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Isolation and Pathogenicity Evaluation of Postharvest Fungal of Some Fruits in Cameroon

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Abstract— *The present work was designed to study the biodiversity of fungal post-harvest decay of banana, mango and safou fruits sold in local markets in the Dschang locality, Western Region of Cameroon. A total of 90 infected fruit samples were collected from different local markets, small pieces of mouldy part were inoculated on prepared plates of Potato dextrose agar (PDA), after 7 days of incubation, pure isolated fungi were identified according to the recommended references. The pathogenicity of the some most prevalent fungi isolated was evaluated on uninfected fruits. Results obtained showed some variations in isolation frequency of the fungi from each fruit. Aspergillus, Colletotrichum, Fasarium and Veticillium were the most common genera that colonized banana, mango and safou fruits with different incidences. Cercospora capsici was present on safou (50%), C. mangiferae on mango (50.9%) and C. musae on banana (51.7%). Colletotrichum gloesosporioides appeared on banana (8.62%), mango (15%) and safou (22.92%); Colletotrichum musae on banana (22%). Cercospora spp caused injuries with lesions diameters that vary depending on the type of fruit and fungal species. Proper measures should be adopted to protect fruits from fungal decay.*

Keywords—Fruits, Post-harvest fungi, Isolation, Pathogenicity, Western Region, Cameroon.

I. INTRODUCTION

Fruits play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. It has been recognized that fruits are commercially and nutritionally important food product. Rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year (Salman, 2005; Parveen *et al*., 2016). Fruits have wide distribution in nature. Tropical fruit production knows more and more increased with fresh bananas which was ranked 1st, with more than 145 million tons produced in 2011 globally (FAO, 2011). Cameroon's production of sweet banana in 2010 was 1 333 851 tons. The mango and the safou, despite their low production, also feature prominently after the banana. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Approximately 20-25% of the harvested fruits are deteriorated by pathogens during post-harvest handling even in advanced countries (Droby, 2006). The postharvest losses are often more harsh in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation and post-harvest stockpile and marketing conditions, or after procuring by the consumer. Fruits incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay (Singh *et al*., 2007). Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors (Valiuskaite *et al*., 2006; Ewekeye *et al*., 2016). Fungi are the most crucial and common pathogens and the main cause of crop diseases. It Infect a wide range of fruits and vegetables during storage and transportation (Sommer, 1985).

The importance of post-harvest diseases is now recognized by fruits producers since serious losses occurred during the transit. In Cameroon, banana and mango are almost produced all over the country (MINADER, 2012). Fruits play an important role in socioeconomic. Surveys conducted by Hartill and Everett (2002), Everett *et al.* (2007) 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 some fruits in Cameroon. This study was aimed at isolating and identifying the fungi associated with post-harvest decay of bananas, mangoes and safou from different localities in the Dschang market, Cameroun.

II. MATERIALS AND METHODS

Collection of samples :

Ninety samples of infected and uninfected fruits were randomly collected from some markets in the city of Dschang in May 2016. Thirty samples of each the fruits

banana, mangos and Safou were collected. Samples were separately kept in clean plastic bags, transferred to the Phytopathology laboratory of the University of Dschang and stored in a refrigerator for mycological analysis.

Isolation and identification of fungi :

The direct plating technique described by Pitt and Hocking (1985) was employed. The fruit samples were surface sterilized for 3 minutes with 1% NaOCl and rinsed in four successive changes of sterile distilled water. Four small pieces from the margin of lesion of each sample were directly inoculated on prepared plates of Potato dextrose agar which contain (g/L): peeled potato100.0g, glucose 20.0g, agar 15.0g, water 1000.0 ml. The medium was supplemented with chloramphenicol (250 mg per liter) as a bacteriostatic agent (Smith and Dawson, 1944). The plates were inoculated at 28 ± 1 °C for 5 to 7 days. Three replicates were prepared for each sample. The resulting fungi were isolated, purified and identified according to their macro and micro characteristics.

Identification of fungal genera and species :

The pure isolated fungi were identified following the most documented keys in fungal identification (Raper and Fennell, 1965; Barnett and Hunter, 1972; Pitt, 1985, Moubasher, 1993; Alexopoulos and Mims, 1996; Klich, 2002; Agrios, 2005).

Pathogenicity test :

The pathogenicity test was done on apparently healthy mature fruits. The method of inoculation by wound of fruits was used Rivera-Vargas *et al*. (2006). The inoculated fruits were kept in laboratory conditions (22 ±2° C) for seven days. Data collected on the lesions developed by the fungus. For this test, the 3 species of *Cercospora* genus isolated from fruits were used, namely *C. capsici, C. mangiferae* and *C. musae*.

Statistical analysis :

Frequency occurrence of isolation of each fungus and diameters of lesions developed on fruits were calculated. Data obtained was analyzed statistically using SPSS (Version 17).

III. RESULTS AND DISCUSSION

The biodiversity of fungal species listed on Table 1 could be regarded as common post-harvest decay agents of various studied fruits. Through this investigation at 28 ± 2°C nine fungal species attributed to six genera were isolated. *Aspergillus, Cercospora, Colletotrichum, Fasarium* and Veticillium were the most common genera that colonized banana, mango and safou fruits with different incidences (Fig. 1). In which *Aspergillus* was represented by *A. niger*, *Cercospora* (3 species), *Fusarium* and *Verticillium* by one spece. *Cercospora* contained 3 species namely *C. capsici, C. mangiferae* and *C. musae. Fusarium* and *Verticillium genera* were represented by one specie for each namely *F. oxysporum* and *Verticillium albo-atrum. Cercospora* was by far the most common genus affecting the different kinds of fruits. It appeared on 50 % each of banana, mango and safou fruits (Fig. 1). *Aspergillus, Colletotrichum, Fusarium* and *Verticellium* were the second most common genus affecting these fruits. A. niger was found on banana (8.62%), and mango (15%) and safou (12%). *Colletotrichum gloesosporioides* appeared with variable incidences on banana (8.62%), mango (15%) and safou (22.92%). Other species showed higher affinity towards certain fruits such as *Rhizoctonia solani* on mango and safou fruits.

Pathogenicity of fungal species:

Table 2 shows the diameters of lesions caused by the 3 species of *Cercospora* genus on the fruits of banana, mango and safou. Different species of *Cercospora* caused injuries with lesions diameters that vary depending on the type of fruit and fungal species. It should be noted that all the types of fruits used presented lesions.

Developed lesions varied from 22 mm to 36 mm on banana fruit, from 19 mm to 45 on mango fruit and from 16 mm to 24 mm on safou fruit. *C. mangiferae* and *C. capsici* caused injury significantly greater than that caused by *C. musae* on bananas. On mango fruit, *C. mangiferae* and *C. musae* caused injuries to 45 and 36.5 mm respectively while that caused by *C. capsici* was 19 mm.

It should be noted that damage caused by fungi on the safou fruits were weak compared to those caused on the banana and mango. However the safou fruits showed more likely *C. capsici* with a lesion of 24 mm. *C. mangiferae* confirmed its pathogenicity to the mango fruits. Figure 2 presents some of the lesions caused by species of genus *Cercospora* on the fruits.

This investigation embraces an extensive survey of the fungi associated with post-harvest rot of fruits in samples collected from markets in Dschang. The tested samples comprised of banana, mango and safou fruits. In this respect, Akinmusire (2011) and Chukwuka *et al.* (2010) mentioned that fruits can be affected by a wide range of microorganisms such as fungi which have a serious threat to production of fruits. Spoilage attributed to any change in the condition of food makes it less palatable, or even toxic; these alterations may be accompanied by changes in taste, smell, appearance.

During the first part of this investigation, it was possible to isolate 9 species belonging to 6 fungal genera from the samples of fruits. 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 *et al.,* 2005; Regnier *et al.,* 2010; Onyeani *et al*., 2012; Didy *et al*., (2013); Amadi *et al.,* 2014 ; Djeugap *et al*., 2015)

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During this study, *Colletotrichum musae* was also isolated and it is known as the causative agent of banana rot. Scott, (2001) found that the two primary post-harvest rots of banana fruits in Hawai'i were crown rot and anthracnose caused by the fungus *C. musae*. Raut and Ranade (2004) and Ranasinghe *et al.* (2005) reported that, banana suffer from serious post-harvest losses caused by fungal infections, especially *C. musae*.

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).

Species such as *Cercospora musae* or *Mycophaerella musae*, *Cercospora mangiferae* and *Cercospora capsici* were the most frequent compared with other fungal species isolated. These three species are generally reported to cause significant damage to fruits.

A. niger, *R.solani, F. oxysporum* and *F. solani* were relatively less important on these in respect to their low isolation frequencies. These fungi however have been reported as pathogenic in some fruits including mango, apple, banana and grape in other part in the tropics (Kortsen *et al*., 1994; Bashar *et al.,* 2012). Several reports showed the implication of *A. niger* 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).

IV. CONCLUSION

Several fungal species belonging to 6 fungal genera could be regarded as the most common causes of post-harvest deterioration of banana, mango and safou fruits in the Dschang markets, Western region of Cameroon. Results suggested the need of developing appropriate management strategy to control post-harvest diseases caused by *Cercospora* spp, especially since their pathogenicity has been proven on these fruits.

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Fig.1: Lesions caused by Cercospora spp on banana and safou fruits

REFERENCES

[1] Agrios, G.N., 2005. Plant pathology. 5th Ed. Elsevier Academic Press, California, USA, 922p.

[2] Akinmusire, O.O., 2011. Fungal Species Associated with the Spoilage of Some Edible Fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology*, 2011; 5(1): 157-161.

[3] Alexopoulos, C.J., C.W. Mims and Blackwell, M., 1996. Introductory Mycology. 4th Ed. Wiley, New York.

[4] Amadi, J.E., P. Nwaokike, G.S. Olahan and Garuba, T., 2014. Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka Metropolis. *Inter J Eng Appl Sci*, 4: 7-12.

[5] Bali, R.V., Bindu, M.G., Chenga, R.V., Reddy, K., 2008. Post-harvest fungal spoilage in sweet orange (*Citrus sinensis*) and acid lime (*Citrus aurentifolia* Swingla) at different stages of marketing. *Agric. Sci. Digest*., 28:265-267.

[6] Barnett, H.L. and Hunter, B.B., 1972. Illustrated genera of imperfect fungi. 3rd Edition. Burgess Publishing Company, 200 p.

[7] Bashar, M.A., S. Shamsi and Hossain, M., 2012. Fungi associated with rotten fruits in Dhaka Metropolis. Bangladesh *J Bot*, 41: 115-117.

[8] Chukwuka, K.S., Okonko, I.O., Adekunle, A.A., 2010. Microbial ecology of organisms causing pawpaw (*Carica Papaya L.*) Fruit decay in Oyo State, Nigeria. *American-Eurasian J. Toxicol. Sci*., 2 (1): 43-50.

[9] COLEACP (Comité de Liaison Europe-Afrique/Caraïbes/Pacifique). 2008. Guide de bonnes pratiques phytosanitaires pour l'avocat (*Persea americana*) issu de l'agriculture biologique en pays ACP.

[10] Didy O., Odimba A., Legreve B. and Dhed'A, D., 2013. Caractérisation des populations de *Mycosphaerella jiensis* et épidémiologie de la cercosporiose noire du bananier dans la région de Kisangani. Thèse de Doctorat Université Catholique de Louvain, French.72p.

[11] Djeugap, F.J., G.N. Tsompbeng, K.E. Keuete, A. Yaouba and Serferbe, S., 2015. Isolation and Identification of Fungi Associated with Avocado fruits from Local markets of the West Region of Cameroon. *Inter J Agri Biosci*, 4(2): 64-68. www.ijagbio.com

[12] Droby, S., 2006. Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticul*., 709: 45–51.

[13] Ewekeye, T.S., Oke, O.A. and Esan O.O., 2016. Studies on post harvest rot of apple (*Malus domestica* Borkh). *Indian Journal of Plant Sciences* ISSN: 2319–3824. Vol.5 (1) January-March, pp.36-41

[14] Everett, K.R., L.M. Boyd, H.A. Pak and Cutting, J.G.M., 2007. Calcium, fungicide sprays and canopy density influence postharvest rots of avocado. *Australasian Plant Pathology* 36: 223-230.

[15] Everett, K.R., S.G. Owen and Cutting, J.G.M., 2005. Testing efficacy of fungicides against postharvest pathogens of avocado (*Persea Americana var* Hass). *New Zealand Plant Protection* 58: 89-95.

[16] FAOSTAT., 2011. Statistiques des productions, d'exportations et d'importations fruitières du Cameroun.

[17] Hartill, W.F.T. and Everett, K.R., 2002. Inoculum sources and infection pathways of pathogens causing stem-end rot of Hass avocado (*Persea americana*). New Zealand *J Crop Hortic Sci*, 30: 249-260.

[18] Klich, M.A., 2002. Identifiction of common *Aspergillus* species. United state Department of Agriculture. Agriculutre Research Service, Southern Regional research center New Oluisiana USA, 116.

[19] Korsten, L., G.M. Sanders and Grosse-Weischede, E., 1994. Isolation and pathogenicity of avocado post-harvest pathogens from Westfalia and other avocado producing areas. South African Avocado Growers' Association Year book 17: 46-48.

[20] MINADER, 2012. Ministère de l'Agriculture et du Développement Rurale. Annuaire Statistique du Cameroun 2010.

[21] Moubasher, A.H., 1993. Soil fungi in Qater and Other Arab Countries. *The Scientific and Appl. Res. Center* Univ.of Qater. 670.

[22] Onyeani, C.A., S. Osunlaja, O.O. Oworu and Olufemi, S., 2012. First report of fruit anthracnose in mango caused by *Colletotrichum gloeoesporioides* in Southwestern Nigeria. *Inter J Sci Technol Res*, 1: 30-34.

[23] Parveen, S., Wani, A.H., Bhat, M.Y., Koka, J.A., Wani, F.A., 2016. Management of postharvest fungal rot of peach (Prunus persica) caused by Rhizopus stolonifer in Kashmir Valley, *India. Plant Pathology & Quarantine* 6(1): 19–29. doi 10.5943/ppq/6/1/4.

[24] Pitt, J.I., Hocking, A.D., 1985. Fungi and food spoilage. Sydney: *Acad. Press*, 1- 413.

[25] Ranasinghe, L., Jayawardena, B., Abeywickrama, K., 2005. An integrated strategy to control post-harvest decay of Embul banana by combining essential oils with modified atmosphere packaging. *Int. J. Food Sci. Technol*., 40: 97–103.

[26] Raper, K.B., Fennell, D.J., 1965. The genus *Aspergillus*, Williams and Wikins, Baltimore USA.

[27] Raut, S.P., Ranade, S., 2004. Diseases of banana and their management. In: Naqvi, S.A.M.H. (Ed.), *Diseases of Fruits and Vegetables*, Kluwer Academic Publishers, Netherlands, 2: 37–52.

[28] Regnier, T.S. Combrinck, Y. Du Plooy and Botha, B., 2010. Evaluation of *Lippia scaberrima* essential oil and some pure terpenoid constituents as postharvest mycobiocides for avocado fruits. *Postharvest Biolog Technol*, 57: 176-182.

[29] Rivera-Vargas, L.I., Y. Lugo-Noel, R.J. McGovern, T. Seijo and Davis, M.J., 2006. Occurrence and distribution of *Colletotrichum* spp. on mango (*Mangifera indica* L.) in puerto rico and Florida, USA. *Plant Pathol. J*., 5: 191-198.

[30] Salman, M.A.M., 2005 – Biological Control of *Rhizopus* Soft Rot on Apple, Pear and Peach by Trichoderma harzianum. Doctoral Thesis, National University, India.

[31] Scott, P.M., 2001. Analysis of agricultural commodities and foods for *Alternaria* mycotoxins. *J. Am. Oil Chem. Soc*., 6: 1809–17.

[32] Singh, R. P., Dhania, G., Sharma, A. and Jaiwal, P. K., 2007. Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Environmental bioremediation technologies, Singh, S. N.;Tripahti, R. D. (Eds) Springer, 223-258

[33] Smith, N.R., Dawson, V.T., 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. *Soil Sci.,*58: 467- 471.

[34] Sommer, N.F., 1985. Strategies for control of post-harvest disease of selected commodities. In: Post-harvest Technology of Horticultural Crops. University of California Press,. 83-98.

[35] Tafinta I.Y., K. Shehu, H. Abdulganiyyu, A.M. Rabe and Usman, A., 2013. Isolation and

Identification of Fungi Associated with the Spoilage of Sweet Orange (Citrus Sinensis) Fruits In Sokoto State. *Nigerian Journal of Basic and Applied Science*, 21(3): 193-196

[36] Valiuskaite, A., Kvikliene, N., Kviklys, D. and Lanauskas, J., 2006. Post-harvest fruit rot incidence depending on apple maturity. *Agronomy Research* 4(Special Issue) 427-431

[37] Wharton, S.J., Basu, S.P., Ashe, H.L. 2004. Smad affinity can direct distinct readouts of the embryonic extracellular Dpp gradient in Drosophila. *Curr. Biol.* 14(17): 1550-1558.

Table.1: Different types of fungal species isolated from deteriorated fruit samples during this investigation.

Genus	Species	Banana	Mango	Safou
Aspergillus	*A. niger*	+	+	+
Cercospora	*C. capsici*	-	-	+
	C. mangiferae	-	+	-
	C. musae	+	-	-
Colletotrichum	*C. gloesosporioides*	+	+	+
	C. musae	+	-	-
Fusarium	*F. oxysporum*	+	+	+
Rhizoctonia	*R. solani*	-	+	+
Verticellium	*V. albo-atrum*	+	+	+
No. of species		6	6	6

+ = Present; - = absent

Table.2: Diameters (mm) of the lesions developed on fruits by Cercospora genus

	Diameters (mm) of the lesions			
Fruit	*C. musae*	*C. mangiferae*	*C. capsici*	Witness
Banana	22.12 ± 4.82^{b}	36.00 ± 5.84^{a}	31.75 ± 2.33^{a}	0
Mango	36.50 ± 7.58^{a}	45.00 ± 11.22^{a}	19.88 ± 3.42^{b}	0
Safou	16.12 ± 6.70^{b}	16.37 ± 4.46^{b}	24.31 ± 5.50^{b}	0

Values followed by the same aphabetical letter in the same column are not significantly different according to Duncan test.

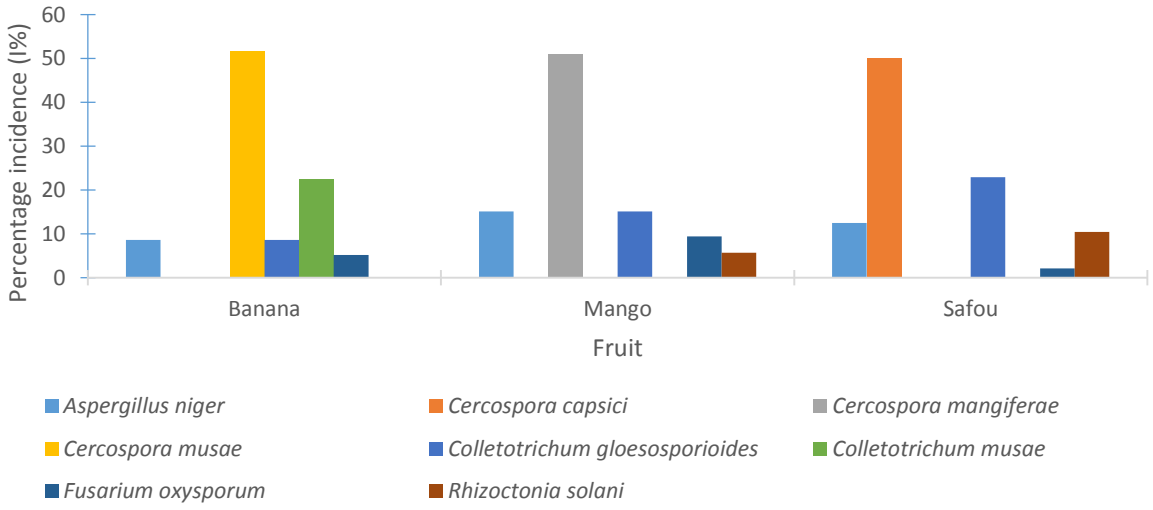

Fig.2 : Prevalence of the most common fungal species isolated from different fruits