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Research Article

An Investigation on Fusarium Wilt Disease of Tea Caused by *Fusarium oxysporum* in Southwest Ethiopia

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ABSTRACT

Tea *(Camellia sinensis)* production is constrained by fusarium wilt disease in Ethiopia. Despite this, there is lack of information on the status of the disease and its associated factors, and lack of knowledge on features of the pathogen associated with the disease as there have been no any systematic assessments conducted so far. Therefore, the current works were designed with the objectives to assess the distribution of the disease and its associated factors, identify the associated pathogen and determine the pathogenicity of the pathogen isolates. For this purpose, field surveys were carried out across three tea estate farms and tea out growers surrounding them in Southwest Ethiopia during the 2019 season. Causative pathogen of the disease was identified using cultural and morphological features. The average fusarium wilt incidence varied from (0 to 20%). The disease intensity was positively and strongly associated with altitude (r=0.83) and positively and intermediately associated with rainfall (r=0.85), but the disease intensity was negatively and strongly associated with maximum temperature (r=-0.84) and negatively and weakly associated with minimum temperature (r=-0.31). The colony growth rate of the isolates was ranging between 8.38 and 9.00 mm/day. F. oxysporum isolates produced mycelia with floral white front and back side color, circular in form, flat in elevation, filiform margins and produced in abundance. The macroconidial shape of all the isolates was slightly curved with curved apical shape, footed basal shape and an average of 3 septa per macro conidia whereas micro conidial shape of all the isolates was fusiform without septation. The present study revealed the economic importance of fusarium wilt disease of tea in Southwest Ethiopia. Future research should be directed towards the investigation and determination of management options for the control of this important disease of tea in the country.

Keywords: Chewaka; Gumaro; Intensity; Root rot; Tea Bushes; Wushwush

INTRODUCTION

Tea (*Camellia sinensis* (L.) Kuntze) is one of the most commonly consumed beverages next to water by a wide range of age groups in all levels of society [1]. However its production and productivity is declining due to unusual weather and large number of insect pests and diseases. Fungal diseases are one of the major constraints of tea production and productivity in many tea growing countries in the world. About 5-10% crop loss has been estimated due to disease incidence, while crop loss has been increasing which is as high as 15-25% [2]. Pest damage in China decreased the yield by 10-20% in an average a year [3]. The report indicated that 10-15% crop loss due to disease in normal condition and as high as 100% in severe cases [4].

Tea root rot disease is one of the major constraints in tea production specifically. With a 1% loss of bushes which is owed to root disease, in the 10th years the loss of the potential crop would be 10% [5]. Root rot disease in tea bushes can be caused by several pathogens. Some of the pathogens are host-specific and some have a broader range of hosts. Reported root rot pathogens can belong to different groups such as bacteria [6] virus [7], oomycetes [8] and fungi [9]. The transmission of these pathogens is facilitated by root nematodes and other parasites which made wounds to the plant and enabling the entrance of other pathogens. Fungi are the most abundant causal agents of root rot and they can stay dormant and overwinter in debris and infested soil for several years [10].

Tea root rot disease is becoming an important and epidemic disease in tea estate farms in Ethiopia. Tea is produced in Southwest part of Ethiopia where the damage by tea root rot disease is severe. Yet the disease is recurring every year and spreading to the neighboring tea fields of tea estate farms of the country. Although, the disease becomes very important disease in Ethiopia, there is limitation of quantified data that reflect the extent of its distribution across tea estate farms in the country. Even though, root rot disease on tea

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lasts many years in Ethiopia, the features of the causative pathogen have not yet been characterized in Ethiopia. Therefore, the present study was initiated with the following objectives:

- To assess the distribution and significance of tea root rot in Southwest parts of Ethiopia
- To identify the major factors associated with Fusarium wilt disease of epidemics.
- To identify root rot causing pathogen in southwest parts of Ethiopia
- To determine the pathogenicity of the pathogen isolates

MATERIALS AND METHODS

The present study was conducted in Southwest Ethiopia during 2019 cropping season. The study consisted of four components viz. Disease survey to assess the occurrence, distribution and relative importance of fusarium wilt disease of tea; assessment of factors associated with the disease intensity; a laboratory work to characterize the disease causing pathogen; and determination of the pathogenicity of the isolates.

Description of the study areas

A survey was carried out during July to last August 2019 at the major tea producing farms of southwest Ethiopia to ascertain the intensity of fusarium wilt diseases on tea bushes. Ilubabor zone from Oromia regional state; and Sheka and Kaffa zones from SNNPRS were included in the survey program. Wushwush, Gumaro and Chewaka tea estate farms and their surrounding newly emerging tea fields (Figure 1 and Table 1).

Assessment of Fusarium Wilt of tea

The assessments were carried out on selected 10 tea farms of each tea estate farms and 10 tea gardens from each tea estate farms. Based on farm or field size 30-50 tea bushes per farm or field were selected. Data on fusarium wilt disease of tea was recorded on the incidence of the disease by observing the typical symptom.

$$\text{Disease incidence} = \frac{\text{Number of infected plant parts (twigs or leaves or berries)}}{\text{Total number of plant parts (twigs or leaves or berries) assessed}} \times 100$$

Identification of factors associated with thread blight disease

Altitude : Altitude in Meters Above Sea Level (MASL) was recorded, using Ground Positioning System (GPS) at a central point for each farm surveyed.

Meteorological data : Such as annual cumulative rainfall, mean temperature and relative humidity of the surveyed areas were obtained from the nearest meteorological stations (Table 2). The monthly weather data (rainfall, minimum temperature and maximum temperature) of the surveyed districts were summarized into yearly time scale. The missing daily data were filled by multiple imputation method by using XLSTAT statistical software [11].

Isolations and identifications of pathogens

The research was conducted in June-September 2019 at the Laboratory of Coffee and tea Pathology, Jimma Agricultural Research Center. The diseased tea plant part samples were taken from three tea estate area of Wushwush, Chewaka and Gumaro tea farms.

Pathogen was isolated from diseased tea plant samples collected from the main tea growing plantations in Southwest Ethiopia between July and August 2019. The isolates were obtained from infected plants by plating mycelial fans removed from beneath the bark of roots on Potato Dextrose Agar (PDA) for root diseases. The isolations of leave diseases were carried out by following standard isolation techniques. Rose Bengal and streptomycin were used to avoid contaminants. The isolates were stored on slants of the same medium and maintained in the dark at room temperature (21-23°C).

Pathogenicity tests

The pathogenicity tests for the root rot causing pathogen isolates were performed under greenhouse condition at Jimma Agricultural Research Center. Pure cultures of each fungal isolates growing on PDA medium were used as inoculum. Plates were incubated for 10-15 days at 22-25°C. Inoculum suspensions were prepared by mixing the contents of six agars plates (9 cm diameter) with 600 ml of sterile distilled water in a blender for 3 minutes at high speed. Plant material for pathogenicity tests were obtained from wushwush nursery.

Young rooted cuttings (6 months old, clones BB-35, 1156, 6-8 and 11-4) were inoculated as follows: roots were carefully cleaned under tap water and submerged for five minutes into the inoculum suspension. Then, they were transplanted in plastic pots (12 cm diameter × 9 cm high, one plant per pot) containing 600 ml of previously autoclaved soil (Top soil: sub-soil, 3:1) plus 50 ml of inoculum. Five inoculated plants per fungal isolate plus five control ones were placed in the greenhouse (10-30°C, 40-95% RH) and watered twice a week.

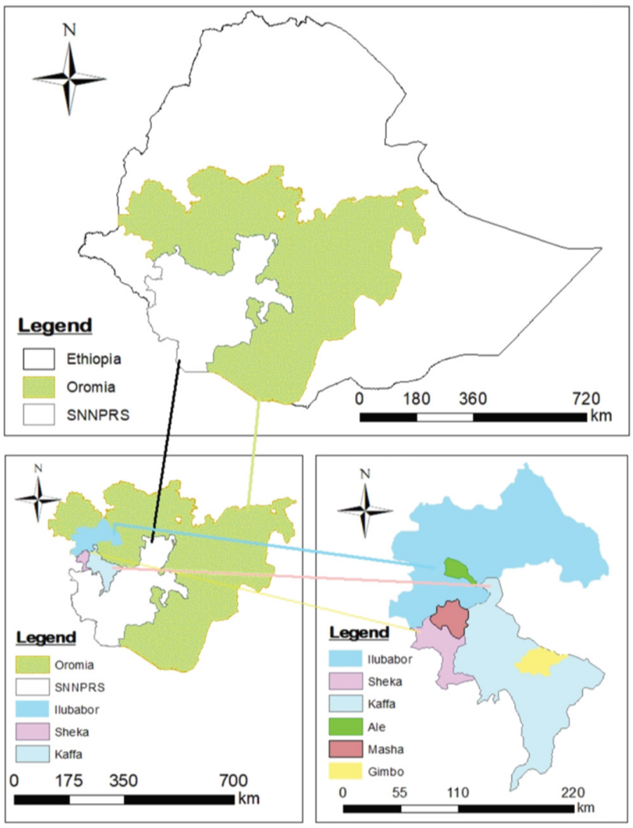

Figure 1: Map of Ethiopia showing regions, zones and districts of Southwest Ethiopia included in the survey program.

Table 1: Geographical information of the surveyed tea plantations in Southwest of Ethiopia.

Region	Zone	District	Plantation/ farms/	Altitude (masl)	Rainfall (mm)	Temperature	
						Min. °C	Max. °C
SNNPR	Kaffa	Gimbo	Wushwush tea plantation	1900	1820	12	24
			Out growers garden	1875	1820	12	24
SNNPR	Sheka	Masha	Chewaka tea plantation	1743	2035	10	23
			Out growers garden	1808	2035	10	23
Oromia	Ilubabor	Alle	Gumaro tea plantation	1718	1811	12	27
			Out growers garden	1689	1811	12	27

SNNPR = Southern Nation Nationalities Peoples Representatives, Min= Minimum, Max = Maximum

Table 2: Intensity of Fusarium wilts of tea at Southwest Ethiopia in 2019.

Parameters	Disease Intensity	Altitude	Rainfall	Minimum Temperature
Disease Intensity				
Altitude	0.83*			
Rainfall	0.35^{ns}	-0.90^{ns}		
Minimum Temperature	-0.3^{ns}	0.12^{ns}	-0.99**	
Maximum Temperature	-0.84*	-0.60^{ns}	-0.72^{ns}	0.69^{ns}

* = correlation is significant at p< 0.05, ** = correlation is significant at p < 0.01, ns = correlation is insignificant at p < 0.05.

Severity of aerial symptoms was periodically assessed for each plant on a 0-4 scale, according to the percentage of foliage with yellowing or necrosis: (0=0%, 1=1-33%, 2=34-66%, 3=67-100%, 4=dead plant). At the end of each experiment (after three months), root rot was assessed by using 0-4 scale [12].

RESULTS AND DISCUSSION

Intensity of Fusarium Wilt of tea across Southwest parts of Ethiopia

Fusarium wilt of tea was prevalent in southwest Ethiopia as evidenced from the tea bushes uprooted as infected by the disease (Figure 2A) and tea fields on which tea bushes infected by the disease were uprooted at the study areas (Figure 2B). During the field survey, it was observed that the tea plantations and gardens at Wushwush, Gumaro, Chewaka areas were mostly affected by fusarium wilt disease of tea which attacked about 19.75% of total tea bushes assessed. The disease was the primary disease of tea bushes at all tea growing areas of Southwest Ethiopia which causes death of healthy tissues or bushes even under the best conditions. Tea bushes of all ages were susceptible to this disease. The disease is most damaging if orchards are established in old tea plantations or newly cleared forest sites but not observed in tea gardens of out-growers previously planted with annual crops and appears to be related to the plantation's history.

Factors affecting Fusarium Wilt of tea disease

Altitude: Altitude is also one of the factors positively associated with fusarium wilt disease of tea. There was significant ($p < 0.05$) and strong positive correlation between altitude on one hand and Fusarium wilt of tea disease intensity (r=0.83) on the other (Table 3). The disease became more intense as altitude increases. Accordingly, the highest mean disease intensity (19.75%) was recorded at Wushwush tea estate farms and its surroundings having relatively highest Altitude. Whereas the lowest mean disease intensity (4.84%) was recorded at Gumaro tea estate farms and its surroundings having relatively lowest Altitude. This might be

Figure 2: (A) Tea bushes uprooted as infected by root rot disease at Gumaro tea plantation. (B) Tea field on which tea bushes infected by root rot disease were uprooted at Wushwush tea plantation.

Table 3: Pearson correlation coefficients between disease intensity and associated factors.

Location	Isolate	Colony					Growth rate (cm)$^{-1}$day On PDA
		Color		Form	Elevation	Margin	
		Front	Back				
Wushwush	RtRt1	F. white	F. white	Circular	Flat	Filiform	8.38
Chewaka	RtRt2	F. white	F. white	Circular	Flat	Filiform	9.00
Gumaro	RtRt3	F. white	F. white	Circular	Flat	Filiform	8.67

because of prolonged rainfall and storms accompanied by hail in the tea growing areas of Wushwush.

Rainfall: Rainfall is also one of the factors positively associated with fusarium wilt disease of tea. There was a positive intermediate correlation between rainfall and disease intensity (r=0.35) of fusarium wilt disease of tea (Table 3). The highest mean disease intensity (19.75%) was recorded at Wushwush tea estate farms and the surrounding tea fields, whereas percent of disease intensity was very low (4.84%) at Gumaro tea estate farms and the surrounding tea fields.

The result of this study indicated that the decreased level of rainfall may lead to decreased intensity of fusarium wilt disease on tea. Whereas the periods of high and prolonged rainfall, which result in prolonged periods of plant parts wetness, are the most important meteorological factor influencing the occurrence and further development of the disease in the field.

Environmental changes can also indirectly influence the biology of a pathogen by changing the plant architecture, thereby altering the microenvironment. For example, canopy density and structure can affect the temperature, moisture and availability of ultraviolet light at an infection site. Increased plant and leaf densities tend to increase leaf wetness, thus promoting the development of pathogens that prefer humid conditions [13].

Maximum and minimum temperature: There was significant ($p < 0.05$) and strong negative correlation between maximum temperature on one hand and fusarium wilt disease of tea intensity (r=-0.84) on the other (Table 3). The disease became more intense as maximum temperature level decreases. Accordingly, the highest mean disease intensity (19.75%) was recorded at relatively coolest tea growing areas, where as the lowest disease intensity (4.84%) was recorded at relatively hottest tea growing areas. However, there was insignificant and weak negative correlation between minimum temperature on one hand and fusarium wilt disease of tea intensity (r=-0.31) on the other.

Identification of *Fusarium Oxysporum*

Symptomological identification: Affected bushes occur in patches (Figure 3A), usually around old tree stumps, but sometimes isolated bushes are affected. Plants become weaker and their leaves begin to turn yellow and finally wilt and defoliate, eventually leading to death of the plant (Figure 3B). Longitudinal cracks were usually present on the collar above the soil level but also on the tap root and lateral roots (Figure 3C). Scrapping of the bark at the collar region revealed sheets of creamy white mycelia (Figure 3D) and the wood had a strong mushroom like-smell. This was observed at Wushwush Estate in Kaffa zone in 2019.

Macroscopic and microscopic identification: The basic keys for identification of Fusarium oxysporum fungus were growth rate, colony color, colony elevation, colony margin on culture media, macroconidial and microconidial morphology and number of septation. In the current work, pure cultures of the isolates were observed under compound microscope and identification was carried out based on morphological characteristics using standard references the fusarium laboratory manual. On the basis of morphological and cultural characters, the fusarium wilt causing fungus was identified as *Fusarium oxysporum.*

Macroscopic identification

Growth rate of mycilia: The study revealed that there were no considerable colony growth rate variations among Fusarium oxysporum isolates collected from different Tea producing regions of Ethiopia. The growth rate of the isolates was ranging between 8.38 and 9.00 mm/day, with a mean of 8.68 mm/day in diameter (Table 4). Isolates in the present study depicted periodic change in their growth rate. All the isolates showed an increasing trend in growth rate from 3 days to 8 days but growth rate decreased afterwards.

Colony color, elevation and margin: Colony color, form, elevation and margin did not differ very much among the tested isolates of *F. oxysporum* on PDA medium (Table 4 and Figure 4). *F. oxysporum* isolates produced mycelia with floral white front and back side color, circular in form, flat in elevation, filiform margins and produced in abundance.

Microscopic Identification

The result of this study indicated that the macroconidial shape of all the isolates of *F. oxysporum* was slightly curved with curved apical shape, footed basal shape and an average of 3 septa per macroconidia. Microconidial shape of all isolates of F. oxysporum was fusiform without septation (Table 5).

Pathogenicity tests

To ascertain the pathogenic ability of the isolates, *F. oxysporum* (wushwush, Chewaka and Gumaro) were tested for pathogenicity with six month young tea seedlings. Observation of the

Figure 3: (A) Patches of tea bushes affected by Fusarium wilt of tea.
(B) Turning of tea leaves to yellow and weakened tea bush.
(C) Longitudinal cracking of tea stem at collar.
(D) Creamy white mycelia on tea stem.

Table 4: Cultural characteristics of *Fusarium oxysporum* isolates.

Location	Isolate	Colony					Growth rate (cm)$^{-1}$day On PDA
		Color		Form	Elevation	Margin	
		Front	Back				
Wushwush	RtRt1	F. white	F. white	Circular	Flat	Filiform	8.38
Chewaka	RtRt2	F. white	F. white	Circular	Flat	Filiform	9.00
Gumaro	RtRt3	F. white	F. white	Circular	Flat	Filiform	8.67

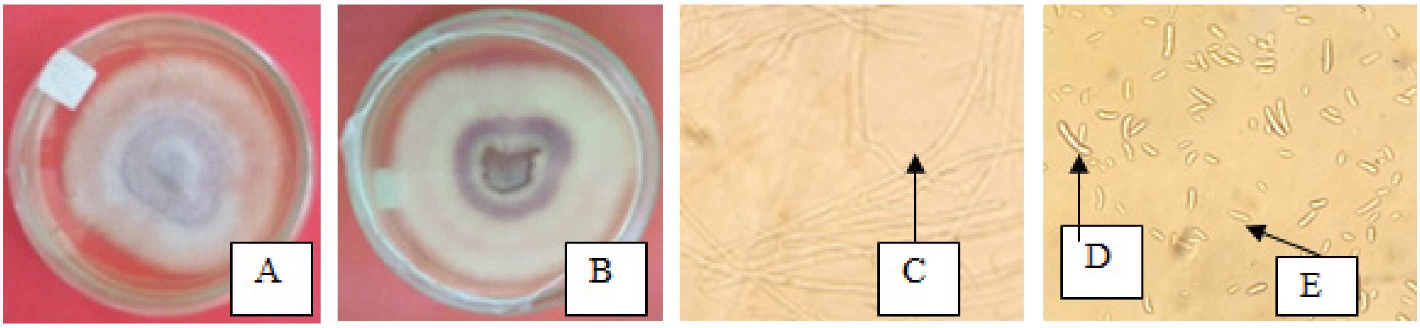

Figure 4: Colony morphology of a 7-day-old culture of isolate from Wushwush. A) Front side, (B) Back side, (C) Hyphae of *F. oxysporum* under microscope, (D) Macro conidia and (E) Micro conidia under 40 X objective compound microscope of isolate from Wushwush.

Table 5: Morphological characteristics of *F. oxysporum* isolates collected from tea producing areas of Ethiopia in 2019 cropping season.

Location	Isolates	Macro conidial					Micro conidial	
		Morphology			Septa		Morphology	
		Conidial shape	Apical	Basal	Range	Average	Shape	Septa
Wushwush	RtRt1	Slightly curved	Curved	Foot	2-4	3	Fusiform	0
Chewaka	RtRt2	Slightly curved	Curved	Foot	2-4	3	Fusiform	0
Gumaro	RtRt3	Slightly curved	Curved	Foot	2-4	3	Fusiform	0

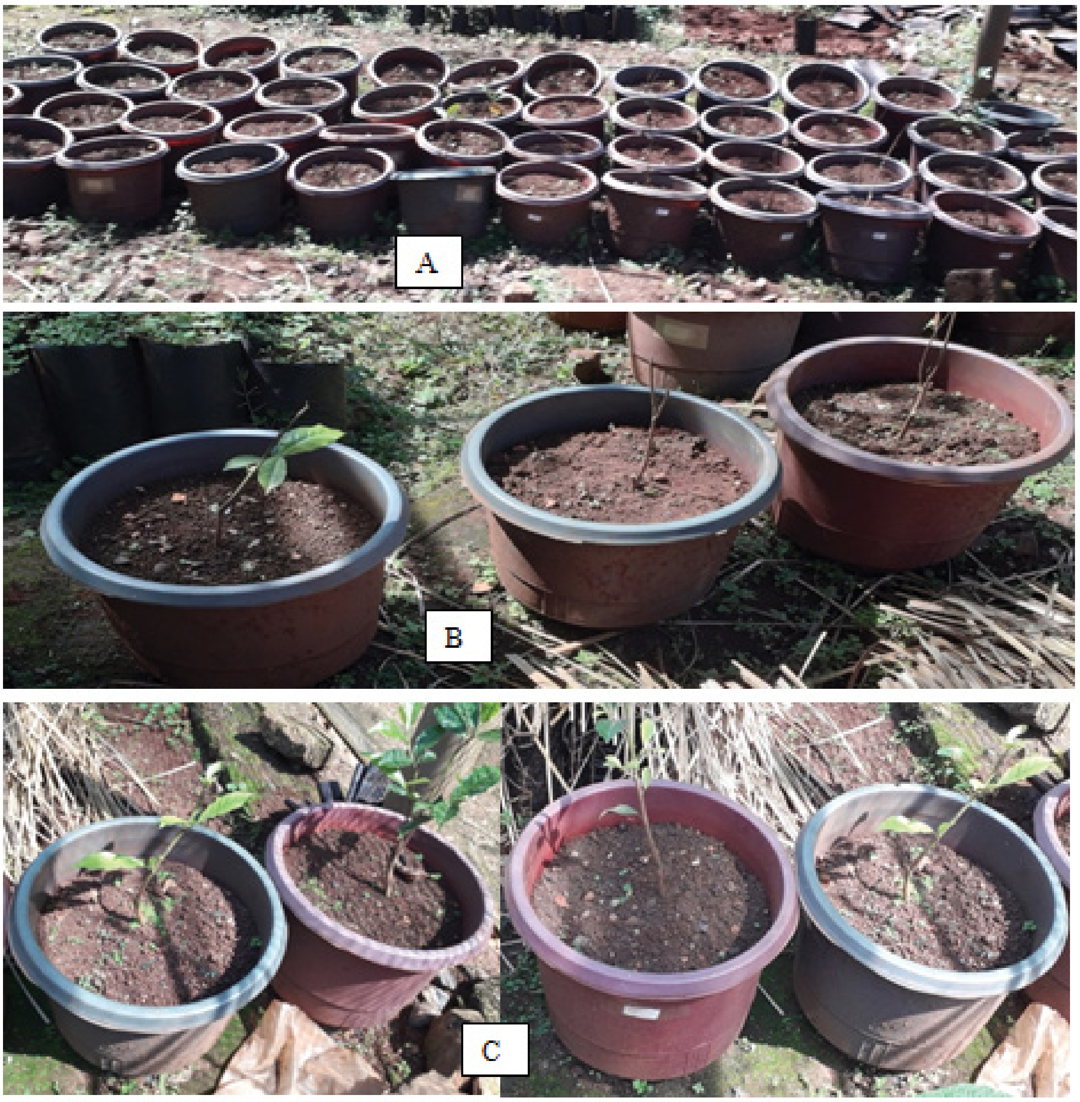

Figure 5: Pathogenic reactions of Fusarium wilt isolates on seedlings of different tea clones.
(A) Susceptible tea clones inoculated with aggressive isolates.
(B) 6-8 tea clones inoculated with Chewaka isolate.
(C) Water sprayed tea seedlings of clone 11-56.

Table 6: Pathogenic reactions symptom of *F. oxysporum* isolates on seedlings of different tea clones.

Tea Clone	Isolates from Different Farms			Water Sprayed
	Wushwush	Chewaka	Gumaro	
BB-35	***	**	***	NA
11-56	***	***	***	NA
6-8	**	*	**	NA
11-4	***	**	***	NA
11-56	NA	NA	NA	-

symptoms after 60 days of inoculation showed that leaf color of the inoculated tea plants turned from green to yellowish-green and wilting symptom of the tea leaves occurred. After 90 days of inoculation, whole tea leaves of the inoculated plants became completely wilted; most of the tea feeder roots became rotten with black wood discoloration (Figure 5). The pathogen was then re-isolated from the infected roots of the inoculated tea seedlings and its morphological characteristics (Figure 4) were compared with the original *F. oxysporum* isolates.

There was significant difference among tea clones tested for fusarium wilt isolates in disease development and severity. Tea clone seedlings 11-56, BB-35 and 11-4, exhibited greater diseases incidence which indicates highly susceptible reactions to fusarium wilt disease under laboratory conditions (Table 6). However, few seedlings of 6-8 tea clone showed lowest diseases incidence as compared to other tea clones. With regarding to F. oxysporum isolates, the isolate from Wushwush and Gumaro were the most aggressive isolates, whereas the isolate from Chewaka was the least aggressive one.

CONCLUSION

Many diseases caused by fungal pathogens are important constraints of tea production and productivity at tea producing Southwest of Ethiopia. The present study revealed the economic importance of fusarium wilt of tea disease in Southwest Ethiopia. Fusarium wilt of tea caused by *Fusarium oxysporum* was identified on tea bushes in southwest Ethiopia. The average fusarium wilt intensity varied from (0 to 20%). Fusarium wilt of tea directly related to tea yield loss. Altitude, cumulative rainfall, maximum temperature and minimum temperature have considerable impact on the intensity of fusarium wilt disease. High altitude and increased level of rainfall were associated with an increased risk of fusarium wilt disease development on tea bush. Besides, disease intensity was the highest on tea bushes planted on deforested soils other than farm lands. The colony growth rate of the isolates was ranging between 8.38 and 9.00 mm/day. *F. oxysporum* isolates produced mycelia with floral white front and back side color, circular in form, flat in elevation, filiform margins and produced in abundance. The macroconidial shape of all the isolates of *F. oxysporum* was slightly curved with curved apical shape, footed basal shape and an average of 3 septa per macroconidia whereas microconidial shape of all the isolates was fusiform without septation. Pathogenicity tests indicate that the isolates from wushwush and Gumaro were very aggressive on tea clones BB-35, 11-56 and 11-4 as compared to other isolates and tea clones whereas no any disease symptom was observed on water sprayed 11-56 tea clone (negative control).

THE WAY FORWARD

Since the present status of fusarium wilt disease of tea is remarkably on increasing trend, it is recommended to:-

1) Further investigate the impacts of weather variables and other factors on fusarium wilt disease intensity under different agro-ecological conditions in multi-location and multiyear trials to come up with conclusive results.

2) Study on safe, effective and environmentally friendly disease management options such as cultural practices, biological control and integrated disease management.

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