

Full Length Research Paper

Comparative fungal profile of tea leaves from highland and lowland in Nigeria

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A study to determine the fungal profile in leaves of both highland and lowland tea in Nigeria was performed. Healthy leaves were harvested from nineteen tea clones: T-14, T-19, T-33, T-35, T-61, T-68, T-74, T-108, T-143, T-228, T-229, T-236, T-238, T-318, T-354, T-355, T-367, T-368 and T-369 on the highland and lowland areas. Potato dextrose agar (PDA) was used as the growth medium to culture associated mycoflora from the tea leaves in five replications. The healthy tea leaves of the clones from the highland and the lowland areas show the presence of ten and six fungi respectively which include *Pleurothecium recurvatum*, *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Itersonilia perplexans*, *Penicillium italicum*, *Lasiodiplodia theobromae*, *Trichordema viride*, *Rhizopus stolonifer* and *Botrytis cinerea*. Clones T-369, T-14, T-108 and T-368 have the highest (4) mycoflora load found on the tea leaves but three were found associated with each of clones T-228, T-68 and T-236 on the highland. Only one fungus was isolated from each of the clones T-108, T-61, T-229 and T-318, two fungi from each of T-355, T-19 and T-236 while the highest mycoflora load on the lowland tea were found in clones T-368 and T-228.

Key words: Tea, two and a bud, clones, highland, lowland, mycoflora.

INTRODUCTION

Tea (*Camellia sinensis* (L) Kuntze) belongs to the family Theaceae; this plant is an evergreen shrub or small tree. Tea is a popular beverage produced from the young leaves of commercially cultivated tea plant and has become one of an important revenue source for tea producing countries in the world (Bandyopadhyay, 2011). Originally cultivated in East Asia, India and China or perhaps both of these countries are native home of tea plant. Today, tea is grown over a wide range of tropical and subtropical region in more than 50 countries throughout Asia and Africa (Balasubramanian et al., 2011).

Tea was introduced into Nigeria by de Bouley from

West Cameroon in 1952 (Kassboll -Smith, 1965).

However, the first commercial clones were imported into the Mambilla Plateau in 1975 (Hainsworth, 1981). Since then, more clones have been imported to establish large commercial plantations. Due to limited land area for tea on Mambilla Plateau, tea adaptation trial plots were established in seven lowland locations in Nigeria (Omolaja et al., 2000).

Tea production is greatly hindered due to a number of pests and diseases. Perennial habit of tea plant, peculiar culture condition and warm humid climate of the tea growing areas are highly conducive for disease development (Ponmurugan and Baby, 2007) and majority

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of the diseases are of fungal origin (Muraleedharan and Chen, 1997).

Microorganisms live in association with plants (endophytic microbes) and are commonly observed in nature (Bacon and White, 2000). They are commonly found in Coniferaceae, Gramineae, dan Ericaceae (Okane et al., 2001a), bakau *Bruguiera gymnorrhiza* (Okane et al., 2001b, c), *Theobroma cacao* (Rubini et al., 2005), and *Camelia sinensis* (Agusta et al., 2006). Array of fungi have being isolated from tea plants parts; *Helminthosporium* sp. and *Pestalotiopsis theae* (synonym: *Pestalotia theae*) from the leaves, *Fusarium*, *Helminthosporium*, *Cercospora*, *Botryodiplodia* and *Rhizoctonia* spp. and *Pestalotiopsis theae* from the shoot, *Rigidoporus microporus* (synonym: *Fomes lignosus*), *Colletotrichum coccodes* (synonym: *Colletotrichum camelliae*, *Nemania serpens* (synonym: *Hypoxylon serpens*, *Macrophoma theicola* and *Lasiodiplodia theobromae* (synonym: *Botryodiplodia theobromae*), *Fusarium* sp. and *Amyloflagellula pulchra* (synonym: *Marasmius pulcher*) Olunloyo, 1985; Filani et al., 1989; Anonymous, 1991; Otieno, 1996; Adedeji, 2006) among others as causing diseases of different tea parts. Fungal infestations and association with tea are of utmost importance as the leaves can be infused and consumed directly.

MATERIALS AND METHODS

Collection of tea leaves

The Mambilla Plateau on coordinate 6.7138N, 11.2500E and Mayo Selbe on 7.283N, 11.133E were the study locations where nineteen clones of tea representing the Indian/China types were collected. Average altitude in Mambilla is 1600 m (5000 ft), mean rainfall 1800 mm and average annual temperature of 16°C. Mayo Selbe has an average altitude 455 m (1496 ft), average temperature of 25.8°C and rainfall of 1518 mm.

The clones of the tea types were T-14, T-19, T-33, T-35, T-61, T-68, T-74, T-108, T-143, T-228, T-229, T-236, T-238, T-318, T-354, T-355, T-367, T-368 and T-369 on the Mambilla Plateau on the highland while T-19, T-61, T-108, T-228, T-229, T-236, T-318, T-355 and T-368 are from the lowland, Mayo Selbe. The healthy two tea leaves immediately below the bud, known in the tea trade as "two and a bud" (TAB), and use for the production of tea were harvested from each of the clones in the selected locations. The tea leaves were kept in a transparent polyethylene bag and transferred to the laboratory for further studies.

Isolation from tea leaves

The tea leaves samples were treated separately on clone basis. The medium, potato dextrose agar (PDA) acidified with 10% lactic acid use for culturing fungi from the leaves samples. The tea leaf samples were cut into small pieces of about 3 x 4 mm using a sterile scalpel; surface sterilized in 1% sodium hypochlorite, washed in three changes of sterile distilled water and aseptically sandwiched to dry in between sterile Whatman No 1 filter paper (150 mm). The tissue pieces were aseptically inoculated into the solidified PDA medium in 90 mm Petri dishes and replicated five times. The inoculated plates were incubated at ambient

temperature (28±2°C) and the plates observed daily for colony growth. The fungal colonies were hyphal-tip transferred into new plates to obtain pure cultures and the colonies were thereafter identified base on their morphological features (Pitt et al., 1992).

RESULTS AND DISCUSSION

The selected 17 healthy tea leaf clones from the Mambilla Plateau (Taraba state) and 9 clones from Mayo Selbe in the lowland area of the state show the presence of ten and six fungi respectively. The isolated mycoflora include *Pleurothecium recurvatum*, *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Itersonilia perplexans*, *Penicillium italicum*, *L. theobromae*, *Trichordema viride*, *Rhizopus stolonifer* (synonym: *Rhizopus nigrican*) and *Botrytis cinerea* (Table 1). Clones T-369, T-14, T-108 and T-368 have the highest (4) mycoflora load found on the tea leaves but three were found associated with each of clones T-228, T-68 and T-236 on the Mambilla Plateau respectively. Each of clones T-318, T-354 and T-33 have only one fungus isolated while two fungi were culture from each of clones T-35, T-19, T-74, T-61, T-367, T-143 and T-238 on the highland (Table 1).

The six fungi species from the nine tea clones in the lowland include *L. theobromae*, *I. perplexans*, *P. recurvatum*, *A. niger*, *C. albicans* and *R. stolonifer* (Table 2). Only one fungus was isolated from each of the clones T-108, T-61 and T-229 two fungi from each of T-355, T-19, T-368 and T-228 while the highest mycoflora load on the lowland tea were found in clones T-318 and T-236 (Table 2).

In the highland tea clone samples, the incidences of *L. theobromae* was most prevalent as it was recorded 58.82% of the highland clones and closely followed by *P. recurvatum* having 52.94% incidence which distant from 29.41% occurrence of each of *A. niger* and *R. stolonifer*. The nine lowland clones of tea have both *L. theobromae* as the most common flora with 66.67% occurrence, followed by *R. stolonifer* having incidence of 55.56%. *P. recurvatum* was isolated from 3 clones and each of the rest isolated fungi was found on one clone only and some other flora have zero incidence in the lowland tea leaves but occurred though at low percent incidences in the highland tea clones (Figure 1).

The isolation of *L. theobromae* from ten and six tea clone on the highland and lowland areas respectively corroborates the existence of the fungus as reported by Saha et al. (2008) that *L. theobromae* causes severe damage of tea gardens in the nurseries. It has also been reported as a common pathogen of tea, causing diplodia disease which affects roots, stems and leaves of tea plants of all ages (Sarmah, 1960; Chandramouli, 1999; Singh, 2005). Mbenoun et al. (2007), however, reported *L. theobromae* as most commonly associated with dieback and consistently isolated from various tissues (twigs, bark, vascular tissues and fruits) of symptomatic

Table 1. Frequency of occurrence of fungi associated with leaves of tea clones collected from highland, the Mambilla Plateau.

Clones	<i>Pleurothecium recurvatum</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Itersonilia perplexans</i>	<i>Penicillium italicum</i>	<i>Lasiodiplodia theobromae</i>	<i>Trichoderma viride</i>	<i>Rhizopus stolonifer</i>	<i>Botrytis cinerea</i>
T-369		+	+				+			+
T-35						+	+			
T-19							+		+	
T-14	+	+						+		+
T-74			+				+			
T-318	+						+			
T-61	+						+			
T-354							+			
T-367	+								+	
T-228		+	+				+			
T-108	+				+		+		+	
T-143	+		+							
T-68			+	+			+			
T-33								+		
T-236	+					+	+			
T-238	+								+	
T-368	+			+	+				+	

Presence (+); Absence (-).

Table 2. Frequency of occurrence of fungi associated with leaves tea clones collected from lowland, Mayo Selbe.

Clones	<i>Pleurothecium recurvatum</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Itersonilia perplexans</i>	<i>Lasiodiplodia theobromae</i>	<i>Rhizopus stolonifer</i>
T-236	+	+	-	+	+	+
T-318	+	-	+	-	+	+
T-108	-	-	-	-	+	-
T-61	-	-	-	-	+	-
T-229	-	-	-	-	-	+
T-355	+	-	-	-	+	-
T-19	-	-	-	-	+	-
T-368	+	-	-	+	-	+
T-228	-	+	+	-	-	+

Presence (+); Absence (-).

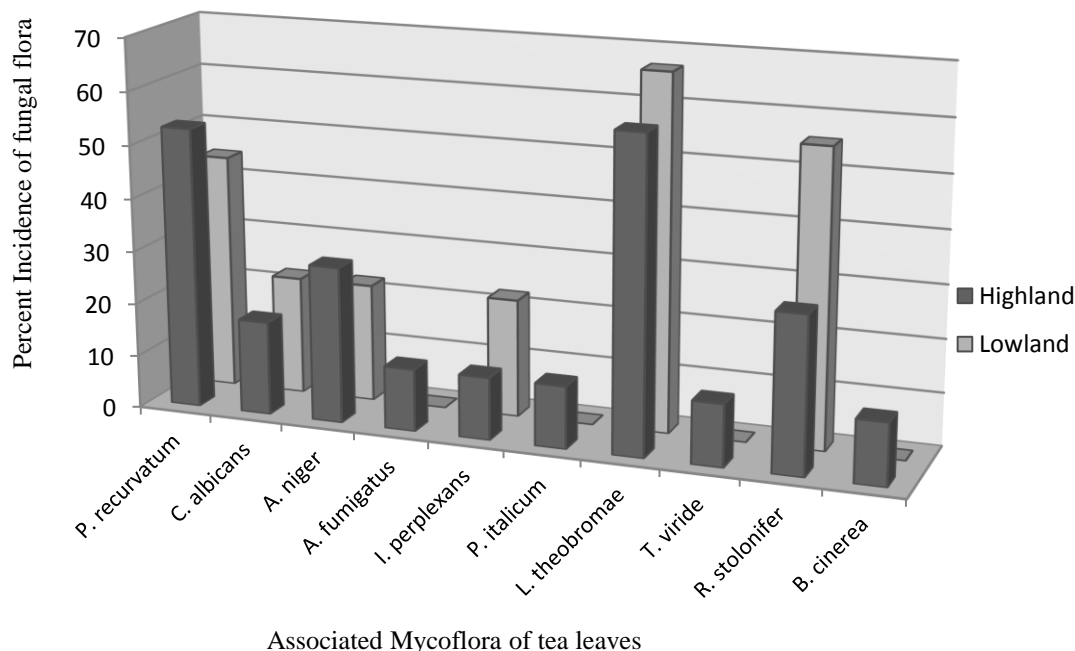


Figure 1. Incidences of Mycoflora in tea clones of the highland and lowland areas.

cacao (*Theobroma cacao*) plants.

This study also agrees with the early findings of Olunloyo et al. (1987) which reported the isolation of *L. theobromae*, *Penicillium tamari* and *A. niger* among others from the tea cuttings. Adedeji (2000) also reported the incidences of *L. theobromae*, *Fusarium* spp. and *A. niger* on fresh leaves of tea plants in the lowland. However, the tea leaf samples used in this study did not exhibit any disease symptom when they were collected and processed.

Conflict of Interest

The authors have not declared any conflict of interest.

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