Short Research Article

The Prevalence of Leaf Blight Diseases of Tea (*Camellia sinensis*) In Hobigonj and Moulvibazar District: Botryodiplodia *theobormae* an Important Obstacle

Abstract

Among soft and non-alcoholic beverages, tea is the most popular beverage worldwide. Tea is the daily favourite drink of low-income Bangladeshi people. The tea industry market in Bangladesh is quite large and active. Especially Habiganj and Moulvibazar districts of Sylhet division are a big source of the tea industry. But every year in this Habiganj-Moulvibazar tea region there is huge loss in the tea industry due to various diseases, especially fungal diseases. This affects the people who depend on this industry for their livelihood. Tea leaf blight disease is one of the major causes of loss in the tea industry in this tea region.The main objective of my research was to identify the fungal pathogen that causes blight of tea leaves and and to determine the prevalence rate of this disease, diseased tea leaves were collected from two tea gardens of Madhabpur upazila of Habiganj district several times and The data on the prevalence of tea leaf blight disease was taken from 50 units of 19 tea plantations in 2 districts of Habiganj and Moulvibazar districts. How much area of tea leaf is affected, how many affected areas are there in the leaves, how much is affected in new tea leaves or old leaves, the color of the affected area, whether the entire tea leaf is curled, all these data are collected from 50 units of 10 tea gardens.

First at the Moist chamber and then cultured or isolated in PDA media at specific temperature and systematically. Pathogen growth was monitored in them regularly. After a certain period of time, slides were prepared from special parts of the fungus and observed under an electron microscope, a fungal organism clearly identified this was Botryodiplodia theobromae. Data was collected to determine the prevalence rate of blight disease .By analysing the data which we collected from 10 tea gardens regarding the spread of tea leaf blight disease, we came to know the rate of spread of the disease.

Such research on tea leaf blight disease can play a helpful role in disease identification, rate determination and expansion of Tea industry in Habiganj-Moulvibazar tea region.

Keywords

Botryodiplodia theobromae; *Camellia sinensis*; Leaf Blight Disease

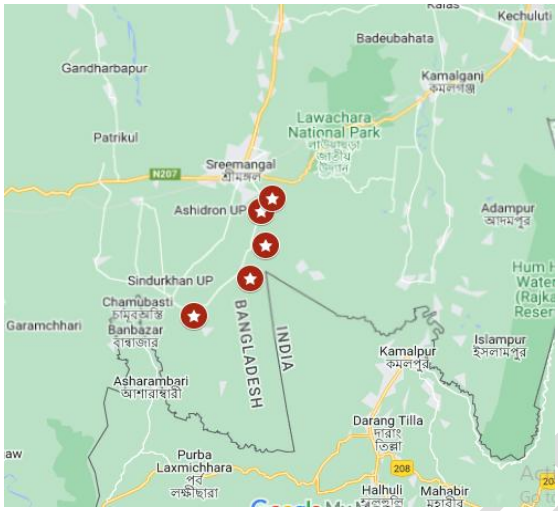
Introduction

Tea is the second largest cash crop in Bangladesh. Currently there are 167 tea gardens in Bangladesh (Tea journal of Bangladesh,
2019) and Tea Gardens on 2,79,507.88 acres of land in Bangladesh (Businessinspection.com.bd,2022), Some of these gardens are counted among the largest tea gardens in the world ("Tea Gardens in Bangladesh" bangladesh.com. Retrieved 24 March 2015.) The Tea industry of Bangladesh accounts for 3% of the world's tea industry (Sajjad Hossan March 26, 2022). More than 40 million people depend on this industry for their livelihood (Tea Scribd.com,15 March 2015). High altitude, warm-humid climate and high rainfall areas (FAO,2016) are ideal for good quality tea. But every year due to various diseases of tea, this industry is not able to play a more active role in the national economy (Pandey et al., 2021). As a result, the country, workers and companies are suffering a lot. If the diseases of tea plants or leaves can be identified or if a disease is spread by any pathogen and those pathogen can be identified and controlled by biological or artificial means, the production of tea can be increased.There is a close relationship between the climate and the environment with the spread of disease germs.Tea leaf blight disease is one of the various diseases of tea leaves or plants.There is 91 tea gardens in Moulvibazar district and 25 tea gardens in Habiganj district(bdnews24.com,2021).Every year in the Habiganj-Moulvibazar region this disease causes considerable loss to the tea industry, which then affects the life and livelihood of the tea workers. At certain times of the year, the germs are spread with the help of different media.If the tea leaf blight disease and its germs were identified in this region, the spread of the disease could have been prevented. For this, it is also important to know the incidence rate of this disease in tea gardens at a specific time. *Diplobotridium theobromae*, the fungal pathogen of tea leaf blight, has been identified in the laboratory, which is one of the major constraints to tea production in Habiganj-Moulvibazar district. By observing the various changes (colour, texture, shape) of the leaves affected by this organism (Diplobotridium theobromae), diseased tea leaves of 50 units of 10 tea plantations in these 2 districts were observed and the prevalence and spread rate of the disease was determined.Therefore research on this subject can lead to the development of the tea industry in this region.

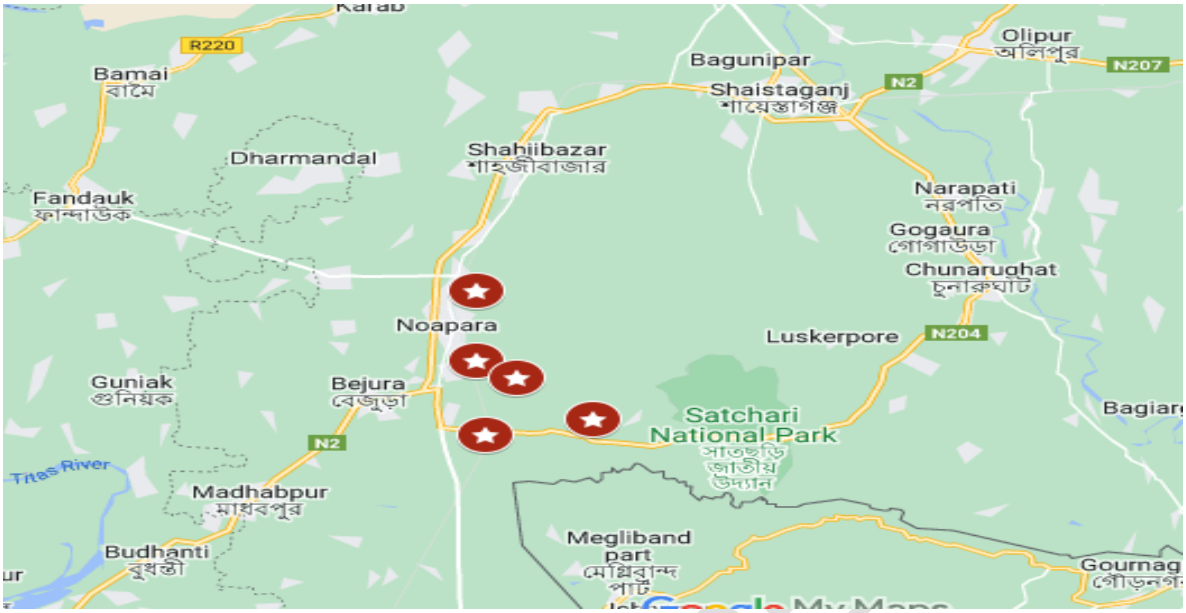
Materials and Methods

1.1. Study area - Study area was the North-east region of Bangladesh,Habiganj and Moulvibazar district. Out of 10 tea gardens, 5 are located in Sreemangal upazila of Moulvibazar district and remaining 5 tea gardens are located in Madhabpur upazila of Habiganj district. The 5 tea gardens of Habiganj are Noapara tea garden, Jagdishpur tea garden, Baikunthpur tea garden, Teliapara tea garden and Surma tea garden. 5 tea gardens in Moulvibazar district are - Pulchra tea garden, Kakia chara tea garden, Balisira tea garden, Lakhai tea garden and Khejurichra

1.2. tea garden.



Map 1: Tea gardens of Moulvibazar district (source of the data for analysing)



Map 2: Tea gardens of Hobiganj district
(source of the data for analysing).

1.1(A) _Sample collection of blight infected tea leaves

First, Collected diseased tea leaves from Noapara tea garden and Jagdishpur tea garden in Madhabpur upazila of Habiganj district on 14th November 2022. Blister blight, grey blight, and brown blight are particularly important as they adversely affect the bud and the two youngest leaves(Pandey et al, 2021) So by the examination of diseased tea leaf samples shows that there are reddish-brown small irregular round spots on the surface of the leaves.The underside of the leaf is not separately infected, although the surface of the tea leaf is infected with the underside.Some tea leaves have spots of different shapes. The edges of some tea leaves are dry or the entire leaf is curled. All the leaves of some tea plants are affected and some leaves of some tea plants are affected. The rate of infection is higher in new leaves.

Later in December 2022 and lastly on 10th April 2023 I collected diseased tea leaves again. Cut the leaves with scissors and immediately put them in polythene bags and tie them. Then took them to the laboratory of Habiganj Brindavan Government College. Next time collected tea leaves from that garden in the same way and took them to the laboratory.

1.3.Isolation of fungal pathogens from blight infected tea leaves: -

A. **Moist Chamber :-** Some diseased tea leaves are taken from polythene bags in the laboratory and cleaned and disinfected. Ethanol was used for this purpose.The tea leaves were left for a short time and the diseased parts of the specific leaves were cut into small pieces with scissors.

B. **PDA media** :-Unsterilized leaves with blister blight lesions were randomly selected and were placed at the center of sterile Petri dishes (90 mm) with PDA with streptomycin to inhibit bacterial growth and incubated at 28 °C for 3 days. The pure culture of the isolates was subcultured in PDA and further stored at 4 °C. Each isolate (Barman, et al.,2020).On the other hand, It was done- PDA (Potato-, Dextrose-Agar) media was prepared (Potato extract 500ml + 10g + 08g)+ Antibiotic . After preparing the media, 15 test tubes (10 small + 5 large), PDA media, petri dish (Obagwu et al., 1997) , some amount of cotton, bandage For yarn, cloth etc. the autoclave machine is sterilised all for a specified time. Before removing all the sterile materials from the autoclave machine, a table was thoroughly cleaned with ethanol. Then two spirit lamps were lit on both sides. Then some of the small pieces of diseased tea leaves were placed in 5 petri dish without PDA media, A few pieces were placed in sterile cotton inside several test tubes and the test tube mouths were well sealed with cotton.A piece of diseased tea leaf is placed right in the middle of the rest 5 petty dis and test tube(5 large and 5 small) with PDA media.. Later they were kept at specific places for observation for 28 degree c.for 21 days(Barman and Tamuli, 2017). Observation was done daily. After 12 days, fungal colonies were correctly observed in petri dish containing PDA media but not colonies were formed on petri dishes without PDA media.

1.4.**Identification** :-colony colour, pigmentation, texture, and growth

A. **General view of Pathogen colony - Color, pigmentation, texture and growth of pathogen colonies were observed.**

About 13 days of keeping infested tea leaves on PDA media, Top and bottom view of Petri dish shows that the fungal colony is black in centre with brown mycelium on one side. And white mycelium surrounds the dark-brown color.And the fungal mycelium was branched and spreading.But not all petri dish fungal colonies are created equal.Care is taken from the beginning that no other organisms enter the fungal colony.

B. **fungal isolates under light microscope** :-First the pcynidia were cut and transferred to a clean slide with a sterile needle. The slide was carefully teased with a drop of lactophenol cotton blue to release the conidia and covered with a cover slip. The prepared slides were then observed using a x25 microscope (Adeniyi,et al.,2016) Slide were prepared by taking portions of central black mycelium on slides and staining them with dyes. The slides were observed under an electron microscope.The branching mycelium is spreading and contains grape-like conidia from the brownish placenta.Conidia of light brown colour were clearly visible.Some mature and some immature conidia were clearly identified.Conidia were single septate. Thin slender hyphae of normal B.theobromae. Microscopic description of Botryodiplodia theobromae is presented in Figure 1. Mycelium was hyaline and branched. Major hyphae were up to 6-8 µm wide. Average radial growth was measured in PDA media - about 11 mm per day at 28°C.The fungus grows at temperatures between 40 and 36 degrees Celsius, with optimum growth at 28 degrees, (A Saha, et al 2008). Sporangia globose to be slightly cylindrical, measuring 15-55 diameter µm and length 65 µm.

Fig 1A: Pathogen under light microscope,dark- brown walled, single septate.

Fig 1B.- Upper surface of Pathogen colony and branched mycelium growth **(Botryodiplodia theobromae)**

Statistical analysis

One-way ANOVA (Microsoft Excel) was performed for statistical analysis with the data retrieved from at least three independent replicates.

2. Results and discussion

Leaf blight prevalence in Habiganj and Moulvibazar district. The information about the number of affected trees of 10 trees was collected by random selection from 5 units of each tea garden in 10 tea gardens of Habiganj and Moulvibazar districts. Table 1 presents data on the infection of tea leaves by the pathogen *Botryodiplodia theobromae* in various tea gardens within Moulvibazar district. The infection levels are categorized into different sections within each tea garden. A total of 103 infections were reported across all sections.

2.1. Infection of Tea Leaves by *Botryodiplodia theobromae* in Moulvibazar District Tea Gardens

Table 1: Report related to destruction of diseased tea trees in 5 tea gardens of Moulvibazar district.

A tea garden dazed report of infection of tea leaves by Botryodiplodia theobromae							
District Name- Moulvibazar							
Sl. No.	Tea garden name	Section no					Total
		1	2	3	4	5	
1	Fulchara tea estate	2	4	10	1	0	17
2	Kakiachara tea estate	3	9	6	1	2	21
3	Balisera tea estate	0	1	6	2	8	17
4	Lakhai tea estate	7	1	9	3	6	26
5	Khajurichara tea estate	0	3	6	6	7	22
				Grand Total			103

2.1.1. Distribution of Infections by Tea Garden and Section:

Fulchara Tea Estate: The highest number of infections was reported in Section 3 (10 infections), followed by Section 2 (4 infections). Sections 1 and 4 had lower infection rates of 1 and 0, respectively. No infections were reported in Section 5.

Kakiachara Tea Estate: In this garden, Section 3 had the highest infection count (9 infections), followed by Sections 1 and 5 with 6 and 2 infections, respectively. Sections 2 and 4 had minimal infections, with 1 and 2 cases, respectively.

Balisera Tea Estate: Section 5 reported the highest number of infections (8 infections), while Sections 2 and 3 had 6 and 1 infections, respectively. Sections 1 and 4 remained infection-free.

Lakhai Tea Estate: The highest infection count was recorded in Section 4 (9 infections), followed by Sections 5 and 3 with 6 and 3 infections, respectively. Sections 1 and 2 reported 7 and 1 infection(s), respectively.

Khajurichara Tea Estate: The infection distribution was relatively even, with Sections 4 and 5 each having 7 infections. Sections 2 and 3 reported 6 and 3 infections, respectively, while Section 1 remained infection-free.

2.1.2. Comparative Analysis and Implications:

By monitoring the infestation rate of tea plants in 5 tea gardens of Moulvibazar district, it is seen that a total of 103 trees were infected and maximum 26 trees were found infected in Lakhai tea garden (out of 50 trees) (Fig. 2). The variation in infection levels among different tea gardens and sections underscores the heterogeneous nature of pathogen spread. Factors such as microclimate, plant density, and management practices could contribute to these differences. Sections with higher infection rates might require focused attention in terms of disease management strategies, including fungicide application, pruning infected parts, and maintaining proper sanitation. The absence of infections in certain sections suggests successful preventive measures or a less conducive environment for the pathogen's growth.

Continuous monitoring and early detection are crucial for preventing outbreaks and minimizing yield losses. Implementing integrated disease management practices can contribute to sustainable tea cultivation. It is important to note that the table provides only a snapshot of the infection situation; a longer-term analysis would provide more insights into disease trends and potential patterns.

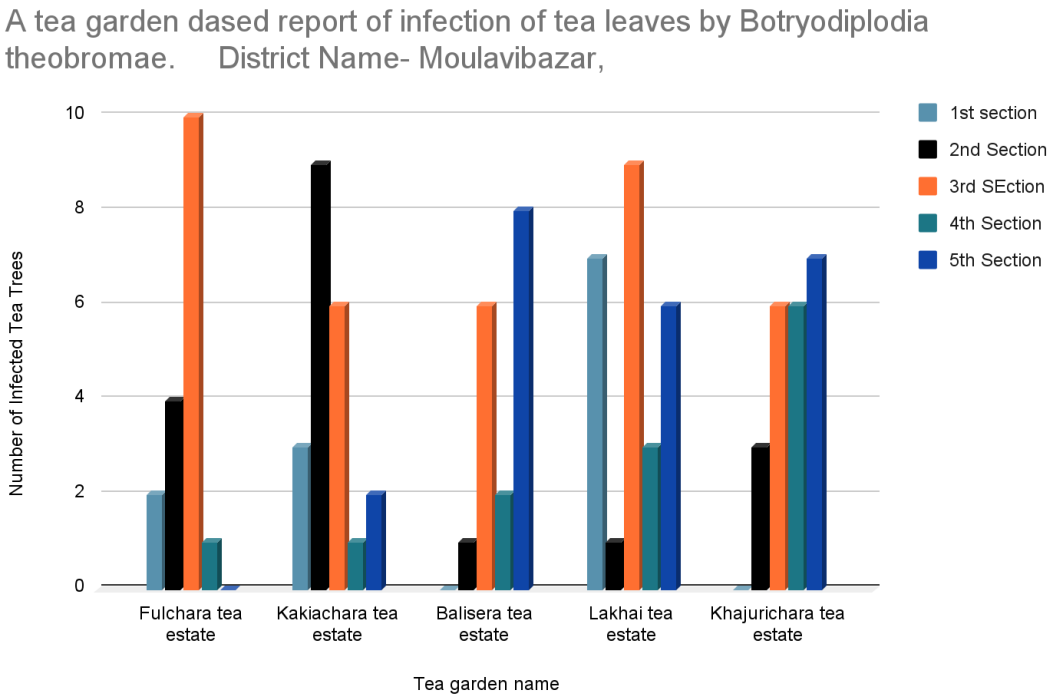


Fig 2. Comparison among 5 tea states of Moulvibazar district.

2.2. Infection of Tea Leaves by Botryodiplodia theobromae in Habiganj District Tea Gardens

Table 2 represents insights into the extent of infection caused by the pathogen Botryodiplodia theobromae in various tea gardens located in the Habiganj district. The infection data are segmented by tea garden, section numbers, and the number of infected tea trees in each section. The "Grand Total" column indicates that a total of 143 infected tea trees were reported across all tea gardens and sections.

Table 2. Report related to destruction of diseased tea trees in 5 tea gardens of Habiganj district.

A tea garden dazed report of infection of tea leaves by Botryodiplodia theobromae							
District Name- Habiganj							
serial no	Tea garden's name	Number of Infected Tea Tree					
		Section no					Total
		1st	2nd	3rd	4th	5th	

1	Noapara tea estate	9	8	6	10	8	41
2	Boikunthapur tea estate	0	2	1	7	9	19
3	Jagadishpur tea estate	9	1	1	6	3	20
4	Surma tea estate	10	9	5	9	5	38
5	Taliapara tea estate	0	4	7	6	8	25
				Grand Total			143

2.2.1. Infection Distribution across Tea Gardens and Sections:

Noapara Tea Estate: The highest number of infected tea trees was found in the 4th section (10 trees), closely followed by the 1st and 5th sections, each with 9 and 8 infected trees respectively. The 2nd and 3rd sections reported 8 and 6 infections, respectively.

Boikunthapur Tea Estate: The 4th section had the highest infection count (7 trees), trailed by the 5th and 2nd sections with 9 and 2 infected trees, respectively. The 1st and 3rd sections reported no infections.

Jagadishpur Tea Estate: Both the 1st and 4th sections exhibited the highest infection levels with 9 trees each. The remaining sections reported lower infection counts, ranging from 1 to 6 trees.

Surma Tea Estate: The 1st section reported the highest number of infected tea trees (10 trees), followed by the 2nd section with 9 infections. The 3rd and 4th sections each had 5 infected trees, while the 5th section reported 5 infections.

Taliapara Tea Estate: The 3rd section had the highest infection count (7 trees), followed by the 5th and 4th sections with 8 and 6 infected trees, respectively. The 1st and 2nd sections remained infection-free.

2.2.2. Analysis and Implications:

By monitoring the infestation rate of tea plants in 5 tea gardens of Habigonj district, it is seen that a total of 143 trees were infected and maximum 41 trees were found infected in Noapara tea garden (out of 50 trees) (Fig 3). The variation in infection levels across tea gardens and sections suggests different susceptibility levels of tea trees to Botryodiplodia theobromae.

The higher infection in specific sections might be due to various factors, such as microclimatic conditions, soil health, and the presence of other stressors affecting the tea trees. Tea estates with lower or zero infections could potentially be implementing effective disease management practices, including regular monitoring, pruning infected parts, and maintaining proper hygiene. The variation in infection levels underlines the importance of targeted strategies for disease management based on specific garden sections and their unique conditions. The cumulative "Grand Total" illustrates the collective impact of Botryodiplodia theobromae on tea tree health within the Habiganj district.

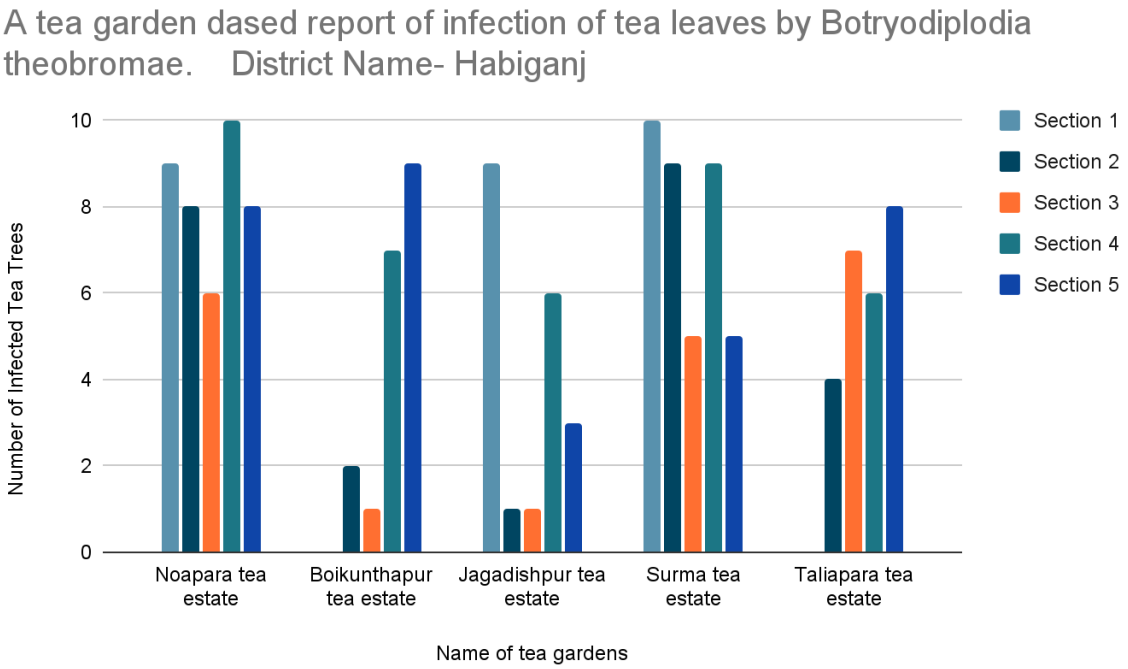


Fig. 3. Comparison among 5 tea states of Habiganj district.

2.3. Comparative study between Moulvibazar and Habiganj districts tea garden

Table 3 represents a comparative analysis of the infection of tea leaves caused by Botryodiplodia theobromae in tea gardens across two districts, Moulvibazar and Habiganj. The infection data are categorized by tea garden, section numbers, and the total number of infected tea trees in each section. The "Grand Total" column indicates a combined total of 246 reported infections across all tea gardens and sections.

Table 3. Comparative study between Moulvibazar and Habiganj districts tea garden.

A tea garden based report of infection of tea leaves by Botryodiplodia theobromae							
District Name- Habiganj & Moulavibazar							
sl	Name of Tea Garden	section no					Total
		1	2	3	4	5	
1	Noapara Tea Estate	9	8	6	10	8	41
2	Boikunthapur Tea Estate	0	2	1	7	9	19
3	Jagadishpur Tea Estate	9	1	1	6	3	20
4	Surma Tea Estate	10	9	5	9	5	38
5	Taliapara Tea Estate	0	4	7	6	8	25
6	Fulchara Tea Estate	2	4	10	1	0	17
7	Kakiachara Tea Estate	3	9	6	1	2	21
8	Balisera Tea Estate	0	1	6	2	8	17
9	Lakhai Tea Estate	7	1	9	3	6	26
10	Khajurichara Tea Estate	0	3	6	6	7	22
				Grand Total			246

2.3.1. Moulvibazar District:

The highest infection count was reported in the Lakhai Tea Estate, with a total of 26 infected tea trees. Other tea estates such as Kakiachara, Balisera, Fulchara, and Khajurichara also reported infection counts ranging from 17 to 22. Overall, Moulvibazar district recorded a cumulative total of 103 infections.

2.3.2. Habiganj District:

The highest number of infected tea trees was reported in the Noapara Tea Estate, with a total of 41 infections. Other tea estates such as Surma, Taliapara, and Boikunthapur reported infection counts ranging from 19 to 38. In Habiganj district, the cumulative total of reported infections was 143.

2.3.3. Comparative Insights:

Infection Levels: The combined total infections in Habiganj district were notably higher than those in Moulvibazar district. This could be attributed to varying environmental conditions, management practices, and disease control efforts in the two districts.

Impact on Specific Tea Gardens: Both districts had tea estates with relatively higher infection levels. In Habiganj district, Noapara Tea Estate had the highest infections, while in Moulvibazar district, Lakhai Tea Estate had the highest number of infections.

Geographical Factors: The difference in infection rates between the districts could be influenced by factors such as climate, altitude, and soil types. These factors can create distinct conditions that may favor or hinder the growth and spread of the pathogen.

Diseased tea plants in 5 of Moulvibazar district and 5 of Habiganj district out of a total of 10 tea gardens were monitored to determine the spread of the disease. It can be seen that the prevalence of blight disease is higher in the tea gardens of Habiganj district as compared to the tea gardens of Moulvibazar district.

2.4.Microbiological study:

Microscopic description of Botryodiplodia theobroma is presented :- mycelium was hyaline and well branched. Major hyphae were found up to 6–8 µm wide. Average radial growth 11 mm per day on PDA at 25°C. Sporangia globose to slightly cylindrical, measuring 15-55 diameter µm and length 65 µm. The morphological description of Botryodiplodia theobromae in this study is in line with the general description of Botryodiplodia theobromae as reported by Shah et al. (2010) and Twumasi et al. (2014). Triest and Hendrickx (2016) observed that crown rot infection begins at harvest with the mycelium developing on dead banana stalks or leaf. The results of various studies confirm that B. Theobroma is a broad-spectrum pathogen and host range reported in various studies(Pitt and Hocking, 2009; Opoku et al., 2007; Domsch et al., 2007; French, 2006; Khanzada et al., 2004a; Sutton, 1980).

Conclusion:

This study emphasizes the importance of localized disease management strategies, taking into account the unique conditions of each district. Continuous monitoring, research, and adaptive management approaches are crucial to sustain tea cultivation in the face of pathogenic challenges in Habiganj and Moulvibazar district of Bangladesh. Further research and analysis could delve deeper into the underlying factors influencing the observed infection patterns.

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