Antifungal activity of some alternative control against mango anthracnose in Senegal

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Abstract

Mango production in the South of Senegal is exposed to intensive rainfall from late May to October, with high temperature and moisture levels. These conditions are conducive for the development of anthracnose caused by Colletotrichum gloeosporioides (sensu lato) and leading to an absolute necessity for adequate control measures for good quality mango production. Anthracnose disease causes both pre- and postharvest fruit spots and fruit rot as well as premature fruit drop. The purpose of this study was to test the efficacy of several fungicide alternatives (fertilizers and biological control agents) against mango anthracnose in Senegal comparatively to standard fungicides. Field trials were set up in contrasting climates conditions and involved three orchards in the Northern part of the country with a short rainy season and dry climate over 9 to 10 months a year and three other orchards in the Southern part of Senegal with in a humid tropic environment. No anthracnose was recorded in the orchards in northern Senegal. In the south, in contrast, the disease was actual. Among the treatments tested for the control of anthracnose, Sodium molybdate were found effective after fungicides (azoxystrobin and thiophanate methyl). All the alternative treatments to fungicides provided a statistically significant control to the disease as compared to the control.

Keywords: *Mangifera indica*; anthracnose control; *Colletotrichum gloeosporioides*; fungicides; Sodium molybdate; biocontrol products;

1. Introduction

In Senegal, the main mango producing areas are located in the regions of Dakar, Thies, Kaolack, Saint-Louis, Fatick, Kolda, Sedhiou and Ziguinchor. The Niayes area (the coastal area between Dakar, Thies, and Saint-Louis) in the north is the main provider of export mango (Mbaye *et al*, 2006) and accounts for 80% of export volume. International mango trade is operated in an increasingly competitive environment governed by high-level standards. In order to meet consumer's expectations targeting flawless, healthy and beautiful fruits without pesticide residues, growers must resolve conflicting issues to produce the desired quality, in accordance with regulations and specifications. The yield issue is still one of the top priorities to guarantee profitability. Attacks by pathogens in the field and/or after harvest interfere negatively with the ambition of substantially increasing the production and export volume more despite high delivery potential.

Fungi cause most of fruit diseases of mango with early infection stages occurring in the field, before harvest. They cause many types of fruit rotting during storage and and/or upon onset of ripening resulting in significant financial losses. In Senegal, fungal diseases play an important role after harvest in the mango business (Mbaye et *al*, 2006; Diedhiou et *al*., 2007). Among the fungal diseases causing fruit rots, anthracnose is the most important mango disease. The importance of losses due to this disease depends on the agro-climatic zone, with a 100% fruit rot in rainfall rich ecologies like the one in the southern part of Senegal (Diedhiou *et al*, 2014b). The consequences are a shortening of the export window, and high risks of destruction of export mangoes in foreign markets. This often results in huge financial losses aggregating both direct losses due to expected sale price and penalties for the destruction as well as longer lasting difficulties linked with the loss of the market. Efficient systemic fungicides for with a protective effect as well as curative action have become available (Kumar et al, 2007). Pre-harvest sprays of these fungicides are used to prevent the establishment of quiescent infections before harvest and reduce disease pressure, thereby improving considerably the effectiveness of postharvest fungicide treatments. There are many reports on the efficacy of azoxystrobin and thiophanate methyl against anthracnose on mangoes (Diedhiou *et al*., 2007; Diedhiou *et al.* 2014a; Sundravadana, 2006). However, for environmental safety, some alternative approaches (resistant cultivars, cultural practices, and biofungicides) have been developed to control diseases (Conway *et al.*, 1991; Sugar *et al.*, 1997; Wilson *et al.*, 1997; Janisiewicz*et al*., 2002; Hewajulige *et al*., 2010; Diallo *et al*., 2017; Jenny *et al*., 2019). However, reports on efficacy in the field have been limited until Govender *et al* (2006) demonstrated the ability of *Bacillus licheniformis* to control anthracnose of mango. Senghor *et al* (2007) showed that *B. subtilis* significantly reduces anthracnose incidence on ripening fruits. Moreover, Peralta (2004) showed that a biological control product named Virtuoso 10 AS (*B. subtilis* QST 713 strain) had excellent activity in suppressing or reducing the severity of anthracnose on mango.

This study was carried out to examine the efficacy of several fungicide alternatives (fertilizers and biological control agents) against mango anthracnose in contrasting climatic conditions in Senegal comparatively to standard fungicides.

2. Materials and Methods

2.1. Field experiment in 2015 and 2016

The field studies were conducted in two regions of Senegal. In the North (region of Thies), the experiments were set up in three orchards. Each orchard harbored commercial mango varieties (Kent and Keitt) on over more than 100 ha. The mango trees were planted at high density (10 cm x 10 cm) and trees kept small through grafting and intensive pruning. All three orchards were engaged in organic farming and consequently only biofungicides and fertilizers were allowed for testing**.** The first treatments were applied before onset of the rainy season, generally in June. A treatment calendar with a 16-days interval, applied twice was adopted. The first treatment was carried out on June 9, 2015 and on June 8, 2016. The second application was on June 25, 2015 and on June 27, 2016.

In the South (region of Ziguinchor), the experiment was also set up in three orchards. All three orchards were conventional and allowed synthetic chemicals. In that region, two fungicides - azoxystrobin and thiophanate methyl-, were used beside the two biofungicides (Sonata and Serenade), and sodium molybdate (fertilizer) as a reference. The three orchards are operated traditionally, with mango varieties, citrus and papaya trees grown mixed in the same field. The treatments started here also before the first rains of the season. The test products were applied twice at a 16-day interval. The first treatment was applied on June 16, 2015 and on June 14, 2016. The second on July 2, 2015 and on July 12, 2016.

2.2. Experimental design

Within each orchard, a completely randomized experimental design was used. Trees to be treated, representing the experimental unit, were selected in a way they were surrounded by untreated trees. Each experimental unit was repeated three times. The concentration of the products was calculated based on the assumption of 121 trees/ha, (Table 1).The products were Ortiva® (azoxystrobin), Fongsin® (thiophanate methyl), Sonata® and Serenade®. Sodium molybdate was purchased from Alfa Aesar (USA).

Table 1: Field treatments and rates applied in the north and the south

Treatment	Formulation	a i/hectare	a i in 10 L water for 3 trees
Azoxystrobin	Ortiva 250 SC	425 ml	11ml
Thiophanate methyl	Fongsin 450 SC	1,134 ml	30 ml
Bacillus pumilus QST 2808	Sonata	4,732ml	117 ml
Bacillus subtilis QST 713	Serenade Optimum	992 g	25 g
Sodium molybdate		90 g	2 g

2.3. Sampling

Mango fruits were collected at maturity, on July 22nd in the north and July 29th in the south. In each orchard, five fruits per experimental unit were randomly picked, in a total of 15 fruits per treatment summing the contributions of the three replicates. The fruits were placed in a plastic bag each, and transported to the laboratory.

2.4. Ripening fruit and disease assessment

The fruits were maintained in a clean and ventilated area at room temperature (26-29° C) in the laboratory. Every 2 days, each fruit was assessed for anthracnose lesions over a 8 days period.

2.5. Disease assessment and statistical analysis

The collected data was used for statistical analysis. When the data fails the normal distribution test, it went under the GLIMMX procedure of SAS version 9.4. GLIMMIX is a generalized linear mixed model assuming a Poisson distribution. The means were separated using a 95% confidence interval and the t-test pair comparison.

3. Results

3.1. Field experiment in Senegal in 2015

In all orchards located in the north of the country, zero fruit was infected by anthracnose. For mango orchards in the south in contrast, anthracnose lesions were recorded on fruits. Namely, in Badjo's orchard, all the treatments provided significant disease control compared to mangoes from non-treated trees. Serenade, sodium molybdate, thiophanate methyl, and azoxystrobin performed significantly better than Sonata, although the latest reduced significatively diseases severity as compared to the control (Table 4).

In Jean's orchard, sodium molybdate, thiophanate methyl, and azoxystrobin were more effective than Serenade and Sonata. However, all the treatments reduced significantly disease severity as compared to the control.

In Toure's orchard, despite differences in extent of performance, all the treatments provided significant disease control compared to mangoes from non-treated trees.

There was a homogeny between the tree orchards in the south since the analysis of variance showed significative difference (P=0.4979).

With respect to market value, the number of disease-free mangoes was assessed. It showed that in Badjo's orchard, treatment with azoxystrobin and thiophanate methyl allowed to obtain six disease-free mangoes out of 15 (40%) and 5 disease-free mangoes out of 15 for Serenade (30%).

In Jean's orchard, all the treatments had many disease-free mangoes (9 to 11 fruits, e.g. 60% to 73%) except for Sonata that gave only 40% of disease-free mangoes.

In Toure's orchard, the treatments protected effectively between 8 and 12 fruits meaning that from 53% up to 80 % of fruits could have a market value.

Table 2: Influence of field treatments with biological control products and two fungicides as well as sodium molybdate (fertilizer) on fruits anthracnose 8 days after harvest and incubation at room temperature in the laboratory. Treatments with the same letter are not significantly different (n = 15 $p \leq 0.05$).

	Number of lesions per fruit						Disease-free mangoes			
Treatment	Badjo		Jean	Toure		Mean	Badjo	Jean	Toure	Mean
Control	143	A	123A	132	A	133 A	0	0	0	0.0
Sonata	54	B	63 B	31	DC	49 B	2	6	9	5.7
Serenade	34	D	46 C	53	B	44 C	5	9	8	7.3
Sodium molybdate	38	C	26 D	33	C	32 C	2	8	10	6.7
Thiophanate methyl	37	DC	30 D	27	D	31 D	6	11	12	9.7
Azoxystrobin	34	D	30 D	22	E	29 D	6	11	11	9.3

3.2. Field experiment in Senegal in 2016

Like for 2015, zero fruit was infected by anthracnose in orchards from the northern part of the country in 2016. During that same period, fruits from orchards in the south displayed some lesions of anthracnose.

In Badjo's orchard, all the treatments provided significant disease control compared to mangoes from non-treated trees. Azoxystrobin, serenade, thiophanate methyl, and sodium molybdate performed significantly better than Sonata (table 3).

In Jean's orchard, sodium molybdate, thiophanate methyl, and azoxystrobin were more effective than Serenade and Sonata as in 2015. However, all the treatments reduced significantly disease severity as compared to the control.

In Toure's orchard, azoxystrobin and thiophanate methyl were effective than sodium molybdate, serenade and sonata. Sodium molybdate came in the second position of effective compared to serenade and Sonata. Despite differences in extent of performance, all the treatments provided significant disease control compared to mangoes from non-treated trees.

There was a homogeny between the tree orchards in the south since the analysis of variance showed significative difference (P=0.4979) as in 2015.

With respect to market value, the number of disease-free mangoes was assessed. It showed that in Badjo's orchard, the number of disease-free mangoes is low for all the treatments.

In Jean's orchard, the treatment with sodium molybdate, thiophanate methyl and azoxystrobin had many disease-free mangoes (10 to 13 fruits, e.g. 67% to 87%) except for Sonata and Serenade that gave respectively 27% to 13% of disease-free mangoes.

In Toure's orchard, the treatment with sodium molybdate, thiophanate methyl and azoxystrobin had many disease-free mangoes (10 to 14 fruits, e.g. 67% to 93%) except for Serenade and Sonata that gave respectively 13% to 20% of disease-free mangoes.

Table 3: Influence of field treatments with biological control products and two fungicides as well as sodium molybdate (fertilizer) on fruits anthracnose 8 days after harvest and incubation at room temperature in the laboratory. Treatments with the same letter are not significantly different (n = 15 $p \leq 0.05$).

	Number of lesions per fruit					Disease-free mangoes			
Treatment	Badjo		Jean	Toure	Mean	Badjo	Jean	Toure	Mean
Control	150	A	132 A	130 A	137 A	0	0	0	0.0
Sonata	50	B	75 B	58 B	61 B	2	4	3	3.0
Serenade	35	C	54 C	53 B	47 C	3	2	2	2.3
Sodium molybdate	38	BC	30 D	30 C	33 D	4	10	10	8.0
Thiophanate methyl	37	BC	27 D	24 D	29 D	4	11	13	9.3
Azoxystrobin	35	C	25 D	20 D	27 D	3	13	14	10.0

4. Discussion

This study provides the first data on biological control and other alternatives control of anthracnose on mangoes in Senegal.

During the field trials in 2015 and 2016, the orchards in the north registered no anthracnose during the experiments. This should be in relation with the climatic conditions because the pathogen requires warm and humid conditions to infect and mango fruit. In that part of Senegal however, the rainy season starts often in August allowing mangoes to ripen before the moisture level becomes too high. Anthracnose disease developed already at the same period in the orchards in Ziguinchor (south) were rainfall starts in May confirming the requirements for anthracnose to develop.

The systemic fungicides azoxystrobin and thiophanate methyl were good for the control of mango anthracnose in southern Senegal. These results are consistent with those obtained in several studies like those of Diedhiou *et al*. (2014a) who reported excellent control of mango anthracnose in Senegal through treatments with azoxystrobin and thiophanate-methyl (benzimidazoles). This shows that systemic fungicides were more effective than non-systemic ones in controlling mango anthracnose as already reported by McMillan, (1984). Sundravadana *et al.,* (2007) reported that azoxystrobin inhibits 100 % of mycelial growth of *C. gloeosporioides* the causal agent of mango anthracnose. Diallo *et al*., (2021) reported that in Senegal the causal agent of mango anthracnose is *Colletotrichum siamense* and not *C. gloeosporioides*.

Systemic fungicides are of great importance in the local conditions of Casamance (Ziguinchor area) where rainfall can be very frequent (700-1250 mm) between late May and October and the mango trees can be very tall, reaching more than 15m height. Optimal conditions for infection and dispersal of spores of Colletotrichum are met during the humid period with an average temperature of about 28 °C (Dodd et al, 1997), prevailing in the Ziguinchor region during the rainy season.

The height of the trees and their huge canopy make it difficult to achieve a complete coverage of the mango trees when applying a contact fungicide. Systemicity of the product is therefore a great importance in these conditions as shown by the level of protection achieved in the 2 years experiments.

Several reports have shown that controlling anthracnose on mango relies heavily on the use of fungicides. However, the use of a specific group (benzimidazoles or strobilurin) of fungicides has declined drastically due to development of resistance by *Colletotrichum* species. According to Kumar *et al.* (2007), *Colletotrichum gloeosporioides* was moderately resistant to thiophanate-methyl in Andhra Pradesh, India. In addition, Meng-Jun Hu *et al.* (2015) showed that resistance to azoxystrobin and thiophanate methyl existed in *Colletotrichum siamense* from peach and blueberry in South Carolina.

The treatments with biofungicides *Bacillus pumilus* QST 2808 (Sonata) and *Bacillus subtilis* QST 713 (Serenade Optimum) were statistically significantly less effective than the two fungicides. It is however to point out that all the biofungicides were also very effective and provided significant protection for mangoes against anthracnose far better than the “do nothing option” represented by the control. Diallo *et al*, (2017) reported that the protective treatments with the biofungicides allowed reducing significatively the incidence of the disease as well as its severity. The good efficacy of the tested alternatives to fungicides is very encouraging for a sustainable control of mango anthracnose.

The field experiment in Senegal showed the efficacy of sodium molybdate which just behind the fungicides (azoxystrobin and thiophanate methyl). This product performed well, with performances just a bit below azoxystrobin and thiophanate methyl. It performed a bit better than the biocontrol products Sonata and Serenade. The efficacy of molybdate in reducing the intensity of diseases of bean and tomato have been reported (Dutta *et al*, 1981; Miller *et al*, 1983 and Júnior *et al*, 2004). In addition, Polanco et *al* (2014) speculated on a direct effect of molybdate (NaMo) on *C. lindemuthianum*, and thereby and increase of yield as interpretation of the decrease of the area under the disease progress curve through application of that product. Sodium molybdate should be considered a promising product to control mango anthracnose.

References

W.S. Conway, C.E. Sams, J. Abbott, and B.D. Bruten (1991). Post-harvest treatment of apple fruit provides broad-spectrum protection against post-harvest pathogens. *Plant Disease* 75, 620–622.

Y. Diallo, P. M. Diedhiou, E. Bush, M. Nita and A. Baudoin (2021). Characterization of *Colletotrichum* species causing mango anthracnose in Senegal. *International Journal of Science and Research*. 10(1), 365 - 370.

Y. Diallo, P. M. Diedhiou, E. Bush, M. Nita and A. Baudoin (2017). BIOFUNGICIDES AN EFFICIENT ALTERNATIVE CONTROL STRATEGY AGAINST MANGO ANTHRACNOSE IN SENEGAL. *Int. J. Adv. Res. 5(6)*, 990-995.

P. M. Diedhiou, N. Mbaye, A. Dramé, and P. I. Samb (2007).Alteration of post-harvest disease of mango (*Mangiferaindica*) through production practices and climatic factors. *African Journal of Biotechnology*, 6: (9), 1087-1094.

P. M. Diedhiou, Y. Diallo, R. Faye, A. A. Mbengue, and A. Sene (2014a). Efficacy of different fungicides against mango anthracnose in Senegalese Soudanianagroclimate.*American Journal of Plant Sciences*, 5, 2224-2229.

P.M. Diedhiou, S.A.G. Diop, N. Mbaye, I. Diedhiou, Y. Diallo, R. Faye and P.I. Samb (2014b). Mango rotting in southern Senegal, a big phytosanitary challenge.*International Journal of Biosciences*, 5: 183-188.

J.C. Dodd, D. Prusky, and P. Jeffries (1997). Fruit diseases. In: Litz, R.E. (ed.) *The Mango:Botany, Production and Uses*. CABI. pp. 257-280.

B. K. Dutta, and E. Bremmer (1981). Trace elements as plant chemo therapeutants to control Verticillium wilt. *Z. Pflanzenkrankh. Pflanzenschutz* 88:405-412.

V. Govender, and L. Korsten (2006). Evaluation of different formulations of *Bacillus licheniformis*in mango packs house trials. *Biological Control* 37:237-242.

I.G.N. Hewajulige, W.R.S. Wigeratnam (2010). Alternative postharvest treatments to control anthracnose disease in papaya during storage. *Fresh produce*, 4:15-20

M.J. Hu, A. Grabke, M.E. Dowling, and G. Schnabel (2015). Resistance in *Colletotrichum siamense* from peach and blueberry to thiophanate methyl and azoxystrobin.*Plant Disease*.99:806-814.

F. Jenny, N. Sultana, M. M. Islam, M.M. Khandaker and M. B. A. Bhuiyan (2019). A review on anthracnose of mango caused by Colletotrichum gloeosporioides. *Bangladesh J. Plant Pathol*. 35(1&2):65-74

A. S. Kumar, N. P. E. Reddy, K. H. Reddy, and M. C. Devi (2007). Evaluation of fungicidal resistance among *Colletotrichum gloeosporioides* isolates causing mango anthracnose in Agri Export Zone of Andhra Pradesh, *India Plant Pathology Bull*. 16: 157-160.

Mc. Millan (1984). Control of mango anthracnose with foliar sprays.*Proc. Florida State Horticulture Society* 97:344-345.

G.A. Peralta (2004). Field evaluation of *Bacillus subtilis* (QST 713 strain) for the control of mango anthracnose**.** Philippines Univ. Los Banos, College, Laguna (Philippines). National Crop Protection Center.

L. R. Polanco, F. A. Rodrigues, E. N. Moreira, H. S. S. Duarte, I. S. Cacique, L. A. Valente, R. F. Vieira, T. J. Paula Júnior, and F. X. R. Vale (2014). Management of anthracnose in common bean by foliar sprays of potassium silicate, sodium molybdate, and fungicide. *Plant Disease* 98:84-89.

A.L. Senghor, W.J. Liang, and W.C. Ho (2007). Integrated control of *Colletotrichum gloeosporioides* on mango fruit in Taiwan by the combination of *Bacillus subtilis* and fruit bagging, *Biocontrol Science and Technology*, 17:8, 865-870.

S. Sundravadana, D. Alice, S. Kuttalam, and R. Samiyappan (2006). Control of mango anthracnose by azoxystrobin. *Tunisian Journal of Plant Protection* 1: 109-114.

C.L. Wilson, J.M. Solar, A. El-Ghaouth, and M.E. Wisniewski (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea. Plant Disease* 81, 204–210.