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Research Article

A Preliminary Study on the Diversity of *Fusarium solani*, Causing Dieback Disease of Tea (*Camellia sp*) and its Alternate Hosts

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ABSTRACT

This study deals with the diversity among isolates of *Fusarium solani* of different locations of Dooars tea growing areas of West Bengal and its alternate host range. This pathogen causes dieback disease of tea and adversely affects crop production to the great extent. The pathogen was isolated from diseased tender shoot samples of various locations. These isolates were further, *in vitro* studied to assess their cultural and morphological variability, using potato dextrose agar medium. Results revealed that isolates exhibited huge variation in mycelial growth rate, texture, color, and sporulation. They produced dull-white, off-white, light pink, and violet-colored colonies. Some isolates produced fluffy whereas others produced flat colonies in plates. KBN-7 showed the highest mycelial growth rate followed by KBF-3, 2, 9, and 1, however, isolates KBF-5, 6, 8, and 4 were found to be slow growers in plate culture. Isolates showed differences in sporulation, as well. Isolate KBF-8 and 9 produced higher numbers of conidia, whereas KBF-1, 2, 5, and 6 produced moderate numbers of conidia. Isolate KBF-3 produced a fair number of conidia whereas isolate KBF-4 and 7 produced the least conidia and hence categorized them as poor sporulator. There was significant variation for germination of conidial among the isolates, also. To find out the alternate hosts of this pathogen, out of the seven other host plants studied, none was found to be an alternate host in support of its life cycle.

Keywords: Tea; Crop; Morphology; Variability; Sporulation; Host plant

INTRODUCTION

Tea, (*Camellia* sp) is an important plantation crop of India, mainly cultivated in North East and South India on an approximate area of In India, tea is cultivated in about 6.36 lakh hectares with a production of about 1,338 million kg made teas [1]. Among various diseases [2,3] dieback of the shoot, incited by *F. solani* [4] is considered as one of the most economically important foliar diseases of this crop responsible for huge crop losses because it directly infects the young shoots which are the fundamental input of manufactured teas [5]. The disease is characterized by necrosis of apex younger leaves and buds that gradually extend up to adjacent lower internodes and nodes (Figure 1a). The pathogen flourishes well under humid temperate climates, prevalent in North East India and West Bengal. Its incidence, generally, occurs from mid-April to the end of September in the Dooars area of West Bengal.

F. solani (*teleomorph Haematonectria haematococca* [6]; syn. *Nectria haematococca*) is a complex genus that has wide distribution as well as host range [7]. In addition to causing soil-borne diseases, it may also cause diseases of aerial parts of various host plants like ber, *Ziziphus jujuba* [8], plum [9], shisham [10], mango [11] and date palm [12]. Variability, in form of cultural, morphological, and or pathogenic, is the common phenomenon of this genus [13] due to wide variations in climatic conditions. This genus also has great adaptability depending upon the environmental situations. The present study was undertaken to generate preliminary information in connection with variation in the existing population of the pathogen in different tea growing localities of the Dooars region of West Bengal, with the ultimate aim to plan further strategies for successful management of disease by utilizing all possible approaches of integration.

MATERIALS AND METHODS

Sample collection and isolation of *Fusarium sp*

During the year 2013-14, dieback diseased samples (Figure 1b) were collected from different tea gardens of the Dooars region of West Bengal (Figures 1c and 1d), brought to the laboratory, and soon used for isolation of pathogen by adopting standard technique [14] with minor modifications. Samples were washed with distilled sterilized water, cut into small pieces (1 cm length), surface sterilized with 0.1% mercuric chloride solution for 15-20 seconds,

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followed by two subsequent washing with sterilized water and then plated into potato dextrose agar solidified plates. Plates were then incubated at room temperature for 5-7 days. Developed colonies were purified by hyphal tip culture technique and identified based on colony characteristics and conidial morphology, tentatively. Their identity was further reconfirmed with the available literature. Pure cultures were maintained on PDA slants in the refrigerator for further studies.

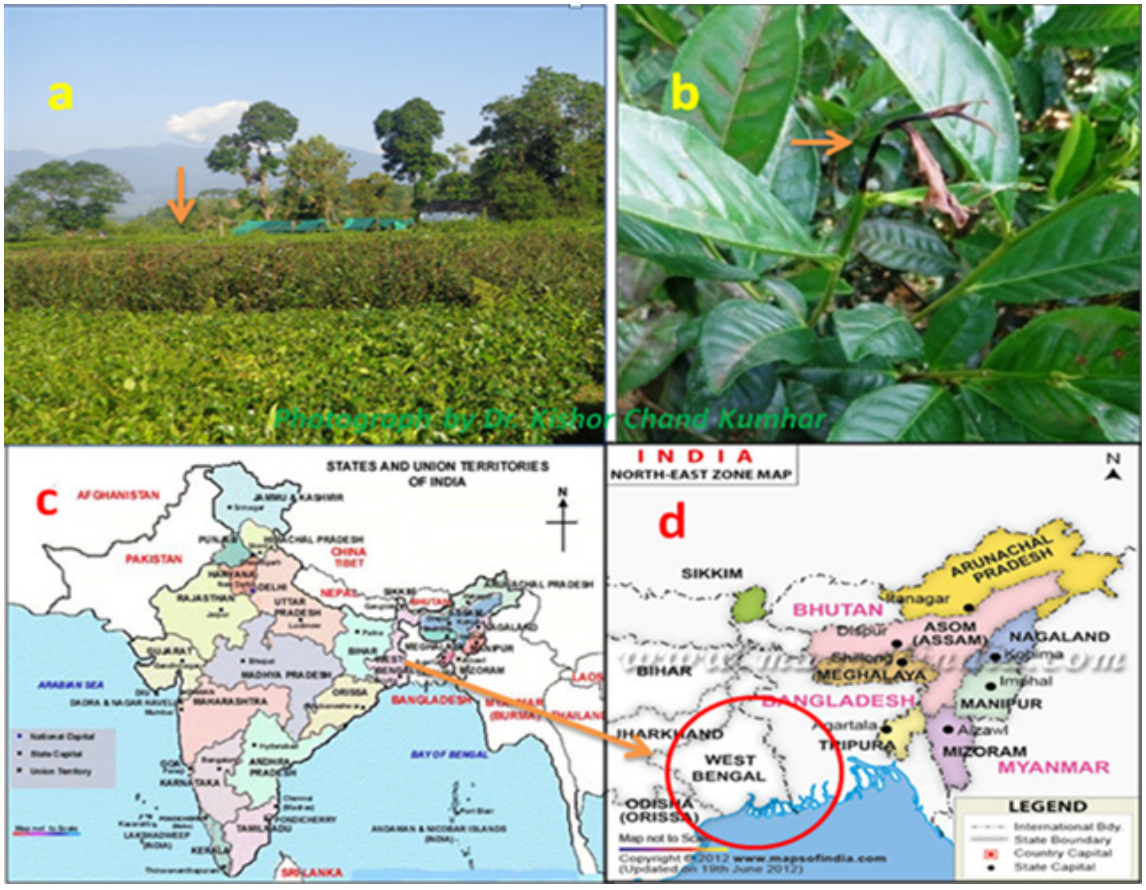

Figure 1: a) Tea field severely infected with die back disease; b) Diseased tender tea shoot; c) Map of india showing west Bengal; d) Locations of sampling.

In vitro cultural and morphological variability

The pure cultures of *F. solani* isolates were inoculated, separately, in the center of plates (90 mm diameter) containing potato dextrose agar as it is a suitable medium [15,16] and incubated at room temperature for 15 days. Colony characteristics such as colony diameter, texture, colony color, colony edge, etc. were recorded.

In vitro variability in conidiation

One month after inoculation, the conidial production efficiency of the isolates was assessed. For this purpose mycelial disc (5 mm diameter) were taken and dipped in conical flasks containing 10 mL sterilized water, homogenized by manually shaking. From this suspension, the sample was taken with the help of a surgical syringe and one drop was mounted on a glass slide, covered with a glass cover slide, finally viewed under a compound microscope (Olympus-BX-51) at 10 × zoom. Based on the number of viewed conidia, isolates were categorized as abundant, moderate, good, and poor spore producers as categorized by the earlier workers [13].

In vitro variability in conidial germination

A bit of 5 mm diameter of fungal culture (one-month-old) was taken with the help of a sterilized cork borer and transferred in conical flasks containing 100 mL distilled water and agitation was done properly. Two hundred fifty µL from it was inoculated in plates containing 2% agar, spread uniformly by spreader, and incubated at room temperature for 5 hours. Conidial germination was assessed by counting the germinated conidia from 5 locations in the plates, under the compound microscope at 10× zoom. Percent germination of conidia was calculated by the following formula.

Percentage of conidial germination=(Number of germinated conidia in the microscopic field ÷ Total number viewed conidia in the microscopic field) × 100

Alternate host plants of dieback pathogen

During the off-season (December–February 2014-15), weeds and other plants, available in and around the tea plantation area were surveyed, and their leaves, infected with leaf spot disease were collected. Isolation of pathogen was done by employing standard methodology [17] followed by their cultural and morphological identification.

Statistical analysis

Statistical analysis of data was done using the online statistical package OPSTAT of Chaudhary Charan Singh, Haryana Agricultural University, Hisar, Haryana, India.

RESULTS AND DISCUSSION

Identification of *Fusarium sp*

OIsolates produced dull white to pinkish colonies in PDA plates (Figure 2a). The micro-conidia were born on mono-phialides and long conidiogenous cells. They were oval-shaped, having 0-1 septa. However, macro-conidia were bigger and straight with a slight curvature. Although, there were 3-4 well-developed transverse septa, however, majority of them had 4 septa (Figure 2b), rarely 5 septate with pointed apical cell and barely notched basal cell. Based on these characteristics, the isolates were identified as *F. solani* and cross-check was done with available literature [18].

In vitro cultural and morphological variability

Isolates of *F. solani* showed mycelial growth variability in PDA slants as well as the plate culture method. In slant culture, all the isolates exhibited variation in mycelial growth rate and its coloration. Some isolates were multiplied at a very fast rate whereas some at a slower rate and produced dull white to light pinkish colored colonies. Cultural variability of *F. solani* isolates on PDA medium (Figure 3a).

When they were subjected to plate culture, variations in mycelia growth rate, pattern, and colony color were noted again. In plates, they produced flat to fluffy colonies of dull white to pinkish purple color with even or uneven edges (Table 1). Isolate KBF-4, KBF-5 produced dark creamish and violet-colored colonies. Isolates exhibited significant variability in mycelial growth rate that ranged from 50.3 mm to 84.3 mm on the 15th day of inoculation. Among the isolates, KBF-7 showed the fastest-growing behavior followed by KBF-3 and KBF-2, however, isolate KBF-5 was the slowest grower. None of the studied isolates could develop concentric rings (Table 2, Figures 3b and 3c).

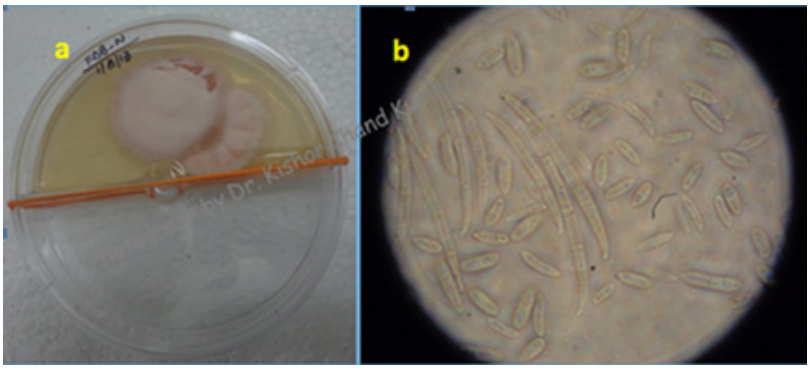

Figure 2: a) *F solani* pure culture on PDA plate; b) Macro and microconidia.

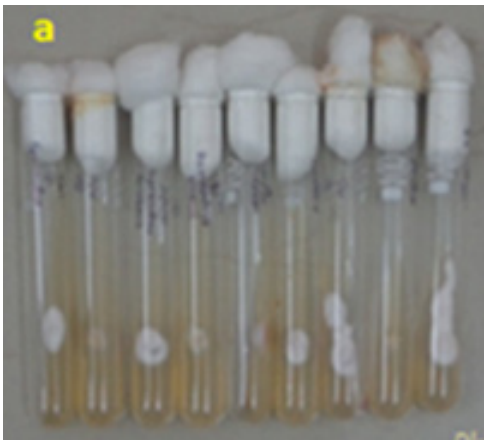

Figure 3a: Pure culture on PDA slant.

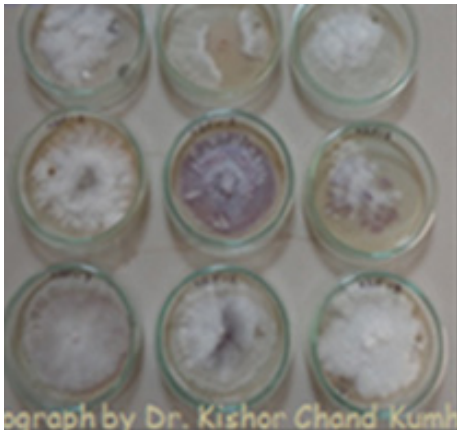

Figure 3b: Front view of culture plate.

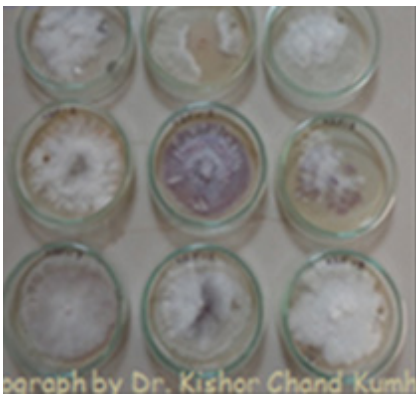

Figure 3c: Backside view of the cultural plate.

Table 1: Cultural and morphological variations among *F. solani* isolates.

F. solani isolate	Mycelial colony color	Concentric ring development	Mycelial colony edge/margin	Mycelial growth pattern
KBF 1	Purplish	-	Uneven	Fluffy
KBF 2	White	-	Uneven	Fluffy
KBF 3	White	-	Wavy even	Fluffy
KBF 4	White	-	Wavy even	Fluffy
KBF 5	Pinkish purple	+	Smooth even	Flat
KBF 6	Purple	+	Uneven	Flat
KBF 7	Pinkish	-	Wavy even	Fluffy
KBF 8	Pinkish	-	Wavy even	Fluffy
KBF 9	Creamish	-	Uneven	Fluffy

+ indicates presence of concentric ring and – indicates the absence of the ring

In vitro variability in conidiation

Results of conidial production efficiency revealed that all isolates produced the variable number of conidia. The highest number of conidia was produced by strain KBF-8 and KBF-9, whereas the least conidia could produce by KBF-6 (Table 2).

In vitro variability in conidial germination

As far as conidial germination is concerned, there was great variation among the isolates. The conidial germination ranged from 49.5% to 99.6%. The highest germination was noted in the case of isolate KBF-1 (99.6%) followed by KBF-9 (99.0) and KBF-7 (96.0). Isolate KBF-4 and KBF-5 exhibited comparatively less germination among tested isolates of *F. solani* (Figure 4).

Table 2: Mycelial growth variability of *F. solani* isolates of the tea crop.

F. solani isolate	Colony diameter (mm) after 15 days	Grade of sporulation after one month
KBF-1	79.0 (62.7 ± 1.0)	+++
KBF-2	80.7 (63.9 ± 0.6)	+++
KBF-3	81.0 (64.2 ± 1.5)	++
KBF-4	77.7 (62.1 ± 3 .8)	+
KBF-5	50.3 (45.1 ± 0.5)	+++
KBF-6	66.0 (54.3 ± 1.3)	+++
KBF-7	84.3 (66.7 ± 1.3)	+
KBF-8	70.7 (57.4 ± 4.0)	++++
KBF-9	80.0 (63.4 ± 0.8)	++++
CD	6.3	

Mean of 5 replications, figures in brackets are angular transformed values with ±SE
++++ = Abundant sporulation, +++ = Moderate sporulation ++ =Good sporulation, + =poor sporulation.

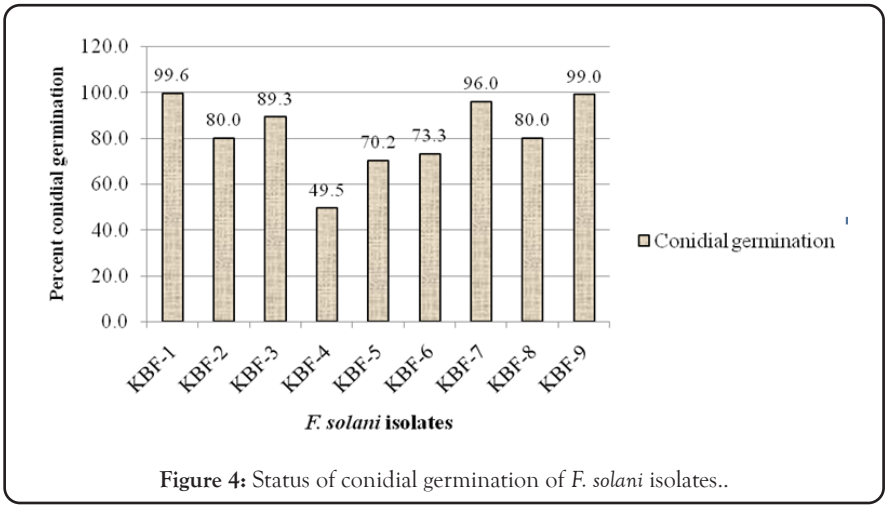

Figure 4: Status of conidial germination of *F. solani* isolates..

Table 3: Detail of weeds and other plants used for isolation of pathogen, *F. solani*.

Sr. No	Name of weed and other plant	Botanical name	Result of isolation of ***F. solani*** from diseased leaves
1	Shisham	*Dalbergia sissoo Rob.*	Negative
2	Indian Indigo	*Indigofera tinctoria Linn.*	Negative
3	Wild Colocasis (weed)	*Colocassia sp*	Negative
4	Weed	*Borarea hispida*	Negative
5	Weed	*Eupatorium odoratum*	Negative
6	Wild tulsi	*Ocimum sp*	Negative
7	Kadi patta	*Morraya koenigii*	Negative

Different *F. solani* isolates used for cultural and morphological variability produced colonies of a different color. Colonies were fluffy or plate on potato dextrose agar plates. Diffusion of pigmentation in media was noted with 2 isolates. All produced oval-shaped, one to two septate microconidia whereas macroconidia were banana-shaped and 3-4 septate. Similar morphological traits of conidia of this genus have been reported by earlier researchers [14, 19] which confirms our observations.

In the present investigation, isolates showed variation in the rate of mycelial growth rate, colony texture, colony edge, colony color, and sporulation. Similarly, earlier researchers investigated the cultural and morphological variability of several isolates of *F. solani* (Mart.) using potato dextrose agar medium and found significant variations in radial growth, size of macroconidia as well as microconidia of the pathogen [20]. Findings of previous workers also support our present results wherein it has been noted that the *F. solani f. sp. phaseoli* isolates exhibited high variability in colony characteristics on PDA and Synthetic Nutrient Agar (SNA) media. The growth of aerial mycelia was luxuriant to scanty with fluffy to fibrous texture having purple, pink, and white color. *F. solani* isolates showed variability in their cultural and physiological attributes when cultured on different culture media [21]. The isolates varied in their colony growth, mycelial biomass, macro-and microconidia production [22]. Morphological and molecular variability of *F. solani* isolates was studied and variations in colony growth, as well as sporulation, were reported [13] which confirm our results.

Alternate host plants of dieback pathogen

In this investigation, sheesham (*Dalbergia sisoo*), *Indigofera tinctoria*, *Colocassia sp*, *Borarea hispida*, *Eupatorium odoratum*, wild tulsi, and kadi patta (*Murraya koenigii*) were used to isolate the *F. solani*, however, the pathogen could not be recovered from any of the host studied (Table 3), indicating the fact that, the pathogen does not survive on these plants.

A few, locally grown plant as well as weed species were investigated to find out whether or not the same pathogen could survive on them during the off-season. Our results revealed that the pathogen did not survive on any of the studied plants; however, some of the earlier researchers could isolate this pathogen from several other plant species [8-12] which is contradictory to our present findings.

CONCLUSION

Pathogenic diversity is a common phenomenon that helps in the survival of an organism under different adverse conditions. The study is conducted to know about variations in cultural and morphological characteristics. The common plant species of the tea ecosystem showed the absence of *F. solani*. Such kinds of investigations would help in creating awareness for finding out the suitable plant protection measures for the management of *F. solani* under diversity.

RECOMMENDATION

This is a preliminary study including various aspects; however, to have a clear-cut idea on various parameters, the study of possible pathogenic isolates on a molecular level could also be undertaken for getting successful management. The tested plant species in

the tea ecosystem did not show the presence of phytopathogenic fungus and hence such plant species do not help in the survival of pathogens, therefore, there is no need to adopt plant protection measures for such plants.

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