads using the tool UCHIME implemented in the tool USEARCH. The number of chimeric sequences and the pre-processed reads of bacteria and fungi are provided in the Table.4.51 and 4.52. These pre-processed sequence or non-chimeric sequences were used for taxonomic classification.

Table 4.51. Pre-processed reads of 16S amplicon obtained after chimera filter

Sample No.	Consensus Reads	Chimeric Sequences	Pre-processed Reads (Non-chimeric sequences)
T2R	348771	10636	338135
T7R	310249	13790	296459
T9R	361198	6268	354912
T11R	458124	14284	443840

Table 4.52. Pre-processed reads of ITS amplicon obtained after chimera filter

Sample No.	Consensus Reads	Chimeric Sequences	Pre-processed Reads (Non-chimeric sequences)
T2R	542721	28414	514307
T7R	382832	9647	373185
T9R	377122	11263	365859
T11R	503544	77259	426285

4.9.4. Taxonomic Assignment

The Operational Taxonomic Units (OTUs) were obtained by the pooling and clustering of the pre-processed reads based on the sequence similarity using Uclust program. The basis of this sequence clustering was 97% sequence similarity and implemented through UCLUST algorithm. Hence, all the sequences from the samples was clustered into OTUs based on their sequence similarity. OTUs consisted of only one sequence ie. singletons were removed thus retaining OTUs having at least 2 sequences**.**

The total number of bacterial and fungal OTUs obtained after the removal of singletons are provided in Table 4.53 and 4.54. It shows that, the maximum number of bacterial (772) and fungal (960) OTUs were obtained from the sample T9R in which tomato plants were treated with PGPM mix which was followed by T11R (control plants) recorded 709 and 917 bacterial and fungal OTUs respectively. From the sample T2R, 613 bacterial and 833 fungal OTUs were obtained while the sample T7R recorded minimum bacterial (385) and fungal (549) OTUs among the four samples. After the singleton's removal, a representative sequence for each of these OTU's was picked and assigned taxonomic names to these sequence at 90% sequence similarity. This was done by UCLUST algorithm, where query was our representative sequences and subjects was the curated sequences at greengenes database for bacteria and UNITE database for fungi. To confirm the accuracy of QIIME taxonomic assignments, rarefaction analysis was performed and it indicated that the sequencing depth had been saturated for both bacterial and fungal samples (fig 4.33 and 4.34).

Table 4.53. Total OTUs of 16S amplicon obtained after singleton removal

Sample No.	Total Reads	Total OTUs Picked	Total Singleton OTUs	Total OTUs After Singleton Removal
T2R	338135	1367	754	613
T7R	296459	873	488	385
T9R	354912	1775	1003	772
T11R	443840	1715	1006	709

Table 4.54. Total OTUs of ITS amplicon obtained after singleton removal

Treatment No.	Total Reads	Total OTUs Picked	Total Singleton OTUs	Total OTUs After Singleton Removal
T2R	514307	1805	922	833
T7R	373185	925	376	549
T9R	365859	1441	481	960
T11R	426285	1431	514	917

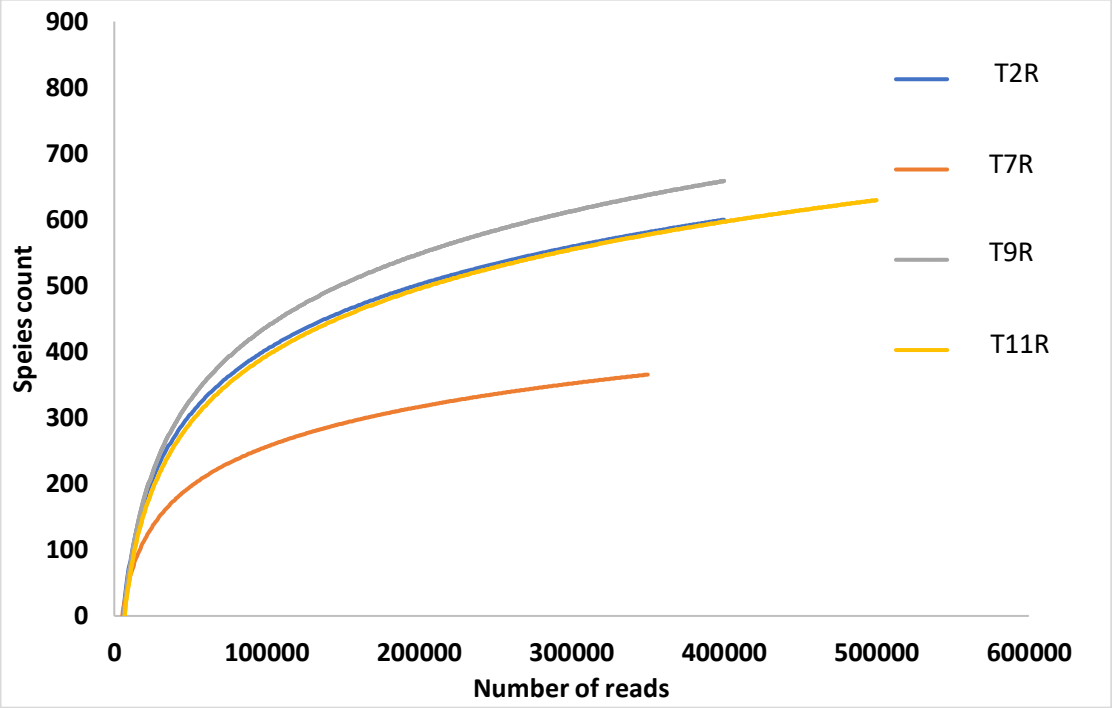


Fig. 4.35 Rarefaction curve for bacterial OTUs clustering at 97% sequence similarity

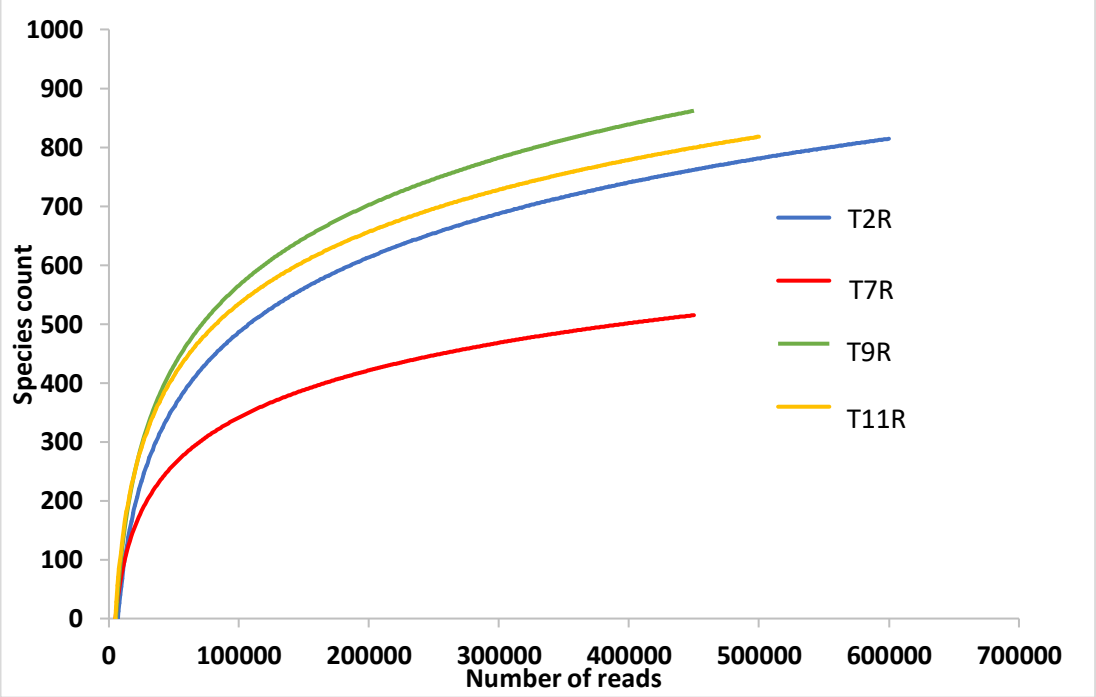


Fig. 4.36 Rarefaction curve for fungal OTUs clustering at 97% sequence similarity

4.9.5 Fungal diversity analysis using One Codex pipeline

The fastq sequences were uploaded into the One Codex pipeline and subjected to taxonomical analysis. The fungal diversity at phylum level was studied. The fungal population present in the four samples were belongs to two phyla; Ascomycota and Basidiomycota (Fig 4.35). Among these two phyla members of the phylum Ascomycota were dominant in all samples ranged from 78 to 90 per cent of the total number of detected sequences. At phylum level, maximum reads were assigned in sample T9R(302765) which was collected from leaves of tomato plant sprayed with PGPM mix followed by T11R (196804), T2R (105409) respectively while the minimum (91363) was observed in sample collected from plants treated with iprodione + carbendazim - 0.2% (T7R) (Table 4.55). So, the above results indicate that fungal population was comparatively less in chemically treated samples than control as well as bioagent treated samples. Moreover, in bioagent treated leaf sample, fungal population was more than control.

4.9.5.1. Fungal diversity in propineb treated tomato leaves (T2R)

The fungal diversity of the 10 most abundant class, order, family and genus in the sample T2R is provided in Table 4.56. Results showed that, Dothideomycetes was most abundant class, occupying 53.38 per cent of the total classified reads of fungal population, followed by Eurotiomycetes (17.51%), Ustilaginomycetes (14.27%) and Agaricomycetes (9.27%). The fifth abundant class was Sordariomycetes occupying 6.39 per cent of the fungal population which was followed by class Malasseziomycetes (1.17%) and Tremellomycetes (1.09%) while the classes like Exobasidiomycete, Leotiomycetes and Orbiliomycetes were found to harbour less than one per cent of fungal population (Fig.4.36).

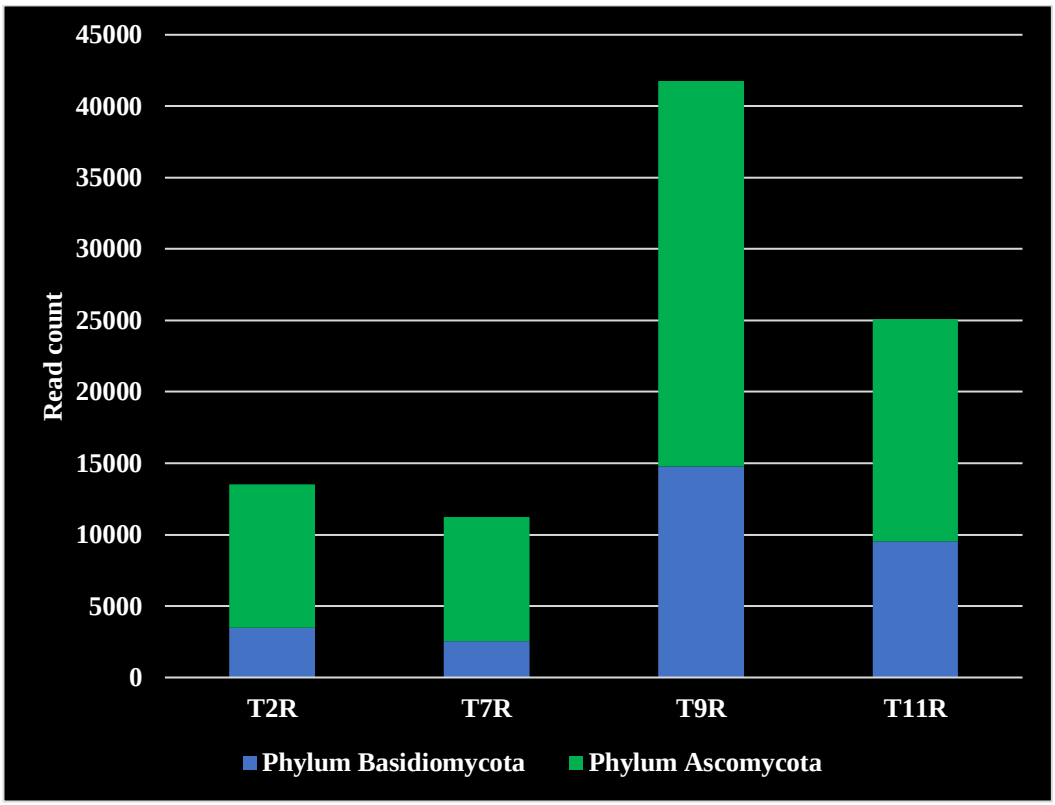


Fig.4.37 Phylum-level fungal diversity obtained using One codex pipeline

Table.4.55. Phylum-level fungal diversity of different samples

Domain	Phylum	No. of reads assigned			
		T2R	T7R	T9R	T11R
Eukaryota	Ascomycota	82372	71509	286700	123026
	Basidiomycota	23037	19854	16065	73778
Total reads		105409	91363	302765	196804

Table 4.56. Genus-level taxonomic assemblage of fungal diversity in propineb treated tomato leaves in rain shelter condition

Class	Read count (% of classified reads)	Order	Read count (% of classified reads)	Family	Read count (% of classified reads)	Genus	Read count (% of classified reads)
Agaricomycetes	19012 (9.27%)	Agaricales	4991 (2.69%)	Agaricaceae	1235 (0.74%)	*Acremonium*	1354 (0.81%)
Dothideomycetes	59351 (50.38%)	Auriculariales	14016 (8.4%)	Aspergillaceae	25633 (15.36%)	*Agaricus*	1061 (0.64%)
Eurotiomycetes	26481 (17.51%)	Botryosphaeriales	186 (0.11%)	Auriculariaceae	14016 (8.4%)	*Alternaria*	45967 (39.53%)
Exobasidiomycetes	139 (0.13%)	Capnodiales	10763 (7.58%)	Cladosporiaceae	2541 (1.52%)	*Aspergillus*	25402 (15.22%)
Leotiomycetes	17 (0.02%)	Eurotiales	25670 (19.38%)	Corynesporascaceae	2621 (1.3%)	*Auricularia*	14016 (8.4%)

Malasseziomycetes	1235 (1.17%)	Hypocreales	2784 (1.67%)	Malasseziaceae	1144 (0.69%)	*Cladosporium*	2421 (1.45%)
Orbiliomycetes	12 (0.01%)	Malasseziales	1144 (0.69%)	Mycosphaerellaceae	8215 (6.79%)	*Corynespora*	3830 (2.3%)
Saccharomycetes	116 (0.34%)	Pleosporales	48588 (41.1%)	Nectriaceae	1143 (0.68%)	*Exserohilum*	3385 (2.03%)
Sordariomycetes	6748 (6.39%)	Saccharomycetales	346 (0.81%)	Pleosporaceae	45967 (39.53%)	*Malassezia*	1144 (0.69%)
Tremellomycetes	1146 (1.09%)	Tremellales	260 (0.16%)	Psathyrellaceae	3052 (1.83%)	*Moesziomyces*	22856 (13.7%)
Ustilaginomycetes	23301 (14.27%)	Ustilaginales	23282 (13.95%)	Ustilaginaceae	23282 (13.95%)	*Pseudocercospora*	8136 (5.79%)
Remaining	240 (1.13%)	Remaining	907 (0.8%)	Remaining	4031 (2.42%)	Remaining	3395 (2.03%)

It was also found that, the fungal population of sample TR2 mainly comes under the order Pleosporales sharing 41.1 per cent of total population. The second dominant order was Eurotiales (19.38%) followed by Ustilaginales (13.95%). The fungal population of class Agaricomycetes mainly divided into two orders; Auriculariales and Agaricales which occupied 8.4 and 2.69 per cent respectively. Order Capnodiales recorded 7.58 per cent and each remaining order contributed only less than one per cent of fungal population (Fig.37). The most abundant order Pleosporales majorly composed of the fungal family Pleosporaceae and Corynesporascaceae which conferred 39.53 and 1.3 per cent respectively. The second predominant family was Aspergillaceae (15.36%) followed by Ustilaginaceae (13.95%), Mycosphaerellaceae (11.79%) and Auriculariaceae (8.4%) respectively (Fig. 4.38).

From the data presented in the Table 4.56, it was observed that, the most abundant genus of the sample TR2 was *Alternaria* which occupied 39.53 per cent of the total fungal population. Next to this, *Aspergillus* was second dominant genus with 15.22 per cent population. The most abundant genus in the family Ustilaginaceae was *Moesziomyces* which shared 13.7 per cent of fungal population in sample TR2 and *Auricularia* (8.4%) was fourth abundant fungal genus in the sample. Similarly, sample also contained genera like *Pseudocercospora* (5.79%) *Corynespora* (2.3%) and *Exserohilum* (2.03%) and many more genera with minute population (Fig. 4.39).

4.9.5.2. Fungal diversity in iprodione + carbendazim treated tomato leaves(T7R)

The taxonomic assemblage of the fungal diversity from class level to genus level for the sample T7R is furnished in Table 4.57. The most dominant class in the sample was found to be Dothideomycetes (48.44%), followed by Eurotiomycetes (16.38%) and Sordariomycetes (12.95%) respectively and these classes comes under phylum Ascomycota. Next dominant classes were Agaricomycetes (10.26%)

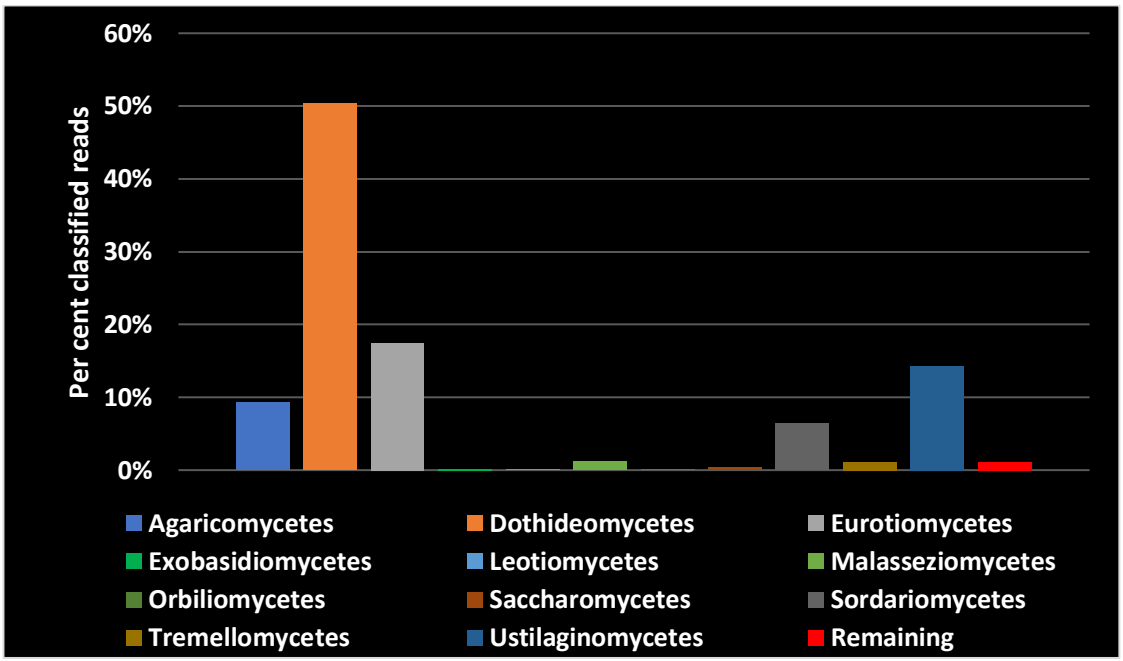


Fig 4.38. Class-level fungal diversity of propineb treated tomato leaves obtained using One codex pipeline

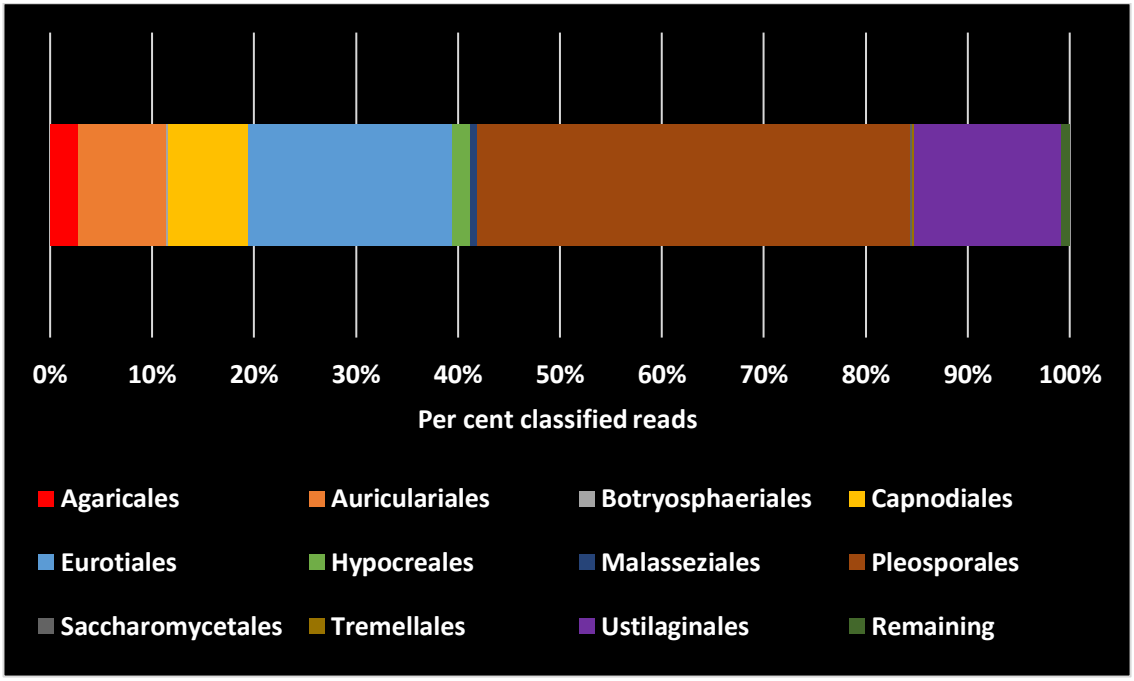


Fig 4.39. Order-level fungal diversity of treated tomato leaves obtained using One codex pipeline

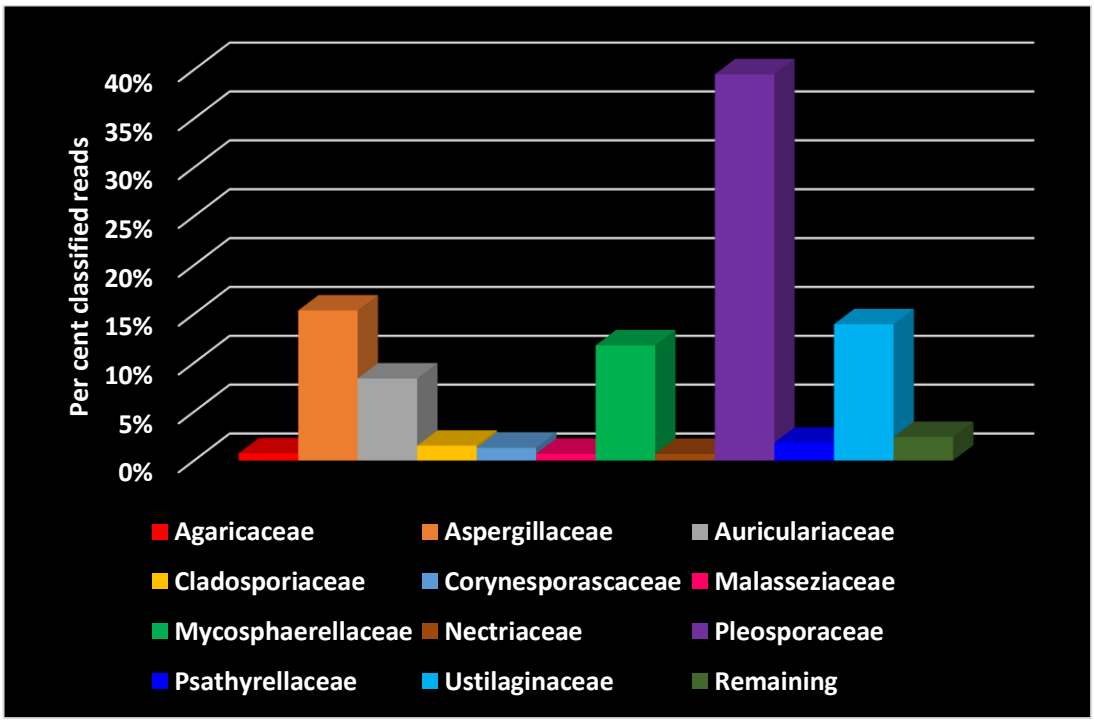


Fig 4.40. Family-level fungal diversity of propineb treated tomato leaves obtained using One codex pipeline

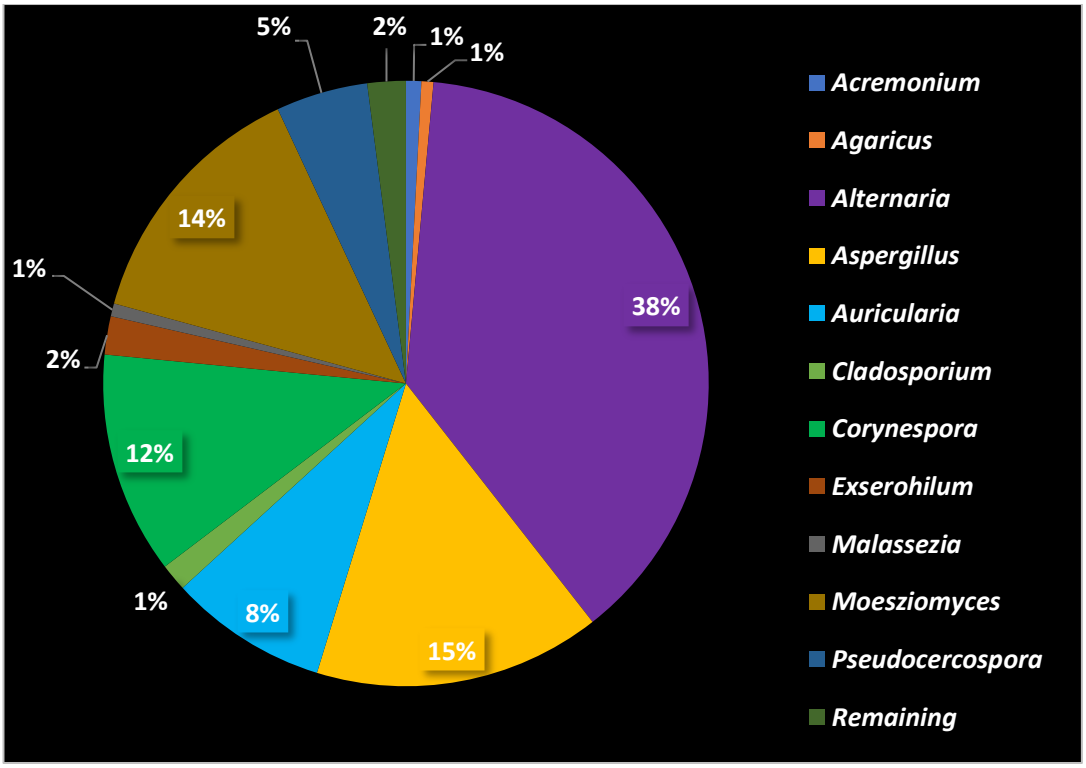


Fig 4.41. Genus-level fungal diversity of propineb treated tomato leaves obtained using One codex pipeline

and Ustilaginomycetes (9.71%) which comes under phylum Basidiomycetes. The sample also contained fungal population from the classes like Exobasidiomycetes, Leotiomycetes, Malasseziomycetes, Microbotryomycetes, Saccharomycetes and Tremellomycetes in minute proportions (Fig. 4.40).

The data also showed that, the organism present in the most abundant class Dothideomycetes was mainly divided into two orders *viz.* Pleosporales and Capnodiales; in which Pleosporales was most dominant order with 41.20 per cent fungal population and Capnodiales contributed 11.22 per cent. The second predominant order was Eurotiales (15.17%). The order Ustilaginales and Hypocreales shared 9.69 and 9.02 per cent of total fungal flora. Similarly, the fungi from the class Agaricomycetes was mainly categorized into three order; Agaricales, Auriculariales and Polyporales with 2.49, 6.89 and 0.76 per cent respectively (Fig. 4.41). The most abundant order Pleosporales majorly composed of the fungus from the family Pleosporaceae, Corynesporascaceae and Massarinaceae which occupied 30.27, 5.37 and 2.11 per cent respectively in the sample. The second predominant family was Ustilaginaceae (9.69%) followed by Cladosporiaceae (7.61%), Auriculariaceae (6.86%), Nectriaceae (5.17%), Mycosphaerellaceae (4.08%) and Didymellaceae (3.69%) respectively and remaining each fungal family contribute only less than one per cent of the total fungal flora. (Fig.4.42).

The most abundant fungal genus identified from the sample was *Alternaria* (29.65%) which is classified under the family Pleosporaceae and this was followed by *Moesziomyces* (7.46%) from Ustilaginaceae and *Cladosporium* (7.43%) from Cladosporiaceae family. The tomato leaf samples from plant treated with iprodione + carbendazim also showed fungal genera *viz. Cladosporium* (7.43%), *Auricularia* (6.86%), *Corynespora* (11.37%), *Fusarium* (4.65%), *Pseudocercospora* (2.63%), *Anthracocystis* (2.1%) and *Agaricus* (1.32%). In addition to these, the sample contained many more genera with less than one per cent proportions (Fig 4.43).

Table 4.57. Genus-level taxonomic assemblage of fungal diversity in iprodione + carbendazim treated tomato leaves in rain shelter condition

Class	Read count (% of classified reads)	Order	Read count (% of classified reads)	Family	Read count (% of classified reads)	Genus	Read count (% of classified reads)
Agaricomycetes	9378 (10.26%)	Agaricales	2279 (2.49%)	Agaricaceae	1441 (1.58%)	*Alternaria*	27100 (29.65%)
Dothideomycetes	43964 (48.44%)	Auriculariales	6298 (6.89%)	Auriculariaceae	6269 (6.86%)	*Anthracocystis*	1917 (2.1%)
Eurotiomycetes	14869 (16.38%)	Capnodiales	10256 (11.22%)	Cladosporiaceae	6958 (7.61%)	*Agaricus*	1206 (1.32%)
Exobasidiomycetes	683 (0.75%)	Eurotiales	13869 (15.17%)	Corynesporascaceae	4765 (5.37%)	*Auricularia*	6269 (6.86%)
Leotiomycetes	71 (0.08%)	Hypocreales	8248 (9.02%)	Didymellaceae	3369 (3.69%)	*Cladosporium*	6945 (7.43%)

Malasseziomycetes	79 (0.09%)	Pleosporales	37664 (41.20%)	Massarinaceae	1925 (2.11%)	*Corynespora*	4765 (5.37)
Saccharomycetes	151 (0.17%)	Polyporales	693 (0.76%)	Mycosphaerellaceae	3733 (4.08%)	*Fusarium*	4249 (4.65%)
Sordariomycetes	11841 (12.95%)	Trichosphaeriales	1159 (1.27%)	Nectriaceae	4722 (5.17%)	*Helminthosporium*	1898 (2.08%)
Tremellomycetes	686 (0.75%)	Ustilaginales	8861 (9.69%)	Pleosporaceae	27665 (30.27%)	*Moesziomyces*	6819 (7.46%)
Ustilaginomycetes	8879 (9.71%)	Xylariales	833 (0.91%)	Ustilaginaceae	8861 (9.69%)	*Pseudocercospora*	2408 (2.63%)
Remaining	132 (0.14%)	Remaining	4546 (4.97%)	Remaining	14551 (15.92%)	Remaining	22480 (24.59%)

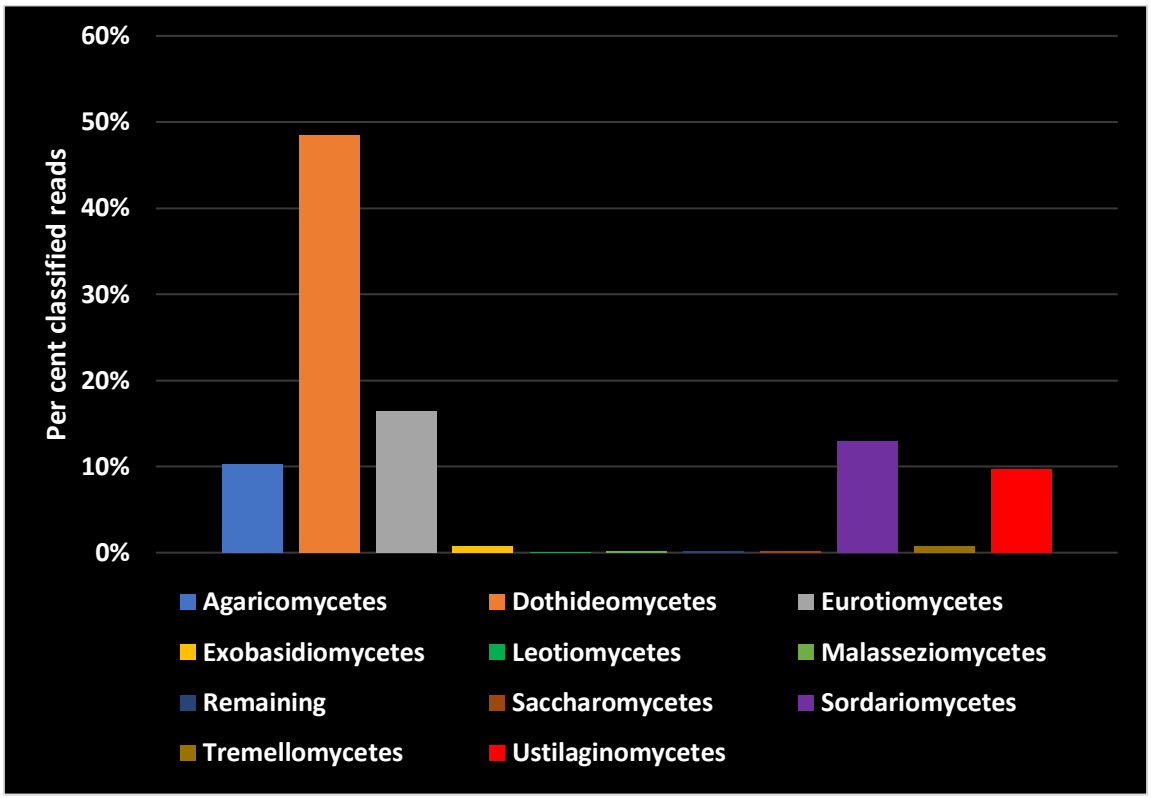


Fig 4.42. Class-level fungal diversity of iprodione + carbendazim treated tomato leaves obtained using One codex pipeline

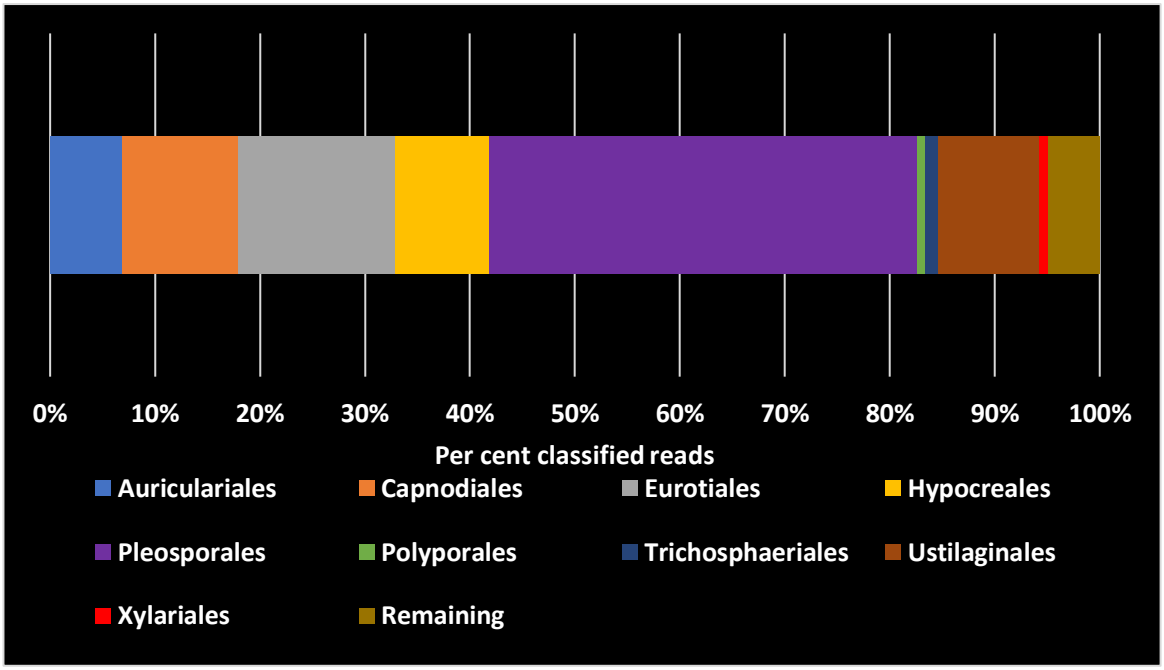


Fig 4.43. Order-level fungal diversity of iprodione + carbendazim treated tomato leaves obtained using One codex pipeline

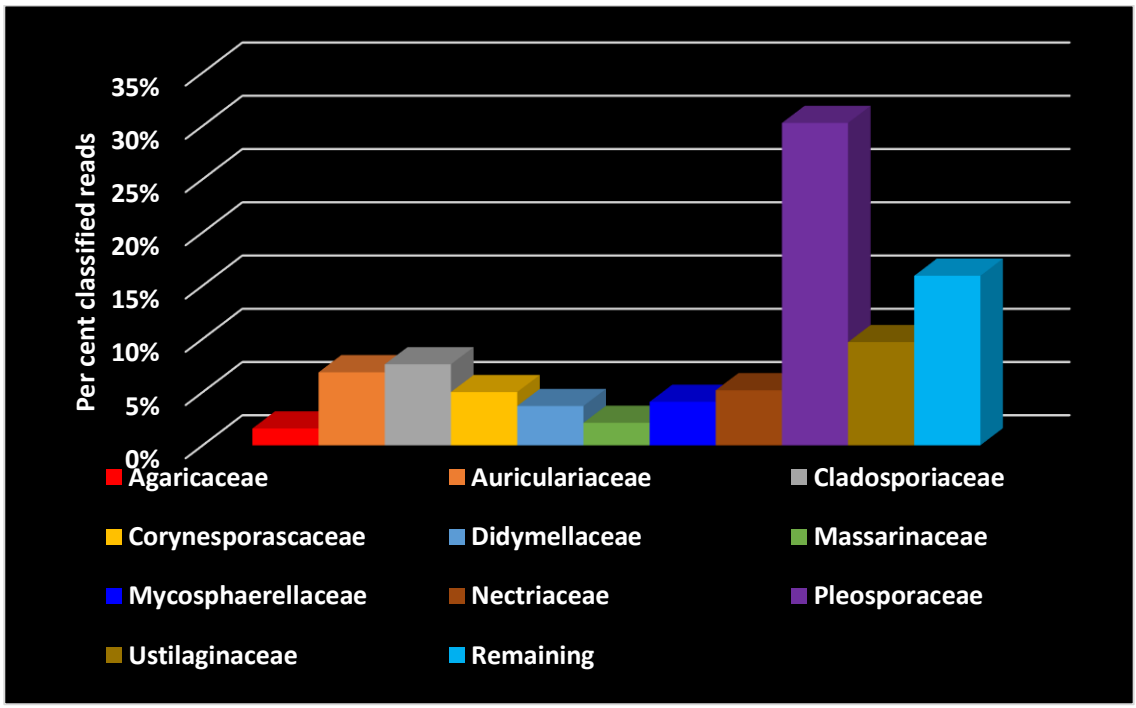


Fig 4.44. Family-level fungal diversity of iprodione + carbendazim treated tomato leaves obtained using One codex pipeline

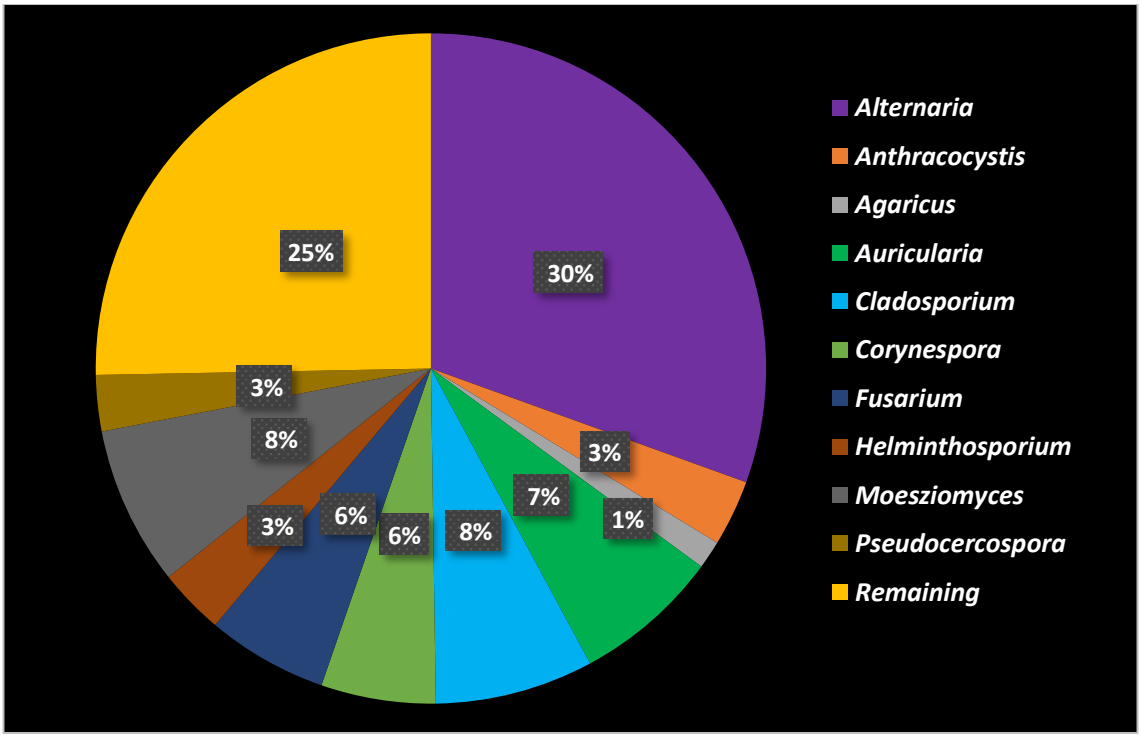


Fig 4.45. Genus-level fungal diversity of iprodione + carbendazim treated tomato leaves obtained using One codex pipeline

4.9.5.3. Fungal diversity in PGPM mix treated tomato leaves (T9R)

The fungal diversity of tomato leaves collected from plant sprayed with biocontrol consortium; PGPM mix (T9R) is enlisted in the Table 4.58. It was observed that, 67.74% per cent fungal population was from the class Dothideomycetes followed by class Sordariomycetes (20.72%), Ustilaginomycetes (2.56%), Eurotiomycetes (2.53%) and Agaricomycetes (1.92%) respectively. Remaining fungal flora was from the classes like Cystobasidiomycetes, Exobasidiomycetes, Leotiomycetes, Malasseziomycetes, Saccharomycetes and Tremellomycetes each with less than one per population (Fig. 4.44).

The fungi from the class Dothideomycetes was categorized into two order *viz.* Pleosporales and Capnodiales in which Pleosporales was dominant order recorded 60.28 per cent of total fungal population. The fungal flora placed under the order Capnodiales was 6.24 per cent. Hypocreales (17.18%) was the second most abundant fungal order in the sample. This was followed by Xylariales, Ustilaginales and Eurotiales which occupied more than two per cent of fungal population. Other orders were Agaricales, Auriculariales, Exobasidiales, Malasseziales each confer below one per cent (Fig.4.45). Fungal population at family level were also studied (Fig.4.46). Among the top ten families studied, Pleosporaceae (50.13%) was dominant followed by Hypocreaceae (22.99%), Mycosphaerellaceae (3.69%), Ustilaginaceae (3.53%), Aspergillaceae (3.5%) and Corynesporascaceae (2.15%) respectively.

It was observed that, the most abundant genus of the sample TR7 was *Alternaria* which occupied 47 per cent of the total fungal population. *Trichoderma* was second dominant genus with 23.92 per cent population and it was followed by genus *Helminthosporium* (4.38%). The most abundant genus in the family Ustilaginaceae was *Meira* which shared 4.14 per cent of fungal population. Similarly, sample also contained genera like, *Aspergillus* (2.46%), *Pseudocercospora* (2.36%), *Moesziomyces* (2.18%), *Corynespora* (1.99%), *Auricularia* (1.35%) and *Cladosporium* (2.55%) and many more genera with minute proportions (Fig.4.47).

Table 4.58. Genus-level taxonomic assemblage of fungal diversity in PGPM mix treated tomato leaves in rain shelter condition

Class	Read count (% of classified reads)	Order	Read count (% of classified reads)	Family	Read count (% of classified reads)	Genus	Read count (% of classified reads)
Agaricomycetes	5832 (1.92%)	Agaricales	1430 (0.47%)	Aspergillaceae	7569 (3.5%)	*Alternaria*	81945 (47.0%)
Cystobasidiomycetes	18 (0.01%)	Auriculariales	4086 (1.35%)	Auriculariaceae	4086 (1.35%)	*Aspergillus*	7458 (2.46%)
Dothideomycetes	104027 (67.74%)	Capnodiales	13963 (6.24%)	Brachybasidiaceae	1091 (0.36%)	*Auricularia*	4086 (1.35%)
Eurotiomycetes	7664 (2.53%)	Eurotiales	7569 (2.5%)	Cladosporiaceae	4716 (1.55%)	*Cladosporium*	4716 (1.55%)
Exobasidiomycetes	1247 (0.41%)	Exobasidiales	1171 (0.39%)	Corynesporascaceae	5621 (2.15%)	*Corynespora*	5621 (1.99%)

Leotiomycetes	16 (0.01%)	Hypocreales	61894 (17.18%)	Hypocreaceae	51324 (22.99%)	*Helminthosporium*	10211 (4.38%)
Malasseziomycetes	475 (0.16%	Malasseziales	475 (0.16%)	Massarinaceae	953 (0.31%)	*Meira*	10091 (4.14%)
Saccharomycetes	355 (0.1%)	Pleosporales	90012 (60.28%)	Mycosphaerellaceae	7247 (3.69%)	*Moesziomyces*	6616 (2.18%)
Sordariomycetes	82547 (20.72%)	Ustilaginales	7674 (2.53%)	Pleosporaceae	83480 (50.13%)	*Pseudocercospora*	7125 (2.36%)
Ustilaginomycetes	7764 (2.56%)	Xylariales	894 (2.9%)	Ustilaginaceae	7674 (3.53%)	*Trichoderma*	51102 (23.92%)
Remaining	273 (0.19%)	Remaining	9522 (4.83%)	Remaining	12863 (9.59%)	*Remaining*	20384 (10.08%)

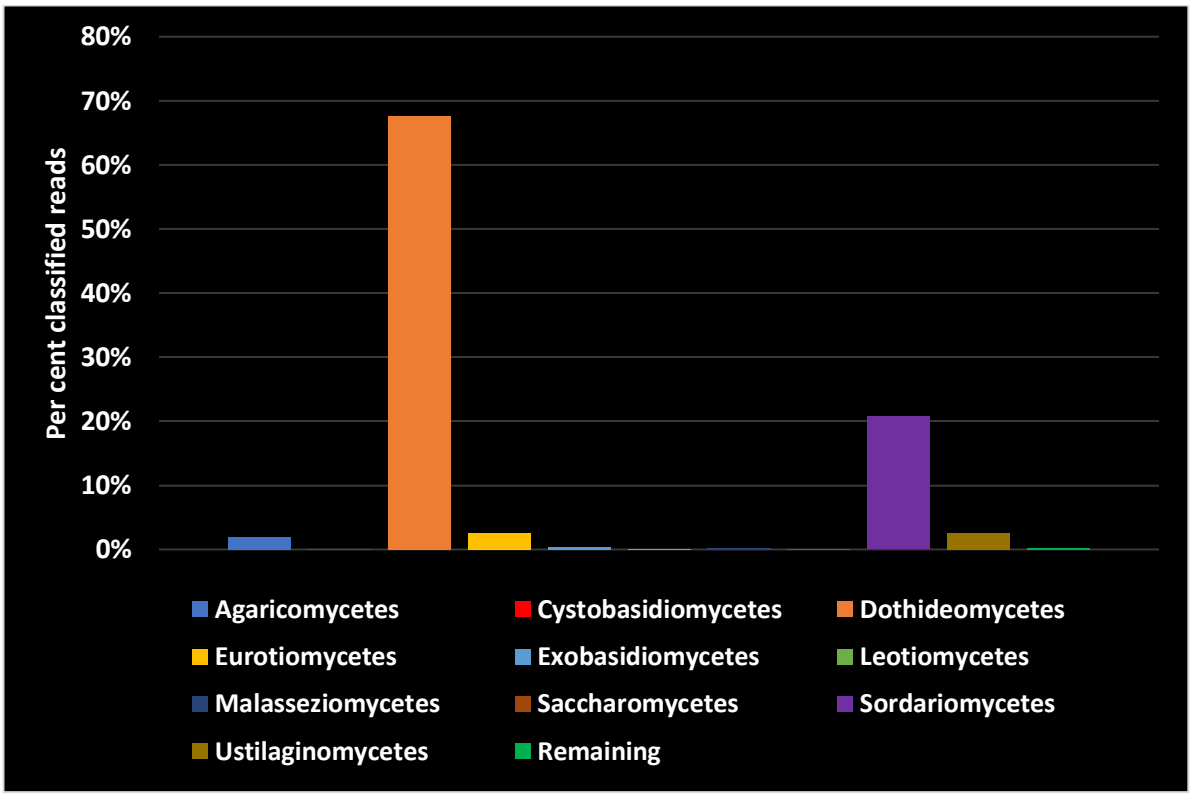


Fig 4.46. Class-level fungal diversity of PGPM mix treated tomato leaves obtained using One codex pipeline

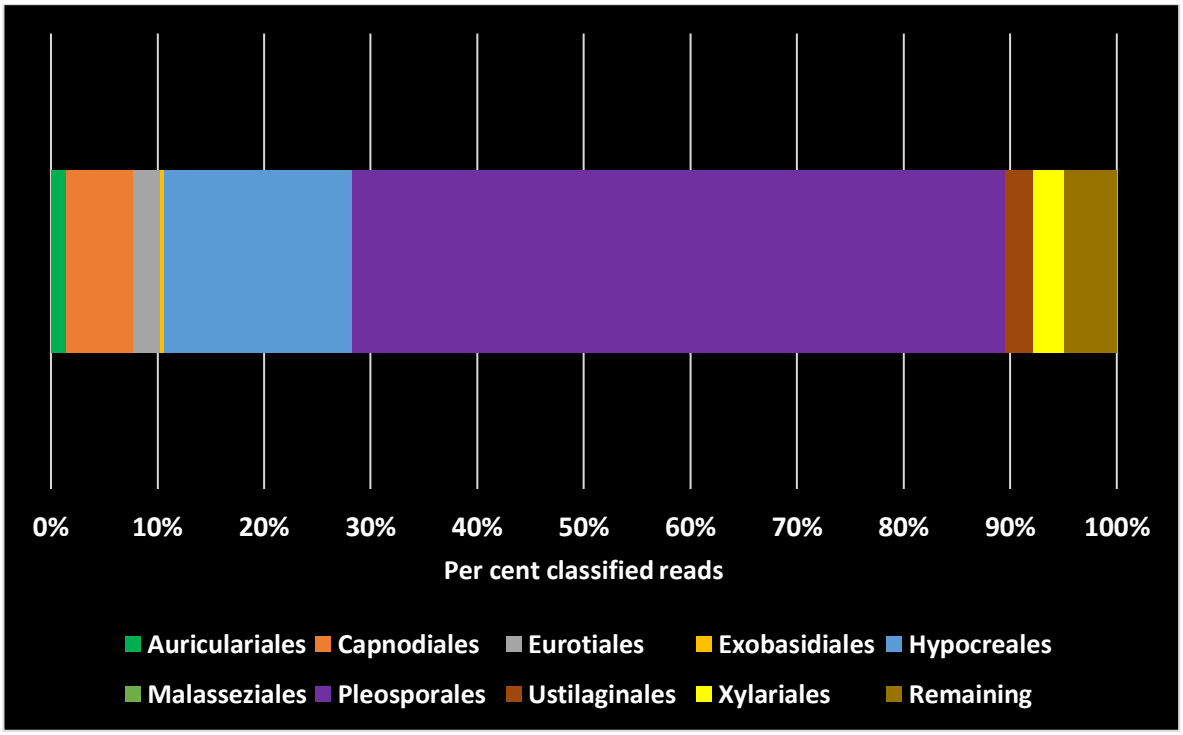


Fig 4.47. Order-level fungal diversity of PGPM mix treated tomato leaves obtained using One codex pipeline

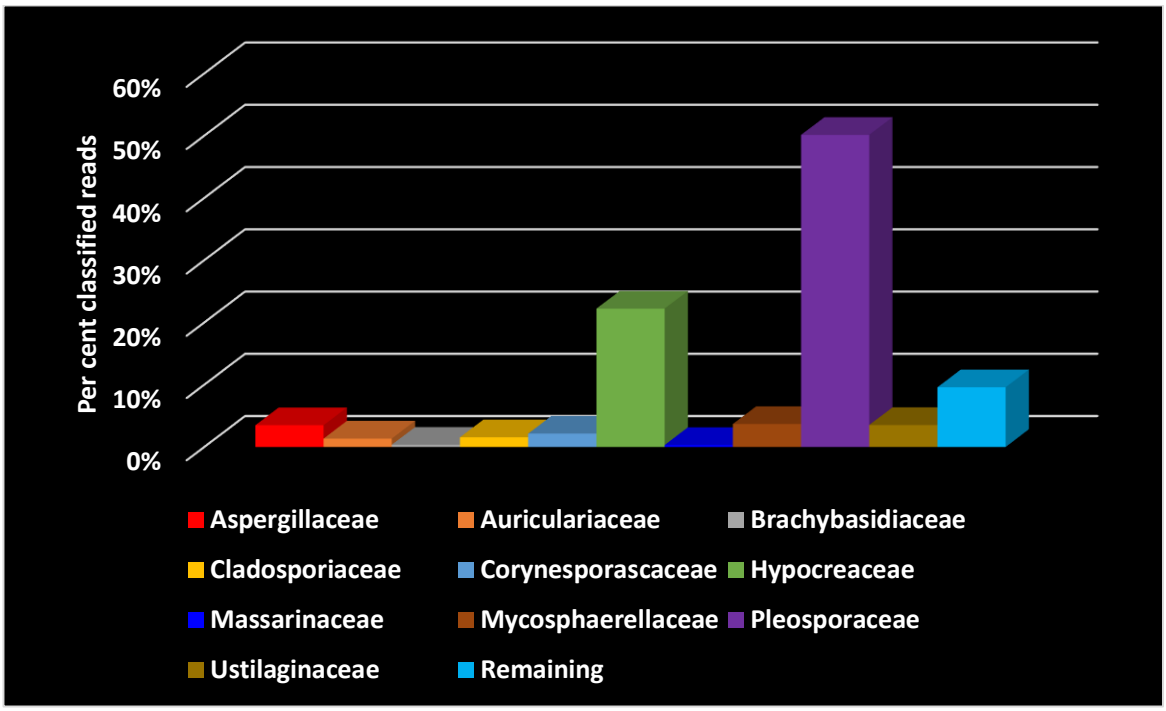


Fig 4.48. Family-level fungal diversity of PGPM mix treated tomato leaves obtained using One codex pipeline

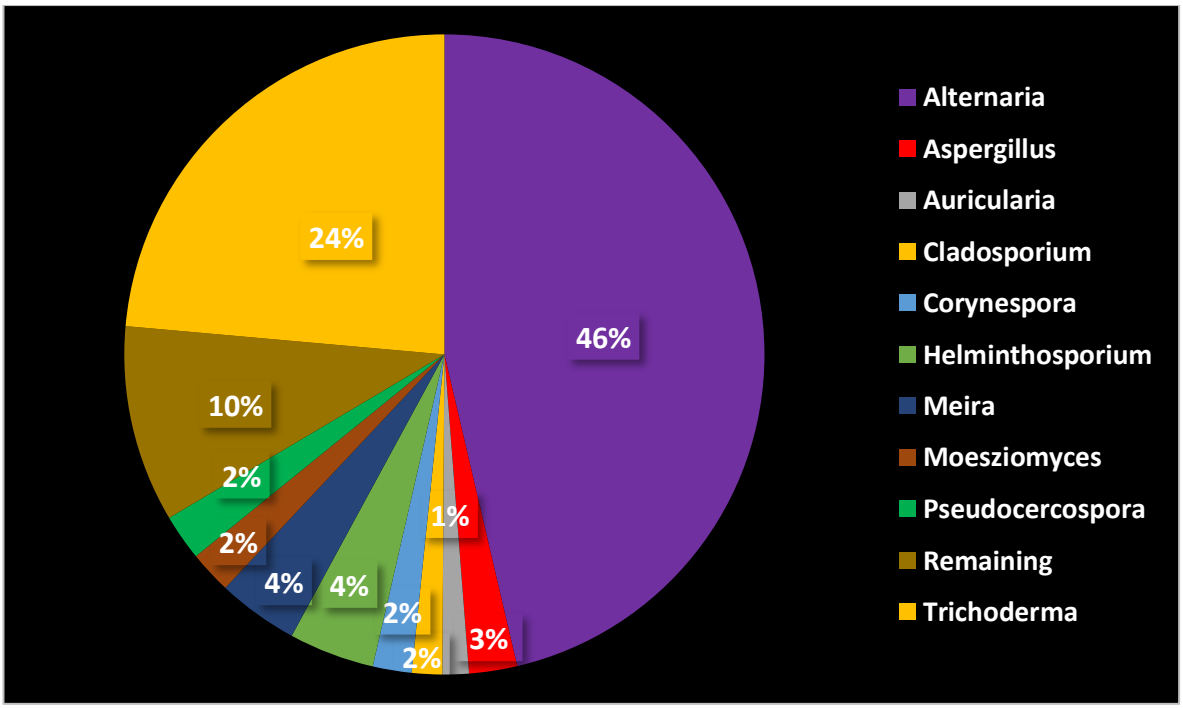


Fig 4.49. Genus-level fungal diversity of PGPM mix treated tomato leaves obtained using One codex pipeline

4.9.5.4. Fungal diversity in untreated tomato leaves (T11R)

The taxonomic assemblage of the fungal diversity in the sample T11R was expressed from class level to genus level and results are furnished in Table 4.59. It was observed that, majority of the fungus present in the sample were placed under class Dothideomycetes (66.16%) which comes under phylum Ascomycota while the second and third abundant class were from phylum Basidiomycota *ie*. Ustilaginomycetes (13.96%) and Agaricomycetes (11.15%) respectively. The class Eurotiomycetes occupied 9.48 per cent whereas 1.92 per cent population was from class Sordariomycetes. Classes like Cystobasidiomycetes, Exobasidiomycetes, Leotiomycetes, Malasseziomycetes, Saccharomycetes and Tremellomycetes represented less than one per cent of fungal flora (Fig.4.48).

The data also showed that, the organism present in the most abundant class Dothideomycetes was mainly divided into two orders *viz.* Pleosporales and Capnodiales; in which Pleosporales was most dominant order with 60.99 per cent fungal population and Capnodiales contributed 5.16 per cent. The second predominant order was Ustilaginales (13.95%). The order Eurotiales and Auriculariales shared 9.48 and 8.4 per cent of total fungal flora (Fig 4.49.). The most abundant order Pleosporales mainly composed of the fungus from the family Pleosporaceae and Corynesporascaceae which occupied 58.67 and 2.32 per cent respectively in the sample. The second predominant family was Ustilaginaceae (13.95%) followed by Auriculariaceae (8.4%), Aspergillaceae (7.49%), Mycosphaerellaceae (3.54%), Corynesporascaceae (2.32%) and Psathyrellaceae (1.83%) respectively and remaining each fungal family contribute only less than one per cent of the total fungal flora. (Fig.4.50).

The most abundant fungal genus identified from the tomato control plants' leaf sample was *Alternaria* (58.47%)) which is classified under the family Pleosporaceae and this was followed by *Moesziomyces* (13.7%) from Ustilaginaceae family and *Auricularia* (8.6%) from Auriculariaceae family. The sample also showed the presence of fungal genera *viz. Aspergillus* (7.49%), *Pseudocercospora* (3.41%), *Corynespora* (2.32%), *Cladosporium* (1.61%), *Coprinopsis* (1.45%), *Acremonium* (0.81%) and *Malassezia*

Table 4.59. Genus-level taxonomic assemblage of fungal diversity in untreated tomato leaves in rain shelter condition

Class	Read count (% of classified reads)	Order	Read count (% of classified reads)	Family	Read count (% of classified reads)	Genus	Read count (% of classified reads)
Agaricomycetes	18602 (11.15%)	Agaricales	4491 (2.69%)	Agaricaceae	1235 (0.74%)	*Acremonium*	1354 (0.81%)
Cystobasidiomycetes	28 (0.02%)	Auriculariales	14016 (8.4%)	Aspergillaceae	12456 (7.49%)	*Alternaria*	100120 (58.47%)
Dothideomycetes	109895 (66.16%)	Capnodiales	8583 (5.16)	Auriculariaceae	14016 (8.4%)	*Aspergillus*	12456 (7.49%)
Eurotiomycetes	15748 (9.48%)	Eurotiales	15748 (9.48%)	Cladosporiaceae	2689 (1.61%)	*Auricularia*	14016 (8.4%)
Exobasidiomycetes	194 (0.12%)	Hypocreales	2784 (1.67%)	Corynesporascaceae	3858 (2.32%)	*Cladosporium*	2689 (1.61%)
Malasseziomycetes	1144	Malasseziales	1144	Malasseziaceae	1144	*Coprinopsis*	2421

	(0.69%)		(0.69%)		(0.69%)		(1.45%)
Saccharomycetes	346 (0.21%)	Pleosporales	108312 (60.99%)	Mycosphaerellaceae	5894 (3.54%)	*Corynespora*	3858 (2.32%)
Sordariomycetes	3201 (1.92%)	Saccharomycetales	346 (0.21%)	Pleosporaceae	107454 (58.67%)	*Malassezia*	1144 (0.69%)
Tremellomycetes	260 (0.16%)	Tremellales	260 (0.16%)	Psathyrellaceae	3052 (1.83%)	*Moesziomyces*	22856 (10.01%)
Ustilaginomycetes	23296 (13.96%)	Ustilaginales	23282 (10.01%)	Ustilaginaceae	23282 (10.01%)	*Pseudocercospora*	5664 (3.41%)
Remaining	237 (0.13%)	Remaining	1167 (0.7%)	Remaining	4031 (2.42%)	Remaining	2643 (1.66%)

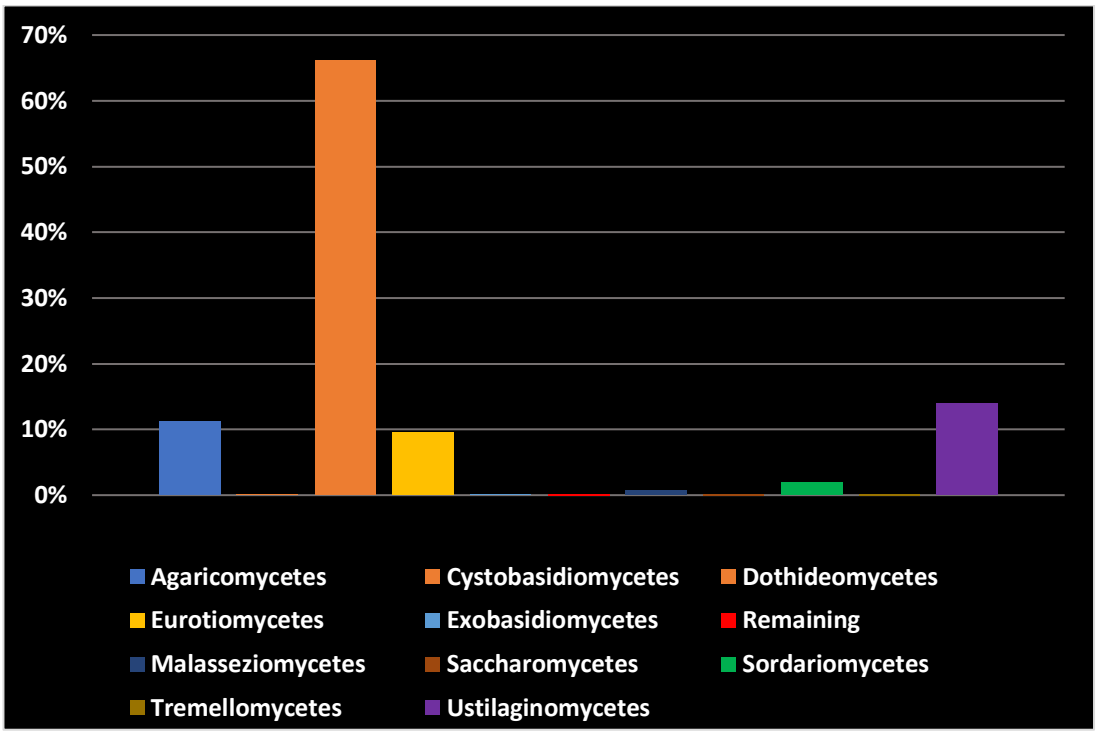


Fig 4.50. Class-level fungal diversity of untreated tomato leaves obtained using One codex pipeline

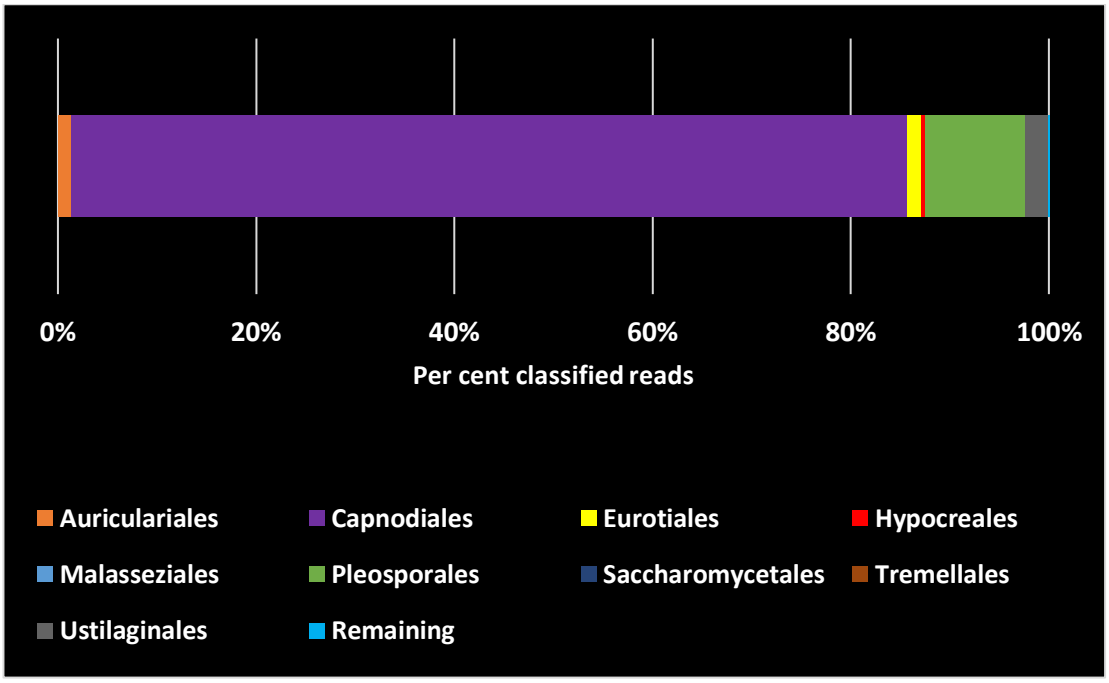


Fig 4.51. Order-level fungal diversity of untreated tomato leaves obtained using One codex pipeline

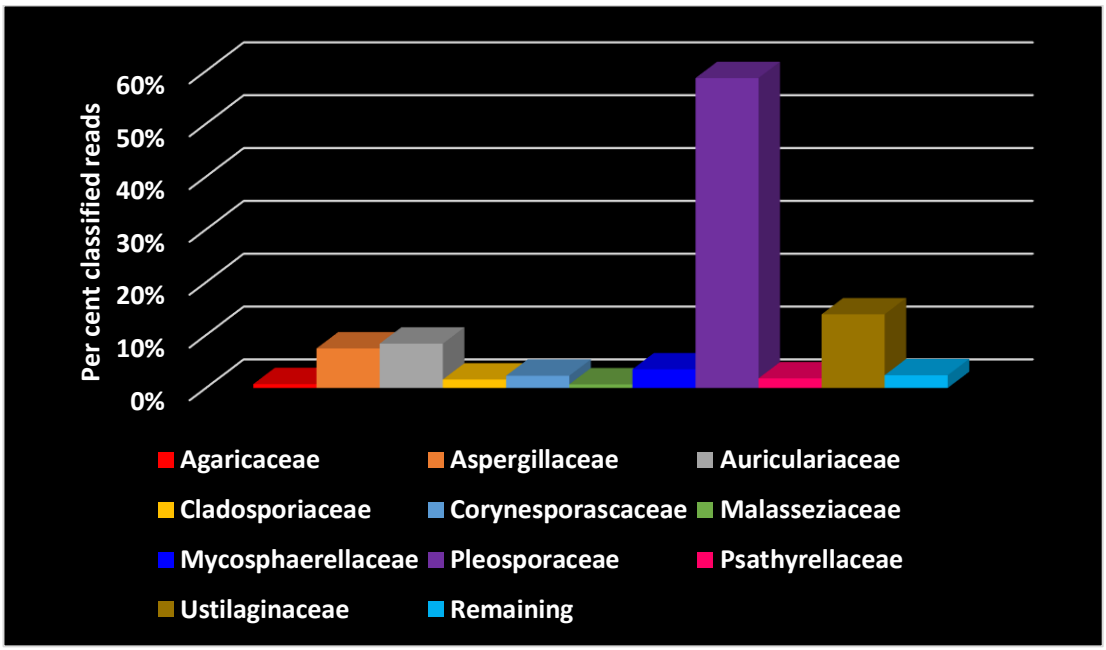


Fig 4.52. Family-level fungal diversity of untreated tomato leaves obtained using One codex pipeline

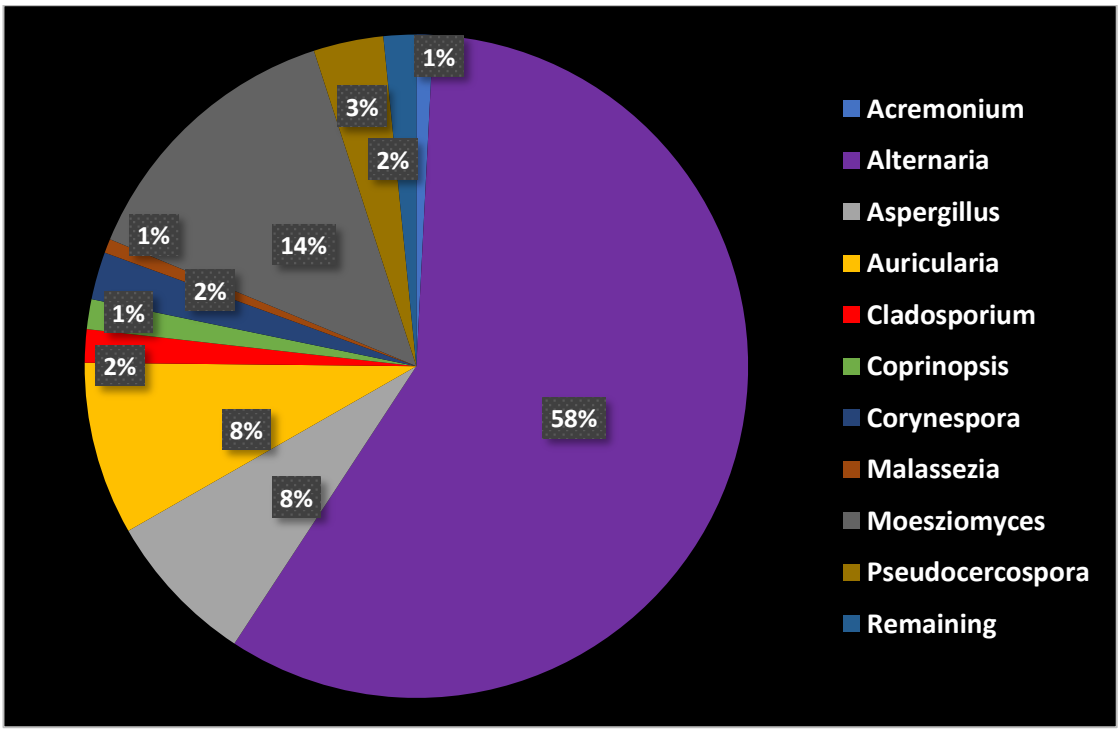


Fig 4.53. Genus-level fungal diversity of untreated tomato leaves obtained using One codex pipeline

(0.69%). In addition to these, the sample also contained hundred more genera each with less than one per cent proportions (Fig.4.51).

4.9.5.4 Comparison of fungal diversity between the samples

The fungal diversity between the four samples were compared up to the first ten predominant one from class, order, family and genus level.

In all the samples, the maximum read count was obtained from class Dothideomycetes which ranged from 43964 to 109895 (Fig. 4.52). Among the four samples, sample collected from control plants (TR11) recorded maximum Dothideomycetes population followed by TR9, TR2 and TR7 respectively. After Dothideomycetes, the dominance was varied in all the samples. Like Eurotiomycetes was second dominant class in TR2 and TR7 where as it was Sordariomycetes in TR9 and Ustilaginomycetes in TR11. Eventhough there was variation in the read count, the fungal classes most commonly observed in all the samples were Agaricomycetes, Dothideomycetes, Eurotiomycetes, Sordariomycetes and Ustilaginomycetes. Read count for the classes like Eurotiomycetes, Malasseziomycetes and Tremellomycetes was comparatively more in the sample collected from propineb sprayed plants (TR2) while Exobasidiomycetes, Sordariomycetes and Saccharomycetes were more in the sample collected from PGPM applied plants. Similarly, sample TR2 and TR11 showed comparably higher population of Agaricomycetes and Ustilaginomycetes fungi. It was also noticed that the total read count or fungal population was maximum in TR9 whereas sample collected from tomato plants treated with iprodione + carbendazim showed minimum fungal population at class level.

From the fig 4.53 it was evident that Pleosporales was the most abundant fungal order in all the samples. The maximum population of Pleosporales was observed in the sample TR11 (108312) followed by TR9 (90012), TR2 (48588) and TR7 (37664). Order Eurotiales was the second most abundant order in TR2 and TR7 while it was Hypocreales and Ustilaginales in the sample TR9 and TR11 respectively. Even though all samples possess fungi from common orders, read count was varied greatly. Moreover, with in the first ten orders, two orders

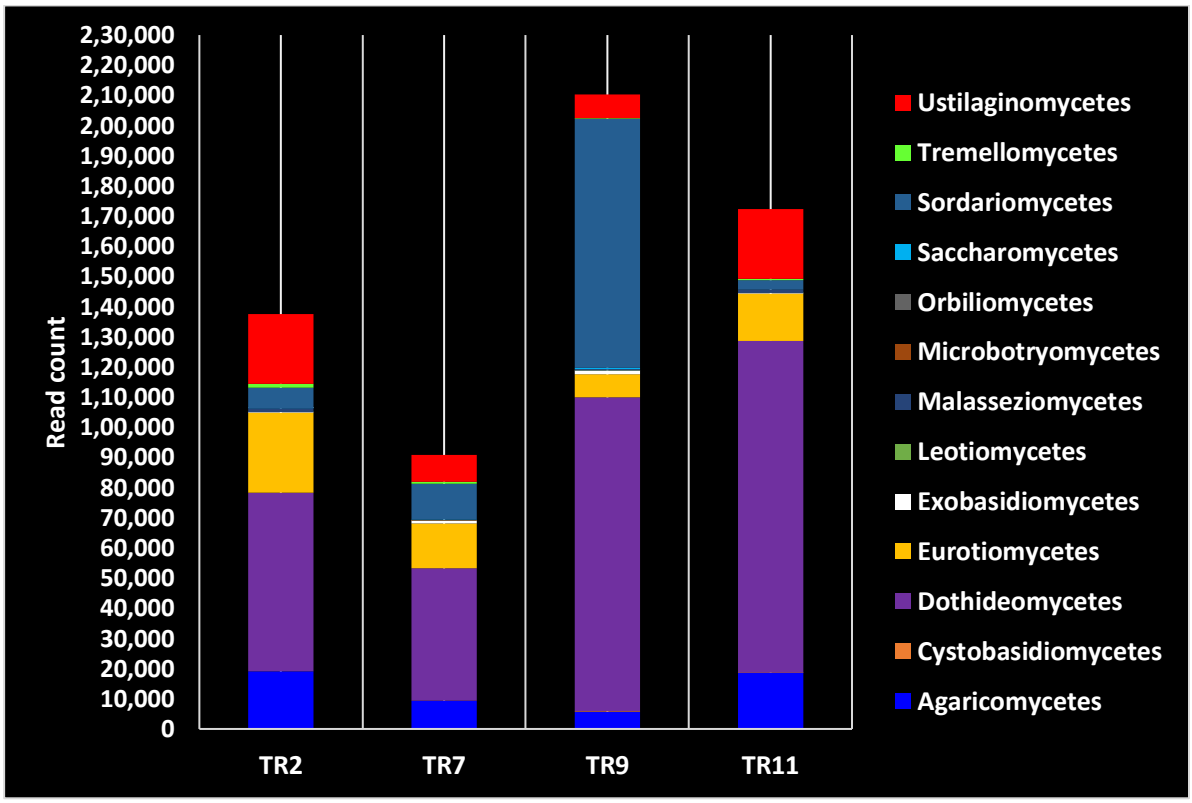


Fig. 4.54 Class-level comparison of different samples using One codex pipeline

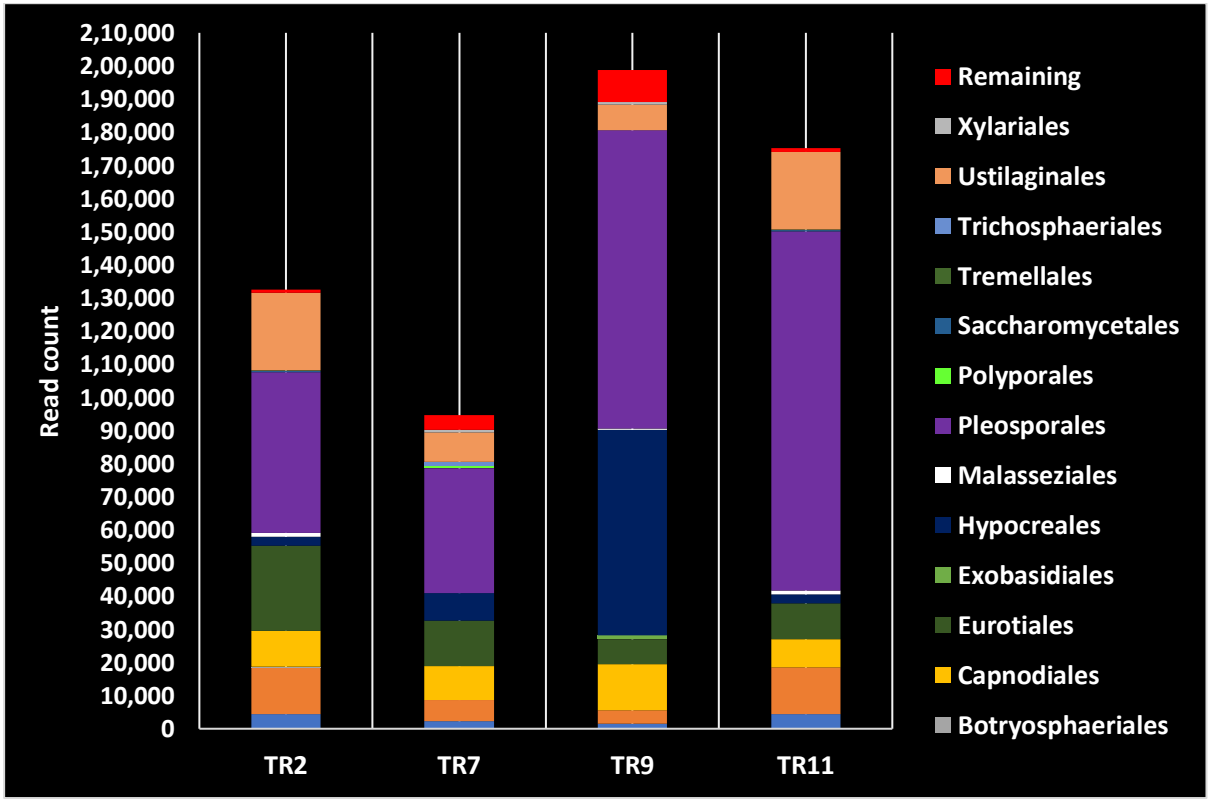


Fig. 4.55 Order-level comparison of different samples using One codex pipeline

such as Botryosphaeriales and Polyporales were unique to the sample TR2 and TR7 respectively.

Fungal diversity was also compared at family level and dominance of fungus from Pleosporaceae family was found in all the samples and maximum count was obtained from the sample TR11 (107454) followed by TR9 (83480), TR2 (55967) and TR7 (27665) respectively (Fig. 4.54). When compared to the other samples, fungal population from the families *viz.* Aspergillaceae, Malasseziaceae and Mycosphaerellaceae was high in TR2. Similarly, Cladosporiaceae and Massarinaceae was comparably more in TR7 and Corynesporascaceae in TR9 while Hypocreaceae was unique to TR9. It was also interesting to note that the read count for the families such as Agaricaceae Auriculariaceae Psathyrellaceae Ustilaginaceae was same for the sample collected from propineb treated plants (TR2) and control plants (TR11).

Genus level comparative study was also carried out to study the changes in the fungal flora due to the spraying of fungicides (propineb and iprodione + carbendazim) and bioagent PGPM on the tomato plants. Since artificial inoculation of early blight pathogen *Alternaria* was given before the treatment application, all the four samples showed the high abundance of genus *Alternaria.* The maximum population was observed in sample collected from control plants (TR11) with a read count of 100120 and it was followed by sample from PGPM (81945), propineb (45967) and iprodione + carbendazim (27100) treated plants respectively. The second abundant genus was varied between the four samples and it was *Aspergillus* (25402), *Coprinopsis* (10395), *Trichoderma* (51102) and Moesziomyces (22856) in TR2, TR7, TR9 and TR11 respectively (Fig. 4.55).

Since *Trichoderma* being one of the components of PGPM mix sample TR9 showed high abundance *Trichoderma* genus. Among the top ten genera studied the common genera present in all the four samples were *Alternaria, Auricularia, Cladosporium, Corynespora, Moesziomyces* and *Pseudocercospora*. It was interesting to noticed that genus *Aspergillus* was present in all the samples except in TR7. Moreover, among the top ten genera, the samples TR7 possessed

two unique genera *Anthracocystis* and *Fusarium*. Similarly, the sample TR2 also had unique genera *Exserohilum* while it was *Meira* in TR9. The genera present in control plants' sample but absent in treated samples were *Acremonium* and *Malassezia* in TR7 and TR9 and *Coprinopsis* in TR2 and TR9.

Comparing the response of different treatment applications on tomato leaf fungal population and diversity it was evident from above results that, there was reduction in total read count or population count in samples collected from fungicides treated (TR2 and TR7) plants while an increase in fungal population was observed with bioagent PGPM treated (TR9) plants. Moreover, difference was also observed with the proportion of different fungal taxa among four samples and presence of unique taxa were also noticed in treated plant samples. These indicates that, there was changes in fungal flora between control plants and treated plants. So, these results were in agreement with the findings of microbial enumeration study by culture depended method. In additions to these, it was found that population of early blight pathogen *Alternaria* was more in control plant sample followed by plants treated with bioagent PGPM, contact fungicide propineb and systemic fungicide iprodione + carbendazim respectively. Hence, this study once again confirmed the effectiveness of PGPM, propineb and iprodione + carbendazim for the management of early blight disease.

4.9.5.4.1 Comparison of fungal diversity using diversity indices

The diversity of the fungi from all the samples was analysed using the diversity indices *viz,* Chao 1 estimator, Shannon index and Simpson index. The indices were used to rank the samples and to compare them based on its diversity. The diversity indices are provided in the table 4.60.

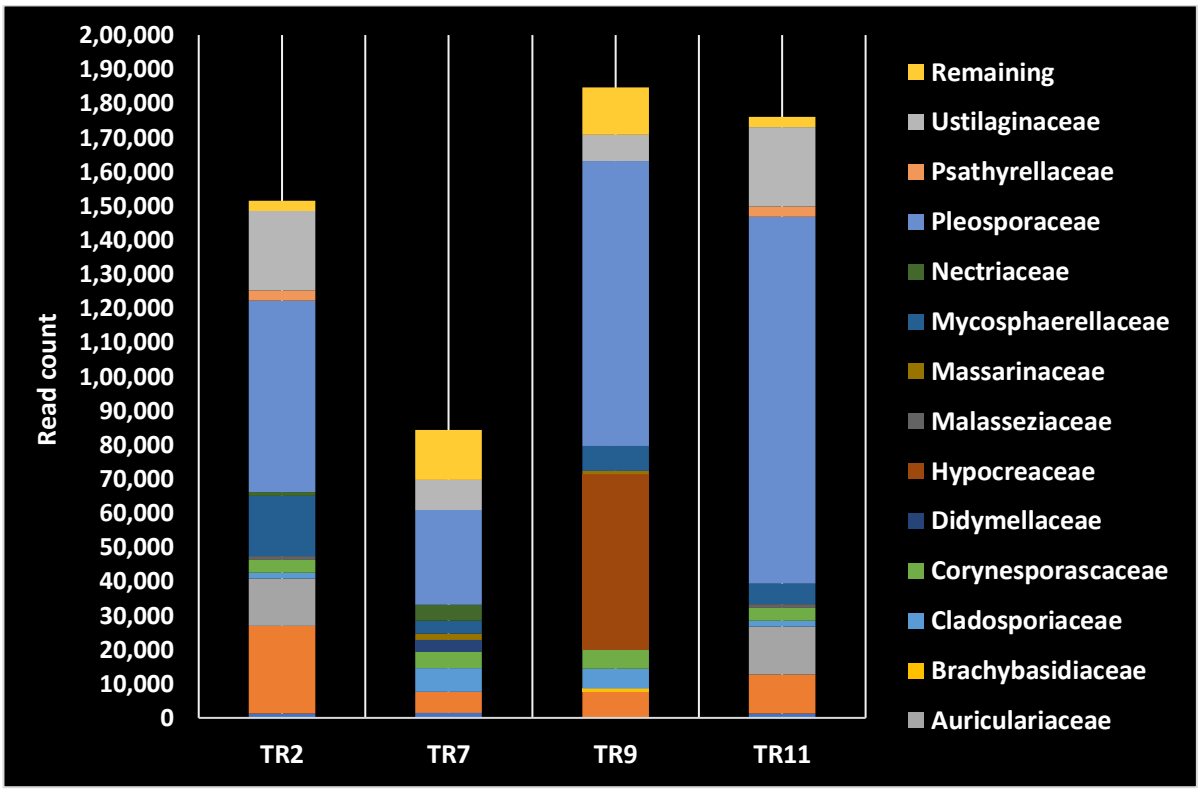


Fig. 4.56 Family-level comparison of different samples using One codex pipeline

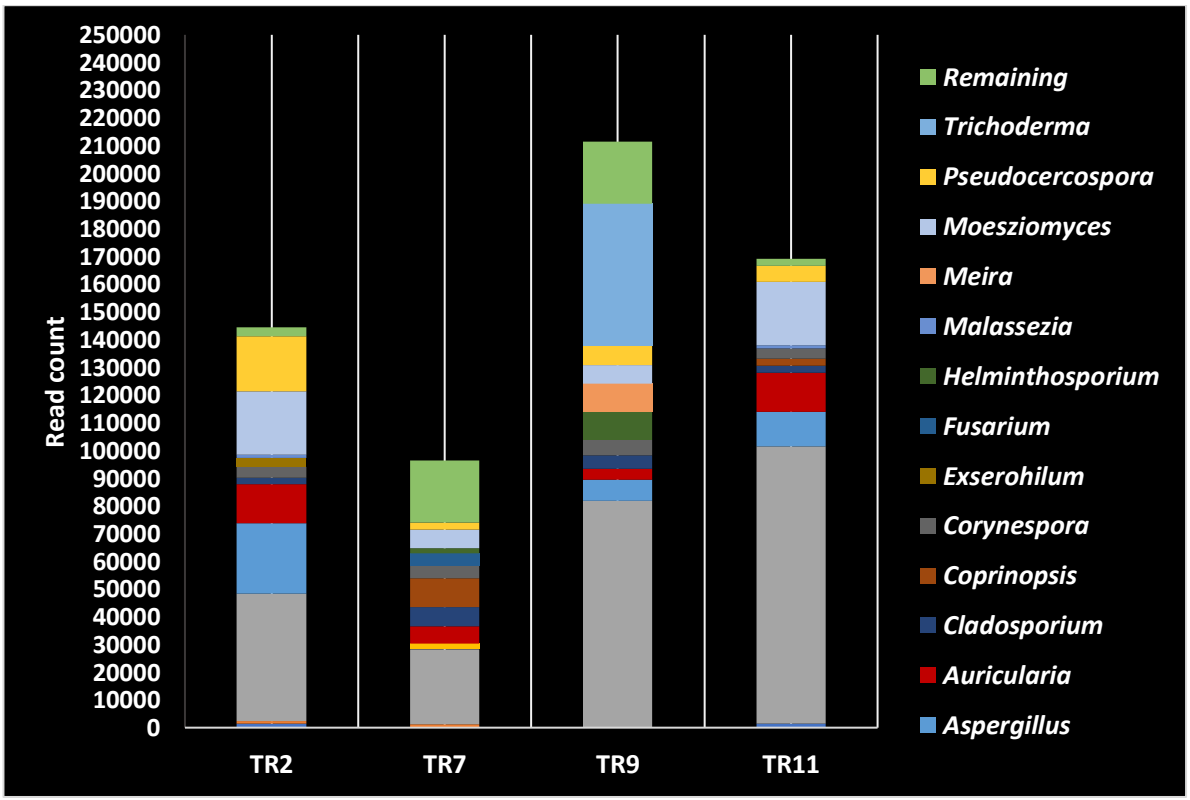


Fig. 4.57 Genus-level comparison of differentsamples using One codex pipeline

Chao 1 estimator includes the observed number of species more specifically the number species singletons (species observed once) and doubletons (species observed twice). The maximum value for Chao 1 estimator was recorded in TR9 followed by TR11, TR2 and TR7 respectively. Shannon's index (H') measures for both abundance and evenness of the species present in sample. The highest value of Shannon index was recorded in the sample TR9, which indicates that the tomato plants sprayed with PGPM occupies a wide range of fungal flora compared to others and it was followed by TR11, TR2 and TR7 respectively. The same trend was also repeated in the Simpson index value which indicated species richness was more in TR9 and less in TR7.

Table 4.60. Diversity indices of fungi collected from tomato leaves

Sample	Chao 1	Shannon index	Simpsons index
TR2 (propineb)	856	3.62	0.95
TR7 (iprodione + carbendazim)	560	2.30	0.75
TR9 (PGPM mix)	965	5.24	0.98
TR11 (control)	930	4.04	0.96

4.9.5.4.2 Comparison of fungal diversity using number of taxa

The fungal diversity was also compared using the number of taxa at each level of the taxonomic classification from class to genus (Table 4.61). The more the number, the more was the diversity. The sample TR9 recorded maximum number of taxa at all taxonomic level except class level where sample collected from control plants score maximum value. The samples collected from fungicide treated plants (TR2 and TR7) exhibited minimum number of taxa compared to control as well as bioagent, PGPM treated plants. Among the samples collected from fungicidal treatments, iprodione + carbendazim treated leaf sample (TR7) showed minimum number of taxa at all level and sample picked from propineb sprayed leaves recorded values next to control. It indicates that contact fungicide propineb has comparably less effect towards the fungal

diversity than systemic fungicide iprodione + carbendazim. Hence, the above study revealed that tomato fungal diversity and population was greatly reduced due to the fungicide application while diversity was increased due to bioagent application.

Table 4.61. Number of fungal taxa at each taxonomic level

Sample	Class	Order	Family	Genus
TR2 (propineb)	14	32	72	101
TR7 **(**iprodione + carbendazim)	14	30	64	89
TR9 (PGPM mix)	15	39	98	199
TR11 (control)	16	35	75	110

4.9.6 Bacterial diversity analysed using MG-RAST pipeline

The fastq sequences were uploaded into the MG-RAST pipeline and subjected to taxonomical analysis. The graphical representations using krona was obtained for easy comparison and tab separated files in csv format were exported for the calculation of population indices.

Based on the metagenomic analysis of the complete 16S data set, at phylum level, maximum reads were assigned in sample TR9 (12653) followed by TR11 (9873), TR2 (9379) and TR7 (7784) respectively (Table 4.62). At phylum level an increased population of Firmicutes was observed in all the four samples and maximum Firmicutes population was obtained from the sample TR9 (4026) followed by TR11 (3109), TR2 (3026) and TR7 (2820) respectively. Proteobacteria was second most abundant phylum in all the samples but its proportion was varied between the samples and number of reads assigned for Proteobacteria were 2163, 1259, 3299 and 2327 for TR2, TR7, TR9 and TR11 respectively.

Table 4.62. Phylum-level bacterial diversity of different sample

Domain	Phylum	No. of reads assigned			
		T2R	**T7R**	**T9R**	**T11R**
Bacteria	Acidobacteria	172	293	7	2
	Actinobacteria	428	254	1730	578
	Bacteroidetes	324	350	636	243
	Chloroflexi	221	174	0	239
	Cyanobacteria	195	138	596	246
	Deinococcus-Thermus	0	0	2	0
	Firmicutes	3026	2820	4026	3109
	Fusobacteria	172	210	350	0
	Gemmatimonadetes	0	0	0	213
	Planctomycetes	230	129	0	0
	Proteobacteria	2163	1259	3299	2327
	Spirochaetes	0	0	130	0
	Synergistetes	0	127	21	0
	Tenericutes	0	0	36	0
	Verrucomicrobia	344	65	3	516
	unclassified (derived from Bacteria)	2104	1965	1817	2400
Total reads		9379	7784	12653	9873

Next to Proteobacteria, Actinobacteria was most abundant in all samples except TR7 in which Bacteroidetes was the third most abundant phylum. The results also showed that, phyla such as Deinococcus-Thermus, Spirochaetes and Tenericutes were unique for the sample T9R. Similarly, Planctomycetes was present only in TR2 and TR7 and Synergistetes in T7R and T9R whereas phylum Chloroflexi was found in all samples except TR9. The unknown categories of bacterial phyla were found in all the samples recorded 2104, 1965, 1817 and 2400 reads in TR2, TR7, TR9 and TR11 respectively.

4.9.6.1 Bacterial diversity in propineb treated tomato leaves (TR2)

Tomato leaf sample which was collected from plants sprayed with propineb (TR2) were subjected to bacterial diversity analysis. The genus level bacterial diversity of the most abundant class from each phylum is provided in Table 4.63. The most abundant known phylum in the sample TR2 was observed to be Firmicutes, occupying 32.26 per cent of the total bacterial population followed by Proteobacteria (23.06%), unknown portion of bacterial phylum (22.43%) and Actinobacteria (4.56%). The remaining eight phyla showed more or less similar proportion of bacteria and among these Acidobacteria and Fusobacteria were least abundant phyla (Fig. 4.56).

In the phylum Firmicutes, class Bacilli was found to be the dominant one being 40.41 per cent of the phylum followed by Clostridia (35.75%). Betaproteobacteria was the dominant class in phylum Proteobacteria occupied 43.89 per cent of the phylum followed by Gammaproteobacteria with 41.26 per cent. Hence, among the top ten bacterial classes of sample TR2 most bacterial population was assigned as unclassified (22.43%) followed by Bacilli (13.03%), Clostridia (11.21%), Betaproteobacteria (10.15%), Gammaproteobacteria (9.54%), Negativicutes (7.5%), Verrucomicrobiae (3.67%), Alphaproteobacteria (3.02%), Fusobacteria (2.05%) and Bacteroidetes (1.82%) respectively. (Fig. 4.57).

Table 4.63. Genus-level taxonomic assemblage of bacterial diversity in propineb treated tomato leaves in rain shelter condition

Phylum	Class	Order	Family	Genus
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	*Acidobacterium* (45)
	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter* (141)
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	*Actinomyces* (45)
			Geodermatophilaceae	*Geodermatophilus* (32)
			Microbacteriaceae	*Curtobacterium* (235), *Frigoribacterium* (1)
			Micromonosporaceae	*Micromonospora* (2), *Polymorphospora* (75)
		Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium*(4)
		Coriobacteriales	Coriobacteriaceae	*Collinsella* (12)
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides* (84)
			Porphyromonadaceae	*Butyricimonas* (1), *Parabacteroides* (10), Porphyromonas (4)
			Prevotellaceae	Prevotella (69)

			Rikenellaceae	Alistipes (3)
	Cytophagia	Cytophagales	Cytophagaceae	*Flexibacter (2)*
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Arenibacter (58), Flavobacterium (45), Riemerella (20)*
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter (2)*
			unclassified	*Terrimonas (35)*
Cyanobacteria	unclassified (derived from Cyanobacteria)	Chroococcales	unclassified	*Gloeocapsa (110), Synechococcus (5)*
		Nostocales	Nostocaceae	*Cylindrospermopsis (32)*
		Oscillatoriales	unclassified	*Oscillatoria (24)*
	Gloeobacteria	Gloeobacterales	unclassified	*Gloeobacter (59)*
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus (23)*
			Paenibacillaceae	*Brevibacillus (10), Paenibacillus (530)*

			Planococcaceae	*Kurthia (317), Planomicrobium (177),*
			Staphylococcaceae	Staphylococcus (12)
		Lactobacillales	Carnobacteriaceae	*Trichococcus* (152)
			Lactobacillaceae	Lactobacillus (2)
	Clostridia	Clostridiales	Clostridiaceae	Clostridium (326)
			Eubacteriaceae	Eubacterium (8)
			Lachnospiraceae	Hespellia (9), Roseburia (10)
			Peptococcaceae	Desulfotomaculum (520)
			Ruminococcaceae	Faecalibacterium(11)
				Ruminococcus(11)
			unclassified	unclassified (164)
		Thermoanaerobacterales	Thermoanaerobacteraceae	Moorella (2)
	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus(3), Phascolarctobacterium (4)
			Veillonellaceae	Megamonas (1), Selenomonas (460),

				Dialister (2), Veillonella (232)
Fusobacteria	Fusobacteria (class)	Fusobacteriales	Fusobacteriaceae	Fusobacterium (57), Leptotrichia (136)
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	Planctomycetaceae (94), *Blastopirellula* (130)
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas (1)
		Rhizobiales	Methylobacteriaceae	Methylobacterium (85)
			Phyllobacteriaceae	Phyllobacterium (1)
		Rhodobacterales	Rhodobacteraceae	Thioclava (51)
		Unclassified	unclassified	unclassified (146)
	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter (1), Burkholderia (632) , Rubrivivax (34)
			Comamonadaceae	Variovorax (18)
		Methylophilales	Methylophilaceae	Methylovorus (25)
		unclassified	unclassified	unclassified (242)
	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfobacterium (84)

		Myxococcales	Myxococcaceae	Anaeromyxobacter (21)
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Citrobacter (1), Pantoea(32)
		Methylococcales	Methylococcacea	*Methylomicrobium* (61)
		Oceanospirillales	Halomonadaceae	*Candidatus Portiera* (283)
			Oceanospirillaceae	*Marinomonas* (45)
		Pseudomonadales	Moraxellaceae	*Acinetobacter*(256)
			Pseudomonadaceae	*Pseudomonas* (12)
		unclassified	unclassified	unclassified (112)
		Vibrionales	Vibrionaceae	*Vibrio* (1)
		Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas* (92)
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Akkermansia* (93), *Prosthecobacter* (45)
			Unclassified	Unclassified (207)
unclassified (derived from Bacteria)	unclassified	unclassified	Unclassified	unclassified (2104)

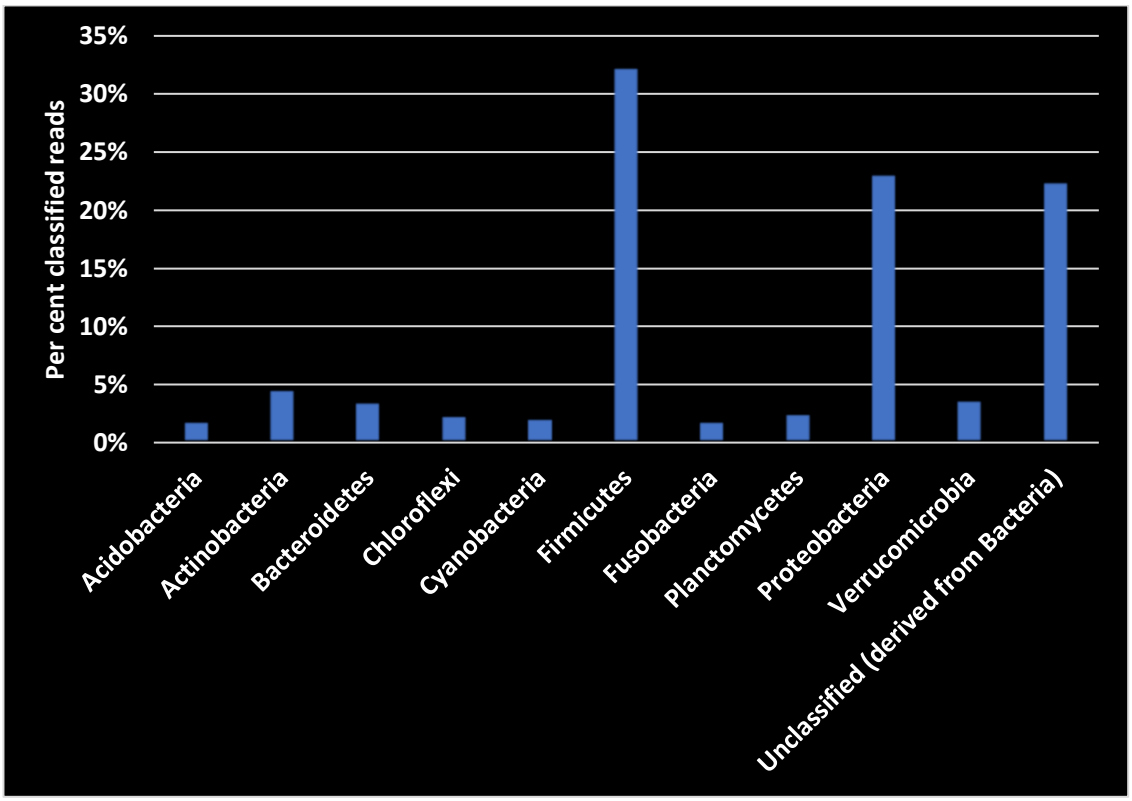


Fig 4.58. Phylum-level bacterial diversity of propineb treated tomato leaves obtained using MG- Rast pipeline

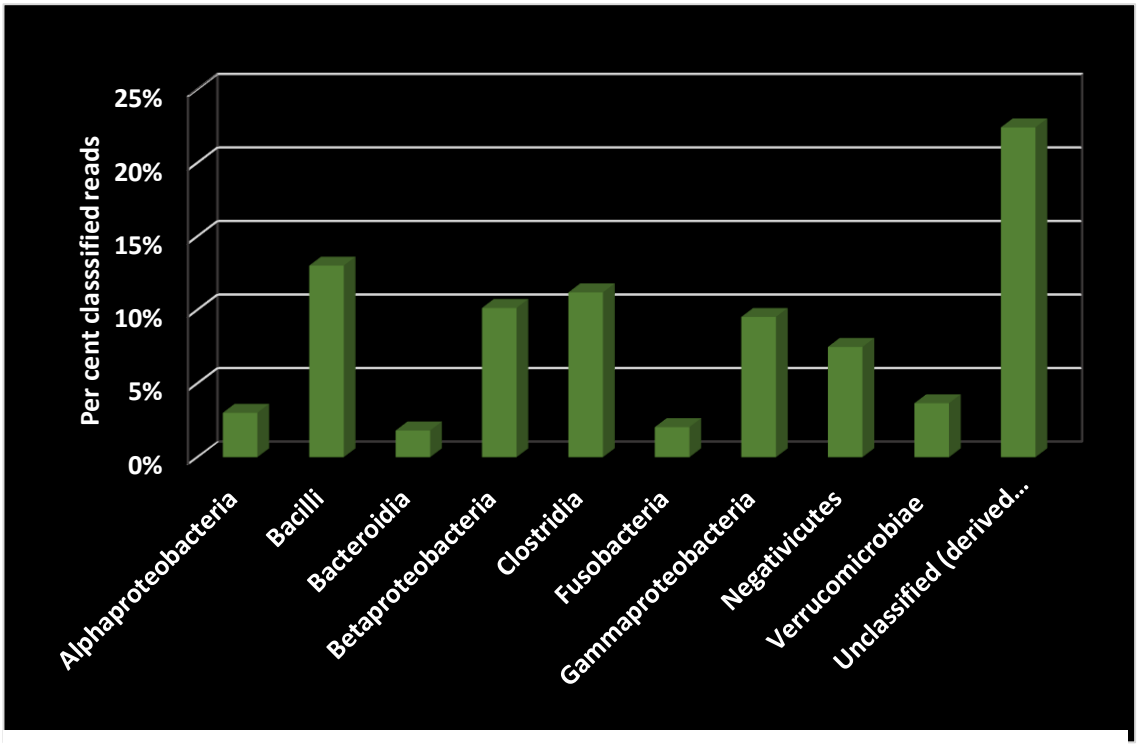


Fig 4.59. Class-level bacterial diversity of propineb treated tomato leaves using MG-Rast pipeline

At order level Selenomonadales (18.16%) secured maximum bacterial population next to unclassified once. This was followed by Bacillales and Clostridiales occupied 11.39 and 11.21 per cent of the total tomato leaf bacteria. Burkholderiales was the fourth abundant order being 7.30 per cent of total bacterial count (Fig. 58). In Selenomonadales, Veillonellaceae was the dominant family contributed 7.44 per cent. The second predominant family was Alcaligenaceae (7.11%) from the order Burkholderiales followed by Paenibacillaceae (5.75%) and Peptococcaceae (5.54%) from the order Bacillales and Clostridiales respectively (Fig. 4.59).

The most abundant known genus of the sample TR2 was *Burkholderia* (6.73%) from the family Burkholderiales and phylum Proteobacteria followed by *Paenibacillus* (5.65%) from the family Paenibacillaceae, *Desulfotomaculum* (5.54%) from Peptococcaceae and *Selenomonas* from Veillonellaceae and these three genera comes under the phylum Firmicutes (Fig.). *Clostridium* (3.47%), *Kurthia* (3.37%), *Candidatus Portiera* (3.01%), *Acinetobacter* (2.72%), *Curtobacterium* (2.50%) and *Veillonella* (2.47%) were the remaining genera among the most ten abundant ones (Fig. 4.60)

4.9.6.2 Bacterial diversity in iprodione + carbendazim treated tomato leaves (TR7)

Bacterial diversity of tomato leaves collected from plants sprayed with iprodione + carbendazim (TR7) was analysed and the results are presented in the Table 4.64. Firmicutes was the most dominant bacterial phylum in the sample and it represent 36.22 per cent of total bacterial population and the sample also occupied unclassified bacterial category in a large proportion (25.24%). The second abundant known phylum was Proteobacteria (16.17%) followed by Bacteroidetes (4.49%) and Acidobacteria (3.76%). Out of the twelve bacterial phyla obtained in the sample TR7, Verrucomicrobia was the least abundant phyla contributed only 0.83 per cent of total bacterial population (Fig. 4.61). The phylum Firmicutes was dominated by class Negativicutes with 7.8 per cent of total tomato leaf bacteria in sample TR7. Actinobacteria was the second abundant class which showed 7.61 per cent followed by class Alphaproteobacteria (7.3%)

from the phylum Proteobacteria and Bacilli (5.72%) from phylum Firmicutes (Fig. 4.62). At order level, Selenomonadales classified under the class Negativicutes possessed maximum bacterial population (7.8%). Order Actinomycetales from the class Actinobacteria also occupied 7.18 per cent and this was followed by Bacillales (5.3%) and Clostridiales from the class Bacilli and Clostridia repectively (Fig. 4.63).

It was also found that, the majority of order Selenomonadales include bacteria from the family Veillonellaceae sharing 6.7 per cent of bacterial population. The second dominant family was Micrococcaceae (4.1%) of the order Actinomycetales. Next comes Fusobacteriaceae (2.9%) and Clostridiaceae (2.6%) from the order Fusobacteriales and Clostridiales respectively (Fig.4.64).

The genus Selenomonas (6.7%) classified under Veillonellaceae family and phylum Firmicutes was found to be the most abundant bacteria in the sample TR7 (Fig.4.65). It was followed by Arthrobacter (4.17%) and Methylobacterium (3.4%) from Micrococcaceae and Methylobacteriaceae respectively. Other genera placed in top ten list were Clostridium (2.6%), Kurthia (2.1%), Brevibacterium (2.04%), Marinomonas (2%), Acidobacterium (1.9%) and Leptotrichia (1.79%).

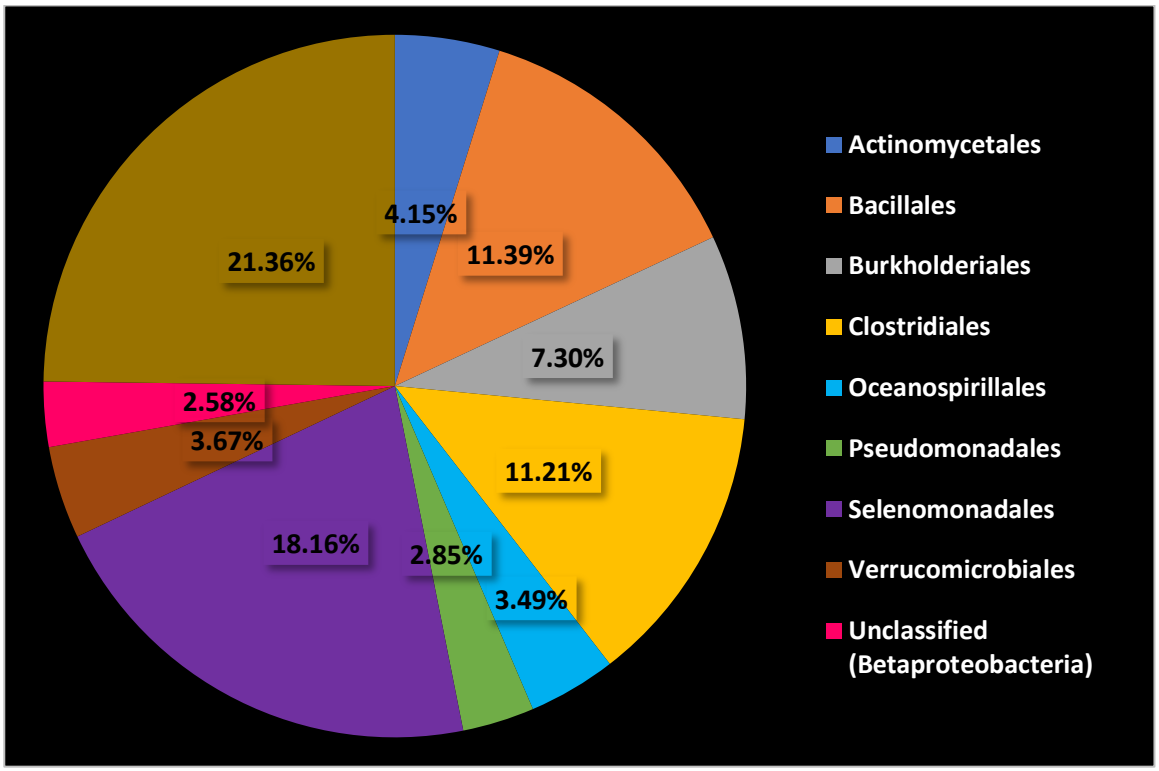


Fig 4.60. Order-level bacterial diversity of propineb treated tomato leaves obtained using MG-Rast pipeline

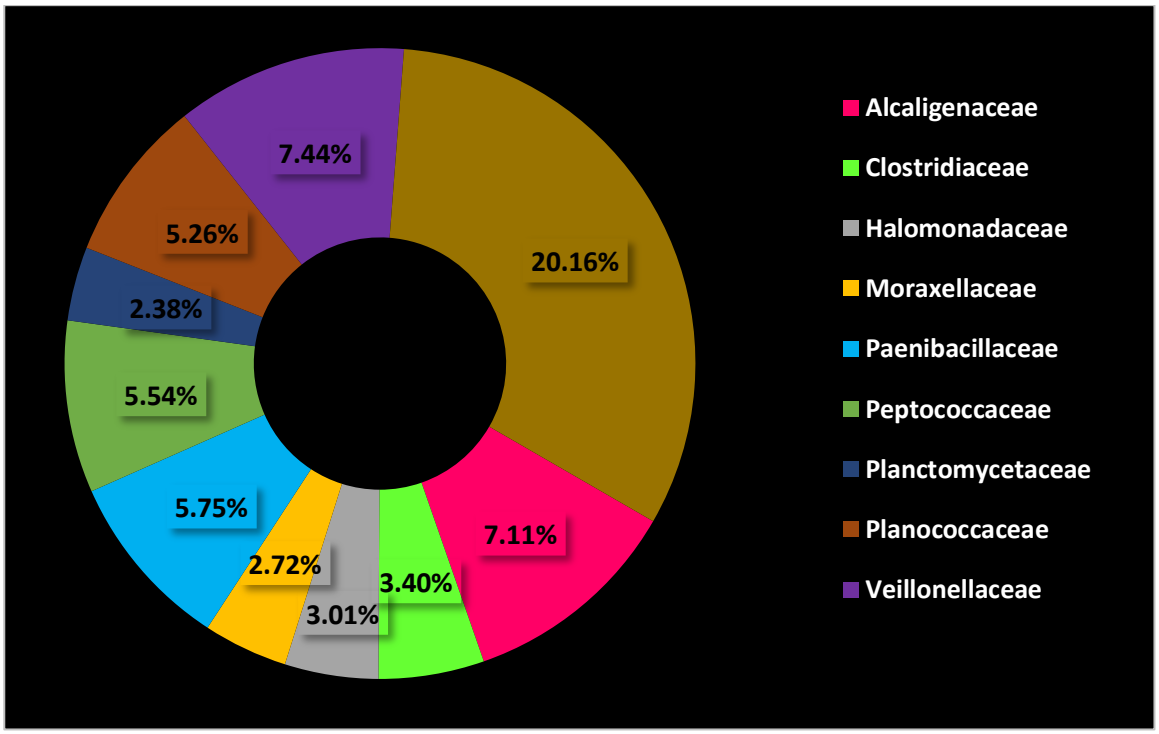


Fig 4.61. Family-level bacterial diversity of propineb treated tomato leaves obtained using MG-Rast pipeline

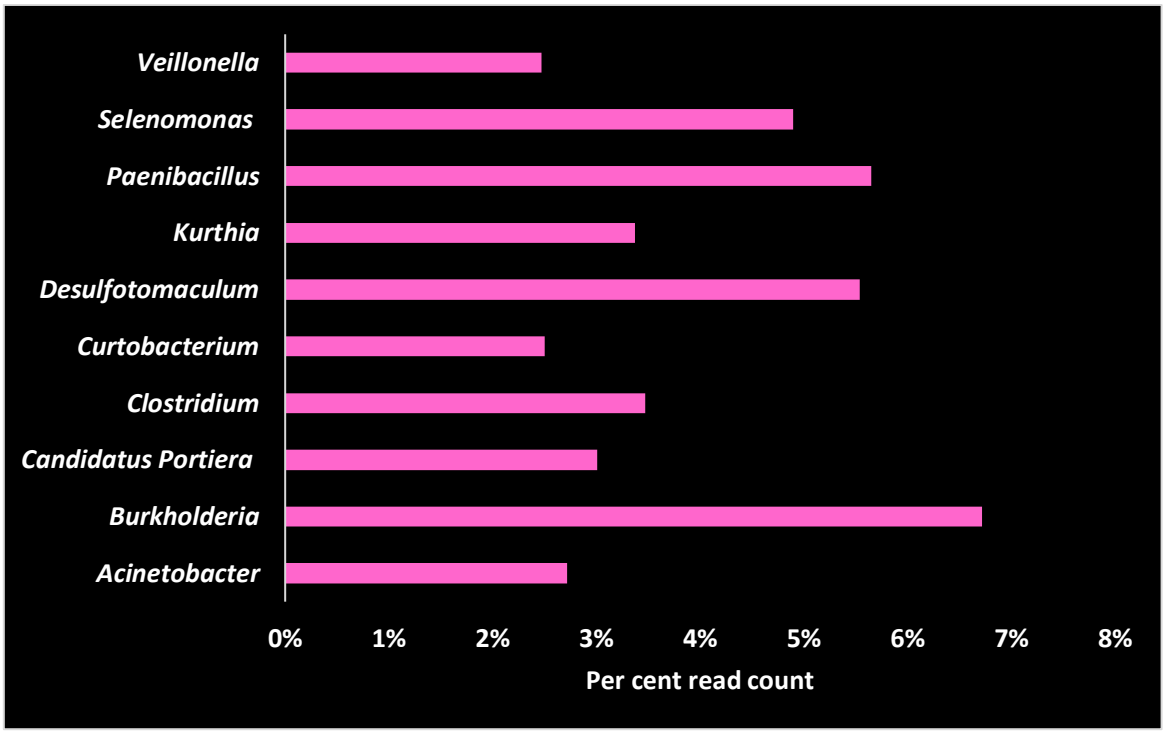


Fig 4.62. Genus-level bacterial diversity of propineb treated tomato leaves obtained using MG-Rast pipeline

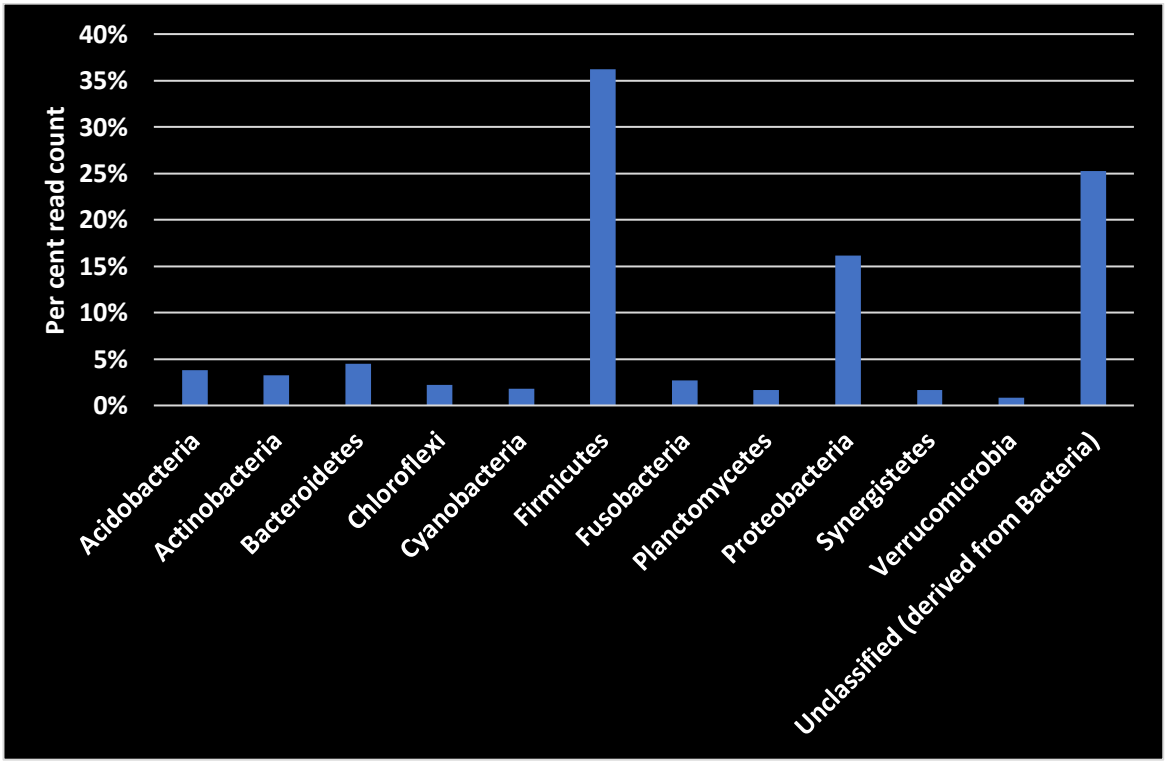


Fig 4.63. Phylum-level bacterial diversity of iprodione + carbendazim treated tomato leaves obtained using MG-Rast pipeline

Table 4.64. Genus-level taxonomic assemblage of bacterial diversity in iprodione + carbendazim treated tomato leaves in rain shelter condition

Phylum	Class	Order	Family	Genus
Acidobacteria	Acidobacteria	Acidobacteriales	Acidobacteriaceae	*Acidobacterium (152)*
	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter (138)*
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	*Acidithiomicrobium (10)*
		Actinomycetales	Brevibacteriaceae	*Brevibacterium (159)*
			Micrococcaceae	*Arthrobacter (325)*
			Nocardioidaceae	*Nocardioides (75)*
		Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium(6)*
		Coriobacteriales	Coriobacteriaceae	*Collinsella (18)*
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides (69)*
			Porphyromonadaceae	*Porphyromonas (2)*

			Prevotellaceae	*Prevotella (45)*
			Rikenellaceae	*Alistipes (2)*
	Cytophagia	Cytophagales	Cytophagaceae	*Cytophaga (10), Flexibacter (98)*
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Riemerella (1)*
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter (2)*
				Sphingobacterium (96)
Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	*Chloroflexus (102)*
		Herpetosiphonales	Herpetosiphonaceae	*Herpetosiphon (37)*
	Thermomicrobia	Sphaerobacterales	Sphaerobacteraceae	*Sphaerobacter (21)*
Cyanobacteria	unclassified (derived from Cyanobacteria)	Chroococcales	unclassified	*unclassified (121)*
		Nostocales	Nostocaceae	*Anabaenopsis (39)*
		Oscillatoriales	unclassified	*Trichodesmium (25)*

Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus (16),* *Lysinibacillus (107)*
			Planococcaceae	*Kurthia (164)*
			Paenibacillaceae	*Brevibacillus (130)*
		Lactobacillales	Lactobacillaceae	*Lactobacillus (29)*
	Clostridia	Clostridiales	Clostridiaceae	*Clostridium (206)*
			Eubacteriaceae	*Eubacterium (10)*
			Lachnospiraceae	*Roseburia (11)*
				Hespellia (8)
			Ruminococcaceae	*Faecalibacterium(11)*
				Ruminococcus(15)
			unclassified	*Unclassified (30)*
	Negativicutes	Selenomonadales	Acidaminococcaceae	*Acidaminococcus(65)*
				Phascolarctobacterium (4)
			Veillonellaceae	*Dialister(10)*

				Megasphaera (5)
				Mitsuokella (1)
				Selenomonas (524)Veillonella (2)
Fusobacteria	Fusobacteria (class)	Fusobacteriales	Fusobacteriaceae	*Fusobacterium (92), Leptotrichia (140)*
Planctomycetes	Planctomycetacia	Planctomycetales	Planctomycetaceae	*Blastopirellula (43), Planctomyces (85)*
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium(270)*
		Sphingomonadales	Sphingomonadaceae	*Sphingomonas (161)*
		unclassified	unclassified	*unclassified (138)*
	Betaproteobacteria	Burkholderiales	unclassified	*Rubrivivax (1)*
		Neisseriales	Neisseriaceae	*Chromobacterium (34)*
		unclassified	unclassified	*unclassified (51)*
	Deltaproteobacteria	Desulfovibrionales	Desulfovibrionaceae	*Desulfovibrio (86)*

	Gammaproteobacteria	Aeromonadales	Aeromonadaceae	*Aeromonas (2)*
		Enterobacteriales	Enterobacteriaceae	*Pantoea(35)*
		Oceanospirillales	Oceanospirillaceae	*Marinomonas (156)*
		Pseudomonadales	Moraxellaceae	*Acinetobacter(120)*
			Pseudomonadaceae	*Pseudomonas (19)*
		unclassified	unclassified	*unclassified (46)*
Synergistetes	Synergistia	Synergistales	Synergistaceae	*Aminobacterium (59)*
				Pacaella (21)
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Akkermansia (34), Prosthecobacter (27)*
unclassified (derived from Bacteria)	unclassified	unclassified	unclassified	*unclassified (1230)*

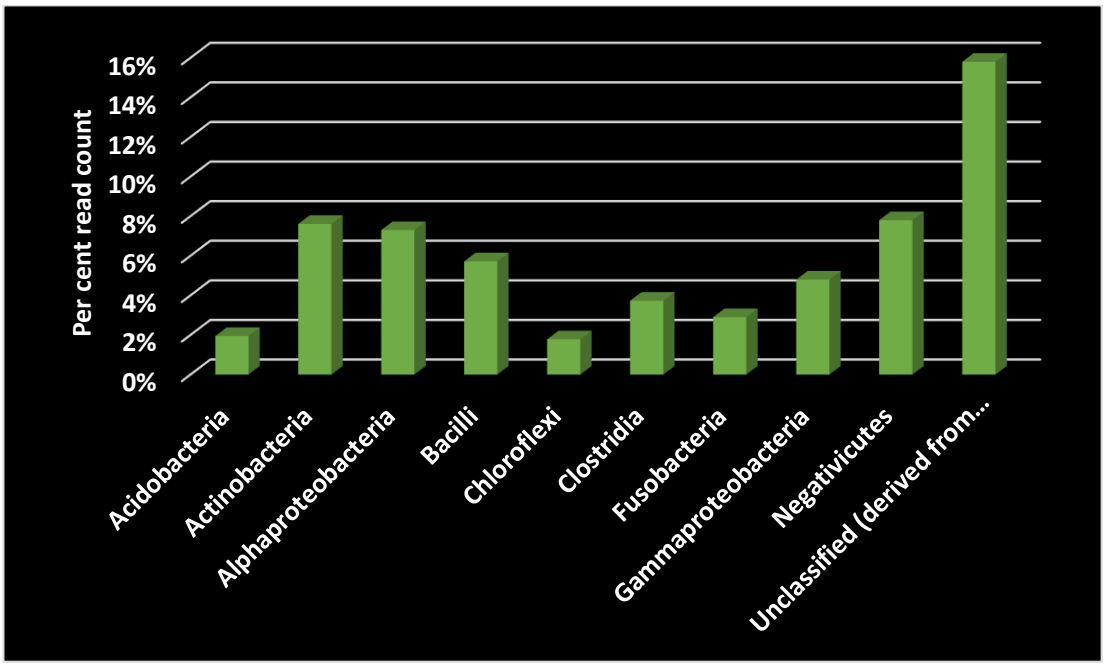


Fig 4.64. Class-level bacterial diversity of iprodione + carbendazim treated tomato leaves obtained using MG-Rast pipeline

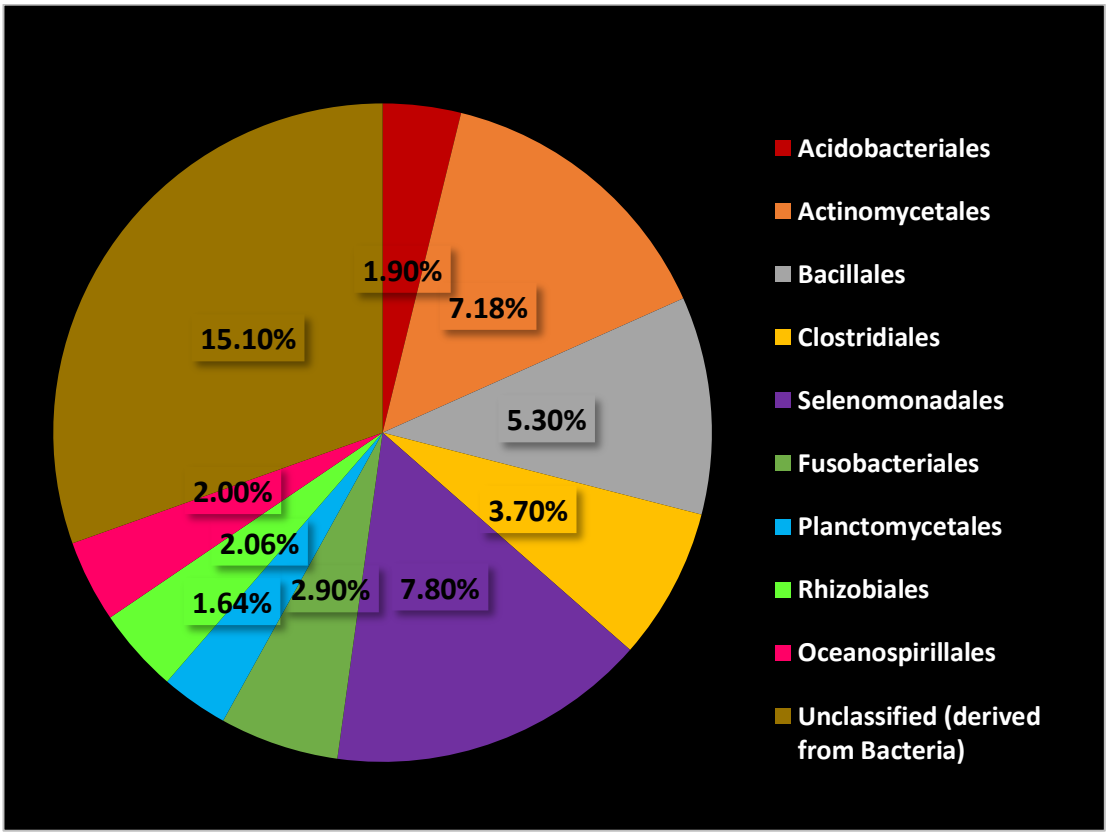


Fig 65. Order-level bacterial diversity of iprodione + carbendazim treated tomato leaves obtained using MG-Rast pipeline

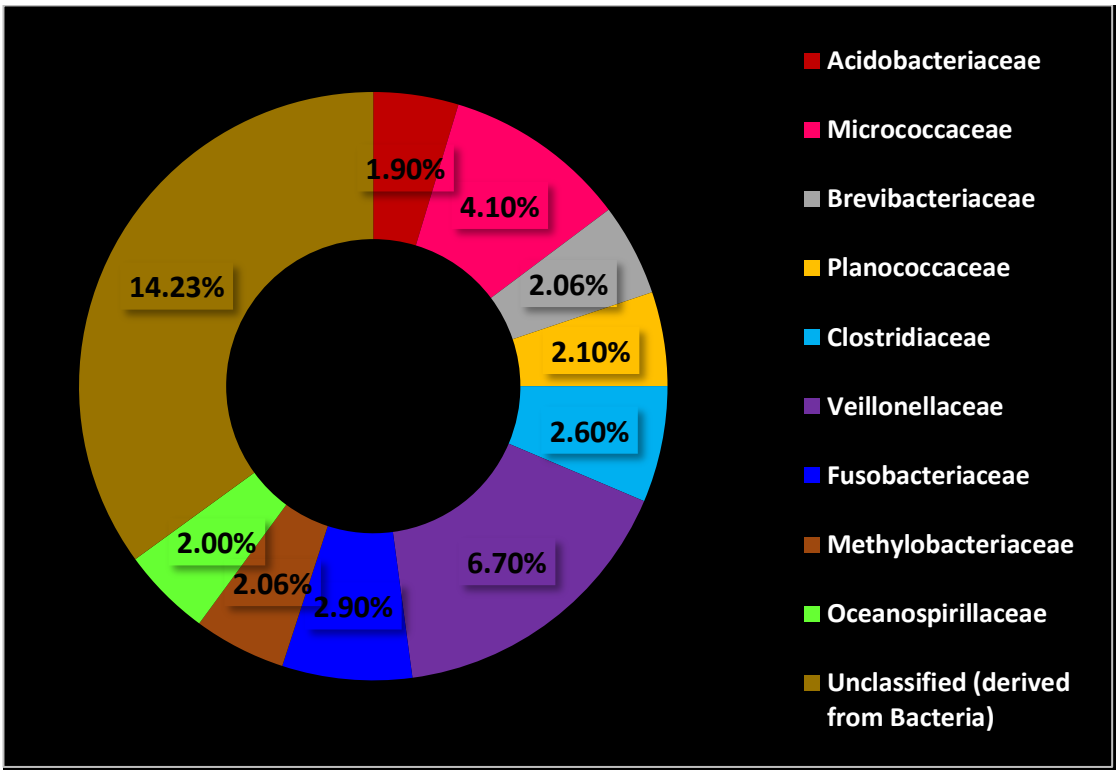


Fig 4.66. Family-level bacterial diversity of iprodione + carbendazim treated tomato leaves obtained using MG-Rast pipeline

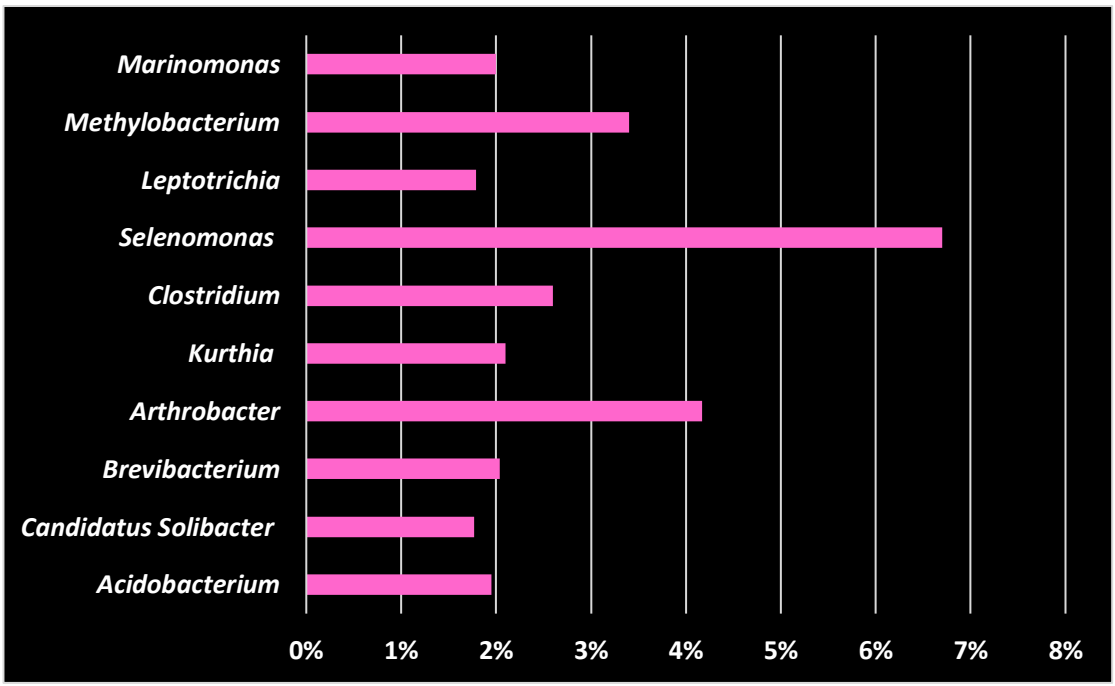


Fig 4.67. Genus-level bacterial diversity of iprodione + carbendazim treated tomato leaves obtained using MG-Rast pipeline

4.9.5.3 Bacterial diversity in PGPM mix treated tomato leaves (TR9)

The taxonomic assemblage of the bacterial diversity of tomato leaves collected from PGPM mix applied plants (TR9) is furnished in Table 4.65. The most abundant known phylum in the sample TR9 was observed to be Firmicutes, occupying 31.8 per cent of the total bacterial population followed by Proteobacteria (26.07%), unknown portion of bacterial phylum (14.36%) and Actinobacteria (13.67%). Among the thirteen bacterial phyla obtained, Synergistetes was the least abundant phyla occupied only 0.16 per cent of total bacterial population (Fig.4.66).

In the phylum Firmicutes, class Bacilli was found to be the dominant one being 23.07 per cent of the phylum. The second abundant class was Actinobacteria recorded 20.8 per cent. Gammaproteobacteria was the dominant class in phylum Proteobacteria occupied 11.60 per cent of the phylum followed by Alphaproteobacteria with 9.90 per cent. Among the top ten classes studied, the minimum bacterial count was noticed from the class Bacteroidetes (1.82%) (Fig.4.67).

At order level Bacillales (22.70%) secured maximum bacterial population. This was followed by Sphingomonadales (9.8%) and Pseudomonadales (5.08%) from the class Alphaproteobacteria and Gammaproteobacteria respectively. Selenomonadales of class Negativicutes was the fourth abundant occupied 4.75 per cent of total bacterial count (Fig.4.68). In Bacillales, Paenibacillaceae was the dominant bacterial family contributed 13.40 per cent. The second predominant family was Micrococcaceae (12.8%) from the order Actinomycetales followed by Bacillaceae (8.2%) and Microbacteriaceae (6.3%) from the order Bacillales and Actinomycetales respectively (Fig.4.69).

The most abundant known genus of the sample TR9 was Sphingomonas (9.8%) from the family Sphingomonadaceae and phylum Proteobacteria followed by Arthrobacter (9.7%) from the family Actinomycetales, Paenibacillus (6.86%) and Brevibacillus (6.63%) from Paenibacillaceae family and phylum Firmicutes (Fig.). Bacillus (4.5%), Pantoea (4.29%), Pseudomonas (3.92%), Lysinibacillus (3.6%), Lyngbya (3.5%) and Micrococcus (2.9%) were the remaining genera among the most ten abundant ones (Fig.4.70).

Table 4.65. Genus-level taxonomic assemblage of bacterial diversity in PGPM mix treated tomato leaves in rain shelter condition

Phylum	Class	Order	Family	Genus
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter (4)*
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	*Acidithiomicrobium (2)*
		Actinomycetales	Brevibacteriaceae	*Brevibacterium (2)*
			Corynebacteriaceae	*Corynebacterium (190)*
			Microbacteriaceae	*Agrococcus (229),* *Curtobacterium (327),* *Microbacterium (246)*
			Micrococcaceae	*Arthrobacter (1231),* *Micrococcus (372),* *Rothia (17)*
			Nocardioidaceae	*Nocardioides (4)*
		Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium(7)*
		Coriobacteriales	Coriobacteriaceae	*Atopobium (6)*

Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides (8)*
			Porphyromonadaceae	*Parabacteroides (4), Porphyromonas (8), Tannerella (5)*
			Prevotellaceae	*Paraprevotella (17), Prevotella (193)*
			Rikenellaceae	*Alistipes (2)*
	Cytophagia	Cytophagales	Cytophagaceae	*Cytophaga (3)*
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Capnocytophaga (119), Ornithobacterium (80), Riemerella (4)*
	Sphingobacteria	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter (3)*
Cyanobacteria	unclassified (derived from Cyanobacteria)	Chroococcales	unclassified	*Gloeocapsa (78), Synechococcus (2)*
		Nostocales	Nostocaceae	*Cylindrospermum (5)*
		Oscillatoriales	unclassified	*Lyngbya (449), Oscillatoria (8)*

Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus (580), Lysinibacillus (466)*
			Paenibacillaceae	*Brevibacillus (839), Paenibacillus (869)*
			Pasteuriaceae	*Haemophilus (6), Pasteuria (88)*
			Staphylococcaceae	*Staphylococcus (28)*
			Thermoactinomycetaceae	*Laceyella (6)*
		Lactobacillales	Aerococcaceae	*Abiotrophia (19)*
			Lactobacillaceae	*Lactobacillus (2)*
			Streptococcaceae	*Lactococcus (3), Streptococcus (14)*
	Clostridia	Clostridiales	Clostridiaceae	*Clostridium (18)*
			Eubacteriaceae	*Eubacterium (22)*
			Lachnospiraceae	*Roseburia (4)*
				Hespellia (6)
			Ruminococcaceae	*Faecalibacterium(7)*

				Ruminococcus(6), unclassified (56)
			unclassified	*unclassified (13)*
	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	*Erysipelothrix (20)*
	Negativicutes	Selenomonadales	Veillonellaceae	*Megasphaera (44), Mitsuokella (20), Selenomonas (259), Veillonella (279)*
Fusobacteria	Fusobacteria (class)	Fusobacteriales	Fusobacteriaceae	*Fusobacterium (131), Leptotrichia (206)*
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium(17)*
		Rhodobacterales	Rhodobacteraceae	*Paracoccus (2)*
		Sphingomonadales	Sphingomonadaceae	*Sphingomonas (1242)*
	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Comamonas (2)*
		Neisseriales	Neisseriaceae	*Kingella (4), Neisseria (4)*
		unclassified	unclassified	*unclassified (2)*
	Deltaproteobacteria	Myxococcales	Cystobacteraceae	*Melittangium (2)*

	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	*Campylobacter (29)*
		Nautiliales	Nautiliaceae	*Caminibacter (2), Nautilia (6)*
		unclassified	Unclassified	*Unclassified (11)*
	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	*Pantoea (543)*
		Oceanospirillales	Halomonadaceae	*Halomonas (2), Candidatus Portiera (76)*
		Pasteurellales	Pasteurellaceae	*Haemophilus (6), Pasteuria (88)*
		Pseudomonadales	Moraxellaceae	*Acinetobacter(139)*
			Pseudomonadaceae	*Pseudomonas (497)*
		Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas (120)*
Synergistetes	Synergistia	Synergistales	Synergistaceae	*Aminobacterium (136)*
Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	*Candidatus Phytoplasma (21)*
unclassified (derived from Bacteria)	unclassified	unclassified	Unclassified	*unclassified (1801)*

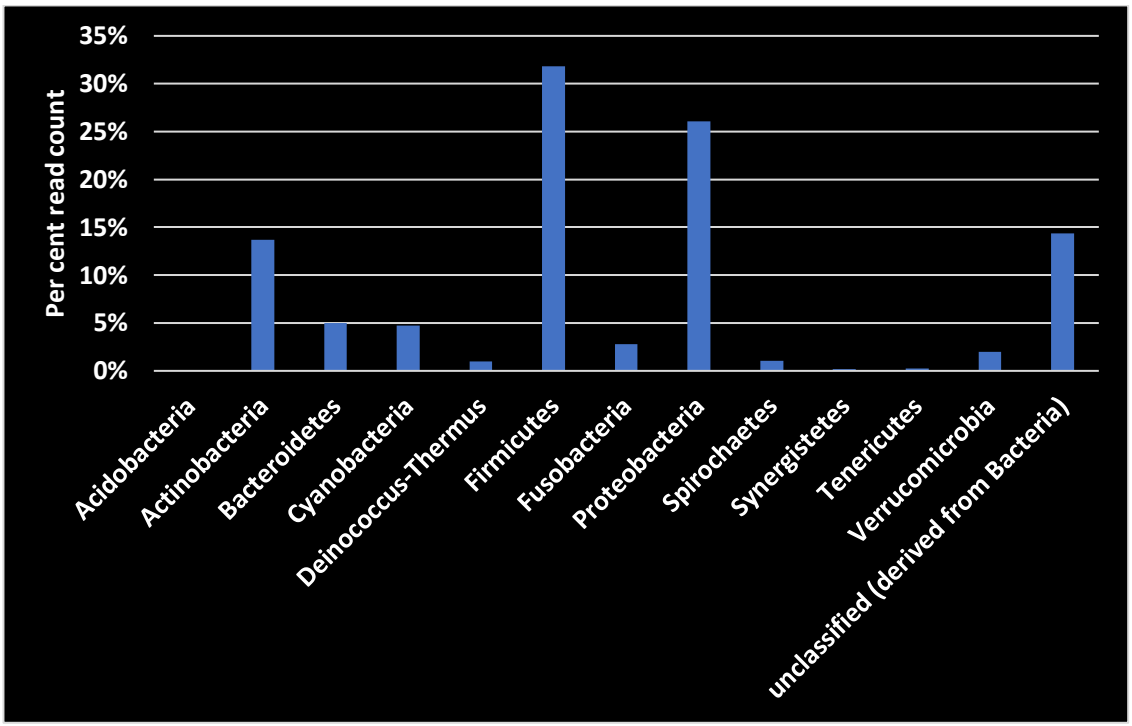


Fig 4.68. Phylum-level bacterial diversity of PGPM mix treated tomato leaves obtained using MG-Rast pipeline

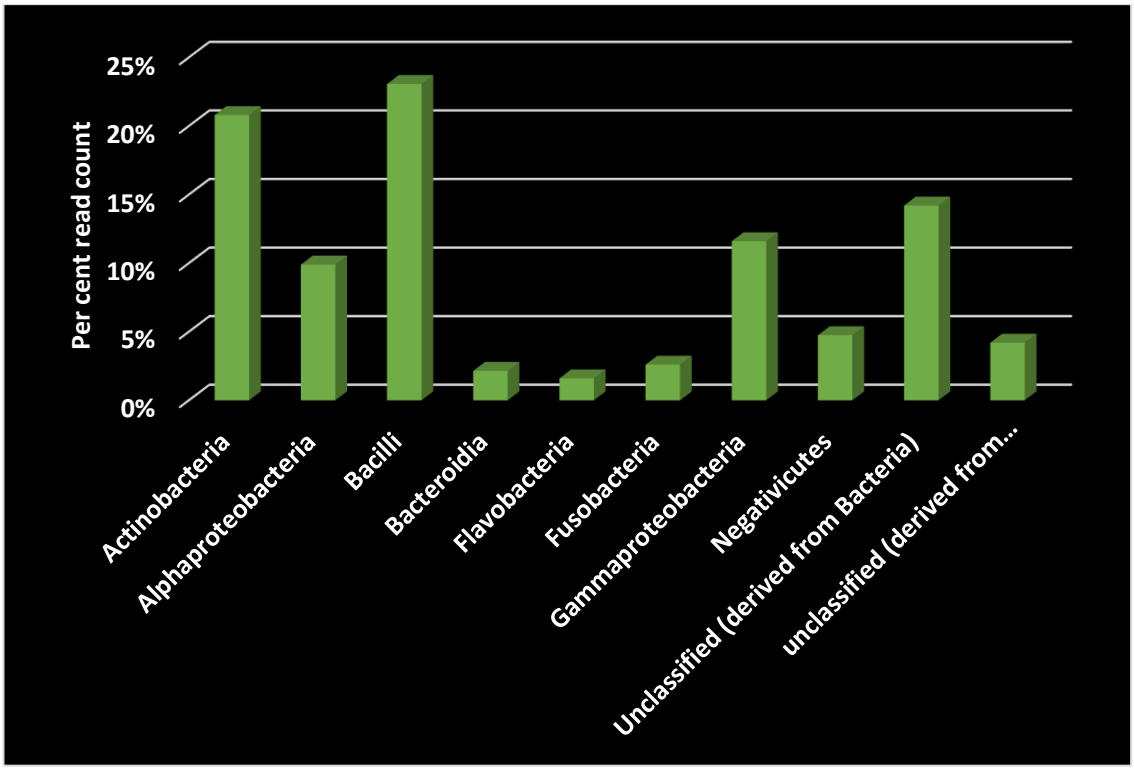


Fig 4.69. Class-level bacterial diversity of PGPM mix treated tomato leaves obtained using MG-Rast pipeline

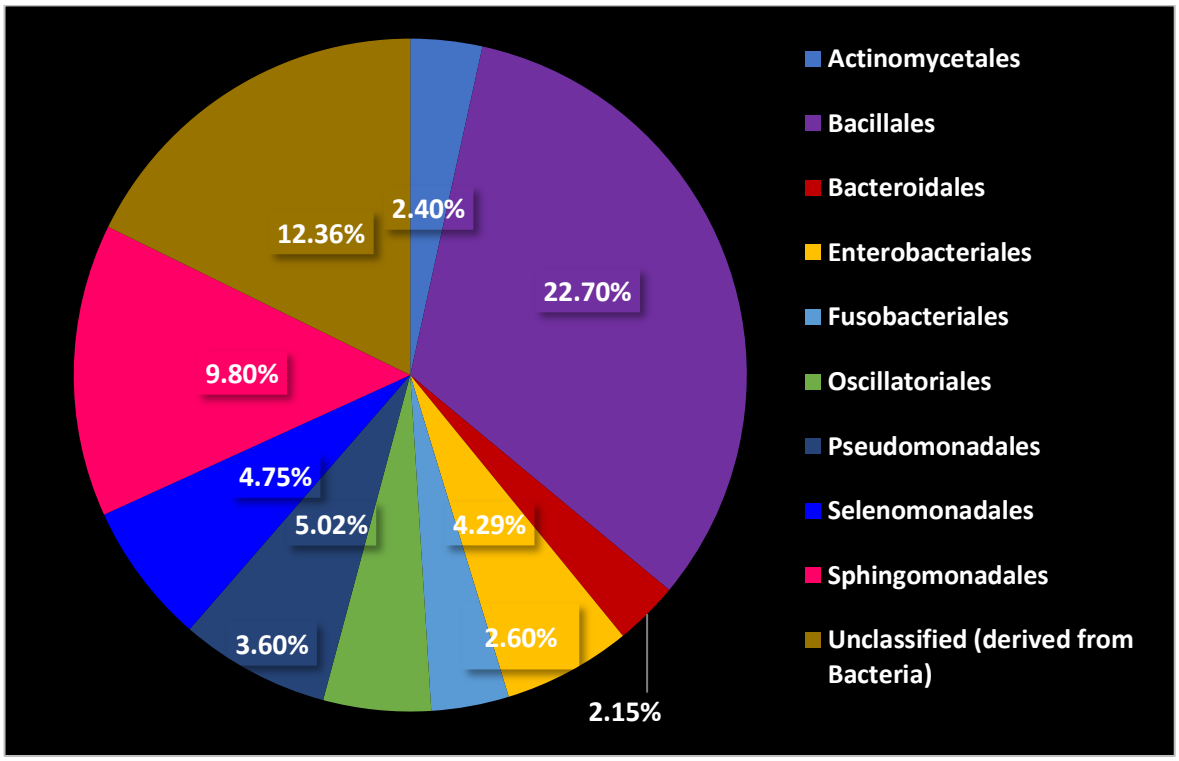


Fig 4.70. Order-level bacterial diversity of PGPM mix treated tomato leaves obtained using MG-Rast pipeline

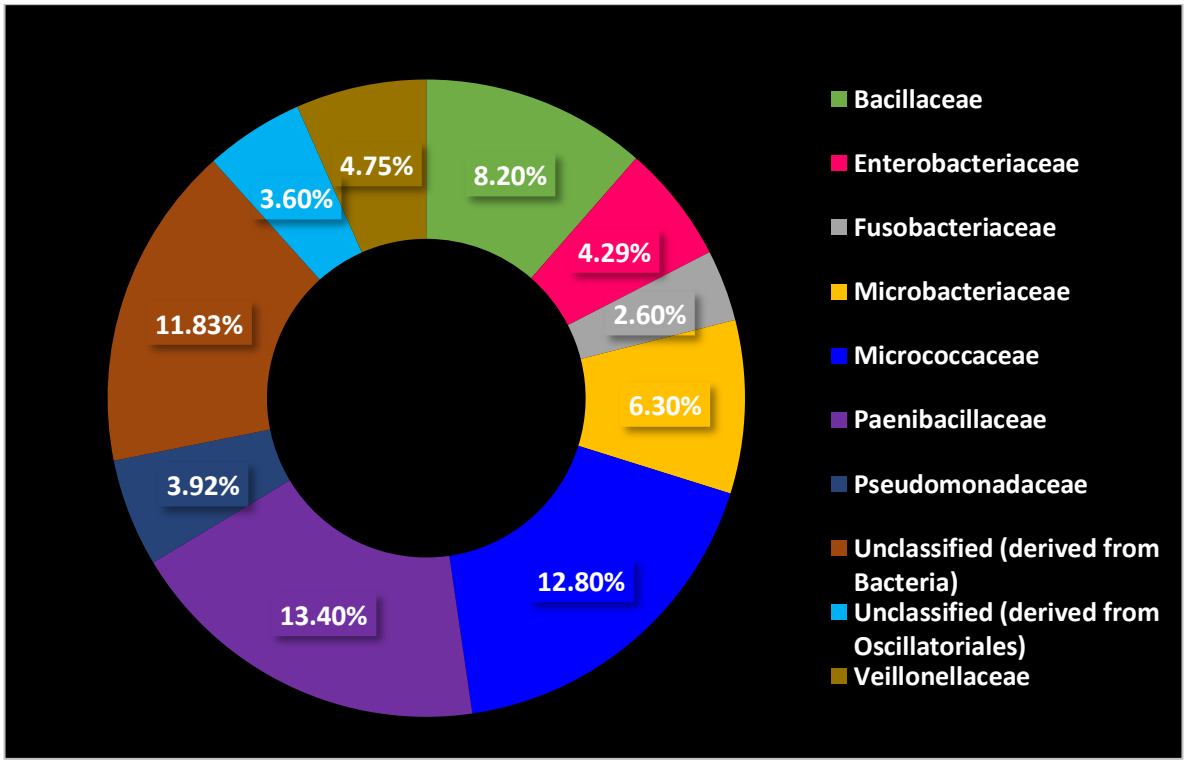


Fig 4.71. Family-level bacterial diversity of PGPM mix treated tomato leaves obtained using MG-Rast pipeline

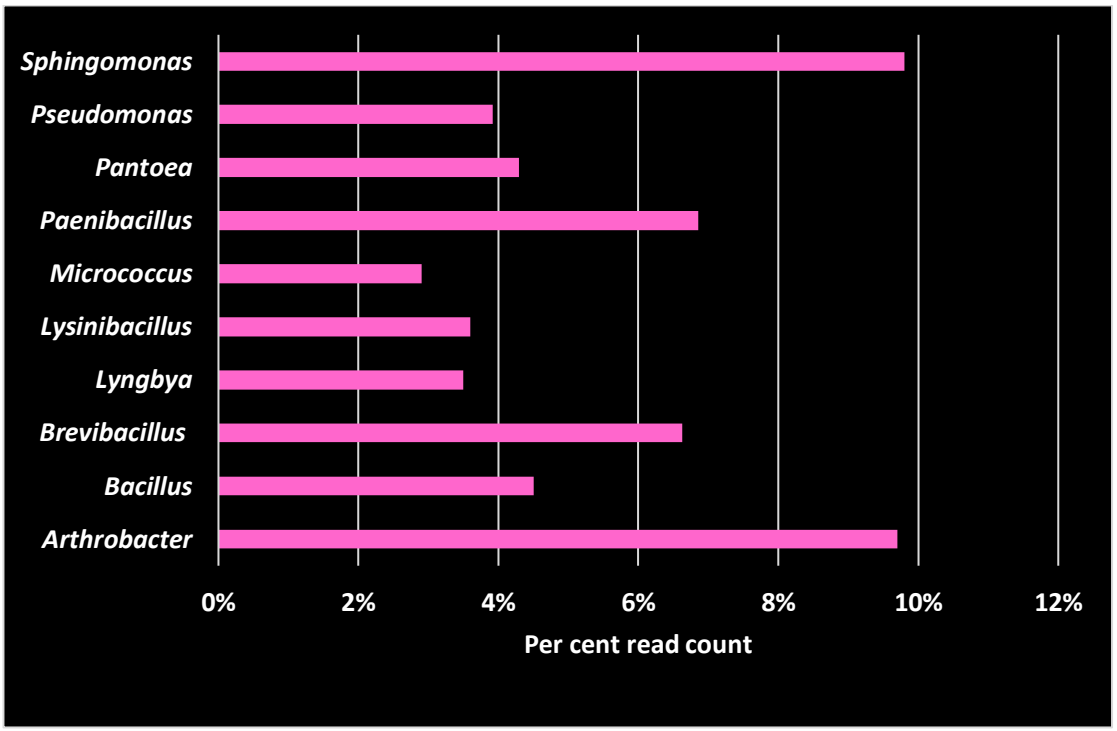


Fig 4.72. Genus-level bacterial diversity of PGPM mix treated tomato leaves obtained using MG-Rast pipeline

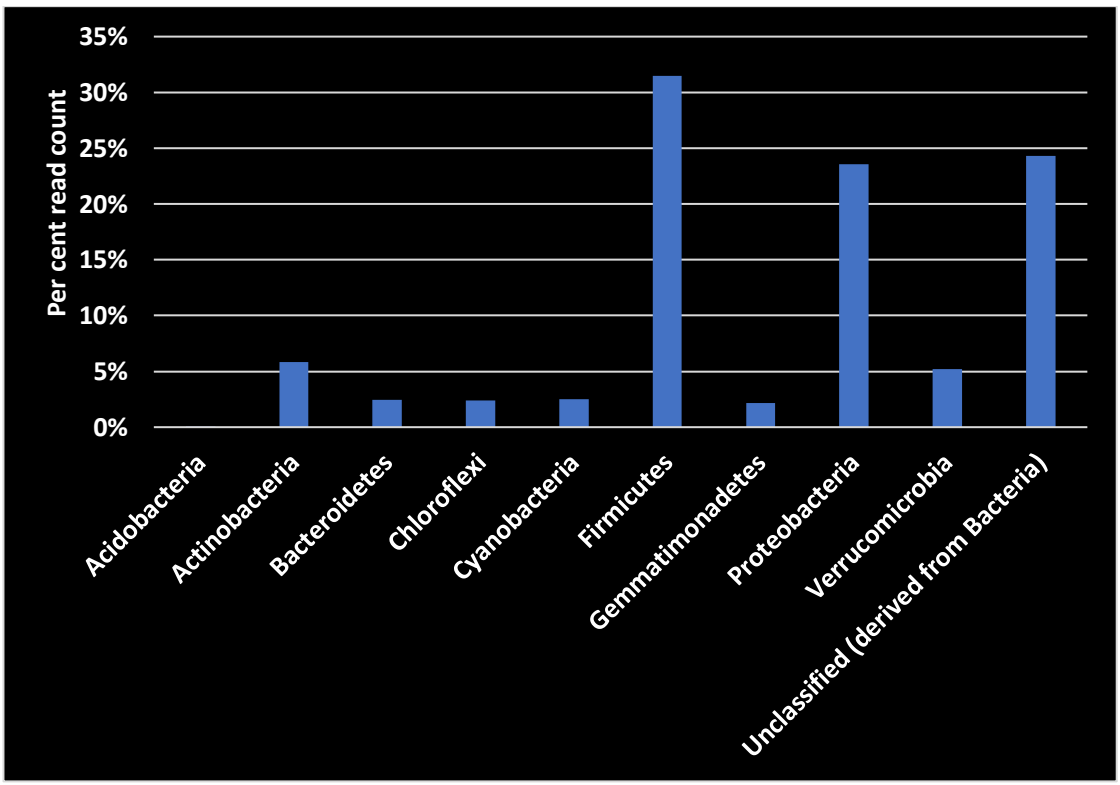


Fig 4.73. Phylum-level bacterial diversity of untreated tomato leaves obtained using MG-Rast pipeline

4.10.5.4 Bacterial diversity in untreated tomato leaves (TR11)

Bacterial diversity of tomato leaf sample collected from control plants were studied and the genus level bacterial diversity of the most abundant class from each phylum is provided in Table 4.66. The results showed that, Firmicutes was the most dominant bacterial phylum in the sample and it represent 31.48 per cent of total bacterial population and the sample also occupied unclassified bacterial category in a large proportion (24.3%). The second abundant known phylum was Proteobacteria (23.56%) followed by Actinobacteria (5.85%) and Verrucomicrobia (5.22%). Out of the ten bacterial phyla obtained in the sample TR7, Acidobacteria was the least abundant phyla contributed only 0.02 per cent of total bacterial population (Fig. 4.71).

The phylum Firmicutes was dominated by class Bacilli with 21.6 per cent and Betaproteobacteria from phylum Proteobacteria was the second abundant class which showed 16.3 per cent followed by class Negativicutes (12.52%) and Clostridia (9.27%) both from phylum Firmicutes (Fig. 72). At order level, Bacillales classified under the class Bacilli possessed maximum bacterial population (12.7%). Order Selenomonadales from the class Negativicutes also occupied 12.52 per cent and this was followed by Burkholderiales (11.5%) and Clostridiales (9.27)from the class Betaproteobacteria and Clostridia respectively (Fig. 4.73).

It was also observed that, the majority of order Selenomonadales include bacteria from the family Veillonellaceae sharing 12.4 per cent. The second dominant family was Thermoactinomycetaceae (11.7%) followed by Paenibacillaceae both from the order Bacillales. Next comes Clostridiaceae (5.3%) and unclassified (3.15%) derived from the order Clostridiales (Fig. 4.74).

The genus *Selenomonas* (12.45) classified under Veillonellaceae family and phylum Firmicutes was found to be the most abundant bacteria in the sample TR11 (Fig. 4.75). It was followed by Thermoactinomyces (8.86%) and Paenibacillus (7.3%) from Thermoactinomycetaceae and Paenibacillaceae respectively. Other genera placed in top ten list were *Clostridium* (5.3%), Blautia (3.15%), Laceyella (2.9%), Brevibacterium (2.7%), Azospira (2.66%), Prosthecobacter (2.32%) and Halobacillus (2.12%).

Table 4.66. Genus-level taxonomic assemblage of bacterial diversity in untreated tomato leaves in rain shelter condition

Phylum	Class	Order	Family	Genus
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter (2)*
Actinobacteria	Actinobacteria (class)	Acidomicrobiales	Acidimicrobiaceae	*Acidimicrobium (120)*
		Actinomycetales	Brevibacteriaceae	*Brevibacterium (267)*
			Corynebacteriaceae	*Corynebacterium (58)*
			Geodermatophilaceae	*Geodermatophilus (40)*
		Bifidobacteriales	Bifidobacteriaceae	*Bifidobacterium(3)*
		Coriobacteriales	Coriobacteriaceae	*Collinsella (8)*
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	*Bacteroides (121)*
			Porphyromonadaceae	*Parabacteroides (14)*
			Prevotellaceae	*Prevotella (78)*
			Rikenellaceae	*Alistipes (6)*
	Flavobacteria	Flavobacteriales	Flavobacteriaceae	*Aquimarina (84), Capnocytophaga (25) Weeksella (35)*
	Sphingobacteria	Sphingobacteriales	unclassified	*Terrimonas (65)*

Chloroflexi	Chloroflexi	Chloroflexales	Chloroflexaceae	*Chloroflexus (120)*
			Oscillochloridaceae	*Oscillochloris (76)*
Cyanobacteria	unclassified (derived from Cyanobacteria)	Chroococcales	unclassified	*Cyanothece(2), Gloeocapsa (32)*
		Nostocales	Nostocaceae	*Aphanizomenon(62)*
		Oscillatoriales	unclassified	*Geitlerinema (43)*
Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus (20),* *Halobacillus (210)*
			Paenibacillaceae	*Brevibacillus (22), Paenibacillus (721)*
			Thermoactinomycetaceae	*Thermoactinomyces (875)* *Laceyella (289)*
		Lactobacillales	Lactobacillaceae	*Lactobacillus (2)*
	Clostridia	Clostridiales	Clostridiaceae	*Clostridium (526)*
			Eubacteriaceae	*Eubacterium (15)*
			Lachnospiraceae	*Roseburia (5)*
				Hespellia (34)
			Ruminococcaceae	*Faecalibacterium(15)*

				Ruminococcus(10)
			unclassified	*Blautia (311)*
	Negativicutes	Selenomonadales	Acidaminococcaceae	*Acidaminococcus(4)*
			Veillonellaceae	*Dialister(3)*
				Selenomonas (1230)
Gemmatimonadetes	Gemmatimonadetes (class)	Gemmatimonadales	Gemmatimonadaceae	*Gemmatimonas (196)*
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	*Methylobacterium(125)*
		Sphingomonadales	Sphingomonadaceae	*Sphingomonas (151)*
		unclassified	unclassified	*Unclassified (130)*
	Betaproteobacteria	Neisseriales	Neisseriaceae	*Neisseria(9)*
		Rhodocyclales	Rhodocyclaceae	*Azospira(263)*
		unclassified	unclassified	*unclassified (210)*
		Burkholderiales	Burkholderiaceae	*Burkholderia (1136)*
	Deltaproteobacteria	unclassified	unclassified	*unclassified (194)*
	Epsilonproteobacteria	unclassified	unclassified	*unclassified (19)*

	Gammaproteobacteria	Alteromonadales	Shewanellaceae	*Shewanella(43)*
		Enterobacteriales	Enterobacteriaceae	*Pantoea(81)*
		Oceanospirillales	Oceanospirillaceae	*Neptunomonas(1)*
		Pseudomonadales	Moraxellaceae	*Acinetobacter(24)*
		Vibrionales	Vibrionaceae	*Vibrio (5)*
		Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas (3)*
		unclassified	unclassified	*unclassified (8)*
	unclassified (derived from Proteobacteria)	unclassified	unclassified	*unclassified (18)*
Verrucomicrobia	Opitutae	Unclassified	Opitutaceae	*Opitutus (106)*
	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Prosthecobacter (230)*
	unclassified (derived from Verrucomicrobia)	Methylacidiphilales	Methylacidiphilaceae	*Methylacidiphilum (91), unclassified (184)*
unclassified (derived from Bacteria)	unclassified	unclassified	unclassified	*unclassified (2218)*

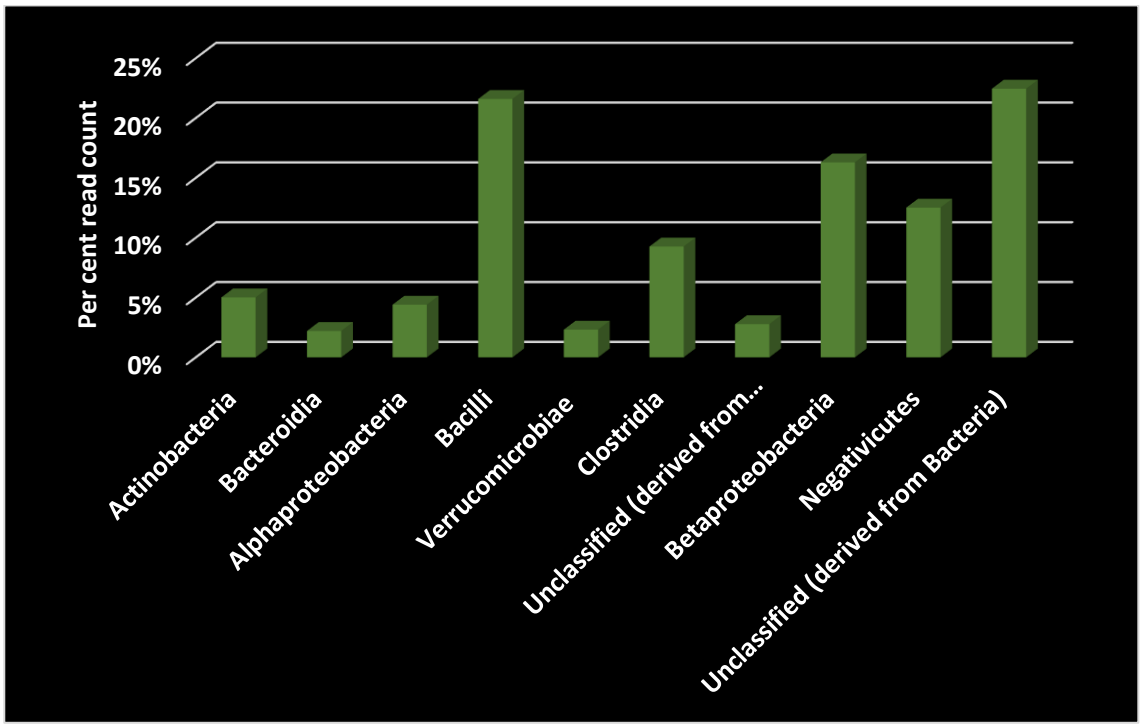


Fig 4.74. Class-level bacterial diversity of untreated tomato leaves obtained using MG-Rast pipeline

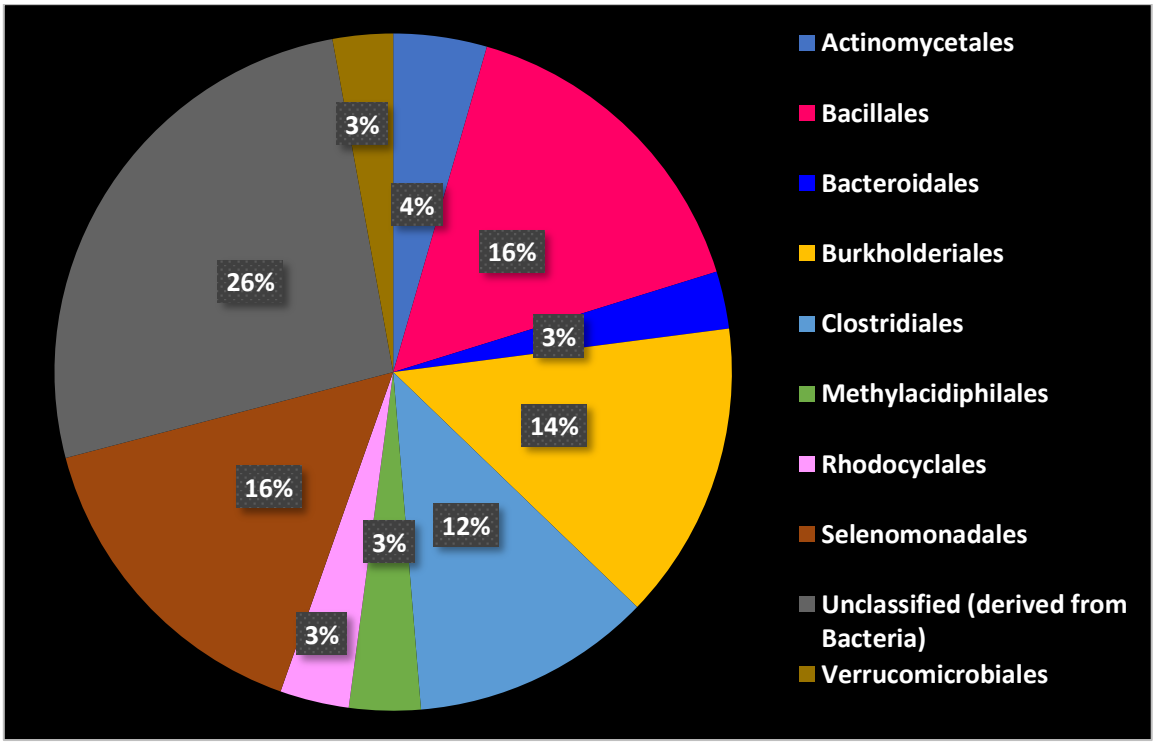


Fig 4.75. Order-level bacterial diversity of untreated tomato leaves obtained using MG-Rast pipeline

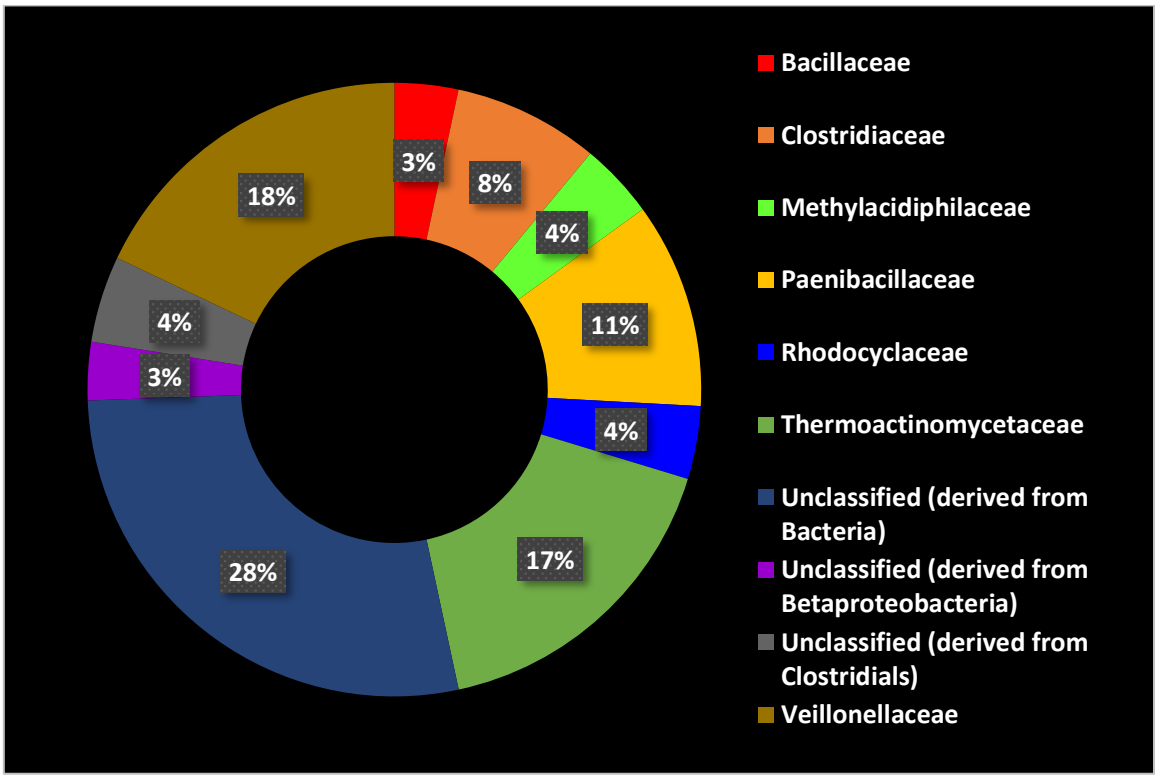


Fig 4.76. Family-level bacterial diversity of untreated tomato leaves obtained using MG-Rast pipeline

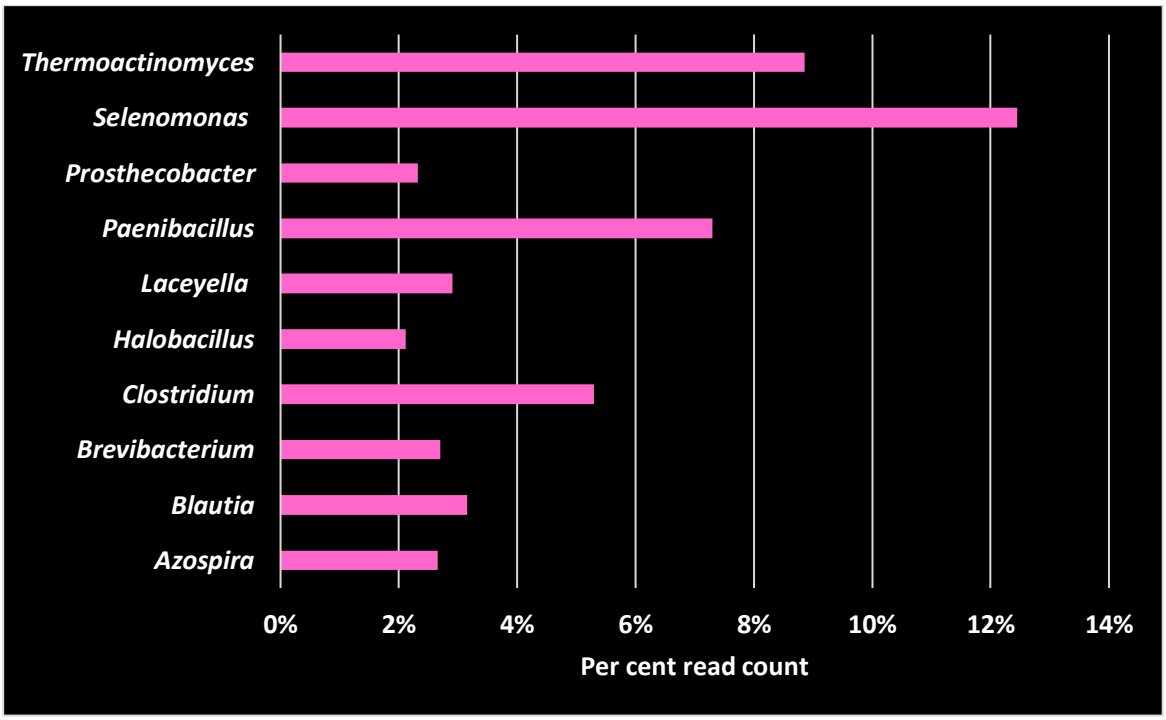


Fig 4.77. Genus-level bacterial diversity of untreated tomato leaves obtained using MG-Rast pipeline

4.9.5.5 Comparison of bacterial diversity between the samples

The bacterial diversity between the four samples were compared up to the first ten predominant one from phylum to genus level. The comparison of prominent phylum among the samples was done and it was noticed that Firmicutes was most abundant phyla which occupied almost equal proportion in all the samples. Proteobacteria was second most abundant phylum in all the samples but its proportion was slightly varied between the samples. Sample TR9 showed maximum per cent of Proteobacterial population while minimum was in TR7. It was also observed that, in TR9 bacterial population of all most all phyla were maximum except in three phyla; Chloroflexi, Gemmatimonadetes and Planctomycetes. These phyla were absent in TR9. Moreover, Planctomycetes was the phylum which was present in fungicidal treated sample (TR2 and TR7) and absent in PGPM treated (TR9) and control (TR11) samples. Similarly, Gemmatimonadetes was only phylum which was present in control (TR11) sample but absent in all the treated samples (TR2, TR7 and TR9) (Fig. 4.76).

At class level also the pattern of abundance was varied between the samples. Bacilli was the most abundant class in sample TR2, TR9 and TR11 while it was Negativicutes in TR7 (Fig.4. 76). Similarly, Actinobacteria was the second dominant class in TR7 and TR9 whereas in TR2 and TR11 it was Betaproteobacteria. Among the top ten categories, the classes which were not present in each samples were Actinobacteria, Chloroflexi, unclassified (derived from Cyanobacteria) and Unclassified (derived from Verrucomicrobia) in TR2; Bacteroidia, Betaproteobacteria, Verrucomicrobiae, unclassified (derived from Cyanobacteria) and Unclassified (derived from Verrucomicrobia) in TR7; Betaproteobacteria, Chloroflexi, Fusobacteria, Verrucomicrobiae and Unclassified (derived from Verrucomicrobia) in TR9; Fusobacteria, Chloroflexi, Gammaproteobacteria and unclassified (derived from Cyanobacteria) in TR11 respectively.

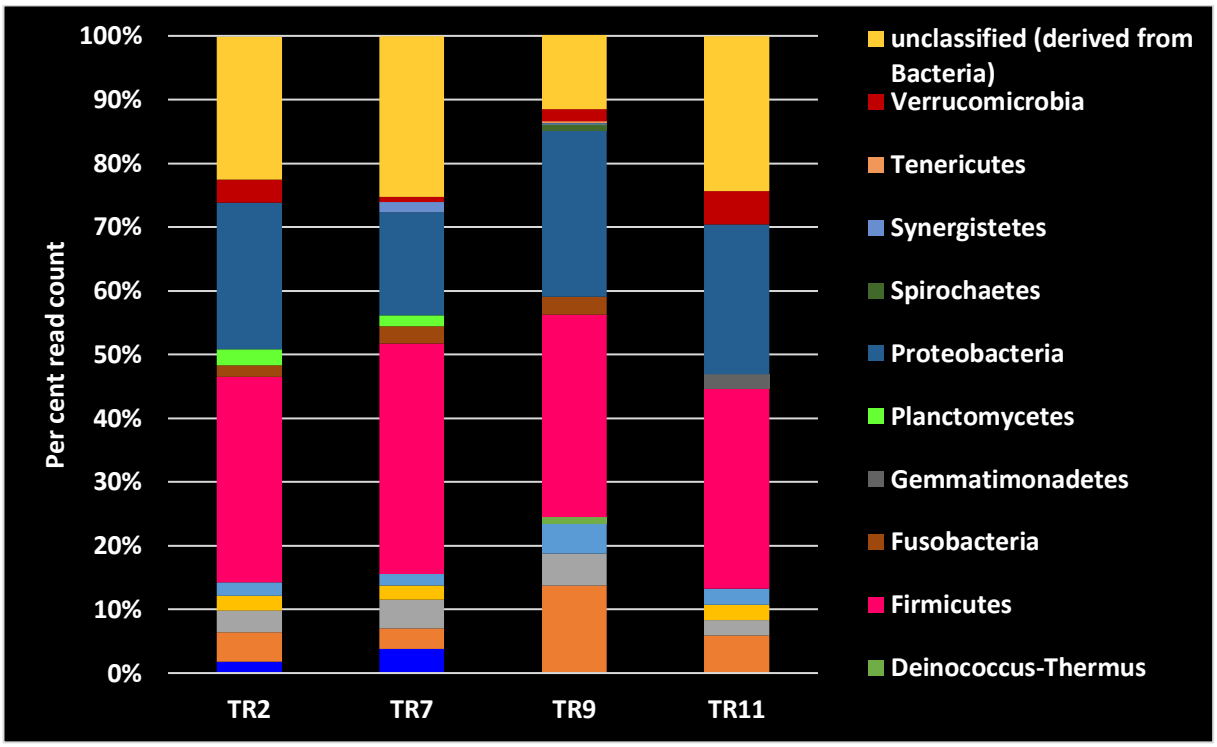


Fig. 4.78 Phylum level comparison of different samples using MG- Rast pipeline

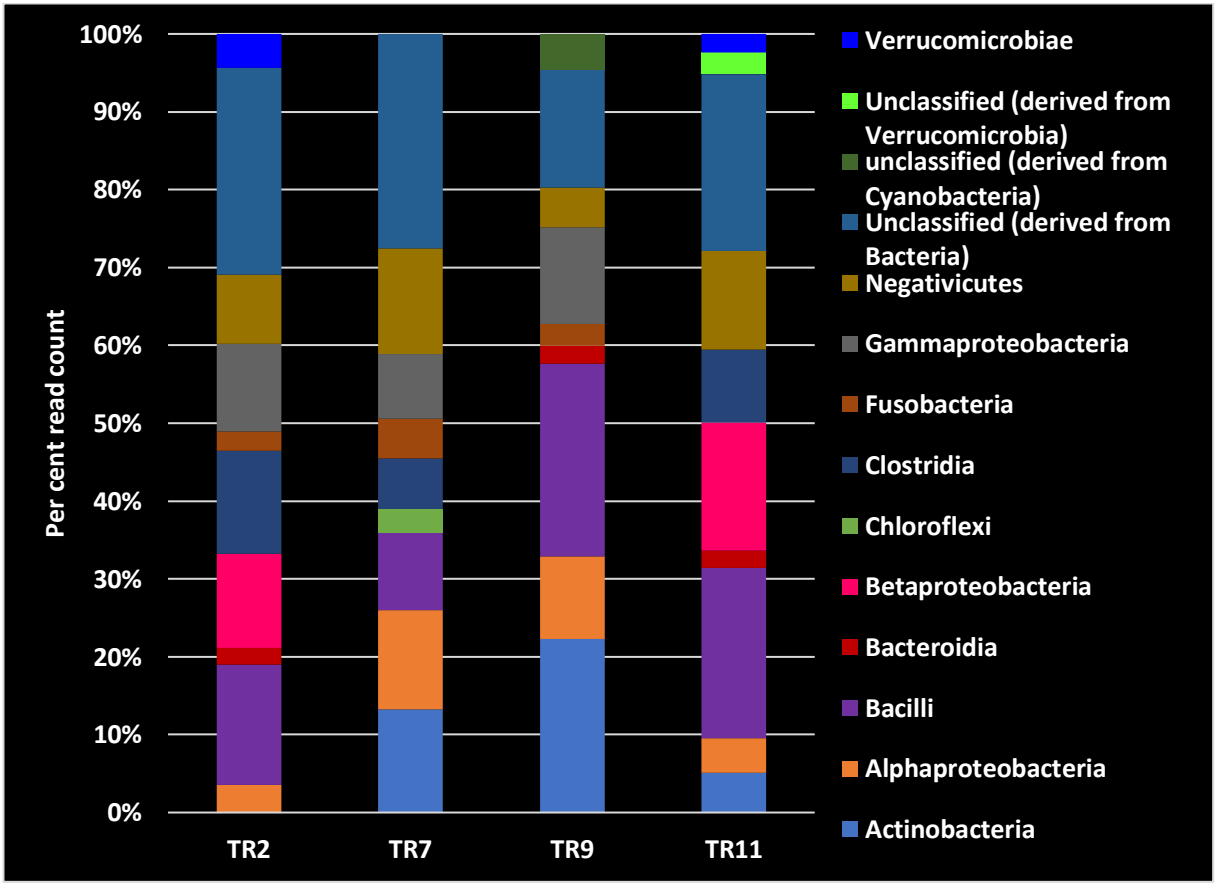


Fig.4. 79 Class level comparison of different samples using MG- Rast pipeline

From the fig 4.78 it was evident that bacteria from the order Bacillales was most abundant in sample TR9 and TR11 and order Selenomonadales in TR2 and TR7. Next comes Bacillales in TR2, Actinomycetales in TR7, Sphingomonadales in TR9 and Selenomonadales in TR11 respectively. Among the top ten orders studied, the orders which present in the four samples were Actinomycetales, Bacillales and Selenomonadales. Similarly, among top ten abundant orders the unique order in each sample were Acidobacteriales and Rhizobiales in TR7, Enterobacteriales and Pseudomonadales in TR9 and Methylacidiphilales and Rhodocyclales in TR11.

Bacterial diversity was also compared at family level and dominance of bacteria from Veillonellaceae family was observed in all the samples except TR9 in which Paenibacillaceae was dominant family. The second abundant family was Alcaligenaceae in TR2, Micrococcaceae in TR7 and TR9 and Thermoactinomycetaceae in TR11 respectively. When compared to the other samples, maximum population bacteria from the family Paenibacillaceae and Micrococcaceae was obtained from the sample TR9 whereas TR11 recorded maximum bacterial count from Veillonellaceae family. Unique families identified from top ten abundant one was Alcaligenaceae and Halomonadaceae in TR2 Brevibacteriaceae and Methylobacteriaceae in TR7, Microbacteriaceae and Unclassified (derived from Clostridials) in TR11(Fig. 4.79).

Genus level comparative study was also carried out to study the changes in the bacterial population and diversity due to the spraying of fungicides (propineb and iprodione + carbendazim) and bioagent PGPM on the tomato plants. *Burkholderia* was the most abundant bacterial genus in the sample which was collected from propineb treated plants (TR2). However, *Selenomonas* was most observed genus in iprodione + carbendazim treated sample (TR7) as well as in control (TR11) while *Sphingomonas* occupied maximum in sample collected from with bioagent PGPM applied plants. The second most abundant genus was *Desulfotomaculum* in TR2, *Arthrobacter* in TR7 and TR9 and *Paenibacillus* in TR11 (Fig.4.80).

It was interest to noticed that, *Clostridium* and *Selenomonas* was present in all the samples except TR9 and their population was higher in sample collected from control plants. Since *Bacillus* and *Pseudomonas* were the two bioagents present in the

PGPM mix, the sample TR9 showed a higher population of these two bacteria compared to other samples. Moreover, out of the top ten abundant genera studied in sample TR9, it was found that eight were unique to TR9. Similarly, among the top ten abundant genera, *Azospira*, *Blautia*, *Halobacillus*, *Laceyella*, *Prosthecobacter* and *Thermoactinomyces* were present in control sample (TR11) but absent in treated samples (TR2, TR7 and TR9) indicated that application of fungicides and bioagents has negative effects on these bacterial population.

Comparing the response of different treatment applications on tomato leaf bacterial population and diversity it was evident from above results that, there was reduction in total read count or population count in samples collected from fungicides treated (TR2 and TR7) plants while an increase in bacterial population was observed with bioagent PGPM treated (TR9) plants. Moreover, difference was also observed with the proportion of different bacterial taxa among four samples and presence of unique taxa were also noticed in treated plant samples. Even though total bacterial diversity in each sample were more or less same, the bacterial genus composition of control plants as well as treated plants were greatly affected. These indicates that, there was changes in bacterial population and composition between control plants and treated plants.

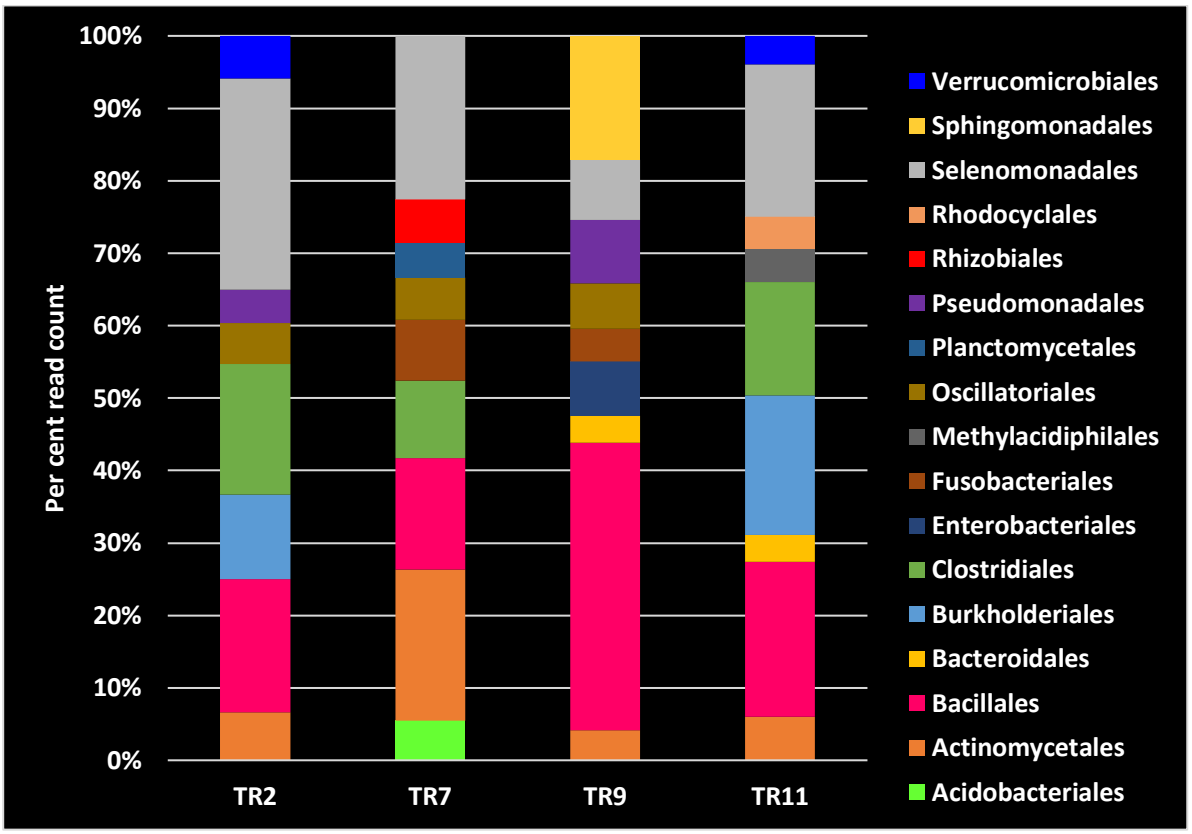


Fig. 4.80 Order level comparison of all the samples using MG- Rast pipeline

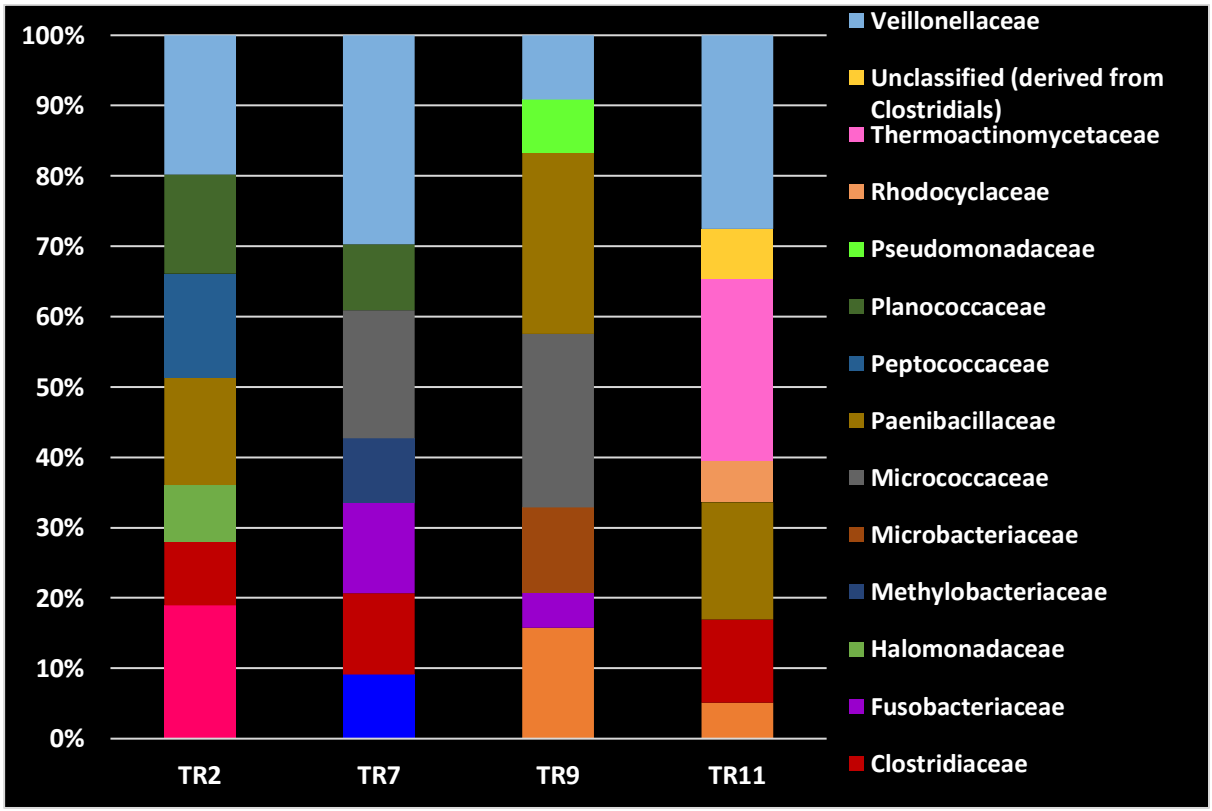


Fig. 4.81 Family level comparison of all the samples using MG- Rast pipeline

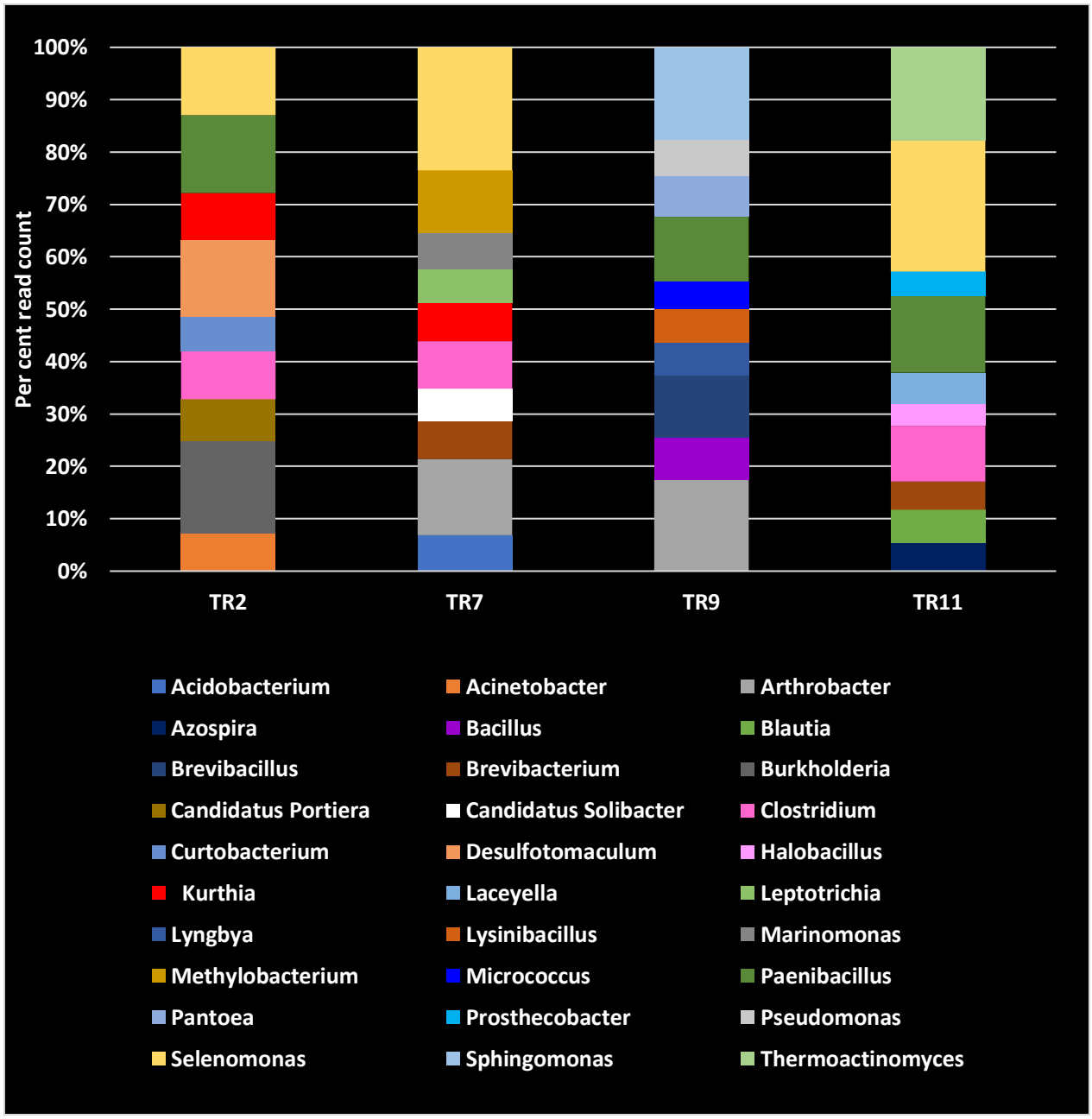


Fig. 4.82 Genus level comparison of all the samples using MG- Rast pipeline

4.9.5.4.1 Comparison of bacterial diversity using diversity indices

The bacterial diversity of four samples was analysed using the diversity indices *viz,* Chao 1 estimator, Shannon index and Simpson index and results were provided in the Table 4.67.

The sample which was collected from PGPM treated tomato plants recorded maximum value in all the diversity indices followed by sample collected from control (TR11), propineb (TR2) and iprodione + carbendazim (TR7) treated plants respectively. Hence, the values of Chao 1 estimator, Shannon's index (H') and Simpson index revealed that species richness and abundance was more in TR9 and less in TR7.

Table.4.67 Diversity indices of bacteria collected from tomato leaves

Sample	Chao 1	Shannon index	Simpsons index
TR2 (propineb)	623	3.96	0.84
TR7 (iprodione + carbendazim)	596	3.05	0.71
TR9 (PGPM mix)	784	5.10	0.92
TR11 (control)	721	4.3	0.86

4.9.5.4.2 Comparison of bacterial diversity using number of taxa

The bacterial diversity was also compared using the number of taxa at each level of the taxonomic classification from phylum to genus (Table 4.68). The more the number, the more was the diversity. The sample TR9 recorded maximum number of taxa at all taxonomic level. The samples collected from fungicide treated plants (TR2 and TR7) exhibited minimum number of taxa compared to control as well as bioagent, PGPM treated plants. Among the samples collected from fungicidal treatments, iprodione + carbendazim treated leaf sample (TR7) showed minimum number of taxa at all level except at phylum level where TR11 scored minimum value. The sample

picked from propineb sprayed leaves recorded values next to control. It indicated that contact fungicide propineb (0.2%) has comparably less effect towards the bacterial diversity than systemic fungicide iprodione + carbendazim (0.2%). So, these results were in line with the findings of microbial enumeration study by culture depended method. Hence, the above study revealed that tomato bacterial population and diversity was reduced due to the fungicide application while diversity was increased due to bioagent application.

Table 4.68. Number of bacterial taxa at each taxonomic level

Sample	Phylum	Class	Order	Family	Genus
TR2	11	39	72	116	162
TR7	12	37	67	111	157
TR9	13	44	90	146	231
TR11	10	39	74	120	195

4.9.6. Comparison of density and diversity of culturable and non-culturable microbes

The total count and diversity expressed by culturable microbes, as shown in serial dilution plating were compared with that showed by the total microflora. The data in the Table. 4.69 indicate that in all the four treatments, maximum number of fungal and bacterial genus was identified by culture independent metagenomic approach than dilution plating method which contribute only 4-8 per cent total tomato leaf fungal and 4-7 per cent of total bacterial genus identified through metagenomic analysis. Hence, the results confirmed the importance of metagenomic approach in microbial diversity analysis over culture dependent method through which only a small fraction of microorganisms can be obtained.

Table 4.69. Comparison of fungal and bacterial diversity analysis using cultural and metagenomic approach

Treatments	Total fungal genera				Total bacterial genera			
	Metagenomic method	Dilution plating method			Metagenomic method	Dilution plating method		
		Phylloplane	Endophytes	Per cent of culturable out of total fungal genera		Phylloplane	Endophytes	Per cent of culturable out of total fungal genera
T2R	101	4	5	8.91	162	5	7	7.40
T7R	89	5	3	8.98	157	4	4	5.09
T9R	199	5	5	5.02	231	5	6	4.76
T11R	110	2	3	4.54	195	3	5	4.10

4.10 Submission of sequences in NCBI database

The metagenomic DNA sequences were submitted in the Sequence Read Archive (SRA) for the accessibility by the public domain. The 16S sequences of the bacterial samples TR2, TR7, TR9 and TR11 were submitted in the SRA portal under the accession numbers SRX7295051, SRX7374604, SRX7374600 and SRP237725 respectively. Similarly, SRA accession number of fungal ITS sequences of the samples TR2, TR7, TR9 and TR11 were SRX7422041, SRX7422047, SRX7422042 and SRP237725 respectively

DISCUSSION

5. Discussion

Tomato (*Solanum lycopersicum* L.) is one of the most common horticultural crops cultivated throughout the world. It can be grown in a wide range of climates from tropical to temperate region. Tomato being a high value vegetable crop, has been more popular for cultivation in protected structures. The main purpose of protected cultivation is to create a favorable environment for the sustained yield as to realize its maximum potential even in adverse climatic conditions. It also offers distinct advantages of quality, productivity and favourable market prices to the growers. However, the commercial production of tomato under protected structures is also hindered by many fungal, bacterial, viral and nematode diseases. Moreover, due to high relative humidity and temperature prevails in protected structures, tomato crop cultivated under these structures are prone to various diseases irrespective of the season. Among the fungal foliar diseases early blight disease caused by *Alterneria solani* is the most disastrous disease leading to heavy economic losses of 78-80 % in yield (Adhikari et al., 2017).

Early blight disease incited by *A. solani* is one of the most destructive disease of tomato both under field and post-harvest stage (Singh and Sirohi, 2010). Under field conditions, it leads to leaf and stem blight which in term causes defoliation of the plants resulting in drastic reduction in fruit yield. The fruit infection in the field and after harvest, during storage and transit results in rotting of tomato fruits in addition to affecting marketable quality. Hence, farmers are commonly used high doses of fungicides in frequent intervals. Intensive usage of these chemicals in its higher doses increases the cost of production, environmental pollution and creating problems on human and animal health. So, the application of fungicides at proper dose and time interval is mandatory. In addition to this, safe and ecofriendly methods like use of biocontrol agents need to be identified and evaluated for continuous search to develop ecofriendly strategies to reduce the dependence on harmful chemicals. However, whether it is chemical or biocontrol methods, most of the disease management strategies are mainly focused on the target pathogen and little is known about their effects on the non-target microbial communities that inhabit inside and

outside the plant. These treatments could potentially affect taxonomic structures and functional properties of the plant's natural microbiota which may lead to increased vulnerability of crops. Hence, it has become necessary to consider the effect of different treatments on target and non-target microbial communities while formulating disease management strategies. Under this circumstance, it is pertinent to have detailed workup on different strategies for the management of early blight disease of tomato grown under protected structures along with their impacts on non-target microorganisms.

5.1 Isolation of the pathogen and pathogenicity test

In the present study, *Alternaria solani* was isolated from the tomato leaves showing typical early blight symptoms of dark brown spots with concentric rings surrounded by discolored tissue using tissue isolation and purified by following hyphal tip method. Isolation of the pathogen from infected leaves samples showed the association of a fungus in culture medium. The pure culture of the fungus obtained was used in further studies. Similar technique was followed by Arunakumara (2006) and Kumar *et al*. (2008) to isolate *Alternaria* from infected tomato leaves.

The pathogenicity test was carried out by spraying spore suspension of *A. solani* on foliage of 30 days old Akshaya variety of tomato. Spore inoculation has been successfully used by several workers to establish the pathogenicity of *Alternaria* spp. on their respective hosts (Coffey *et al*., 1975; Arunakumara, 2006; Tippeswamy *et al*., 2010). Rotem (1994) noticed that wounds/ injury and stomata are targeted by less virulent *Alternaria* species, while more virulent species can penetrate directly to host tissue. In the present study, infection was observed with both inoculation methods *viz.* with/without injury indicating high virulence of the isolated pathogen. However, the symptom expression was found to be delayed with spore inoculation without injury. This is in line with the findings of Thomma (2013) who also observed slow lesion development on leaf when inoculated with the spore suspension without injury as compared to with injury and stated that pathogen has to produce different enzymes for direct penetration than indirect penetration through wounds/ injuries. In both cases typical symptom was observed on tomato leaves.

5.2 Symptomatology

Symptoms are the observable effects that a pathogen causes on the growth, development and metabolism of an infected host plant. Symptomatological studies are important in better understanding of the disease. In the present investigation, detailed studies were carried out to understand the various types of symptoms produced by the pathogen on various parts of tomato plants both under natural and artificial conditions. Symptoms observed on artificial inoculation were almost similar to those produced under natural condition. The first symptoms of the early blight disease caused by *A. solani* on tomato appear a small brown to black necrotic spots on the leaf. Then the symptoms were oval or angular in shape one to four mm diameter in size and usually with narrow chlorotic zone around the spot. Later, the spots enlarged with characteristic concentric rings in the center which eventually coalesced to form large irregular spots leading to drying and defoliation. Older leaves got infection first and later disease progressed upward. The disease appeared as brown to dark brown, elongated to oval cankerous spots on stems and petioles. On fruits, at stem end spots started as black or brown sunken lesions which later enlarged to considerable extent and they covered the whole fruits which ultimately led to their decay. Same type of symptoms on leaves, shoots and fruits of tomato were described by several workers *viz.* Wellman (1949), Ghosh (1998), Mirkova and Konstantinova (2003), Farhood and Hadian (2012).

In general, *Alternaria* species are foliar pathogens that cause a relatively slow destruction of host tissues through the reduction of photosynthetic potential. An infection leads to the formation of necrotic lesions, which sometimes have a target-like appearance due to growth interruptions caused by unfavourable conditions. The fungus resides in the centre of the lesion, which is surrounded by an un-invaded chlorotic halo, a symptom that is commonly observed for the infection process of necrotrophic pathogens. This zone is created by the diffusion of fungal metabolites like toxins (Nagrale *et al.*, 2013 and Thomma, 2013).

5.3 Cultural and morphological characters of the pathogen

Cultural and morphological characters of the pathogen are the important criteria for the correct identification of the pathogen. Hence a study on the cultural and morphological characters of the pathogen was carried out on potato dextrose agar medium. The pigmentation changed from greenish brown to grey colour in later stages and produced profuse septate mycelium with aerial topography. Mycelial growth pattern was irregular rough with concentric zonation and sporulation was noticed 6 days after incubation. Similar patterns were reported by Sodlauskiene *et al.* (2003) and Kumar *et al.* (2008) for *A. solani* isolated from tomato and potato respectively. Woudenberg *et al*. (2014) also noticed profuse mycelium with concentric zonation in isolates of *A. solani* obtained from tomato leaves and he also observed that its pigmentation was varied from yellow, brown or brownish green to grey or black. on potato dextrose agar medium.

The *Alternaria* sp. obtained on cultural medium produced septate mycelium with conidiophores arising singly or in small groups. The conidiophores were straight or flexuous, sometimes geniculate brown to olivaceous brown. The conidia were solitary straight or muriform or ellipsoidal tapering to beak, pale or olivaceous brown, length 40-110 μm and 7-15 μm thick in the broadest part with 2-8 transverse and 1-3 longitudinal septa. The beaks were flexuous, pale and sometimes branched. The above description of the pathogen agreed with the description given for *A. solani* by Ellis (1971) and Alexopoulos *et al.* (1996). The present finding is also in agreement with the findings of Khalaf (2012) and Woudenberg *et al*. (2014) they reported the conidia were beaked, flexuous muriform and dark brown in colour having 2- 10 transverse septa and few or no longitudinal septa. The conidiophores were slender straight or curved and geniculated with one or two septa. Similarly, Rahmatzai *et al.* (2016) reported isolates of *Alternaria solani* with septate mycelium and muriform conidia with 53-120 x 6-17 μm in size, beaked and dark coloured which also supports the present findings. Hence, it is confirmed that the symptom observed on tomato leaves are those of early blight disease caused by *A. solani.*

5.4. *In vitro* evaluation of fungicides against the pathogen

Plant disease control aims at prevention or reduction in the incidence or severity of the disease. Among various methods, use of chemicals offers comparatively more effectiveness and quick action in prevention or reduction of disease. However, the constant use of fungitoxicant chemicals may lead to the occurrence of resistant races of pathogen, phytotoxicity and environmental pollution. Studies conducted on the use of antagonists have opened a new avenue for the control of plant diseases. Besides being safe and non-phytotoxic, antagonists are known to be effective against various plant pathogen and enhance plant growth. In the present investigation, an attempt was made to find out effect of certain selected fungicides and antagonists against early blight pathogen under *in vitro* and field conditions.

In vitro evaluation of all chemicals tested under the study were found effective against the pathogen. However, the efficiency varied with the chemicals. Complete inhibition of the pathogen was observed with all the three concentrations of propineb (0.1%, 0.2% & 0.3%), hexaconazole (0.05%, 0.1% & 0.15%), iprodione 25% + carbendazim (0.1%, 0.2% & 0.3%) and with higher concentration of difenoconazole (0.075%). These results indicated that, even the lower concentration of fungicides like propineb, hexaconazole and iprodione 25% + carbendazim were effective against the pathogen. Similarly, difenconazole was significantly superior over copper hydroxide, pyraclostrobin, azoxystrobin and trifloxystrobin 25% + tebuconazole in inhibiting mycelial growth and recorded 94.99-100 per cent inhibition. The combination fungicide, trifloxystrobin 25% + tebuconazole, was the least effective and recorded only 73.33 per cent inhibition even at the concentration of 0.075 per cent. Similar findings have been recorded by Singh and Singh (2006) who observed complete inhibition of *A. solani* with 0.025% hexaconazole and 0.075% difenoconazole. Efficacy of fungicides like mancozeb, copper oxychloride, copper hydroxide, chlorothalonil, difenoconazole, azoxystrobin and propineb against *A.solani* has also been proved by many workers (Vloutoglou *et al.,* 2001; Patel *et al*., 2007 and Genie *et al*., 2013). The present results also in agreement with the earlier reports of Gaur (2009) and Horsfield *et al*., (2010) who also noticed the reduction in the growth of *A. solani* with iprodione and propineb under *in vitro* condition.

Earlier studies showed that, Azole fungicides like hexaconazole and difenconazole can inhibit the pathogen either by destroying their cell membrane or its permeability or by inhibiting metabolic processes of the pathogen and hence are extremely effective (Cycon *et al*., 2010 and Yang *et al.*, 2011). Similarly, propineb, a contact fungicide which interferes at different locations in the metabolism of the pathogen; on several points of the respiration chain, in the metabolism of carbohydrates and proteins, in the cell membranes and this multi-site mode of action of propineb also prevents development of resistance in the pathogen (Sahu *et al.*, 2013). Strobilurin group fungicides like azoxystrobin, pyraclostrobin and trifloxystrobin are broad spectrum in action and excellent inhibitors of spore germination and comparatively less effective for direct mycelial inhibition than above chemicals. Moreover, strobilurins inhibits mitochondrial respiration by blocking electron transfer at the cytochrome bc_1 complex (Jiang *et al*., 2009). The induction of the alternative oxidase respiratory pathway at the cytochrome bc_1 target site has been proposed as the likely reason for the low mycelial sensitivity to strobilurins displayed by the pathogen. It was also found that Iprodione dicarboximide fungicide, widely used in a variety of crops, inhibits glycerol synthesis and hyphal development by cutting off signal transduction (Minambres *et al*., 2012).

5.5 *In vitro* evaluation of bioagents against the pathogen

In vitro evaluation of all bioagents tested under the study were showed antagonistic activity against the pathogen. Among them *T. viride* (KAU), *T. viride* (PGPM mix), *T. harzianum* (PGPM mix) and plant growth promoting microbial consortium (PGPM mix of KAU) showed complete inhibition of pathogen growth. Among different bacterial antagonists *B. subtilis* 1 (Endophyte from cocoa) recorded maximum growth inhibition while *P. fluorescens,* showed the lowest inhibition (34.44%). Moreover, the study also revealed that among individual microorganism of PGPM mix of KAU, fungal counterpart *viz. T. viride* (PGPM mix), *T. harzianum* (PGPM mix) showed maximum inhibition than the bacterial counterpart against *A.solani*. Efficacy of *Trichoderma* against *Alternaria* sp. have been reported by many workers (Verma *et al.*, 2008; Pandey, 2010; Begum et al., 2010). *Trichoderma* inhibit the growth of the pathogen through its rapid growth potential and competition for food and space (Devi *et al.*, 2012). *Trichoderma* can also inhibit the

pathogen through the production of volatile and non-volatile compounds (Sumana and Devaki, 2012). *Bacillus* species as a group offer several advantages over other bacteria for protection against pathogens because of their ability to form endospores, and broad-spectrum activity of their antibiotics (Abdalla *et al*., 2014). Antagonistic activity of endophytic strains of *B. subtilis* from coconut and cotton against *A. solani* of tomato has been earlier observed by Sundaramoorthy (2013). Similarly, Koley *et al*., 2017 and Ramakrishna *et al.* (2018) also recorded 40-50 per cent growth inhibition of *A. solani* by *B. subtilis.*

5.6 Management of early blight disease of tomato under protected cultivation

It is a well-established fact that, many chemicals and antagonists which are promising against the pathogen under *in vitro* condition may not be effective in the field condition. With this view, an experiment was conducted to study the efficacy of selected fungicides and bioagents under protected structures *viz.* polyhouse and rain shelter. Soil solarization was carried out inside these structures before starting the experiment. Solarization is a chemical-free way of controlling pests such as pathogenic microorganisms (mainly fungi, bacteria, and nematodes), insects, and wild plants in the soil before crops planting (Gill *et al*., 2009). Solarization technology does not release any dangerous chemical residue in the ground; it is a safe, simple, effective, and eco-friendly tool for the home gardens and fields (Kapoor, 2013). Solarizing soil in closed structures like polyhouses or in containers with a limited volume of soil may produce considerably higher soil temperatures. This pre-plant method involves soil heating by capturing solar radiations for 4–6 weeks and soil intercept the energy radiated from the sun and its temperature rise to the level that is deadly to many soil-borne pathogens. It improves soil texture and the nutrients availability in the soil which are essential for plants growth and development (Addabbo *et al*., 2010). Therefore, in the present study solarization in polyhouse and rain shelter was carried out for a period of 90 days before transplanting tomato seedlings.

5.6.1 Effect of seed treatment on seed germination and seedling vigour

Since, treatments were given as both seed treatment and foliar spray, effects of treatments on seed germination and seedling vigour were recorded and from this data vigour index was calculated. Significant difference between treatments was noticed with respect to seed germination after five days of sowing and become nonsignificant after ten days after sowing and almost all seeds were germinated. Thus indicating, there was a difference among treatments in earliness of seed germination rather than germination per cent and early seed germination was noticed in bioagents treatments than control and fungicidal treatments. Similarly, the same trend was observed in case of all biometric characters (shoot length, root length, girth) except number of leaves in which treatments were nonsignificant. Among bioagents, seed treated with PGPM mix showed earliness in seed germination and maximum value in biometric characters and it was followed by *T. viride* of KAU and *Bacillus subtilis* 1 respectively. Hence, highest vigour index also obtained for PGPM treated ones followed by *T. viride* of KAU and *Bacillus subtilis* 1. So, the present result is in accordance with the findings of James (2015) who reported that the treatment with microbial consortium consisted of *T. viride, T. harzianum, B. subtilis* and *Streptomyces thermodiastaticus* showed significantly higher vigour index of tomato seedlings than individual treatments and the control.

There are several mechanisms by which *Trichoderma* influences the seed germination and seedling vigor by secretion of the seed germination stimulating factors and phytohormone (Clear and Valic, 2013; Doni *et al*., 2014) as well as enzymes involved in nutrition absorption (Jiang *et al*., 2011). Evidences also suggest that *Trichoderma* produces plant growth regulatory material and hormone such as Indol acetic acid and their analogous vitamins, and enzyme leading to stronger root and shoot growth (Vinale *et al*., 2011). Similarly, Izzeddin and Medina (2012) and Luna-Martínez *et al*. (2013), who showed that the inoculation of tomato seeds with the *Bacillus* strains increase the germination percentages in 5 or 6 %. Moreover, *Bacillus* can influence in breaking of seed dormancy since the reduction in ethylene levels due to the ACC deaminase activity of

bacteria in the seed increases germination, along with the production of IAA that stimulates cell division in order to promote the growth of the embryo (Cendales *et al.,* 2017).

Effects of treatments on seedling vigour also revealed that, minimum seedling vigour and vigour index was with hexaconazole 5% EC (0.1%) and iprodione 25% + carbendazim 25% WP (0.2%). Moreover, it was also found that, seed treatments with fungicides *viz.* propineb (0.1% & 0.2%) and difenoconazole (0.05%) was superior over control for enhancing seedling vigour index. Reduced seedling vigour in case of seeds treated with higher concentrations of hexaconazole was also observed by Yun *et al* (2008) and Shahid (2018) in tomato and pea respectively. They opined that this may be due to the modification/inhibition of several enzymes involved in the growth, development, physiology and metabolic activities of plants. In a similar study, reduced seedling growth, delays seedling emergence and restricting the lateral growth of main root was seen by combined application of carbendazim and iprodione on cotton seeds (Baniani *et al.,*2016). Rokib and Monji (2017) noted a positive effect of seed treatment with propineb on seed germination and Vigour Index of lentil. Similarly, the research findings of Kumar *et al.* (2018) corroborate with present study, he also recorded an increased seedling vigour for wheat plants after the seed treatment with difenoconazole. Shanmugapriya *et al.* (2018) also found that difenoconazole treatment stimulated carotenoid formation in tomato plants which intern enhances the photosynthesis of the plant. Hence, this fungicide has both fungi toxic and plant growth regulating properties.

5.6.2 Effect of treatments on per cent disease incidence and severity

Next point of investigation was to find out the effect of treatments on per cent early blight disease incidence and severity in tomato plants cultivated under polyhouse and rain shelter. Since artificial inoculation was given, most of the plants both under polyhouse and rain shelter showed the disease incidence irrespective of different treatments. Even though there was no significant difference in per cent disease incidence between different treatments under polyhouse and rain shelter conditions, per cent disease incidence was more in polyhouse compared to rain shelter. This may be due to high humidity and fairly

high temperatures that prevailed for longer duration in polyhouse compared to rain shelter which are more favourable for early blight disease development (Nashwa and Kamal, 2012).

In reviewing the effect of treatments on disease severity all treatments were found superior to control at all intervals of observations, recording 53-73 and 56-74 per cent disease reduction over control in polyhouse and rain shelter respectively. In both condition, foliar application of iprodione 25% + carbendazim 25% WP (0.1% and 0.2%) and propineb 70% WP- (0.1% and 0.2%) were the most effective ones against the early blight disease of tomato. Similar findings have been recorded by Rao (2009) who observed combination product iprodione + carbendazim at 0.2% as effective fungicide for the management of Alternaria blight of sunflower. Horsfield *et al.*, (2010) and Tofoli *et al*. (2010) reported the efficacy of iprodione and propineb in the management of early blight disease of potato and thus supported our findings.

It is also observed that, both the concentration of hexaconazole showed low disease severity compared to difenoconazole foliar spray. Singha *et al.* (2017) also found that among six different fungicides tested against Alternaria leaf spot of wheat, hexaconazole was most effective followed by difenoconazole. Even though, foliar application difenoconazole was least effective among selected fungicidal treatments, it was provided 66-72 per cent disease reduction over control. Difenoconazole has both protective and curative activity and are extensively used for control of leaf spot and leaf blight diseases of cereals, tobacco, tomato, potato, grapevines and sunflower (Gopinath *et al.,* 2006; Horsfield *et al*., 2010; Mesta, *et al.,* 2011; Wang *et al.,* 2017). It is also worthwhile to mention that, in seedling vigour study, propineb and difenoconazole was superior over control for enhancing seedling vigour index compared to other fungicides.

Among bioagents treatments, foliar application of PGPM mix provides better control of early blight disease followed by *T.viride* (KAU) and *B. subtilis* 1 respectively. It is a well-known fact that, application of microbial consortium provides better performance in yield and disease control than application of single antagonist. Because microbial consortium consists of microbes with different biochemical and physiological capabilities,

which permit interaction among themselves, and will lead to the establishment of a stable and effective microbial community. Several researches have observed improved disease control using microbial consortia comprising of various biocontrol organisms like *Trichoderma, Pseudomonas, Bacillus* spp. etc in tomato, chilli, black pepper, ginger, chickpea, wheat and pigeon pea (Mathew, 2008; Srivastava *et al.,* 2010; Sarma *et al.,* 2015 Palmieri *et al.,* 2017). These biocontrol agents suppress the foliar pathogens by the same mechanism as they do in case of soil borne pathogens.

Furthermore, it is known that the genus Trichoderma comprises a great number of fungal strains exert biocontrol activity against fungal phyto-pathogens either directly or indirectly. These antagonistic properties are based on the activation of multiple mechanisms. Its inhibitory effect is attributed to extracellular enzymes, phenylalanine ammonia-lyase defense enzyme and oxidative enzymes such as polyphenol oxidase, peroxidase and superoxide dismutase, antifungal metabolites and antibiotics (Kareem, 2007; Shukla and Ratan, 2014; Altinok and Erdogan, 2015). Recently, the ability of mutualistic bioagents to induce host plant systemic resistance pathways has been successfully tested and showed that Trichoderma were capable to trigger host plant systematic resistance against *A. solani* through altering the gene expression of some involved genes in different systemic resistance pathway (Selim, 2017). Additionally, several studies showed that *Bacillus* spp. including *Bacillus subtilis* exhibit various inhibitory effects against early blight pathogen and can synthesize many biologically active compounds including antibiotics, siderophores, lipopeptides, enzymes and exopolysaccharides (Pane and Zaccardelli, 2015; Awan and Shoaib, 2019; Lastochkina *et al.*, 2019). It is also interested to notice that, in the present study efficacy of fungicides and bioagents showed same trends both under polyhouse and rain shelter conditions indicating constant performance of the selected fungicides and bioagents regardless of variation in temperature and humidity.

5.6.3 Effect of treatments on biometric characters of tomato

A good disease management strategy not only focus the pathogen and disease but it should also improve the overall plant vigour. Hence, effects of selected fungicides and

bioagents on biometric characters of tomato plants *viz.* plant height, collar girth, days to flowering and number of flowers per plant were studied. In general, biocontrol treated plants showed better performance than fungicidal treated and control plants in both structures and significant difference was found between different treatments except in case of number of flowers per plant. Among bioagents treatments PGPM mix sprayed plants expressed better plant vigour. Plant growth-promoting effects by foliar application of biocontrol agents were achieved in several crop species such as wheat (Panwar *et al*., 2014), maize (Costa *et al*., 2015), cucumber (Raj, 2016), canola (Ahmadi-Rad *et al*., 2016) and Capsicum (Pawar, 2017). Foliar spray with combinations of microorganisms were explored by Johnson *et al.* (2017) Sangiogo *et al.* (2018) for the management of leaf blight disease of coconut and common blight of bean respectively and found that combination *Trichoderma*, *Pseudomonas* and *Bacillus* resulted in greater control as well as plant growth characters. Since, these microbes can regulate phytohormone biosynthesis pathways, modulate ethylene levels in plants, and influence the emission of volatile organic compounds (VOCs) and the launch of host plants' systemic resistance/tolerance it will positively affects the physiology of plants and enhance their growth and vigour.

Among fungicidal treatments difenoconazole and propineb showed comparatively good plant vigour but, it was statistically on par with control plants and plants treated with higher concentration of hexaconazole (0.1%) and Iprodione + carbendazim (0.2%) showed comparatively less plant height and girth. It was also noticed that, plant height, earliness in flowering and number of flowers were more in plants cultivated under polyhouse condition. Plant growth promotion properties of difenoconazole were also reported by Gomathinayagam *et al.* (2007) Bhattacharjee *et al.* (2018) in cassava and malabar spinach respectively. They also found a significant increase in chlorophyll, carotenoid and xanthophyll content of the plant. In the same way remarkable increase in the total protein content, amino acid content and phenol content was also observed after difenoconazole application. Similarly, Pan and Lai (2009) observed enhanced growth of rice seedlings in addition to the control of sheath blight after the foliar application of propineb.

The effectiveness of a disease management strategy will be complete, when it coincides with the increase in crop yield. So, effects of selected treatments on tomato yield

parameters such as average number of fruits per plant, fruit weight and yield per plot were studied both under polyhouse and rain shelter. It was found that all treatments under polyhouse and rain shelter were significantly superior to control in all the observed yield attributes. Moreover, trends of selected treatments were more or less same under both conditions. However, tomato yield obtained from polyhouse was very less even though a greater number of flowers recorded in polyhouse than rain shelter. Earlier studies point out several reasons for this. If daily mean temperatures inside the polyhouse is 29 or more then, fruit number, percentage fruit set and fruit weight per plant will decrease. This reduction in yield is mainly due to impaired pollen and anther development and reduced pollen viability (Sato *et al.,* 2002). Sensitivity of the reproductive stage of the flower to above optimal air temperature can cause a reduction in percentage fruit set and thus decrease the fruit yield during commercial tomato growth (Sato *et al.*, 2006). Another factor that might influence pollen viability inside the polyhouse is relative humidity in the air. Relative humidity between the range of 50%–70% is generally considered to be optimal for tomato pollination (Nepi *et al.*, 2010). Trials that tested tomato pollen quality and fruit set at several air humidity levels found that increased humidity (60%–70% RH) improved pollen and fertilization in comparison with 30%–40% RH. (Huang *et al.,* 2011). However, increasing humidity to 90% may increase pollen susceptibility to heat stress. In addition to these, although tomato flower is self-fertile, the structure of the anther core, it's mode of dehiscence and the position of the style make some form of disturbance necessary to ensure adequate pollination in low or high temperatures (Harel *et al.,* 2014). Nazer *et al.* (2015) also found that fruit setting of tomatoes grown in polyhouses was frequently poor and fruit set is very dependent on the use of mechanical aids or Bumblebees.

Under both conditions, the maximum yield was obtained from plants treated with the combination fungicide iprodione 25% + carbendazim 25% WP and this was followed by plants treated with propineb (0.2%). Moreover, all fungicidal treatments were superior to control as well as bioagents with respect to the tomato yield. However, average fruit weight was higher for bioagents treated tomato plants compared to fungicidal treated ones. Increased tomato yield with iprodione + carbendazim treatment was in agreement with findings of Amaresh and Nargund (2002) and Prasad *et al.* (2015). They also reported that

among different fungicides tested, seed treatment with iprodione + carbendazim combined with foliar application of same fungicide 0.2% two times at 15 days interval reduced leaf blight and increased seed yield of sunflower. However, they also mentioned that application of this fungicide gave the highest cost- benefit ratio. Similarly, the minimum early blight disease intensity and maximum yield in tomato plants by the foliar application of propineb was observed by Sharma *et al.* (2018). Moreover, the findings of Hafez *et al.* (2018) were also in line with the present study and he reported that bioagent treatments significantly increased fruit weight in Squash (*Cucurbita pepo* L.) than fungicidal treatments. In addition to this, the experimental results from rain shelter II in which same treatments were superimposed were also confirmed the efficacy of selected treatments against early blight disease as well as yield of tomato.

5.6.4. Meteorological parameters

Temperature and relative humidity are the two factors that closely correlate with the occurrence of the early blight disease of tomato under protected cultivation. Hence, temperature and relative humidity inside the poly house and rain shelter was recorded at 7.30 am and 2.30 pm daily during the experiment. As similar to the earlier reports (Raj, 2016 and Pawar, 2017), compared to rain shelter the maximum temperature and relative humidity were recorded in polyhouse and this may the reason for higher per cent disease severity in plants cultivated under polyhouse. Chothanil *et al.* (2017) also reported that warm, humid environmental conditions are conducive to early blight infection in tomato and *Alternaria* conidia germinate fastly at temperature range of 28-30°C. Moreover, a significant positive correlation was found between severity of early blight disease and temperature in polyhouse and with relative humidity in rain shelter. A similar observation was also made by Devi *et al.* (2017) who stated that maximum temperature ranging between 25.5 and 36.7^0C found to have a significant positive correlation with tomato early blight disease severity.

5.6.5 Residue analysis

Tomato is a one of the most important vegetable components of the diet and are consumed raw, cooked or processed. Hence, fungicidal residues on tomato fruits constitute a possible risk to consumers and have been a human health concern. So, in present study residue analysis was carried out to find fungicide residue level in tomatoes which were harvested from plants treated with hexaconazole, difenoconazole and carbendazim in polyhouse and rain shelter. According to the standards of Food Safety and Standards Authority of India (FSSAI) the maximum residue limits (MRLs) for hexaconazole, difenoconazole and carbendazim were 0.4ppm, 0.5ppm and 0.1ppm. So, the present results indicate that among fungicide tested carbendazim has more residual effect and it was above MRLs value even after seven days of spraying while hexaconazole has least residual effect and its presence were below detectable level three days after spraying. Whereas difenoconazole residue become below detectable level seven days after spraying. Hence, except carbendazim all detected residues from tomato samples were below the maximum residue limits (MRLs). Das *et al.* (2003) and Liu *et al.* (2014) also observed the presence of carbendazim above MRLs value even after seven days after spring in tomatoes as well as in apples. They also observed that techniques washing, blanching, peeling, pureeing, cooking, roasting, frying and boiling of vegetables and fruits will help to reduce the fungicide content in the sample. From the point of hazards due to residues they also suggested a waiting period of ten days after carbendazim application for tomato plucking.

5.7 Effect of treatments on non-target culturable microflora

Fungicides and bioagents have been used extensively for controlling fungal pathogens of plants. However, little is known regarding the effects of these fungicides upon the non- targeted indigenous microbial communities that present on the leaves (Phylloplane microbes) as well as microbes resides within the plant (Endophytic microbes). These microbial communities play a major role in plant functioning and vigour. Hence, an attempt was done to study the impact of selected treatments on culturable phylloplane and endophytic microbes of tomato cultivated under polyhouse and rain shelter by serial dilution plating.

5.7.1 Effect of treatments on phylloplane microflora

Enumeration of phylloplane microbes showed that, before treatment application, natural population of phylloplane fungi and bacteria was comparatively more in rain shelter tomato than in polyhouse whereas phylloplane actinomycetes population was more in polyhouse tomato plants. Such changes in population of phylloplane microbial communities were also observed by many researchers **(**Jurkevitch and Shapira**,** 2000; Lindow and Brandl, 2003; Gu *et al.,* 2010; Ikeda *et al*., 2011). They reported that phylloplane communities are greatly influenced by microclimate of the plant and among which temperature, humidity and availability of free water are important ones. Since temperature and relative humidity were more in polyhouse condition, population of phylloplane fungi and bacteria were comparatively less in polyhouse while actinomycetes prefer higher temperature range (Akond *et al.,* 2016 and Pudi *et al.,* 2016) hence more population observed under polyhouse condition.

However, effects of treatments were reflected on tomato phylloplane microbial population under both conditions. It was found that application of fungicides on tomato plant drastically reduced the tomato phylloplane microflora while bioagent applications response was varied depending up on the type of bioagent. Significant difference was found between different treatments with respect to population of phylloplane fungi and bacteria but treatments were more or less uniform with actinomycetes population. Plants treated with iprodione 25% + carbendazim 25% WP (0.2%) showed maximum reduction of phylloplane microbes and difenoconazole 25% EC (0.05%) treated plants recorded minimum reduction among fungicidal treatments.

The use of foliar fungicide to control diseases can cause major disruption of phylloplane microorganism populations, often reducing the number and diversity of organisms. Today, intensive chemical sprays change the natural balance of the phyllosphere microflora in favors of pathogens. Many studies showed that after chemical spray applications, number of microorganism species decline, composition and diversity of the microorganisms change and some microorganisms even disappear from the phyllosphere microflora (Calhelha, 2006; Elmholt, 2013; Newton *et al.,*2010; Raj, 2016;

Pawar, 2017). Similar to the present result, Cadez *et al.* (2010) and Minambres *et al.* (2012) also reported the non-target effect of iprodione and they state that it can modify the population structure of the soil and phylloplane bacterial and fungal community. Kozak and Erkilic (2018) noticed that iprodione had severe effects on non-target fungal populations of citrus leaves. Moreover, it was also found that carbendazim- very popular MBC fungicides widely used in crop production can also influence the beneficial arbuscular mycorrhiza fungi, bacteria and mammalian cells (Wang *et al.,* 2009: Clement *et al.,* 2010; Yang *et al.,* 2011). Some recent research also reported the association of carbendazim in the inhibition of nitrification in soil, a microbially mediated process and the negative effects of this fungicides last long 6 months (Batool *et al.,* 2016 and Trinidad *et al.,* 2018). The findings of Kozak and Erkilic (2018) were also inconformity with the present result that spraying of propineb caused comparatively more phylloplane microbial reduction in citrus than difenoconazole but, the negative effects of these fungicides disappeared 15 days after application, then the fungal population started to increase again. Hence, these studies showed that more knowledge on the effect of fungicides on phyllosphere microbial communities is important in order to optimise fungicide application strategies.

In general, phylloplane fungal population was increased with the application of bioagent *Trichoderma viride* and PGPM mix, bacterial population was increased with PGPM mix and *Bacillus subtilis* I while population of actinomycetes increased in plants treated with *B. subtilis* I. This increase in fungal and bacterial population may be due to the presence of fungal and bacterial biocontrol agents itself that was given as foliar spray. Since phylloplane is considered as hostile habitat for for survival and colonization by microorganisms (Bulgarelli *et al.,* 2013), then the possible mechanism used by these biocontrol agents may be competition for space and resources so that, they can quickly colonised with a higher colony count (Mueller and Ruppel, 2014). In addition to this, compared to inoculation into soil, spraying biocontrol agents on plant leaves cannot change the soil physicochemical properties, thus the most likely mechanism in increasing the phylloplane fungal and bacterial population by BCAs is changing microbial community in the phyllosphere. Similar observation was also made by Qin *et al.* (2019) while studying

the responses of tobacco phyllosphere microbiota and plant health to application of two biocontrol agents such as *Bacillus* and *Stenotrophomonas* and he found that its application increased the overall phylloplane bacterial population by changing microbial community that is in addition to the population of *Bacillus* and *Stenotrophomonas,* the tobacco leaves showed higher population of *Sphingomonas* and *Pantoea* which are often considered as plant beneficial microbes.

It was interesting to noticed that, under polyhouse and rain shelter condition phylloplane actinomycetes population was reduced in all the fungicidal and biocontrol treatments except in *B. subtilis* treated plants. Andryan *et al.* (2016) and Singh *et al*. (2016) also observed the reduction in phylloplane actinomycetes population due to different fungicidal treatments. Similarly, Boruta and Paluszak (2016) also observed reduction in Actinokineospora *an actinomycetes naturally present in tobacco phylloplane after the application of Trichoderma koningii.* Although the importance of phyllosphere microbes on plants is well recognized, the ecological effects of BCAs on phyllosphere microbes and the relationships between phyllosphere community and plant health are complex and poorly understood. Therefore, the response of microbial interactions within the phyllosphere to BCAs may also be an important aspect to assess the efficacy of BCAs.

5.7.2 Effect of treatments on endophytic microflora

Recently, much importance is given for endophytic microbial population in disease management as well as plant health. Endophytic bacteria reside in specific tissues of the plant and develop a close association with the plant, with exchange of nutrients, enzymes, functional agents, and also signals (Sartori *et al.,* 2005). Endophytes colonize their plant host tissues in which they persist without exerting the negative effects of a pathogen. On the contrary, the presence of these endophytes in the host plant leads to beneficial effects on its health and/or growth. The endophytic community can be affected naturally by changes in environmental conditions and soil types. They can also be affected by the application of agrochemicals (Stuart *et al.,* 2018) which can affect the plant life cycle and

metabolism. Thus, an investigation was done to assess the effects of selected treatments on leaf, stem and root endophyte community associated with tomato.

In case of endophytic fungi and bacteria it is clearly evident that, all chemical treated plants exhibited a population reduction whereas in case of actinomycetes significant difference was not observed. In general, among the chemical treatments spraying of iprodione 25% + carbendazim 25% WP (0.1% and 0.2%) and hexaconazole 5% EC (0.1%) showed maximum reduction of endophytic microbial population while contact fungicide propineb recorded minimum in case of stem and root fungal and bacterial endophytes and difenoconazole treated plants recorded minimum reduction of leaf endophytes. Gaitan *et al*. (2005) also reported drastic reduction in non-targeted endophytic fungal population in *Guarea Guidonia* -a tropical tree after the spraying of Benomyl, a carbendazim fungicide. Pimentel *et al*. (2006) also observed a decrease in the number of endophytes between the first and second sampling events in a study on soybeans planted under field and greenhouse conditions after the foliar application of hexaconazole. The present observation was also in line with John and Charles (2014), they reported 10 to 1000-fold reduction in endophytic microbial populations on fungicide treated apple leaf. Collectively the current results and the emerging microbial ecology model indicate that the development of fungicides especially, systemically active agrichemicals should include assessment of effects on endophytic microbes.

Biological control agents (BCAs) are perceived to have specific advantages over synthetic fungicides, including fewer non-target and environmental effects, efficacy against fungicide-resistant pathogens, reduced probability of resistance development (Cook, 1988), and use in organic farming situations where synthetic fungicides are restricted (Harman, 2000; Tsror *et al*., 2001). However, the micro-organisms involved may have detrimental impacts on other organisms present in the plant system to which they are applied. Brimner and Boland (2003) reviewed the potential non-target effects of BCAs including competitive displacement, toxicity, and pathogenicity. Competitive displacement occurs when a BCA expels or replaces native non-target species through competition for space or nutrients. In the present study, biocontrol treatments showed different responses

towards the endophytic microbial population. Plants treated with biocontrol agents such as *T. viride* and PGPM mix exhibited an increase in fungal endophytes while reduction in fungal endophytes recorded with *B. subtilis.* Similarly, biocontrol agents such as PGPM mix and *B. subtilis* exhibited an increased endophytic bacterial count. However, *T. viride* treated plants showed reduction in leaf and root endophytic bacterial population while it recorded an increase in case of stem bacterial endophytes. Many studies revealed that continuous use of a particular biocontrol agent will cause reduction in the diversity and/or abundance of plants innate microbial community as well as soil microbial community (Pimentel *et al*., 2006; Elmholt (2013); Rachel *et al*., 2014). This may have significant environmental impacts. For example, continuous soil application of *T.harzianum* was shown to reduce germination of resting spores of *Glomus intraradices*- an arbuscular mycorrhizae (Rousseau *et al.,* 2014). Similarly, the continuous application of *T. viride* in peanut caused significant reductions in the number and weight of root nodules formed by *Rhizobium*. This may be due to the ability of *T. viride* to grow quickly in the soil and colonise plant surfaces, thus preventing the subsequent invasion of roots by the bacteria (Naseby *et al.,* 2015). Another finding that is in agreement with the present study was by Ros *et al.* (2017), he also noticed a general increase in endophytic population with the application of two *Trichoderma* strains (*T. harzianum* and *T. asperellum*) in pepper seedlings

The results also revealed that, population of tomato fungal endophytes was more in leaves compared to roots and stem while bacterial and actinomycetes population was more in roots. Moreover, trends of different were same under polyhouse and rain shelter conditions. However, natural population of endophytic fungi and bacteria was more in plants cultivated under rain shelter whereas actinomycetes population was more under polyhouse condition. Since, treatments were given as foliar spray the changes due to different treatment application was more in leaf compared to stem and root bacterial endophytes. Previous studies suggested that some environmental conditions, such as temperature, humidity, illumination, geographic location, and vegetation significantly affected the distribution pattern of endophytic microorganism (Suryanarayanan *et al*., 2005; Song *et al.,* 2007). So, in the present case, difference in temperature, humidity and

illumination between polyhouse and rain shelter may the reason for variation in endophytic microbial population.

5.8 Survival of the biocontrol agents on tomato phylloplane

Insufficient research efforts have been directed towards selection for characteristics of biocontrol agents which survive well, or towards environmental conditions capable of enhancing the survival and activity of the biocontrol agent. It is obvious that a biocontrol organism will not persist and be active unless it is adapted to the plant environment; moreover, to be successful in controlling the pathogen, the introduced biocontrol agent must compete with other microorganisms and establish an active population on the phylloplane (Bonaterra, *et al*., 2009). This is one of the least understood aspects of biocontrol. Hence, the next aspect of investigation was to find out the survival of biocontrol agents sprayed on the tomato leaves. On spraying biocontrol agents like *Trichoderma* and *P. fluorescens,* there was a general increase in this biocontrol agent's population and it gradually decreased after ten to fifteen days of spraying but it never came down to the initial population count that exist before spraying. In addition to this, it was also found out that biocontrol agents once sprayed on leaves survive there for 15 days or more. The ability of this biocontrol agent to survive and to establish an active population in the phylloplane may be affected by phyllosphere inhabitants, nutrients, and microclimatic conditions. Apart from the agent's antagonistic activity, effective biocontrol involves also the ability of the agent to survive in the habitat where it is applied. The results of the present study confirm the findings of Raj (2016) and Pawar (2017) where the survivability of *T. viride* and *P. fluorescens,* applied individually on cucumber and capsicum leaf surfaces respectively, was assed and they also reported more than 15 days of biocontrol agent's survival and observed immediate increase and gradual decrease of *T. viride* and *P. fluorescens* population.

5.9. Metagenomic analysis to assess the impact of foliar spray on non-target microflora

As we discussed earlier, it should be considered that chemical and bioagents applications cause harmful effect not only pathogens but also non-target microorganisms which resides in and around the plant system and play a major role in plant functioning and vigour. Hence, it is necessary to evaluate the impact of fungicides and bioagents on non-targeted microbes while formulating disease management strategies. Although studies of different crops microbial community have been conducted, these were based on isolation/culture techniques. At the same time, it is a well-known fact that, 99% of the total estimated microbial diversity cannot or are extremely difficult to culture, as well as rare taxa that are usually missed by culturing techniques (Chen and Pachter, 2005). Next generation sequencing (NGS), together with the evolution of bioinformatic tools, and the emergence of metagenomic approaches have made it easier to comprehensively analyze microbial communities on or in any type of matrix, including plant tissues. In recent years researchers have widely applied these new technologies in relation to plant pathology and plant microbial ecology studies (Rastogi *et al*., 2013; Romero *et al.*, 2014; Aragón *et al*., 2017). One of the main advantages of metagenomic approaches over culture-dependent methods, is the ability to detect all organisms that possess the targeted barcode gene. The metagenomic toolbox allows accessing, storing, and analysing this DNA and thus can provide an otherwise hard-to-attain insight into the biology and evolution of environmental microorganisms, independent of their culturable status (Bodenhausen *et al.*,2013). Thus, in the present study metagenomic analysis was carried out to access the impact of foliar spray on non-target microflora especially on bacteria and fungi.

Obtaining good quality metagenomic DNA is the major challenge in metagenomic studies. Since fungicides were given as foliar spray, metagenomic DNA was isolated from tomato leaves using DNeasy Plant Mini kit (QIAGEN, Dusseldorf, Germany). This kit procedure included a combined physical and chemical DNA lysis step followed by filtering of impurities after binding the DNA to the inhibitor removal column. The good quality DNA was then eluted using suitable buffers. The metagenomic DNA obtained was of good quality, devoid of RNA contamination and intact band was obtained on agarose gel.

Metabarcoding using 16S rRNA marker is widespread in the studies of various microbial communities. The 16S rRNA gene which encodes the 16S ribosomal RNA, is 1500 bp in size and is composed of nine variable regions interspersed between conserved regions. Delmotte *et al*. (2009) reported that 16S rRNA gene is the best molecular chronometer with greater resolving power than other oligonucleotides used earlier for cataloguing purposes. 16S rRNA gene was used to classify bacteria based on the difference in the hyper-variable regions by designing specific primers to amplify the mentioned hyper-variable regions. The 16S rDNA sequences generated using Illumina sequencing was found to be an effective tool in analyzing the diversity and taxonomic assemblage in environmental metagenomes (Alves *et al*., 2018). Bukin *et al.* (2019) compared the resolution of V2-V3 and V3-V4 16S rRNA regions for the purposes of estimating microbial community diversity using paired-end Illumina MiSeq reads, and show that the fragment, including V3 and V4 regions, has higher resolution for lower-rank taxa (genera and species). It allows for a more precise distance-based clustering of reads into species-level OTUs. In the present investigation also, the hyper-variable V3 and V4 regions were used for investigating the diversity of bacteria from four tomato leaves samples which was collected from plant sprayed with propineb (0.2%), Iprodione + carbendazim (0.2%) and PGPM and also from control plants.

The internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) are the most predominant DNA barcode sequences used for fungal metabarcoding. These regions can be easily amplified and sequenced with universal primers and the corresponding ITS sequence data is highly represented in GenBank and other databases (Pruesse *et al*., 2007). The choice of using either ITS1 or ITS2 is optional since these regions share many properties, and enable similar levels of discrimination (Bazzicalupo *et al*., 2013). However, ITS2 is generally used because it is less variable in length, lacks the problem of co-amplification of a 5`SSU intron, and is better represented in databases than ITS1 sequences (Nilsson *et al.*, 2013). Hence, in the present study ITS 2 was used for fungal barcoding.

In metagenomic analysis, a massive amount of data is generated from NGS, which is anlaysed using various bioinformatics tools. Several platforms are available for NGS, which includes Roche-454, Illumina and Ion Torrent. In the present investigation, we followed Illumina because Illumina platform produced comparatively higher output (number of reads) which resulted in better detection of the microbes per run (Frey *et al.,* 2014). Illumina workflow involves amplification of V3-V4 regions (bacteria) and ITS 2 region (fungi) using suitable primers, ligation of adapters and dual indices, followed by library quantification. The denatured library was then subjected to MiSeq sequencing. The obtained sequences were in Fastq format. The quality of the Fastq sequences were analysed and these were trimmed and filtered to remove mismatches, chimeras and singletons to obtain total number of Operational Taxonomic Units (OTUs). To confirm the accuracy of taxonomic assignments, rarefaction analysis was performed and it indicated that the sequencing depth had been saturated for all the sample. This curve is a plot of the number of species as a function of the number of sequences in a sample. The vertical axis displays the diversity of the community, while the horizontal axis displays the number of sequences considered in the diversity calculation (Romero *et al.,* 2014).

The number of fungal and bacterial OTUs from all the four samples were analysed and it was found that, the tomato leaves sample which was collected from plants treated with PGPM mix had comparatively a greater number of bacterial and fungal OTUs than others. This was followed by control plants sample and propineb treated leaves sample respectively while bacterial and fungal OTUs were comparatively minimum in samples collected from Iprodione + carbendazim sprayed plants. OTUs are clusters of sequences, frequently intended to represent some degree of taxonomic relatedness, each resulting cluster is typically representing a same species. In the metagenomic sense, an OTU is described as a cluster of 16S gene sequence (bacteria) and ITS (fungi) variant with a minimum of 97 per cent identity threshold at genus level. An increased identity threshold at 98 or 99 per cent is considered to be suitable for species level classification. The total number of OTUs was measured to compare the bacterial and fungal population among the samples. It was also found that all the samples occupied more fungal OTUs than bacterial OTUs indicating higher fungal population in tomato leaves compared to bacterial

population. Similar observation was also made by Ogwu and Osawaru (2014) and Luo *et al.* (2019) in okra and cucumber respectively under protected cultivation. They also pointed out that, leaf microbial community structure were affected strongly due to changes in the relative abundance of those "key" microbes induced by abiotic or biotic factors. For example, it can be influenced by the plant species, season, geographical location, and different environmental conditions.

The primary challenge of microbial metagenomic sequence analysis using long reads is the comparison of input sequences against a large reference database of whole genomes from bacteria, viruses, fungi, etc. Although a number of algorithms have been developed for alignment of long, error-prone reads, those sensitive algorithms are not optimized for the challenge of comparison against the large and ever-expanding universe of microbial genomes (Chaisson and Tesler, 2012). The bioinformatic methods used in this analysis were One Codex for fungi and MG-RAST for bacteria, each compare the input reads against their own more concise reference databases, providing an assignment for the most likely origin of each individual sequence.

One Codex, which uses exact *k*-mer alignment to classify sequences against a reference database of ~40 000 complete microbial genomes *viz.* fungi, bacteria, viruses, protists, and archaea (Minot *et al.*, 2015). Based on the analysis of the complete ITS2 data set, members of the phylum Ascomycota were dominant in all samples, collectively accounting for 78 to 90 per cent of the total number of detected sequences. Remaining sequences were assigned to phylum Basidiomycota. Higher proportion of ascomycetes fungi has been reported from various crop's leaves/ phylloplane (Perazzolli *et al.,* 2014; Abdelfattah *et al.,* 2016; Luo *et al.,* 2019). Read data at phylum level revealed that fungal population was comparatively less in chemically treated samples than control as well as bioagent treated samples. Among the two fungicidal samples, fungal population obtained from iprodione + carbendazim treated tomato leaves were less compared to propineb treated ones. Moreover, in bioagent (PGPM mix) treated leaf sample, fungal population was more than control. Similar results were recorded in enumeration study of non-target microbes by dilution plating. Thus, once again confirmed effects of the selected treatments on non-target fungal population.

Since artificial inoculation of *Alternaria* was given in all plants before the treatment application and it is being a member of class Dothideomycetes, in all the samples Ascomycota sequences were largely identified as members of the class Dothideomycetes. Apart from this the dominance was varied between all the samples indicating effects of different treatments on tomato leaves fungal flora. High abundance of Dothideomycetes and Ustilaginomycetes in tomato and cucumber leaves was also reported by Toju *et al.* (2019) and Luo *et al* (2019) respectively. In sample TR2 where propineb was sprayed, Dothideomycetes, Eurotiomycetes, Ustilaginomycetes, Agaricomycetes and Sordariomycetes were the first five dominant class in their respective order. While in iprodione + carbendazim treated leaves, the order was Dothideomycetes, Eurotiomycetes, Sordariomycetes, Agaricomycetes, Ustilaginomycetes respectively. Similarly, in PGPM treated leaves, the pattern was Dothideomycetes, Sordariomycetes, Ustilaginomycetes, Eurotiomycetes and Agaricomycetes whereas in control plants the order was Dothideomycetes, Ustilaginomycetes, Agaricomycetes, Eurotiomycetes and Sordariomycetes. These results indicate that, natural fungal population structure on tomato leaves was greatly changed due to the foliar application of fungicides and bioagent.

Since artificial inoculation of early blight pathogen *Alternaria* was given before the treatment application, all the four samples showed the high abundance of genus *Alternaria.* The maximum population of *Alternaria* was observed in control followed by leaves sample from PGPM mix, propineb and iprodione + carbendazim treated plants respectively. Hence, the present observation once again confirmed the results of disease blight management study. Apart from *Alternaria,* order of dominance was varied between samples. Other predominant genera in propineb treated leaf were *Aspergillus, Moesziomyces, Auricularia, Pseudocercospora, Corynespora* respectively while it was *Moesziomyces, Cladosporium, Auricularia, Corynespora, Fusarium* in iprodione + carbendazim treated leaves. Since *Trichoderma* was one of the components of PGPM mix, this was the second dominant genus in PGPM treated leaf samples followed by *Helminthosporium, Meira, Aspergillus* and *Pseudocercospora*. In control samples after *Alternaria,* first five abundant genera were *Moesziomyces, Auricularia, Pseudocercospora, Corynespora* and *Cladosporium* respectively.

Even though there was variation in fungal flora between different samples, these samples share some common predominant genera like *Alternaria, Auricularia, Cladosporium, Corynespora, Moesziomyces* and *Pseudocercospora.* It was important to noticed that, along with early blight pathogen *Alternaria,* other pathogenic genera *Cladosporium, Corynespora, Pseudocercospora* were also present in tomato leaves and these pathogens will also produce leaf spot and leaf blight symptoms. Das and Bhattacharya (2008) and Tej *et al.* (2013) were observed that leaves collected from the same host and same locality were colonized by different genus of Dothideomycetes fungi like *Alternaria, Cladosporium, Corynespora, Pseudocercospora.* Furthermore, they also found that, close association of these fungi may also help to accelerate the leaf spot and blight symptom in tomato leaves. Similarly, Leye (2013) also reported abundance of *Moesziomyces* within the leaf-associated microbiome of tomato plants. A recent phylogenetic study of teleomorphic (*Moesziomyces*) and anamorphic (*Pseudozyma*) specimens suggested that this Ustilaginaceae taxon could involve not only phytopathogenic species but also species with antifungal properties against the causal agent of cucumber powdery mildew (*Podosphaera fuliginea*) or species that can induce resistance of host plants against fungal pathogens such as *Botrytis cinereal* (Toju *et al.*, 2019). In addition to these genera, genus *Aspergillus* was present in all the samples except in TR7. With the advent of DNA sequence comparisons, a positive correlation between *Alternaria* spp. and *Aspergillus* spp. were reported, but their exact relationships remain poorly resolved and await additional evidence from genome-scale analyses (Hofmann *et al.*, 2010).

In addition, the samples TR7 possessed two unique genera *Anthracocystis* and *Fusarium*. Similarly, the sample TR2 also had unique genera *Exserohilum* while it was *Meira* in TR9. The members of *Anthracocystis* are predominantly tropical species and a negative correlation between the population of *Alternaria* and *Anthracocystis* was recorded by Denchev and Denchev (2013) in wheat leaves . Similarly, previous experiments showed that *Fusarium* and *Alternaria* genera can influences each other's growth and metabolic profile, suggesting complex interactions between them during the infection process, which have been described as competitive (Sab *et al.*, 2012 and Muller *et al.*, 2015). Field studies on plants observed a negative correlation between their infection rates. Subsequently they

have been classified as members of different species clusters, or functional types, which are assumed to have different "lifestyles" or ecological niches. Hence, it was interested to noticed that, even though fungicide application reduces the total fungal population, it will help to modify the fungal composition that will adversely affect the pathogenic population. The present observation was in line with the findings of Balint and Stapleton (2011) they demonstrated that application of streptomycin on leaves of maize changed the microbial community in such a way that it provided greater resistance against the foliar fungus *Cochliobolus heterostrophus*, causative agent of Southern leaf blight. The genera present in control plants' sample but absent in treated samples were *Acremonium* and *Malassezia* in TR7 and TR9 and *Coprinopsis* in TR2 and TR9 indicating these genera was affected by the fungicidal and bioagent treatments.

According to the fungal diversity analysis using diversity indices and number of taxa, it was found that fungicidal and bioagent treatments was changed the structure of indigenous fungal communities on tomato leaves. The biocontrol treatment (PGPM mix) increased the fungal diversity and population while both the fungicidal treatments reduced fungal diversity and population. Systemic fungicide iprodione + carbendazim treated leaves occupies a very narrow range of fungal flora compared to the contact fungicide propineb. Chemical or fungicides applications have been reported to have a significant impact on non-target organisms and reduce overall genetic diversity ((Pinto *et al.*, 2014 and Singh *et al.*, 2015). Karlsson *et al.* (2014) compared leaf fungal population of chemically treated wheat against organically grown wheat and reported that fungicide treatment a significant impact on fungal species richness and evenness on the other hand, fungal species richness and evenness were found to be significantly higher in organically grown wheat leaves. Supporting to the present study, fungal diversity analyses through metagenomics revealed that exposure of strawberries to chemical treatments resulted in a significant reduction on the fungal composition of leaves and flowers while the application of biocontrol treatments also found to shift fungal composition in leaves and flowers but microbial population as well as diversity were enhanced (Abdelfattah *et al.*, 2016).

Effects of fungicidal and bioagent application on non- target bacterial population was also studied by metagenomic approach using MG-RAST tool. MG-RAST

(Metagenomic analysis by Rapid Annotation using Subsystem Technology) is optimized for analyzing short-read, low-error data and it is hosted by Argonne Laboratory was used to taxonomically characterize the OTUs using RDP (Ribosomal Database Project) database. RDP is an rRNA sequence database containing ribosomal information of bacterial and archaeal sequences (Meyer *et al.,* 2008). Based on the metagenomic analysis of the complete 16S data set, an increased population of Firmicutes was observed in all the four samples at phylum level and maximum Firmicutes population was obtained from plants sprayed with PGPM mix followed by control plants, propineb and iprodione + carbendazim treated plants respectively. Proteobacteria and Actinobacteria were the second and third abundant phylum in all samples except in iprodione + carbendazim treated plants in which Bacteroidetes was the third most abundant phylum. Predominance of *Fermicutes*, *Proteobacteria* and *Actinobacteria* as leaf communities of different agricultural crops (e.g. wheat, rice, apple, lettuce, and spinach) and naturally growing plants/trees had been reported by several researchers, although the proportions of individual taxa can vary depending on plant species and phenotype, geographical location, time of year, and human intervention such as application of chemicals and bioagents (Redford, *et al.,* 2010; Lopez-Velasco *et al.,* 2011; Knief *et al.*,2012).

Moreover, PGPM mix treated leaves showed three unique phyla such as Deinococcus-Thermus, Spirochaetes and Tenericutes. Phylum Deinococcus-Thermus and Spirochaetes include several bacteria which helps in plant growth promotion (Yadav *et al.,* 2017) while in contradictory to this Tenericutes is a bacterial Phyla encompasses the phytoplasmas, which are regarded as plant pathogens, infecting up to 98 plant families (Lee *et al.,* 2000). Wang *et al.* (2020) detected habitat-specific functional patterns between Tenericutes and Bacillus spp. where common genes are involved in carbohydrate storage, carbon fixation, mutation repair, environmental response and amino acid cleavage as a result of environmental adaptation. Since Bacillus is one of the components of PGPM mix this may be the reason for presence of Tenericutes in this sample. Similarly, phylum Planctomycetes was observed only in fungicides (propineb and iprodione + carbendazim) treated samples. Zhang *et al.* (2019) also made an important observation on population of Planctomycetes and fungicides. He noticed that Planctomycetes population was more in

different soil animal's gut microbiome which are present in azoxystrobin applied soils compared to non-applied soils. However exact mechanism or relationship between fungicides and Planctomycetes population was not yet studied properly. Gemmatimonadetes was only phylum which was present in control but absent in all the treated samples indicating that bacteria under this phylum are very sensitive to both chemical and bioagent application.

The results also showed that maximum reads were assigned in sample TR9 followed by TR11, TR2 and TR7 respectively indicating higher bacterial population in PGPM mix applied leaves and minimum in combination fungicide iprodione + carbendazim. Gu *et al.* (2010) and Moulas *et al*. (2013) also reported significant changes in the bacterial communities of the wheat and pepper phyllosphere with the spraying of fungicides like enostroburin and metalaxyl. Furthermore, Zhang *et al.* (2010) assessed the effect of *Bacillus thuringiensis* (Bt), a biocontrol agent on the microbial communities within the pepper plant phyllosphere using culture-independent methodologies and found that Bt application enhances abundance of Gammaproteobacteria.

The tomato leaves microbial community composition were affected strongly due to the application of chemical and bioagent. The most abundant known genus present in propineb treated tomato leaves was *Burkholderia* and remaining once among top ten genera were *Paenibacillus, Desulfotomaculum, Selenomonas, Clostridium*, *Kurthia*, *Candidatus Portiera*, *Acinetobacter, Curtobacterium,* and *Veillonella* respectively Members of the genus *Burkholderia* belong to the class *β-Proteobacteria* and are widely distributed in the environment. *Burkholderia* are diazotrophic particularly abundant in soil where they can be associated with a wide range of plants and its presence was detected from different plant parts such as root, stem and leaves (Elliott *et al.*, 2009; Carlier and Eberl, 2012). Both plant growth promotion abilities and biocontrol efficacy against different fungal pathogen were reported for *Burkholderia*. Antagonistic behaviour of *Burkholderia* species is well described and is largely due to the production of multiple antifungal compounds ability of *Burkholderia* to establish a close association with fungi mainly lies in the capacities to utilize fungal-secreted metabolites and to overcome fungal defense mechanisms (Pamela *et al.*, 2015). Similarly, strains of *Paenibacillus*, *Curtobacterium* and *Acinetobacter* play

an important role in plant growth promotion, as certain strains of this genus are known to be involved in phytostimulation based on the production of plant-growth-promoting hormones, solubilization of phosphate and production of siderophores (Sachdev *et al*., 2010 and Bulgari *et al.,* 2014). Hence these indicate that even though foliar application of propineb decreases the total leaf bacterial community it modified the bacterial community in such a way that it provided better plant growth and greater resistance against the foliar fungus and this may one of the reasons for the increased tomato yield obtained during the field experiment.

In iprodione + carbendazim treated as well as in control sample, genus *Selenomonas* was found to be the most abundant bacteria. The sample TR7 also occupies bacterial population from the genera such as *Arthrobacter, Methylobacterium, Clostridium, Kurthia, Brevibacterium, Marinomonas, Acidobacterium* and *Leptotrichia*. Members of genus *Selenomonas* belongs to phylum Firmicutes and class Negativicutes. They are mainly found in the gastrointestinal tracts of animals, in particular the ruminants and helps in fiber digestion. But recent phylogenetic studies revealed that they were actually inhabitants of plants leaves and adapted to animal gut microflora during the course of evolution (Sawanon *et al.,* 2011; Aragón *et al.,* 2017). Pristas *et al.* (2011) observed that population of was induced by the presence of methanol. Methanol is abundantly present on plant leaf surfaces as a byproduct of pectin demethylation during plant cell wall metabolism (Galbally & Kirstine, 2002). Hence this may the reason for presence of this genus in all the samples except in PGPM mix sprayed plants indicating the effects of PGPM mix treatment on *Selenomonas* population. Furthermore, it was found that foliar application of the propineb, iprodione + carbendazim treatments and PGPM reduced the population of *Selenomonas.*

Similarly, the most abundant genus occupied in tomato leaves treated with PGPM mix was *Sphingomonas* followed by *Arthrobacter, Paenibacillus, Brevibacillus, Bacillus, Pantoea, Pseudomonas, Lysinibacillus, Lyngbya* and *Micrococcus* respectively. Vorholt (2012) also identified *Sphingomonas, Bacillus, Pseudomonas* and *Pantoea* as phylloplane bacterial genera in different crops *Sphingomonas* spp. is a Gram-negative, rod-shaped aerobic bacterium that is a highly competitive plant leaf colonizer. Carbon partitioning plays an important role for *Sphingomonas* spp. to be effective antagonists in the

phyllosphere (Delmotte *et al*., 2009) and it promote plant growth by producing phytohormones such as gibberellins and indole acetic acid (Khan, 2014). In a series of experiments, researchers demonstrated that the leaf colonising bacterium *Sphingomonas* spp. could protect plants against the leaf-pathogenic *Pseudomonas syringae* through substrate competition (Innerebner *et al*., 2014). *Pantoea* species can produce *N*-acyl-homoserine lactone (AHL) and the plant-growth hormone indole-3-acetic acid (IAA), fix nitrogen from the atmosphere and establish quorum sensing systems on leaves, which makes them possible to suppress pathogens on leaves (Pusey *et al*., 2015). Hence, the foliar application of PGPM mix increased the bacterial population in a such a way that tomato leaves occupied a greater number of commensal bacterial genera *viz. Sphingomonas, Bacillus, Pseudomonas, Pantoea, Acinetobacter* which are considered as beneficial to the plant, since they have important role in pathogen exclusion, plant growth and productivity.

Genus *Selenomonas* was the most abundant bacteria in control. It also includes bacteria from genera like *Thermoactinomyces*, *Paenibacillus*, *Clostridium*, *Blautia*, *Laceyella*, *Brevibacterium*, *Azospira*, *Prosthecobacter* and *Halobacillus*. It was important to noticed that among the top ten abundant genera, *Azospira, Blautia*, *Halobacillus, Laceyella, Prosthecobacter* and *Thermoactinomyces* were present in control but absent in leaves collected from fungicides and bioagents sprayed plants indicated that application of fungicides and bioagents has negative effects on these bacterial population. Another important observation was presence of *Clostridium* in all the samples except in PGPM treated leaf sample. The well documented habitat of genus *Clostridium* is soil, water and decaying organic matter and most of them are capable to survive as saprophytes. But recent studies reveal plants as hosts for epi or endophytic colonization of *Clostridium* suggesting possibility of plants as alternate host for major human and animal bacterial pathogens (Kirzinger *et al*., 2011and Timmers *et al*., 2012).

The analysis of the bacterial diversity using diversity indices and number of taxa indicated that the diversity was high in PGPM mix sprayed leaves and it was minimum in iprodione + carbendazim (0.2%) sprayed leaves. The highly diverse bacterial assemblage

in the PGPM mix sprayed tomato leaves might indicate that these plants have the ability to support a wide range of bacterial taxa when compared to that of chemically treated plants.

Hence, metagenomic analysis of microbial diversity on tomato leaves collected from different treatment applied plants in the context of early blight disease management revealed that spraying of chemical fungicides reduces microbial population and diversity while bioagent application enhances microbial population and diversity. However, microbial community structure was changed in both cases. Among the selected fungicides foliar application contact fungicide propineb (0.2%) imparted less non -targeted effects than systemic fungicide iprodione + carbendazim. Interestingly, metagenomic results also showed association of *Cladosporium, Corynespora, Pseudocercospora* along with early blight pathogen *Alternaria* on tomato leaves that otherwise remain undetected. Meanwhile this study once again proved the efficacy of the selected treatments against early blight disease. Further system-level analysis of the complex interaction that governs outcomes among community members in the context of the plant host is required, in order to identify beneficial harmful microbial interaction and selection processes for beneficial communities at concentration of fungicides and pathogen pressures.

Recalling back the results obtained from management of tomato early blight disease under polyhouse and rain shelter condition, it is evident that the systemic fungicide iprodione + carbendazim (0.2%) was more effective among different selected treatments. However, apart from the desired effects on the pathogen, an effective disease management strategy should consider its effects on non- targeted effects on beneficial microflora that inhabit inside and outside the plant. Hence, considering the results of effects of treatments on tomato seedling vigour, biometric characters, per cent disease severity both under polyhouse and rain shelter condition, residue analysis, phylloplane and endophytic microbial enumeration study and metagenomics analysis of microbial diversity we recommend spraying of propineb (0.2%) as best treatment among the tested fungicides and spraying of PGPM mix among biocontrol agents for the management of early blight disease of tomato under protected cultivation.

SUMMARY

Protected cultivation is a fascinating technology gaining tremendous importance in India. Government of Kerala also encouraging polyhouse and rain shelter cultivation of vegetable crops. Tomato is one of the most preferred vegetable grown under protected condition. It is the richest source of vitamin A and C and supplies a sufficient amount of the antioxidant lycopene pigment that helps to protect the body against cancer and heart disease. Crop suffer from various diseases and among which one of the major fungal disease is early blight caused by *Alternaria solani* and it contribute yield losses up to 79 and 67 per cent under field and protected cultivation respectively. Fungicides and bioagents are commonly used to manage plant pathogens. But little is known about their effects on the non-target microbial communities that inhabit inside and outside the plant. Hence, it has become necessary to consider the effect of different fungicidal and bioagent treatments on target and non-target microbial communities while formulating disease management strategies. So, the present investigation was carried out with the objectives to formulate suitable management strategies against early blight disease of tomato under protected cultivation and to assess their impact on culturable and non-culturable microflora. The experiment entitled "Management of early blight disease of tomato (*Solanum lycopersicum* L.) under protected cultivation" was conducted in the department of Plant Pathology, College of Horticulture, Vellanikkara during the period 2016-2020.

1. Isolation of the pathogen from infected leaves showed the association of a fungus, which was found to be *A.solani* based on cultural and morphological characters. Pathogenicity was proved Pathogenicity was proved and the spore inoculation with injury showed the early infection as compared to without injury.
2. The characteristic symptoms on tomato leaves were development of light-yellow discolored spots mostly near the leaf margin which later become a small brown water-soaked lesion surrounded by a narrow chlorotic halo. Later, the lesion enlarged with characteristic concentric rings in the center and the adjacent spots

eventually coalesced to form large irregular spots leading to drying and defoliation. The symptom was also observed on stem, petioles and fruits. Symptoms observed on leaves, shoot and fruits were almost same under both natural and artificial conditions.

3. *In vitro* evaluation of fungicides showed complete inhibition of the pathogen with propineb (0.1%, 0.2% & 0.3%), hexaconazole (0.05%, 0.1% & 0.15%), iprodione 25% + carbendazim (0.1%, 0.2% & 0.3%), difenoconazole (0.075%).
4. *In vitro* evaluation of bioagents showed complete inhibition of the pathogen with *T. viride* (KAU), *T. viride* (PGPM mix), *T. harzianum* (PGPM mix) and plant growth promoting microbial consortium (PGPM mix of KAU) and among bacterial antagonists *B. subtilis* (Endophyte from cocoa) showed maximum growth inhibition.
5. Field experiments were conducted simultaneously inside the polyhouse and rain shelter for the management of tomato early blight disease with 11 treatments and three replications. The treatment includes seven fungicidal (propineb - 0.1%, 0.2%, hexaconazole- 0.05%, 0.1%, difenoconazole - 0.075%, iprodione 25% + carbendazim -0.1%, 0.2%) and three biocontrol treatments (*T. viride* - KAU, PGPM mix -KAU, *B. subtilis* - endophyte from cocoa).
6. All the three bioagents treatments showed higher seedling vigour compared to fungicidal treatments and highest vigour index was recorded for PGPM treated ones.
7. Foliar spray with iprodione 25% + carbendazim 25% WP (0.1% and 0.2%) and propineb (0.2%) showed maximum reduction of early blight disease both under polyhouse and rain shelter conditions and spraying of PGPM mix was most efficient among bioagent treatments. Highest yield was recorded from iprodione + carbendazim treated plants
8. Biocontrol treated plants showed better performance in overall plant vigour of which PGPM mix application was more effective.
9. Plant height, earliness in flowering and number of flowers were maximum in plants cultivated under polyhouse condition while maximum yield was obtained from plants cultivated under rain shelter condition.

10. Residue analysis revealed that degradation rate of fungicides was more under polyhouse condition.
11. Correlation analysis on severity of early blight disease with meteorological parameters showed a significant positive correlation with temperature inside the polyhouse and relative humidity inside the rain shelter.
12. Enumeration of phylloplane and endophytic microbial population showed that application of fungicides on tomato plant drastically reduced the tomato phylloplane and endophytic microflora while bioagent applications response was varied depending up on the type of biocontrol agents.
13. Natural population of phylloplane and endophytic fungi and bacteria were more under rain shelter condition while actinomycetes population was more in polyhouse condition.
14. Maximum reduction in phylloplane and endophytic microbial population was in plants sprayed with iprodione 25% + carbendazim 25% WP and minimum in difenoconazole and propineb sprayed plants.
15. Survival of biocontrol agents on the phylloplane of tomato was also studied and it was found that both *Trichoderma* and *Pseudomonas fluorescens* survived on leaf surface up to 15 days after foliar application.
16. Metagenomic studies revealed that spraying of chemical fungicides reduces microbial population and diversity while bioagent application enhances microbial population and diversity. However, microbial community structure was changed in both cases.
17. Metagenomic studies enlightened a new mode of action for fungicides and bioagents besides their direct effect, that is shifting the microbial community structure so that it provides greater resistance against the pathogen.
18. Metagenomic results also showed association of *Cladosporium, Corynespora, Pseudocercospora* along with early blight pathogen *Alternaria* on tomato leaves that otherwise remain undetected.

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Appendix

APPENDIX –I

COMPOSITION OF MEDIA USED

1. Martins's Rose Bengal Agar

Peptone	-5 g
Agar - Agar	- 20.0 g
Dextrose	- 20.0 g
KH2PO4	- 1.0 g
MgSO4	- 0.5 g
Rose Bengal	- 0.03 g
Streptomycin	- 30.0 mg
Distilled water	- 1000 ml

2. Nutrient Agar

Peptone	- 5.0 g
Agar - Agar	- 20.0 g
Beef extract	- 1.0 g
Sodium chloride	- 5.0 g
Distilled water	- 1000 ml
pH	- 6.5 to 7

3. Kenknight's Agar

Dextrose	- 1.0 g
KH2PO4	- 0.1 g
NANO3	- 0.1 g

KCl - 0.1g

MgSO4 - 0.1g

Agar- Agar - 20.0g

Distilled water - 1000 ml

4. King's B Agar

Peptone - 20.0 g

Giycerol - 10.0 ml

KH2PO4 - 10.0 g

MgSO4. 7 H20 - 0.1 g -

Agar - 20.0g

Distilled water - 1000 ml

5. *Trichoderma* Selective Agar

MgSO4 - 2.0 g

K2HPO4 - 0.9 g

NH4NO3 - 1.0 g

KCL - 0.15 g

Glucose - 3.0 g

Metalaxyl - 0.3 g

PCNB - 0.2 g

Rose Bengal - 0.15 g

Chloramphenicol - 0.25 g

Agar - 20.0g

Distilled water - 1000 ml

MANAGEMENT OF EARLY BLIGHT DISEASE OF TOMATO (*Solanum lycopersicum* L.) UNDER PROTECTED CULTIVATION

By

SUMBULA V.

ABSTRACT OF THESIS

Submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the most remunerative and widely grown vegetables all over the world. With the coordinated efforts of central and state governments, protected cultivation of tomato is now gaining popularity in Kerala. Despite being a versatile crop adapted to various agroclimatic regions and seasons, cultivation of tomato is constrained by various fungal, bacterial and viral diseases. Among the fungal diseases, early blight caused by *Alternaria solani* is the most common, destructive and widespread in all the tomato growing tracts. Fungicides and bioagents are commonly used to manage plant pathogens. But little is known about their effects on the non-target microbial communities that inhabit inside and outside the plant. Hence, it has become necessary to consider the effect of different fungicidal and bioagent treatments on target and non-target microbial communities while formulating disease management strategies. So, the present investigation was carried out with the objectives to formulate suitable management strategies against early blight disease of tomato under protected cultivation and to assess their impact on culturable and non-culturable microflora associated with the plant.

Isolation of the pathogen from infected tomato leaf samples revealed the association of the fungus, *Alternaria* sp. and its pathogenicity was established by inoculating on three-month-old tomato seedlings. Symptoms observed on leaves, shoot and fruits were almost same under both natural and artificial conditions. Cultural and morphological characters of pathogen was studied on potato dextrose agar (PDA). Initially, pathogen produced greenish brown mycelium and later turned to grey colour. Hyphae are septate and the colony has aerial topography and irregular rough growth patterns with concentric zonation. Sporulation was observed after six days of incubation and conidiophores were straight or flexuous brown to olivaceous brown in colour. The conidia are solitary straight or muriform or oblong, pale or olivaceous brown, length 40-110 µm and 7-15 µm thick with 2-8 transverse and 0-3 longitudinal septa. The cultural and morphological characters of the pathogen completely fit into the description of *Alternaria solani* by Alexopoulos *et al.* (1996). Hence, it is confirmed that the symptom observed on tomato leaves are those of early blight disease caused by *A. solani*.

In vitro evaluation of fungicides and bioagents showed complete inhibition of the pathogen with propineb (0.1%, 0.2% & 0.3%), hexaconazole (0.05%, 0.1% & 0.15%), iprodione + carbendazim (0.1%, 0.2% & 0.3%), difenoconazole (0.075%), *Trichoderma viride* (KAU), *T. viride* (PGPM mix), *T. harzianum* (PGPM mix) and plant growth promoting microbial consortium (PGPM mix of KAU). Among the bacterial antagonists, *Bacillus subtilis* (endophyte from cocoa) showed maximum growth inhibition of the pathogen. All the three bioagents recorded earliness in seed germination and enhanced seedling vigour compared to the fungicidal treatments and control.

The results of field experiment under polyhouse and rain shelter conditions showed that all the treatments are superior to control in early blight disease management, of which, spraying of iprodione + carbendazim (0.2%) and propineb (0.2%) were the best among fungicides and PGPM mix application was the most efficient among bioagents. Moreover, the highest yield was recorded from iprodione + carbendazim treated plants. Biocontrol treated plants showed better performance in overall plant vigour of which PGPM mix application was the most effective. Residue analysis showed that degradation rate of fungicides was more under polyhouse condition.

Analysis of population of phylloplane and endophytic microflora proved that there was drastic reduction in microbial population after spraying with chemical fungicides whereas population increased after bioagent application. The study on survival of bioagents on tomato phylloplane revealed that both *Pseudomonas fluorescens* and *T. viride*, survived on leaf surface up to 15 days after foliar application. Analysis of fungicidal residue on tomato fruits revealed that, the degradation of fungicides was faster in polyhouse compared to rain shelter.

Metagenomic analysis of microbial diversity on tomato leaves revealed that spraying of chemical fungicides reduces microbial population and diversity while bioagent application enhances the same. However, microbial community structure was changed in both cases. This study also enlightened the new mode of action for fungicides and bioagents besides their direct effect that is shifting the microbial community structure so that it provides greater resistance against the pathogen. Interestingly, metagenomic results also showed association of *Cladosporium, Corynespora, Pseudocercospora* along with early blight pathogen *Alternaria* on tomato leaves that otherwise remain undetected. Another important observation was *Clostridium* in tomato leaf samples except in PGPM mix treatment, suggesting the possibility of plants as alternate host for major human and animal bacterial pathogens.

Hence, considering the effects of treatments on per cent disease severity both under polyhouse and rain shelter condition, residue analysis, phylloplane and endophytic microbial enumeration study and metagenomics analysis of microbial diversity, the present study recommends spraying of propineb (0.2%) as the best treatment among the tested fungicides and spraying of PGPM mix among biocontrol agents for the management of early blight disease of tomato under protected cultivation. Further system-level analysis of the complex interaction that governs outcomes among community members in the context of the plant host is required, in order to identify microbial interaction and selection processes for beneficial communities at different concentrations of fungicides and pathogen pressures.