Annals of Plant Sciences 8.9 (2019) pp. 3603-3615

ANNALS OF PLANT SCIENCES
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Research Article

Incidence and identification of foliar fungal pathogens from tea gardens of Sikkim using foldscope as research tool

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Abstract: Tea (*Camellia sinensis* (L.) O. Kuntze), is a popular beverage worldwide and it is prone to many diseases during its long-life span. A number of the fungal pathogen causes foliar diseases of tea, some of which cause serious damage to the existence of the tea plantations and have an adverse effect on the quality of tea beverages. The survey and reporting of the diseases are important to achieve the target of disease-free and good quality tea production. In the current study, Foldscope the cost-effective paper microscope is used as a research tool for the very first time to study the incidence and identification of fungal leaf spots, leaf blight diseases of Tea plants from Sikkim. After isolation and characterization of the fungal isolates, the incidence of Phytopathogens like *Cladosporium cladosporioides* (Fresen) G.A. de Vries, *Xylaria hypoxylon* (L.) Grev., *Colletotrichum* sp. aff. *C. Musae* (Berk. & M. A. Curtis) Arx/, *Colletotrichum coffeanum* F. Noack., *Colletotrichum Spp., Rhizosphaera oudemansii* Maubl., *Alternaria alternata* (Fr.) Keissl./ *Fusarium sp. aff. F. fusarioides* (Gonz. Frag. & Cif.) C. Booth, *Exobasidium vexans* Massee was found to be associated with the leaf spot and leaf blight disease in the tea garden of Sikkim. And it is the first incidence report of foliar fungal pathogens from Tea plants of Sikkim, India. The result of these findings will help in the formulation of an effective disease management plan by the planters. The foldscope microscope being handy and cost-effective, it was used as an alternative diagnostic research tool for the basic study of microbial pathogens associated with the tea plants. It was found to be a good optional diagnostic/research tool to be carried around in the field in remote and resource-constrained areas.

Key words: Sikkim, *Camellia sinensis*, fungal phytopathogen, leaf spot, leaf blight, Foldscope.

Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze, China variety, *Camellia assamica* (Masters) - Assam Variety and *Camellia assamica* sub spp. *lasiocalyx* (Planchon ex Watt) a popular hybrid commonly known as Cambod variety are mainly grown for tea production. Tea is the most valuable plantation crop and is the highest net foreign exchange earner of India. A very popular beverage known widely for its antioxidant properties and India continues to be the world's largest producer and consumer of tea. Diseases caused by plant pathogens result in considerable losses to agricultural production and may also seriously affect farming, forestry and the environment. A number of the fungal pathogen causes foliar diseases of tea, some of them cause serious damage to the existence of the tea plantations viz., leaf blight, leaf spot and leaf rot are the common diseases of the tea bush in many tea growing countries (Eden, 1976; Hainsworth, 1952; Petch, 1923; Sarmah, 1960). These diseases have direct effect on bush health as the quality and quantity of harvest is reduced when both young and mature leaves are attacked. There are approximately 380 fungal pathogens which attack roots, leaves and branches of tea plants. A majority of tea diseases are of fungal origin, except red rust (*Cephaleuros parasiticus* Karst.) which is caused by an alga. G. Watt was perhaps the first to give a comprehensive account on pests & disease of tea in his memorable book "Pests & Blight of the

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Tea plant (1898), after that several workers documented their contribution in the field, viz. Mann and Hutchinson (1904), Butler and Bisby (1931), Tunstall and Sarmah (1947), Gadd (1949), Goodchild (1952), Agnihothrudu (1963, 64); Ramlogun (1971), Hamaya (1981), Tzong Mao Chen and Shin Funchen (1982) Chen and Chen (1989), Satyanarayana and Barua (1978), Sudoi and Langat (1992), Chandra Mouli (1995), Muraleedharan, Chen (1997). As tea is produced from young shoots of tea bushes and leaves, the foliar diseases are extremely important economically as it reduces the quality and quantity of tea production. The most common leaf diseases Blister blight caused by *Exobasidium vexans* Massee, by far the most important leaf disease, is known to occur in almost all the tea growing areas in India, Sri Lanka and Japan. Red rust caused by *Cephaleuros parasitica* (Karst.) and birds eye spot (*Cercospora theae* Car and Caurzi) occur in tea in several tea growing countries has been reckoned as one of the most problematic secondary diseases of tea in North East India Agnihothrudu (1963).

There is very negligible data on fungal pathogens of tea from Sikkim, Bhutia (2012), it is therefore felt necessary to detect and identify microbial pathogen(s) with reliable diagnosis methods which will enable farmers to administer suitable disease management strategies and restrict further spread of the disease(s). There are many methods of identification of fungal phyto-pathogens; a wide range of diagnostic techniques can be applied for detection and identification of fungal pathogens present in the infected plants. Culturing of the pathogens on selective nutrient media, and morphology examination of the fungal colony using an optical microscopy is among the basic classical methods used for detection and identification of fungi. Most importantly the speed, specificity, sensitivity and cost-effectiveness are the primary factors that determine the suitability and choice of the diagnostic tests. In the present study, the Foldscope microscope was used as diagnostic tool, considering the unique features. Foldscope was invented by Manu Prakash and Jim Cybulski from Stanford University, US in 2014, is cost effective, portable and versatile microscope, which folds like an origami piece, and is made mostly out of water-proof paper. It has the magnification and resolution sufficient for imaging live individual cells, cellular organelles, fungal mycelia/spore and much more Cybulski *et al.,* (2014). It's being the part of frugal science movement, the Foldscope in India is endorsed by Department of Biotechnology, Government of India. In an attempt to bring the Foldscope to the most "resource constrained parts of India" with special emphasis on the North Eastern Region of India, DBT, GoI has initiated and funded this project to understand and document the rich biodiversity, ecology and environment of the region. Most phytopathogens in tea plants are micro fungi and may not be easily seen; the disease symptoms they cause are both highly visual and often occur in epidemic proportions resulting in large yield losses. Keeping the above in view, the present work was taken up to isolate, identify and characterized some of the foliar disease-causing tea pathogens from Temi Tea Garden (TTG), Sikkim. And in future to explore sustainable control measures against the isolated fungal tea pathogens.

Materials and Methods

Study Area: The Temi Tea Garden (27.24°N 88.42°) which is located in Temi, South District of Sikkim, and a northeastern part of India, under Eastern Himalayan region. The garden was established in 1969 by the Government of Sikkim and covers an area of approximately 440 acres, the only commercial organic tea garden of Sikkim. The tea produced here enjoys international repute commanding premium prices in world auctions with its brand name Organic Temi Tea.

Collection of samples and Symptomology: Diseased samples of tealeaves were collected randomly from different plots/sites of the garden during the survey period (2018-2019), placed into labeled zip lock bags and brought to the laboratory for detail study and fungal isolation. Disease symptoms and severity were recorded; leaves showing different degree of

disease symptoms were examined morphologically and compared with healthy leaves. Each diseased sample was studied first with hand lens and then with foldscope microscope in the field itself by preparing slides of diseased portion to assess the anatomical and morphological characteristics of the disease. On the basis of symptomatic study tea leaves were collected for further study in laboratory.

Isolation of the pathogen: Diseased samples were washed thoroughly with running tap water to remove the surface contaminants and the tissues exhibiting clear symptoms and small adjacent unaffected tissues were cut into small pieces (2-5mm) using sterile scalpel blades. These small pieces were then surface sterilized with 0.5% sodium hypochlorite solution (NaOCl) for (30-60 Second) and then washed three-four times in sterile distilled water. These surface sterilized pieces were then placed between sterile blotting papers and aseptically inoculated onto Petri-dishes containing Sabouraud Dextrose Agar (SDA) and Potato dextrose agar (PDA) supplemented with chloramphenicol. The plates were incubated at 27 ± 2° C for 5 to 6 days. Plates were examined for the mycelial growth of fungi at regular intervals for a week. The fungal mycelia growth on the diseased leaf pieces in plates were transferred to different growth media for culture characterization of isolates; (YMA) Yeast mannitol agar, Corn meal agar (CMA), Malt extract agar (MEA), Potato dextrose agar (PDA), PH of the medium was adjusted to 6-7 and autoclaved at 15 psi for 20 minutes. Portion of developing mycelium was transferred to agar slants for axenic culture and further examination.

Identification and Characterization of fungal cultures: The isolated strains were sub-cultured to fresh media to ensure the purity. Genus level identification of fungi was done mainly based on morphological characteristics, observing colonial growth and microscopical observation of spore and mycelia with the help of foldscope using standard slide preparation technique and also by using strip of transparent sticky tape which is firmly pressed onto the surface of a sporulating colony cultivated on an appropriate medium in the petridish. The cellophane tape, with its sticky surface carrying fungal spores and hyphae is carefully placed over drops of lactophenol cotton blue and kept at the center of a clean glass slide pressed gently. Using the foldscope microscope, which can be attached with smartphone to record the images, the characteristics of the spores and sporulating structures was studied. The morphological features and cultural characteristics were scientifically examined as per standard keys; Alexopoulos *et al.,* (1996), Williams-Woodward (2001), Dugan (2006), Aneja (2001), Kirk *et al.,* (2001), Gilman (2001), Ellis (1971), Bilgrami *et al.,* (1991), Nelson *et al.,* (1983, 1994), Leslie and Summerell (2006), Gilman and Joseph (2008), Moretti (2009), Simmons (2007), Barnett and Hunter(2000), Booth (1971), Ellis (1976), Ellis and Ellis (1997), Sutton (1980), Thind K S, Dargan J S (1978), Agrios (2005) and also by referring mycobank website and specific research articles etc. The identification of isolated fungal strains was further confirmed at National Fungal Culture Collection of India, Agharkar Research Institute (NFCCI-ARI), Pune Maharashtra (India) and accession number were procured.

Pathogenicity test: Pathogenicity test of each fungal isolate was tested *in vitro* by detached leaf method. Healthy Tea leaf was rinsed with tap water, surface sterilized with 1% sodium hypochlorite solution (NaOCl) and finally rinsed with sterile water. Artificial pricks on the abaxial/adaxial surface of the leaf were made by sterilized needle and immediately inoculated with fungal disc. Before inoculation, all fungal isolates were cultured on PDA and some on MEA medium at 25^{o}C for 7-10 days. With a sterilized cork borer mycelia were cut to discs and were transferred to the healthy leaf. The discs of PDA medium only were maintained as control. After that, each leaf was placed in sterilized moist chamber (Petriplate) and incubated in environmental test chamber at (25±2° C) for 4-7 days. Symptoms were observed and the disease portion was re-inoculated on media plates for the confirmation of the pathogen.

Results and Discussion

Study of disease symptoms: During the field survey it was observed that the incidence of fungal infection on leaf was highly prevalent in tea garden of Sikkim. The different forms of disease symptoms ranging from leaf spot and leaf blight infection, red rust, twig die-back, stem-canker, cankered lesions, rot and wilting were frequently observed. Leaf blight symptoms ranges from small circular translucent yellow to brown necrosis on leaves. Leaf spot also vary with size and coloration. The incidence of disease was high during the summer and monsoon period, while the average minimum/maximum temperature and humidity during the period was recorded as 14/24°C and 79/90% respectively. Most of the spot and blight causal agent were examined carefully, symptoms with closer anatomical observations of the diseased part are depicted in Figure 1. During the month of July a severe incidence of Blister Blight disease of tea caused by obligate fungal pathogen *Exobasidium vexens* Messee was found to be in peak and as per the observation of the workers it is becoming a recurring phenomenon for past few years. Blister blight caused by fungus *Exobasidium vexans* Massee was not subjected to culture isolation in the present study since it is an obligate biotropic fungus, Subba Rao (1946). This serious foliar disease of tea was reported from Asia especially inIndia, Sri Lanka, Indonesia and Japan. The cool humid climatic condition of Temi tea garden favors the proliferation of the disease and during the monsoon it attains an epidemic proportion. The disease starts appearing at the onset of monsoon in the month of June and disease peak was observed during the months of July and August. The disease symptom initially appears as a pale-yellow translucent spot on young shoots and leaves, which gradually enlarges to a lesion. The upper side of the lesion gets depressed into a shallow cavity and correspondingly the lower side becomes convex forming the typical velvety white blister as shown in Figure 1 (B). Under severe infection, leaves often become irregularly rolled-up, the tender stem are also affected. The fungus reproduces through basidiospores which are disseminated by wind; the spores are hyaline, elliptical and single-celled when immature and two-celled at maturity. The life-cycle of the fungus is completed in a short period of 11-28 days Gadd and Loos (1948), Agnihothrudu and Moulli (1991). Blister blight is a multiple cycle disease as a result different stages of blister lesions can be seen in a single tea bush.

Identification and characterization of fungal isolates: The symptomatic disease leaf spot and leaf blight were collected from the tea garden and were subjected to details morphological studies followed by the isolation. A total of 25 fungal isolates displaying foliar symptoms were recovered from lesions of the diseased leaves, given the code as TF1 to TF25, respectively. Out of which some are non-sporulating which could not be identified by the basic technique of culture characterization. A total of 13 fungal pathogen were identified form the isolated cultures. All the fungal isolates grow well on PDA, SDA and MEA agar plates at 27 ± 2°C but for sporulation the MEA media was found suitable. In the case of *Xyleria sp.*, it was found that the fructification (stroma) in artificial media corresponds with fruiting in natural habitat; it could be presumed that the signal for stroma formation is genetically encoded in this species. The visual characteristics of fungal colony and image data captured through smart phone which was attached with foldscope (microscope) are presented in Figure 1 (H-T). The cultural characteristics of the isolated fungi along with microscopic analysis indicate the association of many other fungal plant pathogens like *Cladosporium cladosporioides* (Fresen) G.A. de Vries, *Xylaria hypoxylon, Colletotrichum* sp. aff. *C. Musae* (Berk. & M. A. Curtis) Arx/, *Colletotrichum coffeanum* F. Noack., *Colletotrichum Spp., Rhizosphaera oudemansii* Maubl./ *Alternaria alternata* (Fr.) Keissl./ *Fusarium sp. aff. F. fusarioides* (Gonz. Frag. & Cif.) C. Booth/ and some non-sporulating dematiaceous fungus.

Table 1: Characterization and Identification of the isolated fungal pathogens.

S. No.	Culture Code	Cultural characteristics	Microscopic characteristics	Fungal isolates name	Disease symptoms on Tea leaf
1	TF 1	Colonies in PDA, 4 cm growth was observed after 7 days, Olivaceous-brown to blackish-brown, floccose, radial furrows. Reverse is dark green to black coloured.	Hyphae, conidiophores and conidia are equally pigmented. Conidiophores are more or less distinct from the vegetative hyphae, being erect. Conidia are produced in branched chains, single-celled, lemon-shaped and smooth-walled.	*Cladosporium cladosporioides* (Fresen) G.A. de Vries,	Black rot
2	TF 2	Colonies are dark brown and black reverse.	Non sporulating dematiaceous form.	Dematiaceous fungi	Leaf spot
3	TF 5	Colonies on MEA, slow growing, 2.5 cm growth after 7 days, distinct mycelium rhizomorph like, *filiform,* filamentous, white cottony growth in the beginning, turned dark grey, reverse is white. Whitish stroma gradually turning to dark at the base in matured fertile state.	Perithecia compact structures, ascospores are present in the stroma beneath the outer black crust. 8 spored, ellipsoid.	*Xylaria* sp. aff. *Hypoxylon* (L.) Grev	Root rot
4	TF 6	Colonies on MEA, mouse gray, floccose, margin irregular, reverse slate buff. Mycelium subhyaline to brown, septate, branched, simple or in bundles.	Chlamydospore subhyaline to olivaceous intercalary, aseptate to 1 septate. Conidiophore not observed. Appressoria terminal, filamentous or knob shaped, dark brown, variable in shape size, 14.7717.10x10.8510.99µm. Conidia cylindrical, tip obtuse, base narrow, pigmented, smooth walled, 24.13x17.68µm.	*Colletotrichum* sp. aff.*C.Musae* (Berk.& M.A.Curtis) Arx	Brown blight
5	TF 7	Colonies in PDA, 4cm growth in 7 days, Greyish to light grey growth with cottony surface, margin irregular, reverse is black.	Spore cylindrical, Sclerotia present in culture. Appresoria circular to slightly irregular, pale to medium brow.	*Colletotrichum* sp.	Leaf spot
6	TF 8	Colonies on PDA, white to grey, reverse grey at the Centre, dense and raised mycelium, orange conidial masses.	Conidiophores wide at the base, hyaline, unbranched. Conidia unicellular, hyaline, ovoid to cylindrical or clavate.	*Colletotrichum* sp.	Leaf spot
7	TF 9	Colonies in PDA, 3cm growth in 7 days, pure white cottony within 10 - 15 days & turn dark grey coloured, irregular growth, raised rough, opaque, reverse is black coloured, margin undulate.	Hyphae smooth, branched, septate, pycnidia present, conidiophores absent, conidia hyaline, 2-3septa, ovoid to ellipsoid.	*Rhizosphaera* sp.aff. *oudemansii* Maubl	Leaf spot canker

8	TF 10	Colonies on MEA, first white later becoming mouse gray, reverse brown.	Conidiomata numerous, globose to subglobose & oval, brown texura angularis, upto 10.5 x 95.65 µm. Hyphae wide pigmented, smooth walled, in parallel bundles, anastomoses, constricted near septa, 5.69-2.17 µm. Conidia rarely present, cylindrical, hyaline, smooth walled, 13.58x4 µm.	*Colletotrichum* sp.	Leaf blight
9	TF 11	Colonies on PDA, 2 cm growth after 7 days, Greyish mycelium, floppy colony, reverse is dark green.	Hyphae simple to branched, septate, light olivaceous, smooth walled, upto 3.4 µm wide. Conidiomata not prominent. Conidiophore not observed. Appressoria not present. Conidia aseptate, hyaline and ellipsoid and cylindrical in shape, smooth walled.	*Colletotrichum* sp. aff.*C. Musae* (Berk. & M.A. Curtis) Arx	Leaf spot
10	TF 13	Colonies on PDA, 3.5 cm growth after 7 days, Black coloured colony with white margin, mycelium with irregular margin, reverse is black.	Hyphae brownish, multicelled, septate and irregularly branched. Conidiophores present, conidia were obclavate to mostly ellipsoidal, muriform having tapered apex and dark brown colour, length varies, both beaked and unbeaked conidia.	*Alternaria alternate* (Fr.) Keissl.	Leaf spot
11	TF 14	Colonies on PDA, 4-5cm growth observed after 7 days, pink coloured colony with white mycelia, floccose, irregular margin, reverse is dark red to black.	Macroconidia were hyaline with 3-4 septate, oval-shaped microconidia, chlamydospores present.	Fusarium sp**.** *sp. aff. F. fusarioides* (Gonz. Frag. & Cif.) C. Booth	Die-back
12	TF 15	Colonies on MEA media mouse grey, floccose, margin irregular, reverse slate buff. Mycelium subhyaline to brown, septate, branched.	Hyphae light olivaceous to dark brown, chlamydospores subhyaline to olivaceous intercalary, aseptate to septate. Appressoria irregular, dark brown varies in shape/size. conidia cylindrical, pigmented smooth walled. 23.35x4.93 µm.	*Colletotrichum*sp. aff. *C. Musae*	
13	TF 16	Colonies on MEA, Offwhite, floccose, later becoming mouse gray, reverse slate buff.	Hyphae simple to branched, septate, light olivaceous, smooth walled, upto 3.4µm wide. Conidiomata not prominent. Conidiophore not observed. Appressoria not present. Conidia cylindrical, pigmented, smooth walled.	*Colletotrichum* sp. aff.*C. Musae* (Berk. & M.A.Curtis) Arx	
14	TF 17	Colonies on MEA, whitish later turned mouse grey, floccose, reverse brown to mouse grey.	Conidiophores branched, phialides, subhyaline to light olivaceous. Appressorium filamentous. Hyphae light olivaceous, smooth walled upto 4.3 µm wide. Conidia cylindrical, straight, hyaline, smooth walled septate. 17.73x4 µm.	*Colletotrichum* sp. aff. *C. coffeanum* F. Noack.	

In the present study most of the fungal isolates belonged to the genera *Colletotrichum sp*. Morphological feature and cultural characteristics of the fungal isolates are summarized in the Table 1. The identification of fungal strains TF1 (NFCCI 4682), TF 5 (NFCCI 4683), TF 6 (NFCCI 4595), TF 7, TF 8, TF 9 (NFCCI 4685), TF 10 (NFCC I9496), TF 11 (NFCCI 4684), TF 13 (NFCCI 4681), TF 14 (NFCCI 4686), TF 15(NFCCI 4679), TF 16 (NFCCI 9497), and TF 17 (NFCCI 4680) were further confirmed by National Fungal Culture Collection of India, Agharkar Research Institute (NFCCI-ARI), Pune (India). Deposited cultures were provided with corresponding accession number. During the field survey the symptoms of other tea disease like the grey blight, twig dieback and thread blight were also found but the culture isolation was not successful.

Most of the above mentioned fungal phytopathogens isolated form Sikkim's tea plant causes major fungal leaf spot and leaf blight diseases in the tea growing countries across the globe, and it was well documented in the work of Sarma (1960), Eden (1976) Ghosh and Hazra, (1991), Heinrich Lehmann-Danzinger (2000), Yu-Chun Wang (2016), Lisa Keith (2006), Mur *et al.,* (2015), Sarmah *et al.,* (2016), Zhou and Xu (2014), Ying-Juan Chen(20180), Ganga D Sinniah (2017), Barthakur *et al.,* (2001). Besides, documentation of these fungal pathogens causing disease in other crop plants was also well evident from the work of Shafique *et al.,* (2019), David Eduardo Torres (2017),Sivakumar and Kathiresan (1990), Hou Y *et al.,* (2016); Parashurama and Shivanna (2013), Myeong Hyeon Nam (2015), Finlay and brown (2007), Hsieh WH (1980), Proffer (1988), Allen Bluhm *et al.,* (2017), Thangavelu and Sundararaju (2004), Ramjegathesh and Ebenezar (2012). Isshiki *et al.,* (2001). In the present study *Colletotrichum sp* was found to be the most dominant fungal pathogen, similarly Wang *et al.,* (2016) reported a total of 106 *Colletotrichum spp.* isolates obtained from the diseased leaves of tea cultivars in China. Since, *Colletotrichum* can inhabit plants as a pathogen, endophyte, epiphyte, or as Saprobe (Canon *et al.,* (2012), Hyde *et al.,* (2009) they were found highest in the isolates. In the case of *Rhizosphaera oudemansii, Cladosporium cladosporioides, Colletotrichum coffeanum, Xylaria* sp. and *Fusarium fusarioides,* the association of these four fungi with the tea plant seems to be the first report from India and the prevalence of these fungi as Tea foliar fungal pathogens from Sikkim seems to be the first report.

Rhizosphaera oudemansii which was reported to be associated with Spanish fir, *Abies pinsapo* as pathogen by Martínez C Ramírez (1983), *Cladosporium cladosporioides,* pathogenic fungi have been found to infect Macademia nuts/Pecan Tree leaf spot as reported by Walker *et al.,* (2016) and *Colletotrichum coffeanum* cause of berry disease of *Coffea arabica*. *Xylaria* spp. are principally known to occur in soil as saprotropes of wood and other plant parts, but they are also common as endophytes, as per the findings of Petrini and Petrini (1985), U'ren *et al.,* (2016) and few species of *Xylaria* are reported as pathogens by Rogers (1979, 84). Lastly, *Fusarium fusarioides* association with cotton plant was mentioned by Shirsath and Patil (2016).

Pathogenicity Test: The symptoms on detached pathogenicity test on tea leaves were almost similar to the natural symptoms. Symptoms of leaf spot infection appeared on fourth day of infestation. Initially, small circular to irregular, spots or necrosis were appeared on the leaf surface surrounding the disc. As the infection progressed, infected area became enlarged, turned into brown to black in colour. The fungi were re-isolated from the infected leaves and were compared with the original cultures. Except few, the result of the pathogenicity test of all the isolated fungi showed significant visible disease symptoms on detached tea leaf when compared against the control as depicted in Figure 1 (U-X)). From these positive tests it can be concluded that the isolated fungal strains are potential phytopathogens and have the capacity to induce infection on tea leaf.

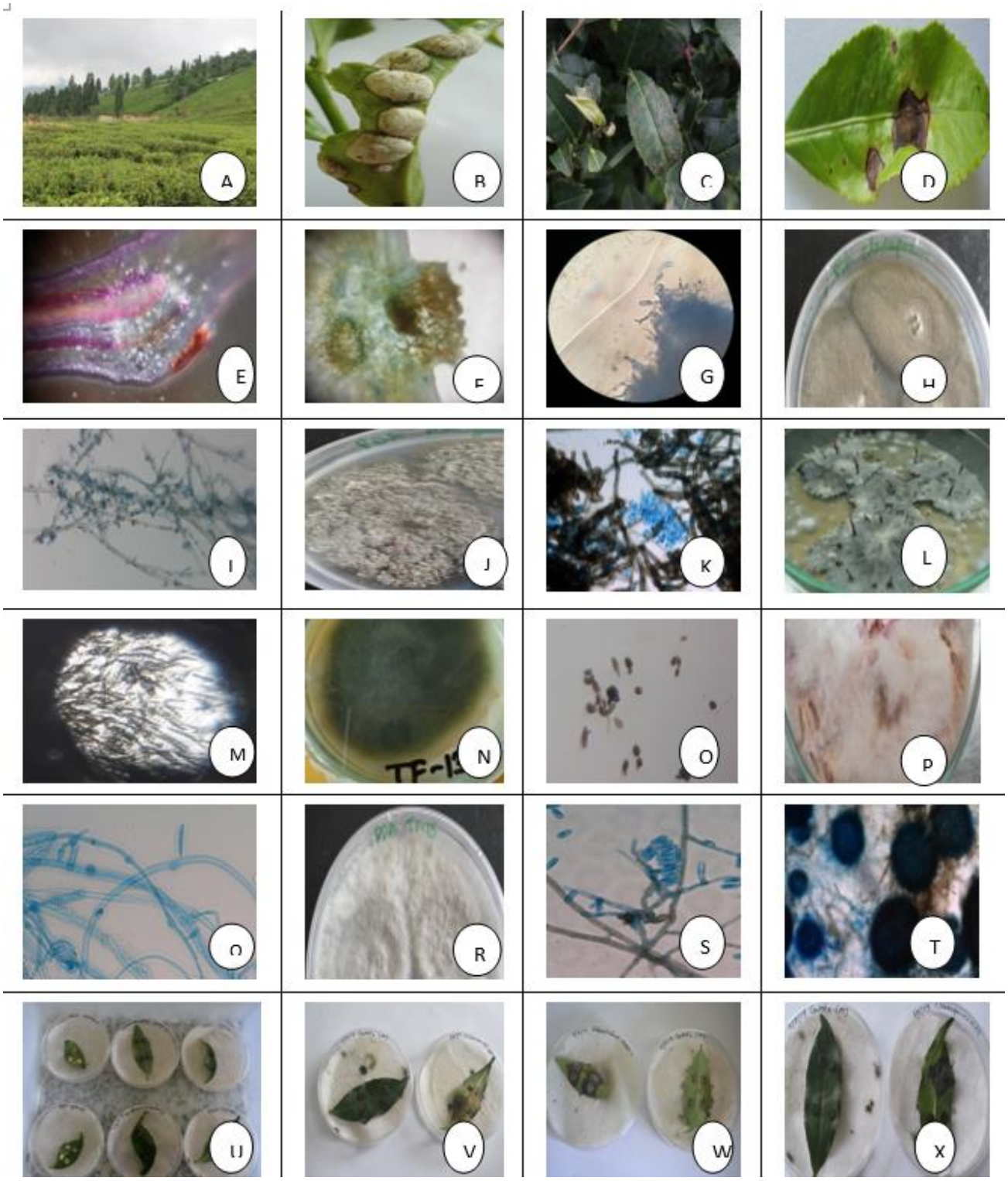

Figure 1. (A) Temi tea garden, Sikkim. (B-D) Tea leaves infected with leaf spot and blight symptoms. (E-G) Sectional view of leaf spot and blight seen through foldscope. (H-T) Fungal isolates on plate and corresponding spores, sclerotia. H-*Cladosporium* sp., J-*Rhizosphaera* sp., L-*Xylaria* sp., N-*Alternaria* sp., P-*Fusarium* sp., R- *Colleotrichum* sp. (U-X) Pathogenecity test on detached tea leaf. U-day one; V- Positive infection after a week with *Fusarium* sp., W-*Colleotrichum sp*., X- *Cladosporium* sp. against the control.

Conclusion

Early detection in the field itself is often critical to success in eradicating new diseases and most of the tea gardens are often located in remote places, foldscope can be a very useful diagnostic tool for the detection and identification of disease in the field itself. With this simple means the detection and identification of foliar fungal pathogens upto genus and to some species level was possible and these findings met the objectives of the present study. Since proper identification is essential for making sound pest management decisions molecular sequencing analysis can be taken up in future. We believe these results can provide researchers, farmers and gardeners with a reference for the detection, prevention and control of leaf spot and blight diseases in tea plant.

Acknowledgements

This work was funded by the Department of Biotechnology, Government of India as a part Indo-US foldscope project. The authors are grateful to the Assistant Manager and his team of Temi Tea Garden Sikkim. Authors are thankful to NFCCI-ARI for accession number.

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Cite this article as:

Lhanjey P. Wangdi, Arpan Pradhan & Srijana Mangar. Incidence and identification of foliar fungal pathogens from tea gardens of Sikkim using foldscope as research tool. *Annals of Plant Sciences* 8.9 (2019) pp. 3603-3615.

http://dx.doi.org/10.21746/aps.2019.8.9.1

Source of support: Department of Biotechnology, Government of India.
Conflict of interest: Nil.