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# Growth habit of Tea pathogens (*Cephaleuros* spp. and *Fusarium solani*) and evaluation of relative susceptibility of selected Tea cultivars

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#### Abstract

Tea diseases are considered as important biotic constraint, leads to significant tea crop losses. Incidence and disease severity of red rust (causal agents: Cephaleuros parasiticus and C. mycoidea) and Fusarium die-back (causal agent: Fusarium solani) in different tea growing areas of Assam was investigated in the present investigation. The results revealed that some of the tea cultivars are relatively susceptible to tea pathogens (Cephaleuros spp., and F. solani). Data within the same column followed by different letters are significantly different from each other (P < 0.05). Tea cultivars and the phenomenon of susceptibility to selected tea pathogens have important consequences for disease epidemiology and the effectiveness of management protocols.

Keywords: Fusarium die-back, Red rust, Relative susceptibility, Resistant cultivars, Tea diseases.

#### Introduction

Indian tea is grown in a wide amplitude of climatic variables at latitudes from  $8^{\circ} 12^{\prime}$  N in Nagercoil in Tamilnadu to  $32^{\circ}13^{\prime}$  E in Kangra in Himachal Pradesh<sup>1</sup>. Tea in India is appreciated as health drink for its unique flavour, aroma and medicinal properties<sup>2</sup>.

Tea plantations usually resemble to "single species forest"<sup>3,4</sup>. Climate plays an important role influencing the productivity of tea<sup>5</sup>. Tea plantations are mostly rain fed and the cropping season needs a moist climate with alternating wet and dry periods<sup>6</sup>. This stable micro climatic condition is suitable for the establishment of major tea pathogens like *Exobasidium vexans*, *Corticium* spp., *Fusarium solani*, *Cephaleuros* spp., *Poria hypobrunnea*, etc. causing tea diseases like blister blight, black rot, *Fusarium* die-back, red rust, *Poria* branch canker respectively. The pest and diseases of tea plants are seriously dealt with as early as late 19<sup>th</sup> century<sup>7</sup>. Incidence and intensity of pathogenic attack, however, varies with change in weather, elevation and the planting material.

Red rust and *Fusarium* die-back are two important diseases in tea growing areas<sup>8</sup> of N. E. India. *Fusarium* emerges as a pathogenic fungus causing serious maladies to seed-barries as well succulent clones<sup>9,10</sup> in and around the tea plantations. In *Fusarium* die-back, blackening of leaf petioles generally occurs that gradually extends to the aerial parts of the tea bushes (nodes and internodes), followed by wilting of the primaries. *F. solani* appears as white cottony growth on dyeing tissues that turns brown at its mature stage. This is accompanied with the formation of *Nectria* (small pink perithecia). Tea seeds are also severely affected in *Fusarium* die-back disease. Blackening of

fruit carp, immature cracking, dropping of tea seeds are usually observed during infection. Tea seeds turn into light pinkish due to fungus infestation. Dropping of immature seeds resulted in reduction of viable seed production. In recent years, die-back in certain succulent TV clones and cracking, pre-mature falling and decay of some biclonal seed stocks of tea were recorded, which causes considerable losses to the tea industry<sup>11</sup>.

The red rust disease was first reported on tea twigs<sup>12,13</sup> at Nagaon district, Assam, India. Red rust in tea is caused by two species of alga, under *Cephaleuros (Cephaleuros parasiticus* and *C. mycoidea*). Due to change in certain predisposing factors like soil pH, potash status of soil, shade and drainage conditions, etc., the incidence of this disease becomes epidemic in different tea growing areas<sup>13</sup>. The pathogen generally penetrates deep into the tissues of the host cells. *Cephaleuros* produces brick red/orange fructifications containing large number of spores in infected stems and leaves of tea bush. Due to the small size of the algal spores it becomes easy to transmit for a long distance<sup>14</sup>. Red rust causes severe damage especially to young tea plants by attacking or killing the stem and leaf tissues resulting die-back of the stem, *thus*, results an extensive annual crop loss.

Since, *in vitro* studies on growth habit of red rust and *Fusarium* die-back pathogens in tea using a culture based approach and field evaluation on relative susceptibility of tea cultivars against these diseases is still in scarce, the present investigation has been carried out for the examination of the growth habit of *Cephaleuros* spp. (*C. parasiticus* and *C. mycoidea*), the causal agents of red rust in tea stem and leaf respectively and *F. solani*, the causal agent of *Fusarium* die-back disease using a wide variety of selective media. Identification of relative

susceptibility among the planting materials (Tocklai and garden series) to these tea pathogens under field evaluation is another objective, the information on which would strengthen tea scientists to categorize and develop future disease management strategies in tea.

### **Materials and Methods**

Isolation and characterization of Fusarium die-back and red rust pathogens: A cultivation-based approach was used for the isolation of the pathogenic strains. For this, the diseased plant materials of the infected areas were first collected. For the isolation of responsible pathogen from the disease infected areas, the infected succulent shoots were collected from various tea estates and treated in accordance with Banerjee  $(1995)^{15}$  for pathogen screening. Infected portions were cut into small pieces and surface sterilized with 0.001% mercuric chloride for two minutes. The processed portions were ringed with sterile distilled water (SDW) for 5-6 times and transferred aseptically to the petridishes containing Potato dextrose agar (PDA) media. Petridishes were incubated at room temperature (25 °C) for 5-6 days and growth of the fungal mycelia was observed on the culture plates. Isolated pathogen was purified by repeated sub culturing using hyphal tip transfer method<sup>16</sup> on PDA. The microbe was preserved at 4 °C in the Culture collection laboratory (CCL), Mycology and Microbiology Department, TTRI, TRA for further use. Different nutritional media like PDA (peeled potato 200 g/l, dextrose 20 g/l, agar 15 g/l), Fusarium armstrong media (KH<sub>2</sub>PO<sub>4</sub> 1.09 g/l, KCl 0.22 g/l, FeCl<sub>3</sub> 6H<sub>2</sub>O 0.2µg/l, Ca (NO<sub>3</sub>)<sub>2</sub>4H<sub>2</sub>O 8.4 g/l, MnSO<sub>4</sub> 0.2µg/l, ZnSO<sub>4</sub> 0.2µg/l, Glucose 20 g/l), Rose bengal chloramphenicol agar (mycological peptone 5 g/l, dextrose 10 g/l, monopotassium phosphate 1 g/l, magnesium sulphate 0.5 g/l, rose bengal 0.05 g/l, chloramphenicol 0.1 g/l, agar 15 g/l), Okons' media (K<sub>2</sub>HPO<sub>4</sub> 6.0 g/l, KH<sub>2</sub>PO<sub>4</sub> 4.0 g/l, NaOH 3.0 g/l, NaCl 0.1 g/l, CaCl<sub>2</sub> 0.02 g/l, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.002 g/l, FeCl<sub>3</sub> 6H<sub>2</sub>O 0.017, Yeast extract 0.5 g/l, Malic acid g/l), Czapek-dox broth (NaNO<sub>3</sub> 3.0 g/l, K<sub>2</sub>HPO<sub>4</sub> 1.0 g/l, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.50 g/l, KCl 0.50 g/l, FeSO<sub>4</sub> 7H<sub>2</sub>O 0.01 g/l, Sucrose 30 g/l, Yeast extract 1.5 g/l) were used to determine the suitability of the growth medium for optimal growth of F. solani. Confirmation of the fungal pathogen was made in accordance with standard taxonomic monographs<sup>17-20</sup>. The fungal pathogen was grown on 100 ml of each of the liquid media mentioned above in 250 ml conical flask. Three replicates were maintained at each case. The biomass of the fungus was recorded after 15 days in vitro.

By adopting Banerjee  $(1995)^{15}$  for pathogen screening, *Cephaleuros* spp. was isolated from the infected portions in Algal culture broth media. To determine the favourable growth media for optimum growth of *Cephaleuros* spp., several algal growth media such as Trebouxia media (bristol's solution 850.0 mL/l, soil extract 140.0 mL/l, glucose 20.0g/l, proteose peptone 10.0, Bristol media (NaNO<sub>3</sub> 250.0 mg/l, K<sub>2</sub>HPO<sub>4</sub> 75.0 mg/l, KH<sub>2</sub>PO<sub>4</sub> 175.0 mg/l, CaCl<sub>2</sub> 25.0 mg/l, NaCl 25.0 mg/l, MgSO<sub>4</sub>.7H<sub>2</sub>O 75.0 mg/l, FeCl<sub>3</sub> 0.3 mg/l, MnSO<sub>4</sub> 4H<sub>2</sub>O 0.3 mg/l,

ZnSO<sub>4</sub> 7H<sub>2</sub>O 0.2 mg/l, H<sub>3</sub>BO<sub>3</sub> 0.2 mg/l, CuSO<sub>4</sub> 5H<sub>2</sub>O 0.06 mg/l), Blue green 11 (H<sub>3</sub>BO<sub>3</sub> 2.68 g/l, MnCl<sub>2</sub> 1.81 g/l, ZnSO<sub>4</sub> 7H<sub>2</sub>O 0.22 g/l, Na<sub>2</sub>MOO<sub>4</sub> 2H<sub>2</sub>O 0.39 g/l, CuSO<sub>4</sub>7H<sub>2</sub>O 0.079 g/l, CO(NO<sub>3</sub>)<sub>2</sub>6H<sub>2</sub>O 0.049 g/l), Proteose media (added 15 g of proteose peptone in Bristol medium; mentioned above), Algae culture broth (NaNo<sub>3</sub> 1.0 g/l, K<sub>2</sub>HPO<sub>4</sub> 0.25 g/l, MgSO<sub>4</sub>.7 H<sub>2</sub>O 0.513 g/l, NH<sub>3</sub>Cl 0.05 g/l, CaCl<sub>2</sub> 0.058 g/l, FeCl<sub>2</sub> 0.003 g/l), Solution for algae (Soil bacteriology-Fred no-77), (MnNO<sub>3</sub> 0.05 g/l, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.2 g/l, CaCl<sub>2</sub> 0.1 g/l, FeSO<sub>4</sub> 7H<sub>2</sub>O 0.01 g/l, K<sub>2</sub>HPO<sub>4</sub> 0.2 g/l), Solution for algae (Soil bacteriology-Fred no-78), CaNO<sub>3</sub> 0.05 g/l, MgSO<sub>4</sub>.7H<sub>2</sub>O 0.2 g/l, KCl 0.1 g/l, FeCl<sub>2</sub> trace g/l, FeSO<sub>4</sub> 7H<sub>2</sub>O 0.01 g/l, KH<sub>2</sub>PO<sub>4</sub> 0.5 g/l), Tea leaf extract medium, (fresh tea green leaf 200.0 g/l, peptone 1.5 g/l, NaCl 0.5 g/l), Tea root extract (fresh tea root 175.0 g/l, peptone 1.5 g/l, NaCl 0.5 g/l), Beneck's media (KH<sub>2</sub>PO<sub>4</sub> 8.75 g/l, CaCl<sub>2</sub> 1 ml/l, MgSO<sub>4</sub> 3.75 g/l, NaNO<sub>3</sub> 12.5 g/l, K<sub>2</sub>HPO<sub>4</sub> 0.5 g/l, NaCl 1.0 g/l, MgSO<sub>4</sub> 0.2 g/l, EDTA 10 g/l, H<sub>2</sub>SO<sub>4</sub> 1 ml/l) were used in vitro. Confirmation of the algal pathogen was made using taxonomic monographs<sup>21</sup>. The pathogen, C. parasiticus was grown on 50 ml of each of the liquid media mentioned above in 100 ml conical flask. Three replicates were maintained at each case. The dry weight of the algae was recorded after 180 days of growth in vitro.

Determination of relative susceptibility under field evaluation: A number of TV clones as well seed stocks are selected to determine their relative susceptibility to Fusarium *die-back* and red rust pathogens. Relative susceptibility of the planting materials was evaluated against the target pathogens by conducting survey and field assessment in different tea estates of Assam, N. E. India. The experimental clones were screened for possible disease incidence of red rust and Fusarium dieback. The intensity of infection was measured in accordance with a modified 0-4 scale method<sup>22</sup>, on the basis of percent area of infections/bush or /leaves<sup>23</sup>. The 0-4 scale method was used as follows; 0 = No infection,  $1 = 1 \sim 25\%$  infection,  $2 = 25 \sim 50\%$ infection.  $3 = 50 \sim 75\%$  infection and 4 = >75% infection. Scoring is calculated on the basis of 0-4 scale method of disease severity, where scoring of individual bushes were recorded through direct field monitoring for disease incidence and by totalling every individual score the final score per clone is measured. Here, hundred numbers of tea bushes was considered for every clone under observation.

**Data analysis:** Duncan's Multiple Range Test (DMRT) comparisons were made for different treatments to test the significance at 5% level using SPSS software 16.0. Biomass data were analyzed where necessary using MS-Excel.

#### **Results and Discussion**

Tea plants harbour an ideal host for the red rust and *Fusarium* die-back pathogens. Figure-1 represents the photographs of *Cephaleuros* infected tea stems and leaves that were observed during field evaluation for disease incidence and further identified as red rust pathogen. Active algal fructifications were

observed in *Cephaleuros* infected tea stems and leaves during disease severity.



Cephaleuros parasiticus



# Cephaleuros mycoidea

#### Figure-1

#### Infection of red rust on tea stems. a. active fructifications of *C. parasiticus* b. Infection of *C. mycoidea* on the tea foliages showing orange-red large spots bearing the alga fructifications

Figure-2 represents the die-back symptoms of *Fusarium* dieback disease. Microscopic observation indicates the formation of microconidia, macroconidia, chlamydospores and Nectria. In the present investigation, maximum disease incidence was recorded during humid conditions both in plains and hills. A similar experiment to determine the susceptibility of apple cultivars to apple scab disease caused by *Venturia inaequalis* was made by Biggs et al. (2010)<sup>24</sup>. They noticed that relative differences in apple scab incidence among different planting groups remained consistent at different locations. Relative susceptibility of *Vitis vinifera* cultivars to vector-borne *Xylella fastidiosa* through time was investigated<sup>25</sup>. Variability in

symptom development among different tea cultivars with changing time and location indicates the role of incubation periods as well topographical alterations influencing the occurrence of *Fusarium* die-back and red rust disease incidences. Topography might influence the quantity and diversity of microbial populations<sup>26</sup>. Growth stage of the planting material might play vital role influencing the pathogen infection<sup>27</sup>, although it may vary with plant species. It is observed in the present investigation that succulent young tissues of the tea plants (leaves and shoots) were generally more susceptible to the infection by the pathogenic genera *Cephaleuros* and *Fusarium* respectively.

A cultivation based approach was used in the present investigation to screen out the microbial pathogens as the approach permit *in vitro* culture of microorganisms<sup>28</sup>. A wide variety of culture media were used to find out the maximum growth of target pathogens. Table-1 represents the biomass of *F. solani* in different growth media. Out of the media used for isolation and growth of *F. solani*, CDB supported maximum vegetative growth of the pathogen (3.74 gm) at pH 6.5. PDA and Armstrong media showed moderate growth of the fungal pathogen. Thus, it is evident that the pathogen grows variably on different nutritional medium. Isolation of *F. solani* using different nutritional media was investigated<sup>20</sup>.

 Table-1

 Biomass of F. solani in different growth media

Different media	*Biomass of F. solani (in gm)
Potato dextrose agar	2.59
Potato sucrose agar	0.66
Armstrong media	1.33
Rose bengal media	0.16
Okon's media	0.24
Bilays media	0.04
Glucose water	0.03
Pikovskaya media	0.35
Coon's media	0.06
Czapek dox broth	3.74
CD	0.142
CV	9.13
SEM(±)	0.0486

\*Data are the mean of three replicates, ±S. D.

The frequency of fungal isolation using different media was also practiced<sup>29</sup>. V8 juice agar media was examined as most effective during their investigation for maximum recovery of fungal species. Table-2 represents biomass data of *C. parasiticus* and *C. mycoidea* in different nutritional media. There are remarkable growth variations in the algal pathogens.

The result corroborates Sarmah et al.<sup>20</sup> who too observed significant variations in radial growth of F. solani at differing media compositions. Optimum growth of Cephaleuros spp. was observed in Algal culture broth followed by Bristol broth and Blue Green 11 media respectively at pH 7.5-7.9 in ambient temperature. Alterations of one or more number of nutrients might have promotional or detrimental effects on the recovery of microbial species. Growth habit of the test pathogens in nutrient media is shown in Figure-3(a-c). A clear mat of fungal mycelium was observed in the conical flask inoculated with F. solani. Cephaleuros spp., produce green pigmentations in the culture broth. Variations in susceptibility of selected tea cultivars against the pathogenic strains, F. solani, C. parasiticus and C. mycoidea were evaluated (Table-3a-c). Values within the same column followed by different letters vary significantly from each other (P < 0.05).

Table-4 indicates the relative susceptibility of selected tea cultivars against the tested pathogens. The data was generated based on the variations in susceptibility levels under field evaluation. A similar experiment on banana cultivars to *Xanthomonas campestris* pv. *Musacearum* was worked out<sup>30</sup>. The *in vitro* as well field trials to determine the relative susceptibility of selected apple cultivars against *Colletotrichum acutatum* was performed<sup>31</sup>. They classified the apple cultivars into four relative susceptibility groups such as most susceptible; highly susceptible; moderately susceptible and least susceptible.

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Table-2
Biomass of C. parasiticus and C. mycoidea in different
nutritional media

Different media	*Biomass of <i>C.</i> <i>parasiticus</i> (in gm)	*Biomass of <i>C.</i> <i>mycoidea</i> (in gm)
Trebouxia media	0.09	0.08
Bristol broth	0.19	0.13
Blue green- 11	0.15	0.10
Proteose media	0.08	0.08
Algae culture broth	0.22	0.14
Solution for algae (Soil bacteriology- Fred no-77)	0.04	0.06
Solution for algae (Soil bacteriology- Fred no- 78	0.02	0.08
Tea leaf extract	0.10	0.07
Tea root extract	0.09	0.06
Beneck's media	0.15	0.09
CD	0.0251	0.0174
CV	13.13	11.37
SEM(±)	0.0086	0.0059

\*Data are the mean of three replicates, ±S. D.



# Fusarium solani infected tea twigs

Figure-2 Fusarium die-back disease caused by Fusarium solani



Figure-3 Growth pattern of tea pathogens. a. *F. solani*; (b-c). *Cephaleuros* sp.

Var	iations in relative suscep	ptibility among differe	nt tea cultivars agains	t red rust (stem) dise	ase
Sl. No.	TV Clones	TG I	TG II	TG III	TG IV
1	TV 1	63abc	64ab	91a	83a
2	TV 8	57abcd	58bc	58bc	65ab
3	TV 9	52bcd	53bd	57bc	54bc
4	TV 17	46bcd	47cde	52c	46c
5	TV 18	40d	41de	46c	52bc
6	TV 23	43cd	44cde	45c	51c
7	TV 25	57abcd	55bcd	61bc	54bc
8	TV 26	62abc	63ab	58bc	56bc
9	Teenali 17	75a	73a	68b	74ab
10	P126	65ab	64ab	66b	62bc
11	TS446	52bcd	51bcde	58bc	46c
12	P7	54abcd	52bcde	45c	61bc
13	S3A3	37d	38e	48c	52bc
14	TS397	37d	41de	47c	43c

Table-3a Variations in relative suscentibility among different tea cultivars against red rust (stem) dise

TG I: Tea Garden I; TG II: Tea Garden II; TG III: Tea Garden III; TG IV: Tea Garden IV.

Values within the same column followed by different letters are significantly different from one another (P < 0.05).

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	Table-3b	
Variations in relative susce	otibility among different tea	cultivars against red rust (leaf) disease

Sl. No.	TV Clones/seed stock	TG I	TG II	TG III	TG IV
1	Teenali	73a	70ab	65a	70a
2	P 126	36b	67ab	62a	61a
3	446	38b	40b	42a	47a
4	T3E3	39b	46ab	56a	61a
5	Р7	54ab	44ab	48a	52a
6	S3A3	61ab	63ab	70a	56a
7	TA17	37b	44ab	56a	50a
8	TS397	37b	48ab	42a	47a
9	TV1	54ab	81a	55a	62a
10	TV9	47ab	67ab	34a	58a
11	TV17	61ab	73ab	63a	60a
12	TV18	73ab	35b	47a	50a
13	TV20	51ab	67ab	44a	49a
14	TV23	37ab	66ab	57a	56a
15	TV25	52b	67ab	37a	41a
16	TV26	37ab	62b	44a	56a
17	TV30	40ab	34ab	46a	52a

TG I: Tea Garden I; TG II: Tea Garden II; TG III: Tea Garden III; TG IV: Tea Garden IV. Values within the same column followed by different letters are significantly different from one another (P < 0.05).

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Varia	Variations in relative susceptibility among different tea cultivars against <i>Fusarium</i> die-back disease				
Sl. No.	TV Clones	TG I	TG II	TG III	TG IV
1	TV1	62abcd	65ab	79a	72a
2	TV9	53bcdef	54bcdef	53cd	53bcd
3	TV17	42def	34g	0e	0e
4	TV18	45cdef	50bcdefg	50cd	Obc
5	TV20	38ef	45cdefg	51cd	45cd
6	TV22	42def	46cdefg	0c	0cd
7	TV23	45cdef	Oh	50cd	0e
8	TV25	42def	48bcdefg	0e	0b
9	TV26	56abcde	58abcde	48cd	4cd
10	TV28	48cdef	47bcdefg	42d	0e
11	TV29	53bcdef	48bcdefg	0e	0e
12	TV33	63abc	63abc	74ab	0e
13	Teenali17	73a	Oh	0e	0e
14	P126	64abc	72a	68ab	75e
15	446	68ab	61abcd	75a	0e
16	T383	74a	64ab	61bc	0e
17	TA17	61abcd	Oh	0e	0e
18	P38	35f	Oh	0e	0e
19	Nokhroy	Of	Oh	0e	0e
20	Betjen	39ef	39fg	0e	0e
21	S3A3	51bcdef	40efg	0e	0cd
22	NP4	51def	43defg	49cd	0d

 Table-3c

 Variations in relative susceptibility among different tea cultivars against *Fusarium* die-back disease

 22
 NP4
 51def
 43defg
 49cd
 0d

 TG I: Tea Garden I; TG II: Tea Garden II; TG III: Tea Garden III; TG IV: Tea Garden IV. Values within the same column followed by different letters are significantly different from one another (P < 0.05).</td>
 0d

<b>Relative susveptibility</b>
,TV 9, TV 17, TV 18, TV 20, TV 23, TV 25, TV 26, TV 30, Teenali 17, P 126, TS 446, T3E3 P7, S3A3, N436, TA 17, TS397
V 1,TV 8, TV 9, TV 17, TV 18, TV 23, TV 25, TV 26, Teenali17, P 126, TS 446, P7, S3A3, TS397
1,TV 9, TV 17, TV 18, TV 20, TV 22, TV 23, TV 25, TV 26, TV 28, TV 29, TV 33, Teenali17, P 126, TS 446, T3E3, TA 17, P-38, Nokhroy, Betjan, S3A3, NP4.

 Table 4

 Relative susceptibility of tea cultivars against red rust and fusarium die back diseases

# Conclusion

*In vitro* studies on growth behaviour of tea pathogens are important for better understanding of the nutritional habit of tea pathogens. The present field survey and data analysis serves as a preliminary source in documenting the resistant tea cultivars against red rust and *Fusarium* die-back pathogens, the knowledge on which is important to select resistant tea varieties. Susceptibility determination among tea cultivars against major tea pathogens are essential steps towards development of systemic acquired resistance. However, within individual cultivars the degrees of resistance are not regular across different sampling points and time.

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