In vivo efficacy of new molecules of fungicides against Eumusae leaf spot disease of French plantain cultivar Nendran (*Musa* AAB)

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Abstract

The French plantain cultivar, Nendran (*Musa* AAB) is the most widely and commercially cultivated variety of banana in Kerala. But this cultivar is highly susceptible to foliar fungal diseases especially leaf spot diseases. Among the leaf spot diseases, Sigatoka leaf spot caused by *Mycosphaerella* spp. poses a serious threat to banana cultivation. The studies on characterization of the pathogen associated with Sigatoka leaf spot disease complex in Kerala revealed that the predominant leaf spot disease is Eumusae leaf spot disease caused by *Mycosphaerella eumusae*. With an objective to develop a fungicidal recommendation for the sustainable management of this disease, a field experiment was conducted during 2016-17 at Banana Research Station, Kannara by evaluating the efficacy of six chemical fungicides *viz.,* trifloxystrobin (25%) + tebuconazole (50%) (Nativo® – 0.4g/L), propiconazole (Tilt® – 1ml/L), copper hydroxide (Kocide® – 2g/L), pyraclostrobin (Headline® – 1g/L), hexaconazole (5%) + captan (70%) (Taqat® – 2g/L) and Bordeaux mixture (1%). All the chemical fungicides used in the study were found to be superior compared to unsprayed control. However, there was a variation in the efficacy between the treatments. The foliar application of the systemic fungicide trifloxystrobin + tebuconazole (0.4g/L) recorded the lowest per cent disease severity (PDS) of 15.43 per cent with the eighth leaf of the plant as the youngest leaf spotted (YLS) and the maximum disease development time (DDT) of 50.66 days. Among the contact fungicides evaluated, the lowest per cent disease severity of 16.65 per cent was recorded in plants sprayed with copper hydroxide which recorded DDT of 49.33 days and YLS of 7.29. The analysis of fungicide residue in the fruits revealed that there was no residue of the chemicals left in the fruit. The study revealed that four times foliar spraying of systemic fungicide, trifloxystrobin + tebuconazole (0.4g/L) or contact fungicide, copper hydroxide (2g/L) starting from the initial appearance of the disease on the lowest leaves of 75 per cent plants was safe and effective for the management of Eumusae leaf spot disease of banana.

Keywords: Disease development time, Eumusae leaf spot, Per cent disease severity, Youngest leaf spotted

Introduction

Banana is one of the most important fruit crops grown in tropical and subtropical countries as a source of livelihood security and as an export commodity. The multifaceted uses of banana as a source of food, fibre, fuel and for therapeutic purposes have made it a crop of wide popularity. Also, it is a rich source of vitamins and minerals like calcium and magnesium, with trace quantities of iron and zinc. These aspects clearly place banana as one among the healthiest fruits. In India, the cultivation of banana covers an area of 860 thousand ha with an average productivity of 30.47 million tons (FAOSTAT, 2017). The state of Kerala is endowed with warm humid tropical climate and is the hub of biodiversity of edible banana especially the plantain variety Nendran (AAB). However, the commercial cultivation of banana var. Nendran in the state is often challenged by the incidence of pests and diseases. The diseases that pose threat to *Musa*

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biodiversity and also to commercially cultivated fields include leaf spot diseases, wilt and viral diseases. Among the fungal diseases, Sigatoka leaf spot disease complex caused by *Mycosphaerella* spp. is a serious constraint to banana cultivation as it destroys the functional photosynthetic leaf area and causes considerable reduction in yield. Globally three related ascomycetous fungi *viz., M. fijiensis*, *M. musicola*, and *M. eumusae* were reported to cause black Sigatoka, yellow Sigatoka and Eumusae leaf spot respectively (Arzanlou et al*.,* 2007). The studies on characterization of the *Mycosphaerella* spp. associated with Sigatoka leaf spot disease in Kerala revealed that the predominant leaf spot disease occurring in Kerala was Eumusae leaf spot disease caused by *Mycosphaerella eumusae* (George et al*.,* 2018). The presence of Eumusae leaf spot caused by *Mycosphaerella eumusae* (anamorph: *Pseudocercospora eumusae*) was also reported from South India earlier by Carlier et al. (2000).

Eumusae leaf spot disease caused by *M. eumusae* destroys the photosynthetic green leaf tissue through the development of necrotic leaf lesions and results in a yield loss of 11 to 80 per cent (Shanthiyaa et al., 2013). Thus, this disease limits the cultivation and production of the popular but susceptible cultivars like Nendran. The geographical position and climatic conditions prevailing in the state of Kerala are highly favorable for the development of this fungal disease throughout the year. Also, in the wake of the recent ban of many currently recommended plant protection chemicals, there was an urgent need to identify alternate new molecules of fungicides which were effective, environmentally safe and economical for the management of the disease without leaving any residues in the fruits. In this context, a field trial was taken up to evaluate the efficacy of selected new molecules of fungicides against Eumusae leaf spot disease of banana.

Materials and Methods

A field experiment was undertaken to evaluate the efficacy of six chemical fungicides *viz.,* trifloxystrobin (25%) + tebuconazole (5%) (Nativo® – 0.4g/L), hexaconazole (5%) + captan (70%) (Taqat® – 2g/L), propiconazole (Tilt® – 1ml/L), pyraclostrobin (Headline® – 1g/L), copper hydroxide (Kocide® – 2g/L) and Bordeaux mixture (1%) against Eumusae leaf spot disease of banana. The trial was conducted at Banana Research Station, Kerala Agricultural University, Kannara, Thrissur, Kerala which enjoys a humid tropical climate which was favourable for natural incidence of the disease. The experimental site was laid out in the hotspot area with high inoculum pressure. The variety used for the experiment was plantain cultivar Nendran (*Musa* AAB) and morphotype, Nedunendran.

The experiment was laid out during May 2016-2017, in Randomized Block Design (RBD) with seven treatments replicated thrice with nine plants per replication. Pits of size 50 cm x 50 cm x 50 cm were formed and suckers were planted with a spacing of 2 x 2 m. The organic manures, lime and chemical fertilizers were applied as per Package of Practices recommendations of Kerala Agricultural University (KAU, 2011). The details of the treatments are given in Table 1.

The treatments were imposed as foliar sprays using

Table1. Details of fungicidal treatments evaluated against Sigatoka leaf spot disease

Treatment No.	Treatments
T_1	Foliar spraying of pyraclostrobin (1g/L)
T_2	Foliar spraying of copper hydroxide (2g/L)
T_3	Foliar spraying of propiconazole (1ml/L)
T_4	Foliar spraying with hexaconazole (5%) + captan (70%) (2g/L)
T_5	Foliar spraying with trifloxystrobin 25% + tebuconazole (50%) (0.4g/L)
T_6	Foliar spraying of Bordeaux mixture (1%)
T_7	Control

a rocker sprayer and the first spray was given when ten leaf spot stage (approximately 55 DAP) was visible on the lowest leaves of 75 per cent of plants and subsequently three sprays were given at fortnightly intervals. The observations on per cent disease severity (PDS), youngest leaf spotted (YLS), disease development time (DDT), agronomic parameters such as plant height, plant girth and number of functional leaves, and yield parameters *viz.,* bunch weight, number of hands, number of fingers, length of fingers, circumference of fingers, fresh weight of fingers, ripe weight of fingers and peel to pulp ratio were recorded.

The disease severity was assessed at critical stages of the crop *viz*., six months after planting and at flowering stage using Gauhl's modification of Stover's severity scoring system score chart (Carlier et al*.,* 2002) following 0 - 6 scale as mentioned below:

Score	Symptoms
0	No symptom
1	< 1 per cent of leaf lamina with symptoms
2	1 to 5 per cent of leaf lamina with symptoms
3	6 to 15 per cent of leaf lamina with symptoms
4	16 to 33 per cent of leaf lamina with symptoms
5	34 to 50 per cent of leaf lamina with symptoms
6	51 to 100 per cent of leaf lamina with symptoms

The per cent disease severity was calculated using the formula:

$$\text{Per cent disease severity (PDS)} = \frac{\Sigma\, nb \times 100}{(N-1)T}$$

where,

n= number of leaves in each grade,

b= grade

N=Number of grade used in the scale

T= total number of leaves

Statistical analysis

The data obtained from the field experiment was subjected to two way analysis of variance using the statistical package MSTAT.

Results and Discussion

Effect of treatments on per cent disease severity (PDS)

The statistical analysis of data on PDS at vegetative (six months after planting) and at shooting stage revealed that all the fungicidal treatments were significantly superior compared to unsprayed control but the efficacy varied with the chemicals (Table 2). The severity of the disease was assessed at vegetative and shooting stage, as these are critical stages of plant growth. The reduction of green leaf area at these stages would affect the crop yield. A series of literature citing the effectiveness of different chemical fungicides in reducing disease severity of leaf spot disease is available (Stover, 1969; Ramsey et al*.,* 1987; Foure et al*.,* 1988; Cherian et al*.,* 2002 and Marin et al*.,* 2002).

At vegetative phase, the severity of the disease in

Table 2. Effect of fungicidal treatments on per cent disease severity of Eumusae leaf spot of banana

Treatment details	*PDS at 6MAP**	* PDS at shooting	Mean PDS	Per cent disease reduction over control
T_1: Pyraclostrobin (1g/L)	17.49 ^{b}	20.69 ^{bcd}	19.09	42.00
T_2: Copper hydroxide (2g/L)	16.12^{bc}	17.18 ^{e}	16.65	49.08
T_3: Propiconazole (1ml/L)	15.55^{bc}	21.93 ^{b}	18.74	42.69
T_4: Hexaconazole + Captan (2g/L)	16.04^{bc}	18.50 ^{cde}	17.27	47.18
T_5: Trifloxystrobin + Tebuconazole (0.4g/L)	13.90^{c}	16.96 ^{e}	15.43	52.81
T_6: Bordeaux mixture (1%)	17.81^{b}	18.02 ^{de}	17.92	45.19
T_7: Control	31.33^{a}	34.03 ^{a}	32.70	-
CD (0.05)	3.41	3.06	-	-

*Per cent disease severity

terms of PDS was lowest (13.90%) in plants sprayed with T_5 (trifloxystrobin + tebuconazole, 0.4g/L) followed by foliar application of T_3 (propiconazole, 1ml/L), T_4 (hexaconazole + captan, 2g/L), T_2 (copper hydroxide, 2g/L) having PDS of 15.55, 16.04 and 16.12 per cent respectively and these treatments were on par with each other. The highest PDS was in unsprayed control (31.33%) plants.

During shooting stage also, the lowest PDS (16.96%) was recorded in plants sprayed with T_5 (trifloxystrobin + tebuconazole, 0.4g/L). This was followed by foliar application of T_2 (copper hydroxide, 2g/L), T_6 (Bordeaux mixture, 1%) and T_4 (hexaconazole + captan, 2g/L) with PDS of 17.18, 18.02 and 18.50 per cent respectively.

Comparing the mean values of PDS at vegetative and at shooting stage revealed that among the systemic fungicides, the foliar application of T_5 (trifloxystrobin + tebuconazole, (0.4g/L)) recorded the lowest PDS of 15.43 per cent, followed by T_4 (hexaconazole + captan, 2g/L) while the highest PDS of 19.09 per cent was recorded in plants sprayed with T_1 (pyraclostrobin, 1g/L). The results were in agreement with the findings of Paul (1998) who reported that banana plants sprayed with trifloxystrobin + tebuconazole (0.7g/L) recorded least disease severity of Sigatoka leaf spot disease (8.6%). Four to five times foliar spraying of strobilurin + triazole (0.063 kg a.i./ha) fungicides provided effective control of early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*) of peanut than strobilurin and triazole fungicides used alone (Hagan et al., 2004). Ruth and Nagalakshmi (2017) observed that banana plants sprayed with propiconazole (1ml/L) and hexaconazole + captan (2g/L) were effective for the management of Sigatoka leaf spot disease. Hermanto et al. (2010) evaluated the efficacy of new generation molecules against Sigatoka disease and reported the effectiveness of azoxystrobin against *Mycospharella* leaf spot diseases. Among the contact fungicides used, the lowest disease severity of 16.65 per cent was recorded in plants sprayed with T_2 (copper hydroxide, 2g/L) followed by T_6 (Bordeaux mixture, 1%). Evans et al. (1961) reported that aerial spraying of copper based fungicides was effective in controlling Sigatoka leaf spot disease by inhibiting the sporulation of the pathogen. Five to six sprays of Bordeaux mixture (1%) at fortnightly intervals soon after the appearance of the initial symptom were observed effective in reducing the disease severity of leaf spot disease of banana (KAU, 2011).

Effect of treatments on disease development time (DDT)

Disease development time (DDT) is the time taken by the youngest cigar leaf to develop at least ten mature necrotic leaf spots. When DDT is more, it indicates that the fungicide is effective against the disease. Data presented in Table 3 revealed that the fungicide sprayed plants took 47.33 to 50.66 days to develop about ten mature necrotic lesions on the cigar leaf compared to control, which took only

Table 3. Effect of fungicidal treatments on youngest leaf spotted (YLS) and disease development time (DDT)

Treatment details	*YLS at 6MAP**	YLS at shooting	Mean YLS	DDT***
T_1: Pyraclostrobin (1g/L)	7.50 ^{b}	6.75 ^{b}	7.12	48.00 ^{b}
T_2: Copper hydroxide (2g/L)	7.50 ^{b}	7.08 ^{ab}	7.29	49.33 ^{ab}
T_3: Propiconazole (1ml/L)	7.83 ^{a}	7.08 ^{ab}	7.45	47.43 ^{b}
T_4: Hexaconazole + Captan (2g/L)	7.50 ^{b}	6.83 ^{ab}	7.16	49.33 ^{ab}
T_5: Trifloxystrobin + Tebuconazole (0.4g/L)	8.91 ^{a}	7.66 ^{a}	8.00	50.66 ^{a}
T_6: Bordeaux mixture (1%)	7.33 ^{b}	6.83 ^{ab}	7.03	47.33 ^{b}
T_7: Control	6.43 ^{c}	5.25 ^{c}	5.18	32.66 ^{c}
CD(0.05)	0.91	0.84	-	2.25

*Youngest leaf spotted, ** Months after planting, ***Disease development time

32.66 days to develop the symptom. The maximum DDT (50.66 days) was recorded in plants sprayed with T_5 (trifloxystrobin + tebuconazole, 0.4g/L) followed by T_2 (copper hydroxide, 2g/L) and T_4 (hexaconazole + captan, 2g/L) which recorded DDT of 49.33 days for both the treatments.

Effect of treatments on youngest leaf spotted (*YLS*)

Youngest leaf spotted (YLS) denotes the leaf number of the fully unfurled leaf with at least ten necrotic leaf spots. Higher value of YLS reflects the effectiveness of the treatment. Statistically significant differences in YLS were noticed among treatments both at vegetative and shooting stage (Table 3).

At vegetative stage, the highest YLS (8.91) was recorded in plants sprayed with trifloxystrobin + tebuconazole (T_5) and was statistically on par with T_3 (propiconazole) with YLS of 7.83, while the other treatments, T_1 (pyraclostrobin), T_2 (copper hydroxide), T_4 (hexaconazole + captan) and T_6 (Bordeaux mixture) were found to be statistically on par.

At shooting stage also, the YLS was found to be the highest (7.66) in plants sprayed with T_5 (trifloxystrobin + tebuconazole), followed by T_2 (copper hydroxide), T_3 (propiconazole), T_4 (hexaconazole + captan) and T_6 (Bordeaux mixture). Analyzing the mean values of YLS at vegetative and shooting stage revealed that the highest YLS (8.00) was recorded in T_5 (trifloxystrobin + tebuconazole). Foliar spraying of banana plants with strobilurin + triazole fungicides resulted in YLS of 9 to 10 on AAA or ABB type of banana cultivars (Rodriquez et al., 2002) This treatment was followed by T_3 (propiconazole), T_2 (copper hydroxide) and T_4 (hexaconazole + captan) with YLS of 7.45, 7.29 and 7.16 respectively. The lowest YLS (5.18) was recorded in T_7 (unsprayed control). Hermanto et al. (2010) reported that application of chemical fungicides reduced the aggressiveness of the pathogen which resulted in higher YLS when compared with the untreated control.

Effect of treatments on agronomic characters

The statistical analysis of the agronomic characters *viz.,* plant height, plant girth and number of functional leaves revealed that there was no statistically significant difference between the treatments (Table 4). However, the highest plant height and plant girth were recorded in plants sprayed with T_3 (propiconazole, 1ml/L) followed by T_2 (copper hydroxide, 2g/L). The lowest plant height (199.16 cm) was recorded in plants sprayed with T_5 (trifloxystrobin + tebuconazole). However, the maximum number of green leaves was recorded in plants sprayed with T_5 (trifloxystrobin + tebuconazole) followed by T_3 (propiconazole). The minimum number of green leaves was recorded in T_7 (unsprayed control).

Effect of treatments on yield attributes

Statistical analysis of the yield attributes such as bunch weight, number of hands, number of fingers, length of fingers, circumference of fingers, fresh weight of fingers, ripe weight of fingers and peel to pulp ratio revealed that there was no statistically significant difference between treatments except in

Table 4. Effect of fungicidal treatments on agronomic characters

Treatment details	Plant height (cm)	Plant girth (cm)	No. of green leaves	B:C ratio
T_1: Pyraclostrobin (1g/L)	217.50	55.08	5.91	1.35
T_2: Copper hydroxide (2g/L)	220.00	56.70	6.00	1.50
T_3: Propiconazole (1ml/L)	223.33	57.66	6.33	1.50
T_4: Hexaconazole + Captan (2g/L)	210.00	53.50	5.83	1.50
T_5: Trifloxystrobin + Tebuconazole (0.4g/L)	199.16	52.41	7.00	1.71
T_6: Bordeaux mixture (1%)	218.83	53.50	5.83	1.75
T_7: Control	216.00	55.21	5.41	1.14
CD (0.05)	NS	NS	NS	

bunch weight, circumference of fingers and fresh weight of fingers. Per cent increase in yield of 16.89 to 44.90 per cent was recorded in all the treatments compared to control.

The higest yield in terms of the bunch weight (9.52 kg) was recorded in plants sprayed with copper hydroxide (T_2) and Bordeaux mixture (T_6). These treatments were found to be statistically on par with T_5 (trifloxystrobin + tebuconazole) and T_4 (hexaconazole + captan) which recorded bunch weights of 9.49 kg and 9.46 kg respectively (Table 5a). The lowest bunch weight (6.57) was recorded in control plants without any chemical treatments. The timely application of fungicides reduced the disease severity, thereby increasing the photosynthetic efficiency of the leaves which in turn increased the yield of the crop (Jones, 2000). Shinde (2013) reported that azoxystrobin (0.15 per cent) and tebuconazole (0.1 per cent) were effective in reducing black Sigatoka with concomitant increase in yield.

The highest peel to pulp ratio (0.19) was noticed in control plants without any treatment followed by the plants sprayed with T_1 (pyraclostrobin) (0.18). However, the lowest value (0.16) was observed in T_2 (copper hydroxide). The highest value for total soluble solids (TSS) was recorded in fingers taken from plants sprayed with copper hydroxide having 29.66^0 Brix. While the lowest value (27.66^0 Brix) was recorded in plants without spraying. No significant differences were observed between the treatments (Table 5b). The results on TSS revealed that the application of chemical fungicides did not affect the quality of the fruits.

The fruit samples were collected from the most effective treatments *viz.,* trifloxystrobin + tebuconazole, hexaconazole + captan during harvest and were subjected to residue analysis at Pesticide Residue Research and Analytical Lab, College of Agriculture, Vellayani. It was found that no residue of the fungicide was observed in the fruits sprayed with trifloxystrobin + tebuconazole and

Table 5a. Effect of fungicidal treatments on yield attributes

Treatment details	Bunch weight (kg)	No. of hands	No: of fingers	Per cent increase in yield over control
T_1: Pyraclostrobin (1g/L)	7.68 ^{bc}	5.91	59.91	16.89
T_2: Copper hydroxide (2g/L)	9.52 ^{a}	6.50	65.75	44.90
T_3: Propiconazole (1ml/L)	8.89 ^{ab}	6.33	63.50	26.09
T_4: Hexaconazole + Captan (2g/L)	9.46 ^{a}	6.58	66.08	43.98
T_5: Trifloxystrobin + Tebuconazole (0.4g/L)	9.49 ^{a}	6.30	65.08	44.44
T_6: Bordeaux mixture (1%)	9.52 ^{a}	6.16	63.08	44.90
T_7: Control	6.57 ^{c}	5.75	55.25	-
CD(0.05)	1.49	-	-	-

Table 5b. Effect of fungicidal treatments on yield attributes

Treatment details	Length of fingers (cm)	Circumference of fingers (cm)	Fresh weight of fingers (g)	Ripe weight of fingers (g)	Peel to pulp ratio	TSS (^{0}Brix)
T_1: Pyraclostrobin (1g/L)	23.02	12.27 ^{c}	130.55 ^{c}	117.48	0.18	28.66
T_2: Copper hydroxide (2g/L)	23.46	12.92 ^{ab}	152.23 ^{a}	135.73	0.12	29.66
T_3: Propiconazole (1ml/L)	24.11	12.45 ^{bc}	140.12 ^{abc}	124.43	0.13	28.66
T_4: Hexaconazole + Captan (2g/L)	24.01	13.15 ^{a}	140.92 ^{abc}	124.01	0.17	27.66
T_5: Trifloxystrobin + Tebuconazole (0.4g/L)	23.05	12.52 ^{bc}	147.32 ^{ab}	114.13	0.16	28.33
T_6: Bordeaux mixture (1%)	23.01	12.61 ^{bc}	148.07 ^{ab}	123.13	0.15	27.66
T_7: Control	20.27	12.26 ^{c}	120.11 ^{d}	105.71	0.19	27.66
CD(0.05)	-	0.515	15.29	-	22.73	-

hexaconazole + captan. Hence, it is concluded from the study that the plants sprayed with the new molecule of fungicides *viz.,* trifloxystrobin + tebuconazole and hexaconazole + captan were efficient for the management of the disease as well as being safe for consumption of harvested fruits. Similar observations were reported by Mohapatra and Ajithakumar (2014) on banana fruits and soil sprayed with trifloxystobin + tebuconazole, suggesting that this chemical could be used for the management of the disease considering the efficacy and safety of the fungicides.

Economic analysis

The benefit to cost ratio of different chemical treatments was calculated and data are presented in Table 4. The highest B: C ratio was observed in T_6 (Bordeaux mixture) with the ratio of 1.75 and was followed by T_5 (trifloxystrobin + tebuconazole) (1.71), while the lowest B: C ratio (1.14) was observed in T_7 (control).

Considering the efficiency, safety and economic benefit, the foliar application of systemic fungicide trifloxystrobin (25%) + tebuconazole (50%) @ 0.4g/ L or the contact fungicide copper hydroxide @ 2g/ L starting from the appearance of ten leaf spots on the lowest leaves of 75 per cent plants and subsequently three sprays at fortnightly intervals could be effectively used for the management of Eumusae leaf spot of banana without leaving any chemical residues in the fruits.

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