

RESEARCH ARTICLE**Isolation and Identification of Fungi Associated with Avocado Fruits from Local Markets of the West Region of Cameroon**

Djeugap Fovo Joseph^{1*}, Tsopmbeng Noumbo Gaston², Keuete Kamdoum Elie², Yaouba Aoudou¹ and Serferbe Signaboubo²

¹Laboratory of Phytopathology, Department of Plant Protection, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222 Dschang, Cameroon; ²Laboratory of Applied Botany, Department of Plant Biology, Faculty of Sciences, University of Dschang, P.O. Box 67 Dschang, Cameroon

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***Corresponding Address:**

Djeugap Fovo Joseph

jdjeugapfovo@yahoo.fr

ABSTRACT

A survey of postharvest fungi associated with avocado fruits from markets of four divisions of the West region of Cameroon was carried out with samples collected between March and April 2013. Samples collected were brought to the laboratory for isolation in Potato Dextrose Agar (PDA) medium. Results obtained show some variation in isolation frequency of fungi from each division. *Colletotrichum gloeosporioides* (23.70%), *Botryosphaeria dothiorella* (18.52%) and *Cercospora purpurea* (15.18%) were frequently isolated. *Alternaria* sp (2.78%), *Sphaceloma persea* (4.63%) and *Pestalotiopsis guepinii* (2.77%) were not isolated from fruits collected respectively from Noun and Mifi divisions. The least frequently occurring fungi included *Aspergillus niger* (5.92%), *Colletotrichum acutatum* (6.85%), *Fusarium solani* (5.74%), *Phytophthora citricola* (4.82%) and *Rhizopus nigricans* (8.89%). Although pathogenicity tests are still to be carried out to confirm the virulence of these phytopathogenic fungi on avocado fruits, there is a need of seeking appropriate management strategy to handle fungal pathogens of economic importance on avocado fruits in Cameroon.

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INTRODUCTION

Post-harvest fungi are one of the major causes for the postharvest loss of horticultural fresh produce including avocado fruits during the supply chain and their incidence can affect the quality and restrict the shelf life of fresh produce. Although diseases caused by these organisms had received much attention on avocado (*Persea americana* Mill.) in other countries such as the USA and Israel (Ken and Marlatt, 2007) they remained fairly neglected in Cameroon. The importance of post-harvest diseases is now recognized by avocado producers since serious losses occurred during the transit. For instance, losses to avocado fruits caused by anthracnose had been reported to be more than 80% (Darvas and Kotze, 1987). Widely cultivated in many tropical and subtropical regions of the world, avocado fruits are rich in lipids, vitamins, fiber, and are essential for intestinal transit (Ternisien and Bellec, 2002). In Cameroon, avocado is mainly produced in the west region (MINADER, 2010). Avocado plays an important role in the socioeconomic

development of the region which was previously based on coffee cultivation. Formerly grown for local consumption, it is nowadays geared towards exportation. National production in Cameroon was estimated at 52,000 t with 530.3 t and 83.2 t exported respectively to Gabon and Equatorial Guinea in 2010 (MINADER, 2010). Despite the economic importance of avocado in the revenue of the population in the region, the quantity of the produce decreased drastically due to a number of factors among which are fungal pathogens that depreciate the quality.

Surveys conducted by Hartill and Everett (2002), Everett *et al.* (2007) and COLEACP (2008) showed that anthracnose, stem rot, galls, fruit spot and fruit rot were the most important fungal diseases. The incidence of these diseases can be up to 90% in areas with high relative humidity (COLEACP, 2008). Little information is available on the fungi associated with avocado fruits in Cameroon. The objective of this study was to isolate and identify fungi associated with avocado fruits in the west region of Cameroon.

MATERIALS AND METHODS

Study area

The area has an average altitude of 700-1400 m; falls between 5°30' N latitude and 10°30' E longitude and the climate is tropical sudanian humid type with two seasons; one dry season from October to November and one raining season from March-April to October-November. The average rainfall is 670.9 mm and the mean monthly temperature ranged between 15° to 30°C.

Collection of samples and isolation of fungi

During the survey infected fruits were collected from different markets of Bamboutos, Menoua, Mifi and Noun divisions of the West Region of Cameroon at weekly intervals for 2 months during the peak avocado production season in 2013 (March and April), and put in a polyethylene bags (Malik, 1996). Samples were brought to the laboratory of Phytopathology, University of Dschang for fungi isolation. The fruits samples were surface sterilized for 3 minutes with 1% NaOCl and rinsed in four successive changes of sterile distilled water. The surface sterilized fruits showing symptoms of diseases were then sliced into 2 mm² pieces and plated on to sterile PDA in Petri dishes supplemented with 250 mg Chloramphenicol to discourage bacterial contamination (Korsten *et al.*, 1994; Djeugap *et al.*, 2009). The plates were incubated in an inverted position at 20±1°C for 2-3 days and observed for fungal growth and later sub cultured into fresh PDA medium.

Identification of fungi

Pure isolates of fungi obtained were identified on the basis of macro and micro morphological characteristics. Morphological characteristics of the fungi (mycelium coloration or pigmentation, presence or absence of septate, spore morphology) were recorded. In some cases the infected tissues were stained by cotton blue and Lactophenol (McClenny, 2005) and observed under microscope. Morphological identification of fungi was based on the morphology of the fungal culture colony or hyphae, the characteristics of the spores and reproductive structures (Barnett and Hunter, 1972; Alexopoulos and Mims, 1996; Agrios, 2005).

Data analysis

Frequency occurrence of isolation of each fungus were calculated using the following formula $F = (NF/NT) \times 100$, where F represents the frequency of occurrence (%) of a fungus, NT is the total number of samples from which isolations were carried out, and NF is the total higher number of sample from which a particular fungus was isolated (Yamaoka *et al.*, 1997; Iqbal and Saeed, 2012).

RESULTS AND DISCUSSION

A total of 540 isolations were made from avocado fruits. After identification, 11 different fungi belonging to 8 genera were isolated from avocado fruits collected from markets of the 4 divisions of the West region of Cameroon. These fungi were identified as *Colletotrichum gloeosporioides*, *Botryosphaeria dothiorella*, *Cercospora purpurea*, *Rhizopus nigricans*, *Colletotrichum acutatum*, *Aspergillus niger*, *Phytophthora citricola*, *Sphaceloma persea* and *Alternaria* sp. (Figure 1). A wide range of fungi was isolated from avocado fruits in the West Region of Cameroon. Some of these fungi are reported by several authors to be commonly implicated in the postharvest deterioration of many fruits and vegetables in the Tropics (Hartil and Evertt, 2002; Everett and Pak, 2002; Everett *et al.*, 2005; Djeugap *et al.*, 2009; Regnier *et al.*, 2010; Onyeani *et al.*, 2012; Oyetunji *et al.*, 2012; Amadi *et al.*, 2014).

The mean isolation frequency of *C. gloeosporioides* (23.70%) was the highest, followed closely by *Botryosphaeria dothiorella* (18.52%) and *Cercospora purpurea* (15.18%). *Aspergillus niger* (5.92%), *Colletotrichum acutatum* (6.85%), *Fusarium solani* (5.74%), *Phytophthora citricola* (4.82%) and *Rhizopus nigricans* (8.89%) have the less isolation frequency. *Alternaria* sp. (2.78%) and *Sphaceloma persea* (4.63%) were not isolated from fruit collected from Noun division while *Pestalotiopsis guepinii* (2.77%) were absent in Mifi division (Table 1). Among these fungi *C. gloeosporioides*, and *B. dothiorella* were more prevalent in Bamboutos and Menoua divisions while *C. gloeosporioides* and *C. purpurea* were prevalent in Mifi division. The most prevalent fungi in Noun division were *C. gloeosporioides* (Figure 2). *C. gloeosporioides*, *B. dothiorella* and *C. purpurea* are generally reported to be important pathogens of avocado fruit in several parts of the world causing pre and post-harvest diseases of avocado fruits (Muirhead *et al.*, 1982; Darvis and Kotzé, 1987; Darvis *et al.*, 1987; Hartil, 1990; Willis and Duvenhage, 2003). These fungi cause significant losses during transportation and storage

Table 1: Isolation frequency (%) and number of isolates of fungi associated with avocado fruits from four divisions of the West Region of Cameroon

Fungus isolated	Divisions				Number of isolates	Mean (%)
	Bamboutos	Menoua	Mifi	Noun		
<i>Alternaria</i> sp.	1,48 (2)*	7,41 (10)	2,22 (3)	0	15	2,78
<i>Aspergillus niger</i>	4,44 (6)	4,44 (6)	7,41 (10)	7,41 (10)	32	5,92
<i>Botryosphaeria dothiorella</i>	22,22 (30)	20 (27)	13,33 (18)	18,52 (25)	100	18,52
<i>Colletotrichum acutatum</i>	5,19 (7)	8,15 (11)	11,85 (16)	2,22 (3)	37	6,85
<i>C. gloeosporioides</i>	27,41 (37)	20,74 (28)	22,22 (30)	24,44 (33)	128	23,70
<i>Cercospora purpurea</i>	11,85 (16)	13,33 (18)	17,78 (24)	17,78 (24)	72	15,18
<i>Fusarium solani</i>	5,19 (7)	2,22 (3)	8,89 (12)	6,67 (9)	31	5,74
<i>Pestalotiopsis guepinii</i>	2,96 (4)	2,22 (3)	0	5,92 (8)	15	2,77
<i>Phytophthora citricola</i>	5,19 (7)	5,19 (7)	3,7 (5)	5,92 (8)	27	4,82
<i>Rhizopus nigricans</i>	7,41 (10)	11,11 (15)	5,92 (8)	11,11 (15)	48	8,89
<i>Sphaceloma persea</i>	6,67 (9)	5,19 (7)	6,67 (9)	0	25	4,63
Total number of isolates	135	135	135	135	540	/

*Numbers in brackets represent the number of fungal isolates; N= 135 isolates per division.

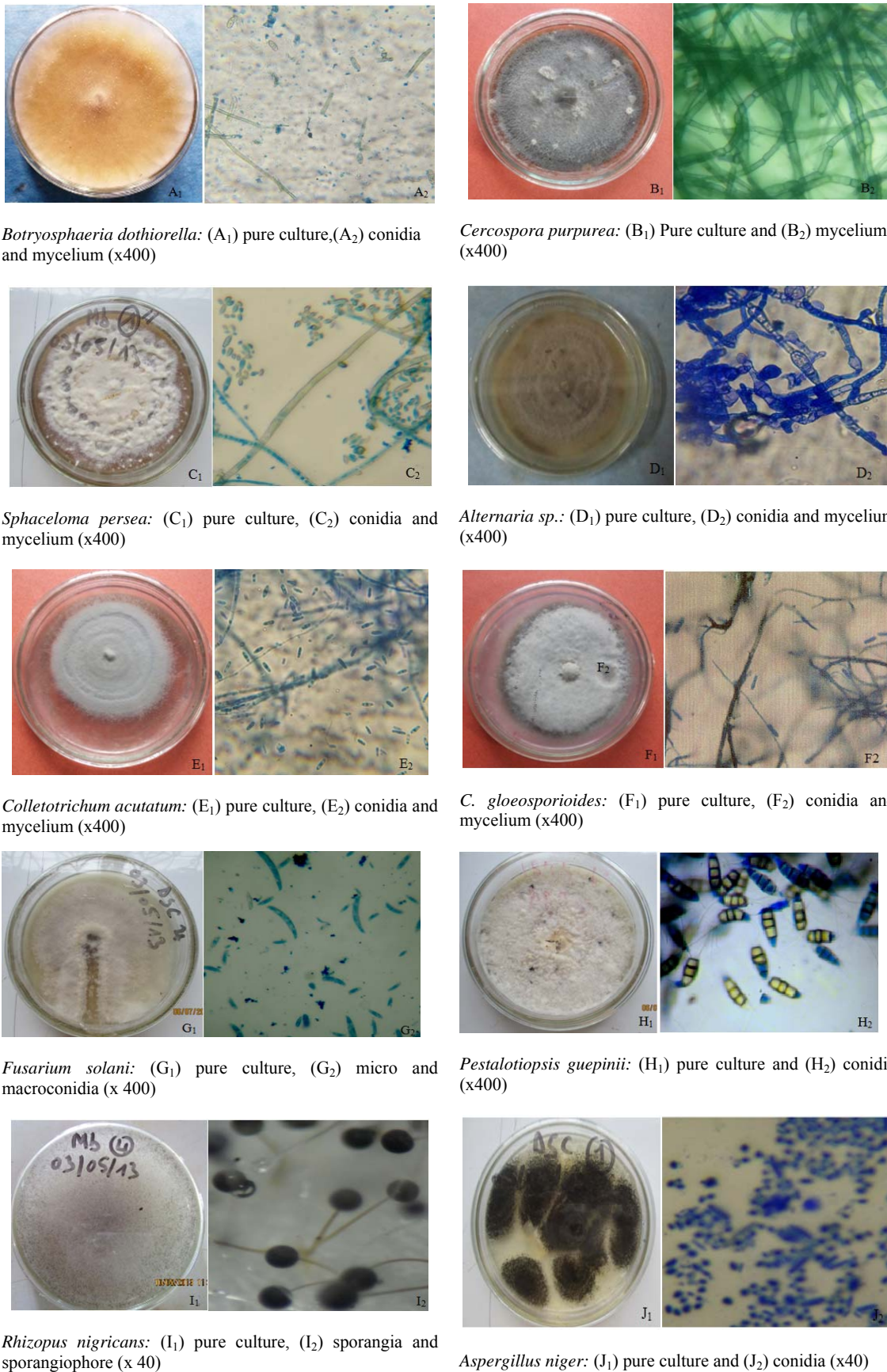


Fig. 1: pure culture, mycelia and/or conidia of isolated fungi associated with avocado fruits on PDA medium, 12 days after plating.

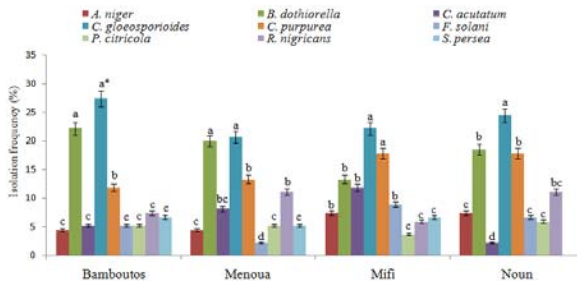


Fig. 2: Isolation frequency of some fungal species associated with avocado fruit from markets of four divisions of the West region of Cameroon.

*Means followed by the same letter within a division are not significantly different at 5% Duncan's multiple range test.

by infecting the fruit either through the side of the fruit (body rots) or through the picking wound (stem-end rots) (Malil *et al.* 2014). The high prevalence of *C. gloeosporioides* on fruits from all the division in this study is in agreement with the report of Darvas and Kotzé (1987). However, contrary to the latter author's findings, this study indicated that in addition to *C. gloeosporioides*, *B. dothiorella* and *C. purpurea* are the most prevalent fungi in avocado fruits in the West region of Cameroon. *Colletotrichum* spp have been reported to affect fruits, causing disease on immature and growing fruits in the field conditions, and damage fruits during transportation and storage (Wharton *et al.*, 2004). *A. niger*, *P. citricola*, *S. persea*, *R. nigricans*, *Alternaria* sp, *F. solani* and *P. guelpinii* were relatively less important on avocado in respect to their low isolation frequencies. Although these fungi are considered as opportunists in avocado (Everett *et al.*, 2005; Ogbo and Oyibo 2008), they have however been reported as pathogenic in some avocado varieties and other fruits including mango, apple, banana and grape in other part in the tropics (El-Hamalawi and Menge, 1994; Kortsen *et al.*, 1994; Avila-Quezada *et al.*, 2003; Djeugap *et al.*, 2009; Bashar *et al.*, 2012). Although, *A. niger* was less frequent, several reports showed its implication in spoilage of many fruits and vegetables (Bali *et al.*, 2008; Tafinta *et al.*, 2013). The origin of fruit contamination by fungi is difficult to determine. Generally, contamination of agricultural product is a function of many factors including infestation in the field prior to harvest, handling during harvesting and methods of packaging and transportation of the product to the market (Amadi *et al.*, 2014). The variation in fungal loads of avocado fruits observed in this study can be attributed to the differences in the level of sanitation in handling and of the market environments. Wounds are also known to be the major pre-disposing factor of fruits and vegetables to microbial attack both in transit and in storage (Amadi *et al.*, 2014).

Conclusion

This study has provided useful information about fungi species associated with avocado fruits from local markets of the western region of Cameroon and their isolation frequency in four divisions of this region. Results suggest the need of developing appropriate management strategy to control post-harvest diseases

caused by the most prevalent fungi such as *C. gloeosporioides*, *B. dothiorella* and *C. purpurea*. However, pathogenicity tests are still to be carried out to confirm and to characterize their virulence on avocado fruits.

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