

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.511.013>

**An *in vitro* Assessment on *Fusarium* Decomposition in Tea**

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**ABSTRACT**

Tea [*Camellia sinensis* (L.) O. Kunze] is an aromatic beverage yielding perennial plant. The manufactured tea is commonly prepared by pouring hot or boiling water over cured leaves of the *Camellia sinensis*, an evergreen shrub native to Asia. After water, it records the most widely consumed drink all over the world. India is the second largest producer of tea in the world after China, including the famous Assam tea and Darjeeling tea. However, as the Indian subcontinent is mostly rain fed and the cropping season also needs a moist climate with alternating wet and dry periods, the micro-climate prevailing the region is, thus, prone for the establishment of major tea pathogens like *Fusarium solani*, responsible for *Fusarium* die-back disease. Present findings illustrated the impact of an incubation period of 60 days for maximum decomposition (up to 17.4% over untreated control) of the *Fusarium* treated tea twigs. *Nectria* formation after 60 days of incubation and softening of stem tissue indicated the decomposition of the fungus treated twigs due to the prominent growth of *F. solani*. Three replicates were maintained at each case and all the data were statistically designed.

**Keywords**

*Fusarium* die-back,  
micro-climate,  
*Nectria*,  
tea,  
tea pathogens.

**Article Info**

Accepted:  
04 October 2016  
Available Online:  
10 November 2016

**Introduction**

Tea diseases are considered as important biotic constraint that leads to significant crop losses all over the world (Sharma *et al.*, 2016). The stable micro climatic condition in Indo-Burma Mega Biodiverse region is suitable for the establishment of major tea diseases such as blister blight, black rot, *Fusarium* die-back, red rust, *Poria* branch canker etc.

*Fusarium* die-back is an important disease in the tea growing areas of N. E. India (Barthakur, 2011). *Fusarium* as a pathogenic

fungus, responsible for creating serious maladies to seed as well as the succulent clones (Sharma *et al.*, 2010) in and around the tea plantations. The severities of *Fusarium* infestation in tea is presently creating devastation among some of the tea sections of Assam and Darjeeling, as the pathogenic attack is sometimes reported as clone specific. Blackening and die-back of tea twigs due to *Fusarium* infection is becoming a common scenario at most of the tea gardens. In *Fusarium* die-back, blackening of the leaf petiole occurs that

gradually extends to the nodes and internodes, followed by wilting of the primaries. According to Barthakur and Debnath, (2011), tea dieback disease caused by *F. solani* is one of the most destructive diseases in North East India responsible for huge crop loss, as it infects the young pluckable shoots. Fungicides namely Copper oxychloride @1:400, Carbendazim @1:400, Copper hydroxide @1:400, Mencozeb@1:400, Hexaconazole@1:1000, Propiconazole @1:1000 were found as effective in controlling the *Fusarium* die-back disease in tea caused by *F. solani* (Sharma *et al.*, 2010). Kumhar *et al* (2015) found *Trichoderma virens* as an effective biocontrol agent against this pathogen and utilized it for the management of die-back. Some planting materials (clones) such as the series of clones of Tocklai Vegetatives such as TV1, TV 19, TV 20, TV 23, TV 25, TV 26, TV 29 and S3A3, S3A1, Tinali17 etc., are more susceptible to the disease. *Fusarium* may cause extensive damage to tea bushes, even in serious cases leads to bush death. Tea seeds mainly TS 491 and TS 520 and their parent clones are recorded susceptible, resulting in blackening of fruit carp, followed by immature cracking. In affected seeds, the symptom turns into light pinkish due to fungus infestation followed by seed drop. Thus, seed cracking, decaying of some biclonal seed stocks, pre-mature falling of leaves, dyeing of the aerial parts of the productive tea bush due to *Fusarium* infestation causes considerable losses to the tea industry.

Keeping the point under consideration, an *in vitro* approach has been designed to understand the effect and severity of *Fusarium solani* on tea bushes. The time period required by the pathogenic fungus to decompose the planting material is also discussed here. Plant protection code (PPC) for disease management in tea introduced by

Tea Board, Govt. of India, is also mentioned here as part of the control strategies for this emerging disease.

## **Materials and Methods**

### **Sample collection**

Twigs of mature tea, Tocklai Vegetative clone (TV1) were collected from experimental garden of Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat 785008, Assam, India. *Fusarium solani* strain was taken from the Culture Stock Laboratory (CSL) of the Mycology and Microbiology Department, TTRI, TRA, Jorhat. The pure culture was sub-cultured and used separately for the present experimental purpose.

### ***In vitro* assessment of *Fusarium* decomposition**

The method described by Wei *et al.*, (2013) was followed to determine the fungal decomposition. For this, the collected tea twigs were properly washed and chopped into pieces of approximately 1.0 cm in size and soaked in 4% sugar solution for 3 hrs. The sugar solution was drained out, keeping only the treated twigs. Nine consecutive conical flasks (500 ml) each containing 100 gm of treated twig pieces were sterilized in an autoclave at 121 °C (equivalent to 15 lb/inch<sup>2</sup>) for 15-20 mins. After sterilization, the treated pieces were inoculated with fresh strain of *F. solani*, the responsible pathogen for causing *Fusarium* die-back disease in tea. The flasks were further incubated at 26 °C in an incubator and observations for changing weight of the flasks as well spore development were recorded after 30, 45 and 60 days of incubation. Another set of flask was taken as control, which consists of cut tea twigs without *Fusarium* inoculation. Finally, the decomposition rate of the tea

twigs with *F. solani* was calculated and compared with control. The data were statistically analyzed.

**Results and Discussion**

Maximum decomposition of the *Fusarium* treated tea twigs were observed after 60 days of incubation (Table 1). The decomposition was up to 17.4% over untreated control. The initial weight of the treated tea twigs also showed a progressive reduction with increase in incubation period over untreated control. On the other hand, the untreated twigs (control) showed minimum weight loss due to evaporation and, thus, they remained as dry and healthy. Plant material decomposition is often considered as microbial composting activities (Awasthi *et al.*, 2012). Fig. 1 shows the photographs of *Fusarium* treated tea twigs after 45 days of incubation compared with untreated control. *Fusarium* infestation was comparatively less on the tea twigs that were initially kept for 30 days of

incubation period.

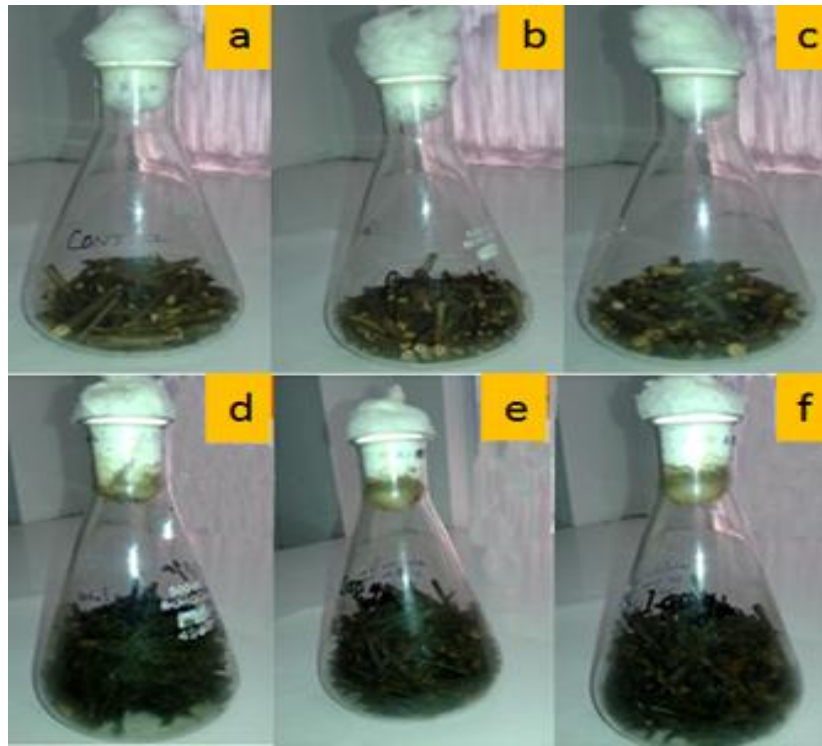
However, there are appearances of small orange colored fructifications after incubation of the tea samples for 45 days. Softening of the stem tissue followed by mature *Nectria* development (orange colored fruiting bodies) after 60 days of the incubation experiment indicated the decomposition of the fungus treated twigs due to the prominent growth of *F. solani* (Fig. 2a-c). Variations in the *Fusarium* decomposition in tea might be attributed to the change in incubation periods and developed moisture content in the stored samples. It is inferred that the temperature and incubation time period considered in microbial decomposition process is a good indicator of the bio-oxidative phase till its time of completion (Buyuksnomez *et al.*, 2000). Decomposition process gets matured when the temperature remains more or less constant and does not vary with the turning of the material.

**Table.1** *Fusarium* decomposition in tea in different time intervals.

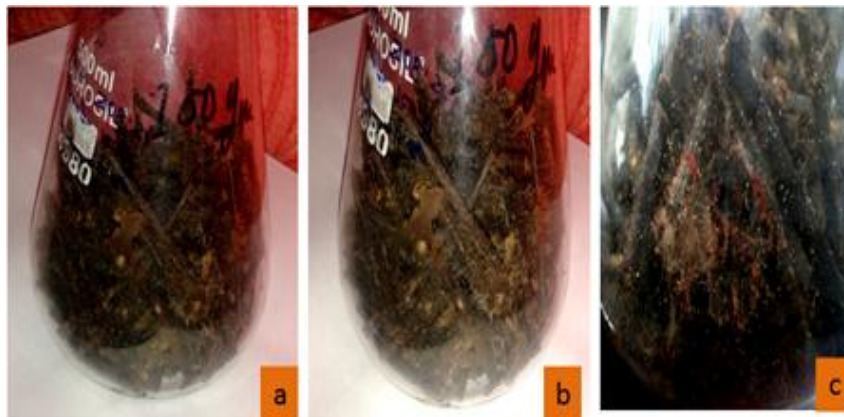
Target Specimen	Initial weight (g)	Final weight (g)		
		Incubation time		
		30 Days	45 Days	60 Days
<i>Fusarium</i> inoculated tea twigs	100	97.2	86.3	80.5
Control	100	99.85	99.24	98.13
Rate of decomposition over control (%)		2.65	13.0	17.4
	CD at 5%	0.0863	0.6233	0.0853
	CV (%)	0.0521	0.3996	0.057
	SEM (±)	0.030	0.21	0.030

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

**Fig.1(a-f)** Photographs showing control (a-c) and *Fusarium* treated tea twigs (d-f) after 45 days of incubation.



**Fig.2(a-c)** Photographs showing stages of Nectria development after 60 days of incubation.



### Management practices

The concept of tea disease management has undergone a considerable change over the last few decades. Presently, spraying with Hexaconazole @1:1000, two rounds at 15 days interval have been suggested for

application on *Fusarium* infected tea bushes for effective management. While, in case of seed infection, application with Hexaconazole @1:1000, in the affected seed baries in every month starting from July to December is recommended. As part of integrated disease management programme,

efforts have also been generated and popularized by TTRI, TRA to adopt non-chemical control approaches. The infected shoots should be cut and burned properly away from the diseased sections. Infected sections should preferably undergo shorter pruning cycles. There is also potentiality in using herbal extracts as well as antagonistic microbials as an alternative safer technology in reducing the die-back disease severity in tea plantations of Assam, N. E. India (Sharma *et al.*, 2009).

### Acknowledgements

The authors are thankful to the Director, Tocklai Tea Research Institute (TTRI), Tea Research Association (TRA), Jorhat, Assam, India for providing the necessary facilities to undertake the study.

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### How to cite this article:

Pranaba Nanda Bhattacharyya, Prasanta Dutta, Satya Ranjan Sarmah, Amarjyoti Tanti, Mausomi Madhab and Rofika Begum. 2016. An *in vitro* Assessment on *Fusarium* Decomposition in Tea. *Int.J.Curr.Microbiol.App.Sci*. 5(11): 121-125.  
doi: <http://dx.doi.org/10.20546/ijcmas.2016.511.013>