Original Research Article

STUDY ON THE SURVIVAL CONDITION OF BLACK ROT (*Corticium theae*) DISEASE OF TEA IN DIFFERENT HABITANT

ABSTRACT

The experiment was conducted to find out the survival condition of Black Rot disease (*Corticium theae*) of tea. Samples and data were collected from experimental field, BTRI. From the samples causing pathogen of Black Rot (*Corticium theae*) was detected. In dormant period after pruning, *Corticium theae* can be still found in plant debris and pruning litres as fruiting body which contains spores. Spores are the reproductive unit of fungi. These spores are often the dusty and colored part of a fungus that is easily moved in the wind or in water. These spores adhere to and germinate on the tea plant surface, produce germ tubes, and the tip of the germ tube developed and sent an infection peg through the cuticle. Following penetration, these fungi initiates sub-cuticular intramural colonization and spreads rapidly throughout the tissue with both inter- and intracellular hyphae that kill cells and tissue as they advance.

Key words: Black Rot, Tea, Habitant and Diseases

1. INTRODUCTION

Tea [*Camellia sinensis* (L.) O. Kuntze] is one of the most common and cheapest beverages in the world which is manufactured from the crop shoots comprising a bud and subtending two or three leaves [1, 2]. Tea is commercially produced in near about 80 countries and becomes the leading cash crop in world agriculture [3, 4]. Like many other countries Bangladesh earns a substantial amount of foreign exchange and contributing to GDP [5]. In Bangladesh, 169 tea estates consist of 59.61 thousand hectares of land in tea plantation and production of made tea about 63.86 million kg during 2014 (PDU 2015) [22].

Being a plantation crop, tea provides a relatively stable microclimate and food supply for different pests and diseases which cause serious damages in tea with a significant impact on productivity and quality [6-8]. In world tea, 1034 species of arthropods and 82 species of nematodes are associated with tea plants which cause crop loss annually worth US$ 500 million to $ 1 billion [9]. Due to the agro-ecological environment of Bangladesh tea garden possess a large number of pest and diseases which includes 25 insects, 4 mites, 12 nematodes and 1 algal and 18 fungal disease respectively [10, 11]. The majority of the diseases in tea are of fungal origin and cause various diseases in tea like foliage, stem and root diseases all over the world [12, 13, 21]. Since the leaves are the harvested product in tea, foliar diseases are of most concern as it leads to direct crop loss and quality deterioration of the final product [14]. Among the foliar disease, black rot is one of the most

prominent and endemic foliar diseases in tea growing areas of Bangladesh which is caused by *Corticium theae* [15, 20]. Inhabitants of saprophytic micro-organisms in soil surface, leaf surface of tea and air-borne propagules have been premeditated by different specialists. Apart from this, the report of the intensive studied on tea leaf surface mycoflora has been also reported by other scientists [16].

The disease attacks the maintenance leaves just below the plucking table. Infected leaves do not fall off but remain hanging and attached to the next leaf through small chords of mycelium at the point of contact. Dense shade, bad drainage, and sanitation, high humidity, etc. are usually considered as predisposing factors for the prevalence of the disease. They produce slightly brown, yellowish to chocolate brown and grey patches on the upper surface and evenly brown or grey on the lower surface on the tea leaves. The fungus produces basidiospores in fructifications on the lower surface of leaves, which appear dusty due to the spore production and easily can move in the wind or water and also by contact through pluckers where by the diseased leaves and spores get transferred into the uninfected portions. The infected leaves turn black as they rot during wet weather. During the unfavorable weather condition, they transformed into the sclerotial stage for survival. Thus they persist from season to season. When favorable condition prevails, they germinate and re-infect [17].

The prevalence of black rot disease depends on the fruiting body, plant parts, variety, location, age of the plant, soil pattern, climate condition, ventilation facility, and shade condition, etc. However, no systematic study has been carried out concerning the survival condition of the Black rot (*Corticium theae*) of tea in Bangladesh, up to now. Therefore, present research work was undertaken to detect black rot (*Corticium theae*) in different potential sources, assessing their capability to infect the tea plants and finding out the survival condition of this disease in different habitats.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in experimental field of Bangladesh Tea Research Institute (BTRI), Srimongal, Bangladesh located between 24.3083°N North latitude and 91.7333°E East longitude in Sylhet zone [18]. This is one of the major tea growing area in Sylhet zone situated on an elevation of about 26 m above sea level with maximum average temperature is 29°C, while the minimum average temperature is 19°C. The total annual average rainfall is 3876 mm [19].

2.2. Sample Collection

This experiment had conducted at Department of Food Engineering and Tea Technology, Sylhet, Bangladesh and Bangladesh Tea Research Institute (BTRI), Srimongal, Moulvibazar, Bangladesh during November to February of pruning season. The Black Rot disease affected tea plant parts were collected from some randomly selected sections of experimental field. The selected plant parts and surface soil were collected from the field. Every time some plant parts were selected and the whole injured plant was carefully pruned. Pruning liters from the tea field,

debris associated with tea plants and surface soils from the infected sections were collected separately and were taken in the laboratory for further experiments.

2.3. *Corticium theae* Detection

For the detection of causing agent *Corticium theae* an *in vitro* experiment was done. For analysis some equipment and materials were needed.

2.4. Materials

Potato dextrose agar (PDA), Petri dish, Measuring flask, Autoclave, Laminar air flow Chamber, Niddle, Compound microscope, Incubator, Colony counter etc.

For the *in vitro* experiment or the detection of the pathogen, Potato Dextrose Agar (PDA) medium was prepared in the laboratory. Medium and required glassware were sterilized in an autoclave. After pruning the operation of tea pruning liters from the tea field, debris associated with tea plants and surface soils from the infected sections were collected separately. In the laboratory, the fungus of Black rot disease was isolated from pruning liters and debris by using direct and washing plating methods. The fungus was isolated from soil by a series dilution method (10^{-3}, 10^{-4} and $10^{-5}$and then dispensed uniformly with media. After that, the Petri dishes were kept in the incubator for 4-7 days. Then using the colony counter, colony forming pathogen was counted. Also using the compound microscope the figure of the fruiting body of *Corticium theae* was found. Colony-forming unit was expressed as (-) = 0, (+) = 1-10%, (+ +) = 11-20%, (+ + +) = >20% in CFU [16].

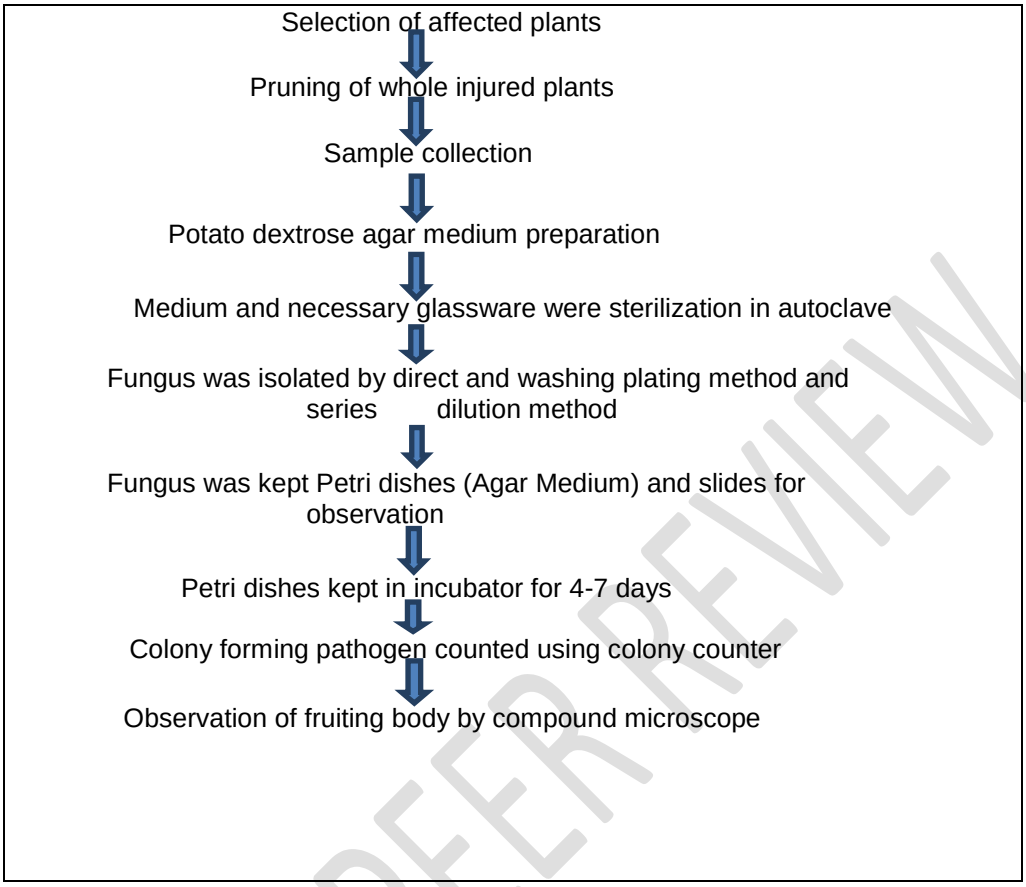
2.5. Cell and Tissue Study

Some black rot affected tea plant parts which included leaves & stems collected from the experimental plot several times. These affected plant parts were taken to the laboratory for further experiments. Thin affected parts were prepared for observation. These affected parts were prepared by the freehand sectioning method. Affected parts were wetted a little and sectioned carefully with the help of potato and razor blade. After that, these sections were cleared in Ethanol, taken into a slide and observed in a compound microscope. In every observation, the picture of the plate is captured and studied for further details.

2.6. Working Flow Charts of the Study

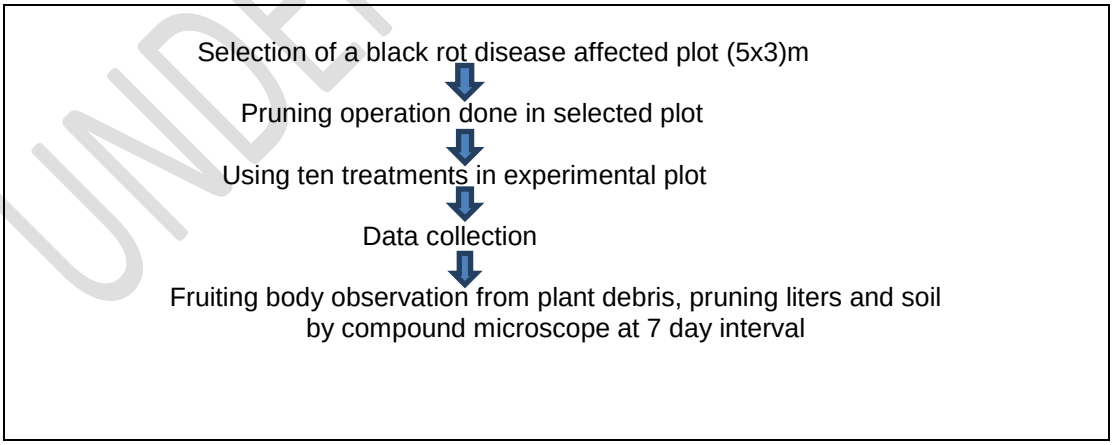
2.6.1. Flow Chart of *Corticium theae* Detection and Observation of Fungal Fruiting Body in Different Sources of Infection (Habitants)

Chart 1: Flow Chart of Corticium theae Detection and Observation of Fungal Fruiting Body in Different Sources of Infection (Habitants)



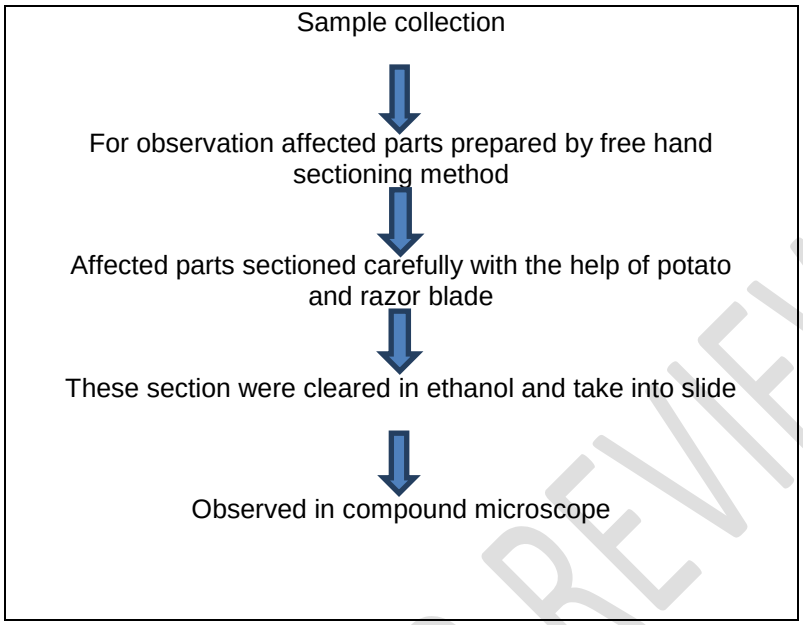
2.6.2. Working Flow Chart of Field Experiment

Chart 2 : Working Flow Chart of Field Experiment



2.6.3. Flow Chart of Cell and Tissue study

Chart 3: Flow Chart of Cell and Tissue study



3. RESULTS AND DISCUSSION

The present investigation was carried out for the study of habitant of the pathogen (*Corticium theae*) and analysis of infected green leaves of tea plants. Details of the results for observation of pathogenicity obtained from the experiment were described under the following heads;

3.1. Detection of *Corticium theae* in different sources of infection (habitants)

Black Rot disease pathogen (*Corticium theae*) was found in pruning liters, plant debris and also in soil. Pathogen (*Corticium theae*) from these sources formed colony in Petri dishes that was count with the help of the colony counter.

Table 1: Level of *Corticium theae* in different sources of infection during dormant period in experimental plot

Sources	Percentage of *Corticium theae* in different habitants	Mode of characteristics
Pruning liters	(a) Leaves contain 12% (b) Stems contain 8%	Both active hyphae and fruiting bodies were found in moderate numbers.
Plant debris	(a) Dead leaves contain 16% (b) Dead stems 11%	Only fruiting bodies are found in maximum numbers
Soil	Soil contain 8%	Soil contain small amount of fruiting bodies.

Table 1 revealed that *Corticium theae* was present in different habitants. They were present as mainly active fruiting bodies and some time as active hyphae in those habitants.

Table 2: Presence of *Corticium theae* in different sources of infection during dormant period in *in vitro*

Sources of infection	**Direct method**	**Dilution method**		
		10^{-3}	10^{-4}	10^{-5}
Pruning liters	+ + +	+ + +	+ + +	+ +
Plant debris	+ + +	+ + +	+ + +	+ + +
Soil	+ +	+ +	+ +	+ +

(Colony forming unit was expressed as - = No CFU, + = CFU: 1-10%, + + = CFU: 11-20%, + + + = CFU: >20%)

As it can be seen from table 2, the highest number of viable and vigorous pathogenic propagules (> 20%) was found in plant debris in all diluted concentrations. Few numbers of pathogenic propagules (20%) was found in pruning liters and in all diluted condition. In surface soils the amount of pathogen (*Corticium theae*) (11-13%) was less than pruning liters and plant debris. From these sources pathogen increases the disease gradually.

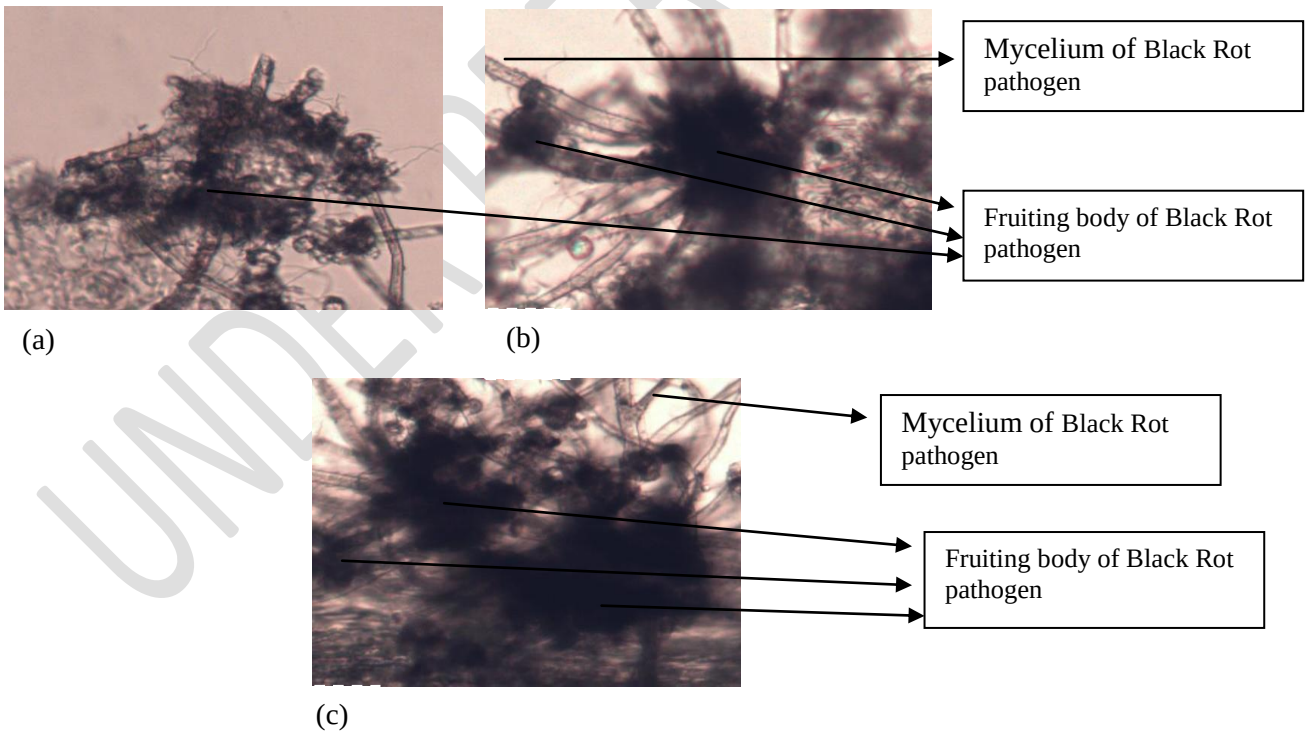


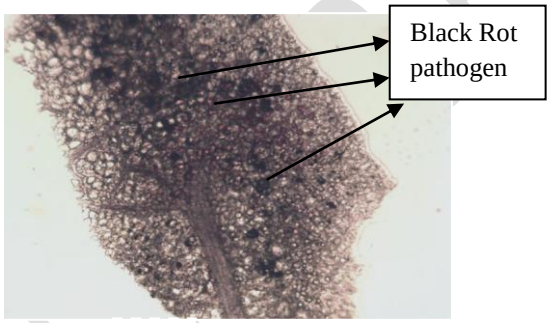
Fig 1: Observations of fruiting body of Black Rot pathogen (*Corticium theae*) into the leaf, Longitudinal Section (LS) under compound microscope (x400). (a) First Observation (b) Second Observation (c) Third Observation.

From the table 3, it is clear that after pruning, pruning liters and plant debris were observed with a compound microscope. The first observation scored a very few numbers of the fruiting body. The age of these fruiting was 7-9 days and fruiting bodies were in the growth stage. The second observation showed a few numbers of the fruiting bodies with the age of 11-15 days, and the pathogen started to produce more fruiting bodies. Third observation scored plenty of fruiting bodies. The age of these fruiting was 21-24 days and the pathogen started to produce a lot of fruiting bodies. Fig. 1 & 2 indicates the gradual increasing of the fruiting bodies.

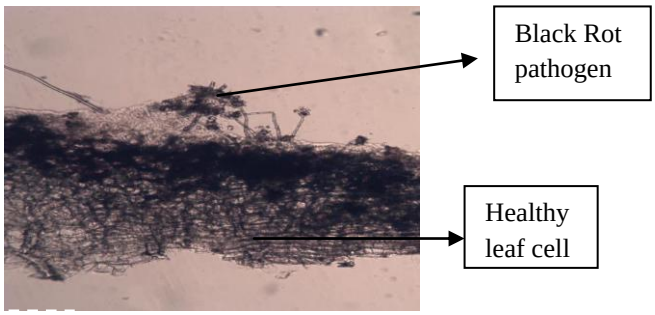
Table 3: Growth rate of Black Rot pathogen fruiting body

Observation	Age of the fruiting body	Number of the fruiting Bodies	Remark
1st observation	7-9 days	Very few numbers	Initial stage of pathogenic growth of fruiting body
2nd observation	13-15 days	Few numbers	*Corticium theae* started to produce fruiting body
3rd observation	21-24 days	Plenty of numbers	*Corticium theae* produced lot of fruiting body

Hence, it can be concluded that the numbers of fruiting body increased with the passing of time. The reason might be due to the availability of plenty of food and plenty of hyphae of the pathogen.



(a)



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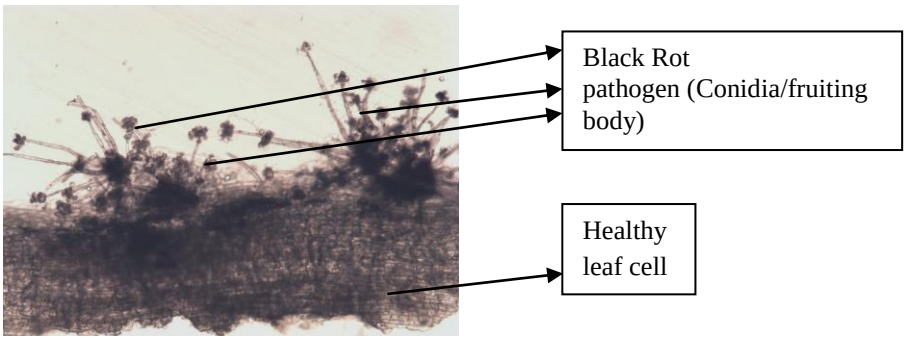

(c)

Fig 2: Detection the pathogen (*Corticium theae*) in infected tea plant part/leaf, (LS) under compound microscope(x400). (a) Detection of Black Rot pathogens in damaged tissue in infected plant part (leaf), (b) Showing low intensity of Black Rot pathogens in infected tea leaf tissues, (c) Showing high intensity of Black Rot pathogens (Conidia/fruiting body)in infected tea leaf tissue.

Fig 2: (c) showed the high intensity *of Corticium theae* in leaf tissue. There were many colonies and only a few parts of tissue were unaffected. (b) Showed low intensity of black rot pathogen and most of the tissues were healthy and unaffected. Pathogen initiates disease on the surface. (a) Showed the infection of whole section by *Corticium theae* pathogen. Smaller red dots represent the pathogen in tissue. A maximum cell was killed by a pathogen.

Spores are the reproductive unit of fungi, equivalent to plant seeds and can easily move in the wind or in water. Consequently they adhere and germinate on the tea plant surface and produce germ tubes. After penetration, this fungus initiates sub-cuticlular intramural colonization and spreads rapidly throughout the tissue with both inter and intracellular hyphae that kill cells as they advance. Thus they may survive in the dormant period and re-infect the tea plants at their loyal condition.

4. CONCLUSION

This experiment showed that *Corticium theae*, the causal pathogen of Black Rot, was present in affected tea plant parts. In the dormant period after pruning plant parts like leaves, plant debris, pruning liters and soil under the affected tea plant contained this pathogen. Pruning liters contain 20%, plant debris 27% and soil 8% of *Corticium theae* as the volume in a petri dish.

Information on plant pathogen's (*Corticium theae*) surviving condition in different habitants would be useful to design appropriate management strategies. Further studies should be done to inactive or increase the dormancy of the fruiting body.

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