ANTAGONISTIC POTENTIAL OF NAGA KING CHILLI RHIZOBACTERIA AGAINST ANTHRACNOSE PATHOGEN

Thesis

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of

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in

Plant Pathology

by

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Affectionately

Dedicated

To my beloved

Azu, Ava and all my family members

for believing in me even

when I was doubtful.

DECLARATION

I, Tiaienla, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis had not been submitted by me for any research degree in any other university/institute.

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CERTIFICATE - I

This is to certify that the thesis entitled **"Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen"** submitted to the Nagaland University in partial fulfilment of the requirements for the award of degree of Doctor of Philosophy in Plant Pathology is the record of research work carried out by Miss. Tiaienla, Registration No. Ph.D./PPL/00349, under my personal supervision and guidance.

The results of the investigation reported in the thesis have not been submitted for any other degree or diploma. The assistance of all kinds received by the student has been duly acknowledged.

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"I can do all things through Christ who strengthens me".

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LIST OF ABBREVIATIONS AND SYMBOLS

@	:	at the rate of
%	:	per cent
/	:	per
a.i.	:	active ingredient
BOD	:	biological oxygen demand
bp	:	base pair
^{o}C	:	degree Celsius
CD (p=0.05)	:	critical difference at 5 per cent probability
cm	:	centimetre
CFU/ml	:	colony forming unit per millilitre
CRD	:	completely randomized design
CV	:	co-efficient of variation
CTAB	:	cetyl trimethyl ammonium bromide
dNTP	:	deoxynucleotide triphosphates
DAS	:	days after sowing
DAT	:	days after transplanting
DNA	:	Deoxyribo Nucleic Acid
et al.	:	et alia (and others)
etc.	:	etcetera
EDTA	:	ethylene diamine tetra acetic acid
Fig	:	figure
g	:	gram
ha^{-1}	:	per hectare
h	:	hour
hrs	:	hours
i.e.	:	that is

in vivo	:	in a living thing
in vitro	:	in laboratory
K	:	potassium
kg	:	kilogram
kg ha^{-1}	:	kilogram per hectare
kg^{-1}	:	per kilogram
L	:	litre
L^{-1}	:	per litre
m	:	meter
m^2	:	square meter
ml	:	millilitre
mg	:	milligram
min	:	minutes
N	:	nitrogen
NS	:	non significant
No.	:	number
O.D.	:	optical density
P	:	phosphorous
PCR	:	polymerase chain reaction
Zn	:	zinc
PDA	:	potato dextrose agar medium
PDI	:	per cent disease index
rpm	:	revolution per minute
R.H.	:	relative humidity
SEm (±)	:	standard error of mean
sp., spp.	:	species (singular and plural)
t ha^{-1}	:	tonnes per hectare
viz.	:	namely
wt.	:	weight

ABSTRACT

The present investigation under the topic “Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen” was conducted in the laboratory and experimental field of the Department of Plant Pathology, School of Agricultural Sciences, Nagaland University, Medziphema campus. For obtaining better insight in the antagonistic potential of native rhizobacteria, 27 rhizobacteria were isolated from the farmers field of Peren district, Nagaland during the flowering stage where Naga king chillies are widely grown and were characterized morphologically and biochemically. The isolates were observed to be predominantly smooth, round, either orange or milky white in colour, smooth surface, convex with entire margin and translucent. Most of the isolates were observed to be gram negative, five isolates were recorded to utilize lactose. All the isolates recorded positive reaction for catalase and gelatine liquefaction activity but, eight isolates were unable to hydrolyse starch. *In vitro* antagonistic potential of the isolates were tested against *Colletotrichum gloeosporioides* and results suggested that T_7 showed highest per cent inhibition of 48.89 % which was statistically at par with T_{25}, T_{18} and T_{16} with 47.41, 46.66 and 42.96 % respectively. The rhizobacteria T_7 and T_{25} showed maximum volatile metabolite production with highest inhibition of 32.50% which was found to be statistically *at par* with and T_{18} (31.67%.) and T_{16} (30.87) over the control. Most of the isolates were reported to produce siderophore (16 isolates) and HCN (15 isolates) but none of the isolates were recorded to synthesize chitinase enzyme nor produce biosurfactant. All the isolates were observed to be positive for ammonia production while most of the isolates (22 isolates) were observed to show positive reaction for IAA production, GA_3 (26 isolates) and could solubilize phosphate (19 isolates), zinc (ZnO) (22 isolates) in varying degree. The best three performing rhizobacteria from dual culture assay *i.e.,* T_7, T_{16} and

T_{25} and their combinations were evaluated for plant growth promotion *in vitro* and the results suggested that T_2 gave highest germination of 76.67 % at 10 DAS and the highest vigor index of 447.11 and maximum root and shoot length of 3.9 cm and 3.18 cm was recorded from T_8 (T_1 + T_2 + T_3) at 15 DAS. These three isolates were further taken for *in vivo* trials where the plants treated with a consortium of rhizobacteria R_7 + R_{16} + R_{25} (T_8) performed significantly better than the other treatment with maximum plant height (85.67 cm), maximum increase in plant biomass (55.41 %), maximum number of fruits per plants (103.67), highest yield (580.17 g per plant, 333.29 kg/ha) with minimum disease incidence (9.00%) and disease severity (10.33 %) with highest per cent disease reduction (72.23 %) against *C. gloeosporioides at* 150 DAT. The sequence of the three potential rhizobacteria were analysed and identified by 16S rRNA gene region as *Pseudomonas oryzihabitans* (T_7), *Chryseobacterium s*p. (T_{16}) and *Bacillus* sp. (T_{25}).

Key words: Naga king chilli, rhizobacteria, *C. gloeosporioides, Pseudomonas oryzihabitans, chryseobacterium sp., Bacillus sp.*

CHAPTER I

INTRODUCTION

INTRODUCTION

Chilli (*Capsicum annuum* L.) a member of the family Solanaceae is the most popular and widely grown spice crops globally while more popular in Asia (Makari *et al*., 2009). It is the second most dominant solanaceous vegetable after tomato grown worldwide both as a spice or vegetable crop (Hasan *et al*., 2014). India is the largest exporter and consumer of chilli and contributes about 36% of total world chilli production. The production of chilli was reported to be 2059.36 MT in the fiscal year 2022-23 and Andhra Pradesh was the leading producer with a production of 767 MT and amongst the North Eastern states Assam is the leading producer with 21.07 MT followed by Mizoram with 10.92 MT (Anon, 2023).

Chilli fruits constitute large amounts of beneficial compounds including antioxidants, carbohydrates, minerals, phytochemicals, proteins, amino acids, and vitamins (Olatunji and Afolayan, 2018). Chilli has anti-mutagenesis, hypocholesterolemic and antioxidant properties (El-Ghoraba *et al*., 2013) and also inhibits bacterial growth and platelet agglomeration (Wahyuni *et al.,* 2013).

The Northeast region of India is considered as centre for chilli variant (Mathur *et al*., 2000) with numerous landraces reported from this part of India (Kumar *et al.,* 2011b). Amongst the various chilli landraces that are grown in this region, Naga king Chilli (*Capsicum chinense* Jacq.) is one of the best known worldwide. It is regarded as one the world's hottest chilli which entered in the Guinness book of world record in the year 2006. It is known as U-Morok in Manipur, Bhut Jolokia in Assam and Naga king chilli in Nagaland (Verma *et al.,* 2013). It is a self-pollinated plant; however, cross pollination (upto 10%) may take place to some extent when the population of the insects are high. Under favourable condition it acts as a semi perennial herb (Borgohain and Devi, 2007).

Naga king chilli is popularly cultivated in the state of Manipur, Nagaland, and Assam and in some parts of Meghalaya, Mizoram and Arunachal Pradesh. The Government of Nagaland has acquired the Geographical Indication (GI) of Goods tag for Naga king Chilli in the year 2008 (Registration and Protection Act, 1999) considering its economic significance (Anon, 2008). It is amongst the most important crops of the Nagaland as well pride for the state. Naga king chilli is traditionally cultivated in hilly jhum fields along with rice. Two growing seasons are practised *viz., kharif* and *rabi*. The crop is sown during February – March as a *kharif* crop in the hill states whereas it is grown during September-October as a *rabi* crop in Assam (Baruah *et al*., 2014). It is ingested as fully ripe or green fruits, either cooked or raw with vegetables and mainly used in the preparation of spicy curries and pickles. It has been reported that the hot principal of Naga king chilli can reduce asthma, prevent gastro- intestinal abnormalities, soothes muscle ache and toothache and the young leaves are useful for the extraction of puss from boils (Bhagowati and Changkija, 2009).

The demand for Naga king chilli has been increasing both in domestic as well as international market. It is cultivated in all the districts of Nagaland where, Peren has the highest production followed by Zunheboto, Wokha, and Dimapur district. The estimated area under Naga king chilli cultivation in Nagaland is about 604 ha with a production of 35279.50 MT in the year 2020-21. The Peren district, which produces around 1697.00 MT of king chillies annually on a total area of 285 hectares under cultivation (Anon, 2022), is the state's leading producer and has the potential to grow economically due to its good agroclimatic conditions.

Anthracnose disease in chilli is amongst the chief limiting factors for the production of chilli worldwide, particularly in tropical and sub-tropical regions which causes both qualitative and quantitative yield loss (Than *et al*., 2008). It is caused by *Colletotrichum* spp. and is amongst the diseases with the largest economic impact causing 10-80% of economic losses depending on cultivation

area which severely affects the marketability of the harvest (Diao *et al*., 2017). *Colletotrichum* spp. can cause disease on all parts of chilli plant during any stage of plant growth and survives on seed as acervuli and micro sclerotia (Suthin *et al*., 2014). The fungus affects both unripe and matured chilli fruits and under conducive environment for disease development, up to 50% of the harvest gets infected causing yield loss up to 50% in India (Sharma *et al*., 2005).

Colletotrichum gloeosporioides is a cosmopolitan pathogen widely disseminated as a common plant pathogen globally (Peralta-Ruiz *et al*., 2023). It is a seed or air-borne fungus attacking fruits, stems and leaves apart from affecting seedling emergence and seedling growth (Gowtham *et al.,* 2018). It has been demonstrated that the pathogen overrun the seed coat and peripheral endosperm even in partially colonized seeds. The pathogen can remain alive in crop residues and grow saprophytically after being dispersed by rain splashes and air transmission (Romero *et al*., 2021).

The initial infection by *Colletotrichum* spp. includes fastening of conidia to the surface of the plant, conidia germination, synthesis of adhesive appressoria, puncturing of plant epidermis, growth and colonisation of plant tissue, acervuli production, and sporulation. Naga king chilli is vulnerable to several biotic stressors due to *viz.,* fungus, bacteria and virus and is attacked by several diseases throughout its growth and development but the most prevalent one being ‘leaf curl’, ‘die-back’ and ‘anthracnose’ (Borgohain and Devi, 2007). Mature fruits are the primary target of anthracnose pathogen causing field and storage fruit rot leading to significant financial losses (Bosland and Votava, 2003). In Nagaland huge losses due to *C. gloeosporioides* causing fruit rot was reported in Naga king chilli (Ngullie *et al*., 2010).

The narrow zone of soil directly surrounding the root is called as rhizosphere (Walker *et al*., 2003). As compared to bulk soil this zone is packed with nutrients because of collection of a various of plant exudates, like sugars

and amino acids, providing nutrients and energy for bacterial growth (Gray and Smith, 2005). The rhizosphere is inhabited by a various population of microbes and the bacterial population found in this area are called rhizobacteria (Schroth and Hancock, 1982).

Plant Growth Promoting Rhizobacteria (PGPR) constitute approximately 2-5% of the total rhizomicrobial population (Kloepper *et al*., 1980). The PGPR can maximize crop harvest and reduce disease development and hence is regarded as one of the most promising agent for the production of cash crop (Raguchander *et al*., 1997). They have been documented to reduce the incidence of a wide range of phytopathogens *viz*. viruses, fungi, bacteria, nematodes, *etc*. (Salem and Abd El- Shafea, 2018). The beneficial effects of PGPR *viz*. ability to produce metabolites and peptides/ enzymes, which may be responsible for plant growth stimulation, availability of nutrients, suppression of phytopathogens, induction of systemic resistance or tolerance against biotic and abiotic stress (Niranjana and Hariprasad, 2014). The PGPR are also reported to increase seed germination since they contribute to the production of metabolites (siderophores and hydrocyanic acid), the production of antibiotics, enzymes, and phytohormones (auxin, cytokinin, and gibberellic acid), and other associated activities such as a greater solubilization of phosphates in soil and root colonization (Swarnalakshmi *et al.,* 2020) which increases the absorption capacity of nutrients and protection against phytopathogens (Pérez-García *et al*., 2023).

A deeper understanding of the local bacterial population their identification is important to know the distribution and diversity of native rhizobacteria of specific crops (Chahboune *et al*., 2011). With the rise in consciousness about the ill effects of chemical fertilizers based agricultural practices, it is of value to search and develop region- specific strains which can be used as a growth promoting/enhancing inoculum to achieve desired crop production (Deepa *et al*., 2010). Keeping in mind of the aforesaid realities the

investigation with the title “Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen” was carried out with the following objectives:

1. Isolation and characterization of rhizobacteria from Naga king chilli.
2. Testing antagonistic potential of the rhizobacteria against anthracnose pathogen *in vitro.*
3. Evaluation of plant growth promoting mechanisms of potential rhizobacteria *in vitro*.
4. Field assessment of suppressive potential of selected rhizobacteria against Naga king chilli anthracnose.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Studies on the "Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen" are reviewed under the following heads:

2.1 Symptomatology

Anthracnose symptoms on chilli fruits includes sunken necrotic tissues, with concentric rings of acervuli, often wet and produce pink to orange conidial masses. Under severe disease pressure, lesion may coalesce. Conidial masses may also occur scattered or in concentric rings on the lesion (Than *et al*., 2008). In die back phase, the disease causes necrosis of tender twigs from the tip backwards. The entire branch or the entire top of the plant may wither away. Dead twigs become grayish white to straw coloured in advanced stage of the disease; a large number of the black dots (acervuli) are found scattered all over the necrotic surface of the affected twig (Singh, 2005).

Ngullie *et al.* (2010) reported that the symptoms of anthracnose on Naga king chilli were mostly recorded on unripe fruits. Symptoms showing bleaching and concentric ring were observed on fruits and tissue that were infected formed a depression leading to fruit shrinking. The spots were 20-40mm in diameter.

Rahman *et al*. (2011) observed that the symptoms on the leaves, initially appears small-circular spots and the severely infected leaves fall off leading to defoliation of plant. The infection starts from growing tips (necrosis of apical branch, dieback) followed by leaves and branches and then fruits. Among the plant parts, most susceptible stage is ripe fruit stage.

Oo and Oh (2016) stated that typical fruit rot symptoms are circular or angular sunken lesions with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease conditions, lesions may coalesce. Conidial masses may also occur scattered or in concentric rings on the lesions.

Manda *et al*. (2020) reported that the symptoms of anthracnose on chilli leaves appears as small brown or black water-soaked spots which are surrounded by light brown or yellow hallo margin. These spots were small at first, develop larger and get coalesced with each other to form a large lesion. These lead to necrotic spot and at later stages defoliation of leaves takes place from the crop, on the stem were brown spots which leads to necrosis of twigs finally ending up in die back of the plants at severe situation. On the fruits lesion turned into concentric rings and the black pin head size acervuli are seen on the concentric lesion. Conidial mass also occurs on severe stage. The fruit become wrinkled, deformed, shrivelled and dried when it is affected completely.

2.2 Causal organism

Anthracnose, derived from a Greek word meaning ‘coal’, is the common name for plant diseases characterized by very dark, sunken lesions, containing spores (Isaac, 1992). Anthracnose disease is one of the major constraints that restrict profitable production of chilli. It is caused by *Colletotrichum* species. Genus *Glomerella* belongs to Kingdom- Fungi, Phylum- Ascomycota, Class Sordariomycetes, Order- Phyllochorales and Family- Phyllochoraceae. *Colletotrichum* is a large genus of Ascomycetes fungi, containing species that cause anthracnose disease on wide range crops of economic value (Sahitya *et al*., 2014). Several *Colletotrichum* species have been reported as causal agents of chilli anthracnose disease worldwide (Than *et al*., 2008).

Kanchalika *et al.* (2010) collected 34 isolates of *Colletotrichum* spp. from anthracnose of bell pepper which included two species that were *C. gloeosporioides* and *C. capsici*.

Talukdar *et al*. (2012) studied the occurrence of fungal and bacterial disease in 'Bhut Jolokia' in Assam and observed that the plant is susceptible to several biotic and abiotic stress and reported five fungal diseases including anthracnose caused by *C. capsica*, dieback caused by *C. gloeosporioides*, stem rot and wilt caused by *Sclerotinia sclerotium*, collar rot caused *by Rhizoctonia solani* and leaf spot caused *by Corynespora cassicola* were identified.

Li *et al*. (2021) isolated anthracnose causing pathogen from chilli and reported that the conidia were cylindrical, aseptate with obtuse to slightly rounded ends and measured 10.1 to 16.9 x 4.7 to 7.0 µm. Molecular identification was done following CTAB method to confirm that it was *C. gloeosporioides*.

Achumi (2022) studied the variability of anthracnose pathogen of Naga king Chilli collected from different districts of Nagaland and reported that *C. gloeosporioides* isolates produced cylindrical conidia with their size 13.5 µm × 4-4.5 µm in length and width respectively.

2.3 Disease occurrence and yield loss

Sharma *et al*. (2005) mentioned that anthracnose disease cause by *C. capsici* (Syd) is the most economically important factor which restricts the production of chilli in major chilli growing regions of India, accounting up to 12-25 per cent yield losses.

Ramachandran *et al.* (2008) reported that anthracnose disease has been identified in all the Naga king chilli growing regions of Nagaland and has

become a serious constraint in its production wherever the crop is grown. Anthracnose incited by *Colletotrichum* spp. is one of the serious diseases of chilli and different species, *viz, C. capsici, C. gloeosporioides and C. acutatum* are known to cause anthracnose of chilli in India. However, *C. capsici* is reported as the predominant species in the major Naga king chilli growing areas of Nagaland. The fungus being both internal and external seed-borne also survives on the stems and branches which causes die back symptoms.

Anand *et al*. (2010) stated that amongst several agents known to cause diseases in chilli, it was observed that chilli anthracnose is the most significant one causing major damage, imposing intense quantitative and qualitative losses in India.

Pezangulie and Banik (2020) studied the occurrence of different Naga king chilli disease in Nagaland and concluded that the most serious infection of the chilli by *C. capsici* was reported from Dimapur district with a disease incidence of 44.39%.

2.4 Rhizobacteria

Burr *et al*. (1978) reported that PGPR act as frontline defence against the pathogen and antagonize them prior to and during the primary infection. The antagonistic bacteria isolated from the rhizosphere of a particular crop gets well adapted to that crop and may provide efficient disease suppression than the rhizobacteria isolated from the rhizosphere of other plant species.

Antoun and Prévost (2006) classified rhizobacteria as being neutral, deleterious or beneficial. The presence of the neutral group might be insignificant to the host plant, while deleterious rhizobacteria produce metabolites that are averse to plant health. The beneficial categories of rhizobacteria are able to

promote plant growth and development, and are generally further grouped according to their physical interaction with the host plant. Beneficial rhizobacteria may form symbiotic interactions which involve modification of the morphology of the host plant root through nodule formation. Other beneficial rhizobacteria are free-living in the soil and employ associative relationships with the host plant. These free-living rhizobacteria are defined as PGPR and form associations with many different plant species (Kloepper *et al*., 1989).

Vejan *et al.* (2016) stated that the use of PGPR facilitate plant growth through direct or indirect mechanism. The mechanisms of PGPR include regulating hormonal and nutritional balance, inducing resistance against plant pathogens, and solubilizing nutrients for easy uptake by plants. In addition, PGPR show synergistic and antagonistic interactions with microorganisms within the rhizosphere and beyond in bulk soil, which indirectly boosts plant growth rate.

Jiao *et al.* (2021) stated that the use of PGPR is proven to be an eco-friendly way of controlling plant diseases and increase crop yield. The PGPR supresses disease by directly synthesizing pathogen antagonizing compound, as well as by triggering plant immune response.

2.5 Characterization

Banerjee *et al*. (2011) isolated 15 bacteria from chilli rhizosphere and elucidated their morphological, biochemical, plant growth promoting and biocontrol characteristics. The study showed that chilli rhizosphere was dominated by gram positive bacterial population with white, irregular, opaque colonies. Majority of the bacterial isolates were able to produce amylase and catalase enzymes and showed negative response to other biochemical tests.

Geetha *et al*. (2014) selected six antagonistic plant growth promoting bacterial isolates from 140 PGPR isolates and reported that all the colonies were unpigmented, round shaped, raised having rough surface with undulated to erosive margins or smooth shiny surface with smooth margin. Out of the six isolates five were gram positive and remaining one was found to be gram negative.

Patel *et al*. (2014) isolated 298 rhizobacteria from sugarcane rhizosphere with bio-control potential against *Rhizoctonia solani* and stated that the isolates were predominantly round shaped while few of them were slightly curved and amoeboid. The colony colour of the isolates varied from white/off white to bright/dark yellow; with few having green and orange.

Patel and Desai (2015) studied 63 paddy rhizobacteria and observed that all the isolates were positive for catalyst test indicating that all isolates are aerobic in nature. Catalase production has been reported as important traits of PGPR isolates that indirectly influence the plant growth.

2.5.1 Hypersensitive response test

Abdeljalil *et al*. (2016) observed that all 200 rhizobacteria isolated from rhizosphere of healthy tomato showed negative response for hypersensitive response test on tobacco leaves as there was no development of necrotic lesion 24 hours after inoculation.

Miladiarsi *et al*. (2017) studied hypersensitivity test by injecting isolated chilli rhizobacteria into tobacco leaves using *P. syringae* as positive control and recorded that all the rhizobacteria were negative for hypertensive response test as there was no development of necrotic symptom after 48 hours.

Yanti *et al.* (2017b) reported that only one rhizobacteria isolated from rhizosphere of healthy chilli plants showed positive for hypersensitive response test by the development of chlorotic symptom on *Mirabalis jalapa* 24 hours after inoculation.

Vasseur-Coronado *et al*. (2021) tested 18 rhizobacterial isolates antagonistic against *Pythium ultimum* for their plant pathogenicity on tobacco leaves and observed that one isolate showed hypersensitive reaction after 24 hours.

2.6 *In vitro* disease inhibition

2.6.1 Antagonism *in vitro*

Soylu *et al*. (2005) screened 113 rhizosphere associated bacterial isolates from tomato and chilli rhizosphere for its antagonistic potential against two important soils borne pathogen *Sclerotina sclerotiorum* and *R. solani in vitro* and reported that isolates belonging to *Bacillus* spp. and *Pseudomonas* spp. were highly efficient against these cited pathogens, significantly suppressing its hyphal growth by 75.3% and 83% respectively.

Ann *et al*. (2015) studied the antagonistic activity of endophytic *Bacillus* species against *C. gloeosporioides* for the control of anthracnose disease in black pepper (*Piper nigrum* L.) and reported that *Bacillus* strain CBF were found to be the most effective isolates in suppressing the growth of *C. gloeosporioides* with an inhibition percentage of 50.1, followed by *Bacillus* strain YCA5593 and YCA0098 with the inhibition percentage of 45.9 and 44.7 respectively.

Abdeljalil *et al*. (2016) screened 200 rhizobacteria isolated from healthy tomato plant for their capacity to suppress *Sclerotinia sclerotiorum* and *R. solani*

in vitro and reported that 69 out of the 200 isolates were shown to significantly inhibit the mycelial growth of target pathogen by 11-62% relative to control. Sequencing of 16S rRNA and rpoB genes identified the isolates as belonging to four genera *viz. Bacillus, Chryseobacterium, Enterobacter*, and *Klebsiella* and the most frequent species were reported to be *B. amyloliquefaciens, B. thuringiensis, B. megaterium, B. subtilis, E. cloacae, C. jejuense* and *K. pneumoniae* respectively.

Horuz and Aysan (2016) reported that the bacteria *Curtobacterium flaccumfaciens* (Antg-198), *Microbacterium oxydans* (Antg-57), *Pseudomonas oryzihabitans* (Antg-12), and *P. fluorescens* (Antg- 273) were highly efficient for treating watermelon seeds and in biological control of watermelon seedling blight caused by *Acidovorax citrulli*. Seed treatments with individual antagonistic bacteria reported in a significant reduction in disease incidence (DI) and severity (DS) ranging between 14.06–79.47% and between 4.57–41.49 %, respectively.

Syafriani *et al*. (2016) isolated 101 rhizobacteria from different plant rhizosphere and reported that 17 isolates were able to inhibit *C. gloeosporioides in vitro* ranging between 7.5% to 43.3 %. The highest inhibition was recorded from UBCR-12 which was later identified as *Serratia plymuthica*.

Sang *et al*. (2018) reported that *Chryseobacterium* sp. significantly reduces the disease severity in pepper plants inoculated with *Phytophthora capsici* and promoted plant growth compared with plants treated with *Escherichia coli* DH5a as negative control or $MgSO_4$ solution as untreated control.

Darmadi *et al*. (2020) isolated 1040 rhizobacteria and tested for their antagonistic activity against *C. scovillei* and reported that 10 isolates inhibited the growth of the pathogen by more than 80%.

El-Sersawy *et al*. (2021) stated that seven isolates out of isolated 23 rhizobacteria from the rhizosphere of healthy faba bean plant were able to inhibit the growth of *F. oxysporum in vitro* with varying degrees ranging between 39.06 ± 0.3% and 59.1±0.2%. Amongst the seven isolates, three isolates Vb1, Vb3, and Vb6 showed the highest efficacy to inhibit *F. oxysporum* growth with inhibition percentages of 59.1±0.2, 46.4±0.3, and 52.5±0.3, respectively. These isolates were identified as *B. velezensis* (Vb1), *B. paramycoides* (Vb3), and *B. paramycoides* (Vb6). These strains also showed positive results for ammonia, HCN, and siderophores production.

2.6.2 Volatile compound production

Farmer (2001) identified low-molecular weight plant volatiles such as terpenes, jasmonates and green leaf components as potent signal molecules for living organisms in different trophic levels.

Vespermann *et al*. (2007) reported that the microbial volatile organic compounds belong to different chemical classes including alkenes, alcohols, ketones, benzenoids, pyrazines, sulfides and terpenes.

Vagelas and Gowen (2012) evaluated *P. oryzihabitans* against the soil-borne pathogens *F. oxysporum* f. sp. *lycopersici* and root-knot nematodes (*Meloidogyne* spp.) and stated that the bacterial cells were able to produce diffusible secondary metabolites *in vitro.* Bacterial cells applied to soil was reported to significantly increased tomato shoot and root dry weights compared with the pathogen treatment.

Guevara-Avendaño *et al.* (2019) isolated 45 rhizobacterial isolates emitting volatile organic compounds with antifungal activity against *Fusarium solani, Fusarium* sp. associated with Kuroshio shot hole borer, and *C. gloeosporioides* from avocado plant and reported that ten, three and two isolates were able to reduce the mycelial growth of *F. solani*, *Fusarium* sp. and *C. gloeosporioides* with an inhibition percentage ranging from 25- 54.6%, 37.00-38.7%, 25.4-32.6% respectively. Most of the isolates were identified as *Bacillus* and *Pseudomonas* sp.

2.6.3 Siderophore production

Siderophore have been recognised as an important tool for antagonistic fungi and bacteria by binding iron with high specificity and affinity making it unavailable for pathogen and limiting their growth.

Kamensky *et al*. (2003) reported that siderophore production are corelated with antifungal activity

Yu *et al*. (2011) stated that the control of *Fusarium* wilt caused by *F. oxysporium* f. sp. *capsici* was due to the production of siderophore by *B. subtilis* CAS15.

Bhakthavatchalu *et al.* (2013) observed changes in colour of the Chrome Azurol Sulfonate (CAS) agar medium from blue to orange red by the isolates and confirmed the ability of *P. aeuriginosa* FP6 to produce siderophore. The maximum production of siderophore of 85.70 µm was recorded after 36 hrs of incubation.

Kumar *et al*. (2014) recovered 114 rhizobacteria from healthy plantation and vegetable crops of Andaman Islands and reported that 110 isolates were able

to utilize a significant amount of iron by the development of yellow zone on CAS agar medium plate. While, 107, 67 and 59 isolates were able to produce ammonia, solubilize P and produce IAA. Six isolates performing better *in vitro* test were identified as *B. stratosphericus*- NFB3, *B. cereus*- NpB6, *B. cereus*- MKP3, *B. cereus* MNB1, *P. fluorescens*- NNB4 and *P. simiae*- NTB2 and were observed to produce significant amount of siderophore chelating rhizospheric iron.

Huang *et al*. (2017) studied the ability of rhizospheric *B. subtilis* strain SL-44 against *R. solani* in pepper and results suggested that the isolate was able to inhibit the growth of the pathogen by 42.3% and was observed to produce IAA, siderophore and fix nitrogen.

Fridayati *et al*. (2020) reported that siderophore compounds are produced by rhizobacterial microorganisms and fungi which had very high inhibitory ability and have the ability to utilize iron, especially in conditions where iron is deficient. Siderophore production is reported to be one of the rhizobacterial mechanisms in suppressing pathogens through the competition of iron.

El-Sersawy *et al*. (2021) studied three rhizobacterial isolates from faba beans showing the highest *in vitro* antagonistic activity against *F. oxysporum* and reported that these strains were also positive for ammonia, HCN, and siderophores production test through the appearance of an orange halo around the bacterial growth. It was also stated that the inhibitory effect of siderophores against phytopathogenic fungi is due to converting Fe3+ (inorganic form) to Fe_2+ (organic form), and hence making it available for plant and unavailable for phytopathogens. These isolates were identified as *Bacillus velezensis* (Vb1), *B. paramycoides* (Vb3), and *B. paramycoides* (Vb6).

2.6.4 Hydrogen cyanide (HCN) production

Kremer and Souissi (2001) characterized rhizobacterial strains by their ability to synthesize hydrogen cyanide and having effects on seedling root growth of various plants. It was observed that approximately 32% of the rhizobacteria from a collection of over 2000 isolates were cyanogenic, evolving HCN from trace concentrations to >30 nmoles/mg cellular protein. Cyanogensis was observed to be predominantly associated with *Pseudomonas* and was enhanced when glycine was provided in the culture medium.

Mahesh (2007) suggested that the production of siderophore and HCN inhibits the growth of pathogenic fungus.

Manikandan *et al*. (2010) recorded the HCN production by *P. fluorescens* Pf1. They tested Pf1 cultures of different ages where the yellow colour of the filter paper change to dark brown compared to the control.

Geetha *et al*. (2014) reported that out of six potential plant growth promoting rhizobacteria isolated from the rhizosphere of *Vigna radita* two isolates were capable of synthesizing hydrogen cyanide. They also stated that the increased production of HCN by the efficient strain of *P. fluorescens* contributed as a factor for efficient and effective inhibition of mycelial growth of *R. solani in vitro*.

Dixit *et al*. (2015) evaluated 11 isolates of fluorescent *Pseudomonas* for HCN production and observed colour change in the picric acid soaked filter paper from yellow to dark brown in most of the isolates, except Pf4, Pf6 and Pf25. While, Pf20, Pf27 and Pf28 were highly HCN producing isolates as compared to other isolates.

Fridayati *et al*. (2020) studied six red chilli rhizobacteria against seed borne pathogens of eggplant and inferred that the difference in effectiveness of rhizobacteria as a biocontrol agent lies in its ability to produce antimicrobial compounds such as HCN. All the isolates were able to produce HCN with a high inhibitory activity of > 75% on the growth of test pathogen *F. oxysporum.* The six isolates were identified as *P. dimuta, B. bodius, B. laterophorus, B. laterophorus, B. larvae* and *B. stearothermophillus*.

2.6.5 Chitinase synthesis

Mubarik *et al.* (2010) studied a total of 25 rhizobacteria isolated from chilli rhizosphere forming a clear zone on solid chitin media and reported that isolates, I.5 and I.21 had the highest chitinolytic index. Maximum production of chitinase was observed from I.21 after 36h of inoculation at 55°C and pH 7.0. Based on sequence of 16S rRNA gene, the isolates I.5 and I.21 were identified as *Bacillus* sp. and *B. cereus* respectively.

Joshi and Joshi (2017) obtained a total of 100 bacterial isolates form 45 different samples from the rhizosphere and parts of sugarcane plant and results suggested that 59, 61, 39, 57 and 65 isolates were able to produce IAA, solubilize phosphate, produce siderophore, fixes nitrogen and produce protease respectively. However, out of eight isolates showing multiple plant growth promoting activities only two isolates were recorded to be positive for chitinase activity.

Dukare *et al*. (2020) isolated 53 rhizobacteria from the rhizosphere of pigeon pea and reported that 13 isolates were able to hydrolyse chitin ranging from 2.6-8.8mm. The highest zone (8.80 ± 0.10 mm) was formed around colonies of CDB NS-17, followed by CDB NS-1 and CDB NS-22 respectively.

2.6.6 Biosurfactant production

Borgio *et al*. (2009) evaluated three bacterial strains, *Bacillus subtilis* NCIM 2063, *Pseudomonas aeruginosa* NCIM 2862 and *Streptococcus mutans* MTCC 1943 for their exopolysaccharide (EPS) producing ability *in vitro* and recorded highest EPS production in *P. aeruginosa* (226 µg ml -1) followed by *S. mutans* and *B. subtilis* (220 and 206 µg ml -1 respectively) in nitrogen free medium after 7 days of incubation at 37°C.

Patowary *et al*. (2016) reported that only three isolates among the five bacteria selected from 23 bacteria based on their ability to degrade crude oil could collapse drop of crude oil indicating the presence of biosurfactant in the culture media.

Yanti *et al.* (2017b) studied antagonistic plant growth promoting *Bacillus* spp. isolated from chilli and reported that all strains had ability to produce variable concentrations of IAA, while, *B. subtilis* strain CIFT-MFB-4158A was able to produce siderophore, all isolates could not produce HCN and biosurfactant.

Kalita *et al.* (2018) studied the diversity and distribution of biosurfactant producing plant growth promoting bacteria (PGPB) from hydrocarbon contaminated soils of upper Assam for the growth of Muga host plant Som (*Persea bombycina*) following three different methods and reported that all the 40 isolates were able to produce EPS to some degree.

2.7 Plant growth promoting activity *in vitro*

2.7.1 IAA production

Sumera *et al*. (2004) isolated 12 plant growth promoting *Bacillus* strains rhizobacteria from rice and reported that nine isolates produced indole acetic acid ranging from 20.0 - 90.8 mg/L. Inoculation with these bacterial isolates resulted in higher plant biomass, root area and total N and P contents in Tanzannian rice variety BKWPRAT 3036B under controlled condition.

Ashrafuzzaman *et al*. (2009) reported that PGPRs are beneficial bacteria that colonize plant roots and enhance plant growth through various mechanisms. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. Most of isolates significantly increased plant height, root length, and dry matter production of shoot and root of rice seedlings. Seed germination was also observed to be increased when seeds were pretreated with PGPR isolates. Amongst the 10 isolates six isolates were recorded to produce IAA while, two could solubilize phosphate.

Datta *et al*. (2010) isolated and characterized 36 rhizobacteria from chilli rhizosphere and recorded that six isolates were able to produce ammonia, 14 could solubilize phosphate, 12 were able to produce siderophore and 31 isolates were able to produce IAA. The rhizobacteria also showed biocontrol activity against plant pathogen *Pseudomonas, Xanthomonas* and *Fusarium*.

Malik and Sindhu (2011) reported that *Pseudomonas* isolated from rhizosphere of chickpea and green gram were found to produce significant amount of IAA when grown in LB medium broth supplemented with L-tryptophan. Coinoculation of chickpea with IAA producing *Pseudomonas* strain

increased nodule number and nodule biomass. The plant dry weights of coinoculated treatments showed 1.10 to 1.28 times increase in comparison to Mesorhizobium- inoculated plants alone and 3.62 to 4.50 times over unioculated control at 100 days of plant growth. The results indicated IAA in enhancement of nodulation and stimulation of plant growth in chickpea.

Ahmed and Kibert (2014) described that biocontrol agents are able to produce plant growth promoting substances including IAA and other antifungal substances which inhibits the growth of pathogenic fungi and favours the growth of crop plants. They facilitate the plant growth by directly modulating the plant hormone levels or indirectly by decreasing the inhibitory effects of various pathogens.

2.7.2 Gibberellic acid (GA_3) production

Joo *et al*. (2005) reported that the growth of red pepper plants was enhanced by the treatment with rhizobacterium *B. cereus* MJ-1 increasing the shoot and root fresh weight by 1.38- and 1.28-fold gain respectively. *B. cereus* MJ-1, *B. macrolides* CJ-29 and *B. pumulis* CJ-69 were reported to produce gibberellic acid.

Patel and Desai (2015) studied 63 paddy rhizobacteria and observed that all the isolates were able to produce IAA and GA_3 to varying degree ranging from 5.79µg/ml to 43.03µg/ml and 118.41µg/ml to 198.18µg/ml respectively.

Gusmiaty *et al.* (2018) collected rhizobacteria from various private forest and isolated 35 rhizobacteria showing positive reaction for both IAA and gibberellin production.

Lofti *et al*. (2022) characterized plant growth promoting rhizobacteria from the rhizosphere of drought-stress tolerant Persian walnut trees and results suggested that different strain produce significant difference in the amounts of GA_3. Significantly higher amounts of GA_3 were observed from drought-stressed strains as observed in Cha41 (94.3), Haw20 (86.7) and ZM39 (85.4) (μg mL^{-1})**.**

2.7.3 Ammonia production

Joseph *et al*. (2007) characterized plant growth promoting rhizobacteria associated with chickpea and reported that all the isolates of *Bacillus, Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of Rhizobium was able to produce IAA. Production of ammonia was detected in the isolates of *Bacillus* (95.0%) followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45.0%). The isolates of *Pseudomonas* spp. were strong siderophore producers (74.2%) while few isolates of *Bacillus* were able to produce siderophore (12.5%).

Malleswari and Bagyanarayana (2013) studied 219 bacterial strains isolated from the rhizosphere of different medicinal and aromatic plants and tested for specific plant growth promotion activities *in vitro*. Results revealed that four isolates showed maximum plant growth promoting activities such as ammonia production, IAA production, phosphate solubilization, HCN production and antifungal activity.

Prasad *et al*. (2017) isolated 24 fungal BCAs and 12 bacterial isolates from the rhizosphere of tomato and evaluated for its ability to produce ammonia. Results suggested that all the BCAs including rhizobacteria and fungal BCAs were able to produce ammonia except for one fungal BCA.

Kashyap *et al.* (2020) stated that out of 32 rhizobacteria isolated from chilli rhizosphere 35.4 % rhizobacteria showed antagonistic ability to inhibit growth of *R. solanacearum*. *In vitro* screening of the rhizobacteria for PGP activities showed phosphate solubilization (64.2%), production of IAA (78.5%), production of ammonia (78.5%), production of HCN (35.7%) and siderophore production (50%).

El-Sersawy *et al*. (2021) studied three rhizobacterial strains identified as *B. velezensis* Vb1, *B. paramycoides* Vb3, and *B. paramycoides* Vb6 isolated from faba bean plant showing *in vitro* antagonistic activity against F. *oxysporum* and it was observed that all the three isolates were able to produce ammonia in varying quantity with Vb3 producing the highest quantity followed by Vb1 and Vb6.

2.7.4 Phosphate solubilization

Datta *et al.* (2010) reported that out of the 36 rhizobacteria isolated from chilli rhizosphere many isolates showed multi-functional plant growth promoting activity where, 14 isolates could solubilize phosphate on Pikovskaya's medium

Kumar *et al.* (2011a) tested a total of 10 rhizobacteria showing antagonistic and plant growth promoting activity on chilli and reported that all the ten isolates showed antagonistic activity against *S. rolfsii*, *F. oxysporum, C. capsici, R. solani, Macrophomina* spp. and *Pythium* spp. In the context of plant growth promoting properties, all isolates exhibited production of indole acetic acid whereas, five isolates produced siderophore and solubilized inorganic phosphate The isolates were identified using Microbial Identification System (Biolog) which revealed four isolates of *Bacillus* spp., two each of *Pseudomonas* spp. and *Enterobacter* spp. and one each of *Alcaligenes faecalis* and *Klebsiella* spp.

Bhakthavatchalu *et al*. (2013) recorded that *P. aeruginosa* FP6 was able to solubilize phosphate on Pikovskaya agar medium after three days by forming a clear halo zone around the colonies.

Abdeljalil *et al*. (2016) reported that 18 isolates out of the 25 tested belonging to *Bacillus, Enteroabacter, Klebsiella* and *Chrysobacterium* were able to solubilize inorganic forms of phosphorous making it available to the plants by solubilizing mineral phosphate through the production of organic acids or phosphatases.

Moustaine *et al*. (2017) studied three PGPR isolated from tomato for their influence on their growth and recorded a remarkable increase in root growth and total chlorophyll. All the strains showed plant growth promotion activities and were able to produce ammonia, produce IAA and fix nitrogen, and one isolate was found to solubilize phosphate.

Vasseur-Coronado *et al.* (2021) isolated 200 bacterial strains from tomato rhizosphere and recorded that 39% were able to grow in the presence of humic acid and were able to solubilize phosphate. Five of the bacterial strain were able to promote seedling vigor *in vitro*.

Syamsuddin *et al*. (2022) isolated 18 rhizobacteria to study the effect of plant growth promoting rhizobacteria on germination and seedling growth of chilli and reported that four rhizobacterial isolates were able to produce a significant amount of IAA, while 13 rhizobacteria isolate produced IAA, 13 isolates showed ability to dissolve phosphate and 12 rhizobacterial isolates were capable of producing siderophore. All rhizobacteria isolates were able to increase germination (100%) compared to the control treatment.

2.7.5 Zinc solubilization

Bapiri *et al*. (2012) evaluated zinc solubility of 40 *Pseudomonas fluorescent* using zinc oxide, zinc carbonate and zinc sulphide in both plate and broth media assays at 0.1% chemical source and reported that eight out of 40 were able to solubilize zinc by forming a clear zone in plate assay with zinc oxide and zinc carbonate plates.

Sharma *et al*. (2012) isolated 134 putative *Bacillus* isolates from soybean rhizosphere of Nimar region and screened for their *in vitro* zinc solubilization ability on Tris-minimal agar medium supplemented separately with 0.1% zinc in the form of zinc oxide, zinc phosphate, and zinc carbonate. It was reported that 31%, 27%, and 19% isolates were found positive towards zinc solubilization on different zinc supplementation respectively.

Ghevariya and Desai (2015) isolated 309 rhizobacteria associated with sugarcane and recorded that 141 isolates were able to solubilize zinc ranging from 116-366 for 0.1% zinc oxide incorporation by the formation of a halo zone around the bacterial colony.

Sultana *et al*. (2020) checked the ability of *Pseudomonas*, *Bacillus* and *Azospirillum* species for their zinc solubilizing ability and reported that these strains were able to solubilize ZnO and ZnCO.

Rion *et al.* (2022) screened zinc solubilizing plant growth promoting rhizobacteria from rice rhizosphere and reported a clear halo zone surrounding the colony in a solid medium containing ZnO for isolates solubilizing zinc with a solubilizing index ranging from 1.08-2.25 after 5 days of incubation.

2.8. *In vitro* compatibility

Thakkar and Saraf (2014) selected three promising antagonistic bacteria from 30 isolates and studied their compatibility *in vitro.* Results suggested that the interaction between bacterial isolates on agar plates at 30ºC were found to be compatible as the study revealed no mutual growth inhibition. The three isolates were identified as *P. aeruginosa, B, amyloliquefaciens* and *B. cereus*.

Ajaz *et al.* (2019) tested the compatibility of lactic acid bacteria, actinomycetes, *Pseudomonas* spp. and *Bacillus* spp. by dual and consortia inoculations *in vitro* and reported to be coexisting and complimentary.

Jaiswal *et al*. (2023) studied six promising PGPR for their compatibility by streaking on nutrient agar plates vertically in the center and horizontally at the plate's periphery and incubating at 28 ± 1°C for 48-72 hours. The result suggested that all the isolates were compatible as the absence of inhibition zones surrounding the colonies indicated compatibility, signifying that these colonies did not exhibit antagonistic behaviour.

Padhan and Jena (2023) tested *in vitro* compatibility between different native fluorescent *Pseudomonas* and *Bacillus* spp. and results suggested that almost all the isolates were compatible with each other as there was no formation of zone of inhibition between the isolates except for some isolates where zone of inhibition was observed indicating its incompatibility.

Bessa *et al.* (2023) studied the compatibility of antagonistic rhizobacteria (*Enterobacter asburiae* and *B. cereus*) isolated from the rhizosphere of *Butia archeri* and results suggested that chemicals, Avicta, Captan, Cropstar, Cruiser, Derosal, Fortenza, and Vitavax, were found to negatively affect the growth of all these rhizobacteria.

2.9 *In vitro* evaluation of plant growth promoting ability

Seleim *et al.* (2011) isolated *P. fluorescens*, *P. putida*, *B. subtilis* and *Enterobacter aerogenes* from tomato rhizosphere and recorded that all the isolates except *E. aerogenes* increase seed germination percentage upto 15% over untreated control while *P. fluorescens* showed maximum disease reduction under field condition.

Agrawal and Agrawal (2013) isolated 28 bacterial isolates from different rhizospheric soil of tomato, from which five efficient isolates were selected to test their efficiency for plant growth promotion and results suggested that the seed germination percentage increased from 90 per cent (uninoculated control) to 97.5 per cent. All rhizobacterial isolates were reported to produced IAA *in vitro* and were able to increase shoot and root length in comparison to control with an enhanced vigour index of 115.50, 714.35 after 6 and 16 days respectively.

Kabir *et al.* (2013) tested five isolates of PGPR where all of the isolates (BI 06, BI 09, BI 11, BI 15 and BI 18) were capable of enhancing different growth parameters *viz.* shoot and root length, fresh biomass and dry matter in comparison with uninoculated control plants.

Kumar *et al*. (2014) recovered 114 rhizobacteria from healthy plantation and vegetable crops and selected six promising isolates based on *in vitro* trials. It was reported that seed treatments with these six promising rhizobacteria increases seed germination (20%), vigor index (408%, 235%, 510%), radicle (2.11cm, 1.03cm, 4.06cm) and plumule length (2.43cm, 1.44cm, 1.24cm) of brinjal, chilli and okra seedlings. The six isolates were identified to be *B. strastosphericus, B. cereus, P. fluorescens* and *P. simiae.*

Majeed *et al.* (2015) purified nine native bacterial isolates from the rhizosphere and root-endosphere of wheat to study their PGP characteristics and evaluated for their beneficial effects on the early growth of wheat. Amongst the nine bacterial isolates, seven were able to produce indole-3-acetic acid, seven were nitrogen fixer, and four were able to solubilize inorganic phosphate *in vitro*. Plant inoculation studies indicated that these plant growth-promoting rhizobacterial (PGPR) strains provided a significant increase in shoot and root length ranged within 25–45% and 29–52%, respectively, over the uninoculated control whilst the corresponding increase in the shoot and root biomass ranged between 2–62% and 100–172%, respectively.

Jayapala *et al*. (2019) reported that beneficial rhizobacteria *Bacillus* spp. was found inhibitory to *C. capsici.* Further, upon seed priming, it reduced the seed-borne incidence of *C. capsici* (2%) and improved seedling vigor (1374 ± 7.15 vigor index) and germination (98 ± 0.57 %) of chilli seedlings. Under greenhouse conditions, seed priming resulted in reducing the anthracnose disease incidence up to 20%.

Charumathi and Raj (2020) evaluated 20 native PGPR isolate of chilli against *C. capsici* and reported that different strain of *P. fluorescens* showed 76.81-93.41% inhibition of the pathogen *in vitro*. The culture filtrate of these selected rhizobacteria were also able to increase germination percentage, shoot length, root length and vigor index of chilli. It was observed that Pf1 showed highest germination percentage of 97.60%, with shoot length of 9.25 cm, root length of 3.94 cm and vigour index of 1287.34 followed by Pf 11 and Pf 2.

Pérez-García *et al.* (2023) evaluated plant growth promoting rhizobacteria for different characters to improve germination in cucumber seedling and observed that treatment with rhizobacteria increases germination

percentage, length of plumule and radicle, germination index and vigor index by 20, 11, 48, 50 and 60% respectively over control.

2.10 Plant growth promotion *in vivo*

Aliye *et al*. (2008) evaluated 120 rhizobacteria *in vivo* against *R. solanacearum* causing bacterial wilt of potato and reported a reduction in wilt incidence by 82.7, 66.2 and 65.7 % by three isolates namely PFMRI, BS-DFS, and PF9 respectively. These rhizobacteria were also reported to increase plant height by 66, 50, and 48.2%, and dry matter by 153.8, 96.8, and 92.5%, respectively compared to the pathogen treated control.

Nguyen and Ranamukhaarachchi (2010) reported that treatment with TR6 (*B. megaterium*) resulted in the highest pepper and tomato fruit weight with significantly higher plant heights than control. The tallest capsicum plant was recorded from TR12 (*E. cloacae*) of 45.3 cm and the tallest tomato plant (70.4cm) was obtained with the treatment LR10 (*Pichia guillermondii*).

Anandaraj and Bini (2011) isolated around 170 rhizospheric and endophytotic bacteria from coriander, cumin, fennel and fenugreek from Rajasthan and Gujarat and recorded increased yield ranging from 10 to 20 per cent for consecutive two years.

Banerjee *et al.* (2011) isolated 15 rhizobacteria from chilli rhizosphere and reported that all the isolates were able to promote plant growth of local chilli cultivar '*Suryamukhii'* in pots. Increase in plant height was not observed in plants treated with individual isolates however, a significant increase in plant height was recorded from plants treated with a mixture of the isolates with maximum plant height of 54.17 cm recorded from a combined treatment of

PGPR C2 and C32. Maximum number of fruits/plant (129.77) and yield/plant and yield (386g) was recorded from a mixture of C2 and C25.

Kumar *et al*. (2011a) isolated and studied ten plant growth promoting rhizobacteria on the growth of chilli and result suggested that all the isolates were able to stimulated the growth of chilli seedlings under pot culture corresponding to a significant increase in the root and shoot biomass in bacterized seeds as compared to control.

Seleim *et al*. (2011) observed that rhizobacteria treated tomato plants grew significantly better than control plants. Plants treated with *P. flurescens* exhibited the highest biomass increase of 166.7% followed by *P. putida* with 157.8%, while plant treated with a mixture of different rhizobacteria gave the lowest biomass increase of 18.6%.

Hahm *et al*. (2012) reported that peppers treated with a mixture of PGPRs containing E681 and KUDC1013, either in a two-way combination, showed greater effect on plant growth than those treated with an individual treatment *in vivo*. Collectively, the application of mixtures of PGPR strains on pepper might be considered as a potential biological control under greenhouse and field conditions. The ability to increase growth and yield of chilli by rhizobacterial isolates is because rhizobacteria are effective root colonizers that survive and proliferate along with plant roots, resulting in enhanced plant growth

Lamsal (2012) isolated 125 rhizobacteria and selected seven strains based on *in vitro* assay against *C. acutatum* and reported that all the isolates were able to significantly increase growth parameters. AB17 was observed to increase the plant height by 39%, root length by 40.44%, fresh shoot weight by 42% and 47% in root biomass as compared to control plants which were observed to have physically retarded growth and pale colour when compared to other treatments.

Jadon *et al.* (2016) reported the effects of 3 PGPR (*B. pumilus, B. licheniformis*, and *P. fluorescens*) on chilli and tomato seedlings germination and observations suggested that all the PGPRs were able to increase the height of seedlings and the number of leaves in sterile and nonsterile conditions.

Odoh and Kenneth (2017) observed that seeds or soil application of PGPRs inoculant directly enhances phosphates solubilization, atmospheric nitrogen fixation and secretion of plant hormones (indole acetic acid, gibberellins, cytokinins and ethylene) needed for growth and adaptation in stressed environment.

Yanti *et al*. (2017a) screened rhizobacteria isolated from rhizosphere of healthy chilli for the control of bacterial wilt disease and for the promotion of growth and yield. Two strains of rhizobacterial isolates were found to show high potential for disease suppression and plant growth promotion activity. It was also observed that not all the selected rhizobacteria were able to accelerate the time to initiate flowering. However, the flower phase was reported to be earlier on two rhizobacterial introduced chili at 47.00-47.33 DAT.

Yanti *et al*. (2017b) characterized the mechanism of selected *Bacillus* spp. as biocontrol agents of *R. solanacearum* and as biofertilizer of chilli *in vitro* and observed that all the strains had the ability to produce variable concentrations of IAA, but not all the isolates can produce siderophore, HCN, biosurfactant and protease. Based on the ability to colonize roots, *B. subtilis* strain CIFT-MFB-4158A was found to be the best root surface colonizer (4.17×10^5 CFU/g root), while *B. subtilis* BSn5 had the best ability to colonize internal roots (2.08×10^5 CFU/g).

Fernandes *et al.* (2018) reported that PGPR could increase plant height (21.67cm), number of leaves (32.7), the number of tubers per hill (17.69g), the

wet weight of tuber per plot highs (3460g) and dry weight of tuber per plot (3010.2g) as well as control *Fusarium* wilt on red onions.

Fatimah (2018) isolated indigenous antagonistic rhizobacteria against *C. gloeosporioides* of chilli plant and reported that 19 of the isolates were capable of stimulating the growth of chilli seedlings and three isolates capable of reducing the disease intensity to 0-28%. The rhizobacteria were also observed to increase plant height from 27.3 to35.6 cm with an effectiveness of 0.4 to 30%. All rhizobacterial isolates were reported to increase the yield of chili crops from 99.1 to 491.5% with maximum yield recorded from seeds treated with isolate B2.11 yielding 213.4 g/plant, equivalent to 6.92 ton/ha) as compared to positive control with a yield of 35.1 grams/plant equivalent to 1.17 tons/ha.

Gowtham *et al*. (2018) evaluated 59 PGPR to study their effect on induction of resistance in chilli (*Capsicum annum* L.) against anthracnose disease and reported that only eight isolates of *Achromobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Ochrobactrum* and *Providencia* genera were able to be both non-pathogenic and possessed the ability to colonize the host plant. Among the PGPR isolates, seed treatment with *B. amyloliquefaciens* resulted in maximum enhancement of seed germination (84.75%) and seedling vigor (1423.8) with an increase in vegetative growth parameters. Significant disease protection of 71% against anthracnose disease was also observed in plants pretreated with *B. amyloliquefaciens* followed by *B. cepacia* and *P. rettgeri*, under greenhouse condition.

Yanti *et al*. (2018) studied 15 antagonistic endophytic rhizobacteria from healthy tomato roots against *R. solanacearum* and results suggested that 12 isolates could increase seed germination, number of leaves and seedling height ranging between 20.3 -61.13% than control in seedling stage. Also six isolates

were recorded to promote early flowering time and increase yield as compared to control.

Fridayati *et al*. (2020) reported that the treatment of eggplant seed with rhizobacteria significantly increased number of fruits/plant, fruit length, fruit diameter, fruit weight and yield over control.

Castillo-Aguilar *et al*. (2021) observed an increase in the yield of habanero chilli pepper by a difference of 4081.50 kg ha^{-1} with *Pseudomonas* treated plants giving an average yield of 16636.30 kg ha^{-1}, compared to control with a yield of 12554.80 kg ha^{-1}.

El- Sersawy *et al*. (2021) concluded that treatment of faba bean seeds with rhizospheric bacterial strains are able to supress the disease caused by *F. oxysporum* and significantly increase plant heights, number of pods/ plants and fresh and dry weight as compared with those planting into infected soil without bacterial inoculation. It was also observed that the plant height increased by 21.3-24.3% when treated with a single species but treatment with a consortium resulted in better plant height with the highest significant value recorded from V-mix treatment (Vb1+Vb3+Vb6) as compared with the infected plant percentages of 28.3% - 48.3%. Moreover, the number of pods/ plants were recorded to be highly reduced in the infected plant (12.3±1.5) as compared with a healthy plant (21.0±1.0). The presence of plant growth-promoting rhizobacterial strains causes a significant increase in the number of pods with the highest number of pods recorded for the bacterial consortium (V-mix) of 37% and 20.4% in the fresh and dry weight respectively over control in presence of the pathogen.

Ramirez-Carino *et al*. (2023) observed better growth in the seedling of tomato inoculated with rhizobacteria UTMR1, UTMR1, UTMR3 and UTMR4 after four days of first inoculation than control. The inoculated plants also had

higher plant height with an average plant height increase, seedling root length, shoot length than control.

2.11 Disease suppression by rhizobacteria *in vivo*

Ghonim (1999) reported that *B. subtilis* reduced the harmful effect of *F. oxysporum*, the causative agent of tomato wilt disease. Tomato seeds treated with biocontrol agent, *B. subtilis* and sown in soil infested with *F. oxysporum* produced less infected plants compared to those treated with the pathogen only.

Anjaiah *et al.* (2003) reported that *P. aeruginosa* PNA1 from chickpea rhizosphere, protected pigeon pea and chickpea plants from *Fusarium* wilt disease, caused by *F. oxysporum* f. sp. *ciceris* and *Fusarium udum*. Inoculation with strain PNA1 significantly reduced the incidence of *Fusarium* wilt in pigeon pea and chickpea on both susceptible and moderately tolerant genotypes.

Silva *et al*. (2003) tested 500 rhizobacteria isolated from tomato rhizosphere for their ability to induce systemic resistance against *Pseudomonas syringae* pv. *tomato.* It was reported that 28 of the isolates were reported to reduce infection to less than 40%.

Lamsal *et al.* (2012) investigated seven rhizobacterial isolates, AB05, AB10, AB11, AB12, AB14, AB15 and AB17 for plant growth promoting activities and inhibition against anthracnose caused by *C. acutatum* in pepper and reported that all seven bacterial isolates were capable of inhibiting *C. acutatum* to various degrees. Moreover, four isolates, AB10, AB12, AB15, and AB17, were identified as the most effective growth promoting bacteria under greenhouse conditions, with AB17 inducing maximum growth of pepper plant. It was stated that phytohormone IAA and phosphate solubilization capacity is a factor inducing Systemic Acquired Resistance (SAR) in various plants.

Loganathan *et al.* (2014) reported that *B. amyloliquefaciens* BA1 and *B. subtilis* BS2 isolated from rhizosphere of tomato, demonstrated substantial wilt reduction as compared to the untreated control. It was also observed that it served as a better agent for wilt control than chemical treatment with a per cent wilt reduction of 45.39 and 58.40 respectively. Pre-treatment of tomato plants with plant growth promoting *B. subtilis* BS2 significantly induced the activities of defence related enzymes *viz*. peroxidase, polyphenol oxidase, chitinase and phenylalanine ammonialyase and phenolics when challenged with the pathogen under field condition.

Islam *et al*. (2016) isolated and identified plant growth promoting rhizobacteria from cucumber rhizosphere to study their effect on plant growth promotion and disease suppression. They concluded that cucumber seeds treated with PGPR strains displayed significantly higher level of germination, seedling vigour, growth and N content in root and shoot tissue compared to non-treated control plants. Moreover, treating cucumber seeds significantly supressed *Phytophthora* crown rot caused by *Phytophthora capsici*.

Boukerma *et al*. (2017) evaluated the potential of *P. fluorescens* PF15 and *P. putida* PP27 to protect tomato plants against *Fusarium* wilt of tomato and recorded a significant inhibition of the pathogen growth (47%) by PF15 *in vitro*. *In situ* experiment also observed a reduced disease severity by 37–72%, and the levels of infection (incidence) by 7–36%.

Veerapagu *et al*. (2018) screened seven PGPR isolates from the agricultural field of chilli and characterized for its plant growth promoting characters, to which three isolates were identified as *Enterobacter* sp., *Bacillus* sp. and *Pseudomonas* sp. showing plant growth promoting characters such as IAA production, phosphate solubilisation, ammonia and exopolysaccharide production. PGPR isolates *Enterobacter* sp., *Bacillus* sp. and *Pseudomonas* sp.

produced 0.76 ± 0.15mg/ml, 1.37 ± 0.25mg/ml and 0.43 ± 0.15mg/ml of exopolysaccharide.

Yanti *et al*. (2020) reported that the occurrence of anthracnose disease in chilli plants treated with consortia of *Bacillus* spp. was lower (5.00-30.00%) compared to controls (70.00%) with effectiveness of 57.14-92.86%. The disease severity was also observed to be lower in treated plants (5.00-25.00%), compared to controls (40%) with an effectiveness of 37.50 to 87.50%. Maximum plant height (2.91-4.09 cm) was recorded from treated plants than from control (2.58cm) with effectiveness of 12.79-58.52%. All the treatments increased field emergence and increased the growth during both nursery stage and after transplanting. It was observed that the *Bacillus* spp. consortium is more profitable than a single isolate because of its more varied species so that the colonization and domination of the rhizosphere and roots are faster, and the mechanism of action of biocontrol agents is more diverse.

Yoo *et al*. (2020) reported that out of the pre-selected 17 potential biocontrol strains, T16E-39 and T20E-257, were selected as biocontrol agents against *P. capsici* and *R. solanacearum* in tomato plants and found that treatment with these strains increased plant growth; especially strain T16E-39 significantly increased fruit fresh weight in a plastic-house test and strain T20E-257 had antifungal activity against *S. sclerotiorum*, *Botrytis cinerea*, P. *capsici, F. oxysporum, Alternaria alternata*, and *R. solanacearum*. The two strains were identified as *Chryseobacterium soldanellicola* and *B. siamensis*, respectively.

Horuz (2021) studied the effects of 14 different antagonistic bacteria isolated from the phyllosphere of melon and watermelon against bacterial fruit blotch of cucurbits caused by *Acidovorax citrulli* under greenhouse conditions and revealed that *P. oryzihabitans* Antg-12, isolated from watermelon leaf,

reduced both disease severity (55.85%) and the area under the disease curve (AUDPC) (66.85%), and increased yield by about 41%.

2.12 Identification of rhizobacteria

Malleswari and Bagyanarayana (2013) reported that four isolates which showed maximum plant growth promoting activities were identified on the basis of colony morphology, gram staining and biochemical tests. These PGPR isolates were further characterized through 16S rRNA gene sequencing which led to their identification as *Pantoea* sp. (Cf 7), *Bacillus* sp. (Cf 60) and *Pseudomonas* sp. (Te1, Av 30) respectively.

Ahmadloo *et al*. (2014) reported that rhizobacteria are largely represented by the species of *Pseudomonas* and *Bacillus* genus and some bacteria belonging to the family of Enterobacteriaceae such as *Serratia, Pantoea* and *Enterobacter* strains found in the area of the rhizoplane of leguminous plants.

Kumar *et al*. (2014) identified six most promising isolates on the basis of microbial identification system and 16S rDNA and results suggested that the rhizobacteria were *B. cereus, B. stratosphericus and P. fluorescens*.

Abdeljalil *et al*. (2016) identified 25 rhizobacteria associated with tomato based on 16S rRNA gene sequencing and reported that 20 isolates were identified as belonging to genus *Bacillus,* three as *Enterobacter cloacae*, one as *Chryseobacterium jejuense* and one as *Klebsiella pneumoniae*. It was also stated that *Chryseobacterium* spp. are commonly found in soil and water and exhibits plant-growth promoting activities besides being able to suppress some soilborne diseases

Horuz and Aysan (2016) identified rhizobacteria exhibiting antagonistic activity against *Acidovorax citrulli* on the basis of 16S rRNA and reported that the rhizobacteria belonged to the genre *P. oryzihabitans* (Antg-12), *Microbacterium oxydans* (Antg-57), *Curtobacterium flaccumfaciens* (Antg-198), and *P. fluorescens* (Antg-27).

Hyder *et al*. (2020) studied the native plant growth promoting rhizobacteria of chilli antagonistic to *Phytophthora capsici* and the 16S rRNA sequence analysis of tested bacterial strains showed 98–100% identity with *P. putida, P. libanensis, P. aeruginosa, B. subtilis, B. megaterium,* and *B. cereus* sequences available in the National Center for Biotechnology Information (NCBI) GenBank nucleotide database. Greenhouse studies also concluded that all tested bacterial strains significantly suppressed *P. capsici* infections (52.3–63%) and enhanced the plant growth characters in chilli pepper.

Fasusi *et al*. (2021) characterized plant growth-promoting rhizobacterial isolates associated with food plants in South Africa and results suggested that rhizobacterial isolates with multiple plant growth-promoting potentials were identified as belonging to *Bacillus* spp. (80.77%), *Rhodocyclaceae bacterium* (3.85%), *Enterococcus* spp. (3.85%). *Massilia* spp. (3.85%) and *Pseudomonas* spp. (7.69%) based on their 16S rRNA molecular characterization.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

All the experiments related to the research work entitled “Antagonistic potential of Naga king Chilli rhizobacteria against anthracnose pathogen” were completed in the laboratory and experimental farm of the Department of Plant pathology, School of Agricultural Sciences (SAS), Nagaland University, Medziphema Campus, Nagaland during the year 2021-2023. The details of materials used and the research methodologies followed during the investigation and analysis are described below:

3.1 General information

3.1.1 Site of experiment

The proposed plan of work was carried out in the laboratory and experimental field of Department of Plant Pathology, School of Agricultural Sciences, Nagaland University, Medziphema, Nagaland and situated at 25° 45′ 45′′ North latitude and 93° 51′ 45” East longitudes at an elevation of 310 m above mean sea level.

3.1.2 Climatic conditions

The experimental location lies in humid sub-tropical zone with moderate temperature and medium to high rainfall. The mean temperature ranges from 12^{o}C to 32^{o}C during summer with an average annual rainfall from 200 cm to 300cm and a relative humidity of 70-80 %. The meteorological data recorded during the field experiments are presented in Table 3.1. The soil is sandy loam in nature with a pH ranging from 4.5-6.5.

Table 3.1 Meteorological data recorded during the field experiments (September 2021-June 2022 and September 2022-June 2023)

Month	Temperature (°C)		Relative humidity (%)		Rainfall (mm)
	Max.	Min.	Max.	Min.	
2021-2022					
October, 2021	32.1	22.1	95	68	130.0
November, 2021	28.5	14.8	96	51	0.0
December, 2021	25.1	11.3	95	51	16.4
January, 2022	22.7	10.1	96	56	34.6
February, 2022	23.2	9.6	95	48	56.3
March, 2022	32.2	15.5	90	40	2.3
April, 2022	30.9	19.9	90	68	175.7
May, 2022	30.5	21.9	92	71	224.4
June, 2022	32.0	23.9	95	72	160.8
2022-2023					
October, 2022	30.5	21.3	94	69	94.8
November, 2022	28.4	14.8	96	58	0.0
December, 2022	25.7	11.7	96	53	15.4
January, 2023	24.4	8.2	95	48	0.0
February, 2023	27.4	11.7	92	48	0.0
March, 2023	29.1	14.7	92	53	76.0
April, 2023	32.7	17.7	87	50	68.0
May, 2023	32.8	20.8	86	56	86.3
June, 2023	33.4	24.0	89	70	267.9

Source: ICAR, Research Complex for NEH Region, Nagaland Centre

3.2 Preparation of culture medium

The following culture media were prepared and distributed equally into 250ml conical flasks @ 200 ml/flask and autoclaved at 121°C (15 lb psi) for 15 min.

Potato dextrose agar (PDA) medium (HIMEDIA)

Ingredients	Grams/Litre
Potatoes	200g
Dextrose	20g
Agar-agar	15g
Distilled water	1000ml

Potato dextrose broth (HIMEDIA)

Potatoes	200g
Dextrose	20g
Distilled water	1000ml

King's B medium (HIMEDIA)

Peptone	20g	Glycerol	15ml
K2HPO4	1.5g	Agar-agar	20g
MgSO4.7H2O	1.5g	Distilled water	1000ml

Chrome Azurol Sulfonate (CAS) agar* medium

Chrome azurol sulfonate 60.5mg/50ml distilled water

CTAB 72.9 mg/40 ml distilled water

Nutrient agar (HIMEDIA) 42.23g

Distilled water 900ml, pH 6.8±0.2

*Chrome Azurol S solution (50ml) and CTAB solution (40ml) were mixed and to it 10 ml of 1mM $FeCl_3.6H_2O$ solution prepared in 10 mM HCl was added. Then, the final solution (100ml) was mixed with 900 ml of nutrient agar medium to make up the volume to 1000ml.

3.3 Collection, isolation and identification of the pathogen

3.3.1 Collection of disease specimens

Diseased fruit specimen showing typical symptoms of anthracnose fruit rot were collected from the farmers field of Jalukie, Peren district of Nagaland and were brought back to the laboratory for isolation, purification and further study.

3.3.2 Isolation and purification of the pathogen

Naga king chilli fruits showing typical anthracnose symptom were cut along with 50% healthy and 50% diseased tissue with the help of a sterile razor blade, and were then surface sterilized with 1% Sodium Hypochlorite (NaOCl) solution for one minute and washed three times with sterilized distilled water. Then the bits were inoculated onto sterilized Petri plates containing sterile solidified PDA medium aseptically and then incubated at room temperature (25±2°C) for five days. After which the pure cultures were transferred in test tube slants to be preserved at low temperature in the refrigerator (4°C). These isolates were used for pathogenicity test.

3.3.3 Identification of the isolated pathogen

The purified isolated pathogen was identified based on the morphological characters *viz.* conidial shape, conidial size, conidial length, breadth and colour observed under the microscope at objective lens 10x and 40x by comparing with the information available in literature and also by molecular identification.

3.3.4 Pathogenicity test

To prove the Koch's postulate, detached fruit method described by Thind and Jhooty, 1990 was followed. Here, the fruits were pin pricked with a sterilized needle and the pathogen was introduced by placing a uniform drop of spore suspension on the fruits. The spore suspension was prepared prior by harvesting conidia from a seven days old culture in sterilized distilled water by scrapping the PDA slants with a sterile spatula. The spore suspension was adjusted to $5X10^5$ spores/ml using a haemocytometer. The culture obtained from re-isolation was kept on PDA slants for further investigation.

3.3.5 Molecular identification of the isolated pathogen

3.3.5.1 Fungal growth condition

After being cultured for six days, four mycelial discs (5 mm in diameter) were transferred to 100 ml of potato dextrose broth medium (HIMEDIA) and left to incubate for another six days at 28 °C. The mycelial disc was filtered using a sterile Whatman filter paper-42, and then washed with sterile distilled water. After draining the mycelial disc onto filter paper, it was pulverised in liquid nitrogen using pestle and mortar.

3.3.5.2 Extraction of DNA

Two hundred mg of pulverised mycelia were used to extract the pathogen's genomic DNA using a commercial DNA isolation kit (HiPurA Fungal DNA purification Kit). A spectrophotometer (Shimadzu UV-160) was used to evaluate the quality and content of the genomic DNA at UV absorbance of 260 and 280 nm and calculate the 260/280 absorbance ratio. DNA was resuspended in 50 µl of TE buffer, and ethidium bromide fluorescens was used to measure the genomic DNA concentration.

3.3.5.3 PCR amplification

The universal primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') was employed for amplifying and sequencing the pathogen ITS region (White *et al*., 1990). Amplification reaction was arranged in a total volume of 50µl containing 4µl of 10 x Taq polymerase Assay buffer, 1µl Taq DNA polymerase enzyme (3 U μl^{-1}), 4µl dNTPs (2.5mM each), 30µl of water. 2µl each of forward (ITS1) and reverse (ITS4) primers and 1 µl of 183ng DNA template.

Thirty cycles were completed with 3min first denaturation at 94^{o}C, followed by denaturation at 94^{o}C for 1min, annealing at 50^{o}C, for 1 min and extension at 72^{o}C for 2min and ending with a last extension at 72^{o}C for 7min.

3.3.5.4 Agarose gel electrophoresis of PCR products

A 1.5-kb ladder (Biokart India) was used as a standard molecular marker. The PCR products were separated by electrophoresis (at 75 V cm^{-1} for 50 min) with 1x Tris acetate EDTA buffer on 1.5 per cent agarose gel. Then, ethidium bromide (0.5 µg ml^{-1}) was used to stain the gels prior pouring. Using a gel documentation system, the ethidium bromide-stained gel was examined and an image was taken.

3.3.5.5 Phylogenetic analysis of *Colletotrichum* sp.

After sequencing was done, the sequence was aligned by using BioEdit Sequence Alignment Editor Version 7.0.5 software by Hall (1999) to obtain consensus sequences. The consensus sequences were then compared with other DNA sequences in GenBank using Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Identification of the isolate was done based on the highest similarity of the BLAST search. Phylogenetic tree was constructed using the MEGA11 software and the nucleotide sequences of ITS 18S rRNA

was deposited in NCBI GenBank for acquiring accession number (Kai-li *et al*., 2019).

3.4 Collection and isolation of Naga king chilli rhizobacteria

3.4.1 Collection of rhizobacteria from Naga king chilli

A field investigation was conducted for the collection of rhizobacteria from the farmer's field of Nchangram and Leikieram village under Peren district, Nagaland. Rhizospheric soil were collected from the roots of healthy plant at flowering stage and were isolated and maintained on the petri plates and test tubes slants during the course of investigation (Plate. 1).

3.4.2 Isolation and maintenance of bacteria

Soil dilution plate technique described by Waksman, 1927 was followed for the isolation of rhizobacteria. For which, the soil adhering to the roots from the plant sample were collected to get 0.5g of the soil and then was added to 4.5 ml of sterilized distilled water and mixed vigorously at 140 rpm for 2 minutes. Then the suspension was diluted serially to 10^{-8} and 0.1 ml of the last 3 serial dilution were spread with a glass spreader on nutrient agar and King's B medium plates and incubated for 48h at 26±1°C. To obtain pure colonies, colonies with distinct morphological appearances were chosen from the countable plates and re-streaked on a fresh plate with the same media (Patel *et al*., 2014).

3.5 Screening through HR (Hypersensitive Response) for pathogenicity test

The rhizobacterial isolates were screened for Hypersensitive response on 4 O'clock plant (*Mirabilis jalapa*) for pathogenicity test. Two days old rhizobacterial pure culture water suspension at 10^8 cfu/ml was made by adding loop full of culture in sterilized distilled water and compared its density with McFarland standard 1. The culture suspension was infiltrated by injection carefully with the assistance of sterilized syringe and needle into the leaves tissue of *M. jalapa*. The development of HR was observed until 24 hours and only the

HR negative rhizobacterial isolates were used further in the experiment (Yanti *et al*., 2017a). For confirmation the rhizobacteria performing better at the antagonistic test were subjected for another pathogenicity test on tobacco following the same procedure.

3.5.1 Preparation of Standard McFarland Standard

To prepare McFarland standard 1 0.1ml of 1% Barium Chloride and 9.9 ml of 1% of hydrochloric acid were mixed which will be comparable to an approximate cell count density of 3.0 X 10^8 cells (McFarland, 1907).

3.6 Characterization of rhizobacteria

3.6.1 Morphological characterization

Morphology of bacterial colonies were characterized based on various traits such as size, shape, elevation, surface, optical properties, margin, and pigmentation. This macro-morphological characterization was performed, following Patel *et al*. (2014) protocol, on bacterial cultures previously grown for 48 h on NA medium and incubated at 28°C.

3.6.2 Biochemical characterization

A series of biochemical tests including Gram staining, KOH test, catalase, starch hydrolysis, gelatine hydrolysis and lactose utilization test were conducted using the criteria of Bergey's Manual of Systemic Bacteriology (Bergy *et al*., 1994).

Gram staining

A 24 hour old culture was thinly smeared onto a glass slide, allowed to air dry, and then heated by running the slide over a spirit lamp flame. The smear was immersed with crystal violet and let stand for 30 seconds, then it was gently rinsed with distilled water using a wash bottle. Then, the smear was flooded with

Plate 1. Sample collection from farmer's field Peren, Nagaland

a. Farmer's field
b. Fruit rot
c. Soil sample collection

Gram's iodine for 1 minute and gently washed with distilled water. The smear was then washed with 95% ethanol by adding drop by drop until no more colour flowed from the smear. Then, the smear was soaked in safranin counter stain and left for 1 minute. The smear was again rinsed with distilled water and blot dried. The appearance of purple colour indicated Gram positive bacteria and the appearance of pink colour indicated gram negative bacteria (Rahman *et al*., 2010).

Catalase test

To detect the production of catalyst a loop full of 24-48 hours old test bacterium was smeared on the slide and then covered it with a few drops of 3% hydrogen peroxide. The smear was examined for bubble production. (Koche and Gade, 2013)

KOH test

A loopful of bacteria was mixed in a drop 3% aqueous KOH solution for not more than 10 seconds with the aid of an inoculating loop. The inoculating loop was raised a few centimetres from the microslide and was observed for the formation of a mucoid thread. Gram positive bacterium do not produce strands even on repeated strokes of the inoculating loop.

Starch hydrolysis

Starch hydrolysis of the isolated rhizobacteria were evaluated using the medium with a composition of Peptone-10g; Beef extract-5g; Starch -2g; Agar -20g and Distilled water-100-ml. The medium was sterilized by autoclaving and the test cultures were inoculated and incubated at 28^{o}C for 7 days. The agar plates were then immersed with Lugol's iodine and let to act for few minutes. The cultures showing a clear zone was considered to be positive reaction.

Gelatine liquefaction test

Stab method described by Koche and Gade. (2013) was followed for gelatine liquefaction test (Peptone-10g; Beef extract-5g; Gelatin-20g and Distilled water -1000ml). The ingredients were combined and heated over a water bath. The medium was administered in tubes to about 5cm height and sterilized. The tubes were cooled and allowed to stand at 20^{o}C for 2 days. Inoculation was done by stabbing a straight inoculating needle charged with 48 hours old growth of the test bacterium. The tubes were incubated at 20^{o}C and observation were taken for liquefaction of gel column.

Lactose utilization test

The 24-hour test cultures were inoculated in sterilized glucose-phosphate broth tubes and incubated at 28 ±1^{O}C for 48 hours. Following incubation, 4-5 drops of Methyl red indicator were added to each tube and gently shaken. Development of red colour was taken as positive and development of yellow colour after addition of reagent was taken as negative.

3.7 Antagonistic effect of rhizobacteria against anthracnose pathogen *in vitro*

For determining the antagonistic potential, the bacterial isolates were streaked on Petri plates with PDA medium at a distance of 3.5 cm from the rim of individual plate. A 5 mm mycelial disc from a 7-day old PDA culture of *C. gloeosporioides* were then placed on the other side of the plates. The plates were then incubated at 28°C for 4-7 days (Rabindran and Vidyasekaran, 1996). A control plate without the rhizobacteria were also be maintained as control. The per cent of pathogen growth inhibition were computed utilising the subsequent formula given by Vincent, (1927):

$$\text{Per cent inhibition (PI)} = \frac{R - r}{R} X100$$

Where, PI = Per cent inhibition

R= Radial growth of pathogen in control plate

r= Radial growth of the fungal colony in dual culture plates

The experiment was conducted in a Complete Randomised Design (CRD) with three replications for each treatment. The treatment combinations for the experiment were laid as follows:

T_0 - Control- *C.* sp. alone

T_1 - *C.* sp. + R_1

T_2 - *C.* sp + R_2

T_3 - *C.* sp. + R_3

T_4 - *C.* sp + R_4

T_5 - *C.* sp.+ R_5

T_6 - *C.* sp.+ R_6

T_7 - *C.* sp.+ R_7

T_8 - *C.* sp. + R_8

T_9 - *C.* sp. + R_9

T_{10} - *C.* sp.+ R_{10}

T_{11} - *C.* sp.+ R_{11}

T_{12} - *C.* sp. + R_{12}

T_{13} - *C.* sp. + R_{13}

T_{14} - *C.* sp. + R_{14}

T_{15} - *C.* sp. + R_{15}

T_{16} - *C.* sp. + R_{16}

T_{17} - *C.* sp. + R_{17}

T_{18} - *C.* sp. + R_{18}

T_{19} - *C.* sp.+ R_{19}

T_{20} - *C.* sp. + R_{20}

T_{21} - *C.* sp. + R_{21}

T_{22} - *C.* sp. + R_{22}

T_{23} - *C.* sp. + R_{23}

T_{24} - *C.* sp. + R_{24}

T_{25} - *C.* sp. + R_{25}

T_{26} - *C.* sp. + R_{26}

T_{27} - *C.* sp. + R_{27}

Note: *C.* sp = *Colletotrichum* sp.
R_1-R_{27} = Rhizobacteria 1 to Rhizobacteria 27

3.8 Evaluation of biocontrol mechanisms by rhizobacteria *in vitro*

3.8.1 Detection of volatile metabolites production

Two lower lids of Petri plates inoculated with the bacterial isolate and the pathogen separately were kept inverted over each other and sealed air-tight with parafilm. The plates inoculated with the pathogen itself was sealed with an uninoculated plate to be marked as control (Denis and Webster, 1971). After 96 hours, colony diameter of the pathogen was measured in all the treatments. When the test pathogen on the control plate was fully covered, the mycelial growth was measured, and the percentage of the pathogen's growth that was inhibited by volatile metabolites over the control was computed as per the formula given in 3.6 (Vincent, 1927). The treatment combinations for the experiment were similar as laid in 3.6.

3.8.2 Qualitative assay on siderophore production

To find siderophore production, a modified Chrome azurol sulfonate (CAS) agar medium test plate was used, following the method described by Milagres *et al*. (1999). After plating the blue medium (CAS agar; Schwyn and Neilands, 1987) on Petri plates, a loop containing rhizobacterial isolates was spot-inoculated and incubated for three days at 28ºC. The colonies with yellow/orange zones were considered as siderophore producing isolates.

3.8.3 Qualitative assay on Hydrogen cyanide (HCN) production

Production of Hydrogen cyanide (HCN) was evaluated by streaking the rhizobacterial isolates on nutrient agar medium amended with glycine modified procedure of Miller and Higgins (1970). Whatman No.1 filter paper saturated in picric acid were placed in the lid of each Petri plate. The plates were then sealed air-tight with parafilm and incubated for 48 h. A shift in the filter paper's colour from deep yellow to a reddish-brown hue was considered as a sign of HCN production.

3.8.4 Qualitative assay on chitinase synthesis

Activity of chitinase was detected on 1% (w/v) colloidal chitin agar plates. Briefly the colloidal chitin was prepared by treating 10g of chitin powder with 100 ml of 37 % w/w hydrochloric acid in a glass beaker kept in a tray filled with cold water which was mixed manually by stirring every 5 mins with a glass rod for 30 min. The chitin HCL mixture was filtered through 8 layers of muslin cloth and was repeated until the pH of the colloidal chitin was neutral. The colloidal chitin medium was then prepared containing 15g of colloidal chitin, 0.5g of yeast extract, 1g of $(NH_4)_2SO_4$, 0.3 g of $MgSO_4.6H_2O$, 1.36 g of KH_2PO_4, 14g of agar agar and make up the volume to 1000ml with distilled water. A single colony of each bacterium was streaked and incubated for up to 8 days. Chitin hydrolysis was detected qualitatively after the culturing period by pouring 0.1% Congo red solution onto culture plates and checking for formation of a clear zone (El-Hamshary *et al*., 2008).

3.8.5 Qualitative assay on biosurfactant production

Biosurfactant production was assayed using Nutrient Broth (NB) medium. Isolated rhizobacteria were incubated for 48h without shaking and the development of biofilm on the medium's surface were considered as an indication of biosurfactant production (Yanti *et al*., 2017b).

3.9 Evaluation of mechanism of plant growth promotion by rhizobacteria *in vitro*

3.9.1 Qualitative assay on Indole Acetic Acid (IAA) production

Production of the phytohormone, indole-3-acetic acid (IAA) by the selected isolates was assessed following Ghodsalavi *et al*. (2013) protocol. Briefly, each bacterium was grown onto NB and incubated at 28°C for 48h in an incubator shaker at 170 rpm. Then, 50µL of each bacterial liquid culture was transferred to NB containing 50µg/mL of L-tryptophane. After 48h of incubation, the bacterial suspensions were centrifuged at 10000rpm for 10min. About 1 mL

of supernatant was mixed with 4 ml of Salkowski reagent. Reagent mixed with SDW was used as control. Following 30 min of incubation at room temperature, the appearance of pink colour was considered as a sign for the production of Indole Acetic Acid.

3.9.2 Qualitative assay on Gibberellic acid (GA_3) production

Bacteria were cultured on 100ml Murashige and skoorg (MS) medium amended with 1000 µg/ml of tryptophan and incubated at 25°C for 6 days. Amount of GA_3 present in the cultures were determined following the standard protocol described by Uthandi *et al.* (2009). 30ml of 6 days old cultures were taken from each flask and then centrifuged at 3000 rpm to remove the particulate matters from which 25ml of culture supernatant were taken into a conical flask to which 2ml of zinc acetate (1 M) were added. After 2 minutes 2ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To 5ml of this supernatant, similar volume of 30% HCl was added slowly and incubated at 20°C for 75 minutes. An uninoculated broth was also maintained as control

3.9.3 Qualitative assay on production of ammonia.

Rhizobacterial isolates were examined for the production of ammonia in peptone water. Ten ml of peptone water was used to culture freshly grown rhizobacterial isolates and incubated for 48-72h at 28 ± 2°C. Nesseler's reagent (0.5ml) was added in each tube and the change in the colour of the medium to brown/ yellow colour was regarded as a positive indicator of ammonia production (Cappucino and Sherman, 1992).

3.9.4 Qualitative assay on Phosphate solubilizing activity

The rhizobacterial isolates were streaked on plates with Pikovaskya's medium and cultured for 7days at 28°C (Pikovaskya, 1948). Phosphate solubilization activity was determined by the growth of the clear zone surrounding the bacterial colony

3.9.5 Qualitative assay on Zinc solubilisation activity

The isolates were introduced into modified PKV medium (Pikovskaya, 1948) (Glucose-10.0g; Ammonium sulphate-1.0g; Potassium chloride-0.2g; Dipotassium hydrogen phosphate-0.2g; Magnesium sulphate-0.1g; Yeast-0.2g; Distilled water -1000ml, 0.1% insoluble zinc compounds- ZnO). The rhizobacteria were inoculated on these media and maintained at 28ºC for 48 hours. Zinc solubilization activity were analysed by the development of the clear zone around bacterial colony (Bapiri *et al.,* 2012).

3.10 Compatibility test

3.10.1 Compatibility of bacterial cultures *in vitro*

Bacterial cultures were streaked on nutrient agar plates so that, for each single colony in the centre of the plate, there were other cultures streaked outward from it. The plates were cultured at 27°C for 48h and the zone of inhibition was observed and noted. The absence of zone of inhibition was considered as a sign of compatibility (Prasad and Babu, 2017).

3.10.2 Compatibility test of bacterial cultures with chemical

Disc diffusion method described by Bauer *et al.* (1959) was followed to test the compatibility of the rhizobacteria with fungicide captan. Where, sterilized filter paper disc soaked with fungicide were placed in Petri plates containing nutrient agar mixed with the selected bacterium. The appearance of clear zone of inhibition was considered incompatible

3.11 Preparation of rhizobacterial inoculum

Two days old culture of the selected strain of rhizobacteria was harvested from agar plates by flooding with sterile deionized water. 10^8 cfu mL^{-1}

concentration of the inoculum was maintained spectrophotometrically at 600 nm (Seleim *et al*., 2011).

3.12 *In vitro* plant growth promotion

3.12.1 Test for seed germination

Blotter method was followed for seed germination test, where 60 numbers of seeds in three replications were treated with rhizobacteria. The seeds were initially surface-sterilized with 1% sodium hypochlorite and soaked in a double volume of sterile distilled water and then in bacterial suspension for 15 minutes. The bacterial suspensions were drained and the seeds were shade dried for 30min before sowing (Longchar, 2019). Each treatment were maintained in three replications with 20 numbers of seeds in each replication and a control plate was also maintained by dipping the seeds in sterile water for comparison. The treated seeds along with the control were placed separately in the moist blotter inside the Petri plate and incubated at room temperature at 28±1°C. The blotter was periodically re-wetted to prevent drying. Seed germination was determined on the tenth day from the treatment and the number of germinated and un-germinated seeds was recorded. The following formula was used for calculating per cent germination:

$$\text{Per cent germination} = \frac{No.of\ seeds\ germinated}{Total\ no.of\ seeds} \times 100$$

The experiment was conducted in a Complete Randomised Design (CRD) with three replications for each treatment and the data were statistically analysed. The treatment combinations for the experiment were laid as follows:

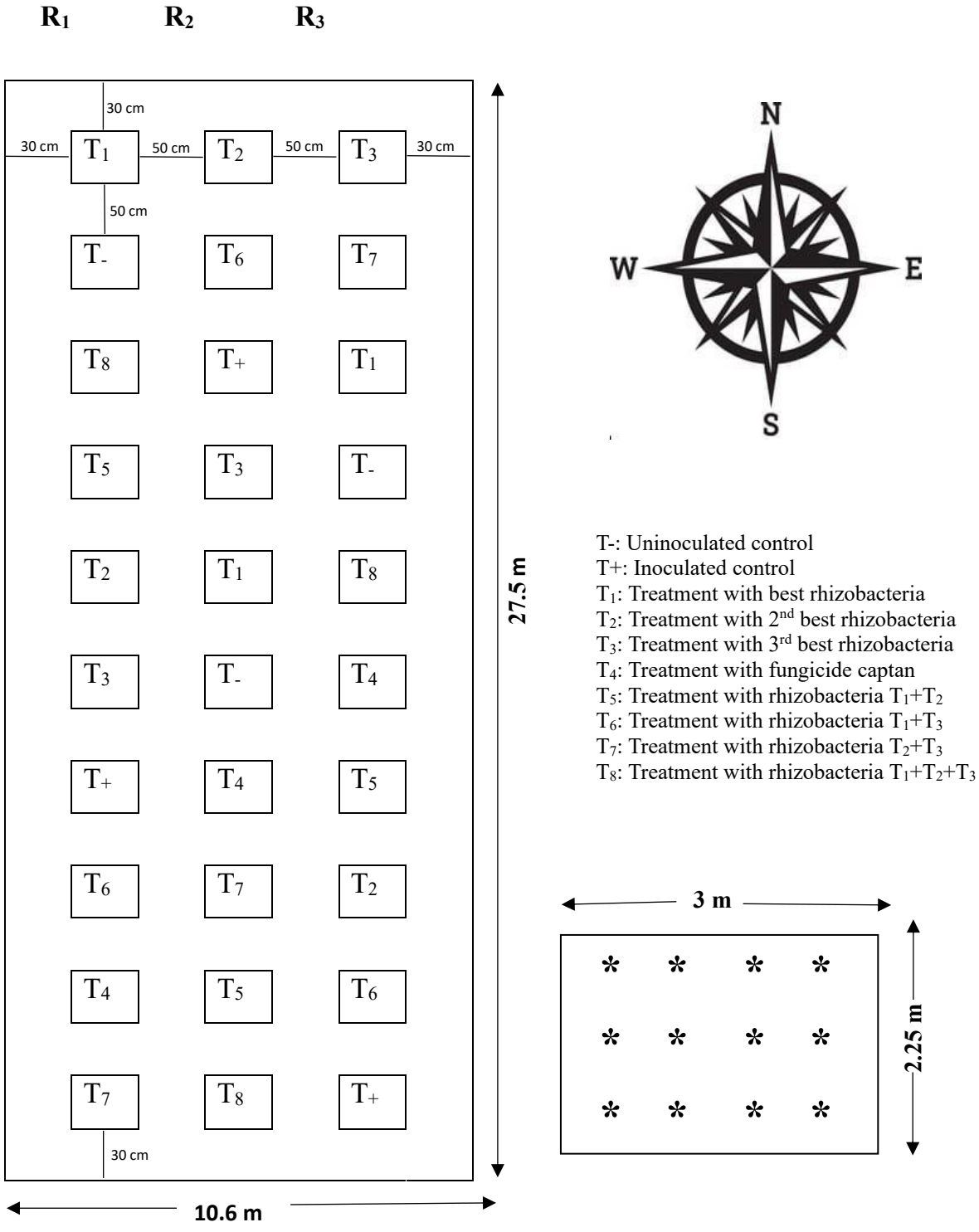


Fig. 3.1 Field layout of the experimental field in Randomised Block Design

Plate 2. *In vivo* trial

a. Seedling stage
b. Naga king chilli plant with fruits
c. General view of experimental field

T_0- control (sterile distilled water)

T_1- Treatment with best rhizobacteria

T_2- treatment with 2nd best rhizobacteria

T_3- Treatment with 3rd best rhizobacteria

T_4- Captan fungicide

T_5 - $T_1 + T_2$

T_6 - T_1+T_3

T_7 - T_2+T_3

T_8 - $T_1+T_2+T_3$

3.12.2 Seedling growth

Seedling growth was determined on the 15th day after sowing. Root and shoot length (cm) of the germinated seed was measured separately along with the control plates and the data were recorded.

3.12.3 Vigor index

Vigor index of the king chilli seedlings was computed by using the following formula (Long *et al*., 2008)

Vigor index (VI) = [Mean of root length (cm) + mean of shoot length (cm)] × per cent germination.

3.13 Field assessment of suppressive potential of selected rhizobacteria against Naga king chilli anthracnose

The best three performing rhizobacteria from the *in vitro* studies, their combinations along with fungicide captan were further studied under field condition keeping an inoculated and an uninoculated control plots (Fig 3.1 and Plate 2).

3.13.1 Seed treatment

The seeds were initially surface-sterilized with 1% sodium hypochlorite and soaked in a double volume of sterile distilled water and then in bacterial suspension for 15 minutes. The bacterial suspensions were drained and the seeds were shade dried for 30min before sowing (Yanti *et al*., 2017b).

For chemical treatment, surface sterilized seeds were treated with captan 50% WP (Seed dressing @ 0.3% or 3mg/1g see) (Srinivasan and Mathhivanan, 2009).

Whereas, for control surface sterilized seeds were soaked in sterilized distilled water (@1ml/1g seed) and dried inside laminar air flow (Srinivasan and Mathhivanan, 2009).

3.13.2 Seedling treatment

The rhizobacterial isolates were prepared following the procedure described by Seleim *et al*. (2011) and inoculated on chilli seedlings when they attain 5-6 leaves stage by root dip technique for about half an hour and dried under shade before transplanting (Batool and Altaf, 2017).

For chemical treatment the seedlings were treated with captan 50% WP (Seed dressing @ 0.3%). Whereas for control, seedlings were dipped in sterilized distilled water and shade dried before transplanting.

3.13.3 Artificial inoculation of pathogen *in vivo*

Spray inoculation of the pathogen was done with a conidial suspension @ 5x 10^5 conidial ml^{-1} water evenly distributing on the plant surface at 45 days after transplanting using a hand sprayer.

3.13.4 Spraying of rhizobacterial solution

Two sprays of the rhizobacteria were imposed after the appearance of initial disease symptoms at an interval of 15 days. Water sprayed plot were served as uninoculated control.

3.13.5 Details of *in vivo* study

Treatment combinations:

T-: Uninoculated control

T+: Inoculated control

T_1: Treatment with rhizobacteria 1

T_2: Treatment with rhizobacteria 2

T_3: Treatment with rhizobacteria 3

T_4: Treatment with fungicide captan

T_5: Treatment with rhizobacteria 1+2

T_6: Treatment with rhizobacteria 1+3

T_7: Treatment with rhizobacteria 2+3

T_8: Treatment with rhizobacteria 1+2+3

Treatment details

Crop : Naga king chilli

Experimental Design : RBD

No. of replication : 3

Spacing : 75 cm x 75 cm

Plot size : 3 m x 2.25 m

No. of plants per plot : 12

Plot to plot spacing : 50 cm

No. of experiment : Twice

3.14 Observation and recording procedures

Three randomly selected plants were tagged for recording of observations from each plot.

3.14.1 Days to first flowering

The days required by the plants to first flower was recorded from three randomly selected plants per plot.

3.14.2 Plant height (cm)

The height of the tagged plants were recorded in cm with the help of a measuring scale from the base of the stem to the tip of the longest leaf from three randomly selected plants per plot.

3.14.3 No. of fruits per plant

The numbers of fruits harvested from three randomly tagged plants were counted from which total number of fruits per plant were calculated.

3.14.4 Yield per plant (g)

The yield from a three randomly tagged plant throughout the course of the investigation was recorded in grams on fresh weight basis.

3.14.5 Projected yield per hectare (kg/ha)

The projected yield from the experimental field was worked out on the basis of yield per plot by using the given formula

$$Y = \frac{\text{A x } 10{,}000}{\text{S}}$$

Where, A = Yield/ plot (Kg)

Y = Yield/ha

S = Plot area (m^2)

3.14.6 Plant growth promotion assessment

At the end of the experiment, the entire plant with the roots were harvested and fresh weight of root and stem was recorded to determine the effects of the bacterial treatments on plant biomass increase. Untreated control plants were also uprooted and their weight were recorded to measure growth promotion, compared with untreated control (Lim and Kim, 1997).

$$\text{Biomass increased} = \frac{\text{Average fresh weight of treated plant} - \text{average weight of control plants}}{\text{average weight of control plants}} \text{X100}$$

3.14.7 Disease incidence (%)

The disease incidence was documented by recording the number of plants showing the symptoms at 60, 75, 90, 105 and 120, 150 days after transplanting. In all the plots each tagged plants were examined and scored for disease incidence using the formula given by Nene (1972).

$$\text{Per cent disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} X100$$

3.14.8 Disease severity

The disease severity was measured on six randomly selected plants by following the scales (0-5 scales) given by Bediako *et al*. (2015) at 150 DAT and the disease severity grades were converted into per cent disease index (PDI) for analysis (Wheeler, 1969).

$$PDI\ (\%) = \frac{\text{Sum of numerical rating}}{No.of\ plant\ scored\ X\ maximum\ score\ in\ scale} \text{X100}$$

Disease reading scale (Bediako *et al*., 2015)

Rating scale	Description
0	Healthy/healthy fruit on whole plant
1	1-5% of mature leaves with chlorotic and necrotic symptoms/ fruit sunken, light coloured lesions on exposed fruits lesion can enlarge that may extend to sides.
2	6-15% of mature leaves with chlorotic and necrotic symptoms/dark
3	15-50% of young shoots and stem waters oaked lesions and minor die back/ water-soaked, dull green spots covered with cream mould growth.
4	51-95% of water-soaked lesions with lots of mycelia growth and pervasive dieback of shoots/water soaked sunken lesions that expand cloudy, yellow blotches directly below skin
5	Dead plant/ pods soften and quickly collapse.

3.14.9 Per cent disease reduction over control

The per cent disease reduction was calculated using the formula:

$$Disease\ reduction\ (\%) = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \text{X}100$$

3.15 Identification of potential antagonistic rhizobacteria

The bacterial cultures were sent to Agharkar research institute, Pune for DNA isolation, amplification and identification. Briefly, the total genomic DNA was extracted using GeneElute Genomic DNA isolation kit (Sigma, USA) as per the manufacturer's instructions and utilised as template for PCR. 27F and 1492R primers were used to amplify entire 16S rRNA gene, as described previously. The PCR was performed using a mixture of 8.5 µl MilliQ, 12.5 µl of PCR Master Mix (PCR buffer, dNTp's and Taq polymerase), 0.5 µl of forward and

reverse primer and 3 µl of Template DNA making up the total volume to 25 µl following the Eppendorf Gradient Master cycler system with a cycle of 94 °C for 3 min; 32 cycles of 94 °C for 45 sec, 51 °C for 1 min and 72 °C for 1.30 min and final extension at 72° C for 10mins, and the mixture was held at 4°C. The PCR product was cleaned using Magnetic bead-based method. BigDye™ Terminator v3.1 Cycle Sequencing Kit (applied biosystems) was used for the sequencing of the PCR product. A combination of universal primers was chosen to sequence the nearly complete gene. The products after cycle sequencing were cleaned using BigDye XTerminator™ Purification Kit (applied biosystems). Seqstudio 232002103 (applied biosystems) was used to ran the samples. The out of sequencing was analyzed using the accompanying DNA Analyzer computer software version 1.1.4 (applied biosystems). The sequence was compared with NCBI for sequence identity. Clusters were constructed using Mega X software.

3.16 Statistical analysis and data interpretation

The data recorded were statically analysed by following the standard procedures described by Panse and Sukhatme, 1985. The laboratory experiments were analysed for significance employing a Completely Randomized Design (CRD) and the field experiment were analysed following a Randomize Block Design (RBD). The recoded values were subjected to arc sine and square root transformation wherever required. Results of the measurements were subjected to analysis of variance (ANOVA) by Least Significant Difference (LSD).

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Observations and results recorded during the course of the investigation on "Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen" were analysed to evaluate the effectiveness of the treatment applied and the results secured are presented and discussed under the following heads.

4.1 Symptomatology

The typical symptoms of anthracnose were witnessed on all above ground parts and the severity of the development of characteristic symptom of circular or angular sunken lesions with concentric rings of acervuli producing pink to orange conidial masses was observed highest during fruiting period with prominent fruit rot and dieback. The symptoms observed are described below.

4.1.1 Leaves

On the leaves, initially small-circular spots appear with chlorosis around the spot which later coalesce to form larger necrotic spots and the fatally infected leaves drop causing defoliation of plant (Plate 3a). Similar symptoms were also recorded by Rahman *et al*. (2011); Manda *et al.* (2020).

4.1.2 Die back

In die back phase, the tender twigs of the infected plants become necrotic from tip backwards which later the entire top twig of the plant may wither and in severely infected plant the entire plant may wither away. Dead twigs become grayish white to straw coloured in advanced stage of the disease; large number of the black dots are found scattered all over the necrotic surface of the affected twig (Plate 3b) Similar symptoms were also reported by Singh (2005).

4.1.3 Fruit rot

Typical anthracnose symptoms on the fruit include sunken necrotic tissue with concentric rings of dark coloured acervuli on both green as well as matured red fruits were observed. Under severe disease condition the lesion may coalesce, covers the entire fruit and eventually rot off the plant (Plate 3c). Similar symptoms were also reported by Ngullie *et al.* (2010); Oo and Oh. (2016).

4.2 Pathogenicity test, characterization and identification of the pathogen

4.2.1 Pathogenicity test

The pathogenicity test was carried out by detached fruit method following the procedure described by Thind and Jhooty (1990) and observations recorded revealed that the isolated pathogen is pathogenic to Naga king chilli as there was development of lesion on the inoculated plants seven days post inoculation. Similar results were also reported by Achumi (2022) when different isolates of *Colletotrichum* spp. were tested on Naga king chilli by detached fruit method (Plate 3d_1 and d_2).

4.2.2 Characterization and identification of the pathogen

Seven days old culture grown on PDA medium was studied for the cultural characters and it was observed that the colonies appeared pale grey with moderate arial mycelium which becomes darker with age (Plate 3e). Microscopic characters from a 14 days old culture revealed that the mycelium consist of septate, branched, hyaline, smooth walled hyphae. Conidia were hyaline, aseptate, smooth, prominently guttulate. Similar findings were also recorded by Achumi (2022) who reported that *C. gloeosporioides* isolated from Naga king chilli produced cylindrical conidia with size ranging 13.5 µm × 4-4.5 µm in length and width respectively (Plate 3f).

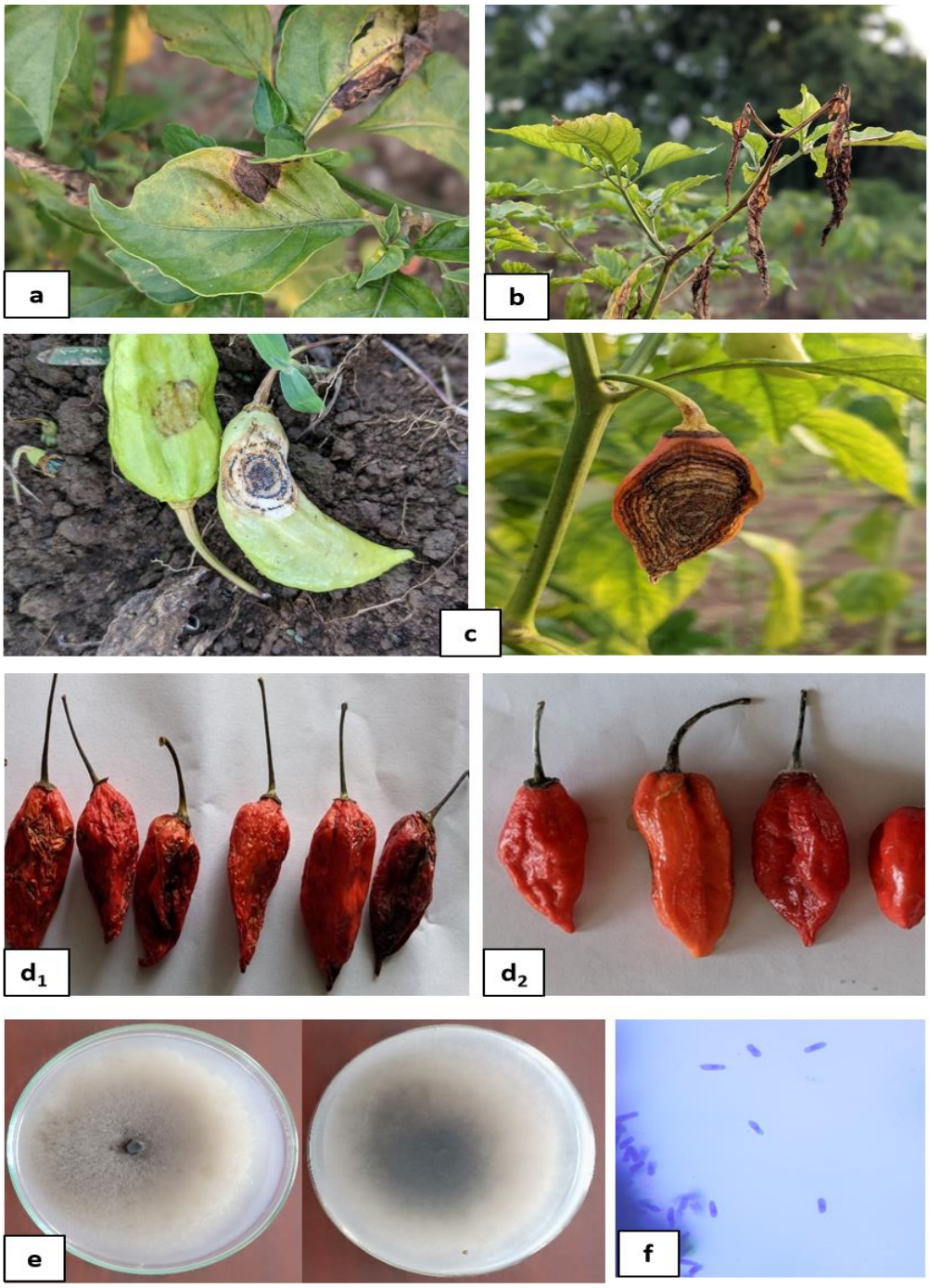


Plate 3. Typical symptoms on plant and pathogen morphology

a. Leaves
b. Die back
c. Fruit rot
d. d_1 inoculated and d_2 uninoculated control
e. *C. gloeosporioides* on PDA front and back view
f. Microscopic view of the spores under 40X

4.2.3 Molecular identification of the pathogen

Molecular identification of the was performed using Internal Transcribed Spacer (ITS) region of 18S rDNA. Sequence analysis of the pathogen was done to confirm the species identity. The forward (ITS1 Forward-5' TCCGTAGGTGAACCTGCGG-3') and ITS4 Reverse 5'TCCTCCGCTTATTGATATGC-3' oligonucleotide pairs amplified a single DNA fragment of approximately 564 bp amplicon size. ITS sequence was submitted to NCBI Gene bank (PP494232) which showed 99.64% similarity with *Colletotrichum gloeosporioides* with 99% query coverage during BLAST analysis (Table 4.1). This information was used to construct a phylogenetic tree using Mega X following bootstrap method and subsequently shown to be closest with *C. gloeosporioides* submitted from Meghalaya isolated from *Piper betel* (Plate 4).

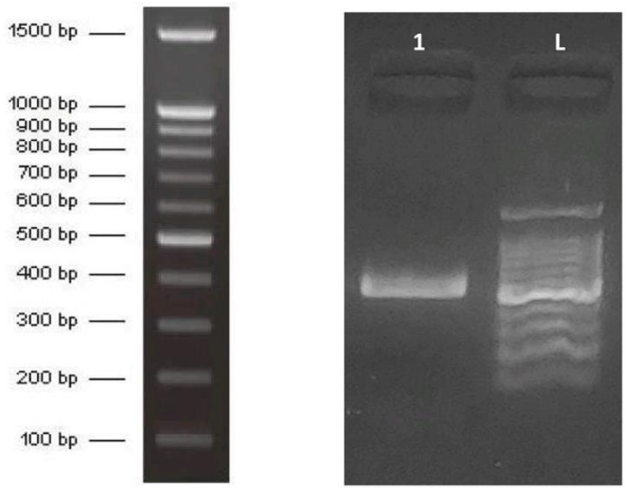
This might be due to the geographical proximity between Nagaland and Meghalaya and *C. gleosporioides* being able to infect a variety of host. The present findings are comparable with the findings of Li *et al.* (2021) who also molecularly identified the pathogen isolated from chilli causing anthracnose to be *C. gloeosporioides*. Taludar *et al.* (2012) has also reported the occurrence of *C. gloeosporioides* in Naga king chilli causing die back from Assam.

4.3 Isolation and maintenance of rhizobacteria

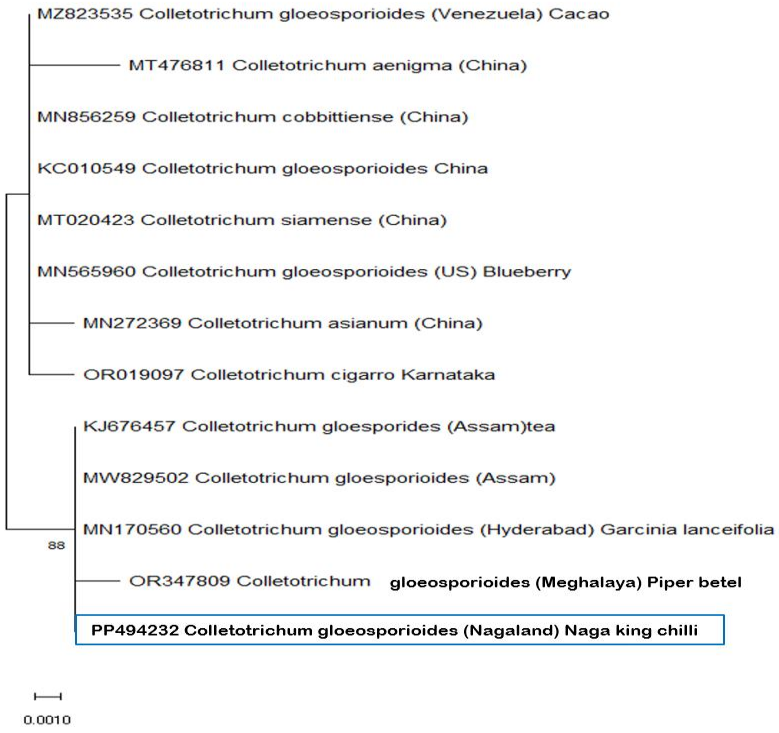
A total of 27 rhizobacteria were isolated from the farmer's field of Peren district of Nagaland which is famous for the cultivation of Naga king chilli. The rhizobacteria were isolated from plant at flowering stage and was maintained on nutrient agar and king's B agar media during the course of the entire investigation.

Table 4.1. Internal Transcribed Spacer (ITS) region of rDNA sequence of *Colletotrichum gloeosporioides*

Accession No.	Primer & their sequence	Sequence	Base pair	Similarity	Query coverage
PP494232	ITS1 Forward-TCCGTAGGTGAACCTGCGG ITS4 Forward-TCCTCCGCTTATTGATATGC	ACCTGCGGAGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATTACTGTTGCTCGGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCCGGGCGGGTCGGCGCCCGCCGGAGGATAAACAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAACCGGAAGAA	563	99.64%	99%



a. PCR amplification of ITS1 and ITS4 gene of rDNA sequence



b. Multiple clustering based on ITS region of rDNA sequences

Plate 4. PCR amplification and clustering analysis of pathogen

4.4 Morphological and biochemical characterization of rhizobacteria

Morphological characters of the rhizobacterial isolates are presented in Table 4.2 and Plate 5. The isolates were observed to be predominantly smooth, round, either orange or milky white in colour, smooth surface, convex with entire margin and translucent.

Result on biochemical characterization are shown in Table 4.3 and depicted in Plate 6. It was recorded that out of the 27 isolated rhizobacteria six were gram positive and 21 Gram negative, five isolates were recorded to utilize lactose whereas, six isolates were negative for KOH test. All the isolates recorded positive reaction for catalase and gelatine liquefaction activity. However, eight isolates could not hydrolyse starch.

The above findings are in contrary to the observations made by Banerjee *et al*. (2011) who reported that the population of bacteria in chilli rhizosphere was dominated by gram positive bacteria with white, irregular, opaque colonies as the Naga king chilli rhizosphere was found to be dominated by Gram negative, orange coloured, opaque colonies. This difference in observations may be due to the difference in ecological and environmental conditions favouring the growth, development and establishment of different bacterial population and hence reflected in the population of the rhizobacteria. The results on catalase productions are similar with the observations of Patel and Desai (2015) who observed that all rhizobacterial isolates were positive for catalyst production and hence, are aerobic in nature.

4.4.1 Screening through HR (Hypersensitive Response) for pathogenicity test

The results on the Hypersensitive response tested is presented on Table 4.4 and depicted on Plate 7.

Table 4.2. Morphological characterization of rhizobacteria

Isolates	Size	Shape	Colour	Surface	Elevation	Margin	Opacity
T_1	Small	Round	White	Smooth	Flat	Erose	Translucent
T_2	Small	Round	White	Smooth	Convex	Entire	Translucent
T_3	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_4	Small	Irregular	Light yellow	Smooth	Raised	Undulated	Translucent
T_5	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_6	Small	Round	Light orange	Smooth	Convex	Entire	Opaque
T_7	Small	Irregular	Orange	Corrugated	Raised	Undulated	Opaque
T_8	Small	Round	Reddish	Smooth	Convex	Entire	Opaque
T_9	Small	Round	Yellow	Smooth	Pulvinate	Entire	Opaque
T_{10}	Small	Round	Yellow	Smooth	Convex	Entire	Translucent
T_{11}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque
T_{12}	Small	Round	Greenish yellow	Smooth	Convex	Entire	Opaque
T_{13}	Medium	Round	White	Smooth	Convex	Entire	Opaque
T_{14}	Small	round	Yellow	Smooth	Raised	Entire	Opaque
T_{15}	Small	Round	Milky white	Smooth	Convex	Entire	Translucent
T_{16}	Medium	Round	Orange	Smooth	Convex	Entire	Opaque
T_{17}	Medium	Round	Dull white	Smooth	Convex	Entire	Translucent
T_{18}	Small	Round	Orange	Corrugated	Raised	Undulated	Opaque
T_{19}	Small	Round	Light orange	Smooth	Flat	Entire	Translucent
T_{20}	Small	Round	Pale white	Smooth	Convex	Entire	Translucent
T_{21}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{22}	Small	Round	Yellow	Smooth	Flat	Entire	Opaque
T_{23}	Small	Round	Orange	Smooth	Convex	Entire	Opaque
T_{24}	Small	round	Pale white	Smooth	Flat	Entire	Translucent
T_{25}	Small	Round	Creamy white	Smooth	Convex	Entire	Opaque
T_{26}	Medium	Round	Dull white	Smooth	Convex	Entire	Opaque
T_{27}	Small	Round	Pale yellow	Smooth	Convex	Entire	Opaque

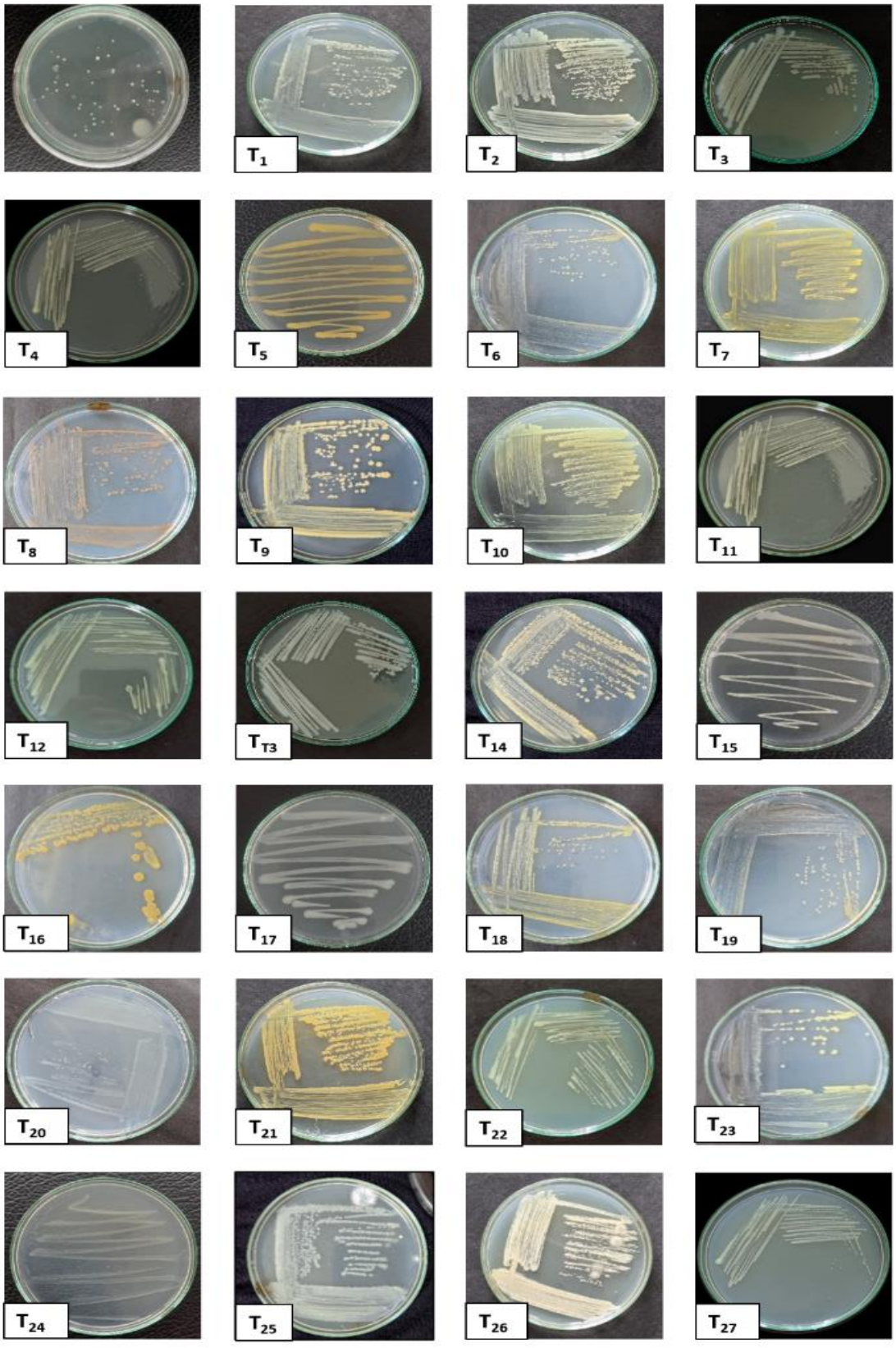


Plate 5. Rhizobacteria isolated from Naga king chilli rhizosphere

Table 4.3. Biochemical characterization of the rhizobacteria

Isolates	Gram staining	Lactose utilization	KOH test	Gelatine liquification	Catalase test	Starch Hydrolysis
T_1	-	+	+	+	+	-
T_2	-	-	+	+	+	-
T_3	-	-	+	+	+	+
T_4	-	-	+	+	+	+
T_5	+	-	-	+	+	-
T_6	-	-	+	+	+	+
T_7	-	-	+	+	+	+
T_8	-	+	+	+	+	+
T_9	-	-	+	+	+	+
T_{10}	-	-	+	+	+	+
T_{11}	-	-	+	+	+	-
T_{12}	-	-	+	+	+	-
T_{13}	-	+	+	+	+	+
T_{14}	-	+	+	+	+	+
T_{15}	+	-	-	+	+	+
T_{16}	-	-	+	+	+	-
T_{17}	+	-	-	+	+	+
T_{18}	-	-	+	+	+	+
T_{19}	-	-	+	+	+	-
T_{20}	+	-	-	+	+	+
T_{21}	-	-	+	+	+	-
T_{22}	+	-	-	+	+	+
T_{23}	-	-	+	+	+	+
T_{24}	-	-	+	+	+	+
T_{25}	+	-	-	+	+	+
T_{26}	-	+	+	+	+	+
T_{27}	-	-	+	+	+	+

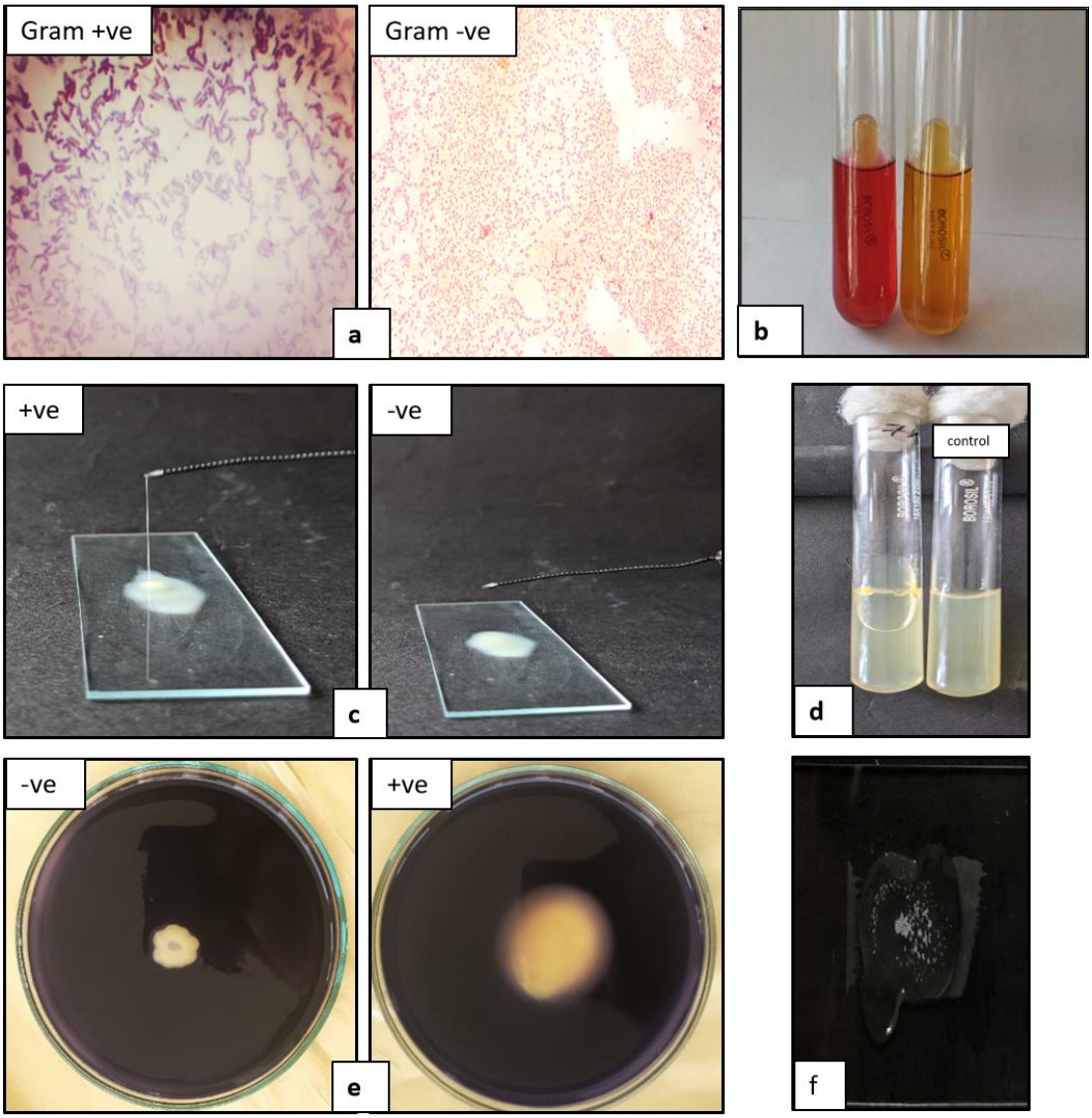


Plate 6. Biochemical test

a. Gram reaction
b. Lactose utilization test
c. KOH test
d. Gelatine liquification test
e. Starch Hydrolysis test
f. Catalase test

Table 4.4. Screening through HR (Hypersensitive Response) for pathogenicity test

Treatments	*M. jalapa*	Tobacco
T_1	-	
T_2	-	
T_3	-	
T_4	-	
T_5	-	
T_6	-	
T_7	-	-
T_8	-	
T_9	-	
T_{10}	-	
T_{11}	-	
T_{12}	-	
T_{13}	-	
T_{14}	-	
T_{15}	-	
T_{16}	-	-
T_{17}	-	
T_{18}	-	
T_{19}	-	
T_{20}	-	
T_{21}	-	
T_{22}	-	
T_{23}	-	
T_{24}	-	
T_{25}	-	-
T_{26}	-	
T_{27}	-	

Note: -= Negative

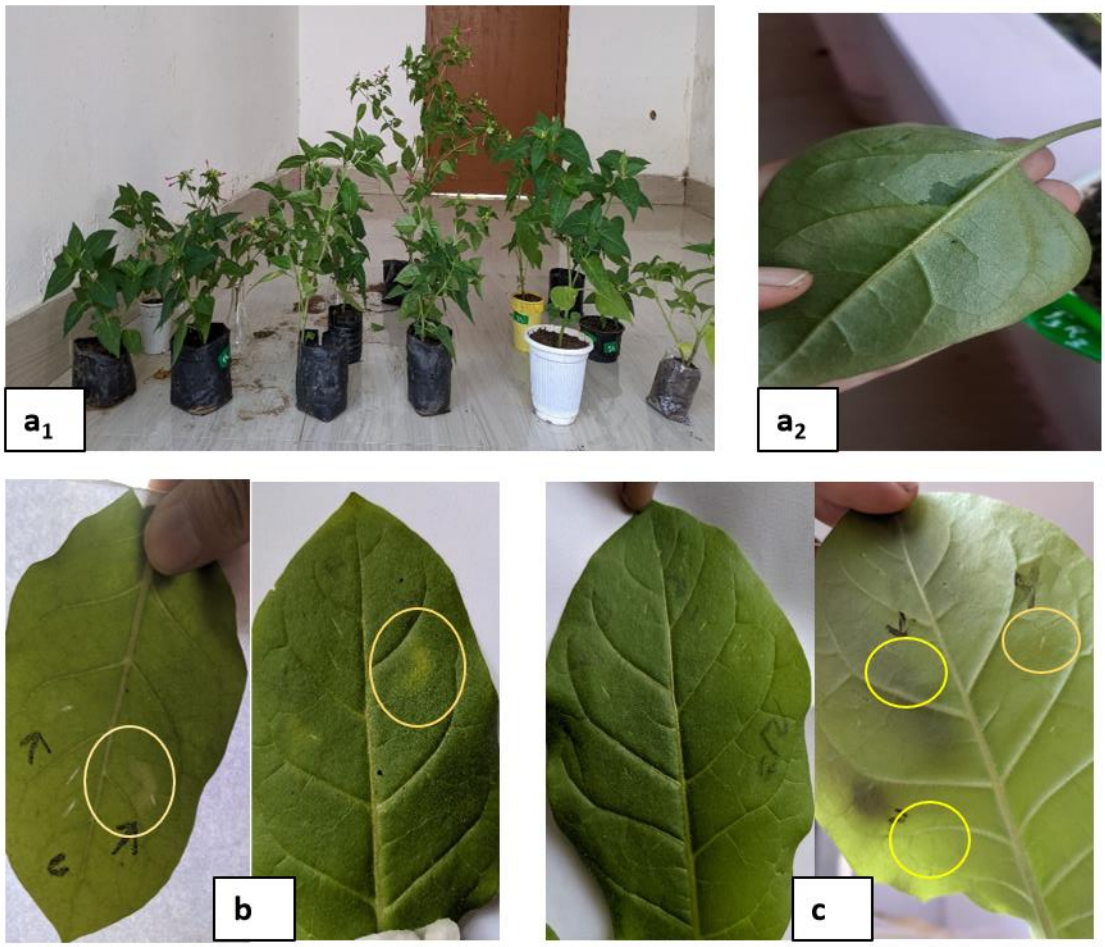


Plate 7. Hypersensitivity Response test

a. 4 O'clock plant (*Mirabilis jalapa*)
b. Control- tobacco leaf (*Nicotiana* sp.) injected with *Xanthomonas axonoposis* pv *citri* after 24h
c. Test rhizobacteria injected on tobacco after leaf 24h

None of the isolated rhizobacteria induced any symptom of hypersensitive response on *Mirabilis jalapa* plants after 24 hours (Plate 7) and hence were concluded as negative for HR.

To further confirm the non-pathogenicity of the rhizobacteria the three best performing rhizobacteria from *in vitro* antagonism test were tested for its HR on tobacco plant using *Xanthomonas axonopodis* pv. *citri* as inoculated control. Where, the inoculated control developed a faint chlorosis after 24 hours while the rhizobacteria inoculated leaf remained green therefore, confirming non-pathogenicity of these rhizobacteria.

Similar observations were made by Abdeljalil *et al*. (2016) who stated that all 200 tomato rhizobacteria showed negative response for hypersensitive response on tobacco leaves as there was no development of necrotic spot 24 hours after inoculation. Similar results were also made by Miladiarsi *et al.* (2017) on tobacco leaves; Yanti *et al*. (2017b) on *M. jalapa* leaves.

4.5 *In vitro* antagonism of rhizobacteria against *C. gloeosporioides*

Results on *in vitro* antagonism of isolated rhizobacteria against *C. gloeosporioides* following dual culture is displayed in Table 4.5 and depicted in Fig 4.1, Plate 8.

All the 27 isolates were subjected to preliminary screening against the pathogen and result suggested that antagonism by the rhizobacteria ranged from 0.00-48.89%. Highest per cent inhibition was recorded from T_7 with 48.89% inhibition which was found to be statistically at par with T_{25}, T_{18}, T_{16} with a per cent inhibition of 47.41, 46.66 and 42.96 % respectively. No sign of inhibition was observed on T_5, T_8, T_9, T_{14}, T_{17}, T_{19}, T_{20}, T_{22}, T_{23} and T_{24}.

In the present investigation the inhibitory activity of the rhizobacteria against *C. gloeosporioides* may be attributed to the production of toxic

Table 4.5. *In vitro* antagonism of rhizobacteria against *C. gloeosporioides*

Treatments	Inhibition of *C. gloeosporioides*	
	Radial growth (cm)	Inhibition %
T_1	4.40	02.29 (7.24)
T_2	4.33	03.78 (9.94)
T_3	4.40	02.29 (7.81)
T_4	2.80	37.78 (37.92)
T_5	4.50	00.00 (0.99)
T_6	4.17	07.41 (15.53)
T_7	**2.30**	**48.89 (44.36)**
T_8	4.50	00.00 (0.99)
T_9	4.50	00.00 (0.99)
T_{10}	2.97	34.07 (35.70)
T_{11}	4.23	06.00 (12.38)
T_{12}	5.97	11.85 (20.12)
T_{13}	4.50	14.07 (21.94)
T_{14}	4.50	00.00 (0.99)
T_{15}	3.47	22.96 (28.63)
T_{16}	2.57	42.96 (40.95)
T_{17}	4.50	00.00 (0.99)
T_{18}	2.40	46.66 (43.08)
T_{19}	4.50	00.00 (0.99)
T_{20}	4.50	00.00 (0.99)
T_{21}	3.10	31.11 (33.87)
T_{22}	4.50	00.00 (0.99)
T_{23}	4.50	00.00 (0.99)
T_{24}	4.50	00.00 (0.99)
T_{25}	2.37	47.41 (43.51)
T_{26}	3.13	30.37 (33.42)
T_{27}	3.63	19.26 (25.97)
T_0	4.50	00.00 (0.99)
SEm±	0.38	1.74
CV (%)	16.72	17.90
CD (p=0.05)	1.07	4.90

Note: Figures in the table are mean values and those in parenthesis are angular transformed values

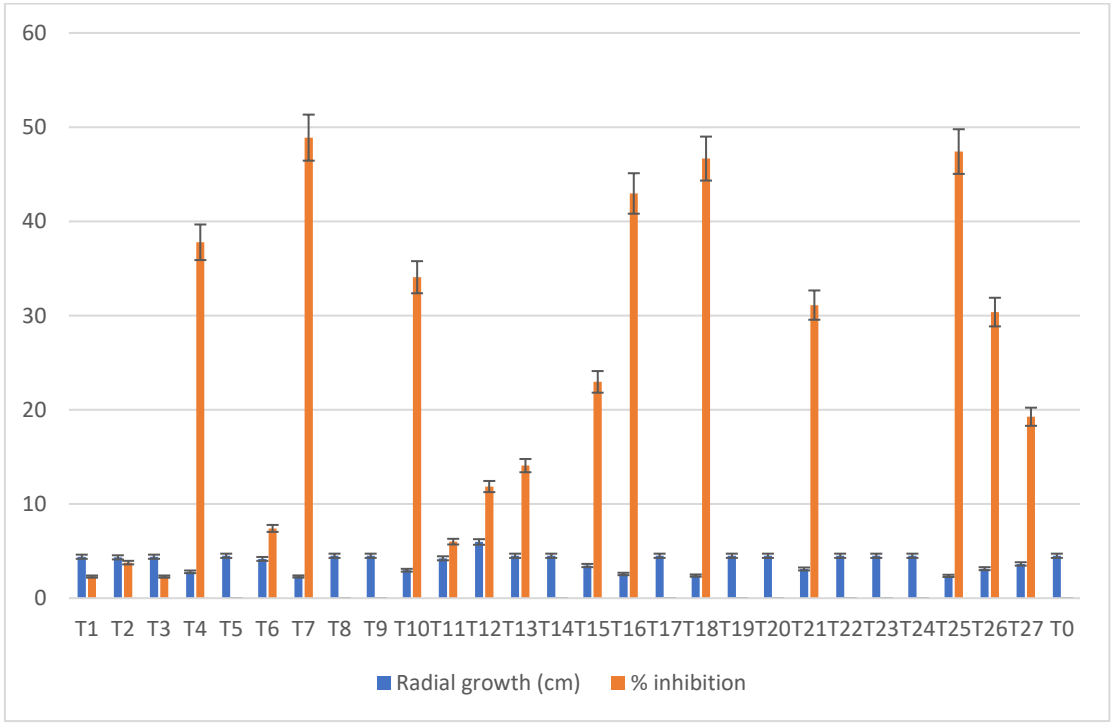


Fig. 4.1 *In vitro* antagonism of rhizobacteria against *C. gloeosporioides*

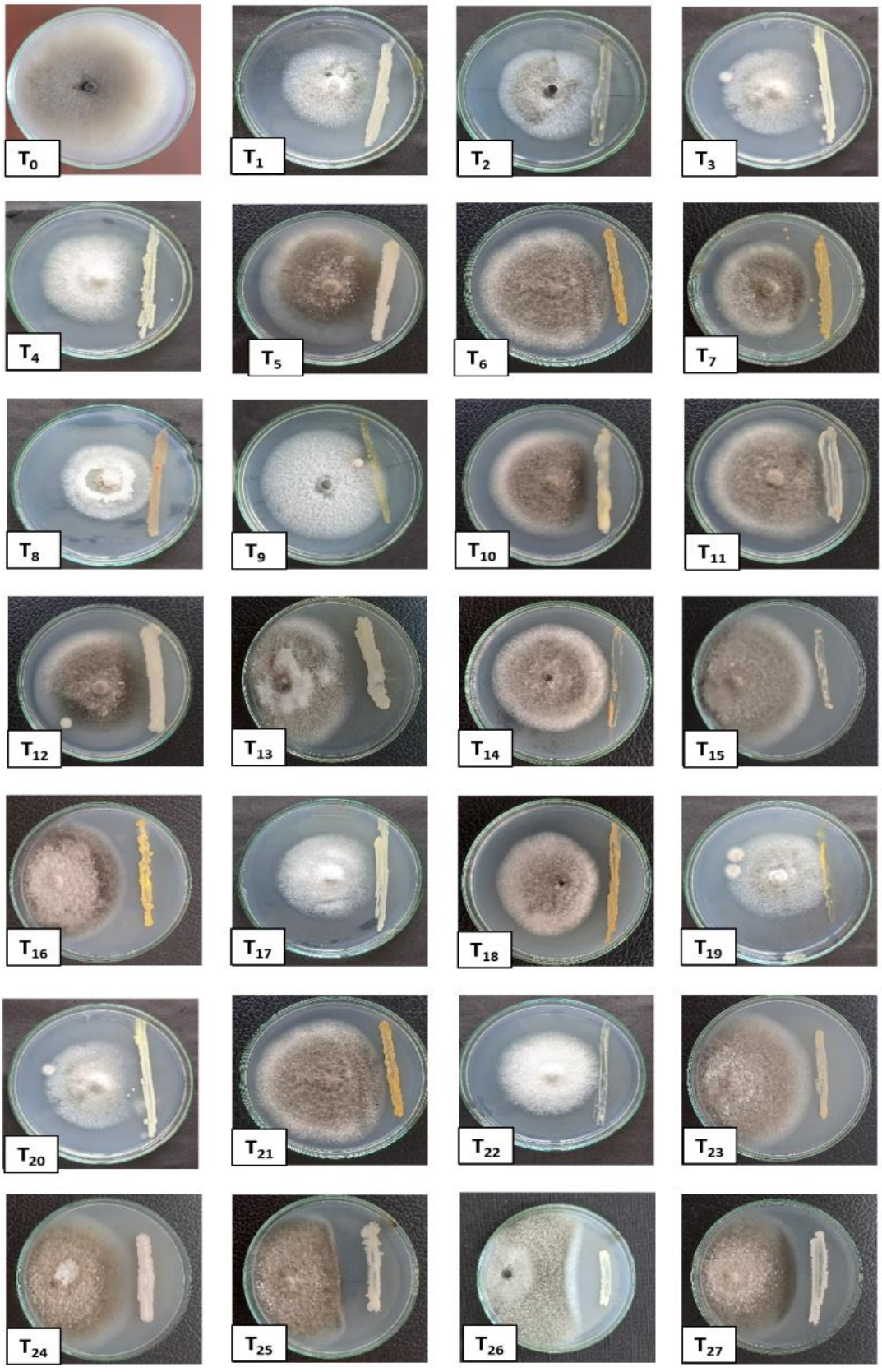


Plate 8. *In vitro* antagonism of rhizobacteria against *C. gloeosporioides*

metabolites, volatile gases as well as other antagonistic enzymes against the pathogen.

The findings of the present experiment are found to be in agreement with the findings of Ann *et al*. (2015) who reported that *Bacillus* strains are most effective against *C. gloeosporioides* with an inhibition of 44.7- 50.1%. Furthermore, Abdeljalil *et al*. (2016) also stated that 69 rhizobacteria from tomato was able to restrict the growth of *Sclerotinia sclerotiorum* and *Rhizoctonia solani in vitro* by 11-62% relative to control. Similar inhibition of 7.5% to 43% against *C. gloeosporioides* by 17 rhizobacteria was also reported by Syafriani *et al*. (2016). El- Sersawy *et al*. (2021) also concluded that, 23 rhizobacterial isolates from healthy faba bean plant reduced *Fusarium oxysporum* growth *in vitro* with an inhibition percentage ranging between 39.06 ± 0.3 % to 59.1±0.2%.

4.6 Investigation on the biocontrol mechanisms of rhizobacteria against *C. gloeosporioides*

4.6.1 Detection of volatile metabolites production

The effect of volatile metabolites of the rhizobacteria against *C. gloeosporioides* is presented in Table 4.6, illustrated on Fig 4.2 and Plate 9.

The data recorded revealed that more than half of the isolates did not inhibit the pathogen growth. However, T_7 and T_{25} showed highest inhibition of 32.50% which was recorded to be statistically at par with and T_{18} (31.67%.) and T_{16} (30.83) over the control.

The above findings are found to be similar with the observations made by Vagelas and Gowen (2012) who stated that *Pseudomonas oryzihabitans* cells produced freely diffusible compounds that restricted the growth of *F. oxysporum* f. sp. *lycopersici*. Comparable conclusions were made by

Table 4.6. *In vitro* effect of volatile metabolites of rhizobacteria on mycelial growth and per cent inhibition of *C. gloeosporioides*

Treatments	Inhibition of *C. gloeosporioides*	
	Diameter growth (cm)	Inhibition %
T_1	4.00	00.00 (1.40)
T_2	3.40	15.00 (22.79)
T_3	4.00	00.00 (1.40)
T_4	3.03	24.17 (29.45)
T_5	4.00	00.00 (1.40)
T_6	4.00	00.00 (1.40)
T_7	**2.70**	**32.50 (34.76)**
T_8	4.00	00.00 (1.40)
T_9	4.00	00.00 (1.40)
T_{10}	4.00	00.00 (1.40)
T_{11}	4.00	00.00 (1.40)
T_{12}	4.00	00.00 (1.40)
T_{13}	4.00	00.00 (1.40)
T_{14}	4.00	00.00 (1.40)
T_{15}	3.43	14.17 (22.11)
T_{16}	2.77	30.83 (33.72)
T_{17}	4.00	00.00 (0.99)
T_{18}	2.73	31.67 (34.24)
T_{19}	4.00	00.00.(1.40)
T_{20}	3.20	15.00 (26.57)
T_{21}	4.00	00.00 (1.40)
T_{22}	4.00	00.00 (1.40)
T_{23}	4.00	00.00 (1.40)
T_{24}	4.00	00.00 (1.40)
T_{25}	2.70	32.50 (34.76)
T_{26}	3.27	00.00 (1.40)
T_{27}	3.07	00.00 (1.40)
T_0	4.00	0.00 (1.40)
SEm±	0.04	0.65
CV (%)	1.74	9.95
CD (p=0.05)	0.10	1.84

Note: Figures in the table are mean values and those in parenthesis are angular transformed values

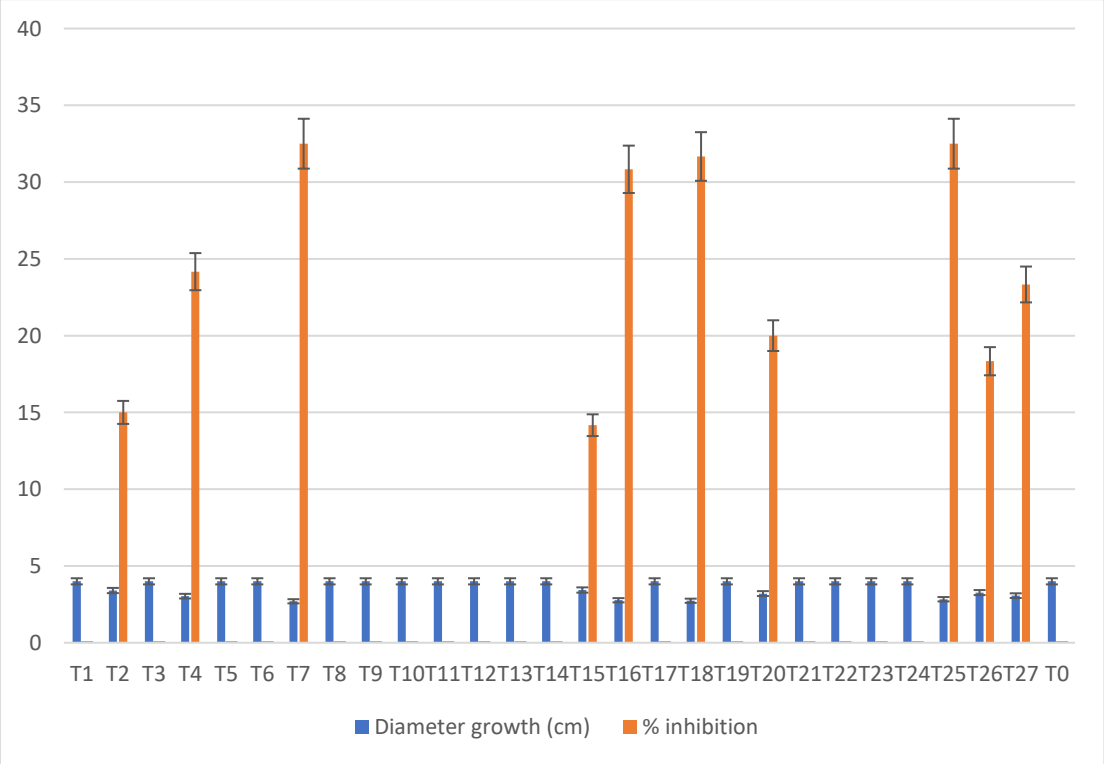


Fig. 4.2. *In vitro* effect of volatile metabolites of rhizobacteria on mycelial growth and per cent inhibition of *C. gloeosporioides*

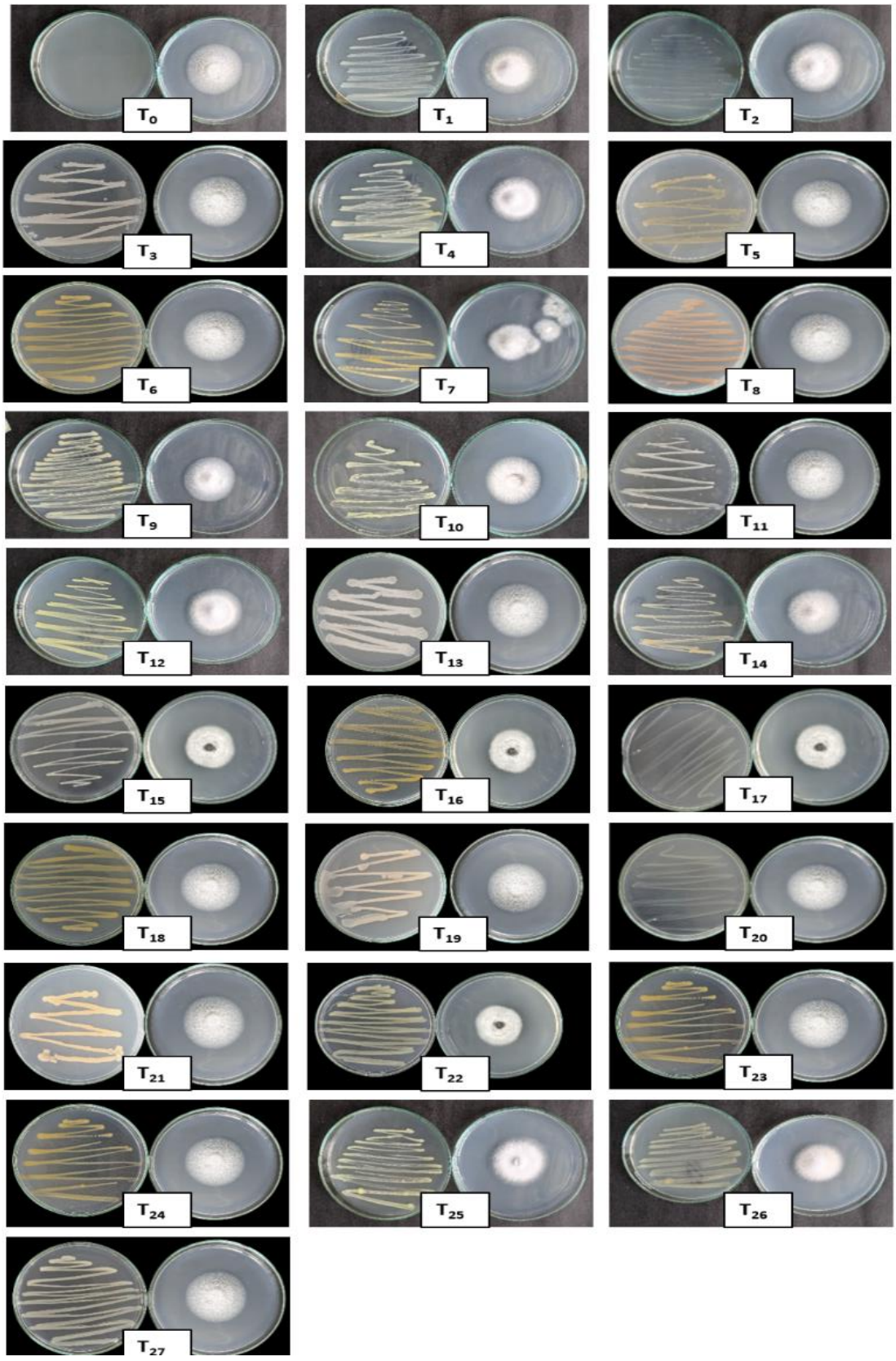


Plate 9. Detection of volatile metabolites production by the rhizobacteria

Guevara-Avendaño *et al*. (2019) who observed that two isolates out of 45 were able to restricts the growth of *C. gloeosporioides* with an inhibition of 25.4-32.6% through volatile compounds production *in vitro*.

4.6.2 Qualitative assay on siderophore production

Results on qualitative assay on siderophore production by the isolated rhizobacteria using Chrome Azurol Sulfonate (CAS) agar medium are displayed in Table 4.7 and depicted on Plate 10.

The data recorded showed that out of 27 rhizobacterial isolates only 16 isolates showed positive reaction by forming a yellow zone around the isolate while the rest showed no orange-yellow zone development. The isolates T_2, T_4, T_5, T_6, T_7, T_9, T_{10}, T_{12}, T_{13}, T_{15}, T_{16}, T_{18}, T_{20}, T_{21}, T_{25} and T_{26} gave positive reaction. Out of which T_7 and T_{16} displayed strong siderophore production while T_1, T_3, T_8, T_{11}, T_{14}, T_{17}, T_{19}, T_{22}, T_{23}, T_{24}, T_{27} did not produce siderophore.

The present findings confirm the observations made by Bhakthavatchalu *et al*. (2013) who reported that *Pseudomonas aeruginosa* FP6 producing siderophore was able to change the colour of CAS agar medium. Similar results were also reported by El-Sersawy *et al*. (2021) who concluded that bacterial strains identified as *Bacillus velezensis*, *B. paramycoides*, and *B. paramycoides* were able to produce siderophores *in vitro* through the appearance of an orange halo zone around the bacterial growth on CAS medium. Production of siderophore helps microbes to effectively compete for available iron with other organisms. Results of present studies also comply to the findings of Kumar *et al*. (2014) who observed that isolates identified as *B. stratosphericus*- NFB3, *B. cereus*- NpB6, *B. cereu*s- MKP3, *B. cereus* MNB1, *P. fluorescens*- NNB4 and *P. simiae*- NTB2 were able to utilize significant amount of iron by siderophore production, indicative of their ability to supress fungal pathogens in the rhizosphere by chelating iron.

Table 4.7. Qualitative assay on siderophore production

Treatments	Siderophore production	Treatments	Siderophore production
T_1	-	T_{15}	+
T_2	+	T_{16}	+++
T_3	-	T_{17}	-
T_4	++	T_{18}	++
T_5	+	T_{19}	-
T_6	+	T_{20}	+
T_7	+++	T_{21}	+
T_8	-	T_{22}	-
T_9	+	T_{23}	-
T_{10}	++	T_{24}	-
T_{11}	-	T_{25}	++
T_{12}	+	T_{26}	+
T_{13}	+	T_{27}	-
T_{14}	-		

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

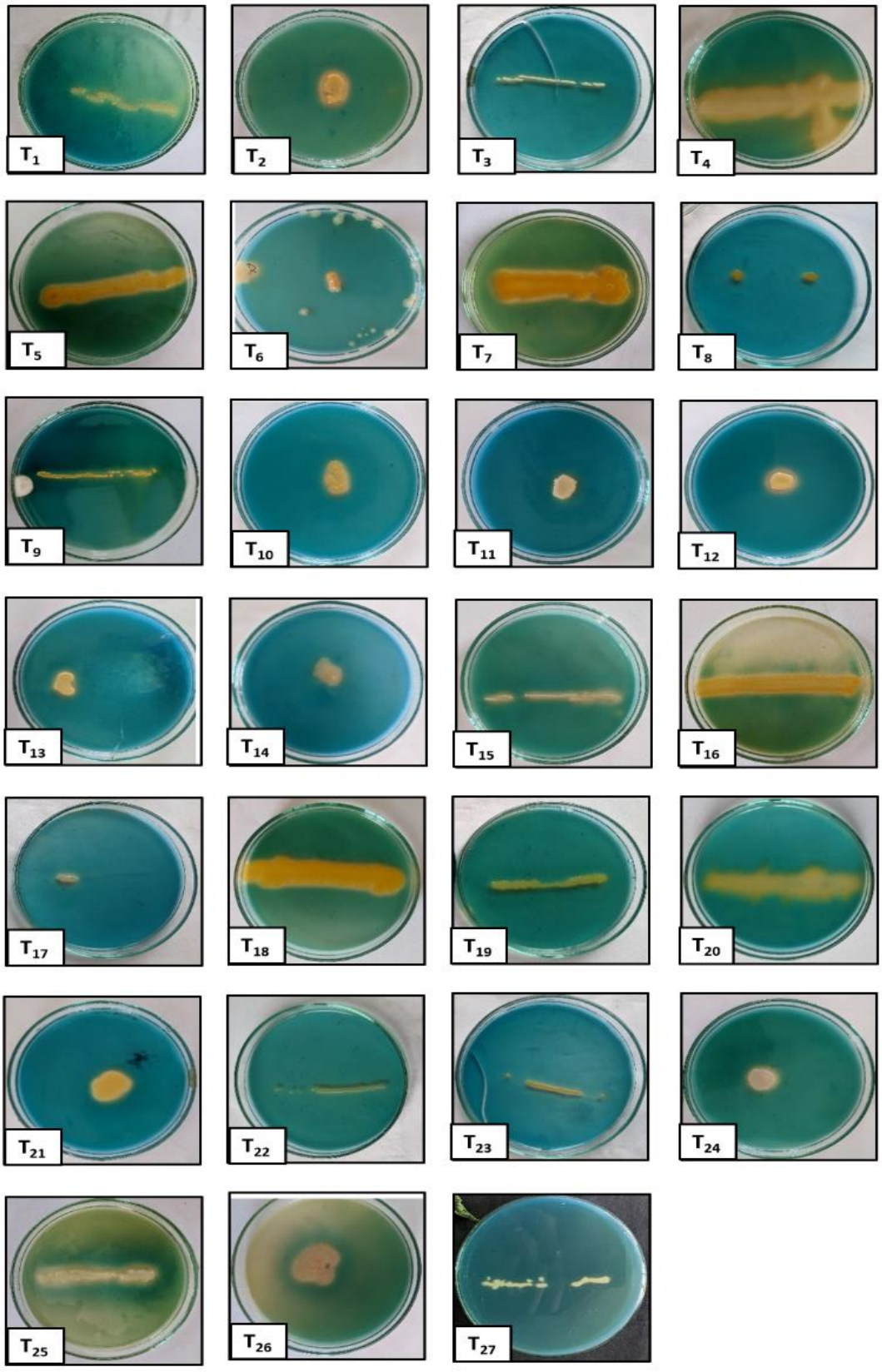


Plate 10. Qualitative assay on siderophore production

4.6.3 Qualitative assay on Hydrogen cyanide (HCN) production

Results on the qualitative assay on production of Hydrogen cyanide (HCN) by the isolated rhizobacteria are presented in Table 4.8 and depicted in Plate 11.

It is evident from the table that 15 isolates out of 27 were able to produce HCN which includes $T_{1,}$ T_4, T_6, T_7, T_{10}, T_{11}, T_{13}, T_{14}, T_{15}, T_{16}, T_{18}, T_{19}, T_{21} T_{22}, T_{23}. Strong production of HCN were recorded from T_7, T_{10} and T_{18}. No sign of HCN production was observed on T_2, T_3, T_5, T_8, T_9, T_{12}, T_{17}, T_{20}, T_{24}, T_{25}, T_{26}, T_{27}.

Similar works done by Kremer and Souissi (2001) reported that 32% of rhizobacteria from a collection of 2000 isolates were able to produce HCN. Dixit *et al*. (2015) also reported that change in colour of the filter paper from yellow to dark brown for positive reaction. Furthermore, Fridayati *et al.* (2020) also stated that HCN produced by rhizobacteria from the *Pseudomonas* spp. and *Bacillus* spp. group inhibits the growth of the pathogenic fungus *F. oxysporum*. They also suggested that the ability of the rhizobacteria to inhibit the growth of pathogenic fungus was positively related to its ability to secrete cyanide acid compounds (HCN). Similar findings were also reported by Geettha *et al*. (2014) who asserted that the production of HCN by the efficient strain of *P. fluorescens* contributed to effective mycelial growth inhibition of *R. solani in vitro* and appears as a major factor in control of soil-borne disease by *P. fluorescence.*

Table 4.8. Qualitative assay on HCN production

Treatments	HCN production	Treatments	HCN production
T_1	+	T_{15}	++
T_2	-	T_{16}	+
T_3	-	T_{17}	-
T_4	++	T_{18}	+++
T_5	-	T_{19}	+
T_6	+	T_{20}	-
T_7	+++	T_{21}	+
T_8	-	T_{22}	+
T_9	-	T_{23}	+
T_{10}	+++	T_{24}	-
T_{11}	+	T_{25}	-
T_{12}	-	T_{26}	-
T_{13}	+	T_{27}	-
T_{14}	+		

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

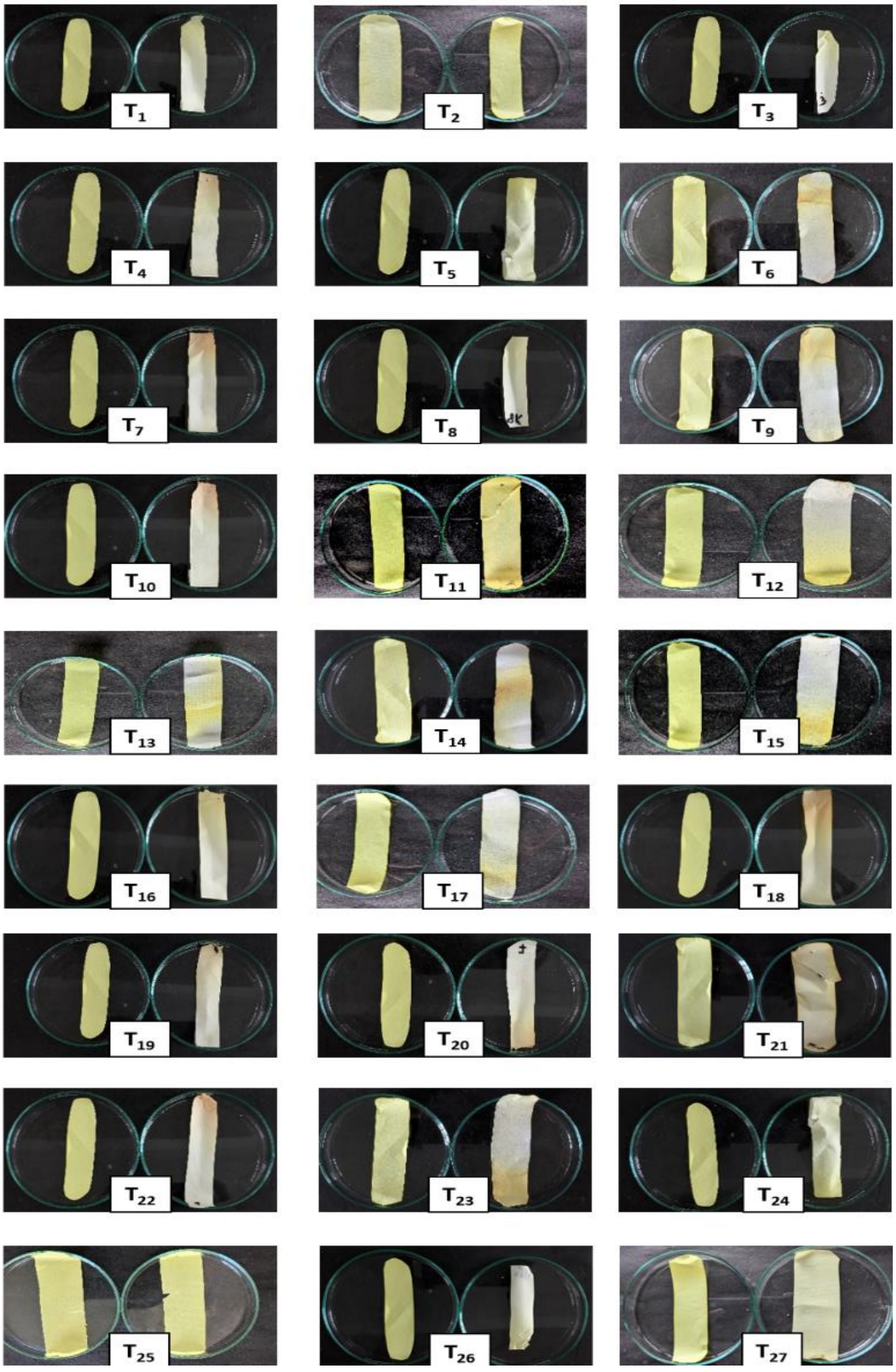


Plate 11. **Qualitative assay on Hydrogen cyanide (HCN) production**

4.6.4 Qualitative assay on Chitinase synthesis

The qualitative assay on chitinase synthesis are presented on Table 4.9 and depicted on Plate 12. In the present study no rhizobacteria were found positive for chitinase enzyme production as no formation of clear zone was recorded.

The findings in the current research are found to be in contrary to the observation made by Mubarik *et a*l. (2010) who stated that 25 rhizobacteria isolated from chilli formed a clear zone around colony on chitin agar. Similarly, Dukare *et al*. (2020) also observed that 13 out of 53 rhizobacteria isolated from pigeon pea rhizosphere were capable of hydrolysing chitin while the remaining showed negligible chitinolytic activity. Furthermore, the results of the present investigation do not comply with the observations made by Joshi and Joshi (2017) who observed that two rhizobacterial isolates were able to synthesize chitinase enzyme *in vitro*.

4.6.5 Qualitative assay on biosurfactant production

Result on the qualitative assay on biosurfactant production by the isolated rhizobacteria are displayed on Table 4.9 and depicted on Plate 13. The result indicated that none of the rhizobacterial isolates were able to produce biosurfactant.

The above findings are in consonance with the observations made by Yanti *et al.* (2017b) where all the *Bacillus* isolates showed negative result for biosurfactant production following the same described procedure. However, the findings of the present experiments are found to be in contrary to the observations made by Kalita *et al*. (2018) where all the 40 Plant growth promoting bacterial isolates from hydrocarbon contaminated soil were able to produce biosurfactant to varying degree. Likewise, Borgio *et al*. (2009) also observe that *Bacillus subtilis*

Table 4.9. Qualitative assay on the production of Chitinase and Biosurfactant

Treatments	Chitinase production	Biosurfactant production
T_1	-	-
T_2	-	-
T_3	-	-
T_4	-	-
T_5	-	-
T_6	-	-
T_7	-	-
T_8	-	-
T_9	-	-
T_{10}	-	-
T_{11}	-	-
T_{12}	-	-
T_{13}	-	-
T_{14}	-	-
T_{15}	-	-
T_{16}	-	-
T_{17}	-	-
T_{18}	-	-
T_{19}	-	-
T_{20}	-	-
T_{21}	-	-
T_{22}	-	-
T_{23}	-	-
T_{24}	-	-
T_{25}	-	-
T_{26}	-	-
T_{27}	-	-

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

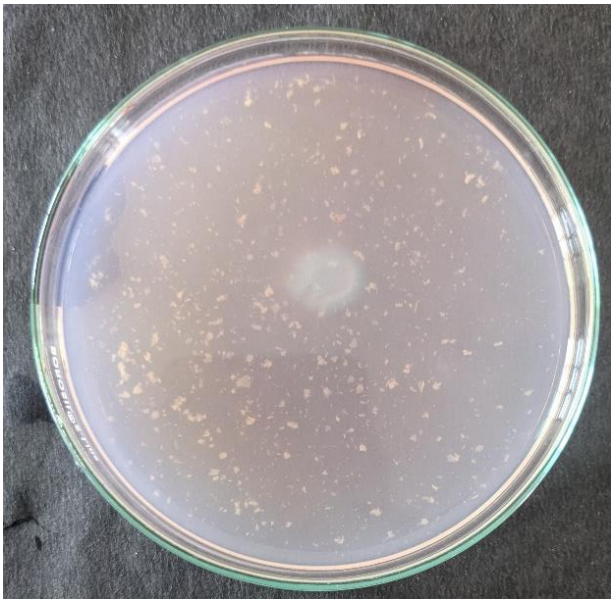


Plate 12. Qualitative assay on chitinase synthesis

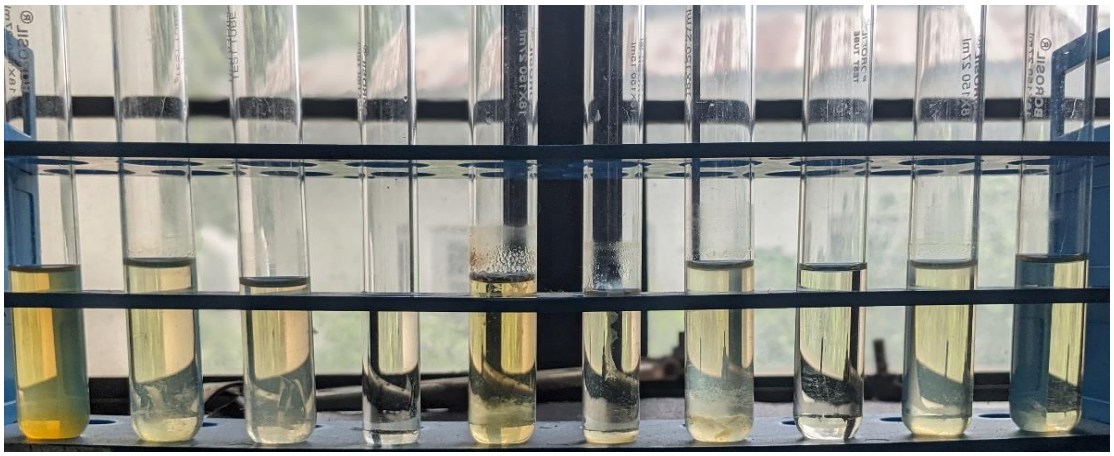


Plate 13. Qualitative assay on biosurfactant production

NCIM 2063, *Pseudomonas aeruginosa* NCIM 2862 and *Streptococcus mutans* MTCC 1943 were able to produce EPS *in vitro.*

4.7 Evaluation of mechanism of plant growth promotion by rhizobacteria *in vitro*

4.7.1 Qualitative Indole Acetic Acid (IAA) production

The results on the qualitative assay on the production of Indole Acetic Acid (IAA) is presented in Table 4.10 and depicted on Plate 14. It is evident from the presented data that 22 rhizobacterial isolates were able to produce IAA in varying quantity depending on the intensity of the colour development. However, T_8, $T_{9,}$ $T_{14,}$ T_{15} and T_{23} were not able to produce IAA. It was observed that T_1, T_2, T_4, T_5, T_{12}, T_{13}, T_{16}, T_{18} and T_{20} elucidated strong production of IAA.

The ability of plant growth promoting rhizobacteria to produce IAA is regarded as one of the important traits as it is regarded as one of the vital phytohormones responsible for promoting plant growth and development. IAA are reported to be involved in numerous physiological processes of plant including seed germination, root development, cell division and differentiation and resistance to stressful conditions etc. The observations made during the present investigations are found to be in acceptance with the works done by Datta *et al*. (2010) who stated that 31 rhizobacteria out of 36 were capable of producing IAA *in vitro*. Likewise, Ashrafuzzaman *et al*. (2009) also reported that IAA functions as vital signal molecule in regulating plant growth and observed that six out of ten isolates were positive for IAA production. They also stated that the generation of IAA by PGPR can change among distinct species and strains, influenced by various factors such as growth stage, culture conditions, and substrate accessibility. Similar findings were also made by Sumera *et al*. (2004); Malik and Sindhu (2011).

Table 4.10. Qualitative assay on IAA production

Treatment	IAA production	Treatment	IAA production
T_1	+++	T_{15}	-
T_2	+++	T_{16}	+++
T_3	+	T_{17}	+
T_4	+++	T_{18}	+++
T_5	+++	T_{19}	++
T_6	++	T_{20}	+++
T_7	+	T_{21}	++
T_8	-	T_{22}	+
T_9	-	T_{23}	-
T_{10}	+	T_{24}	+
T_{11}	+	T_{25}	++
T_{12}	+++	T_{26}	+
T_{13}	+++	T_{27}	+
T_{14}	-		

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

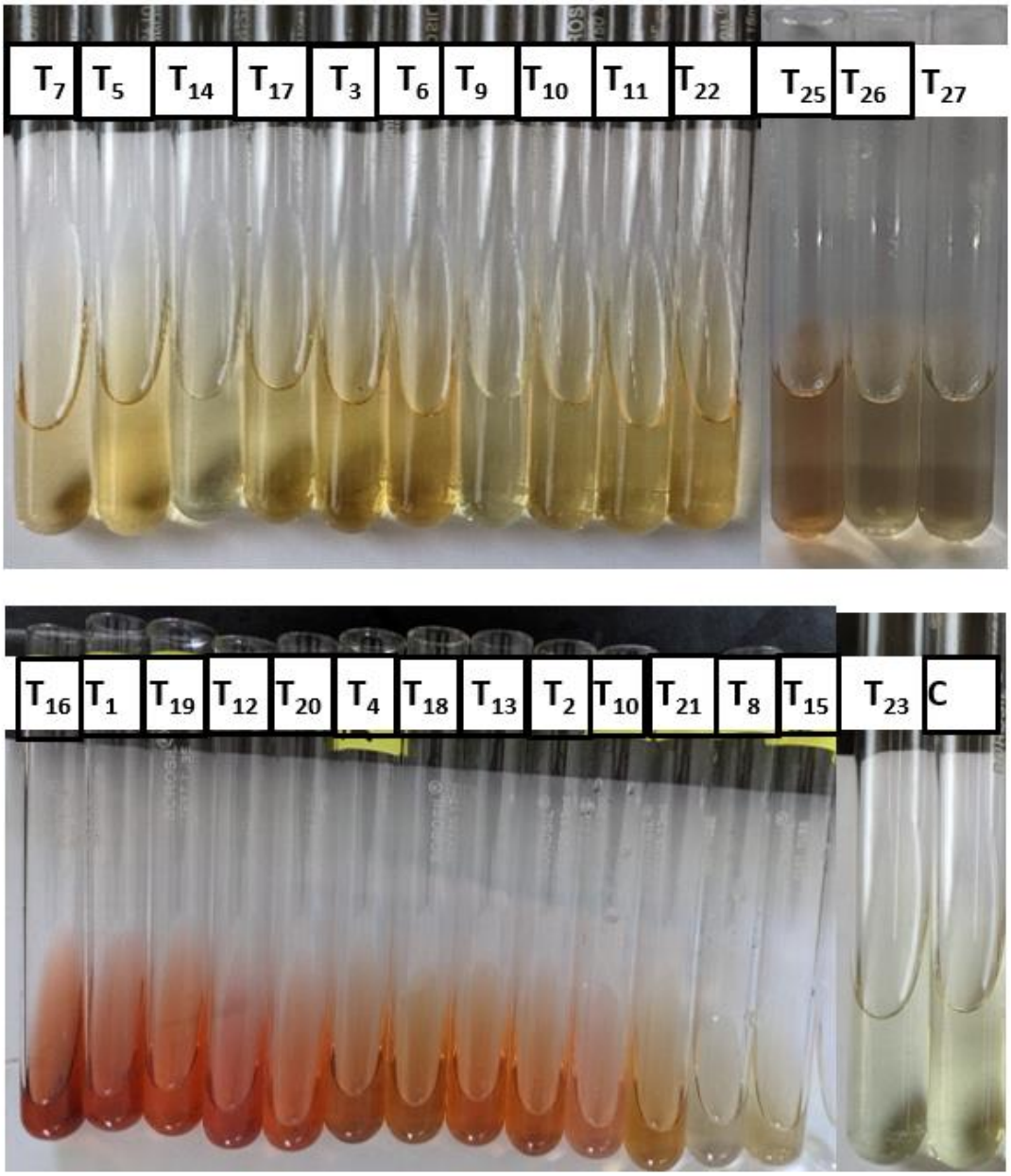


Plate 14. Qualitative assay on IAA production

4.7.2 Qualitative assay on Gibberellic acid (GA_3) production

The production of gibberellic acid by the isolated rhizobacteria is elucidated in Table 4.11 and Plate15. Observation of the recorded data revealed that most of the isolates were able to produce GA_3 in varying concentration except for T_5 which showed no signs of GA_3 production. Amongst the isolates T_{21} was observed to have strong GA_3 production while T_{10} and T_{24} showed medium GA_3 production.

Gibberellins like other plant growth hormones is an important hormone that is important for plant development and are reported to stimulate stem and root growth, promote pollen development, promotes seed germination, enhance α-amylase activity, regulates transition from juvenile to adult plants etc. The finding of the present investigation are in agreement with the observations recorded by Patel and Desai. (2015) who observed that all the isolated rhizobacteria were able to produce GA_3 to some degree. Gusmiaty *et al*. (2018) also reported that all 35 rhizobacterial isolates were able to produce gibberellic acid in various concentrations. Comparable conclusions were also made by Joo *et al*. (2005) who observed that rhizobacteria *B. cereus* MJ-1, *B. macrolides* CJ-29 and *B. pumilus* CJ-69 were able to produce gibberellic acid enhancing the growth of red pepper plants.

4.7.3 Qualitative assay on production of ammonia

Qualitative assay on production of ammonia by the isolated rhizobacteria is displayed in Table 4.11 and depicted on Plate 16. Observation of the recorded data showed that all rhizobacterial isolates are able to produce ammonia with varying degrees depending on the intensity of the colour developed after adding Nessler's reagent. The highest intensity of brownish colour development was recorded for isolate T_7, T_{16}, T_{17}, T_{18}, T_{24}, T_{25}, T_{26} and T_{27}.

Table 4.11. Qualitative assay on GA_3 and ammonia production

Treatments	GA_3 production	Ammonia Production
T_1	+	+
T_2	+	+
T_3	+	+
T_4	+	++
T_5	-	+
T_6	+	++
T_7	+	+++
T_8	+	++
T_9	+	++
T_{10}	++	++
T_{11}	+	+
T_{12}	+	++
T_{13}	+	++
T_{14}	+	++
T_{15}	+	+
T_{16}	+	+++
T_{17}	+	+++
T_{18}	+	+++
T_{19}	+	+
T_{20}	+	++
T_{21}	+++	++
T_{22}	+	+
T_{23}	+	+
T_{24}	++	+++
T_{25}	+	+++
T_{26}	+	+++
T_{27}	+	+++

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

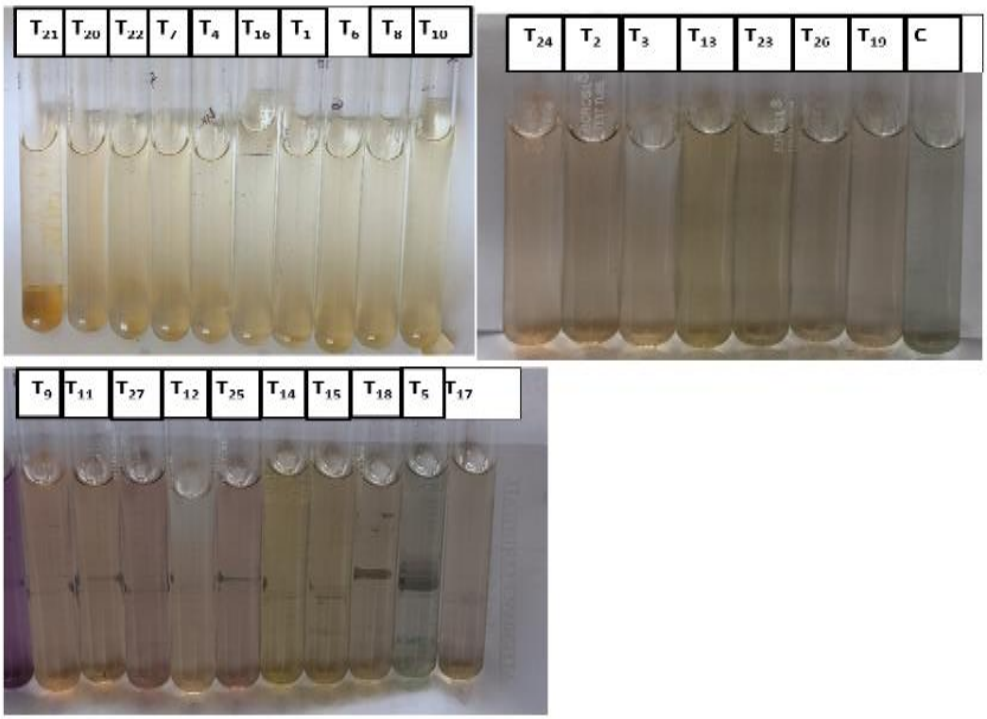


Plate 15. Qualitative assay on GA_3 production

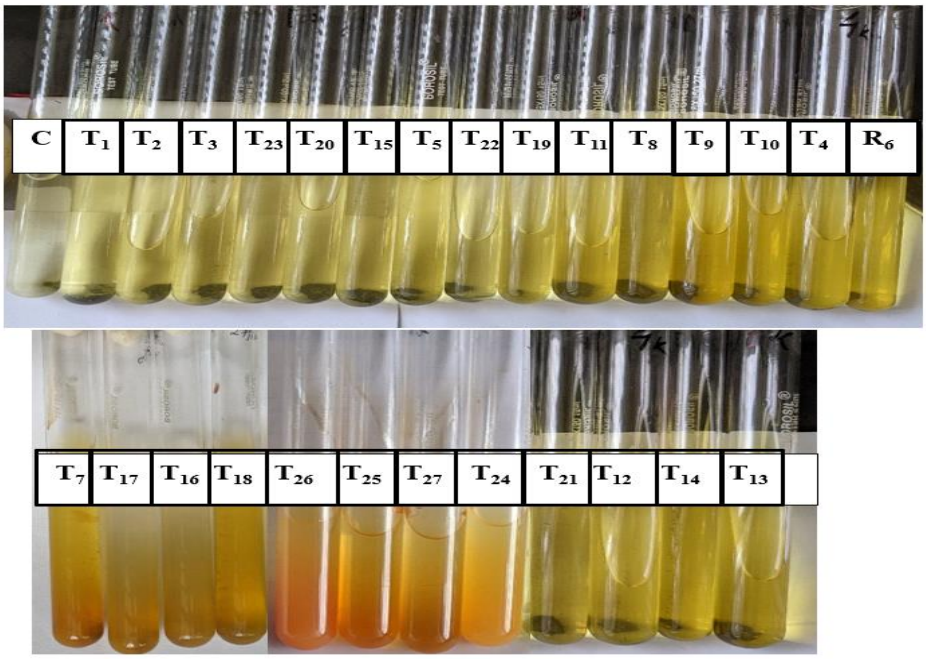


Plate 16. Qualitative assay on ammonia production

Ammonia is amongst the important sources of nitrogen for plants to meet their requirement for their growth and development. Ammonia enhances plant growth by the synthesis of biomolecules containing nitrogen, which are required by the plants for metabolism and photosynthesis. The findings of the present study are found to be in agreement with the findings of El-Sersawy *et al.* (2021) who observed that all three *Bacillus* sp. rhizobacteria has the potentiality to produce ammonia in various quantity by the development of brownish colour on addition of Nessler's reagent. Likewise, Joseph *et al*. (2007) also reported the production of ammonia by 95% of isolates of *Bacillus* followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%) isolates. Similar observations are also made by Kashyap *et al*. (2020) who stated that 78.5% of the antagonistic rhizobacteria against *Ralstonia solanacearum* isolated from chilli rhizosphere were able to produce ammonia.

4.7.4 Qualitative assay on phosphate solubilizing activity

Qualitative assay on phosphate solubilization by the isolated rhizobacteria is displayed in Table 4.12 and depicted on Plate 17

Perusal of data unvail that 70.37% of the isolates were able to solubilize phosphate as 19 isolates were able to solubilize phosphate which includes T_1, T_2, T_4, T_5, T_7, T_9, T_{10}, T_{11}, T_{12}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{20}, T_{21}, T_{22} and T_{23} giving positive reaction by forming a clear halo zone whereas T_3, T_6, T_8, T_{13}, T_{24}, T_{25}, T_{26} and T_{27} gave a negative reaction for phosphate solubilization. Strong phosphate solubilization activity was observed on T_7, T_{16} and T_{18}.

Phosphate is one amongst the three macronutrients required by the plant in high quantity for their normal growth and development but in most cases, it exists in insoluble form. Many genera of rhizobacteria are found to be able to

Table 4.12. Qualitative assay on phosphate solubilizing activity

Treatments	Phosphate solubility	Treatments	Phosphate solubility
T_1	+	T_{15}	+
T_2	+	T_{16}	+++
T_3	-	T_{17}	+
T_4	+	T_{18}	+++
T_5	+	T_{19}	++
T_6	-	T_{20}	+
T_7	+++	T_{21}	+
T_8	-	T_{22}	+
T_9	+	T_{23}	++
T_{10}	++	T_{24}	-
T_{11}	+	T_{25}	-
T_{12}	+	T_{26}	-
T_{13}	-	T_{27}	-
T_{14}	+		

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

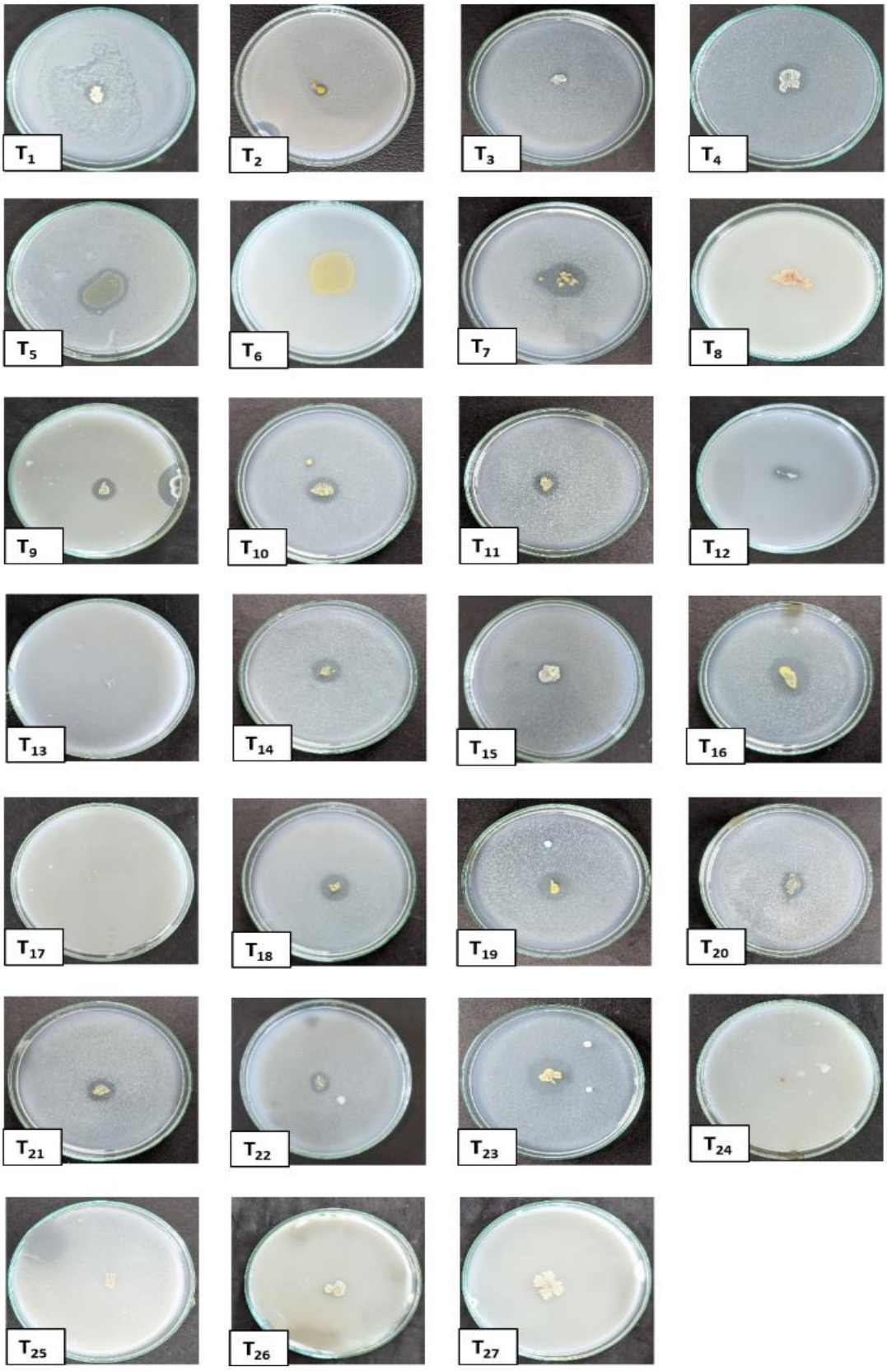


Plate 17. Qualitative assay on phosphate solubilizing activity

solubilize insoluble form of phosphate into soluble form making it available to the plants for utilization. The conclusions of the current investigation are similar with the findings of Datta *et al*. (2010) who reported that 14 rhizobacterial isolates from chilli rhizosphere could solubilize phosphate on Pikovskaya's medium by the development of a clear zone. Similar observations were also made by Abdeljalil *et al.* (2016) who reported that 18 out of 25 rhizobacteria have the ability to solubilize phosphate on Pikovskaya's agar medium. Furthermore, Syamsuddin *et al.* (2022) also reported that 13 out of 18 isolates showed an ability to dissolve phosphate *in vitro*.

4.7.5 Qualitative assay on zinc solubilization activity

Observations recorded on qualitative assay on zinc solubilization by the isolated rhizobacteria is presented on Table 4.13 and depicted on Plate 18. It may be asserted that most of the rhizobacterial isolate displayed positive reaction. T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_9, T_{12}, T_{13}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19}, T_{21}, T_{22}, T_{23}, T_{25}, T_{26} and T_{27} gave positive reaction but T_8, T_{10}, T_{11}, T_{20} and T_{24} gave negative reaction. T_4, T_7 and T_{14} displayed strong zinc solubilization.

The findings of the present work are in consonance with the findings of other researchers where, Sharma *et al*. (2012) reported that 31%, 27%, and 19% rhizobacterial isolates were found positive towards zinc solubilization on the media containing zinc oxide, zinc phosphate, and zinc carbonate, respectively. Furthermore, Ghevariya and Desai. (2015) also stated that out of 309 rhizobacterial isolates, 141 isolates showed positive reaction for zinc solubilization with zinc oxide as an inorganic source by the development of a halo zone around the bacterial colony indicated solubilization of zinc. comparable results were also observed by Rion *et al*. (2022) who observed the formation of a clear halo zone surrounding the colony in solid medium containing ZnO as a source of Zn in varying intensity.

Table 4.13. Qualitative assay on zinc solubilizing activity

Treatments	Zinc solubility	Treatments	Zinc solubility
T_1	+	T_{15}	++
T_2	++	T_{16}	+
T_3	+	T_{17}	+
T_4	+++	T_{18}	++
T_5	++	T_{19}	+
T_6	++	T_{20}	-
T_7	+++	T_{21}	++
T_8	-	T_{22}	++
T_9	++	T_{23}	+
T_{10}	-	T_{24}	-
T_{11}	-	T_{25}	++
T_{12}	++	T_{26}	++
T_{13}	++	T_{27}	++
T_{14}	+++		

Note: - = Negative, + = Low production, ++ = Medium production and +++ = Strong production

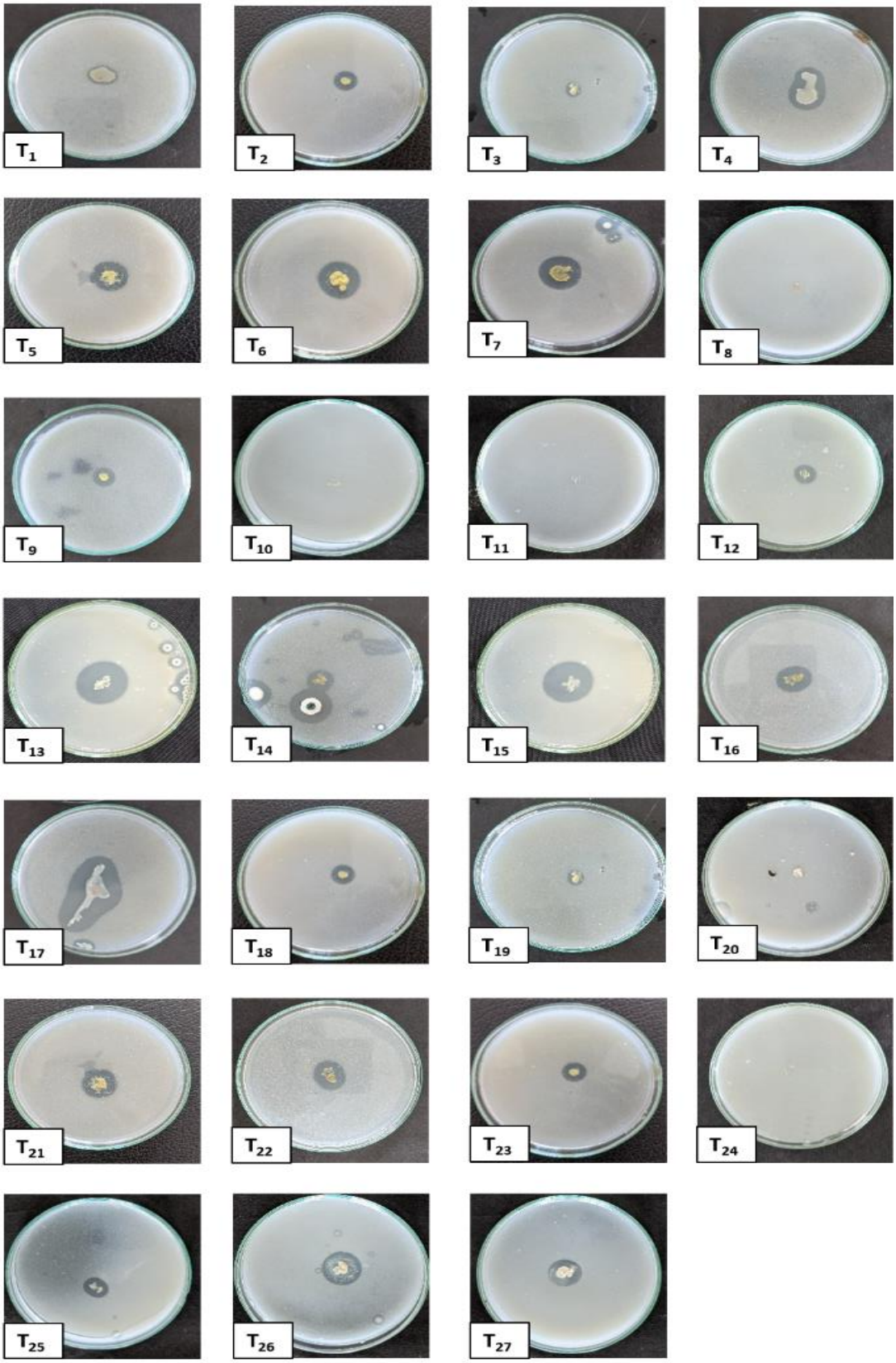


Plate 18. Qualitative assay on zinc solubilizing activity

4.8 *In vitro* Compatibility test of bacterial cultures

Rhizobacteria which performed significantly better on the dual culture assay from Table 4.5 were selected and studied to confirm that they are unique to each other. It was observed that T_7 and T_{18} and similar organism and hence, T_7, T_{16} and T_{25} were selected to be used for further investigation. The three rhizobacteria were further studied for their compatibility with one another and with fungicide captan. Results on *in vitro* compatibility test are presented in Table 4.14 and depicted on Plate 19.

The three selected rhizobacteria were found to be compatible with each other as there was no observation of formation of zone of inhibition around each other when cultured together on petri plate with nutrient agar medium and hence were used to create bacterial consortia.

Results on the compatibility test of rhizobacterial isolates with chemical fungicide captan was found to be incompatible as there was a clear zone of inhibition on nutrient agar medium poisoned with fungicide captan.

Similar conclusions were also made by Ajaz *et al*. (2019) who stated that lactic acid bacteria, actinomycetes, *Pseudomonas* spp. and *Bacillus* spp. are coexisting and complimentary to each other. Likewise, Thakkar and Saraf (2014) also did not observe any mutual growth inhibition between three antagonistic bacterial isolates when grown together on agar plates *in vitro*. Likewise, Bessa *et al*. (2023) also reported that fungicide Captan negatively affects the growth of rhizospheric *B. cereus* and *Enterobacter asburiae*.

4.9 *In vitro* plant growth promotion activity

Results on the effect of rhizobacterial treatment on *in vitro* plant growth promotion of Naga king chilli is displayed in Table 4.15 and depicted in Fig 4.3, Plate 20a and 20b.

Table 4.14. *In vitro* Compatibility test of bacterial cultures

Treatment combinations	Compatible/Not compatible
$R_7 + R_{16}$	+ve
$R_{16} + R_{25}$	+ve
$R_7 + R_{16} + R_{25}$	+ve
Captan + R_7	-ve
Captan + R_{16}	-ve
Captan + R_{25}	-ve

Note: +ve = Compatible, -ve =Not compatible

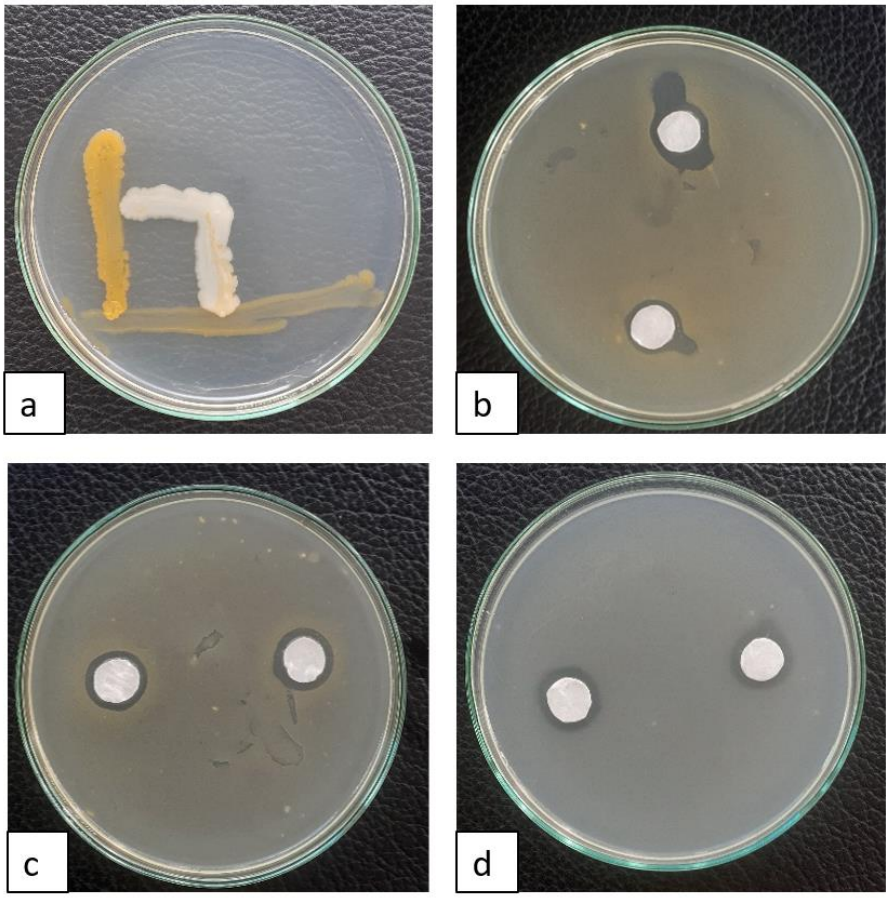


Plate 19. *In vitro* compatibility test

a. Compatibility of rhizobacteria (R_7, R_{16} and R_{25})
b. Compatibility test of rhizobacteria R_7 with fungicide captan
c. Compatibility test of rhizobacteria R_{16} with fungicide captan
d. Compatibility test of rhizobacteria R_{25} with fungicide captan

Table 4.15. *In vitro* plant growth promotion activity

Treatments	Treatment combinations	Germination (%) at 10DAS	Seedling growth at 15DAS		Vigor index (%)
			Root length (cm)	Shoot length (cm)	
T_0	Control	28.33 (32.09)	2.24	2.46	150.45
T_1	R_7	53.33 (46.91)	3.04	2.88	305.05
T_2	R_{16}	76.67 (61.22)	3.28	2.60	446.22
T_3	R_{25}	48.33 (44.03)	2.88	2.70	291.18
T_4	Fungicide	53.33 (46.91)	1.54	2.40	210.12
T_5	T_1+ T_2	65.00 (53.76)	3.76	3.06	444.60
T_6	T_1 + T_3	63.33 (52.78)	2.90	2.22	325.52
T_7	T_2 + T_3	61.67 (51.84)	3.80	2.98	409.49
T_8	T_1 + T_2 + T_3	63.33 (52.74)	3.90	3.18	447.11
SEm±		2.06	0.14	0.12	12.77
CV (%)		7.25	10.21	9.52	8.48
CD (p=0.05)		6.11	0.40	0.33	36.63

Note: Figures in the table are mean values and those in parenthesis are angular transformed values

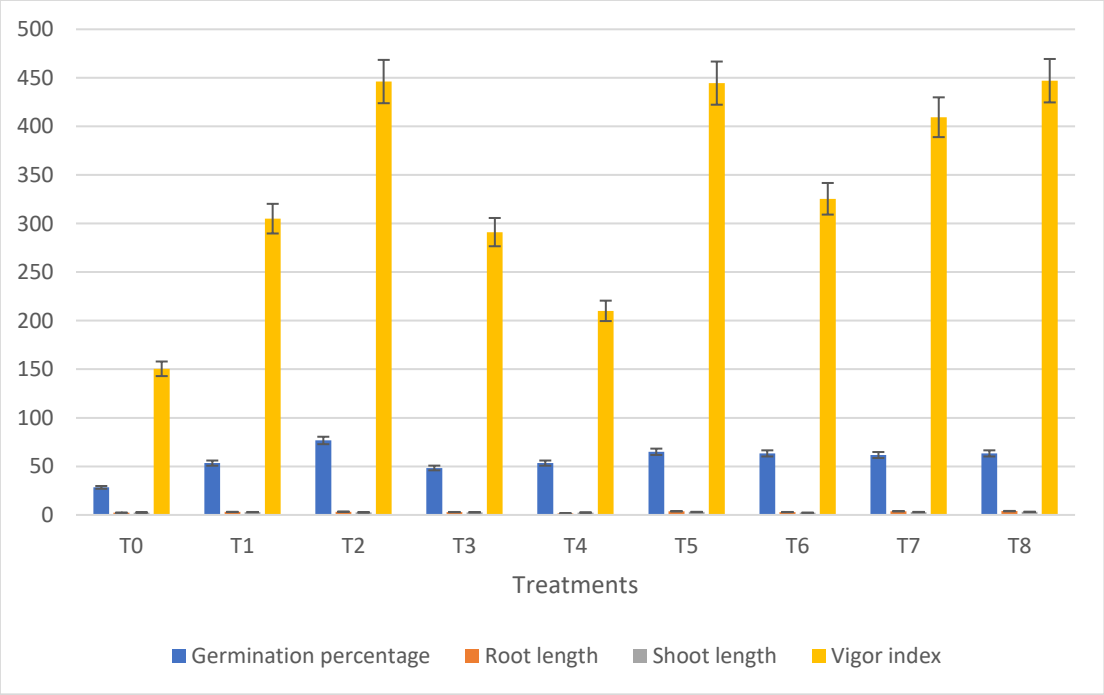
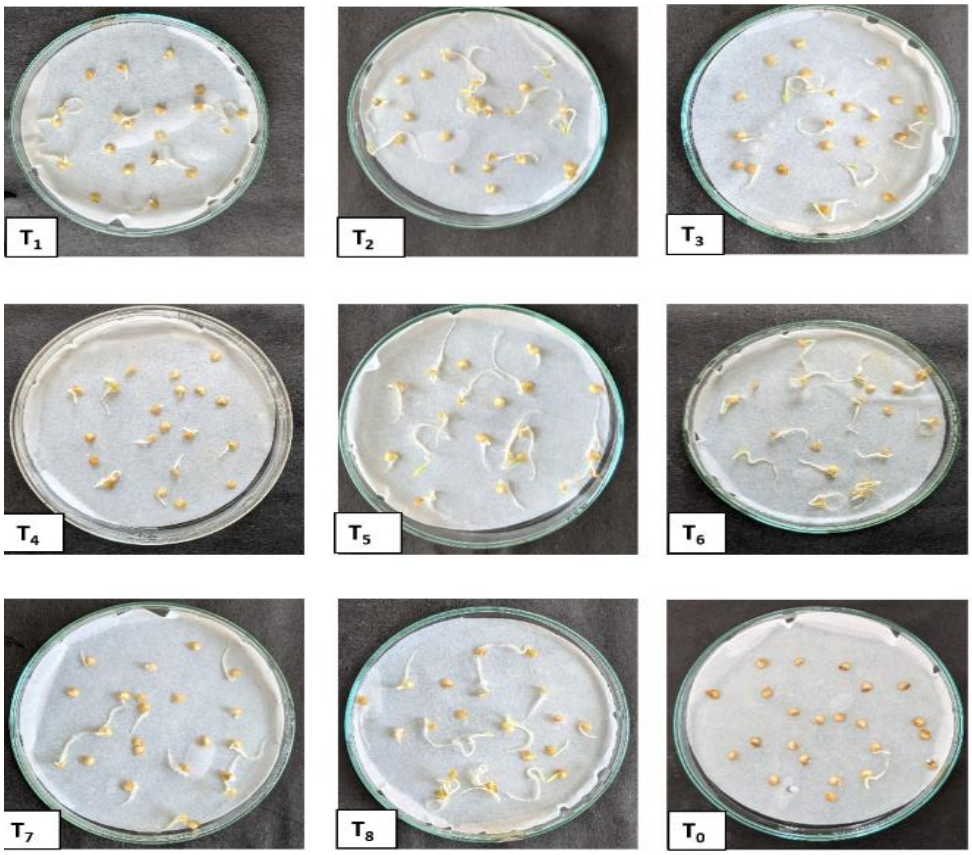
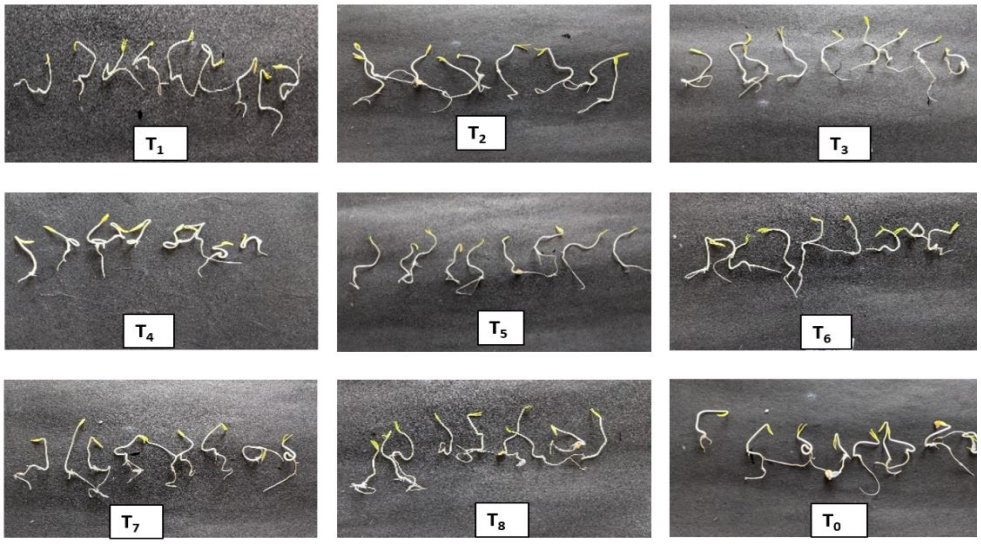


Fig. 4.3. *In vitro* plant growth promotion activity



a. *In vitro* effect of rhizobacteria on seed germination of Naga king chilli at 10 DAS



b. *In vitro* effect of rhizobacteria on seedling growth of Naga king chilli at 15 DAS

Plate 20. *In vitro* plant growth promotion activity

Observation of the data unveils that all the seeds treated with a single rhizobacteria or in mixture performed significantly better than control and chemical treated seeds. A perusal of data revealed that seed treatment with T_2 gave highest germination percentage of 76.67 and the lowest per cent germination was observed from T_0 (control) with 28.33. Maximum root length was recorded from T_8 (3.90cm) which was statistically at par with T_7 (3.80cm) and T_5 (3.76cm) and minimum root length was recorded from T_4 (1.54cm). The rhizobacterial treatment of seed also had a significant influence on shoot length which revealed that the maximum shoot length was recorded from T_8 (3.18 cm). observation on the vigor index suggest that the maximum vigor index was recorded from T_8 (447.11 %) which was statistically at par withT_2 (446.22 %) and T_5 (444.60 %) and minimum vigor index was reported from T_0 (150.45 %).

The improvement in the overall performance of the seeds treated with rhizobacteria maybe due to the production of phytohormones like IAA, GA_3 etc by the rhizobacteria enhancing its germination and growth as all the three rhizobacteria used in the experiments was observed to be positive for the production of the aforementioned plant hormones. The above observations are similar with the findings of Charumath and Raj (2020) who reported that the culture filtrates of rhizobacterial *P. fluorescence* increased germination of chilli seeds and induced plant growth promotion *in vitro* with highest germination percentage of 97.60 %, maximum shoot length, root length and vigour index of 9.25 cm, 3.94 cm and 1287.34 respectively. Similarly, Seleim *et al*. (2011) also stated that all the seed inoculated with different rhizobacteria significantly enhanced seed germination up to 15% over untreated control. Kumar *et al*. (2014) stated that inoculation of seeds with rhizobacteria. significantly increased germination in brinjal, chilli and okra by 20% with increased plumule length, radical and vigor index. Similar findings were also

observed by Agrawal and Agrawal (2013); Majeed *et al*. (2015); Pérez-Garcí *et al.* (2023) who observed that PGPR were able to increases germination percentage, length of plume and radicle, germination index and vigor percentage in cucumber as compared to control.

4.10 Field assessment of suppressive potential of selected rhizobacteria against anthracnose of Naga king chilli

4.10.1 Days to first flowering

The influence of rhizobacterial treatment on time taken by the plant for the appearance of first flower is presented on Table 4.16.

The data recorded revealed that there is no statistically significant influence of the rhizobacterial treatment on the time to first flower.

The findings of present work are in partial agreements with results presented by Yanti *et al*. (2017a) who stated that not all rhizobacterial isolates could accelerate flower phase and enhanced fruit yield of chilli. The synchronicity in days required to flowering may be because all the plants were planted during the same time and a required the same amount of time for vegetative growth to initiate flowering.

4.10.2 Plant height (cm)

The data on the influence of rhizobacterial treatment on the plant height of Naga king chilli is presented in Table 4.16 and depicted on Fig 4.4.

It is evident from the presented data that there is statistically significant difference in the heights of plants treated with rhizobacteria. Pooled data disclosed that plant height was recorded to be maximum from T_8 with 85.67 cm, which was followed by T_5 and was found to be statistically at par with T_6, T_7, T_3, T_1 and T- with 77.67 cm, 77.33 cm, 76.56 cm, 75.78 cm, 75.78 cm and 73.87 cm respectively. Minimum plant height was recorded from T_+ with 66.92 cm.

Table 4.16. Influence of rhizobacteria on days to first flowering (DAT) and plant height (cm)

Treat ments	Treatment combination	Days to first flowering (DAT)			Plant height (cm)		
		2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
T-	Uninoculated control	40.33	41.33	40.83	71.90	75.83	73.87
T+	Inoculated control	47.67	45.33	46.83	68.33	65.50	66.92
T_1	R_7	46.67	44.67	45.67	75.23	76.33	75.78
T_2	R_{16}	45.67	45.67	45.67	73.13	74.33	73.73
T_3	R_{25}	47.67	44.67	46.17	76.67	74.90	75.78
T_4	Fungicide	45.00	45.00	45.00	70.33	72.57	71.45
T_5	R_7+ R_{16}	43.00	46.33	44.67	77.00	78.33	77.67
T_6	R_7 + R_{25}	41.67	42.67	42.17	78.67	76.00	77.33
T_7	R_{16} + R_{25}	41.33	42.33	41.83	76.43	76.68	76.56
T_8	R_7 + R_{16} + R_{25}	39.33	37.33	38.33	86.67	84.67	85.67
SEm ±		2.45	2.96	1.92	2.00	2.61	1.64
C.V (%)		N.S	N.S	N.S	4.59	5.98	5.33
CD (p=0.05)		N.S	N.S	N.S	5.94	7.75	4.71

Note: Figures in the table are mean values

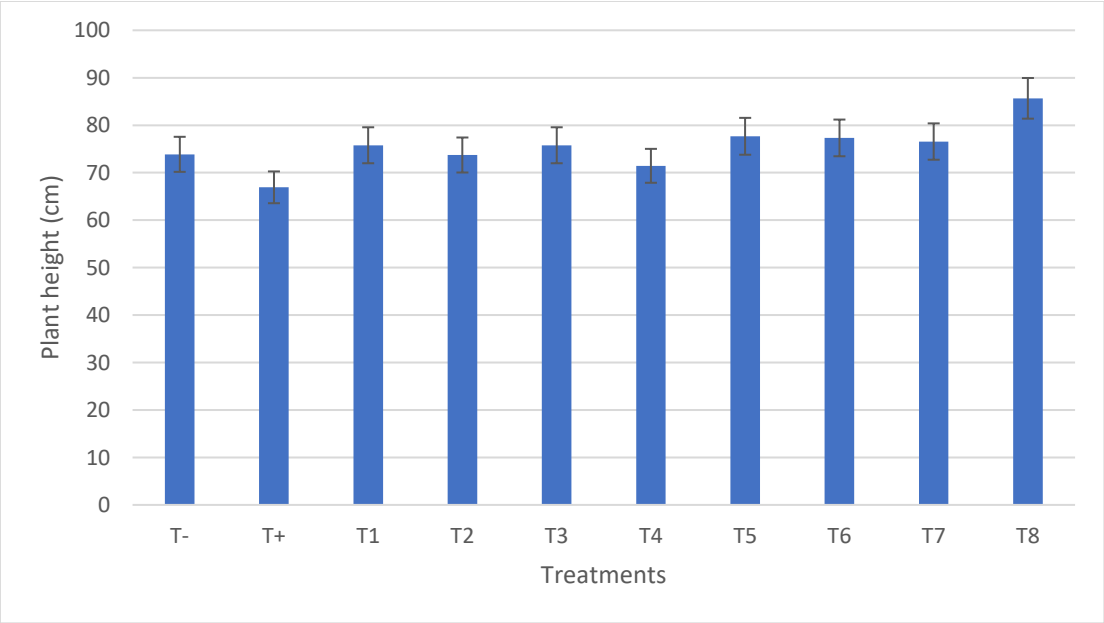


Fig. 4.4. Influence of rhizobacterial treatment on plant height (cm)

The increase in mean plant height in plants treated with rhizobacteria maybe due to the fact that rhizobacterial isolates are capable of producing phytohormones and mobilize reserved food contributing to the improvement in the growth of the treated plants. Similar results were also observed by Gowtham *et al*. (2018) who indicated that rhizobacterial isolates strain *Bacillus* spp. has the ability to increase the height of chili plants up to 37.40 cm compared to control (3.34 cm).

Likewise, Fatimah (2018) also commented that introduction of indigenous rhizobacterial isolate in chilli seeds increase height of the plant from 27.3 to 35.6 cm. Aliye *et al*. (2008) recorded that plant growth-promoting rhizobacteria was able to increase the height of potato plant compared to the pathogen treated control. El-Sersawy *et al*. (2021) reported that rhizobacterial treatment enhanced faba bean plant height with percentages of 21.3% - 24.3% when inoculated with separate bacterial species, whereas when inoculated with the bacterial consortium, plant height increased with percentages of 28.3% - 48.3% over infected control. Similar findings were also made by Lamsal (2012) and Banerjee *et al.* (2011).

4.10.3 Influence of rhizobacterial treatment on yield parameters

Observations recorded on number of fruits per plant and yield are presented in Table 4.17 and depicted on Fig 4.5.

A thorough study of the pooled data revealed that the maximum number of fruits per plant was observed from T_8 with an average of 103.67 fruits per plant followed by T_7 (74.67) which was statistically at par with T_2 (69.67), T_6 (69.00) and T_5 (61.33) and the minimum number of fruits was recorded from T_+ with 34.00 fruits per plant. Highest yield (g) was recorded from T_8 with an average of 580.17 g per plant followed by T_7 (492.67), T_2, (439.50) T_6 (427.00) and T_5 (389.67) and the lowest yield was recorded from T_+ with 287.83 g per plant.

Table 4.17. Influence of rhizobacterial treatment on yield parameters

Treatments	Treatment combination	Number of fruits/plants			Yield per plant (g)			Yield per hectare (kg/ha)		
		2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
T-	Uninoculated control	47.67 (6.94)	46.00 (6.82)	46.83 (6.88)	287.33	288.33	287.83	190.62	192.41	191.51
T+	Inoculated control	36.00 (6.04)	32.00 (5.69)	34.00 (5.87)	227.33	226.67	227.00	135.31	134.32	134.81
T_1	R_7	54.00 (7.37)	53.33 (7.31)	53.67 (7.34)	344.67	346.67	345.67	203.95	204.61	204.28
T_2	R_{16}	69.33 (8.35)	70.00 (8.39)	69.67 (8.37)	439.33	439.67	439.50	260.00	260.53	260.27
T_3	R_{25}	56.67 (7.56)	55.33 (7.47)	56.00 (7.51)	351.33	353.00	352.17	207.90	208.75	208.33
T_4	Fungicide	59.33 (7.73)	59.33 (7.73)	59.33 (7.73)	372.67	372.33	372.50	220.74	220.64	220.69
T_5	R_7+ R_{16}	61.00 (7.82)	61.67 (7.86)	61.33 (7.84)	389.33	390.00	389.67	229.14	231.11	230.13
T_6	R_7 + R_{25}	68.67 (8.31)	69.33 (8.35)	69.00 (8.33)	426.33	427.67	427.00	249.38	242.57	245.98
T_7	R_{16} + R_{25}	74.00 (8.60)	75.33 (8.68)	74.67 (8.64)	492.67	493.00	492.83	285.68	271.26	278.47
T_8	R_7 + R_{16} + R_{25}	105.00 (10.29)	101.67 (10.10)	103.67 (9.27)	580.33	580.00	580.17	333.46	333.12	333.29
SEm ±		0.25	0.28	0.19	14.41	19.77	12.23	8.49	11.37	7.09
C.V (%)		5.49	6.23	5.87	6.38	8.74	7.66	6.35	8.56	7.53
CD (p=0.05)		0.74	0.84	0.54	42.83	58.75	35.09	25.21	33.77	20.34

Note: Figures in the table are mean values and those in parenthesis are square root transformed values

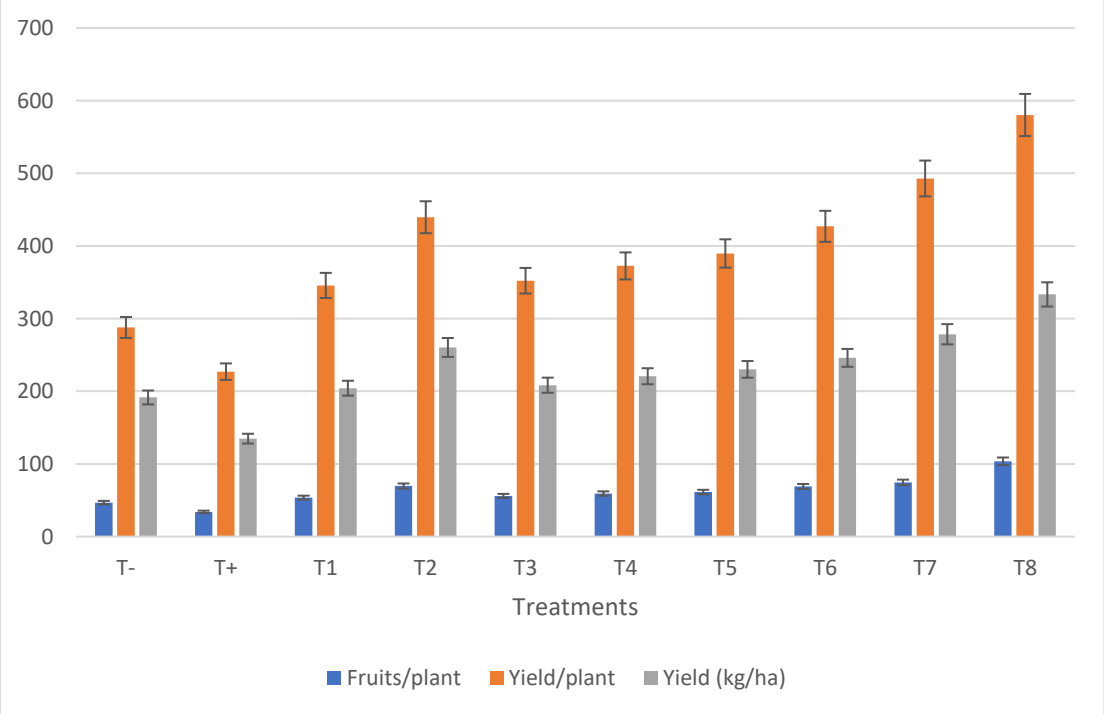


Fig. 4.5. Influence of rhizobacterial treatment on yield parameters

Pooled results on yield per hectare (kg/ha) suggested that highest yield in Kg/ha was recorded from T_8 with an average yield of 333.29 kg/ha followed by T_7 (278.47 kg/ha), T_2 (260.27 kg/ha), T_6 (245.98 kg/ha) and T_5 (230.13 kg/ha) and the lowest yield was recorded from T_+ with 191.51ka/ha.

The noteworthy enhancement in the yield of treated Naga king chilli plants may be assigned to better vegetative growth of the plant due to the increased release of phytohormones and enhanced protection from diseases by the inoculated rhizobacteria through the production of several antimicrobial compounds and activation of plant defence mechanism. The outcome of the present investigation is in accordance with the findings of El-Sersawy *et al*. (2021) who reported that the number of pods per plants were highly reduced in the infected plant as juxtapose to healthy plant. It was also reported that treatment with rhizobacteria significantly increased pods yield as compared to healthy plants and the maximum pods/ plant and maximum yield was recorded from plants treated with the bacterial consortium by 37% and 20.4% in the fresh and dry weight respectively over control. Fridayati *et al*. (2020) also observed that brinjal seeds treated with rhizobacteria significantly increased the number of fruits, fruit diameter, fruit weight and yield over control. Similarly, Fatimah (2018) reported that all the indigenous rhizobacterial isolates introduced in chilli seeds were able to increase the yield of chilli crops from 99.1 to 491.5% as compared to positive control with a yield of 35.1 grams/plant equivalent to 1.17 tons/ha. Similar interpretation were also made by Banerjee *et al*. (2011) who recorded an improvement in plant growth characters in plants inoculated with rhizobacteria and also recorded that plants administered with a combination of isolates performed better than plants treated with individual isolates.

4.10.4 Influence of rhizobacterial treatment on plant growth promotion (% biomass increase)

The data related to plant growth promotion assessments of Naga king chilli plant is presented in Table 4.18 and depicted on Fig 4.6.

The pooled results revealed that highest biomass increase was observed from T_8 with 55.41 % biomass increase which was observed to be statistically at par with T_5 with 53% and followed by T_7 with 46.91 % biomass increase as compared to inoculated control.

The results on plant growth promotion assessments are in acceptance with the findings recorded by Kumar *et al*. (2011a) who recorded an increase in the shoot and root biomass on chilli plants treated with rhizobacteria. Similar inference were also made by Lamsal (2012) who observed biomass increase on plants treated with rhizobacteria.

The drastic increase in the biomass of the plants treated with rhizobacteria may be due to the good vegetative growth owing to the efficiency and synergistic effect of the applied rhizobacteria in supplying required plant growth hormones and facilitating in the uptake of nutrients by making unavailable form of nutrients to available form whilst competing with other harmful organism attributing to better growth of the plants increasing its biomass as compared to control. The findings of present study partially contradicts the findings of Seleim *et al*. (2011) who stated that plants administered with individual rhizobacteria performed better in increasing plant biomass than plants treated with a mixture of different rhizobacteria. Whereas, the observations made during this study are in acceptance with the findings of Hahm *et al*. (2012) who observed that the use of mixtures of plant growth promoting rhizobacterial strains enhanced better growth of pepper plants in the field than plants treated with individual strains. Also, Nguyen and Ranamukhaarachchi (2010) suggested that the improvement in the performance of treated plants is due to the reason

Table 4.18. Influence of rhizobacterial treatment on plant growth promotion (% biomass increase)

Treatments	Treatment combinations	Plant growth promotion (% biomass increase)		
		2021-2022	**2022-2023**	**Pooled**
T-	Uninoculated control	34.00	32.10	33.00
T+	Inoculated control	00.00	00.00	00.00
T_1	R_7	34.86	37.04	35.95
T_2	R_{16}	37.68	35.80	36.74
T_3	R_{25}	35.90	32.10	34.00
T_4	Fungicide	33.20	32.84	33.02
T_5	R_7+ R_{16}	55.13	50.86	53.00
T_6	R_7 + R_{25}	47.26	41.23	44.25
T_7	R_{16} + R_{25}	46.67	47.16	46.91
T_8	R_7 + R_{16} + R_{25}	**58.01**	**52.82**	**55.41**
SEm ±		4.47	3.15	2.73
C.V (%)		20.67	14.89	18.07
CD (p=0.05)		13.27	9.37	7.84

Note: Figures in the table are mean values

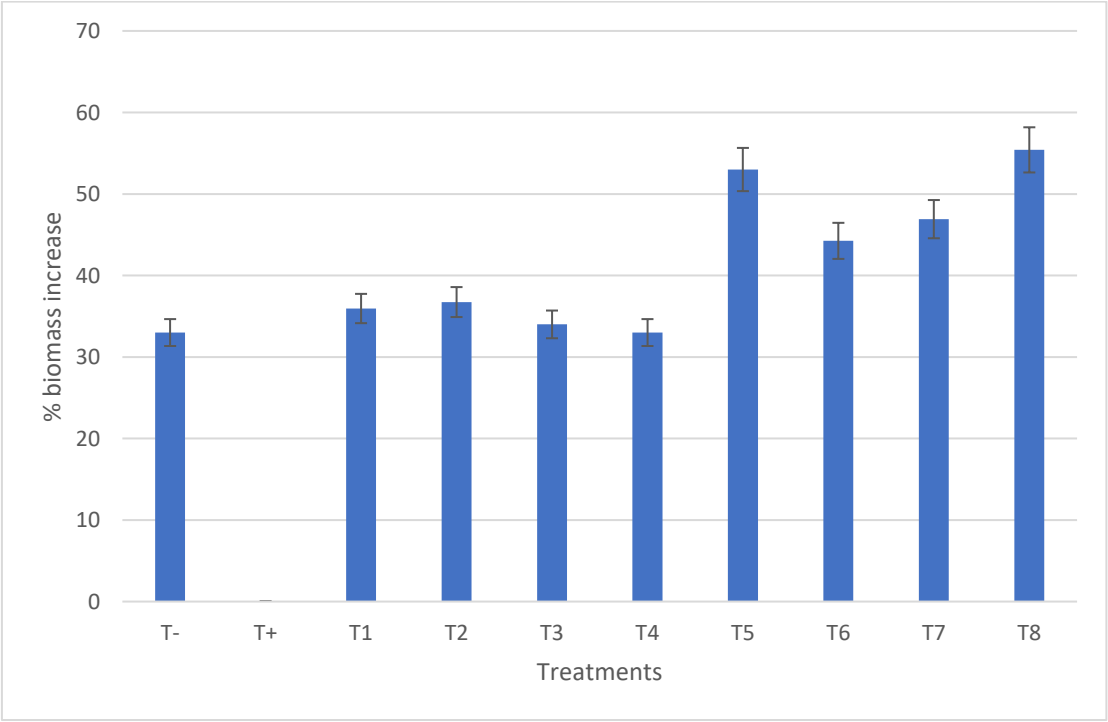


Fig. 4.6. Influence of rhizobacterial treatment on plant growth promotion (% biomass increase)

rhizobacteria being an effective root colonizer and proliferates along with plant roots aiding to its growth and development.

4.10.5 Influence of rhizobacterial treatment on Disease incidence (%) and disease severity (%)

Observations recorded for disease incidence (%) at 60, 75, 90, 105, 120, 135, 150 DAT are presented in Fig 4.7 (Appendix I and II). It is evident from the pooled data presented that the incidence of the disease was recoded to be highest on T_+ with 35.40% and lowest on T_8 with 9.00%.

Data recorded on disease severity at 150 DAT is presented in Table 4.19 and illustrated on Fig 4.8. From the pooled data presented, it is clear that disease severity was recorded to be lowest on T- (uninoculated control) with 10.02% which was statistically at par with T_8 (10.33%) and T_5 (13.99) as compared to T+ (inoculated control) which had the highest per cent disease incidence of 37.20. The per cent disease reduction was found to be highest with T_8 (72.23 %) which was followed by T_5 (62.39 %), T_2 (61.04 %), T_6 (60.67%), T_3 (58.52 %), T_4 (52.04 %), T_7 (51.91 %) and T_1 (50.94 %) over inoculated control.

Increased resistance of the plant treated with rhizobacteria maybe credited to the activation of plant defence system by the release of siderophores, HCN, hydrolytic enzymes and other metabolites antagonistic to the pathogen. The improvement in the overall plant health maybe due to the production of phytohormones including IAA, GA_3 and other essential nutrients like ammonia, phosphate, zinc by the rhizobacteria may also have aided in improving the plant defence system which help in suppression of phytopathogens. Elevated reduction in the occurrence of the disease in the plants administered with a mixture of rhizobacterial combinations suggests that presence of more variety of antagonist bacteria could maximize the resulting inhibitory effect.

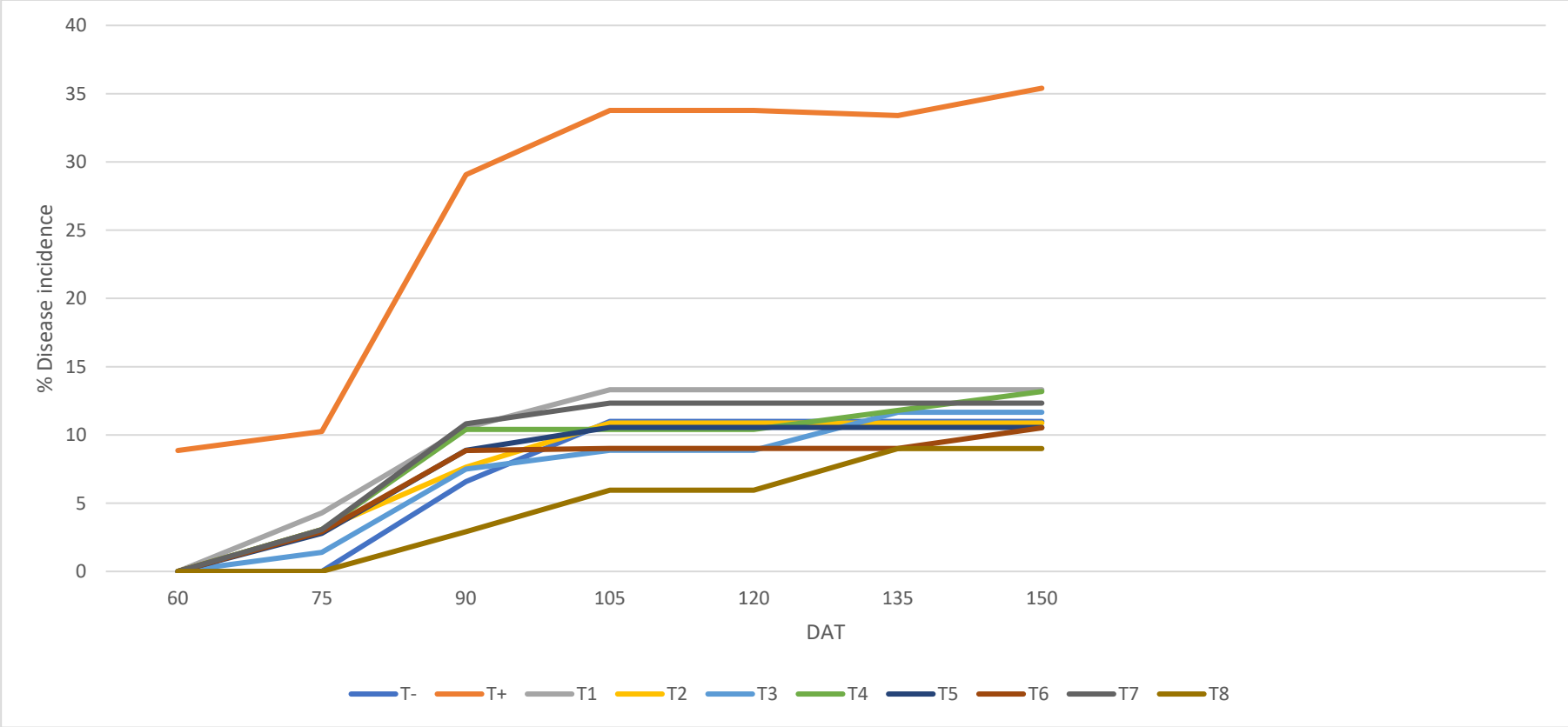


Fig. 4.7. Influence of rhizobacterial treatment on % disease incidence

Table 4.19. Influence of rhizobacterial treatment on disease severity

Treatments	Treatment combinations	Disease severity 150 DAT			
		2021-2022	2022-2023	Pooled	% disease reduction
T-	Uninoculated control	12.26 (19.15)	9.00 (17.44)	10.02 (18.24)	-
T+	Inoculated control	38.67 (38.45)	35.72 (36.69)	37.20 (37.57)	-
T_1	R_7	18.87 (25.68)	17.62 (24.79)	18.25 (25.23)	50.94
T_2	R_{16}	15.93 (23.48)	13.04 (21.08)	14.49 (22.28)	61.04
T_3	R_{25}	15.54 (23.13)	15.31 (22.95)	15 .43 (23.04)	58.52
T_4	fungicide	16.81 (24.06)	18.88 (25.74)	17.84 (24.90)	52.04
T_5	R_7+ R_{16}	14.43 (22.24)	13.55 (21.45)	13.99 (21.84)	62.39
T_6	R_7 + R_{25}	15.43 (23.09)	13.55 (21.82)	14.63 (22.45)	60.67
T_7	R_{16} + R_{25}	18.98 (25.74)	14.33 (21.93)	16.66 (23.83)	51.90
T_8	R_7 + R_{16} + R_{25}	**11.10 (19.01)**	**9.55 (17.87)**	**10.33 (18.44)**	**72.23**
SEm ±		1.85	1.51	1.19	-
C.V (%)		13.05	11.30	12.26	-
CD (p=0.05)		5.49	4.49	3.42	

Note: Figures in the table are mean values and those in parenthesis are angular transformed values

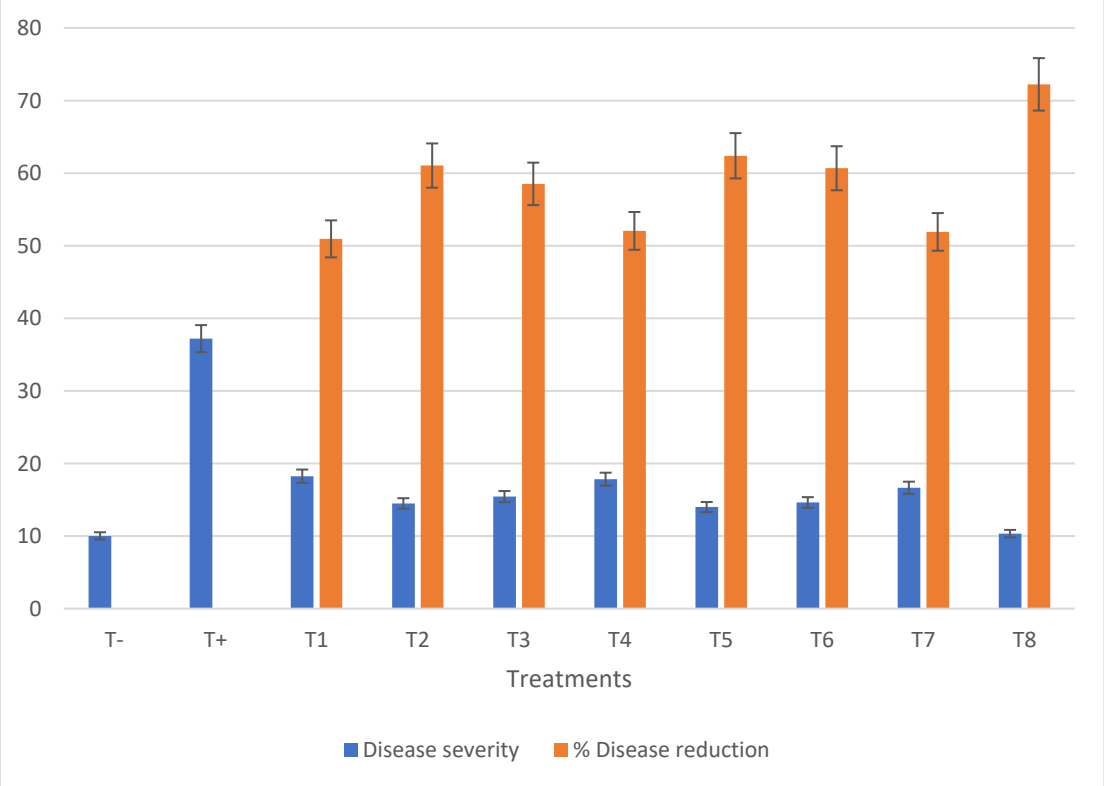


Fig. 4.8. Influence of rhizobacterial treatment on disease severity

Similar conclusions were also made by Yoo *et al*. (2020) who mentioned that considerable species of *Chryseobacterium*, including *C*. *indologenes* and *C. balustinum* promote plant growth and provide resistance against *Phytophthora capsici* and *P. syringae* pv. *tomato* in pepper and Arabidopsis plants, respectively. Similar inference were also made by Horuz (2021) who concluded that *P. oryzihabitans* Antg-12 was able to reduced disease severity (55.85%), area under the disease curve (AUDPC) (66.85%) and increased yield by 41%. Likewise, Yanti *et al*. (2020) also observed reduced occurrence of anthracnose in chilli plants introduced with consortia of *Bacillus* spp. than control with a per cent disease reduction of 57.14-92.86%. It was also noted that used of consortium is more profitable than using a single isolate because of its more varied species which efficiently accelerates colonization and domination of the rhizosphere and the mechanism of action of biocontrol agents is more diverse. Similarly, Boukerma *et al.* (2017) also concluded that treatment of tomato seeds with *P. fluorescens* PF15 and *P. putida* PP27 reduced disease severity by 37–72%, and the levels of infection by 7–36%. Lamsal *et al.* (2012) stated that both phytohormone indole-3-acetic acid and phosphate solubilizing capacity are factors inducing systemic acquired resistance in various plants.

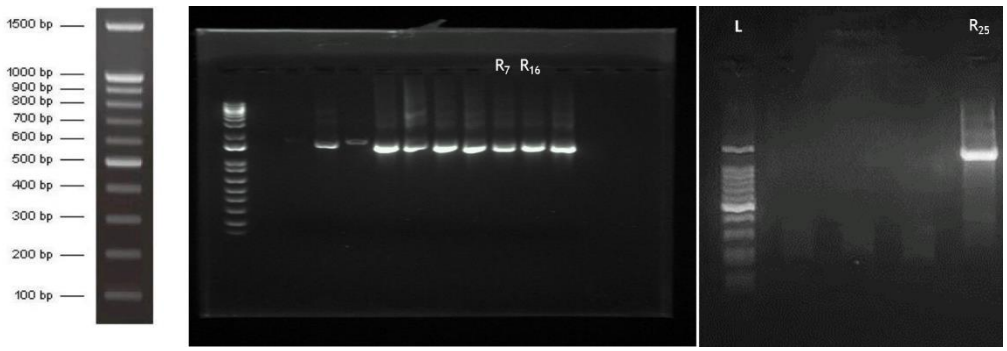
4.11 Molecular identification of rhizobacteria

Molecular identification of potential rhizobacterial isolate (R_7, R_{16}, R_{25}) was performed by using 16S rRNA gene sequences and the sequences of all the isolates were submitted to NCBI Gene Bank. BLAST analysis reported that T_7 (R_7) showed 100% similarity with *Pseudomonas oryzihabitans*; T_{16} (R_{16}) showed 98.73 % similarity with *Chryseobacterium candida* and T_{25} (R_{25}) showed 97.56 % similarity with *Bacillus cereus*. The results obtained were used for the construction of clusters. The 16S rRNA gene sequence for these rhizobacteria are presented on Table 4.20 and Plate 21.

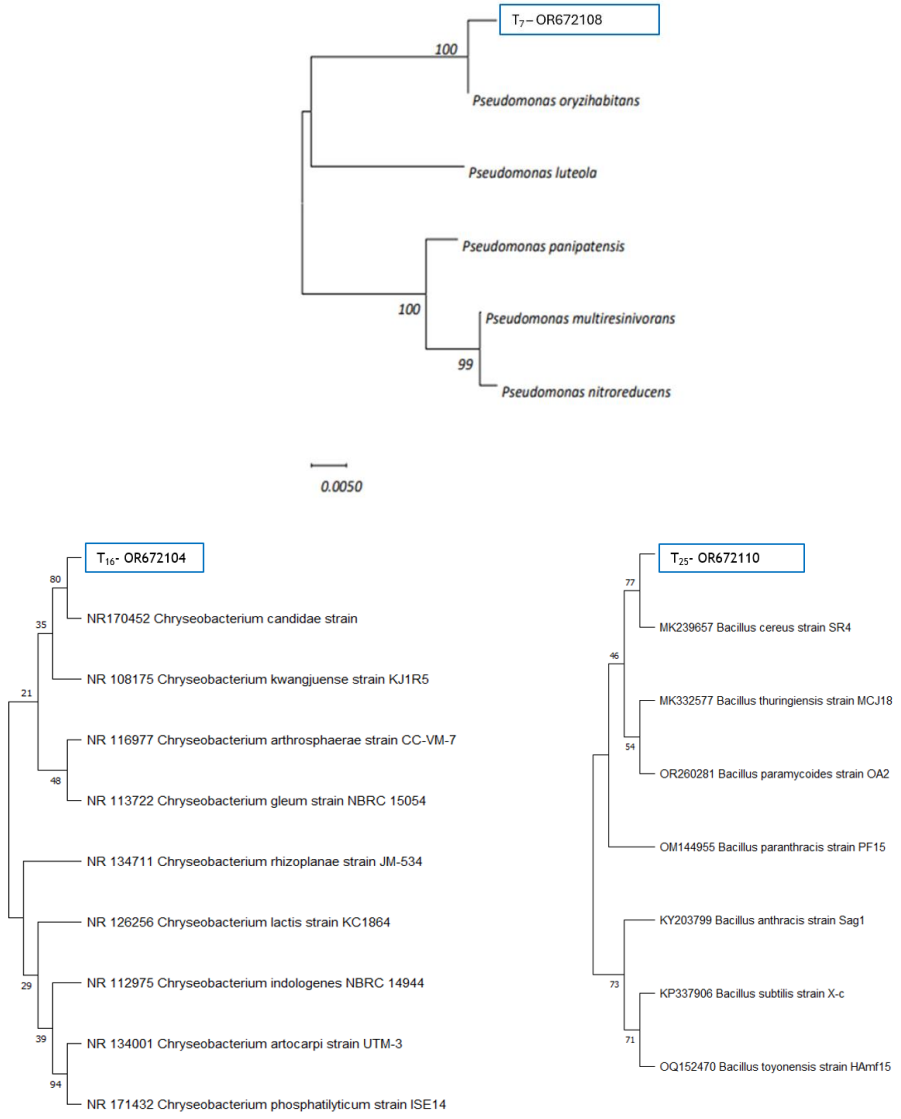
Table 4.20. 16S rRNA gene region of rhizobacteria

Isolate	Accession no.	Sequence	Base pair	Per cent identity
T_7	OR672108	CGAGCGGATGAGAGGAGCTTGCTCCTCGATTCAGCGGCGGACGGGTGAGTAATGC CTAGGAATCTGCCTAGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGC ATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATTAGATGAGCC TAGGTCGGATTAGCTAGTTGGTAGGGTAAAGGCCTACCAAGGCGACGATCCGTAA CTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTA CGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATG CCGCGTGTGTGAAGAAGGCCTTCGGGTCGTAAAGCACTTTAAGTTGGGAGGAAG GGCTCATAGCGAATACCTGTGAGTTTTGACGTTACCAACAGAATAAGCACCGGCTA ACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTAC TGGGCGTAAAGCGCGCGTAGGTGGCTTGATAAGTTGGATGTGAAATCCCCGGGCT CAACCTGGGAACTGCATCCAAAACTGTCTGGCTAGAGTGCGGTAGAGGGTAGTGG AATTTCCAGTGTAGCGGTGAAATGCGTAGATATTGGAAGGAACACCAGTGGCGAA GGCGACTACCTGGACTGACACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAC AGGATTAGATACCCTGGTAGTCCACGCCGTA	746	*Pseudomonas oryzihabitans*-100%
T_{16}	OR672104	CCAGAGAGCGGCGTACGGGTGCGGAACACGTGTGCAACCTGCCTTTATCAGGGGG ATAGCCTTTCGAAAGGAAGATTAATACCCCATAATATTTAGAATGGCATCATTTTAA ATTGAAAACTCCGGTGGATAGAGATGGGCACGCGCAAGATTAGATAGTTGGTGAG GTAACGGCTCACCAAGTCTRCGATCTTTAGGGGGCCTGAGAGGGTGATCCCCCAC ACTGGTACTGAGACACGGACCAGACTCCTACGGGAGGCAGCAGTGAGGAATATTG GACAATGGGTGSGAGCCTGATCCAGCCATCCCGCGTGAAGGATGACGGCCCTATG GGTTGTAAACTTCTTTTGTATAGGGATAAACCTACTCTCGTGAGGGTAGCTGAAGG TACTATACGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGG GTGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGTAGGCGGATCTGTAAG TCAGTGGTGAAATCTCACAGCTTAACTGTGAAACTGCCATTGATACTGCAGGTCTT GAGTGTTGTTGAAGTAGCTGGAATAAGTAGTGTAGCGGTGAAATGCATAGATATTA CTTAGAACACCAATTGCGAAGGCAGGTTACTAAGCAACAACTGACGCTGATGGAC GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGC	713	*Chryseobacterium candidae* strain JC507-98.73%

		CGTAAACGATGCTAACTCGTTTTTGGTATTTCGGTATCAGAGACTAAGCGAAAGTG ATAA GTTAGCCACCTGGG		
T_{25}	OR672110	CCTGAATCAAGTTTTCCAGCCAGGTCTTGTAGGTTCTGAGCGTTTAGGATTAAGAG CTTGCTCTTATGAAGTTACCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCC ATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACC GCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTC GCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACC TGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGA GGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC GTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTG CTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGG CGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAAC CGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAAT TCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGC GACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGG ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTTAGAGGGG TTTTCGCCCCTTTAGTGCTGAAGTTAAACGCATTAAACCCTCCCCCTGGGGAAGTT CGGCCGCAAGGCTTTACTCAAAGGAATTGACGGGGGCCCGCCCAAACGGTGGAG CATGTGGTTTAATTTGAAACAACGCGAAGAACCTTTCCAGGTTTTGGCATCCTTTG ACAACCCTAGAGATAGGGCTTTTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGG TTGTTGTCAGCTTGTGTCGTGAGATGTTGGGTTAAGTTCCGCAACGAGCGCAACC CTTGATTTTAGTTGCCATCATTTAGTTGGGCCCTTTAAGGTGACTGCCGGTGACAA ACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTA CCCCCGTGCTACAATGGACGGTACAAAGAGCTGCAAGGCCGCGAGGTGGAGGTA ATTTCATAAAACCGTTTTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGC TGGAATCGCTAGTAATCGCGGATCAGCATTCCGCGGTGAATACGTTCCCGGGCCTT GTACACCCCGCCCGTCCCCCCCCCGGGGGTTTGTAACCCCCGAAGTCGTAGGGGTA ACTTATCGGAAACAAGCTCGCCGTAGGATCACCTTGGC	1472	*Bacillus cereus*-97.56%



a. PCR amplification of rhizobacteria



b. Clustering based on 16S rRNA region

Plate 21. PCR amplification and clustering analysis of T_7, T_{16}, T_{25}

From the above-mentioned observations we can conclude that T_7 is *Pseudomonas oryzinhabitans* as there were 100% similarity found. However, for T_{16} and T_{25} we can conclude that the two rhizobacteria belongs to the genus *Chryseobacterium* sp. and *Bacillus* sp. respectively as the similarity was found to be low after running BLAST. Similar results were also reported by Kumar *et al*. (2014) who identified *Bacillus cereus* by molecular analysis to be among the isolated rhizobacteria. Horuz and Aysan (2016) also identified *P. oryzihabitans* on the basis of 16S rRNA as one amongst the rhizobacteria exhibiting antagonistic activity against *Acidovorax citrulli.* Similar results were also recorded by Abdaljalil *et al*. (2016) who stated that *Chryseobacterium* spp. are often found in soil and water and exhibits plant growth promoting ability beside being able to supress plant diseases.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The present study on "Antagonistic potential of Naga king chilli rhizobacteria against anthracnose pathogen" was carried out in the laboratory and experimental plot of the Department of Plant Pathology, SAS, Nagaland University, Medziphema Campus, Nagaland following a Completely Randomized Design (CRD) for laboratory experiments and Randomised Block Design for field experiment during the year 2021-2023.

The findings from the present experiment are summarized as follows:

- Typical symptom of anthracnose appeared as sunken necrotic tissue with concentric rings of dark coloured acervuli were observed on fruits and as the disease progresses the infected plants become necrotic from tip backwards which later the entire top twig of the plant may wither away and in severely infected plant the entire plant may wither away.
- The pathogen was characterized as *Colletotrichum gloeosporioides* based on the morphological characters of the pathogen which includes septate, branched, hyaline, smooth walled hyphae. Conidia were hyaline, aseptate, smooth, prominently guttulate. Molecular identification of the pathogen also confirmed the same during BLAST analysis with 99.64% similarity. ITS sequences of *C. gloeosporioides* was submitted to NCBI Gene Bank (PP494232).
- Pathogenicity test was conducted by detached fruit method and was found to be pathogenic by the development of disease after seven days of inoculation.
- Isolated 27 rhizobacteria from the rhizosphere of Naga king chilli. Many isolates were morphologically small, round, orange, smooth, convex, entire and opaque. Biochemical characterization of the isolates showed that most of the rhizosphere was predominated with Gram negative bacteria with 21 isolates being gram

negative and six gram positive, five isolates were recorded to utilize lactose, 21 isolates were observed to give positive reaction for KOH test, 19 isolates were observed to hydrolyse starch while all the isolates were positive for catalase and negative for Hypersensitive Response test on *M. jalapa* and tobacco.

- *In vitro* dual culture assay for antagonism test suggested that antagonism by the rhizobacteria ranges from 0-47.89%. Highest per cent inhibition was recorded from T_7 with 47.89% inhibition.
- The data recorded on volatile metabolites production showed that majority of the isolates does not inhibit the growth of the pathogen. However, some isolates showed significant inhibition over control which includes T_7 and T_{25} which showed highest inhibition of 32.50% followed by T_{18} with 31.67% and T_{16} with 30.83% over the control.
- Qualitative assay on the biocontrol mechanism revealed that 16 isolates showed positive reaction for siderophore where T_7 and T_{16} displayed strong siderophore production. Qualitative assay on HCN production revealed that 15 isolates were able to produce HCN and isolates T_7, T_{10} and T_{18} showed strong HCN production while none of the isolates was recorded to synthesise chitin nor produce biosurfactant.
- *In vitro* evaluation on the mechanism of Plant growth promotion by the rhizobacteria revealed that 22 isolates were able to produce IAA while 26 isolates were recorded to produced GA_3 in varying quantity. Qualitative assay on ammonia production revealed that all the isolates were capable of producing ammonia in varying degree according to colour intensity after adding Nessler's reagent. Nineteen isolates were able to solubilize phosphate by forming a halo zone around the rhizobacteria while 22 isolates were observed to solubilize zinc with T_4, T_7 and T_{14} displaying strong zinc solubilization.
- Compatibility of the three best performing rhizobacteria based on dual culture assay was tested with each other and with fungicide captan and observed that the rhizobacteria were compatible with each other as there was no formation of clear

zone when cultured together on nutrient agar but was found to be incompatible with fungicide captan as there was a formation of clear zone of inhibition on nutrient agar medium poisoned with the fungicide.

- *In vitro* plant growth promotion assay suggested that all the seeds treated with a single rhizobacteria or in mixture performed significantly better than control and chemical treated seeds. Treatment of the Seeds with T_2 gave highest germination percentage of 76.67% and the lowest germination percentage was recorded from T_0 (control) with 28.33%. Maximum root length was recorded from T_8 (3.90cm) and minimum root length was recorded from T_4 (1.54cm). The rhizobacterial treatment of seed also had a significant influence on shoot length which revealed that the maximum shoot length was recorded from T_8 (3.18cm) and the minimum shoot length was recorded from T_4 (2.40 cm). observation on the vigor index suggest that the maximum vigor index was recorded from T_8 (447.11 %) and minimum vigor index was reported from T_0 (150.45 %)
- Treatment with rhizobacteria has little to no influence on days to first flowering as no statistically significant difference within the treatments were recorded.
- Plants treated with a mixture of the three rhizobacteria (T_8) which is a mixture of R_7, R_{16} and R_{25} showed significant improvement on plant height with maximum plant height of 85.67 cm over inoculated control (T_+) with 66.92 cm.
- The influence of rhizobacterial treatments on yield parameter suggest that treatments with a mixture of all the three rhizobacteria (R_7, R_{16} and R_{25}) gave higher number of fruits with an average of 103.67 fruits, highest yield/plant of 580.17 g and yield/ hectare of 333.29 kg/ha over inoculated control.
- *In vivo* plant growth promotion revealed that highest biomass increase was recorded from T_8 with 55.41 % biomass increase as compared to inoculated control.
- Influence of the rhizobacterial treatment on Disease incidence and disease severity (%) concluded that treatment with a mixture of all the three rhizobacteria (T_8) significantly reduce the incidence of disease after 150 DAT

with the lowest disease severity of 10.33% and highest percent disease reduction of 72.23 over inoculated control.

- Molecular identification of the three best performing rhizobacteria from dual culture assay was done using 16S rRNA and were submitted to NCBI GenBank where, R_7 (OR672108) showed 100% similarity with *Pseudomonas oryzihabitans*, R_{16} (OR672104) showed 98.73 % with *Chryseobacterium candidae* strain JC507 and R_{25} (OR672110) showed 97.56 % similarity with *Bacillus cereus* during BLAST analysis.

It can be concluded from the present investigation that the rhizosphere of Naga king chilli is inhabited by various rhizobacteria predominantly gram negative, showing positive reaction for catalase and gelatine liquification test. Majority of the rhizobacteria were also observed to produce plant growth promoting hormone and metabolite like IAA, GA_3, ammonia and were able to solubilize phosphate and zinc *in vitro*. A larger portion of the isolates were also observed to produce siderophore and HCN which are considered to be important metabolites that protect plant against invading plant pathogens. Furthermore, the *in vivo* trials of the present study suggested that treatment of Naga king chilli with a mixture of rhizobacteria *Pseudomonas oryzihabitans* (R_7), *Chryseobacterium* sp.(R_{16}) and *Bacillus* sp.(R_{25}) were able to increase plant height, number of fruits per plant, yield and reduce the incidence of anthracnose disease significantly over inoculated control. However, further studies are required to have a deeper understanding of the isolates. multi-locational field trials are also required to know the full potential of these isolates and their interaction with different environment conditions to confirm its vitality as potential biocontrol agents as strains isolated from one region may not produce similar results in another soil and climatic condition as in case of its original habitat.

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APPENDICES

Appendix 1 Per cent disease incidence

Treatment	60 DAT			75 DAT			90 DAT			105DAT		
	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
T-	0.00 (0.81)	0.00 (0.81)	0.00 (0.84)	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	6.49 (12.38)	6.67 (12.56)	6.58 (12.46)	12.54 (20.51)	9.43 (17.64)	10.99 (19.07)
T+	9.14 (17.59	8.57 (17.07)	8.86 (17.31)	9.13 (17.56)	11.36 (19.59)	10.24 (18.57)	30.09 (33.25)	24.89 (29.72)	27.49 (31.55)	36.45 (37.07)	31.09 (33.68)	33.77 (35.38)
T_1 (R_7)	0.00 (0.81)	0.00 (0.81)	(0.00) 0.81	5.78 (13.86)	2.78 (9.27)	4.28 (11.56)	11.61 (19.60)	9.44 (17.88)	10.53 (18.74)	14.39 (22.28)	12.22 (20.15)	13.31 (21.22)
T_2 (R_{16})	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	2.78 (9.25)	3.35 (10.50)	3.07 (9.88)	6.12 (14.13)	9.14 (17.59)	7.63 (14.81)	12.59 (20.67)	9.14 (17.28)	10.87 (18.97)
T_3 (R_{25})	0.00 (0.81)	0.0 (0.81)	0.00 (0.81)	1.43 (6.82)	1.37 (6.67)	1.40 (6.74)	9.44 (17.88)	5.54 (11.49)	7.49 (14.69)	9.44 (17.70)	8.32 (16.52)	8.88 (17.11)
T_4 (fungicide)	0.00 (0.81)	0.0 (0.81)	0.00 (0.81)	3.35 (10.37)	2.78 (9.24)	3.07 (9.80)	9.14 (17.28)	11.67 (19.77)	10.40 (18.69	9.14 (17.44)	11.67 (19.70)	10.40 (18.57)
T_5 (R_7+ R_{16})	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	2.79 (9.56)	2.78 (9.24)	2.79 (9.58)	8.32 (16.67)	9.43 (17.87)	8.88 (17.32)	11.67 (19.87)	9.43 (17.46)	10.55 (18.66)
T_6 (R_7 + R_{25})	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	3.04 (9.96)	2.78 (9.24)	2.91 (9.75)	8.84 (14.68)	8.88 (17.32)	8.86 (15.80)	9.14 (17.34)	8.88 (16.91)	9.01 (17.13)
T_7 (R_{16} + R_{25})	0.00 (0.81)	0.00 (0.81)	0.00 (0.84)	3.35 (10.52)	2.78 (9.24)	3.06 (10.03)	9.70 (18.14)	11.92 (20.03)	10.81 (19.08)	12.73 (20.61)	11.92 (19.93)	12.33 (20.27)
T_8 (R_7 + R_{16} + R_{25})	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	0.00 (0.81)	3.04 (9.79)	2.78 (9.44)	2.91 (9.62)	6.37 (14.25)	5.54 (13.06)	5.96 (13.66)
SEm ±	0.15	0.08	0.09	0.95	1.09	0.72	2.13	2.22	1.54	2.01	2.55	1.62
CD at 5%	0.46	0.25	0.25	2.83	3.23	2.07	6.33	6.60	4.41	5.96	7.58	4.65
C.V (%)	10.67	5.91	8.69	18.42	21.99	20.21	20.81	21.84	21.35	16.72	22.98	19.87

Note-Figures in the table are mean values and those in parenthesis are angular transformed values

Cont…

Treatment	120 DAT			135 DAT			150 DAT		
	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled	2021-2022	2022-2023	Pooled
T-	12.54 (20.49)	9.43 (17.87)	10.99 (19.18)	12.54 (20.49)	9.43 (17.87)	10.99 (19.18)	12.54 (20.49)	9.43 (17.87)	10.99 (19.18)
T+	36.45 (37.13)	31.09 (33.87)	33.77 (35.50)	36.45 (37.13)	30.33 (33.37)	33.39 (35.25)	36.45 (37.13)	34.34 (35.87)	35.40 (36.50)
T_1 (R_7)	14.39 (22.04)	12.22 (20.32)	13.31 (21.18)	14.39 (22.04)	12.22 (20.32)	13.31 (21.18)	14.39 (22.04)	12.22 (20.32)	13.31 (21.18)
T_2 (R_{16})	12.59 (20.67)	9.14 (17.59)	10.87 (19.13)	12.59 (20.67)	9.14 (17.59)	10.87 (19.13)	12.59 (20.67)	9.14 (17.59)	10.87 (19.13)
T_3 (R_{25})	9.44 (17.88)	8.32 (13.61)	8.88 (15.75)	12.22 (20.32)	11.09 (19.20)	11.66 (19.76)	12.22 (20.32)	11.09 (19.20)	11.66 (19.76)
T_4 (fungicide)	9.14 (17.59)	11.67 (19.77)	10.40 (18.68)	11.92 (20.03)	11.66 (19.76)	11.79 (19.89)	11.92 (20.03)	14.45 (22.21)	13.18 (21.12)
T_5 (R_7+ R_{16})	11.67 (19.77)	9.43 (17.87)	10.55 (18.82)	11.67 (19.77)	9.43 (17.87)	10.55 (18.82)	11.67 (19.77)	9.43 (17.87)	10.55 (18.82)
T_6 (R_7 + R_{25})	9.14 (17.59)	8.88 (17.32)	9.01 (17.45)	9.14 (17.59)	8.88 (17.32)	9.01 (17.45)	12.17 (20.15)	8.88 (17.32)	10.52 (18.73)
T_7 (R_{16} + R_{25})	12.73 (20.70)	11.92 (20.03)	12.33 (20.37)	12.73 (20.70)	11.92 (20.03)	12.33 (20.37)	12.73 (20.70)	11.92 (20.03)	12.33 (20.37)
T_8 (R_7 + R_{16} + R_{25})	6.37 (12.26)	5.54 (11.43)	5.96 (11.85)	9.13 (17.58)	8.87 (17.31)	9.00 (17.44)	9.13 (17.58)	8.87 (17.31)	9.00 (17.44)
SEm ±	2.57	2.11	1.66	1.90	1.59	1.24	2.05	1.41	1.24
CD at 5%	7.65	6.27	4.77	5.66	4.71	3.55	6.08	4.18	3.56
C.V (%)	21.62	18.98	20.440	15.25	13.69	14.56	16.20	11.85	14.33

Note- Figures in the table are mean values and those in parenthesis are angular transformed values