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Study on the Effect of Some Biocontrol Agent in Grey Blight of Tea

Dhritiman Chanda*[1] and Mursida Khatun[2]

[1]Department of Botany, University of Science and Technology, Meghalaya 793 101, India
[2]Department of Botany, West Goalpara College, Assam, India

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ABSTRACT

Tea plantation is covered by 434,000 hectares of land, which is further divided into two distinct regions - the North Indian tea belt and the South Indian tea belt. North-East India produces 75% of the total Indian tea. Present study is carried out to understand the various agro-climatic conditions of Bramhaputra valley like temperature, humidity, rainfall etc which are responsible for occurrence of various diseases in tea plant. The bio control agents are eco-friendly to control the plant diseases with aim of developing a sustainable system in agriculture. It involves in the mechanism of interaction among the antagonists and pathogens, which aid in selection and manipulation to develop an effective control system. It provides protection to the plant throughout its cultivation period and proliferates rapidly in soil and leaves no residue. Tea plants are challenged by several root, stem and foliar diseases. Fungi possess a number of characteristics that make them potentially ideal bio control agents. They can germinate to grow and control the target population there by making continual re-inoculation with the bio control agent. The preliminary investigation reflects the growth of *Pstalotiopsis theae*is controlled by the isolated bacteria which could be a great potential biocontrol agent for the control of various diseases of tea in this region.

Key words*: Biocontrol agents, Grey blight, Foliar diseases*, Bacillus *sp.*

Introduction

Tea is a dicotyledonous perennial plant. Tea is a plantation crop. Tea belonging to the family Theaceae. The scientific name of tea is *Camellia sinensis*. Tea is a species of evergreen shrubs. The leaves and leaf buds are used to produce tea. An evergreen shrub to small tree that can only be grown where frost totally absent and where the temperature never gets high. The most widely used non-alcoholic beverages around the world, or certainly a close second to coffee. It is the most consumed drink other than water.

Tea plants grows up to 15m tall under natural conditions but is maintained at waist height (1.0 to 1.5) in commercial plantations. The small evergreen tree to 16 tall usually cut to shrubs in cultivation, with strong tap root giving rise to surface mat of feeder roots with endortrophic mycorhizae. Leaves alternate longitudinal up to 30 cm long, 2-5cm broad, sometimes becoming glabrous, serrate, acuminate. Tea is propagated from seed, but high yielding clonal material has also become popular. The plants are initially raised in nurseries to produce healthy plants. Twelve to eighteen months. Old nursery seedlings are used for planting in the field. The vegetative propagation of selected tea plant cuttings, each with a leaf and an internode has been standardized (Sarmah *et al.,* 2022)

One of the major climatic factors responsible for

([1] Professor, [2]Assistant Professor)

agricultural production is temperature(both maximum and minimum). According to IPCC 2007 the global average temperature has gone up by about 0.130C per decade since 1950. The study suggests that warming trends in the Himalayan region and nearby is significant, generally higher compared to global averagen (Pandey *et al.,* 2021). The study also suggests that the rate of warming is most often increases with increased elevation (Sharma *et al.,* 2009).

The climate of Assam is favourable for tea plantation, the climatic changes effect more or less on its production. Not only one factor several factors affect variously on the production. Rainfall is one of the most effective factors for the growth of tea plant. Tea plants are challenged by several root, stem and foliar diseases. Foliar disease, blister blight, gray blight and brown blight are particularly important as they adversely affect the bud and two youngest leaves, causing loss of harvestable shoots (Jagessar *et al*., 2020; and Pandey *et al.,* 2021). Over the past several decades, climate change and field management practices have influenced the risk of crop damage by several fungal pathogens, as well as the development and spread of diseases.

Biocontrol has been proposed as a form of pest management. This strategy provides a sustainable method to control plant pests by using natural enemies (example, predators, parasitoids, and pathogens, competitors) to reduce the population of harmful target species (Bahadur *et al.*, 2022). Fungi in particular have been identified as an attractive bio control as they have high reproductive rates, short regeneration times and the ability to survive in the environment during the lean season. Biological control is regarded as a promising alternative and a wide array of microbial biocontrol agents (BCA) have been developed in the pest decades for the management of fungal and bacterial diseases (Priyashantha *et al.,* 2022; Larena *et al*., 2023). Some of the most intensively studied are bacteria belonging of the genus *Pseudomonas* spp., *Bacillus* spp., and *Streptomyces* spp. that have been already registered as commercial products and marketed. The efficacy of a bacterial bio control agent against plant diseases depends on the microbial agent, plant pathogens targets, host and environmental conditions (Jensen *et al*., 2016).

Biocontrol agents for plant disease are gaining stature as viable alternatives to synthetic pesticides given their perceived increased level of safety and minimal negative environmental impacts. It is imperative to continue this line of research, since regulations on the use of new and existing fungicides are becoming more and more stringent (Thabah *et al*., 2022; Nartey *et al.,* 2022. In particular this has to lead extensive researches on the use of microbial antagonists as protective agents and many fungal diseases can now be controlled by microbial antagonists. As a result commercial products containing microbial BCAs have been successfully exploited in modern agriculture. Examples like *Trichoderma* based products and biopesticides based on *Bacillus thuringiensis (*Dhar *et al*., 2018; Bastakoti *et al.,* 2017; Din *et al*., 2019; Babu *et al.,* 2022; Jaffar *et al*., 2023

Materials and Methods

Study Site

The study was conducted in Sonapur Tea Estate, located in the Kamrup District of Assam. The Sonapur is a village in the Dimoria block of Kamrup Metropolitan District in the Indian state of Assam. Sonapur is situated at National Highway 37 on the river Diguru, which flows into the Kalang River close to its embouchure into the Brahmaputra. The place is located between Latitude is 26°07′12.00″N and the Longitude is 91°58′48.00″E. The size of the area is about 3.88 square kilometer. Samples were collected during summer season which took place in the month of Jun-July 2023. All collections took place on Saturday and samples were collected between 10.00 am from Digaru Tea Estate, Sonapur. Samples were handled aseptically and were assigned serial numbers. Samples were kept in the sterile bags and at a storage temperature approximating that of the garden until transferred to the laboratory the next day (Fig. 1).

Isolation of grey blight fungi (*Pestalotiosis theae)*

Tea leaves with symptom spots of tea gray blight were cut into two 4-mm pieces, surface-sterilized with 75% alcohol for 1 min, washed with sterile water, disinfected with 1% sodium hypochlorite for 3 min, and finally washed with sterile water three times. Small pieces were placed on potato dextrose agar (PDA) plates containing 100 μg ml^{-1} chloramphenicol. After 3 days of dark culture at 28 °C, single mycelia were selected and transferred to PDA plates for dark culture at 28 °C for 7-10 days. The isolated and purified strains were identified using morpho-

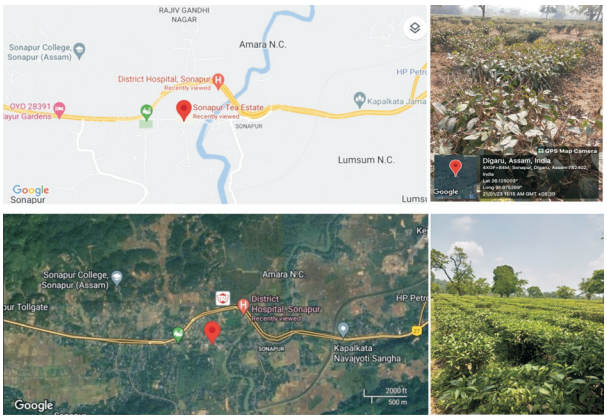


Fig. 1. Study Site (Courtesy: Google source)

logical characteristics like the size (length and width) of conidia, intermediate cells, and apical and basal appendages were measured, and the color of the conidia and the number of apical and basal appendages were observed under a light microscope.

Isolation of bacteria from Tea soil

The serial dilution technique is followed for the isolation and enumeration of bacteria from the tea plant affected with grey blight diseases in nutrient agar (NA) media by following the techniques of Blodgett (2010). The average c.f.u was also estimated and pure culture was carried out for the selected isolate no 1. The morphological and bio chemical characterization were also recorded for the bacterial isolate 1. Gram staining also carried out for morphological and characterization for the bacterial isolate 1(Bartholomew and Mittwer, 1952). Pure culture of isolated bacterial strains were done and biochemical tests i.e., Urease test, H_2S production, starch hydrolysis and catalase test (Table 1).

Identification of Tea Disease

Grey blight disease of tea caused by *Pestalotiopsis thease* has been reported from all tea growing countries of the world. Though the disease appears on bare stalk and young shoots as well, the disease generally affects mature leaves. After the extensive mechanized harvesting had been brought into practice, this disease gained more importance. The mechanized harvesting practice weakened the bushes and also provided sites for infection by this pathogen. Tea being a leafy crop, any damage to the leaf will result in yield and also affect the quality of made tea. A large number of plant diseases have been controlled through bacterial and fungal antagonists. Interaction between bio control agents and plant pathogens has been studied extensively and the application of bio control agents to protect some commercially important crops is promising. Biological control using microorganisms can be economic, self-perpetuating and usually free from residual side effects

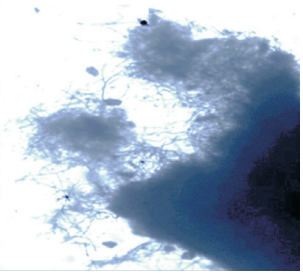
Symptoms

Small, oval, pale yellow-green spots first appear on young leaves. Often the spots are surrounded by a narrow, yellow zone. As the spots grow and turn brown or gray, concentric rings with scattered, tiny black dots become visible and eventually the dried tissue falls, leading to defoliation. Leaves of any age can be affected. The tiny, black spots on the lesions contain the fungal spores. Rain splash transports the spores from one plant or site of infection to another. If the spores land on a leaf, they germinate to start a new leaf spot or a latentinfection.

Results and Discussion

Isolated bacteria from the soil shows positive result against urease test, starch hydrolysis, catalase test and gram positive rod shaped (Table 1). The bio control activity using the isolate shows positive result as the fungal colony growth is found to be decreased from 4.5 cm to 2.1 cm. This preliminary investigation shows effective growth on the fungal growth in the presence of bacteria which is isolated from the

Grey Blight of tea caused by *Pstalotiopsis theae*



Microscopic observation of *Pstalotiopsis theae*

Table 1. Biochemical characterization of bacterial isolates 1

Biochemical tests	Bacterial Isolate 1 (*Bacillus* sp)
Gram staining	Gram positive (rod shaped)
Urease test	+
Starch hydrolysis	+
Catalase test	+
H_2S production	+

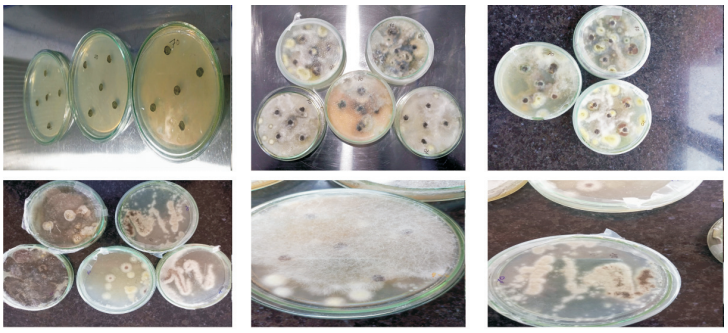


Fig: Pure culture

Fig: Fungal growth (4.5cm)

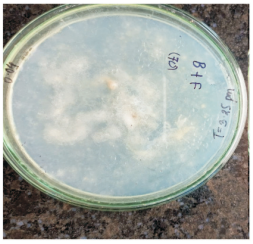


Fig: Fungi + Bacteria(2.1cm)

Photopoplate 1. Biological control of *Pstalotiopsis theae* by the bacterial isolate (*Bacillus* sp.)

rhizospheric soil of the same tea garden. The isolated bacterial strain and fungal was screened for their antagonistic potential against the pathogen, following dual culture technique (Dheeman *et al*., 2023; Pandey *et al*., 2023; Afsan *et al*., 2023). The bacterial strain 1 positive test against the growth of *Pstalotiopsis theae*. A little growth was observed in 24 hours and in 48 hours the growth is also found stable (Photoplate 1). *In vitro* antagonism with dominant bacteria in rhizospheric microflora of *Camellia sinensis* also reported by Priyashantha *et al.* (2023). The significant role of bioformulations for promoting the growth of tea by *Bacillus megatrium* and *Serratia marcescens* also discussed by Chakraborty *et al.* (2012). Jaffar *et al*. (2023) also studied that alternative to chemical agents, biocontrol agents are employed to manage phytopathogens of Tea. Din *et al*. (2019) also reported that application of the inoculum of *Bacillus* sp. Used to provide better protection against the multiple diseases in tomato plant. The antagonistic potential of biocontrol agents against tea pathogens like *Hypoxylon* sp. and *Pestalotiopsis* sp. were also tested to exhibit superior antagonistic potential against the grey blight of tea (Pandey *et al*., 2021; Nistal *et al.,* 2023; Xu *et al*., 2023; Dheeman *et al.,* 2023). Kakoti *et al.* (2023) also reported the beneficial role of bio control agents in tea cultivation for produce long term results without disrupting natural equilibrium.

Conclusion

The present study helps us to suggest the control measures for maintenance of the plants growth under biological method by using bio control agents for generating organic tea production. This study generates the awareness of using bio control agents in tea. Thus, the present study shows the potential use of native bioinoculants to provide protection against horse hair blight to enhance the growth of tea bushes and to reduce the use of pesticide and chemical fertilizer. Our preliminary investigations provided a key concept to use the inoculums of bacterial isolate as bio control equipment in the tea gardens of Sonapur, Kamrup district to get control over grey blight disease of tea. Thus, the transition to consumption of organic tea would become commercially acceptable at a large scale and will increase the acceptability of organic farming of tea. Molecular characterization is also necessary of the bacterial isolate for further detail investigation on the control of grey blight disease of tea and thereby to suggest the future preventive measures for the control of tea disease.

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Conflict of Interest- None

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