Section A-Research paper

BROAD SPECTRUM ACTIVITY OF ACTINOBACTERIA IN TEA ECOSYSTEM

R. Jayanthi[1 2], A.K. Kathireshan[1*], G. Gayathri[1] and P. Nepolean[2]

[1]Vels Institute of Science, Technology and Advanced Studies (VISTAS),
Old Pallavaram, Chennai - 600117

[2]UPASI Tea Research Institute, Valparai- 642127

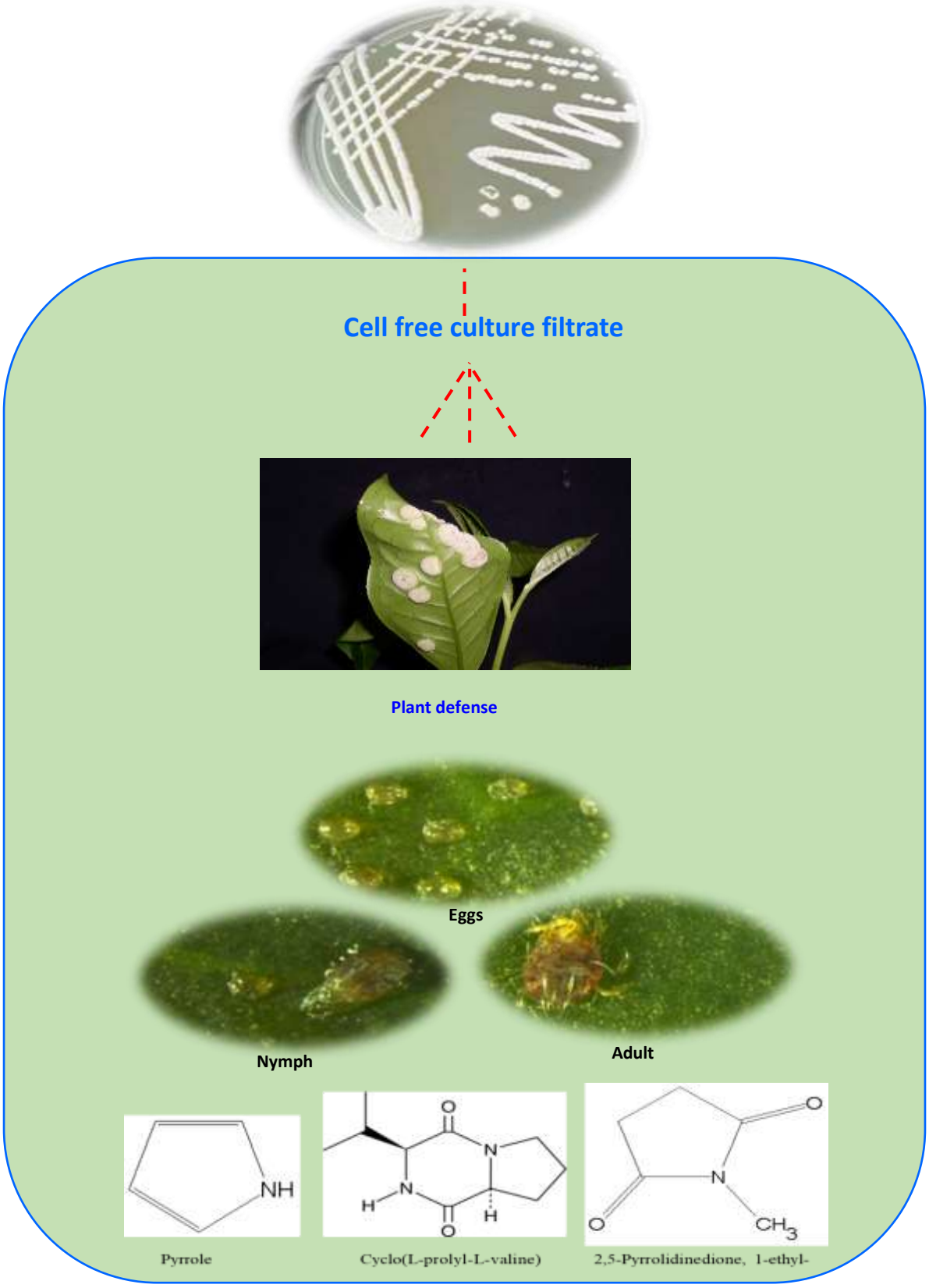
Email id- *Corresponding author: kathirhodmicrobiology@gmail.com

Abstract

While tea is mass-produced from the young shoots of the tea plant, leaf infections are a significant problem. The blister blight caused by the fungus *Exobasidium vexans Massee* is the worst leaf disease in all tea-growing regions. The second most significant foliar disease in tea is grey blight (*Pestalotiopsis* sp.) and brown blight disease (*Glomerella* sp.). Mostly, agrochemicals are used to combat these significant illnesses. Environmental contamination is caused by the continuous use of agrochemicals. In an effort to remove these illnesses in an eco-friendly way, the use of biological agents becomes an alternative strategy. Actinobacteria are gram-positive bacteria that serve a crucial role in the soil ecology. Two potential actinobacteria, AAS7 and CAS4, were discovered in soil samples from the Anamallais and Coonoor. Under in vitro settings, the antagonistic potential of putative actinobacteria against tea pathogens and their acaricidal activities against red spider mite were evaluated. Highest growth inhibition was seen with CAS4 (90%) and AAS7 (82.1%) against foliar pathogens including *Pestalotiopsis* sp. The AAS7 cell-free culture filtrate had the best inhibitory effect against *Glomerella* species (85.3%), followed by CAS4 (65.4%). Using a microscope, the germination of *E. vexans* spores was assessed. The CAS4 culture filtrate suppressed the germination of *E. vexans* spores efficiently. AAS7 and CAS4 are the two actinobacterial isolates with the highest death rate against red spider mites (RSM). AAS7 displayed a 100% mortality rate, followed by CAS4. This study demonstrates that actinobacteria isolated from tea-growing soil may be a particularly rich reservoir for the generation of physiologically active chemicals.

Keywords: Actinobacteria, biological agent, agrochemicals, leaf diseases.

Graphical Abstract



Introduction

Tea is a monoculture crop, and its illnesses may be categorised as leaf, stem, or root diseases. Many pests and illnesses hinder the development and productivity of grown tea. The majority of pathogens that cause illnesses in tea plants are fungi. Tea plants infested with fungi may experience production and quality losses (Pandey et al., 2021). Among plant diseases, leaf diseases provide the greatest concern. The blister fungus is the most significant leaf disease. *Exobasidium vexans*, the causative agent of blister blight disease, is an obligate fungus that completes its whole life cycle in tea. The disease mostly targets young, succulent, and fragile harvestable leaf and stalk, wreaking havoc on the quantity and quality of tea for human consumption. The red spider mite (RSM) is a serious pest of tea (*Camellia sinensis*) in the majority of tea-producing nations. Adults and nymphs of the red spider mite (RSM) lacerate cells, leaving reddish brown spots on the top surface of mature leaves, which become red in severe infestations, resulting in crop loss (Roy et al., 2014)

The usefulness of agrochemicals in managing these primary diseases and pests of tea plants has been shown. Nevertheless, the use of some effective fungicides in tea is prohibited owing to concerns about residual levels in processed tea (Pandey et al., 2021). Constant usage of agrochemicals pollutes the ecosystem. The use of biological agents provides an alternative eco-friendly strategy for eradicating certain illnesses.

Many potential bacterial (*Bacillus* spp. and *Pseudomonas* spp.) and fungal (*Trichoderma* spp.) antagonists against fungal diseases of tea plants have been found as microbial biological control agents (MBCAs). The majority of these MBCAs, however, were evaluated in vitro (Kabir et al., 2016; Mareeswaran et al., 2018) without additional field studies. Actinobacteria are gram-positive bacteria that play an important role in the ecology of soil. Actinobacteria can effectively manage a number of significant agricultural pests and illnesses. Nevertheless, there is little information accessible in tea. An effort has been made in the current work to screen actinobacteria from tea soil for the efficient control of red spider mite and tea illness.

MATERIALS AND METHODS

Survey

Two agro-ecological zones of southern India, the Anamallais and the Nilgiris, were sampled for soil (0-9 inches) samples. Actinomycetes were isolated from the air-dried, sieved soil samples.

Isolation of Actinomycetes

For the isolation of actinomycetes from soil, Starch casein nitrate agar (SCNA medium(Küster & Williams, 1964) with the following composition (g/l) was employed (Starch 10.0g, casein 0.3g, KNO_3 2.0g, $NaCl_2$ 2.0g, K_2HPO_4 2.0g, $MgSO_4.7H_2O$ 0.01g, Agar 20g and distilled water 1000 ml). The medium's pH was adjusted to 7.2, and nystatin and nalidixic acid were added to prevent bacterial and fungal contamination. The soil samples then serially diluted from 10^{-4} to 10^{-7}, and 1ml aliquots from each dilution were transferred to sterile Petri dishes. Around 15ml of the cooled starch casein medium (45°C) was poured to each Petri dish, and the inoculum was blended by gently rotating each Petri dish. Plates were incubated at 37°C for three to four days (for the emergence of aerial mycelium), seven to

fourteen days (for the observation of mature aerial mycelium), and thirty days (for slow growing isolates). Several times, the isolates were sub-cultured on starch casein nitrate agar until they formed single colonies (Jayanthi *et al*., 2016)

Media standardization for antagonistic activity

In several solid media, including PDA, SDA, Actinomycetes isolation agar, Starch casein nitrate agar, and chitin agar, the development of actinobacteria and fungal cultures was compared. In each sterilised petri dish, 15 ml of sterile warm media was poured and allowed to settle. The pathogen was injected in the plate's middle, and actinobacteria were streaked on each plate. The plates were incubated at 25°C for pathogenic fungi and 37°C for actinobacteria. The observation was made five days following the immunisation (Lockwood *et al*., 1975).

Screening of actinomycetes

The UPASI Tea Research Institute provided cultures of fungi. The antifungal activity of these isolated potential organisms was evaluated using a dual culture experiment. Actinobacteria are plated on one side of chitin agar plates, about 2.5 cm from the plate's centre. A disc containing fungi was put on the other side of the plate. On one side of a plate, a fungal disc serves as a control. The antagonistic (inhibition zone) was determined by measuring the distance between the edge of the fungal mycelium and the actinobacteria strain after 5–7 days of incubation at 28°C. The percentage of growth inhibition was computed using the following formula (Charria -Girón *et al*.,2021).

PI = R1-R2/R1x100

where R1 was the radius of mycelial growth in the control group and R2 was the radius of mycelial growth towards the actinobacteria.

Assessment of the effectiveness of actinomycetes against the red spider mite

In order to set up the bioassay, fresh tea leaves were shredded. On tea leaf discs, the culture (10^7 cfu/ml) was sprayed using an atomizer (sprayer). Each set had five treated leaf discs. In the same manner, five leaf discs were sprayed with Quinalphos (0.1ml/L) and put on wet cotton pads on petri plates for the control group. On leaf discs, 10 mites were released in each experimental set. Observations were conducted at 24, 48, 72, and 98 hours. The outcomes were documented (Jayanthi *et al*.,2016). Three times, the experiment was repeated.

Koch's postulate technique

Red spider mites were cleaned for one minute in a 0.1% mercuric chloride solution and again for one minute in sterile distilled water. Thereafter, they were dried on sterile filter paper. They were then transferred to petri plates containing 20ml of the 2% water agar and incubated at 25°C. Daily checks were performed for the first four days. The colonies of actinomycetes arising from the red spider mite were characterised in terms of their shape

before being transferred to SCNA agar in Petri dishes and cultured at 28 ± 1°C for five to seven days (Sarmah *et al*.,2009). After incubation, the findings were documented.

Actinobacteria's influence on spore germination

Fresh branches with well-developed sporulating lesions were gathered from the experimental plots and cultured under 100% humidity in the laboratory for spore collection. The spores generated from a single lesion were captured in a beaker and measured, and a suspension of basidiospores (10^8 spores per ml) was used to evaluate the inhibitory impact of actinobacteria on basidiospores (Ajay *etal*.,2009).

Investigation of actinobacterial strains against the blister blight disease in a nursery

Experiments were undertaken in order to determine the influence of actinobacteria on UPASI-9 nursery plants. The nursery experiment consisted of four treatments, each with three replications and ten plants. In the standard treatment, COC (210g) was administered according to the suggested schedule. Actinobacteria formulations were administered at a rate of 7.5 ml/L each treatment (Borah *et al*.,2020).

Treatment Details:

1. Control (water) -
2. Standard (COC 210g) - 3g
3. CAS4 - 7.5g
4. AAS7 - 7.5g

Molecular identification of actinobacteria

Using the QIAGEN DNA extraction kit (QIAGEN, Valencia, CA), genomic DNA was extracted from overnight cultures of all six actinobacteria antagonists, suspended in 100µl of elution buffer (10 mM/L Tris-HCl, pH 8.5), and quantified by measuring the optical density at 260 nm. 100 ng of template DNA, 20 mol of 16S rRNA primers, 200 M dNTPs, 1.5 mM MgCl2, 1U of Taq DNA polymerase, and 2 L of 10x Taq polymerase buffer were used in the PCR reaction mixture. Initial denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes. 16S rRNA amplicon PCR results were examined on a 1% agarose gel at 100 V (Dilhari *et al*.,2017).

PCR product purification, sequencing, and sequence analysis

The 16S rRNA amplicons were purified using the QIA rapid gel extraction kit (Qiagen, Valencia, CA). According to the manufacturer's recommendations, the purified products were ligated onto the pGEM®-T Easy vector (Promega Corporation, Madison, United States). The ligated products were then transformed into E. coli strain DH5 and plated on Luria Bertaini agar medium containing ampicillin (50 g/mL), X-Gal (20 g/mL), and IPTG (isopropyl—Dthiogalactopyranoside; 0.1 mM/L) (Sambrook et al.,1989). In recombinants, the presence of insert DNA encoding 16S rRNA was confirmed by PCR amplification and

sequencing using an automated DNA sequencer (Model 3100, Applied Biosystems, USA). Sequences have been submitted to GenBank. Using the Basic Local Alignment Search Tool (BLAST) tool (http://www.ncbi.nlm.nih.gov/blast), sequence similarity searches and phylogenetic analyses were conducted online (Bowman *et al*.,2016).

Bioactive metabolite Gas chromatography – mass spectrometry (GC – MS) analysis

To identify the active chemicals in the intracellular extract, a GC-mass chromatography study was done. 1µl of sample was injected into an RT * 5 column (30 * 0.32 nm) of model GC-MS (Perkin Elmer, Clarus 500, USA); helium (3ml/min) was utilised as the carrier gas. The following temperature gradient programme was utilised: 75 °C for 2 minutes, then a rise from 75 to 175 °C at a rate of 50 °C per minute, followed by 7 minutes at 175 °C. Comparing the m/z peaks showing mass to charge ratio features of the antibacterial fractions to those in the mass spectrum library of the respective chemical compounds (Talib Saleh Al-Rubaye et al., 2020).

Results and Discussion:

Survey and Isolation of Actinomycetes

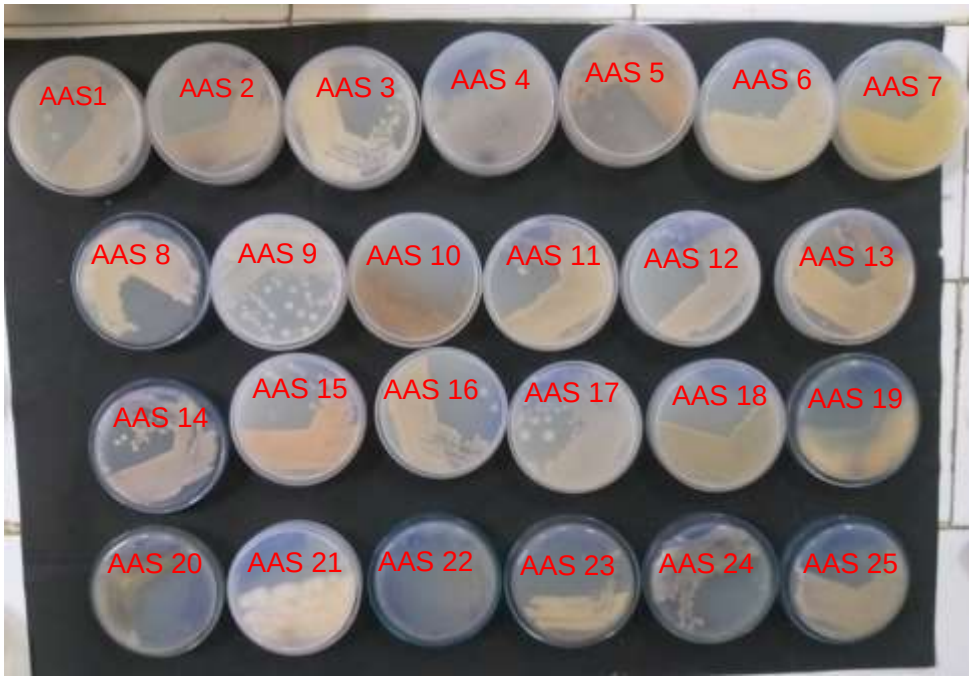
In this study, around 100 strains were isolated in different tea agro-climatic zones of South India. Those purified cultures were involved for screening (Fig.1)

Media standardization:

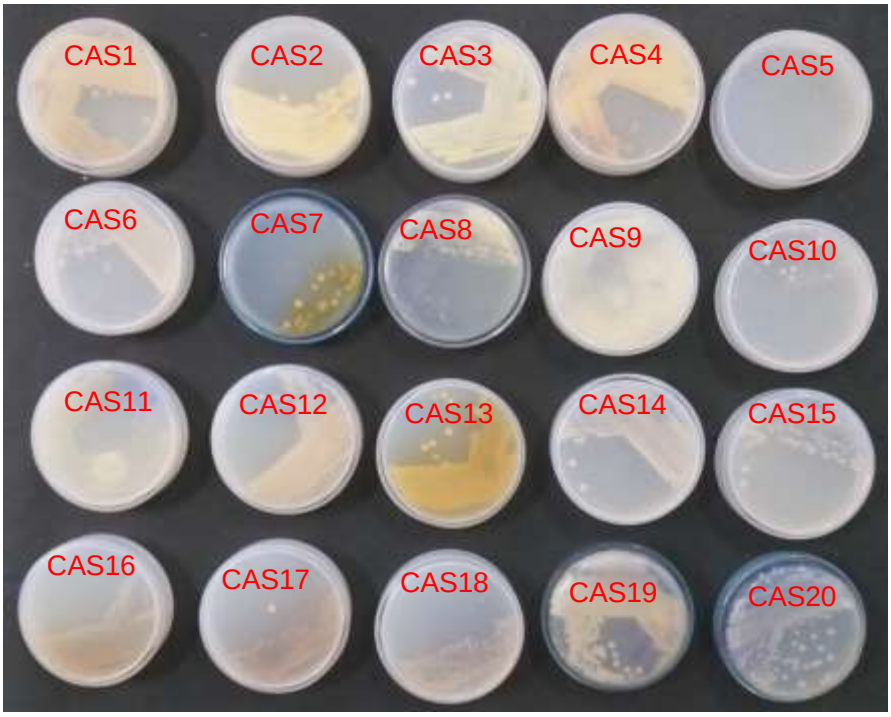
Out of five culture media used to test the growth of the both fungus and actinobacteria, the maximum growth was recorded in Chitin agar. Actinobacteria was no growth in PDA and SDA. Fungal pathogens were low growth in SCNA and Actinomycetes isolation agar. (Fig. 2). In the present study chitin agar supported maximum growth of both fungus and actinobacteria. Chitin agar has been used for the antagonistic study.

Fig 1: Isolation of Actinobacteria

a) Anamallais Isolates



b) Coonoor Isolates



C) Munnar Isolates

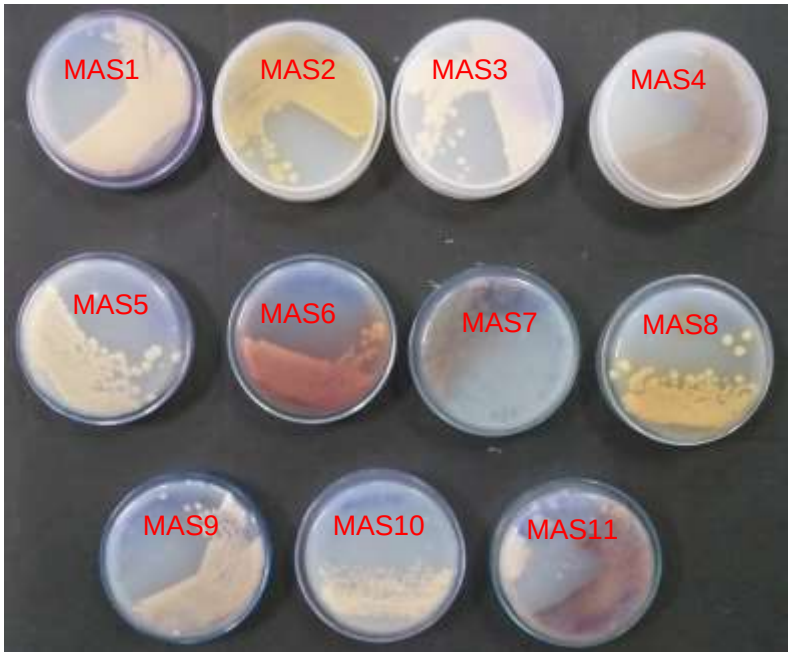
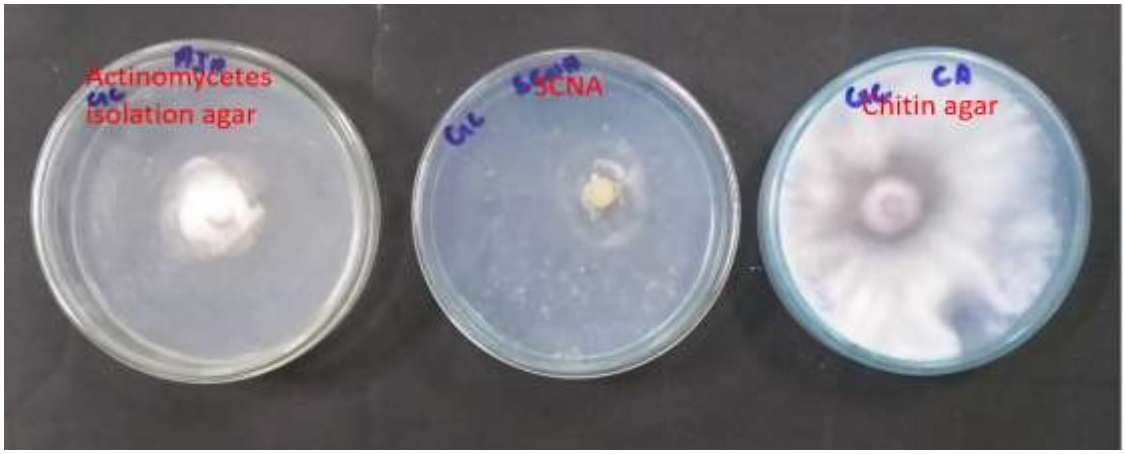
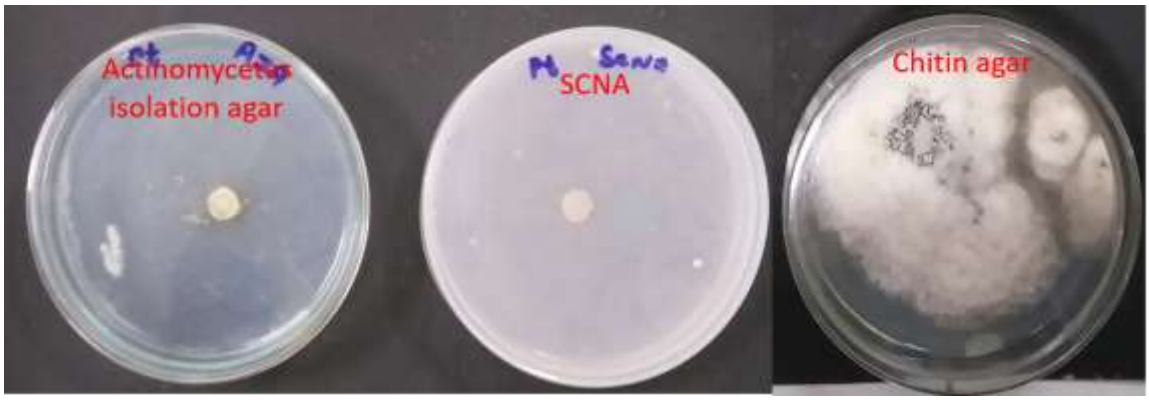


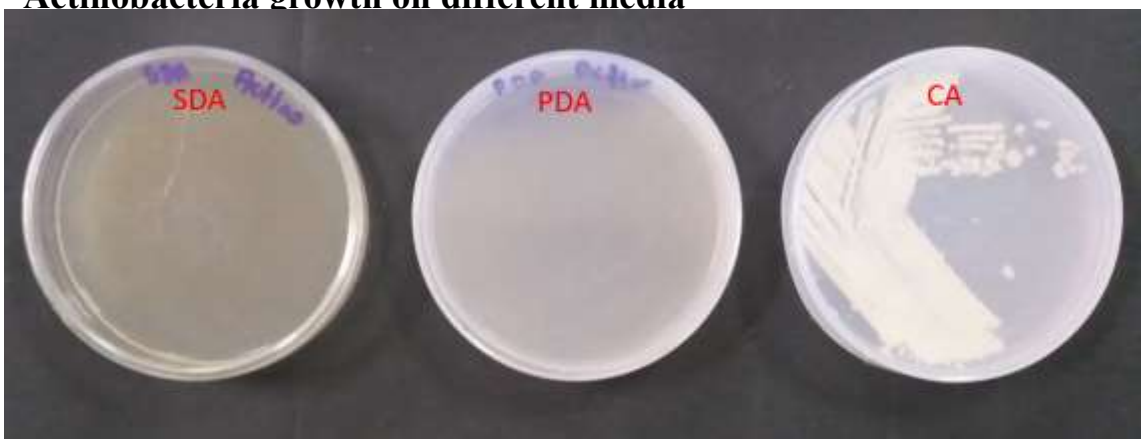
Fig: 2 *Glomerella cingulata growth* on different media



***Pestalotiopsis theae growth* on different media**



Actinobacteria growth on different media



Screening of actinomycetes

Total inhibition of the *Pestalotiopsis theae* was observed in CAS4 (94%), followed by AAS7 (88%), AAS 19 (70%) and AAS 23 (70%). The growth of *Glomerella cingulata* was highly inhibited by the test isolates in the order of CAS4 (90.0 %), AAS7 (88%), AAS19 (70%) and AAS23 (60%). (Table1, Fig 3 & 4)

Table: 1 Screening of Actinobacteria

	Percentage of Inhibition	
Strain	*Pestalotiopsis* sp	*Glomerella* sp
AAS1	53	-
AAS2	67	-
AAS3	**70**	**62**
AAS4	55	42

AAS5	60	40
AAS6	67	40
AAS7	**90**	**88**
AAS8	52	-
AAS9	50	60
AAS10	40	50
AAS11	40	42
AAS12	27	30
AAS13	47	-
AAS14	43	-
AAS17	56	-
AAS18	43	27
AAS19	**80**	**70**
AAS20	44	40
AAS21	40	50
AAS22	53	52
AAS23	**70**	**60**
AAS24	60	-
MAS1	54	50
MAS4	60	52
MAS8	50	-
MAS14	42	-
MAS19	40	40
MAS20	40	60
MAS21	46	-
MAS23	42	-
CAS1	52	-
CAS2	50	-
CAS3	47	-
CAS4	**94**	**90**
CAS5	44	40
CAS6	41	-

Fig 3: Actinobacteria inhibiting the growth of *Pestalotiopsis sp*

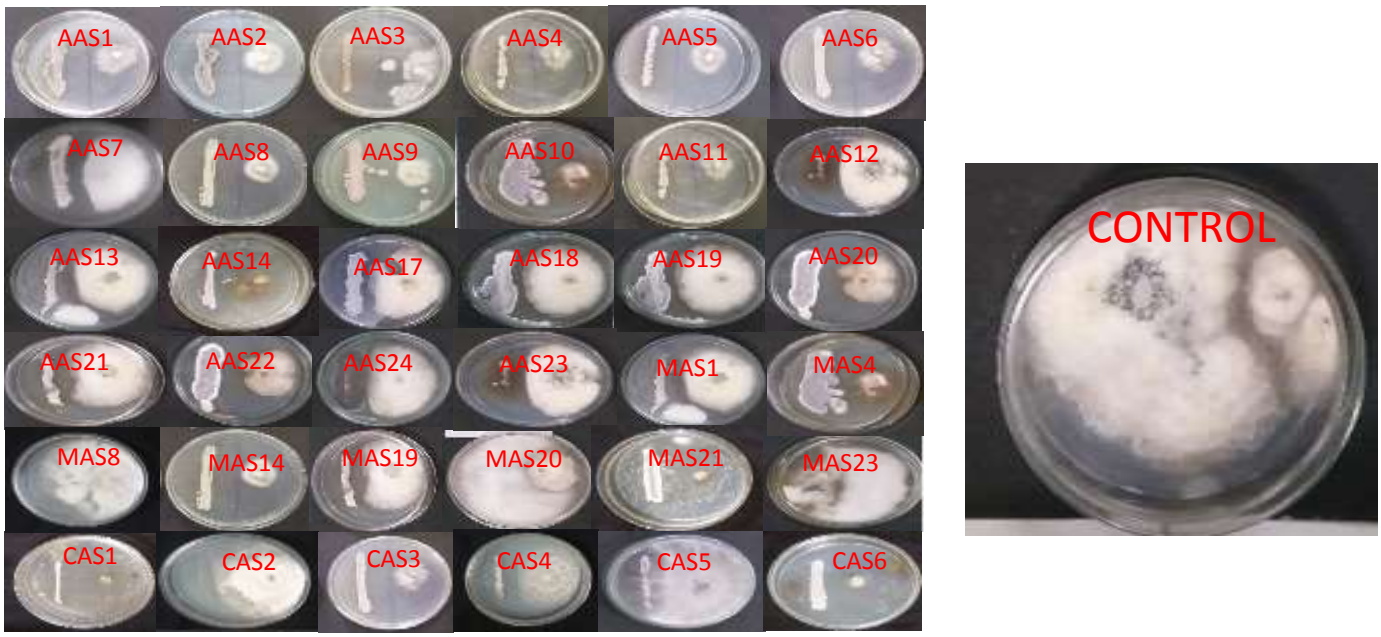
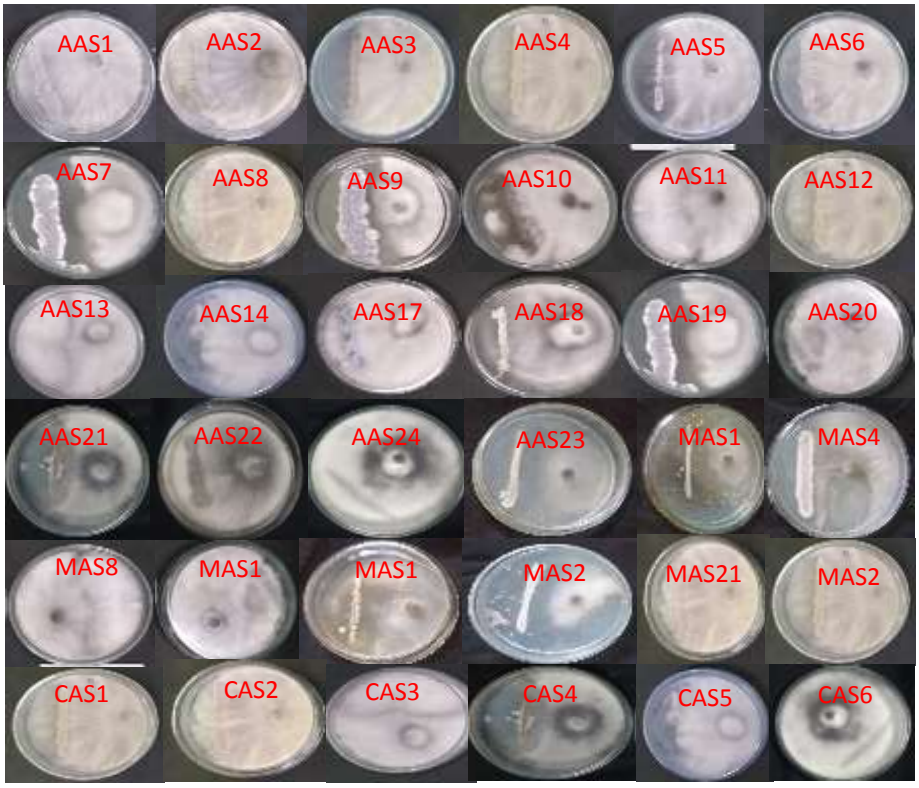


Fig: 4 Actinobacteria inhibiting the growth of *Glomerella sp*



Evaluation of actinomycetes against red spider mite

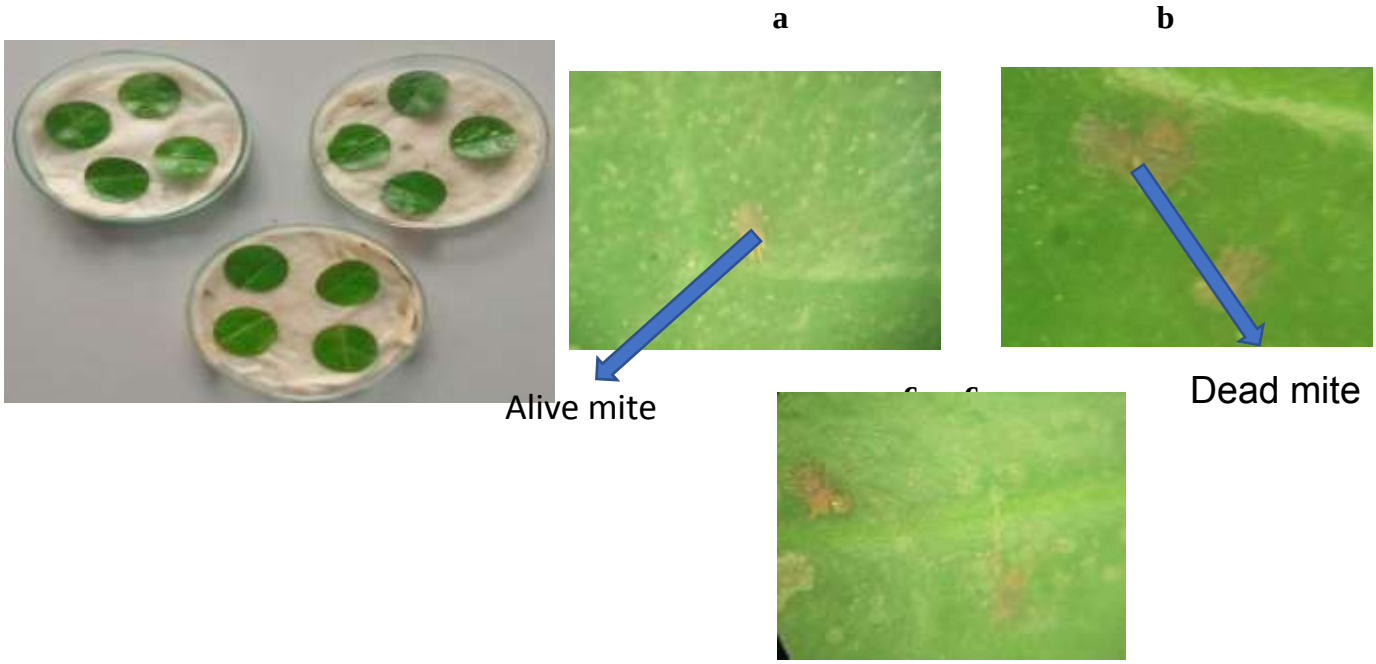
In this investigation, three isolates of actinomycetes induced higher mortality rate of RSM where CAS4 and AAS7 (100%) recorded total mortality followed by AAS17 (82%) and AAS9 (70.0%) after 24 hours (Table 2, Fig. 5).

Table: 2 Evaluation of actinobacteria against RSM

STRAINS	**Mortality (in Percentage)**		
	24 Hrs	**48 Hrs**	**72Hrs**
AAS3	70	74	78
AAS7	**94**	**100**	**100**
AAS9	60	70	70
AAS17	76	80	82
AAS19	60	60	62
AAS23	62	70	70
CAS2	58	62	62
CAS4	**100**	**100**	**100**
MAS1	54	77	78
MAS12	63	63	63

Fig: 5 EVALUATION OF ACTINOBACTERIA AGAINST RSM

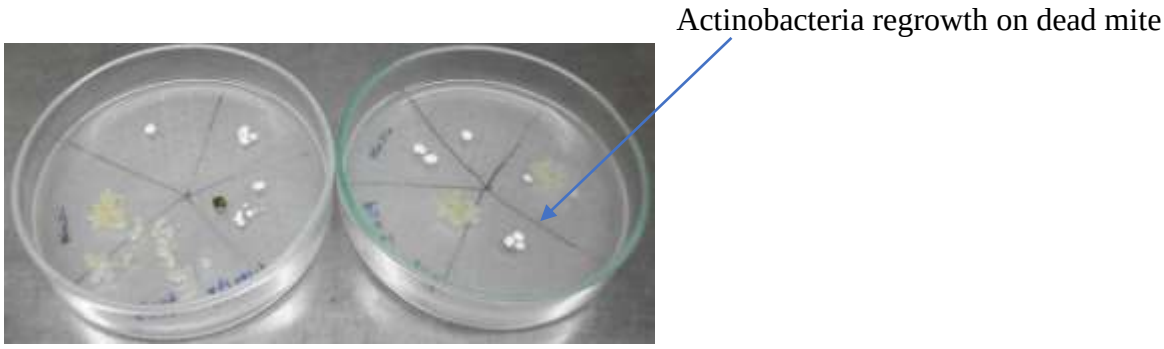
a) Control b) Treated



Koch's postulate

Actinomycetes were reisolated from the dead mites through Koch's postulate method. The results showed that the actinomycetes highly influenced the RSM mortality in tea (Fig. 5).

Fig: 5 Influence of actinomycetes on Red Spider Mite (RSM) mortality in tea



Effect of actinobacteria on basidiospore germination of *Exobasidium vexans*

The basidiospore germination of Exobasidium vexans, the causative agent of blister blight disease in tea, is a crucial step in its life cycle. The spore germination process leads to the formation of primary and secondary infections, which ultimately leads to the spread of the disease. Therefore, controlling the spore germination process is an essential strategy to control the disease (Chaliha *et al.*,2020).

In this study, the effect of actinobacteria on basidiospore germination of E. vexans was examined. The results showed that the spore germination of E. vexans was significantly inhibited by both AAS7 and CAS4 strains of actinobacteria. Additionally, the chemical fungicides used in the study also inhibited the spore germination of E. vexans. This indicates that actinobacteria, as well as chemical fungicides, can be used as an effective means to control the spread of blister blight disease in tea (Thakur *et al*.,2022).

It is important to note that the inhibitory effect of actinobacteria on spore germination was observed under in vitro conditions. Further studies are required to evaluate the efficacy of actinobacteria under field conditions. Additionally, the study did not investigate the mechanism of action of the actinobacteria on spore germination. Future studies should focus on understanding the mode of action of actinobacteria in inhibiting the spore germination process, which will help in developing effective control strategies for blister blight disease in tea (Palaniyandi *et al*.,2013). (Fig.6 & 7 Table.3).

Fig.6. Collection basidiospores under *invitro*

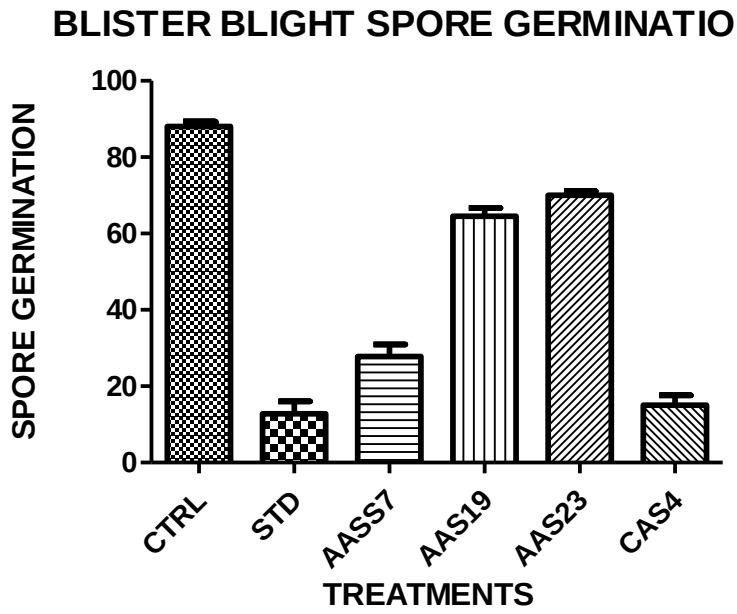
Table : 3 Evaluation of actinobacteria against Basidiospores germination

	Percentage of spore germination (%)			
Treatments	**2hr**	**8hr**	**16hr**	**24hr**
Untreated control	86	86	90	90
Hexaconazole +COC (0.3%)	20	16	10	5
AAS7	35	30	26	20
AAS19	68	63	68	59
AAS23	72	72	68	68
CAS4	20	18	14	8

Fig: 7 Effect of actinobacteria against *Exobasidium vexans*

Control **Actinobacteria Treated**

Fig: 8 Blister blight spore germination

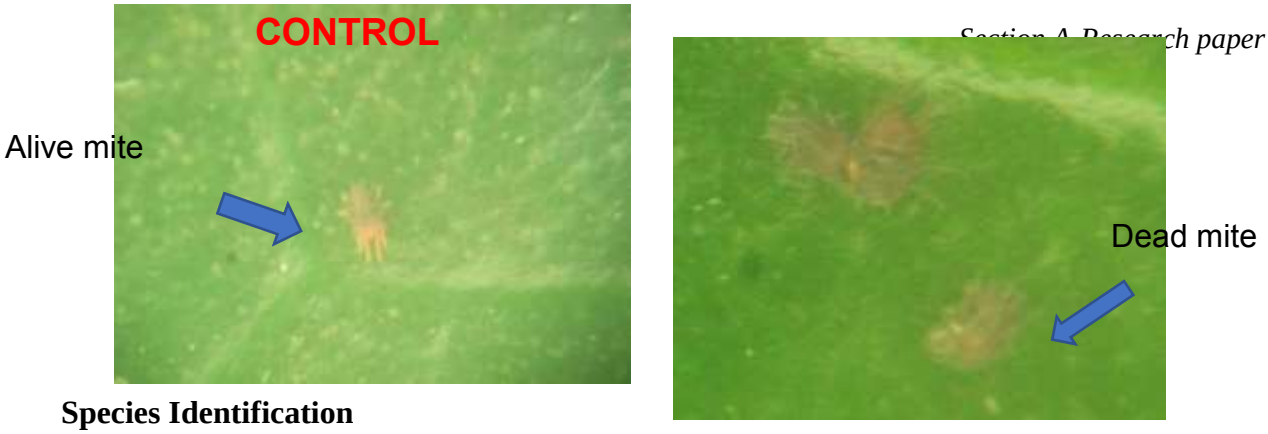


Screening of efficient actinobacteria

Based on acaricidal, Spore germination and antifungal activity of actinobacteria two strains were screened for Blister blight nursery trial. Error represents mean + - standard deviation (Fig. 8)

Fig. 8 Screening of actinobacteria

Effect of actinobacteria against *Pestalotiopsis* sp **Effect of actinobacteria against *Glomerella* sp**



Species Identification

The identification of the two actinobacteria strains, AAS7 and CAS4, using partial 16S rRNA gene sequencing is an important step in understanding their potential for use in controlling tea leaf diseases and red spider mites. The GenBank accession numbers provided in Table 1 will enable other researchers to access and use the sequences for future studies. The identification of these strains as belonging to the genus Streptomyces with at least 99% identity further confirms their potential as a rich reservoir for the production of biologically active compounds. Streptomyces is a well-known genus of actinobacteria that have been extensively studied for their ability to produce a wide range of bioactive compounds with antimicrobial, anticancer, and other pharmacological activities. This identification provides a foundation for further research on these strains to identify the specific compounds produced by them and their efficacy under field conditions, paving the way for their use as an eco-friendly alternative to conventional agrochemicals in tea production (Petti *et al*.,2005).

Table 4: Actinobacteria Isolates

S.No.	Isolates	*Species*	*NCBI accession No.*
2	AAS7	*Streptomyces flavogriseus*	*KM 06711*
6	CAS4	*Streptomyces albus*	*KM067121*

Evaluation of actinobacteria against blister blight disease under nursery condition

A Nursery trial was conducted to study the effect of actinobacteria against blister blight diseases in tea. The experiment was conducted in UPASI-9 clone. It had four treatments with three replications and each 10 nursery plants. In the standard treatment COC (210g) as per the recommended schedule were applied. In the actinobacteria formulations @ 7.5 ml/L per treatment were applied. The disease infection was recorded at 3rd day, 5th day,

7th day and 15th day interval. Among the two strains tested, application of CAS4 strain provided significant control of blister blight disease when compared to the standard recommended schedule (Chandra *et al*.,2014) (Table. 4 and Fig. 9).

The results of the nursery trial demonstrate the potential effectiveness of actinobacteria in controlling blister blight disease in tea. The experiment compared the efficacy of applying two actinobacteria strains, AAS7 and CAS4, to a standard treatment using COC as per recommended schedule. The disease infection was recorded at different intervals, and it was observed that the application of CAS4 provided significant control of blister blight disease compared to the standard treatment (Hazarika *et al*.,2022).

These findings are promising as it suggests that actinobacteria can be a viable and eco-friendly alternative to traditional agrochemicals in controlling tea leaf diseases. The use of actinobacteria can lead to a reduction in the use of agrochemicals, which can result in a safer and more sustainable tea production system. However, it is important to note that the effectiveness of these actinobacteria should be tested under field conditions before they can be adopted as a standard means of controlling tea leaf diseases (Mondal *et al*.,2022).

The study has its limitations as it was conducted under controlled nursery conditions, which may not reflect the same conditions as those found in the field. Therefore, further studies are necessary to determine the effectiveness of actinobacteria in the field conditions. Despite the limitations, the results of the nursery trial provide a promising foundation for further exploration of actinobacteria as a potential alternative to traditional agrochemicals in controlling tea leaf diseases (Al Hamad *et al*.,2021).

Table 5: Disease Incidence

	Disease Incidence in different days Intterval (%)			
Treatments	**3rd day**	**5th day**	**7th day**	**15th day**
Untreated control	96	89.2	84	82.4
Standard (COC 210g/h)	46.4	36	30.4	22.4
AAS7	78.4	73.2	65.2	60
CAS4	40	34.4	29.2	24

Fig 9: Experimental Plants for evaluation of actinobacteria against blister blight disease

Fig 10: CAS4 strain indicating sufficient control of blister blight disease

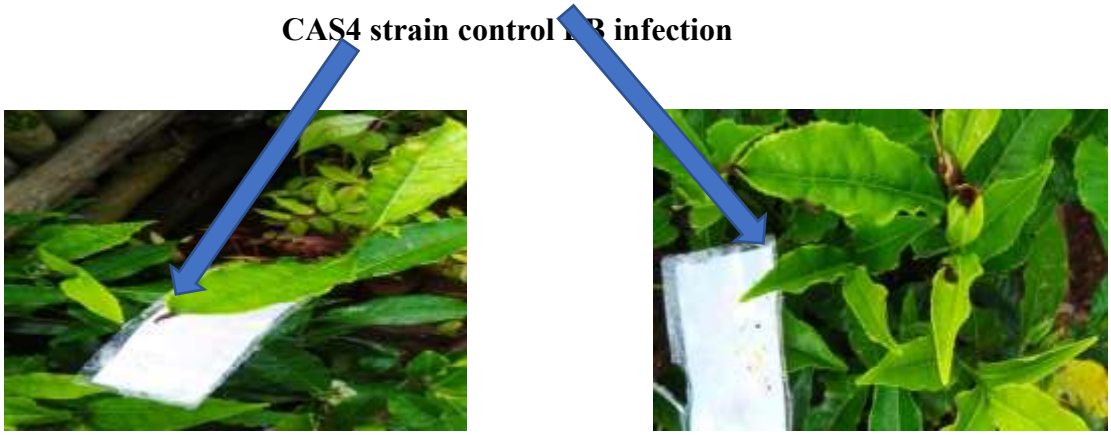
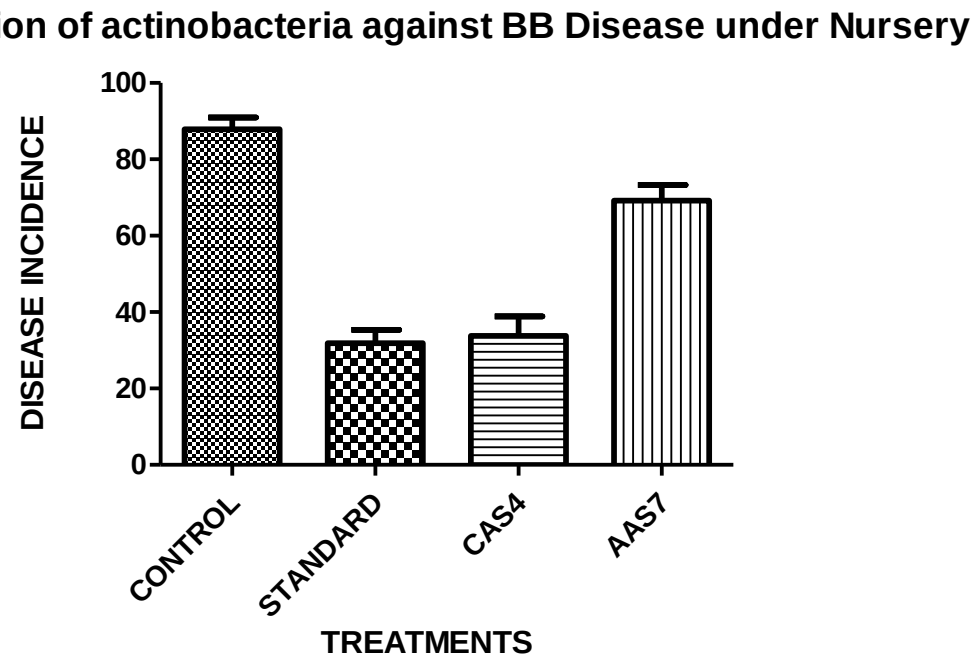


Fig11: Evaluation of actinobacteria against BB disease under nursery



Gas chromatography – mass spectroscopy (GC – MS) analysis of bioactive metabolite

The GC-mass chromatography analysis was performed to identify the active compounds in the intracellular extract of selected CAS4 strain (**Supplementary Table 1).**

Identification of Biologically Active Chemical Compounds in Streptomyces sp. CAS4 Strain by GC-MS Analysis: Potential Candidates for Control of Tea Leaf Diseases and Red Spider Mites is provided in Supplementary Table 2.

The study on the CAS4 strain isolated from tea-growing soil has identified the presence of several biologically active chemical compounds. GC-MS analysis of the strain

revealed the presence of several important chemical compounds, including Piperazinedione, and Pyrrolizidine. These compounds have been reported to exhibit antibacterial, antifungal, anti-inflammatory and antitumour and insecticidal properties, which makes them potential candidates for controlling tea leaf diseases and red spider mites (Anibogwu *et al*.,2021).

The identification of these biologically active chemical compounds in the CAS4 strain through GC-MS analysis provides an insight into the potential of actinobacteria as a source of eco-friendly alternatives for controlling tea leaf diseases and red spider mites. The use of such alternatives can reduce the dependence on agrochemicals, which has resulted in environmental pollution. Further studies are required to understand the efficacy of these compounds under field conditions and their potential for commercial production (Aggarwal *et al*.,2016).

Tea is a popular beverage consumed globally and is an important commercial crop. The production of tea faces several challenges due to various leaf diseases that affect tea plants. The most serious of these diseases is the blister blight, which is caused by the fungus *Exobasidium vexans Massee*. To control these diseases, agrochemicals are widely used, which often leads to environmental pollution. Therefore, there is a need to find eco-friendly alternatives to control tea leaf diseases and pests (Pandey *et al*.,2021).

In this study, actinobacteria were identified as potential eco-friendly alternatives to control tea leaf diseases and pests. Actinobacteria are a group of gram-positive bacteria commonly found in soil ecosystems, and they play a crucial role in maintaining soil health. The study identified two possible actinobacteria, AAS7 and CAS4, which were isolated from soil samples of tea-growing areas in India. Both actinobacteria exhibited antagonistic potential against tea pathogens and acaricidal activity against red spider mites under in vitro conditions (Silva *et al*.,2022).

The results of the study showed that both actinobacteria showed a high percentage of growth inhibition against foliar pathogens such as *Pestalotiopsis sp*. and *Glomerella* sp. CAS4 exhibited the maximum inhibition (90%) against Pestalotiopsis sp., while AAS7 showed the highest inhibitory effect (85.3%) against *Glomerella* sp. The culture filtrate of CAS4 was also found to be effective in inhibiting the spore germination of *Exobasidium vexans*, which is a significant breakthrough in the control of the most serious leaf disease affecting tea production (Kamat *et al*.,2011).

The study also found that both actinobacteria exhibited higher mortality rates for red spider mites, with AAS7 showing 100% mortality and CAS4 following closely. These findings suggest that actinobacteria isolated from tea-growing soil can be a rich reservoir for the production of biologically active compounds that can effectively control tea leaf diseases and red spider mites (Svinningen *et al*.,2010).

The use of actinobacteria as an eco-friendly alternative to control tea leaf diseases and pests has several advantages. Firstly, it reduces the use of agrochemicals, which in turn leads to a safer and sustainable tea production system. The use of agrochemicals has several

harmful effects on the environment, such as soil degradation, water pollution, and the destruction of beneficial soil microorganisms. The use of actinobacteria can help maintain soil health and reduce environmental pollution (Silva *et al*.,2022).

Secondly, the use of actinobacteria can help reduce the cost of production. Agrochemicals are expensive and require frequent application to control tea leaf diseases and pests. In contrast, actinobacteria can be easily cultured and are cost-effective. Therefore, the use of actinobacteria can help reduce the cost of production and increase the income of tea farmers (Sathya *et al*.,2017).

However, it is important to note that the effectiveness of these actinobacteria should be tested under field conditions before they can be adopted as a standard means of controlling tea leaf diseases and red spider mites. The study was conducted under in vitro conditions, which may not accurately represent the actual field conditions. Therefore, it is essential to conduct further research to assess the effectiveness of actinobacteria under field conditions (Liu *et al*.,2019).

In conclusion, actinobacteria isolated from tea-growing soil have the potential to be an effective eco-friendly alternative to control tea leaf diseases and pests. The use of actinobacteria can help reduce the use of agrochemicals, reduce environmental pollution, and increase the income of tea farmers. Further research is required to assess the effectiveness of actinobacteria under field conditions and to develop practical strategies for their application in tea production (Zhang *et al*.,2022).

Conclusion:

In conclusion, the study demonstrates the potential of actinobacteria as an eco-friendly alternative to control major leaf diseases and red spider mites in tea production. The identified actinobacteria, AAS7 and CAS4, showed high efficacy in inhibiting the growth of foliar pathogens and exhibited acaricidal activity against red spider mites under in vitro conditions. The culture filtrate of CAS4 also effectively inhibited the spore germination of the most serious leaf disease affecting tea production, Exobasidium vexans.

These findings highlight the potential of actinobacteria as a promising biological control agent for tea leaf diseases and red spider mites. The use of these eco-friendly alternatives can reduce the dependence on agrochemicals, which can result in environmental pollution, and promote sustainable tea production practices. However, it is important to further investigate the effectiveness of these actinobacteria under field conditions before they can be adopted as a standard means of controlling tea leaf diseases and red spider mites.

Overall, the study provides valuable insights into the potential use of actinobacteria in tea production, which can help in developing sustainable and environmentally friendly pest management strategies. Further research in this area can lead to the development of novel biological control agents that can be used in the tea industry and other agricultural crops.

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Conflicts of interest and financial disclosures:

There are no conflicts of interest to declare.

Supplementary Table 1 : Gas Chromatography- Mass Spectrometry (GC-MS) active conpounds

COMPOUNDS NAMES	RETENTION TIME	COMPOUND	FORMULA
Cpd 1: 2,5-Piperazinedione, 3-methyl-	24.084	2,5-Piperazinedione, 3-methyl-	C5H8N2O2
Cpd 2: 2-Pyrrolidinone, 5-(cyclohexylmethyl)-	25.545	2-Pyrrolidinone, 5-(cyclohexylmethyl)-	C11H19NO
Cpd 3: 3-Isopropyl-2,5-piperazine-dione	25.963	3-Isopropyl-2,5-piperazine-dione	C7H12N2O2
Cpd 4: 1,4-diazabicyclo[4.3.0]nonan-2,5-dione, 3-methyl	26.799	1,4-diazabicyclo[4.3.0]nonan-2,5-dione, 3-methyl	C8H12N2O2
Cpd 5: 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	27.234	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	C13H40O5Si6
Cpd 6: Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	27.645	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	C7H10N2O2
Cpd 7: 3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	29.004	3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	C14H24O3Si2
Cpd 8: Tetracosamethyl-cyclododecasiloxane	29.505	Tetracosamethyl-cyclododecasiloxane	C24H72O12Si12
Cpd 9: Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	30.039	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	C11H18N2O2
Cpd 10: N-(3-(2-Oxopyrrolidin-1-yl)propyl)acetamide	30.33	N-(3-(2-Oxopyrrolidin-1-yl)propyl)acetamide	C9H16N2O2
Cpd 11: 2-(Dimethylamino)-3-methyl-1-butene	30.563	2-(Dimethylamino)-3-methyl-1-butene	C7H15N
Cpd 12: 3-Methylsalicylic acid, 2TMS derivative	30.814	3-Methylsalicylic acid, 2TMS derivative	C14H24O3Si2
Cpd 13: 2-Azacyclooctanone	30.949	2-Azacyclooctanone	C7H13NO
Cpd 14: Hexasiloxane, tetradecamethyl-	31.214	Hexasiloxane, tetradecamethyl-	C14H42O5Si6
Cpd 15: 3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	32.291	3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	C14H24O3Si2
Cpd 16: 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	32.64	2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	C12H22N2O2
Cpd 17: Butyramide, N-allyl-N-hexyl-2-bromo-	32.986	Butyramide, N-allyl-N-hexyl-2-bromo-	C13H24BrNO
Cpd 18: Disiloxane, 1,1,3,3-tetramethyl-1,3-bis(4-methylphenyl)-	33.583	Disiloxane, 1,1,3,3-tetramethyl-1,3-bis(4-methylphenyl)-	C18H26OSi2
Cpd 19: Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-	33.645	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-	C12H25B2NO2
Cpd 20: Tetracosamethyl-cyclododecasiloxane	33.907	Tetracosamethyl-cyclododecasiloxane	C24H72O12Si12
Cpd 21: 3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	34.805	3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	C14H24O3Si2

Cpd 22: Heptasiloxane, hexadecamethyl-	35.169	Heptasiloxane, hexadecamethyl-	C16H48O6Si7
Cpd 23: Cyclononasiloxane, octadecamethyl-	36.537	Cyclononasiloxane, octadecamethyl-	C18H54O9Si9
Cpd 24: Cyclononasiloxane, octadecamethyl-	38.113	Cyclononasiloxane, octadecamethyl-	C18H54O9Si9
Cpd 25: Heptasiloxane, hexadecamethyl-	40.007	Heptasiloxane, hexadecamethyl-	C16H48O6Si7

Supplementary Table 2: Important Chemical compounds detected in CAS4 Strain by GC-MS analysis. RT is retention time.

S.No	Name of the compound	RT (min)	Formulla	Nature of compound	Activity
1	2,5-Piperazinedione, 3-methyl-	24.084	C5H8N2O2	Piperazinedione	antibacterial and antifungal activity
2	3-Isopropyl-2,5-piperazine-dione	25.963	C7H12N2O2	Piperazinedione	Antifungal activity
3	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	27.645	C7H10N2O2	Pyrrolizidine	antibiotics, antitumour, antifungal, anti-inflammatory, antioxidant activity
4	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	30.035	C11H18N2O2	Pyrrolizidine	antioxidant activity, acaricidal activity
5	N-(3-(2-Oxopyrrolidin-1-yl) propyl) acetamide	30.33	C9H16N2O2	acetamide	No activity reported
6	2-(Dimethylamino)-3-methyl-1-butene	30.563	C7H15N	Butene	No activity reported
7	3-Methylsalicylic acid, 2TMS derivative	30.814	C14H24O3Si2	Piperazine	No activity reported
8	2-Azacyclooctanone	30.949	C7H13NO	Azacyclooctanone	No activity reported
9	Hexasiloxane, tetradecamethyl-	31.214	C14H42O5Si6	Hexasiloxane	No activity reported
10	3-Methyl-2-(trimethylsilyl)oxybenzoic acidtrimethylsilyl ester	32.291	C14H24O3Si2	Ester	No activity reported
11	: 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-	32.632	C12H22N2O2	Piperazine	Antifungal activity

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