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Isolation and Identification of Indigenous Bacteria for the Control of Horse Hair Blight disease of tea

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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 Barak valley like temperature, humidity, rainfall etc which are responsible for occurrence of various diseases in tea plant. Six genera of fungi were isolated and identified as *Aspergillus niger, Trichoderma* sp., *Penicillium* sp., *Cladosporium* sp., *Helminthosporium* sp. and *Fusarium*. sp. Percentage of relative abundance of all the isolated fungi also estimated and it is found that *Aspergillus niger* shows high percentage of relative abundance (30%), followed by *Trichoderma* sp. (22%), *Penicillium* sp. (12%), *Cladosporium* sp.(18%), *Helminthosporium* sp. (8%) and *Fusarium* sp.(10%). The isolated bacterial strain (*Bacillus* sp) screened for their antagonistic potential against the pathogen *Marasmius equicrinis* by dual culture technique. The study indicated that biocontrol agents (*Bacillus* sp.) are very effective to control the horse hair blight disease in tea plant under *in vitro* conditions. Our preliminary investigations provided a key concept to use the inoculums of *Bacillus* sp as biocontrol equipment in the tea gardens of N.E. region to get control over horse hair blight disease of tea.

Key words: *Relative abundance, Biocontrol agents, Horse hair blight, Bacillus etc.*

Introduction

Tea plant cultivation is usually done in large commercial operations. Tea is a very demanding beverage all over the world. India is one of the largest teas producing country in the world. From Assam and Darjeeling in east to Tamil Nadu, Kerala and Karnataka in south, the production of Tea is spread and is used for exportation in various countries, resulting in economic growth. So it becomes utmost concern of Government to take care of quality and quantity of production as it will affect the revenue (Ahmed *et al.,* 2018). There are various types of diseases found in tea plant depends on causing source such: Camellia dieback and canker, Camellia flower blight, Root rot, Algal leaf spot, Blister blight, Horse hair blight, Poria root disease (Keith and Sato, 2006). The tea plant is subject to attack from at least 380 fungus diseases. In northeast India alone 190 fungi have been detected. It has been found that 67 million pounds (30 million kg) of tea per annum is lost due to this diseases (Jha *et al*., 2016).

Brahmaputra Valley of Assam which is located at 100 MSL and Barak Valley of Assam is situated 40 MSL) and are considered as the major popular tea growing regions in India. The soil textures of these areas vary from sand to heavy clay type. Annual rainfall of this region is approximately 3000-5000

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mm, though this distribution is uneven and severe-winter droughts generally occurs.

Horse Hair Blight Taxonomy and Descripction

Horse hair blight is caused by *Marasmius equicrinis* Muell., an epiphytic fungus (Division:- Basidiomycota, Family: Marasmiaceae) which appears on leaves of tea bushes as a wiry stipe with a delicate fruiting body. The cap of the fruiting body can be up to 4 mm in diameter, and is pale brown. Black fungal thread resembles like a horse hair which are attached to the upper branches and twigs with small brown disc. The fungi penetrate and infect the twig through the disc and produces volatile substance that causes the rapid leaf drop. Soon after the infection, the pathogen is spread to the healthy twigs by the production of hair like thread which is extended to all the leaf and steam parts.

Description : Pileus 0.15-0.6 cm, convex, pale brown to pale orange colored, glabrous, margin striate. Gills free, distant, white, smooth. Stipe central, 0.3-3 × 0.02 cm, hollow, black, globrous, coriaceous, with long black rhizomorph. Spores fusiform, hyaline, smooth, I-, 12-17 × 4-5 µm. Epidermial cells irregularly branched. Hyphae with clamps.The hair-like rhizomorphs of this species strangle on tea plant, and cause tea-hair disease in tea orchard. Under the hot and humid conditions proliferates in a habitat like tea bush canopies and can cause heavy physical hindrance in major operations of tea as plucking and pruning.

Present study is carried out to understand the various agro-climatic conditions of Barak valley like temperature, humidity, rainfall etc which are responsible for occurrence of various diseases in tea plant. All the optimum climatic condition required are examined with the data obtained. This data is relating that information to pin out causal for the prevalence of Horse hair blight which is of high occurrence in tea estate of Barak Valley. The current study was also attempted to identify the soil micro flora of tea estate soil.

Damodaran *et al.,* (2019) reported the beneficial role of soil microbial community to increase the tea plant growth by breaking down organic and inorganic substance, releasing enzymes. Lie *et al*., (2017) and Dutta and Thakur (2017) studied the micro flora abundance and diversity plant growth promoting rhizobacteria and their antagonistic potential associated with commercial tea plants.Dutta and Thakur, (2021) studied the diversity of culturable bacteria with antifungal metabolites biosynthetic characteristics associated with the rhizospheric soil of tea. Buckley *et al.* (2006) studied the diversity of Planctomycetes in tea soil necessary for the improvement of soil nutrients. Pandey *et al.,* (2013) reported the microbial inoculants to support tea industry for the production of organic tea. Zang *et al.,* (2021) also studied response of soil nutrients and microbial community to develop the intercropping measures for improving microbial structural diversity of tea plantation. Article, (2013) also reported the positive interactions of the intercropping system with Rhizosphere microbial communities with the tea plant production. Gurusubramaniam *et al.,* (2005) worked on integrated pest management of tea and explained the soil microbes to increase the soil mineral nutrients available for host plant.

Isolation of *Marasmius crinisequi*

At first, Horse hair blight samples were taken out from the refrigerator. PDA media was prepared by mixing 9.85 gm of PDA in 250 ml of distilled water in a conical flask (ploughed tightly by cotton) then properly sterilizing them in autoclave at a temperature of 121^{O}C at pressure of 15 pound. Petri dishes were similarly sterilized in Hot Air Oven. After the conical flask containing PDA is cooled enough and the antibiotic (amoxicillin) is mixed with it. After the solidification of media, the Horse hair blight samples were cut into small pieces by sterilized scissor. These samples were inoculated in PDA media using a sterilized forceps. The plates were inocubated at 28 ^{o}C for 48 hours. The isolated colonies are selected and pure cultures were prepared for further experiment.

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 horse hair 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 biochemical characterization were also recorded for the bacterial isolate 1 (Cheesbrough, 1991). Gram staining also carried out for morphological and characterization for the bacterial isolate 1(Bartholomew and Mittwer, 1952).

Results and Discussion

Metrological data of tea garden is also observed and recorded for the different parameters like, Relative humidity (Max/min), Temperature ((Max/min) and average Rainfall. The all parameters have shown a significant increase in the month of July followed by August and May. The different parameters of studied tea garden is mentioned in the Table 1. The average Maximum temperature was recorded 35 °C in the month of July and the minimum average temperature was recorded 10 °C in the month of January. The average Maximum relative humidity was recorded 94% in the month of July and the minimum average relative humidity was recorded as 38⁰C in the month of January. The average rainfall was also recorded 225 mm in the month of July and the minimum average rainfall was recorded as 24 mm in the month of January (Figure 1).

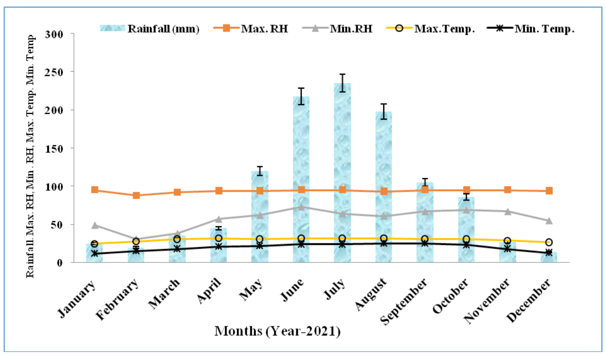


Fig. 1. Metrological data of the study area of Tea Garden for the year 2021

Six genera of fungi were isolated and identified as *Aspergillus niger*, *Trichoderma* sp., *Penicillium* sp., *Cladosporium* sp., *Helminthosporium* sp. and *Fusarium*. sp. Percentage of relative abundance of all the isolated fungi also estimated and it is found that *Aspergillus niger* shows high percentage of relative abundance (30%), followed by *Trichoderma* sp. (22%), *Penicillium* sp. (12%), *Cladosporium* sp.(18%), *Helminthosporium* sp. (8%) and *Fusarium* sp.(10%) (Figure: 2).

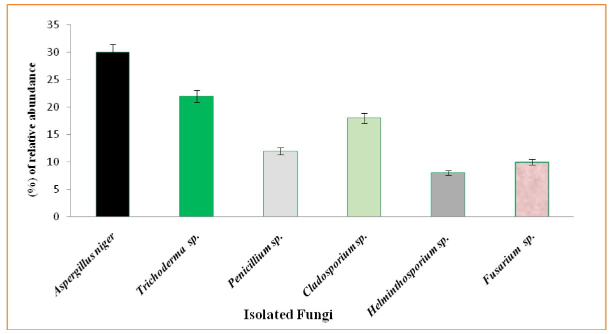


Fig. 2. Relative abundance (%) of isolated fungi from the composite soil sample

The rhizospheric bacteria are also isolated from the tea soil on nutrient agar plate. Pure culture of isolated bacterial strains were done and biochemical tests. i.e., Urease , H_2S production, starch hydrolysis and catalase test were done. The bacterial strain showed positive against H_2S production, starch hydrolysis and catalase test and negative against Urease test. Carbohydrate fermentation test shows positive against glucose, sucrose, xylose and lactose for the isolate. The isolate was found to be multi-antibiotic susceptible to Polymyxin B, Streptomycin and Tetracycline and showed no inhibition against Penicillin antibiotic (Table 2).

The isolated bacterial strains were observed to be gram (+)ve due to the glycoproteins present on the outer site of Gram positive bacterial cells which

Table 1. Various Parameters of Experimented Tea Garden

Different parameters of Tea Garden	
Total Are Under Cultivation	432 Hectares (ha)
Type of Bushes	Seed (upper Assam stock, 402 and 462) and clone (mainly TV9, TV23, TV22)
Ages of Tea Bushes	0-70 years (recent planting -3 year ago)
Diseases of The Leaf, Stem and Root	Red rust, Blister blight, Canker (poria), Horse hair blight, Thread blight, Violet root rot, Brown root rot, Charcoal root rot, Brown blight, Gray blight, Black rot
Production	7 lakhs kg annually
Shade Trees	Neem Tree, *Odoratisama*, *Labac, Derris robusta* etc.
Soil Condition	Carbon-1-1.5%; Potassium- 40 to 200 ppm; Phosphorus-15 to 30 ppm; Sulphur-30 to 40 ppm
pH	4.2 to 5.5

Table 2. Biochemical test and identification of bacterial isolate (*Bacillus* sp 1)

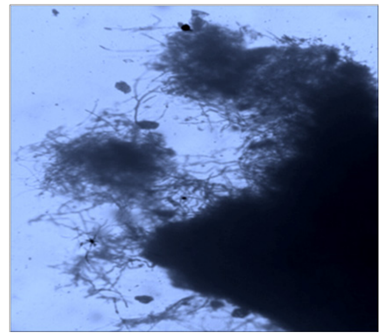
Biochemical test	Isolate (*Bacillus* sp1)
Gram staining	(+)ve; (rod shaped)
Urease Test	-
H_2S production	+
Starch hydrolysis	+
Catalase Test	+
Glucose	+
sucrose	+
Xylose	+
lactose	+
Penicilliin	No inhibition
Polymyxin B	11(I)
Streptomycin	32 (S)
Tetracycline	26(S)

NI=No Inhibition, Diameter of disc =6mm
Letter in parenthesis indicate sensitivity; R = Resistant; I = Intermediate; S = Susceptible.

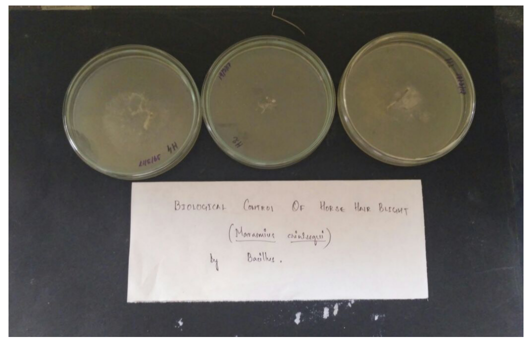
have more potential binding sites than the Gram negative bacteria (Gupta *et al*., 2012; Issazadeh *et al*., 2013). The isolates were showed positive activity Urease, H_2S production, starch hydrolysis and catalase test. The premilinary results of all chemotaxonomic characters revealed that the isolated strains could be *Bacillus* sp. (Kacar and Kocyigit, 2013; Elsilk *et al*., 2014).

The isolated bacterial strain and *Marasmius* strainwere screened for their antagonistic potential against the pathogen, following dual culture technique (Ahmed *et al.,* 2018). The bacterial strain (*Bacillus* sp.) positive test against the growth of *Marasmius.* A little growth was observed in 24 hours and in 48 hours the growth is also found stable (Plate 1). Trivedi *et al.,* (2008) also found that application of *Bacillus* sp. in tea plants could increase the rate of plant growth. *In vitro* antagonism with dominant bacteria in rhizospheric microflora of *Camellia sinensis* also reported by Singh *et al.,* (2008). The significant role of bioformulations for promoting the growth of tea by *Bacillus megatrium* and *Serratia marcescens* also discussed by Chakraborty *et al.,* (2012). Silva *et al.,* (2004) 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 and wood rot pathogens where three strains of *Bacillus*

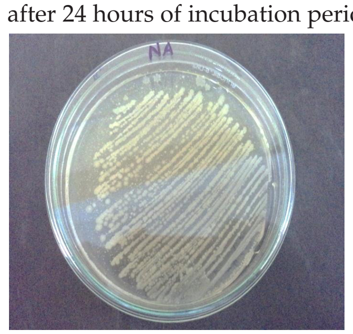


Hyphal growth on leaves by *Marasmius crinisequi*

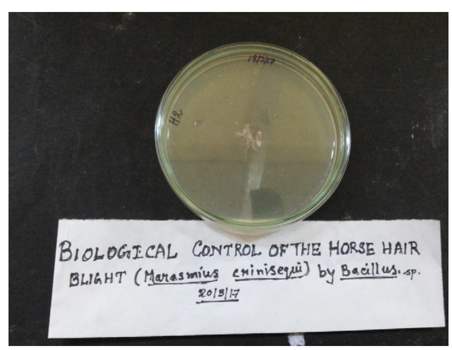


Biological Control of Horse Hair blight (*Marasmius crinisequi)* by (after 24 hours) *Bacillus* sp. (After 24 hours)

Plate 1. Biological Control *Marasmius crinisequi* by *Bacillus* sp. after 24 hours of incubation period



Pure culture of isolated bacteria from rhizospheric soil of tea



Biological Control of Horse Hair blight (*Marasmius crinisequi)* by*Bacillus* sp. (after 48 hours)

Plate 2. Biological Control *Marasmius crinisequi* by *Bacillus* sp. after 48 hours of incubation period

and *Pseudomonas* that showed higher antagonism against branch canker pathogen (Mareeswaran *et al*., 2015).

Conclusion

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 *Bacillus* sp as biocontrol equipment in the tea gardens of Barak valley to get control over horse hair 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.

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