RESEARCH ARTICLE

COMPARATIVE EFFECTIVENESS OF THREE SYSTEMIC FUNGICIDES ON THE FUNGI RESPONSIBLE FOR THE CAVENDISH SUBGROUP GRANDE NAINE BANANA POST-HARVEST DISEASES IN CÔTE D'IVOIRE.

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Abstract
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Post-harvest diseases reduce the market value of bananas and cause huge losses of production intended for export; despite the application of various antifungal molecules in post-harvest treatment. In order to help improve banana quality and reduce the number of fungicidal molecules to be used in chemical treatment, a study on the sensitivity of fungal strains responsible for post-harvest diseases to three systemic fungicides was conducted in Côte d'Ivoire. For two years, the sensitivity of *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicullium* sp. isolates to fungicidal molecules of Azoxystrobin, Boscalid and Imazalil at the recommended doses of 1200 ppm, 300 ppm and 500 ppm respectively, was tested *in vitro* on PDA medium and *in vivo* on Grande Naine banana. The Imazalil molecule inhibited more the growth of the different fungi under controlled conditions. *In vivo*, bananas inoculated with *Botryodiplodia* sp. and *Fusarium* sp. isolates but not treated with fungicides developed crown rot. *Colletotrichum* sp. and *Musicillium* sp. isolates caused epicarp necrosis and distal end rot, respectively, in bananas not treated with fungicides. The bananas inoculated with fungi and treated with Boscalid and Azoxystrobin developed symptoms of post-harvest diseases. However, the bananas inoculated with the different fungi and treated with Imazalil showed no symptoms of post-harvest disease. Imazalil alone is effective against the fungi responsible for postharvest diseases of Grande Naine banana in storage intended for export.

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Introduction:-

Dessert banana is a tropical fruit of socio-economic and nutritional importance which improves the food security of millions of people around the world (Arias *et al*., 2003). The dessert banana sector accounts for 2% of Côte d'Ivoire's gross domestic product. Its cultivation covers 5.49 million hectares with a yield of 113.28 million tons in 2016 (Faostat, 2016). The African continent produced 21 million tons in 2016 over a surface area of 1.94 million hectares. Côte d'Ivoire is the second largest African producer of dessert bananas after Cameroon (Faostat, 2016). With 330 946 tons in 2016 banana ranks third in food crop yields after yam and cassava (Faostat, 2016). The bulk of Ivorian dessert banana yield is intended for export to European Union countries; thus constituting a major

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contribution to the economy and a source of revenue for the players in the sector. To this end, Côte d'Ivoire is planning to reach a yield of 500 000 tons of bananas in 2020 (Le Parisien, 2016).

In order to achieve this goal, it is important to fight against post-harvest diseases which are a real threat to the yield and quality of bananas intended for export. In Côte d'Ivoire, bananas intended for export are constantly subject to crown and distal end rot as well as epicarp necrosis (Nath *et al*., 2014). These post-harvest diseases are responsible for 25 to 30% of banana losses in India (Kachhwaha *et al*., 1991), 5 to 25% in developed countries (Khader, 1992) and 30 to 60% in Senegal (Zakari, 2007). The fungal strains responsible for such post-harvest losses of bananas vary according to production zones and seasons (Lassois *et al*., 2010). In Costa Rica, *Musicillium theobromae, Colletotrichum musae, Ceratocystis paradoxa, Lasiodiplodia theobromae, Nigrospora sphaerica, Cladosporium* sp., *Penicillium* sp., *Acremonium* sp., *Aspergillus* sp., as well as several species of *Fusarium*, namely *F. semitectum*, *F. oxysporum, F. solani*, etc. have commonly been found associated with banana crown rot (Johanson and Blasquez 1992; Anthony *et al*., 2004). The works of Kouakou *et al*. (2017) have helped identify 13 fungal species involved in banana post-harvest diseases in Côte d'Ivoire during rainy seasons.

The failure of post-harvest disease control methods may be due to a lack of knowledge of the pathogenicity of the causal agent. Azoxystrobin is a molecule applied to banana plants in plantation in the fight against fungal foliar diseases such as banana tree leaf spot disease but also in post-harvest. Sensitive fungi may develop resistance to this molecule. Kouakou *et al*. (2017) identified fungal species associated with post-harvest disease symptoms as pathogens based on high isolation frequencies without pathogenicity test.

However, the presence of an agent on a plant organ even infected does not make it the causal agent of the infection. Marin *et al*. (1996) isolated *Musicillium theobromae*, *Lasiodiplodia theobromae* and *Nigrospora sphaerica* on banana in Costa Rica but they were not pathogenic for the crop.

Banana exporting companies use fungicide treatment to control banana post-harvest diseases (Lassois and Lapeyre de Bellaire, 2014). However, a recurrence of those diseases is noticed in Côte d'Ivoire. This situation could be justified by the diversity of fungal populations responsible for post-harvest diseases present on bananas as from plantations.

This work aims at improving the chemical control of producers against post-harvest diseases of bananas intended for export.

Material and methods:-
Sensitivity of fungal strains responsible for banana post-harvest diseases to fungicides used by producers:-
Botryodiplodia sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp. strains provided by the Laboratory of Plant Pathology of the University Nangui Abrogoua were used. The sensitivity of the strains was assessed *in vitro* by conidial culture on 2% of agar medium amended with fungicides at concentrations of 1200 ppm (Azoxystrobin), 300 ppm (Boscalid) and 500 ppm (Imazalil) in Petri dishes. Three repetitions were made per concentration and per strain. The control agar media were prepared without fungicide. The cultures were incubated under laboratory conditions (25 ± 2 °C). This study was conducted during four consecutive crop years (half-year). In each dish, the length of the longest germ tubes was measured with a micrometer after 48 hours of incubation. The average inhibition percentage of conidial germ tube elongation of each strain was calculated according to the following formula (Hasan, 2012):

$$I\ (\%) = \frac{1}{n}\sum_{i=0}^{n} \frac{Lo - Lt}{Lo} \times 100$$

I: percentage of conidial germination inhibition; Lo: average germ tube length of the control; Lt: average germ tube length of the treatment; n: number of repetitions
The behavior of each strain depending on each fungicide was determined using the Kumar *et al* sensitivity scale. (2007).
I = Inhibition percentage; I > 90%: Highly sensitive (S+); 75% < I < 90%: Sensitive (S); 60% < I < 75%: Moderately resistant (R-); 40% < I < 60%: Resistant (R); I < 40%: Highly resistant (R +).

The most effective fungicide identified was the one that inhibited more fungal strains by more than 90%.

A spore suspension inoculum concentrated at 10^6 conidia/ml was prepared to perform the *in vivo* test of fungal strains sensitivity to the different fungicides. To this end, 180 farm-gate bananas of the same age were detached from crowns and disinfected with sodium hypochlorite (12 °chl) at 2% for 5 minutes. The dried bananas were inoculated with 50 µl of the spore suspension at the crown, epicarp and distal end of each finger. Five repetitions were made per inoculated strain. The different fungicides were applied to inoculated bananas according to the following treatments: Uninoculated and untreated (UN+UNT); Uninoculated and treated (UN+T); Inoculated and untreated (I+UNT); Inoculated and treated (I+T). The most effective fungicide was the one in the presence of which bananas developed the least symptoms.

Statistical analyses:-
Data analysis was done using the STATISTICA version 7.1 software. An analysis of variance helped compare the average inhibition rates of conidial germ tube elongation of fungal strains. In case of significant difference at 5% threshold, the Newman Keuls test was used to determine the different homogeneous groups.

Results:-
***In vitro* sensitivity of fungal strains to the fungicides used:-**
Conidial germ tube elongation of fungal strains was inhibited by the different fungicides. The sensitivity of each fungal strain varied according to the inhibition percentages per fungicide used (Fig 1). Azoxystrobin and Boscalid inhibited the growth of post-harvest fungi from 8.29 to 77 % and from 7.86 to 70.23 %, respectively, whereas Imazalil reduced the same fungi from 36.86 to 100 %. Imazalil inhibited more the growth of fungi. Azoxystrobin inhibited *Botryodiplodia* sp. from 21.42 to 64.47 %; *Colletotrichum* sp. from 19.2 to 70.17 % and *Fusarium* sp. from 38.01 to 93.16 %. Imazalil inhibited more the isolates of *Botryodiplodia* sp. Azoxystrobin inhibited the isolates of *Colletotrichum* sp., *Fusarium* sp.; *Musicillium* sp. from 44.14 to 77 %; 8.29 to 48.29 %; 19.71 to 53.86 %, respectively. Azoxystrobin inhibited more the isolates of *Musicillium* sp.

Boscalid inhibited the isolates of *Colletottrichum* sp., *Fusarium* sp. and *Musicillium* sp. from 19.45 to 73.23 %; 7.86 to 50.84 and 20.14 to 45.91 %, respectively. Boscalid inhibited more *Colletotrichum* sp.
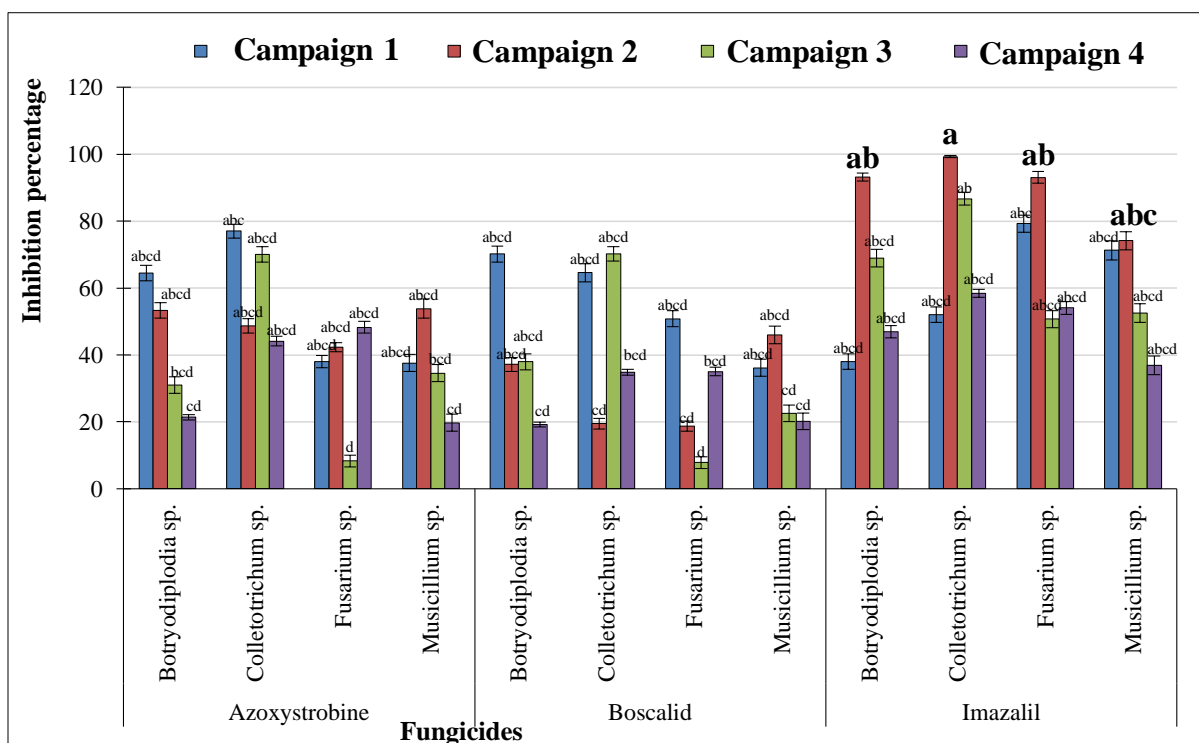
Figure 1:- Inhibition percentage of the conidial germ tube of the fungal strains responsible for post-harvest diseases depending on the fungicides used and the monitoring campaigns carried out

***In vivo* sensitivity of the fungal strains of banana post-harvest diseases:-**
Bananas not treated with Imazalil and not inoculated with post-harvest fungi did not show symptoms of infection (Fig 2). In contrast, bananas not treated with Imazalil and inoculated with *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp. isolates showed crown rot, epicarp necrosis, crown and distal end rot, respectively. Each of the fungi inoculated to bananas were responsible for the observed infections.

Bananas treated with Azoxoystrobin and Boscalid and inoculated with *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp. developed crown rot, epicarp necrosis and distal end rot, respectively (Figs 3 and 4). However, bananas inoculated with the different fungi and treated with Imazalil showed no symptoms. Imazalil protects bananas against post-harvest diseases of bananas in storage.

Uninoculated bananas and not treated (UN + UNT)	Inoculated bananas and not treated (I + UNT)	
	Botryodiplodia sp.	*Colletotrichum* sp.
	Fusarium sp.	*Musicillium* sp.

Figure 2:- Status of uninoculated and untreated bananas, bananas inoculated with fungal strains but not treated with fungicides.

Uninoculated bananes and treated with fungicides (UN + T)		
Azoxystrobin	Boscalid	Imazalil

Figure 3:- Status of bananas uninoculated and treated with the different fungicides

Fungal isolates inoculated	Inoculated bananas and treated with fungicides (I + T)		
	Azoxystrobin	Boscalid	Imazalil
Botryodiplodia sp.			
Colletotrichum sp.			
Fusarium sp.			
Musicillium sp.			

Figure 4:- status of bananas inoculated with fungal strains and treated with the different fungicides

Discussion:-

In this study, the fungal strains responsible for banana post-harvest diseases were partially inhibited by the fungicides commonly used in banana processing and packaging plants in Côte d'Ivoire. This antifungal activity might be due to the modes of action of the molecules of each fungicide used. Imazalil and Azoxystrobin are molecules that inhibit ergosterol biosynthesis. The latter is an important component of fungi membrane. It plays an important role in the fluidity of the membrane and is essential for the aerobic growth of most fungi (Zhang and Rao, 2010). Thus, once applied, fungicidal molecules alter the permeability of fungi cell walls (De Lapeyre de Bellaire and Nolin, 1994). As for Boscalid, the molecule plays a disruptive role in fungi respiration, thus disrupting ATP production by inhibition of the succinate dehydrogenase enzyme (FRAC, 2012).

The *in vitro* sensitivity of fungal strains varied with crop years. This variation might be due to the existence of several populations of fungal strains responsible for banana post-harvest diseases. Indeed, in this study, the fungal strains isolated on banana samples packed in cardboard boxes ready for export were collected every six months (crop year). In each crop year, the fungicides were applied to new fungal populations of the different genera identified, hence the variation in their sensitivity to fungicides.

The variation in the sensitivity of fungal strains is also related to the fungicides commonly used in the processing and packaging stations of bananas intended for export. Repeated and prolonged use of Azoxystrobin in pre- and post-harvest might reduce the sensitivity of fungal strain populations by modifying the sites of action of antifungal molecules (EFSA, 2017). The rates of inhibition, by Imazalil, of *Botryodiplodia* sp., *Colletotrichum* sp., *Fusarium* sp. and *Musicillium* sp. responsible for post-harvest diseases were the highest in time unlike other molecules. In addition to its antifungal activity on *Musicillium* sp. and *Botryodiplodia* sp., the effectiveness of Imazalil was observed by Diedhiou *et al*. (2014) on *Colletotrichum musae*, *Fusarium* sp., *Curvularia* sp., *Thielaviopsis* sp., *Aspergilus* sp. and *Helminthosporium* sp. These authors noticed that Imazalil had the ability to protect bananas against post-harvest diseases for 25 days at low temperatures.

In vivo, in the absence of fungicide, bananas experimentally inoculated with *Botryodiplodia* sp. and *Fusarium* sp. developed crown rot. *Colletotrichum* sp. and *Musicillium* sp. induced epicarp necrosis and distal end rot on bananas, respectively. Each of these fungi is therefore responsible for each post-harvest disease observed. Marin *et al*. (1996) noted that these fungi were also involved in the development of such post-harvest disease symptoms on bananas. The symptoms were more observed on bananas treated with Azoxystrobin and Boscalid compared with Imazalil. Imazalil might be more effective against postharvest fungi of bananas in storage.

Conclusion:-

Bananas treated with fungicides and stored develop post-harvest diseases of crown and distal end rot, as well as epicarp necrosis. The fungi *Botryodiplodia* sp. and *Fusarium* cause crown rot, whereas *Colletotrichum* sp., and *Musicillium* sp. induce epicarp necrosis and distal end rot on bananas in storage, respectively. Azoxystrobin, Boscalid and Imazalil molecules have antifungal activities on post-harvest fungi *in vitro* and *in vivo*. Imazalil is the most effective antifungal molecule against post-harvest fungi of bananas in storage. With a view to having an environmentally friendly chemical control, Imazalil might be the antifungal molecule to be used in production stations of bananas intended for export.

Acknowledgements:-

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