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Effect of different fungicides against *Podosphaera pannosa* causing rose powdery mildew under greenhouse conditions

V. KUMAR AND S. CHANDEL

Department of Plant Pathology
Dr. Y. S. Parmar University of Horticulture and Forestry
Nauni-173230, Solan, Himachal Pradesh

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ABSRACT

Eight fungicides viz. carbendazim, difenoconazole, hexaconazole, propiconazole, tebuconazole, pyraclostrobin, trifloxystrobin + tebuconazole and dinocap were evaluated under in vitro and greenhouse conditions. Difenoconazole was most effective in its efficacy as no conidia germinated and it was followed by the trifloxystrobin + tebuconazole (96.38%), tebuconazole (95.65%) and propiconazole (86.23%) while the carbendazim was least effective with 63.73 per cent inhibition amongst the tested fungicides but found superior to control and most effective fungicide in inhibition of germ tube elongation was difenoconazole followed by trifloxystrobin + tebuconazole (3.78 µm) and tebuconazole (4.10 µm) which showed superiority to other treatments including control while least effectiveness was recorded in carbendazim (29.24 µm). The highest disease control was recorded in difenoconazole of 85.96 per cent followed by trifloxystrobin + tebuconazole (83.60%), tebuconazole (82.07%), hexaconazole (78.99%), propiconazole (77.20%) and dinocap (71.97%). The least effective was carbendazim with 64.00 per cent disease control.

***Keywords*:** Conidial germination, disease control, fungicides, powdery mildew

Rose (*Rosa hybrid* L.), the top ranking cut flower of today, belongs to the subfamily Rosideae within the family Rosaceae. The beauty, fragrance and multiple uses of roses as cut flowers or landscape plants have made roses an appreciated crop since ancient times. From an economical standpoint, roses are the most important plants in ornamental horticulture (Hummer and Janick, 2009). Fruits of the rose are good source of vitamin C; petals are used to make the skin healthy and glowing. It cures dry and patchy skin and its herbal tea use has an advantage of treating cold and cough. Rose water and rose vinegar are also prepared from it and are also considered as major flowers for the perfume industry, medicinal and culinary qualities (Gudin, 1999).

Both pot plants and cut roses either planted outdoors or in glasshouses are susceptible to many phytopathogens. Among the most serious foliar diseases, it is worth mentioning that powdery mildew, downy mildew, black spot, grey mould, leaf spot and rust (Linde and Shishkoff, 2003) causes heavy losses to roses. Powdery mildew fungi are obligate and considered as one of the most conspicuous groups of plant pathogens. They are characterized by the appearance of spots or patches of a white to grayish, powdery growth on the outside of plant organs.

The attacking fungus is most commonly observed on the upper side of the leaves, but it also affects the underside of leaves and every plant parts; young shoots and stems, buds, flowers, and young fruits in many plant species (Gastelum *et al.*, 2014). The powdery mildew fungi seldom kill their hosts but utilize their nutrients, reduce photosynthesis, increase respiration and transpiration, impair plant growth and reduce the yield, ranging between 20 to 40 per cent depending upon the congenial environment favourable for their growth and multiplication (Agrios, 2005). The losses particular in roses ranged between 20 to 25 per cent as reported by Kumar (1998). The present investigation was carried out to found the most effective fungicides against rose powdery mildew caused by *Podosphaera pannosa* under greenhouse conditions.

MATERIALS AND METHODS

In vitro evaluation of fungicides using slide spore germination method

Eight fungicides *viz.* carbendazim, difenoconazole, hexaconazole, propiconazole, tebuconazole, pyraclostrobin, trifloxystrobin + tebuconazole and dinocap (Table 1) were evaluated under in vitro and greenhouse conditions. Double strength solution of each fungicide was prepared in the sterilized distilled water. Simultaneously, the spore suspension (28-30 per microscopic field) was also prepared by dislodging the conidia from the infected leaves. One drop of each (*i.e.* fungicide solution and spore suspension) was separately put in the cavity of cavity slide, cavity slides containing mix were later placed on a glass rod, kept in a Petri plate containing sterilized distilled water at the bottom and

Email: vnarwal777@yahoo.com

sterilized moistened cotton wool lining the inner surface of the upper lid. Petri plates were then kept in the growth chamber for incubation at 25+1^{o}C with 85 per cent relative humidity. The experiment was laid out in a Completely Randomized Design (CRD) and each treatment was replicated three times. The readings on germination of conidia were recorded after 24, 36, 48, 60 and 72 hrs by placing cavity slides under light microscope and per cent inhibition in germination of each fungicide was calculated by adopting the formula as given by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition

C = Germination of conidia in control

T = Germination of conidia in treatment

***In vivo* evaluation of fungicides against powdery mildew of rose**

The fungicides carbendazim (0.10%), difenoconazole (0.05%), hexaconazole (0.05%), propiconazole (0.05%), tebuconazole (0.05%), pyraclostrobin (0.05%), trifloxystrobin + tebuconazole (0.05%) and dinocap (0.05%) were evaluated for their bio-efficacy against powdery mildew on roses grown in greenhouse or polyhouse at the State Department of Horticulture Station, Jhamadu Baag, district Mandi for two consecutive years 2015 and 2016 (Table 1). Each fungicide was sprayed six times with the initiation of disease at an interval of 7 days and plants sprayed with water served as control. Completely Randomized Design was followed to layout the experiment along with three replications in each treatment. The data on disease severity were recorded after every seven days up to the last spray and subjected to statistical analysis. The per cent disease index (PDI) was calculated by using the formula given by McKinney (1923) as follows:

$$\text{Disease index } (\%) = \frac{\text{Sum of all the numerical disease ratings}}{\text{Total number of samples Observed} \times \text{Maximum disease grade}} \times 100$$

Per cent disease control (PDC) was calculated as per the formula given under

$$PDC = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

RESULTS AND DISCUSSION

Efficacy of different fungicides under in vitro conditions

In order to study the effect of different fungicides on the conidial germination, conidial germination inhibition and germ tube length, eight fungicides were tested against the *P. pannosa* by slide spore germination method and the data pertaining to these parameters were recorded and presented in table 2 Perusal of data revealed that all the fungicides reduced the conidial germination of the test pathogen as compared to the control. In all the treatments, per cent conidial germination decreased with the increase in concentration of fungicides. Among the test fungicides, no conidial germination was observed in difenoconazole thereby indicating the 100 per cent inhibition of conidia at all tested concentrations 50, 100 and 150 ppm. Similarly, least conidial germination (2.22%) was revealed in case of trifloxystrobin + tebuconazole followed by tebuconazole (2.67%), hexaconazole (7.33%) and propiconazole (8.44%). Minimum conidial germination inhibition (42.37%) was found in carbendazim followed by pyraclostrobin (56.48%) at 50 ppm concentration. At higher concentration of 150 ppm, least (9.85%) conidia germinated followed by 100 ppm and 50 ppm. Same inference was drawn with respect to inhibition of conidial germination; highest inhibition (83.92%) was registered at 150 ppm compared to 50 ppm (66.34%) and 100 ppm (76.38%).

The overall mean showed difenoconazole to be the most effective in its efficacy as no conidia germinated and it was followed by the trifloxystrobin + tebuconazole (96.38%), tebuconazole (95.65%) and propiconazole (86.23%) in imparting inhibition on conidia germination showing statistically different results while the carbendazim was least effective with 63.73 per cent inhibition amongst the tested fungicides but found superior to control.

The data on the germ tube elongation (Table 2) also indicated that the all fungicides reduced the germ tube elongation as compared to the control. In all the treatments germ tube length decreased with corresponding increase in the concentrations. Amongst tested fungicides, difenoconazole proved most effective at all the concentrations evaluated which completely inhibited germ tube elongation followed by the trifloxystrobin + tebuconazole and tebuconazole which revealed the length of 11.33 µm, and 12.30 µm at 50 ppm, respectively while no germ tube formation occurred at higher concentrations (100 and 150 ppm). Carbendazim was least effective with higher (40.50 µm) germ tube length formation followed by the 35.27 µm in pyraclostrobin but superior to the control (112.60 µm). The overall mean revealed that the most effective treatment was difenoconazole followed by trifloxystrobin + tebuconazole (3.78 µm) and tebuconazole (4.10 µm) which showed superiority to other treatments including control while least effectiveness was reported in carbendazim (29.24 µm).

Table 1: Name of different fungicides used under *in vitro* evaluation against *Podosphaera pannosa*

Sr. No.	Trade Name	Formulation	Chemical name	Concentration (ppm)
1.	Bavistin	50WP	Carbendazim	50, 100, 150
2.	Score	25EC	Difenoconazole	50, 100, 150
3.	Sitara	5EC	Hexaconazole	50, 100, 150
4.	Tilt	25EC	Propiconazole	50, 100, 150
5.	Folicur	250EC	Tebuconazole	50, 100, 150
6.	Insignia	20EG	Pyraclostrobin	50, 100, 150
7.	Nativo	25 WG + 50 WG	Trifloxystrobin + Tebuconazole	50, 100, 150
8.	Karathane	48% EC	Dinocap	50, 100, 150

Table 2: *In vitro* evaluation of different fungicides against *Podosphaera pannosa* using slide spore germination method

Fungicide (s)	Conidial germination (%) at different concentrations (ppm)			Mean	Conidial germination (%) inhibition at different concentrations (ppm)			Mean	Germ tube length (µm) at different concentrations (ppm)			Mean
	50	100	150		50	100	150		50	100	150	
Carbendazim	35.33	21.00	10.33	22.22	42.37	65.77	83.13	63.73				
	(36.46)	(27.26)	(18.73)	(27.48)	(40.59)	(54.17)	(65.73)	(53.50)	40.50	28.13	19.10	29.24
Tebuconazole	8.00	0.00	0.00	2.67	86.95	100.00	100.00	95.65				
	(16.40)	(0.00)	(0.00)	(5.47)	(68.82)	(88.15)	(88.15)	(81.70)	12.30	0.00	0.00	4.10
Hexaconazole	12.67	8.00	1.33	7.33	79.33	86.95	97.83	88.04				
	(20.83)	(16.40)	(6.53)	(14.59)	(62.94)	(68.82)	(81.61)	(71.12)	24.23	17.10	11.33	17.56
Dinocap	21.40	15.67	2.33	13.11	65.09	74.44	96.18	72.99				
	(27.54)	(23.30)	(8.74)	(19.86)	(53.77)	(59.61)	(64.05)	(60.80)	32.47	24.30	17.23	24.67
Trifloxystrobin+	6.67	0.00	0.00	2.22	89.14	100.00	100.00	96.38				
Tebuconazole	(14.94)	(0.00)	(0.00)	(4.98)	(70.73)	(88.15)	(88.15)	(82.34)	11.33	0.00	0.00	3.78
Difenoconazole	0.00	0.00	0.00	0.00	100.00	100.00	100.00	100.00				
	(0.00)	(0.00)	(0.00)	(0.00)	(88.15)	(88.15)	(88.15)	(88.15)	0.00	0.00	0.00	0.00
Pyraclostrobin	26.67	16.67	9.33	17.56	56.48	72.79	84.76	71.34				
	(31.07)	(24.07)	(17.76)	(24.30)	(48.70)	(58.55)	(67.00)	(58.08)	35.27	26.30	18.20	26.59
Propiconazole	13.67	7.66	4.00	8.44	77.70	87.51	93.47	86.23				
	(21.68)	(16.06)	(11.47)	(16.40)	(61.80)	(69.28)	(75.24)	(68.78)	26.37	19.53	12.30	19.40
Control	61.33	61.33	61.33	61.33								
	(51.53)	(51.53)	(51.53)	(51.53)	-	-	-		112.60	112.60	112.60	112.60
Mean	**20.64**	**14.48**	**9.85**		**66.34**	**76.38**	**83.92**					
	(24.50)	**(17.62)**	**(12.75)**		**(55.06)**	**(63.87)**	**(70.31)**		**32.79**	**25.33**	**21.20**	
LSD (0.05)	**Fungicides (F) = 0.39**				**Fungicides (F) = 0.64**				**Fungicides (F) = 0.24**			
	Conc. (C) = 0.68			**LSD**	**Conc. (C) = 1.11**			**LSD**	**Conc. (C) = 0.41**			
	F × C = 1.18			**(0.05)**	**F × C = 1.93**			**(0.05)**	**F × C = 0.72**			

*Note: *Figures in parentheses are arc sine transformed values*

Out of various fungicides evaluated for their fungitoxicity against *P. pannosa* by slide germination method, only one fungicide, difenoconazole proved most effective at all concentrations which resulted in no conidial germination and germ tube formation. Other two, trifloxystrobin + tebuconazole and tebuconazole gave negligible germination as well as germ tube length. The present study results were in conformity with Channaveeresh and Kulkarni (2005) they found the maximum conidial germination inhibition of 90.51 per cent by difenoconazole after azoxystrobin, while studying the powdery mildew of black gram. Efficacy of difenoconazole and carbendazim has also been reported by Cimanowski and Goszczynski (1990) and Sharma and Gupta (1994) in inhibiting spore germination of *Podosphaera leucotricha* and reducing germ tube under *in vitro* condtions. Gupta and Kumar (2008) observed that carbendazim caused complete conidial germination inhibition of powdery mildew (*Erysiphe pisi* DC.) of pea followed by wettable sulphur.

Table 3: Effect of fungicides on rose powdery mildew under greenhouse conditions during 2015 and 2016

Fungicide (s)	Conc. (%)	Disease index (%)			Disease Control (%)		
		2015	2016	Pooled	2015	2016	Pooled
Carbendazim	0.10	29.27 (32.74)	27.07 (31.33)	28.17 (32.04)	62.25 (52.07)	65.75 (54.16)	64.00 (53.11)
Tebuconazole	0.05	13.73 (21.74)	14.33 (22.24)	14.03 (21.99)	82.28 (65.09)	81.86 (64.77)	82.07 (64.93)
Hexaconazole	0.05	17.53 (24.74)	15.33 (23.04)	16.43 (23.91)	77.38 (61.58)	80.59 (63.84)	78.99 (62.69)
Dinocap	0.05	22.33 (28.19)	21.53 (27.64)	21.93 (27.92)	71.19 (57.52)	72.74 (58.03)	71.97 (58.01)
Trifloxistrobin+ Tebuconazole	0.05	12.67 (20.84)	13.00 (21.13)	12.83 (20.98)	83.67 (66.14)	83.54 (66.04)	83.60 (66.09)
Difenconazole	0.05	10.33 (18.74)	11.67 (19.96)	11.00 (19.36)	86.67 (68.56)	85.24 (67.38)	85.96 (67.96)
Pyraclostrobin	0.05	27.73 (31.76)	24.73 (29.81)	26.23 (30.80)	64.23 (53.25)	68.69 (55.95)	66.46 (54.59)
Propiconazole	0.05	19.33 (26.04)	16.33 (23.83)	17.83 (24.97)	75.06 (60.02)	79.33 (62.93)	77.20 (61.45)
Control	-	77.53 (61.68)	79.00 (62.70)	78.27 (62.19)	-	-	-
SEm (±)	**-**	**0.31**	**0.28**	**0.29**	**0.31**	**0.34**	**0.36**
LSD (0.05)	**-**	**0.81**	**0.98**	**0.65**	**1.05**	**0.90**	**0.54**

*Note: *Figures in parentheses are arc sine transformed values*

Difenoconazole and triazole fungicides were highly effective in spore germination inhibition of *Podosphaera pannosa* in the present investigation. Similarly, they were also found to be effective against powdery mildew of different crops like green gram by Venkatrao (1997), rose by Kumar (1998), pea by Biju (2000), okra by Shivanna *et al*. (2006) and fenugreek by Chovatiya (2010).

Evaluation of fungicides under greenhouse conditions

A field experiment was conducted to assess the efficacy of foliar sprays of fungicides against powdery mildew during the months of May and June in the years 2015 and 2016. In total six sprays of each fungicide were applied at 7 days interval during each year. The data pertaining to powdery mildew severity was recorded and is presented in table 3 in both the years of study.

The disease index percentage was minimum in difenoconazole (11.00%) which was followed by trifloxystrobin + tebuconazole (12.83%), tebuconazole (14.03%) and hexaconazole (16.43%) over other treatments and control (78.27%). Similar trend was observed in per cent disease control in the pooled data of two years 2015 and 2016.

All the fungicides significantly reduced and control the powdery mildew disease in comparison to control. The maximum disease control of 86.67 and 85.24 per cent was observed in the plants treated with difenoconazole followed by nativo, combiproduct (trifloxystrobin + tebuconazole) (83.67 and 83.54%), tebuconazole (82.28 and 81.86%) and hexaconazole (77.38 and 80.59%) in 2015 and 2016 years, respectively. The minimum disease control was found in carbendazim with 62.25 and 65.75 per cent followed by pyraclostrobin (64.23 and 68.69%), dinocap (71.19 and 72.74%) and propiconazole (75.06 and 79.33%) in increasing order of their efficacy.

From the pooled data (Table 3) it was also depicted difenoconazole as the most effective fungicide with 85.96 per cent of disease control. Next effective fungicides in order were trifloxystrobin + tebuconazole (83.60%), tebuconazole (82.07%), hexaconazole (78.99%), propiconazole (77.20%) and dinocap (71.97%). However the least effective was carbendazim with 64.00 per cent disease control.

The field trials conducted during two consecutive years revealed that difenoconazole sprays were statistically significant and most effective in reducing the severity of powdery mildew followed by the trifloxystrobin + tebuconazole and tebuconazole. The results were in consonance with those reported by Kakade *et al.* (2006) as they also found that the spray of difenoconazole at eight day interval resulted in 89.17 per cent of disease control for consecutively three years.

Similarly, difenoconazole spray was found highly effective in reducing the disease severity to 1.99 per cent as compared to 66.41 per cent in control, followed by hexaconazole (6.27%) but in present study we have found hexaconazole effective after the trifloxystrobin + tebuconazole, tebuconazole because of the different mode of action or might be due to less exposure to the pathogen (Choudhary *et al.*, 2009). Wei *et al.* (2014) reported control of the disease Chinese rose powdery mildew to 80 per cent after first spray of difenoconazole. Reports of effectiveness of tebuconazole were also found by Wojdya and Jaworska (2005). Similarly, the efficacy of the hexaconazole was reported by various workers on powdery mildew of rose time to time (Dhruj *et al.*, 1994; Perez and Lozoya, 1994 and Lam and Lim, 2008) but during present studies it was placed in fourth position after difenoconazole, trifloxystrobin + tebuconazole and tebuconazole by giving 78.99 per cent disease control as compared to other treatments which ranged between 82.07 to 85.96 per cent. However, the efficacy of dinocap against *Podosphaera pannosa* was also reported by Venkataramana *et al.* (1993) and Sobati *et al.* (2000). Various workers have also reported the efficacy of the carbendazim, propiconazole, pyraclostrobin (Venkataramana *et al.*, 1993; Dhruj *et al.*, 1994; Sobati *et al.*, 2000; Maharshi, 2007 and Kumar *et al.*, 2010). The disease was checked upto the extent of 77.20 per cent giving considerable control of powdery mildew with these fungicides except carbendazim which showed least control (64.00%).

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