INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 15, No. 5, p. 1-11, 2022

Open Access RESEARCH PAPER

Inventory of fungal pathogens of early maturing Mango varieties in the Kounkane area, Southeast of Senegal

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Keywords: *Mangifera indica*, Phytopathogenic fungi, Mango tree diseases, Dry season, Senegal

Publication date: November 05, 2022

Abstract

In Senegal, mango production, in spite of a positive record performance in recent years, is confronted with numerous diseases. The rainfall richer south and south eastern parts of the country, stand among the main contributors of mango production. Fruits are known to by infested and rotten almost totally when they mature in the rainy season. However, a lot of mango varieties mature before onset of the moist rainy season. The present study was therefore undertaken to make an inventory of the causing agents of pre- and post-harvest diseases of mangoes maturing before the rain starts. Samples were taken from the fields and brought to the laboratory for analysis. The results showed that *Colletotrichum spp.*, *Pestalotia sp.*, *Lasiodiplodia sp.*, *Fusarium spp.*, *Curvularia sp.*, *Alternaria sp*. and non- sporulating fungi were associated with diseased organs in tree canopy. Fungal diversity was higher for orchards harboring trees over 15 years of age. For the mangoes, the disease incidence reached 13% after harvest. This infestation was due to by 50% to non-sporulating fungi, 31% to *Colletotrichum spp.*, 13% to *Fusarium spp*. and 6% to *Lasiodiplodia sp*. These results show the pathogens are present in the fields and that their dynamic depend on the climatic conditions.

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Introduction

Mango (*Mangifera indica* L.) is considered one of the most important fruit crops in the tropics. Within the fruit and vegetable sub-group, the mango industry is a promising sector for economic growth. Global mango production is estimated at over 43 million tons (Faostat, 2015). Mango is grown in most West African countries, with an estimated production area of 540,000km², stretching from Senegal throughout to eastern Nigeria according to the Minister of Commerce in 2016 (Ministère du commerce, 2016). Several countries in the sub-region, including Senegal, are currently spearheading their export activities (CARE, 2009). In Senegal mango production represents 60% of the country's fruit production, with an estimated annual production of 150,000 tons harvested from a land area of about 41,000 ha (Diedhiou *et al.*, 2014).

The mango sector is the most dynamic in fruit exports in Senegal (Diouf, 2016). Mango exports have increased from 300 tons in 1998 (Rey, 2011) to 24500 tons in 2021 (Dieye and *al*., 2021). This performance is due to the modernization of traditional orchards and the creation of new plantations for export. The mango producing orchards are located in the regions of Dakar, Thies, Saint-Louis, Fatick, Kolda, Ziguinchor and Sedhiou (Diedhiou *and al.*, 2014). The soil and climatic conditions and land holdings in the country offer great potential for expanding mango production (USAID-PCE, 2006). The improvement of the mango sector and the implementation of improved technologies along the value chain, offer labor and employment opportunities especially to women and rural youth.

However, mango production, despite a positive record in recent years, is still affected by numerous constraints, including phytosanitary problems. The mango tree is susceptible to host a number of diseases agents at all stages of its development from planting to harvest (Alemu *and al.*, 2014). In the field, mango is most often the host of several pathogens especially fungi that significantly down turn production potential (Khanzada *et al.*, 2004). Diverse fungi cause post-harvest rot of mangoes, with the identity and the incidence of species highly depending on the climatic conditions (Diedhiou *et al.*, 2007). The post-harvest mango rotting can affect up to 100% of mangoes produced during the rainy season in southern Senegal in the absence of adequate control (Diedhiou and *al*., 2014). Anthracnose due to *Colletotrichum gloeosporioides* is the almost exclusive causing agents under those conditions while disease incidence is low and results from a diversity of fungi in the Ziguinchor area. Different works have reported various fungi on mango namely *Lasiodiplodia theobromae*, *Colletotrichum spp.*, *Curvularia sp., Pestalotia mangiferae., Alternaria sp.* and *Fusarium spp*. among others (Johnson *and al.*, 1992; Sharma, 1993; Ploetz *and al.*, 1996; Al-Adawi *and al.*, 2003; Dieye and *al*., 2021). It was therefore important for the mango industry in Senegal to make an inventory of causing agents for mango diseases. This study was conducted with the objective of identifying the fungi responsible for mango diseases in the Kounkane area in southern Senegal during the dry season.

Materials and methods

Study site

The orchards are located in Kounkane, 90 km from the city of Kolda in the extreme south of Senegal at 13° 05’ North and 14° 49’ West (Fig. 1). The climate is of the Soudano Guinean type and characterized by heavy rainfall (ANSD, 2013). Samples were collected from orchards with trees aged from 5 more than 15 years.

Sampling in orchards

- Sampling of vegetative organs and flowers

Symptomatic organs of the mango trees (leaves, bark, inflorescence and fruits of mango were collected from 10 plants randomly distributed in each orchard and taken to the laboratory for identification.

- *Sampling of fruits*

Before the rainy season, a total number of 20 mature green mangoes from different trees of the same variety were sampled in each orchard. The fruits were placed in crates for transport to the laboratory. The fruits were numbered from 1 to 20 for each orchard and kept at room temperature in the laboratory. The mangoes were evaluated every 48h until ripeness. For infested fruits isolation was undertaken to identify the causing pathogenic agent.

- Isolation of fungi

Samples (leaves, bark, inflorescence and fruits of mango) showing symptoms were first immersed in 1% chlorine for 1 minute for the other organs. Thereafter, an explant was taken from the symptom front both for fruits and other organs, using a sterile scalpel blade. Under the fume hood, explants were successively cut into smaller pieces, immersed in 70% alcohol and then in sterile distilled water before finally being inoculated at a rate of 3 explants per Petri dish containing the PDA medium.

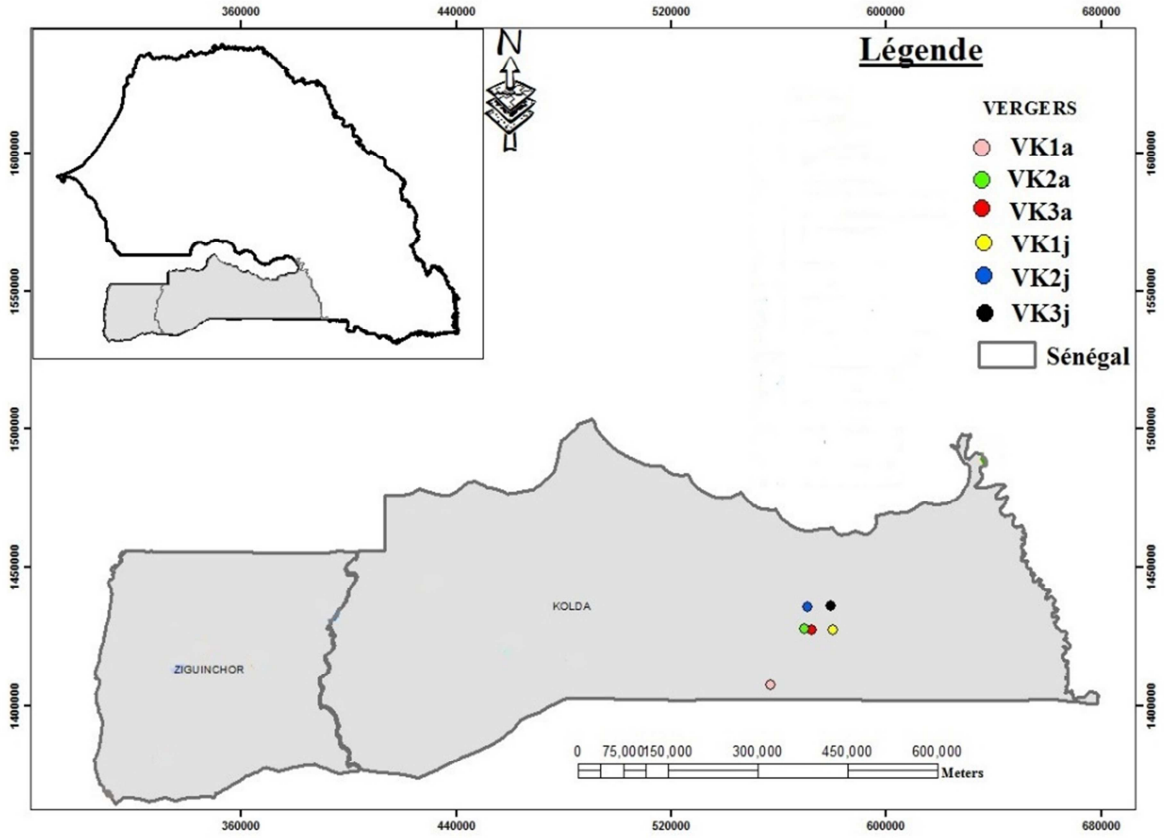


Fig. 1. Location of the orchards.

Legend

Vk1a: first old orchard; Vk2a: second old orchard; Vk3a: third old orchard; Vk1j: first young orchard; Vk2j: second young orchard and Vk3j: third young orchard

For the fruits, the mangoes were first soaked in a 1% NaOCl solution for 15 min and two crossed incisions in the form of V were made at the front of progression of the rotting tissue with a sterile scalpel. A piece of flesh under the peal was taken and placed in a Petri dish containing Potato Dextrose Agar amended with 100 ppm of chloramphenicol.

The plates are incubated in the oven at 28°C and after 2 days an agar slant containing the tips of growing mycelium was transplanted into fresh

plates to obtain pure culture. The latter are left for 2 to 3 weeks for sporulation.

Characterization of fungal cultures

Fungi are characterized on the surface and reverse side of the isolates and according to the spores by referring to the keys of determination of fungi (Barnett and Hunter; Watanabe) for identification (Barnett and Hunter, 2006; Tsuneo., 2002).

Statistical analysis

The data was processed with the Microsoft Office 2013 Excel Spreadsheet. A two-factor analysis of variance was performed using the aov function of the agricolae package. The multiple comparison test of Student Newmann and Keuls (SNK) was done with the function SNK. test of the package agricolae.

Results

Symptomatology encountered in the orchards and Characterization of fungal cultures from symptomatic tissue

A total of 06 genera of pathogenic fungi was identified as responsible for diseases. This group was composed with Colletotrichum, Fusarium, Pestalotia, Lasiodiplodia, Alternaria and Curvularia and some non-sporulating fungi.

Colletotrichum spp.

Colletotichum spp. appeared on leaves as brownish spots with an irregular periphery (Fig. 2A) and blackish spots on fruits with a depression in the center (Fig. 2B). In the petri dish on PDA medium Colletotrichum sp. isolates appeared in 4 groups according to the coloration and appearance of their mycelium (Fig. 4). Microscopic observation showed hyaline cylindrical one cell conidia (Fig. 3).

Fusarium spp.

This fungus was isolated from the floral malformation (Fig. 5A) and the vegetative malformation (Fig. 5B). Microscopic observation showed micro and macro conidia (Fig. 6). The isolates were differentiated into three groups according to the appearance and coloration of the mycelium ranging from whitish, beige to yellowish (Fig. 7).

Pestalotia sp.

This fungus was isolated from whitish spots on leaves (Fig. 8). Microscopically the conidia are hyaline to dark septate and pointed-ended, ellipsoid to fusiform with two or more appendages (Fig. 9). On the PDA medium, a sparse, whitish colored mycelium with regular margins with striae (3 or 4) on which black pycnidia appear with age is built. The underside of petri dish is yellowish with the appearance of pycnidia in the form of black dots (Fig. 10).

Lasiodiplodia sp

Lasiodiplodia sp. has been isolated from split branches and trunks where sap flow (gumming) is sometimes observed (Fig. 11A). This fungus has also been isolated from stem end rotting mango tissues (Fig. 11B). Inside the affected fruit, the pulp is soft and shows browning. Immature conidia are hyaline ovoid to ellipsoid. Mature conidia are dark brown and septate (Fig. 12). *Lasiodiplodia sp.* isolates are characterized by brown mycelium in mat form, sparse and irregularly contoured on the surface. The underside of the boxes is brown. The mycelium fills the dish after 7 to 10 days of incubation (Fig. 13).

Alternaria sp.

This fungus was isolated from branches and trunks showing cracks sometimes accompanied by gumming (Fig. 14).

Microscopic observation showed septate mycelium and brown conidia with transverse (3 to 4) and longitudinal (1 to 2) partitions in the form of baseball whales (Fig. 15). On PDA medium, the mycelium of isolates is dense and grayish in color on the surface of the dishes, while the reverse side of the boxes is blackish with regular margin. The mycelium fills the dish after 4 to 5 days of incubation (fig. 16).

Curvularia sp.

Curvularia sp. appeared on the leaves as dark spots with a blackish outline of different shapes (Fig. 17). Microscopic observation shows dark, more or less fusiform conidia, usually curved in the center with an enlarged central cell (fig. 18). On the surface of the boxes, the mycelium is dense and dark brown. The margin is regular and the underside of the isolates is blackish. The mycelium fills the dish after 5 to 7 days of incubation (Fig. 19).

Fungi isolated from flowers and vegetative organs

In all orchards, a diversity of fungi were responsible for pre-harvest diseases. The causing agents were *Colletotrchum sp.*, *Fusarium sp.*, *Lasiodiplodia sp.*, *Pestalotia sp.*, *Alternaria sp.* and *Curvularia sp*. In terms of population the setup of the species found as well as their level of importance in terms of percentage was variable. *Colletotrichum sp*. was the most frequently identified in the orchards (Fig. 20).

Fungi isolated from infested mangoes

Mango disease was mainly due to *Colletotrichum sp*. (31%), Fusarium spp. (13%) and *Lasiodiplodia sp*. (6%) and for 50% of cases non-sporulating fungi were associated to several symptoms (Fig. 21).

Influence of the age of the orchards on pathogenic fungi of flowers and vegetative organs

For orchards with above 15 years of age, the population of the pathogenic fungi was made of *Lasiodiplodia sp*. (35.7%), *Colletotrichum sp*. (14.3%), *Pestalotia sp*. (28.6%), non-sporing fungi (14.3%) and *Alternaria sp*. (7.1%) (Fig. 22).

In young orchards, only 3 genera of fungal pathogens were found. *Colletotrichum sp*. (53.3%) was dominant, followed by *Fusarium spp*. (20.1%), *Curvularia sp*. (13.3%) and non-sporing fungi (13.3%). *Alternaria sp.* and *Lasiodiplodia sp*. were not detected (fig. 22).

Influence of the age of the orchards on the fungal population of fruit diseases

In aged orchards, *Colletotrichum sp. Fusasium spp*. and other non-sporuling fungi made up the fungal population causing fruit diseases (Fig. 23). In young orchards in contrast, only *Colletotrichum sp.* was found.

Brownish spots on leaves (A) and blackish spots on fruit (B) related to *Colletotrichum sp*.

Fig. 2. Symptoms of *Colletotrichum sp*. on mango leaves and fruit.

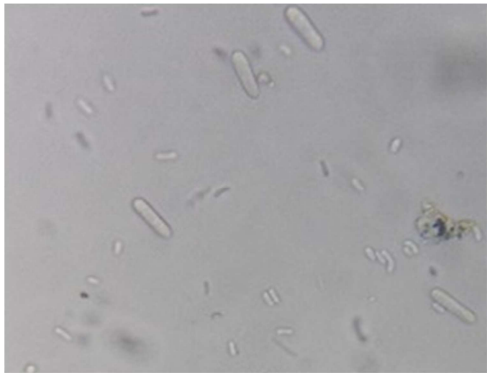


Fig. 3. Spores of *Colletotrichum sp*. (G400).

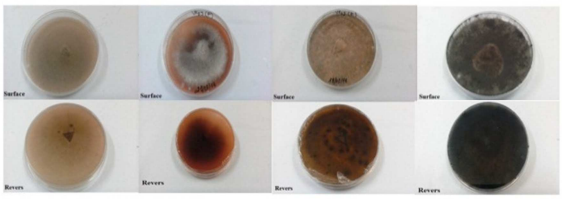


Fig. 4. Morphological appearance of the 4 groups of *Colletotrichum spp* on PDA medium.

Fig. 5. Floral malformation (A) and vegetative malformation of mango.

Fig. 6. Spores (macro and micro conidia) of *Fusarium sp*. (G400).

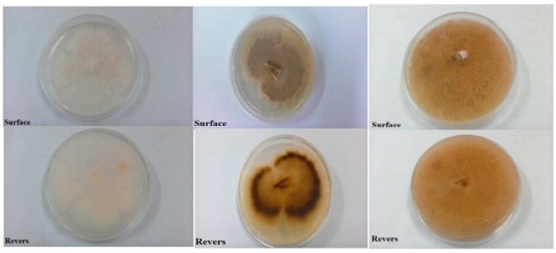
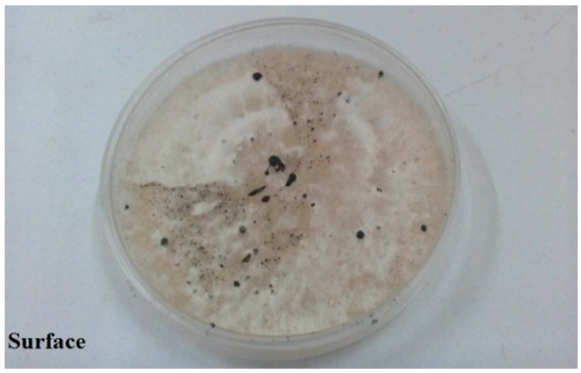


Fig. 7. Morphological appearance of the 3 groups of *Fusarium spp* on PDA medium.

Fig. 8. Whitish spots on mango leaves related to *Pestalotia sp*.

Fig. 9. Spores of *Pestalotia sp*. (G400).



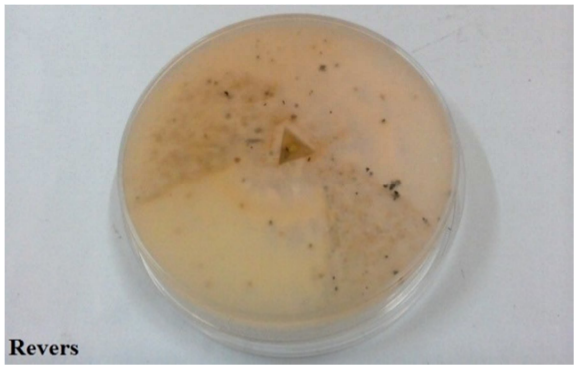


Fig. 10. Morphological appearance of an isolate of *Pestalotia sp.* on PDA medium.

A : Splitting on branch B : Stem end rot

Fig. 11. Symptoms of *Lasiodiplodia sp*. on mango branch (A) and fruit (B).

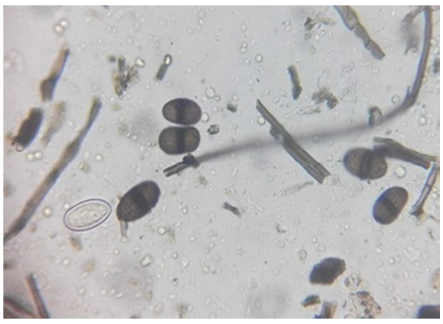


Fig. 12. Spores of *Lasiodiplodia sp*. (G400).

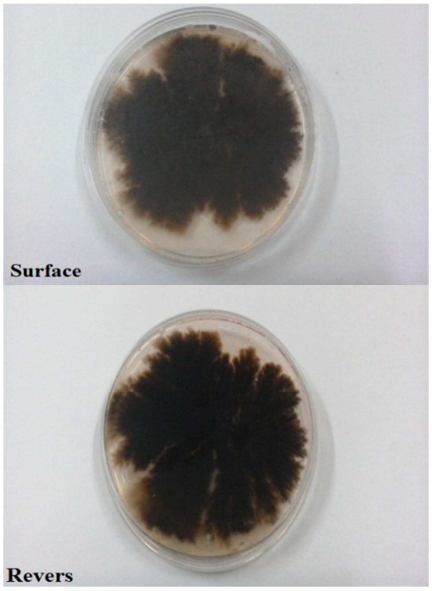


Fig. 13. Morphological appearance of an isolate of *Lasiodiplodia sp*. on PDA medium.

Fig. 14. Splitting on mango branch related to *Alternaria sp*.

Fig. 15. Spores of *Altermaria sp*. a (G400).

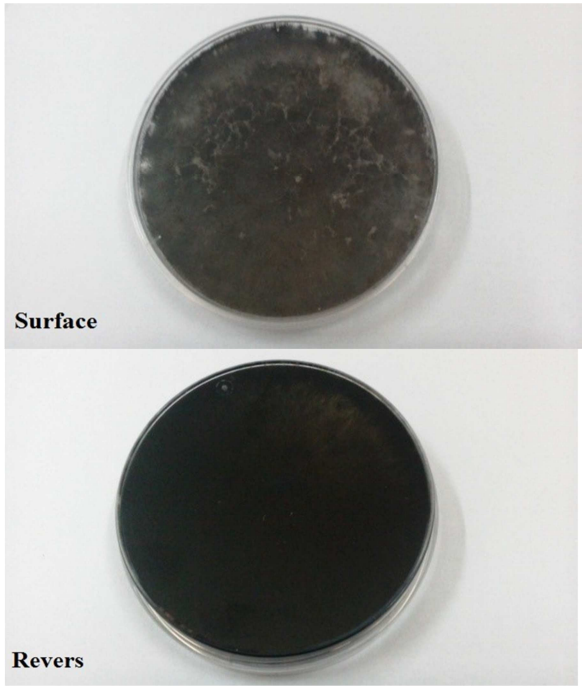


Fig. 16. Morphological aspect of an isolate of *Alternaria sp.* on PDA medium.

Fig. 17. Dark spots on mango leaves related to *Curvularia sp*.

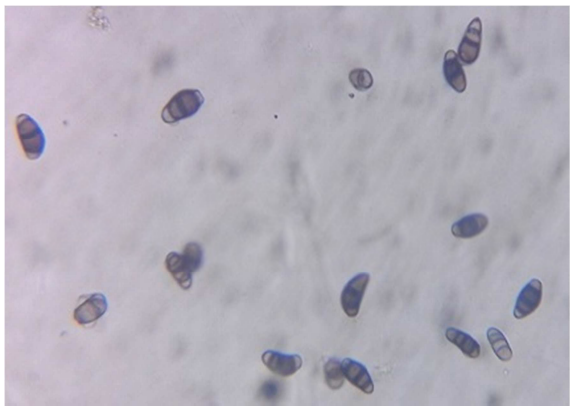


Fig. 18. Spores of *Curvularia sp*. (G400).

Fig. 19. Macroscopic aspect of an isolate of *Curvularia sp*. on PDA medium.

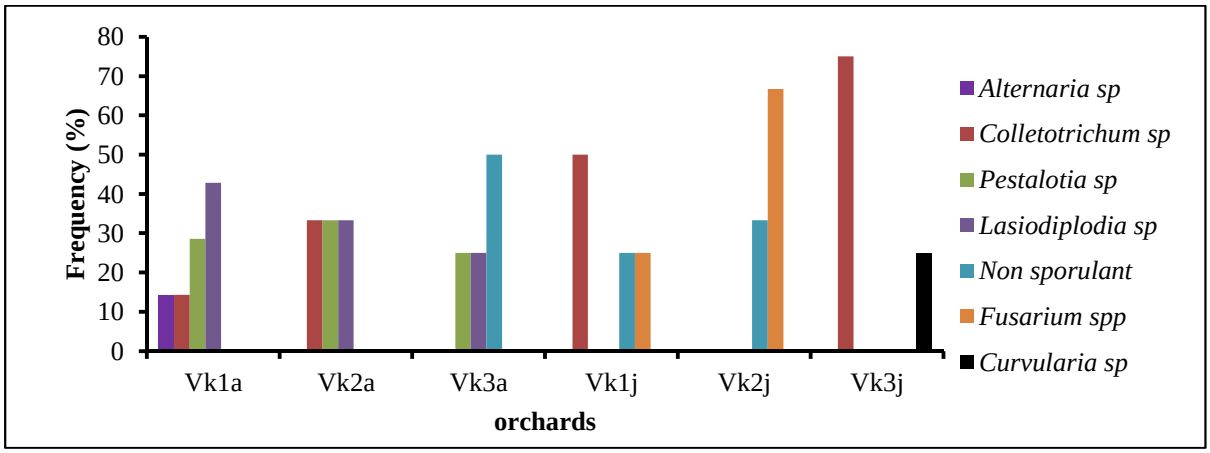


Fig. 20. Frequency of fungi associated with pre-harvest diseases.

Legend

Vk1a : first aged orchard; Vk2a : second aged orchard; Vk3a : third aged orchard; Vk1j : first young orchard; Vk2j : second young orchard and Vk3j : third young orchard

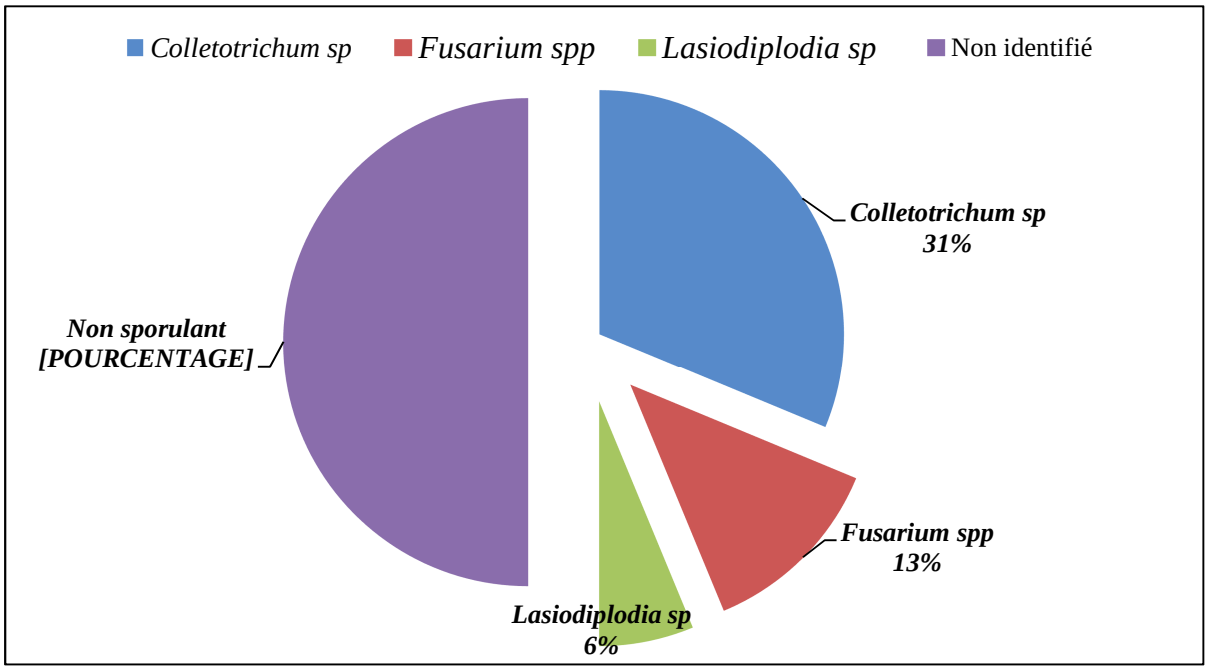


Fig. 21. Fungal pathogens associated with post-harvest diseases.

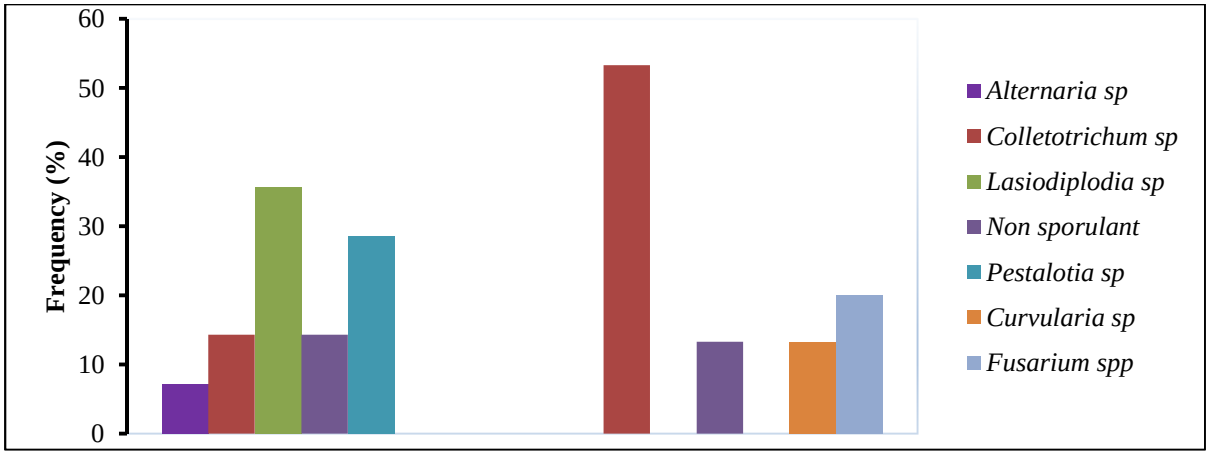


Fig. 22. Fungi isolated on vegetative organs according to the age of the orchards.

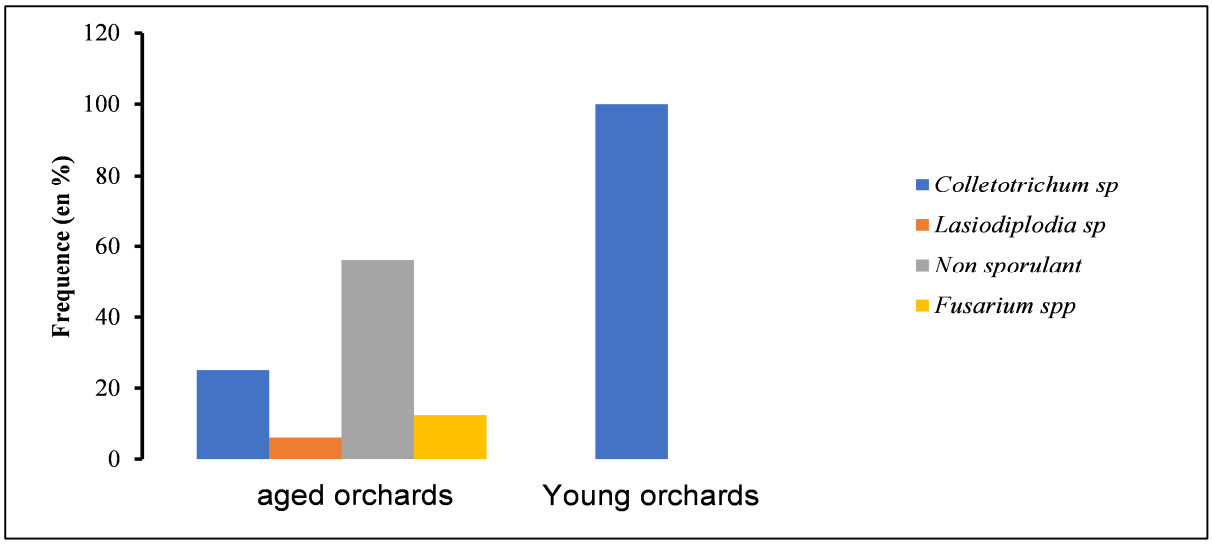


Fig. 23. Fungi isolated on post-harvest spoilage according to the age of the orchards.

Discussion

Mango trees are often confronted with diseases in the field but also, the fruit is known to develop disease symptoms after harvest. The population of the fungi isolated from different organs showing disease symptoms was diverse. The diseases were caused by 6 identified fungal species/genera in addition to non sporulating fungal agents that therefore not be identified. *Colletotrichum sp., Lasiodiplodia sp., Pestalotia sp., Fusarium spp., Culvularia sp.* and *Alternaria sp.* were represented in different proportions depending in the orchard. The presence of these different fungi led to a diversity of symptoms in the orchards. *Colletotrichum sp*. was the most frequently encountered among the fungi. This could be attributed to the close relationship between *Colletotrichum sp*. and the high relative humidity in the southern regions of the country. In fact, in the Kolda region where the orchard are located, annual rainfall is between 700 to 1300mm (ANSD, 2013). Although the work was done at the end of the dry season before onset of the rainy season, Colletotrichum was dominant. This level of importance could be derived from the residual population from its highly dominant status when moisture level is high (Dodd *et al.,* 1997). Although mortality would be at play during the dry season, favoring other species like *Alternaria sp.* and *Lasiodiplodia sp.*, the big size of the mango trees (important height (20 m and above) and huge canopy) could have contributed

to create a high relative humidity in the trees making survival easy for *Colletotrichum sp.* (Dodd and *al.,* 1997).

Lasiodiplodia sp. and *Alternaria sp*. were the only fungi isolated from stems and branches of the trees showing dieback and cracking symptoms as well as gummosis. Similar results were also observed on mango trees from the northernmost part of Senegal (Mbaye *and al.*, 2016).

Foliar diseases were associated with *Colletotrichum sp*, *Curvularia sp, Pestalotia sp*, *Fusarium sp* and non sporulating fungi. The mango malformation disease due to *Fusarium spp* and the floral malformation in particular was also present in the sampling area. This disease was already reported by Senghor and *al*., in 2012 and known to occur in this area (Dieye and *al*., 2021).

As for the incidence of diseases on fruits, the observation of mangoes during ten days, showed that 87% of mangoes ripened disease free. This could be brought in relation with the fact that the fruits were collected from early maturing mango varieties for which harvest takes place before of the rainy season. However, 13% of the mangoes showed post-harvest diseases caused mainly by *Colletotrichum sp. and Lasiodiplodia sp*.

The presence of *Colletotrichum sp*. may be linked to the fact that this fungus, in addition to be able to survive in the tree canopy, can remain in a latent phase for several days or even months, in the form of appressoria below the epidermis of the fruit (Dodd *and al.*, 1991).

Its development resumes during the physiological changes of the mango during ripening and causes the disease symptoms observed during the evaluation. *Lasiodiplodia sp*. was isolated from fruits showing symptoms of stem end rot. This is in line with previous results that reported *Lasiodiplodia theobromae* as the causing agent of stem end rot of mangoes after harvest in the dry season (Mbaye, 2006; Diedhiou *and al.*, 2007; Haggag *and al.*, 2010; Dieye *and al.*, 2020).

The diversity of fungi was higher in the older orchards. This could be explained by the fact that in old orchards the trees are very tall with a huge canopy making available large quantities of debris and dead leaves and twigs. The shade and mild temperatures as well as favorable moisture level in tree canopy build a microclimate that makes the environment suitable for large numbers of microorganisms to survive (Gilhen-Baker et *al*. 2022).

Conclusion

In the south eastern part of Senegal mango trees in the orchards are infested by pathogenic fungi like *Colletotrichum sp., Fusarium spp., Lasiodiplodia sp., Pestalotia sp. Alternaria sp.* and *Curvularia sp.* and other fungi not sporulating on PDA. The diversity of the fungi was higher in the older orchards. In the fruits, only *Colletotrichum sp.,* and *Lasiodiplodia sp.* were causing diseases on fruits maturing in the dry season. The mango malformation disease was more present in this locality.

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