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Kamal Khilari
Department of Plant Pathology, S. V. P. Uni. of Agri. & Technology, Modipuram, Meerut, Uttar Pradesh, India

Sachin Kumar Jain
Department of Plant Pathology, S. V. P. Uni. of Agri. & Technology, Modipuram, Meerut, Uttar Pradesh, India

Prashant Mishra
Department of Plant Pathology, S. V. P. Uni. of Agri. & Technology, Modipuram, Meerut, Uttar Pradesh, India

Dhirendra Kumar Srivastava
Uttar Pradesh Council of Science and Technology, Lucknow, Uttar Pradesh, India

Correspondence
Kamal Khilari
Department of Plant Pathology, S. V. P. Uni. of Agri. & Technology, Modipuram, Meerut, Uttar Pradesh, India

In vitro evaluation of different fungicides against *Fusarium moniliforme*- causing bakanae disease of rice

Kamal Khilari, Sachin Kumar Jain, Prashant Mishra and Dhirendra Kumar Srivastava

Abstract
Bakanae disease is an important disease of rice causing serious threat to basmati production in all basmati rice growing countries including India. It is emerging as major problem in basmati rice producing areas of India. As per report, this disease caused 15% losses in India and 40-50% in Japan. This disease caused by *Fusarium moniliforme* Sheldon (teleomorph- *Gibberella fujikuroi* Sawada) is mainly seed borne but this may be soil borne also. Therefore seed treatment may be an effective method for management of this disease. To know the inhibitory effect of different chemicals, fourteen fungicides (5 contact and 9 systemic) and one antibiotic were evaluated against *F. moniliforme in vitro* through the poison food technique. Contact fungicides at 200 and 500 ppm whereas systemic fungicides and an antibiotic at 20 and 50 ppm were evaluated. It was observed that among the contact fungicides, Manzate was highly effective and suppressed the pathogen growth 33.71 and 39.59% at 200 and 500 ppm respectively. Among the systemic fungicides, four fungicides *viz.* thiophanate (Milduvip), tabuconazole+trifloxystrobin (Nativo), carbendazim (Starbenz) and carbendazim+mancozeb (Saaf) were observed with complete inhibition to pathogen growth at 20 and 50 ppm. Fungicides like propiconazole, tabuconazole+trifloxtrobin and carbendazim also inhibited to spore germination completely. Present investigation concluded that systemic fungicides have the good fungicidal effect against the pathogen. Therefore, fungicides can be use for management of this disease.

Keywords: Rice, Bakanae disease, *Fusarium moniliformae* and *Gibberella fujikuroi,* management

Introduction
Rice (*Oryza sativa* L.) is an important cereal crop and growing all over the world. Growing rice in India is primarily divided into basmati rice and non-basmati rice. India is the major producer and exporter of basmati rice to the world. Many diseases of rice which cause by fungi, bacteria, viruses and nematodes are responsible to reduction the total yield. Among the fungal disease, bakanae disease is an emerging disease of basmati rice causing serious threat to basmati production worldwide including India. This disease is caused by *Fusarium moniliforme* Sheldon, the pathogen was later identified as *F. fujikuroi* Nirenberg. The teleomorph stage of *F. moniliforme* is known as *Gibberella fujikuroi* Sawada (Nirenberg, 1976) [5]. In rice, *F. moniliforme* induces seedling elongation, foot rot, seedling rot, grain sterility, and grain discoloration (Ou, 1985; Webster & Gunnell, 1992) [6, 10]. The pathogen can be both seed-borne and soil borne. Generally, the seed-borne inoculum provides initial foci for primary infection. Precise information on losses caused by this disease 15% was reported in Eastern districts of Uttar Pradesh of India and 40-50% in Japan (Pavgi and Singh, 1964) [7]. Kanjanasoon (1965) [2] found 3.7-14.7% loss in northern and central Thailand. Seed treatment with fungicide such as thiram has been used for management of this disease (Suzuki *et al.*, 1985) [8]. After the emergence of pathogen resistance to these fungicides, propiconazole has become the major fungicide to control the disease (Kumazawa *et al.*, 2000) [4]. Present investigation was carried out to test the efficacy of different contact and systemic fungicides against the *Fusarium moniliforme* because these may be important component in integrated disease management programme.

Materials and Methods
Different fungicides were tested to know the fungicidal property against the *Fusarium moniliforme* through poison food technique.

In fungicides, five contact fungicides [Monceren (pencycuron), Blitox (copper oxychloride), Kocide (copper hydroxide), Nautec (zineb) and Manzate (mancozeb)] at 200 and 500 ppm, nine systemic fungicides [Mirador (azoxytrobin), Milduvip (thiophanate), Tilt (propiconazole), Hexa plus (hexaconazole), Nativo (tabuconazole+trifloxystrobin), Starbenz (carbendazim), Protection (tebuconazole), Saaf (carbendazim+mancozeb) and Curzate (cymoxanil+mancozeb)] and an antibiotic [Vithaj valida (validamycin)] at 20 and 50 ppm were tested.

Required amount of fungicides was added in each 250 ml capacity conical flask, contain 100 ml sterilized potato dextrose medium to obtain 20ppm, 50ppm, 200ppm, and 500ppm concentration. It was mixed thoroughly by shaking the flask prior to pouring in sterilized Petri plates. The medium was allowed to solidify and then 3 mm discs from seven days old culture of *F. moniliforme* was placed in centre of each Petri plate. Control was maintained without any treatment. Three replications were maintained for each concentration for every tested fungicide. Inoculated plates were incubated at 28 ± 2 ˚C in BOD incubator. Observations were recorded on radial growth of test pathogen at regular interval. Percent inhibition over control was calculated by the following formula (Vincent, 1947) [9].

$$\text{Percent inhibition over control} = \frac{C - T}{C} \times 100$$

Where

C = Growth of fungus in control

T = Growth of fungus in treatments

Effect of different contact and systemic fungicides were also tested against pathogen growth inhibition and spore germination of *F. moniliforme*. Systemic and contact fungicides were tested at 0.1 and 0.2% concentration respectively. Required quantity of each fungicide was added in petri plates containing sterilized water to make the required concentration. Control petri plates were maintained with only sterilized water. Pre dipped rice seeds in suspension of *F. moniliforme* were again dip in petri plates having required concentration of fungicides. Rice seeds were dipped in fungicides suspension for 3, 6 and 12 hours. After dipping for proper time, rice seeds were transferred on sterilized blotter paper for remove the extra water. Treated rice seeds then transferred in petri plates containing sterilized PDA medium. Twenty five rice seed were inoculated in each petri plates. Control petri plates were maintained with rice seeds which were dipped only in sterilized water. Three replications were maintained of each treatment. Inoculated petri plates were incubated at 28 ± 2 ˚C in BOD incubator. Data of growth of *F. moniliforme* were taken after 4 days of inoculation.

Results

Efficacy of contact fungicides (Table-1 & Plate-1, 2) indicates that contact fungicides have some toxic effect against *Fusarium moniliforme* at 200 and 500 ppm. At the 200 ppm, maximum inhibition (33.71%) in pathogen growth was found in mancozeb followed by zineb (11.73%) at nine days after inoculation. Pencycuron fungicide was observed with 3.16% inhibition in pathogen growth. At 500 ppm, Mancozeb and zineb was observed with 39.59% and 17.12% inhibition of pathogen growth respectively. Pencycuron, copper oxychloride, and copper hydraoxide were observed with 9.82, 7.76 and 7.08% inhibition in pathogen growth respectively.

Fungicidal effect of systemic fungicides at 20 and 50 ppm was mentioned in Table- 2 & Plate-1, 2. It is clearly indicate that all systemic fungicides have good fungi toxicity against the *Fusarium moniliforme*. Among the systemic fungicides, three fungicides like thiophanate, carbendazim, carbendazim+mancozeb and tabuconazole+trifloxytrobin were observed with completely inhibition to pathogen growth at 20 ppm so far. At 20 ppm, propiconazole and tabuconazole fungicide were observed with 90.52 and 86.43% growth inhibition of pathogen respectively. At the 50 ppm, five fungicides thiophanate, propiconazole, tabuconazole+ trifloxytrobin, carbendazim and carbendazim+mancozeb were observed with complete inhibition to pathogen growth. Tabuconazole, hexaconazole fungicides were observed with 90.35 and 84.23% inhibition in pathogen growth after nine days of inoculation. The lowest inhibition in pathogen growth was observed in cymoxanil+mancozeb. Antibiotic (validamycin) was found with 34.01 and 56.77% inhibition in pathogen growth at 20 and 50 ppm after nine day of inoculation respectively.

Data of Table-3, 4 & Plate-3 indicate that after four days of inoculation, in control petri plate *Fusarium moniliforme* growth was appear around the all inoculated rice seeds. However rice seeds which were dipped in propiconazole and tabuconazole+trifloxtrobin for 3 hours were observed with no fungal growth around inoculated seeds so far. Only 13 and 18 rice seeds out of 75 rice seeds were observed with fungal growth which was dipped in carbendazim and carbendazim+ mancozeb fungicide respectively. When rice seeds were dipped for 6 hours, completely inhibition in fungal growth around rice seeds was observed in propiconazole, tabuconazole+trifloxtrobin and carbendazim (Table-3, 4 and Plate-4). Only 9, 12 and 27 out of 75 rice seeds were observed with fungal growth which was dipped in carbendazim+ mancozeb, tabuconazole and mancozeb fungicides respectively. Rice seeds which were dipped in propiconazole, tabuconazole+trifloxtrobin, carbendazim and carbendazim+ mancozeb fungicides for overnight were observed with no any fungal growth (Table-3, 4 and Plate-5). Whereas 8, 8, and 9 out of 75 rice seeds were observed with fungal growth which were dipped in tabuconazole, mancozeb and cymoxanil+ mancozeb respectively.

Table 1: Efficacy of different contact fungicides against *F. moniliforme*

Treatment	Trade name	Conc. 200PPM		Conc. 500PPM	
		Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
Pencycuron	Monceren	86.67	3.16	84.50	5.76
Copper oxychloride	Blitox	87.83	1.86	85.83	4.28
Zineb	Nautec	79.00	11.73	73.83	17.66
Mancozeb	Manzate	59.33	33.71	54.17	39.59
Copper hydroxide	Kocide	87.67	2.05	85.67	4.46
Control		89.50	0.00	89.67	0.00
CD at 5% Level		0.769		0.967	

Table 2: Efficacy of different systemic fungicides against *F. moniliforme*

Treatment	Trade name	Conc.20PPM		Conc.50PPM	
		Radial growth (mm)	Percent inhibition (%)	Radial growth (mm)	Percent inhibition (%)
Azoxytrobin	Mirador	48.33	46.10	46.83	47.87
Validamycin	Validamycin	59.17	34.01	38.83	56.77
Thiophanate	Milduvip	0.00	100.00	0.00	100.00
Propiconazole	Tilt	8.50	90.52	0.00	100.00
Hexaconazole	Hexa plus	23.67	73.61	14.17	84.23
Tabuconazole+trifloxtrobin	Nativo	0.00	100.00	0.00	100.00
Carbendazim	Starbenz	0.00	100.00	0.00	100.00
Tabuconazole	Protection	12.17	86.43	8.67	90.35
Carbendazim+ Mancozeb	Saaf	0.00	100.00	0.00	100.00
Cymoxanil+ Mancozeb	Curzate	69.67	22.30	60.00	33.21
Control		89.67	0.00	89.83	0.00
CD at 5% Level		0.393		0.846	

Plate 1: Growth of *Fusarium moniliforme* at nine day after inoculation

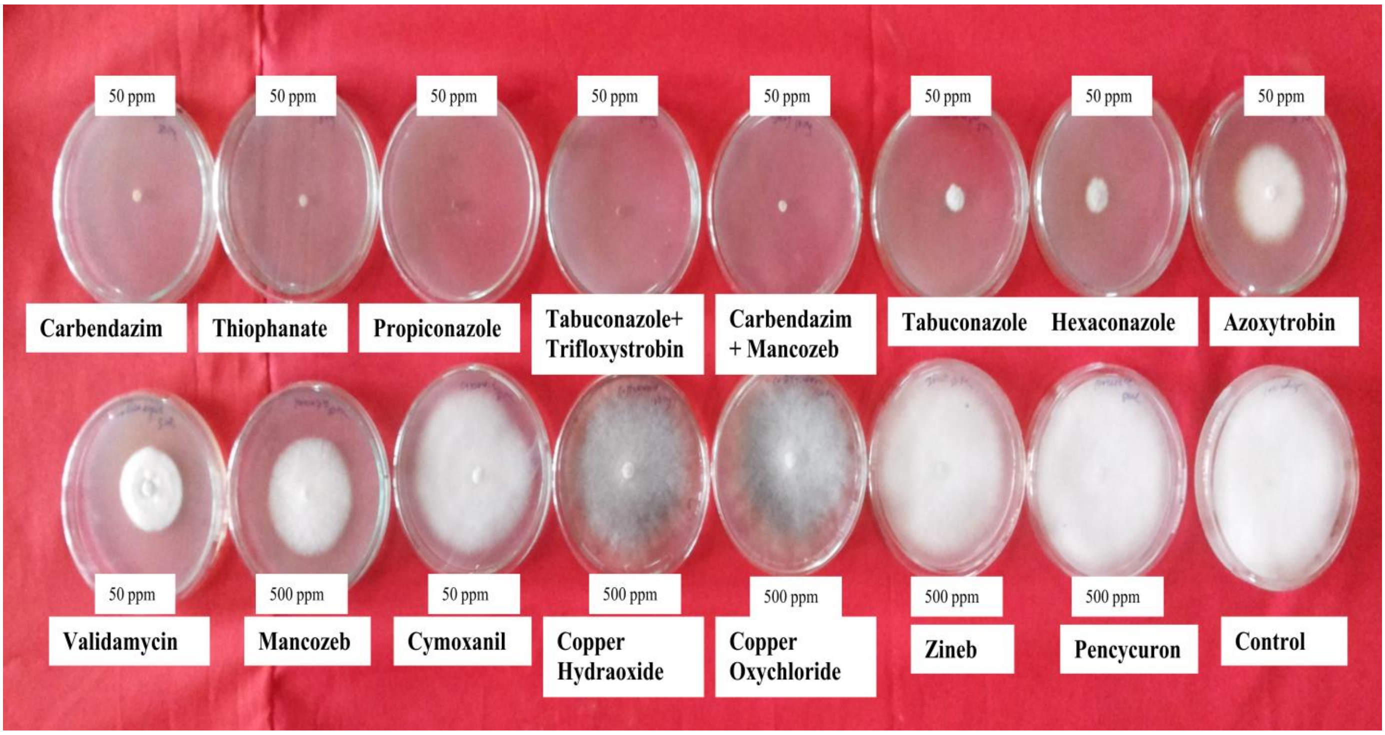

Plate 2: Growth of *Fusarium moniliforme* at nine day after inoculation

Table 3: Efficacy of different contact fungicides against *F. moniliforme*

			3 hours dip		6 hours dip		Overnight dip	
S. No.	**Treatment**	**Trade name**	**Total seed inoculated in three replications**	**Growth appear around the seed**	**Total seed inoculated in three replications**	**Growth appear around the seed**	**Total seed inoculated in three replications**	**Growth appear around the seed**
1.	Pencycuron	Monceren	75	75	75	75	75	75
2.	Copper oxychliride	Blitox	75	75	75	75	75	75
3.	Zineb	Nautec	75	73	75	72	75	56
4.	Mencozeb	Manzate	75	57	75	27	75	8
5.	Copper hydroxide	Kocide	75	75	75	75	75	75
6.	Control		75	75	75	75	75	75
	CD at 5% Level			0.273		0.393		0.846

Table 4: Efficacy of different systemic fungicides against *F. moniliforme*

			3 hours dip		6 hours dip		Overnight dip	
S. No.	**Treatment**	**Trade name**	**Total seed inoculated in three replications**	**Growth appear around the seed**	**Total seed inoculated in three replications**	**Growth appear around the seed**	**Total seed inoculated in three replications**	**Growth appear around the seed**
1.	Azoxytrobin	Mirador	75	66	75	64	75	57
2.	Validamycin	Validamycin	75	75	75	75	75	75
3.	Thiophanate	Milduvip	75	75	75	75	75	64
4.	Propiconazole	Tilt	75	0	75	0	75	0
5.	Hexaconazole	Hexa plus	75	62	75	49	75	44
6.	Tabuconazole+trifloxtrobin	Nativo	75	0	75	0	75	0
7.	Carbendazim	Starbenz	75	13	75	0	75	0
8.	Tabuconazole	Protection	75	70	75	12	75	8
9.	Carbendazim+ mancozeb	Saaf	75	18	75	9	75	0
10.	Cymoxanil+ mancozeb	Curzate	75	75	75	75	75	9
11.	Control		75	75	75	75	75	75
	CD at 5% Level			1.376		1.766		1.258

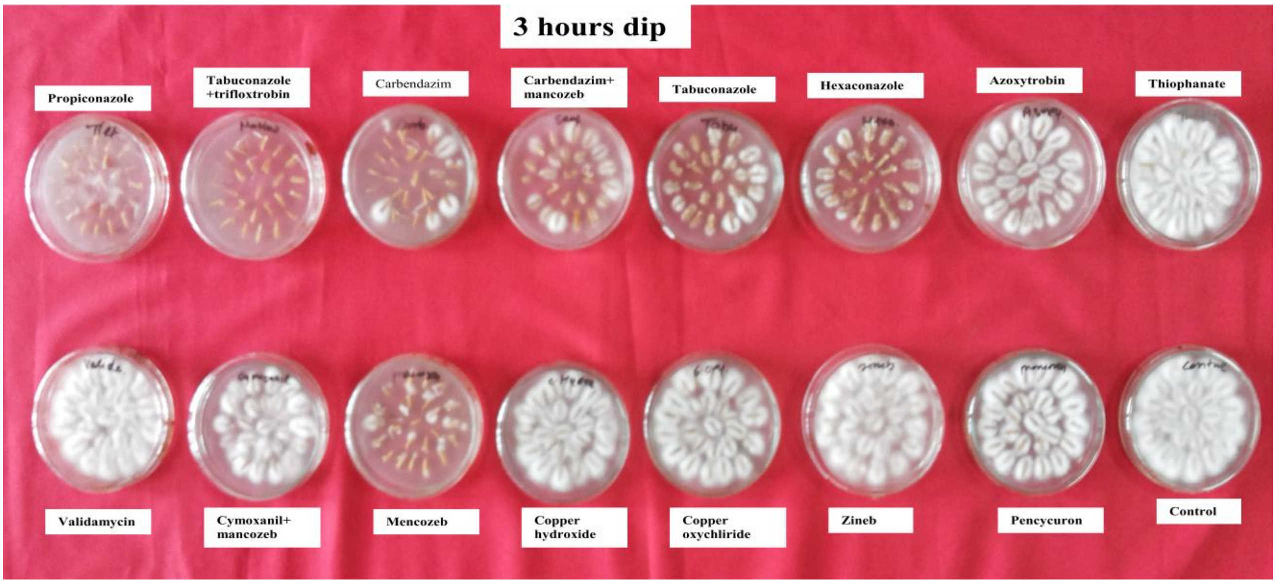

Plate 3: Growth of *Fusarium moniliforme* around the inoculated rice seed at 4 DAI

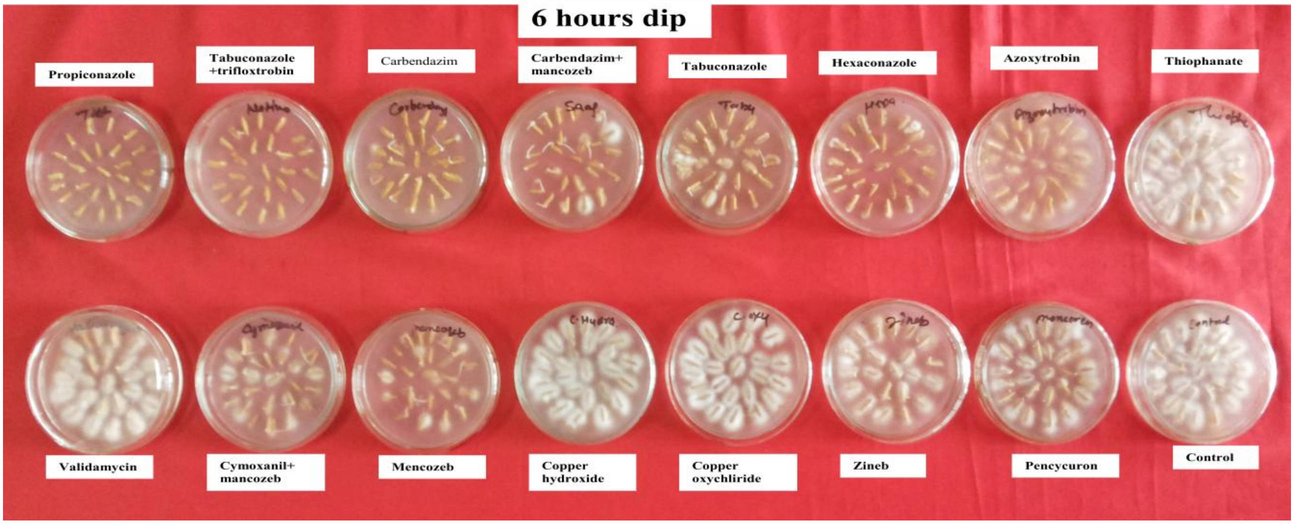

Plate 4: Growth of *Fusarium moniliforme* around the inoculated rice seed at 4 DAI

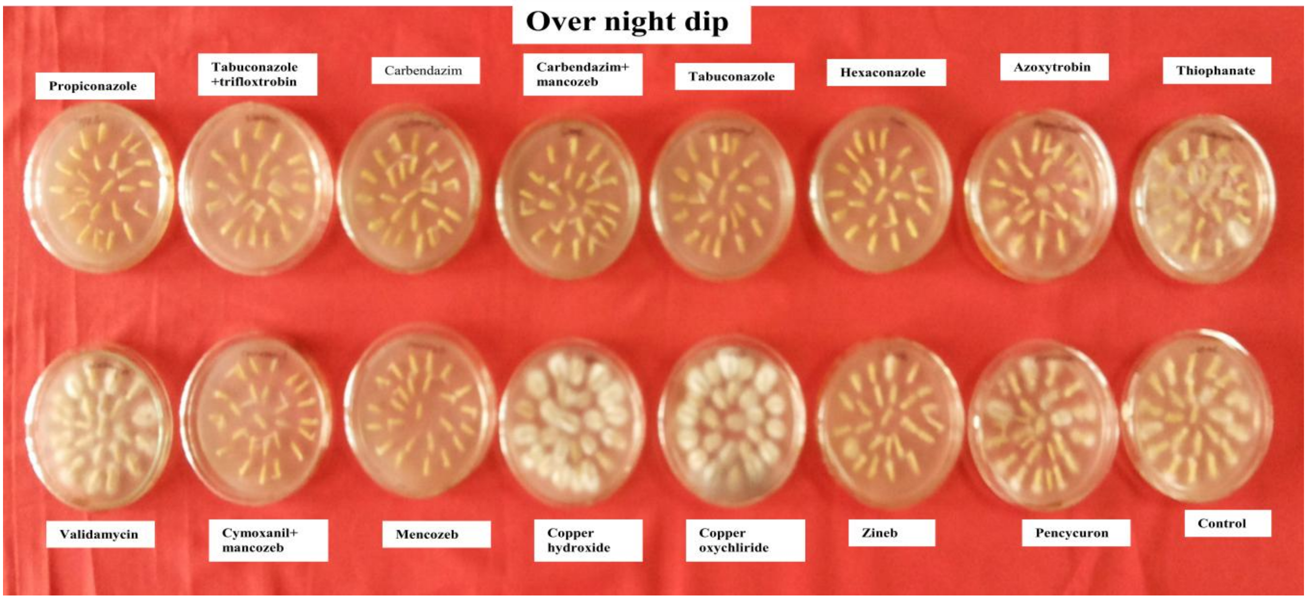

Plate 5: Growth of *Fusarium moniliforme* around the inoculated rice seed at 4 DAI

Discussion

In previous study it has been reported that systemic fungicides have good fungicidal effect against *Fusarium* species. Earlier many researchers have been reported that systemic fungicides have the good fungicidal effect against *Fusarium moniliforme* also. Kapadiya *et al.,* (2013) [3] tested the systemic and non systemic fungicides against the *Fusarium solani* and found that among the systemic fungicide tabuconazole and carbendazim gave cent percent growth inhibition. Jain *et al.,* (2014) [1] reported that carbendazim completely inhibit to pathogen growth at 10ppm and 100ppm. However fungicides such as hexaconazole, tabuconazole and thiophanate inhibited to pathogen growth at 100ppm, 500ppm completely.

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