Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1635-1637
Received: 06-03-2020
Accepted: 10-04-2020

R Guru Nandan Kumar
Department of Plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

JP Mishra
Department of Plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

Rajendra Prasad
Department of Plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

Corresponding Author:
R Guru Nandan Kumar
Department of Plant Pathology, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India

In vitro evaluation of fungicides against *Mycosphaerella musicola* causing sigatoka leaf spot of banana

R Guru Nandan Kumar, JP Mishra and Rajendra Prasad

Abstract
Three systemic fungicides (Difenoconazole, Azoxystrobin, and Carbendazim) and two non-systemic fungicides (Cabriotop, Chlorothalonil) were evaluated against *Mycosphaerella musicola* at 50 ppm 150 ppm and 300 ppm concentration under *in vitro* condition. Among of the five fungicides Carbendazim, Azoxystrobin and Cabriotop showed maximum mycelium growth inhibition of *M. musicola*. Chlorothalonil showed lowest growth inhibition of *M. musicola*.

Keywords: Fungicides, *Mycosphaerella musicola,* Sigatoka leaf spot, Banana

Introduction
A banana botanically an edible berry fruit, produced in the Musa genus, by several varieties of a huge herbaceous flowering plants. In some nations, bananas used in cooking may be labeled "plantains," separating them from bananas used in dessert. The fruit differs for scale, colour and steadiness; nearly all conventional edible seedless bananas (parthenocarp) that came from two wild species, which might be *Musa acuminata* and *Musa balbisiana*, Most cultivated bananas ' scientific names include *Musa acuminata*, *Musa balbisiana*, and Musa al paradisiaca for hybrid *Musa acuminata* al *M. Balbisiana*, based on its genetic makeup. The traditional scientific descriptional name *Musa sapientum* not in use during now a day.
Musa species are local to tropical Indo-Malaya, Australia and are believed to have been domesticated for the first time in country Papua New Guinea. They are cultivated in 135 countries, mainly for their berries, and to a less extent to provide sugar, banana and banana ber and also like ornamental plants. India and China were the world's largest growers of bananas in 2017.
In 2017, the overall global banana production and plantains was stated as 153 million tonnes, driven by India &China with a collective total of 27 per cent of global production [needed to be cited]. The Philippines, Venezuela, Malaysia, Ecuador and Brazil were also major producer
Sigatoka disease or banana leaf spot exists throughout the world and is one of the most damaging diseases of banana. The disease was first reported in Java in 1902 (Zimmerman, 1902) and, its first outbreak occurred in the sigatoka valley of Fiji in 1913, hence the same sigatoka. In India, it is one of the country's major diseases that exist in various banana growing regions.
Sigatoka disease is caused by fungus *Mycosphaerella musicola*. Features of the disease include the presence of abundant discrete spots on older leaf lamina. Young leaves are spot-free. The spots grow to 1 or 2 cm in length after a few days, and turn brown with light gray centers. An equally broad spot on the lower leaves develop, the spots spread and turn yellow and die. There are also black specks on the upper surface of the light-gray patch. On which the conidia of the fungus are raised. The attack on the lamina is a serious degradation of the disease (Agrios, 1997)

Materials and Methods
The research on Sigatoka leaf spot (*Mycosphaerella musicola*) of banana was carried out in the Plant Pathology Lab, Uttaranchal School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India. (2019-2020)

Isolation of the pathogen
The causal organism, *M.musicola* was isolated from banana leaves showing the typical leaf spot symptoms of the disease. The infected leaves were cut in to small leaf bits and surface sterilized with one per cent sodium hypochlorite solution for 2-3 minutes and 3 times

repeatedly washed in sterilized distilled water. Then the infected leaf bits were transferred on to Petri dishes (1-2 leaf bits per Petri plate) containing PDA with the help of a sterile forceps and incubated at 25 °C for 10 days Further purification and sub culturing were done on PDA slants and Petri plates by hyphal tip isolation method.

Hyphal tip isolation

The method was followed for obtaining pure culture of *M.musicola* since the fungus is known to be highly heterozygous. Hyphal tip isolation was done on 2% water agar plates. Diluted hyphal suspensions were prepared in sterile distilled water. One ml of such suspension was spread uniformly on water agar plates and observed for hyphae under microscope. Single isolated hypha was allowed to germinate. Each plate was incubated at 25 ±1 °C and periodically observed for germination under the microscope. Germinating hyphae was marked using marker and were cut by using cork borer and transferred on to the PDA plates incubated at 25±1 °C to get the pure culture. No sectoring was observed in any of the isolates and all of them were found identical in their growth and colony character, Hence it was taken as pure pathogenic culture and was maintained for further studies.

***In vitro* efficacy of fungicides**

Different concentrations of systemic and non-systemic fungicides were evaluated against *M.musicola* following by poisoned food technique. Five different systemic and non-systemic fungicides *viz*., Azoxystrobin, Chlorothalonil, Difenoconazole, Carbendazim and Cabriotop were tested at 50 ppm 150 ppm and 300 ppm concentration.

Stock solution was prepared for each fungicide by dissolving measured quantity of fungicides in a measured volume of sterilized distilled water and added to double concentrated sterilized PDA medium. Four plates were replicated for each treatment and the inoculated plates were incubated at 25±1 °C in BOD incubator. The colony diameter of the pathogen was recorded till the control plates were full with mycelium of the pathogen in 7 days. The growth inhibition percent of mycelium was calculated by following formula.

$$I=100(C-T)/C$$

Where

I - Per cent inhibition in mycelial growth
C - Linear mycelial growth in control (mm)
T - Linear mycelial growth in control (mm)

The experiment was carried out by CRD and data was analyzed after applying the applicable transformation.

Table 1: Name of systemic fungicides and their trade names

S. No	Common name	Trade name
1	Difenoconazole	Score 25% EC
2	Azoxystrobin	Amitsar 25 SC
3	Carbendazim	Bavistin 50WP

Table 2: Name of non-systemic fungicides and their trade names

S. No	Common name	Trade name
1	Cabriotop	Metiram55% WG
2	Chlorothalonil	Kavach 75%WP

Experimental Results

In vitro* evaluation of systemic fungicides against *M. musicola

Three systemic fungicides i.e. Difenoconazole, Azoxystrobin, Carbendazim were tested against *M.musicola at* three concentrations (50ppm, 150ppm, 300ppm) by poison food technique under *in vitro* condition.

Among the three systemic fungicides Carbendazim at all the three concentration maximum inhibited the growth of pathogen and proved to be the most effective, with the growth inhibition of 96.02%, 96.98% and 97.94% respectively. The next best effective fungicides were Azoxystrobin which recorded 93.28% at 300 ppm concentration. The minimum growth inhibition was observed in Difenoconazole at 50 ppm with the growth inhibition of 79.69% and out of the three systemic fungicides Difenoconazole have a low capability to control *M. musicola* at lower concentrations.

In vitro* evaluation of non-systemic fungicides against *M. musicola

Two non- systemic fungicides was tested against *M. musicola* at three concentrations (50 ppm, 150 ppm, 300ppm) by poison food technique.

The maximum inhibition of mycelial growth was observed in Cabrio top 90.78% and 82.47% at 300 and 150 ppm and least inhibition was observed in Chlorothalonil 49.69% at 50 ppm. Chlorothalonil recorded 77.66 per cent inhibition at 300 ppm and after that 71.09 per cent inhibition was recorded in Cabriotop at 50 ppm, 65.16% inhibition at 150 ppm of Chlorothalonil respectively, which is on par with every other.

Table 3: *In vitro evaluation of systemic fungicide on colony growth of M. musicola*

Colony growth (mm) on Fungicides			
Concentration(ppm)	**Difenoconazole**	**Azoxystrobin**	**Carbendazim**
50	16.25	12.13	3.19
150	12.88	8.75	2.41
300	8.63	5.38	1.65
Control	80	80	80
SEM(m)	0.49	0.34	0.07
C D	1.59	1.09	0.22

Table 4: *In vitro* evaluation of systemic fungicide on inhibition of mycelial growth of *M. musicola*

Growth inhibition %			
Concentration(ppm)	**Difenoconazole**	**Azoxystrobin**	**Carbendazim**
50	79.69	84.84	96.02
150	83.91	89.06	96.98
300	89.22	93.28	97.94
Control	0	0	0
SEM(m)	0.61	0.42	0.09
C D	1.99	1.37	0.28

Table 5: *In vitro* evaluation of non-systemic fungicide on colony growth of *M.musicola*

Colony growth (mm) on Fungicides		
Concentration(ppm)	**Cabriotop**	**Chlorothalonil**
50	23.13	40.25
150	14.03	27.87
300	7.38	17.88
Control	80	80
SEM(m)	0.59	1.45
C D	1.91	4.69

Table 6: *In vitro* evaluation of non-systemic fungicide on inhibition of mycelial growth of *M. musicola*

Growth inhibition %		
Concentration(ppm)	**Cabriotop**	**Chlorothalonil**
50	71.09	49.69
150	82.47	65.16
300	90.78	77.66
Control	0	0
SEM(m)	0.74	1.80
C D	2.39	5.86

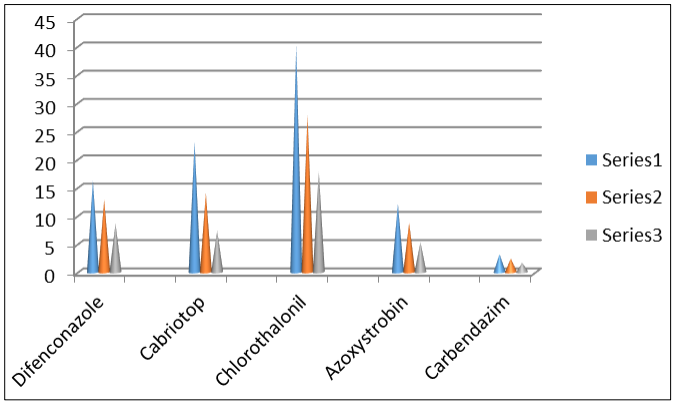

Fig 1: *In vitro* evaluation of systemic & non-systemic fungicide on colony growth of *M.musicola*

Series1 (blue) -- 50 ppm; series2 (red) -- 150ppm; series3 (green) -- 300ppm

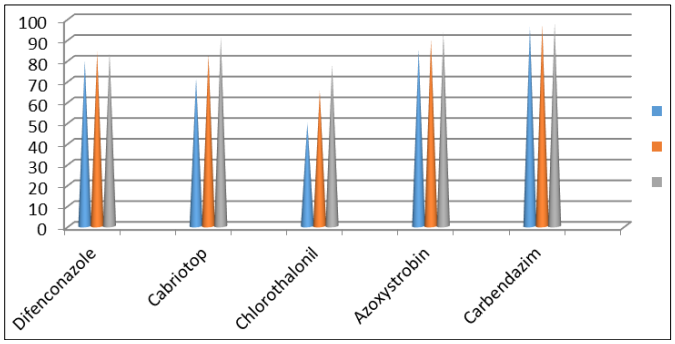

Fig 2: *In vitro* evaluation of systemic& non-systemic fungicides on inhibition of mycelial growth of *M.musicola*

Discussion

Use of fungicides is an alternative method of controlling the diseases of crop in the absence of resistant cultivars or when there is sudden outbreak of disease. Hence, they would continue to be one of the major tools of IDM. Evaluation of fungicides *in vitro* is a handy tool to screen a large number of fungicides and thus can serve as guide for testing. The results of *in vitro* studies reveled that out of 3 systemic and 2 Non systemic fungicides tested, all of them inhibited the mycelial growth of *M.musicola*. The most effecting fungicides were Carbendazim with 97.94% inhibition at all concentrations followed by Azoxystrobin (93.3), Cabriotop (90.7), Difenoconazole (89.2) and Chlorothalonil (49.68) was the least effective.

Aman M and Rai VR (2015) reported that the two systemic fungicides i.e. Carbendazim and Azoxystrobin showed best effective against yellow sigatoka disease causing *Mycosphaerella musicola.* P.R. PATEL (2009) also proved that Carbendazim gave best effective against sigatoka leaf spot (*Mycosphaerella musicola*) of Banana.

Conclusion

In this study we revealed that three systemic fungicides i.e. Carbendazim, Azoxystrobin and Difenoconazole gave best effective against mycelial growth inhibition of *M.musicola* and it may be used for the control of sigatoka leaf spot of banana.

References

1. Aman M, Ravi Shankar R. Antifungal activity of fungicides and plant extracts against yellow sigatoka disease causing *Mycosphaerella musicola*. Current research in Environmental and Applied Mycology. 2015; 5:277-284.
2. PARESH P. Chemical Control of Sigatoka Leaf Spot (*Mycosphaerella musicola*) of Banana. International Journal of Plant Protection. 2009; 2:98-100.
3. Misra DK, Barui FK, Bandyopadhay B, Sarkar S. Management of yellow Sigatoka disease of banana through fungicides under field condition. Journal of Mycopathological Research. 2009; 42:99-101.
4. Khan MAH, Hossain I, Ahmad MU. Impact of Weather on Sigatoka Leaf Spot of Banana (*Musa spp*. L.) and its Eco friendly Management. A Scientific Journal of Krishi Foundation. 2015; 13:44-53.
5. Israeli Y, Slabaugh WR. Effect of banana spray oil on banana yield in the Absence of Sigatoka (*Mycosphaerella* sp.). Scientia Horticulture. 1993; 56:107-177.
6. Vawdrey WR, Peterson RA, Grice KRE. Evaluation of strobilurins, acibenzolar and other chemicals, alone and in spray programs for the control of yellow Sigatoka leaf spot (*Mycosphaerella musicola*) of bananas in far northern Queensland, Australia. International Journal of Pest Management. 2005; 51:245-251.
7. Prasadji JK, Bhagavan BVK, Rao GS, Rao DM. Management of Sigatoka and other leaf spots in banana with fungicides. International Journal of Plant Protection. 2004; 32:151-153.
8. Washington JR, Cruz J, Lopez F, Fajardo M. Infection Studies of *Mycosphaerella fijiensis* on Banana and the Control of Black Sigatoka with Chlorothalonil. Plant disease. 1998; 82:1185-1190.