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## Studies on the post symptom anti-sporulant activity of fungicides, bio-control agents, bio-products and botanicals on formation of conidia of *Podosphaera pannosa* on rose

Vijay Kumar and Sunita Chandel

### Abstract

Anti-sporulant activity of fungicides (carbendazim, difenoconazole, hexaconazole, propiconazole, tebuconazole, pyraclostrobin, trifloxystrobin + tebuconazole and dinocap), bio-control agents (*Ampelomyces quisqualis*, *Pseudomonas fluorescense*, *Trichoderma viride*, *Saccharomyces cerevisiae*, *Bacillus subtilis* and *Verticillium lecanii*), bio-products (vermiwash, chicken manure, FYM, milk, anhydrous milk fat, neem oil, BF-1 [Turmeric (25g)+Chilli (5g)+Onion (25g)], BF-2 [Garlic paste (25g)+Turmeric (5g)] and BF-3 [Reetha (5%) + Cow urine + Neem oil (5%)]) and botanicals (drake (*Melia azedarach* L.), robinia (*Robinia pseudoacacia* L.), bougainvillea (*Bougainvillea glabra* C.), pine needles (*Pinus roxburghii* S.), garlic (*Allium sativus* L.), euphorbia (*Euphorbia resinifera* A.), reetha (*Sapindus mukorossi* L.) and soybean Seed (*Glycine max* L.)) were studied after the appearance of disease. The maximum reduction in conidia production was recorded in difenoconazole giving by 29.98 per cent reduction followed by the hexaconazole (25.56%), tebuconazole (25.14%) and trifloxystrobin + tebuconazole (24.76%) while the least effect was observed with carbendazim (16.55%). Among the bio-control agents the maximum reduction in conidia was found in *Ampelomyces quisqualis* (26.50%) followed by the *Bacillus subtilis* (24.44%) and *Verticillium lecanii* (20.54%) within 8 days of disease observation. However, least reduction in conidia were recorded in *Pseudomonas fluorescense* (9.20%) and yeast (11.10%). From bio-products neem oil, bio-formulation-3, milk and anhydrous milk fat were reported to be superior and while the bio-formulation-1 (8.24%) and vermiwash (9.69%) were less effective in lowering the conidial count. However, among the botanicals best results with regard to conidia reduction was obtained with *Allium sativus* (21.01%) followed by the *Sapindus mukorossi* (18.95%) and *Melia azedarach* (18.01%) both statistically at par in their effectiveness. While least conidial reduction (6.42%) was recorded in *Glycine max*.

**Keywords:** powdery mildew, anti-sporulant activity, conidia, rose

### Introduction

Rose has been admired for its beauty and fragrance since its first cultivation 5000 years ago by ancient civilizations of China, Western Asia and Northern Africa (Gudin, 2000) [1] and brought to India by Baber in the year 1526. Rose is an important ornamental and cut flower crop in India, which is affected by various diseases. Among them, powdery mildew caused by *Podosphaera pannosa* (Syn. *Sphaerotheca pannosa* var. *rosae*) is a more serious and economically important disease found throughout the year reducing the yield to considerable extent. The powdery mildew fungi represent one of the most widely distributed and destructive groups of the plant pathogens worldwide (Braun *et al.*, 2002) [2] which cause serious diseases of the many vegetable, fruits, ornamental and other crops. Powdery mildew causes leaf curling, yellowing, premature defoliation, and in some cases, death of the plant (Pember-ton, 1908; Browne, 1974). Rose powdery mildew severely damages leaves and often causes leaf distortion, curling and premature defoliation. Although leaves normally become resistant to infection as they age, the fungus grows abundantly on the pedicels, sepals and receptacles of many susceptible cultivars, especially when flower buds are unopened, resulting in blooms of poor quality (Longree, 1939; Rogers, 1959; and Mence and Hildebrandt, 1966) [5, 6, 7]. Powdery mildew is the most troublesome of all rose diseases. It can be found on the foliage, buds, stems, and on the petals of the bloom. It can cause the buds to decay at the point of opening. It can also interfere with plant functions when found on the leaves of the plant which might alter the functions and unable the plant to convert carbohydrates into food required to maintain its health (Genders, 1965) [8]. 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,

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ranging between 20 to 40 per cent depending upon the congenial environment favorable for their growth and multiplication (Agrios, 2005) [9]. The losses particular in roses ranged between 20 to 25 per cent as reported by Kumar (1998) [10]. Therefore in the present investigations was carried out to know the anti-sporulant activity, time interval optimization of sprays schedules and effective concentration which helps in disease management programmes.

### Materials and Methods

To determine the post symptom anti-sporulating activity, rose plants were sprayed at different times of disease initiation with fungicides, bio-control agents, bio-products and botanicals. After 2, 4, 6 and 8 days of single spray, five leaves were removed from each treatment and one lesion (5 mm<sup>2</sup>) per leaf was cut with the help of a cork borer and washed into 5 ml of distilled water with the help of a camel hair brush. One drop suspension was placed on a haemocytometer to record the number of conidia and reduction (%) in number of conidia over control was calculated by the method given by Vincent (1947) [11] and Gupta and Gupta (1991) [12].

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

Where,

- I = Per cent inhibition  
C = Germination of conidia in control  
T = Germination of conidia in treatment

### Results and Discussion

#### Post symptom anti-sporulant activity of fungicides

It is evident from the data (Table 1) that the plants sprayed with fungicides, difenoconazole 2 days after first spray developed least (51.00) number of conidia followed by the trifloxystrobin + tebuconazole (57.00), tebuconazole (58.67) and hexaconazole (63.00) while the numerous increase in conidia number was reported in carbendazim (70.67) being

maximum. This was followed by pyraclostrobin (68.67), dinocap (66.33) and propiconazole (64.67) which showed the decreasing order of their efficacy. By 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> days all fungicides continued to reduce the conidial number, but the reduction level came down with the span of time maximum being recorded on 8<sup>th</sup> day.

On the basis of overall mean reduction in conidia production, difenoconazole was rated best giving by 29.98 per cent reduction followed by the hexaconazole (25.56%), tebuconazole (25.14%) and trifloxystrobin + tebuconazole (24.76%) however first two fungicides were at par with each other while the least effect was observed with carbendazim (16.55%). The overall mean reduction of conidial formation at 4<sup>th</sup> duration indicated that there was significant decrease after the 2<sup>nd</sup> day which further decreased and became minimum on 8<sup>th</sup> day of spray. This trend also showed that with the increase in number of days, there was corresponding decrease in conidia production giving thereby a negative correlation between number of days on conidia production.

Maximum conidial reduction (42.26%) after one spray was observed in difenoconazole followed by trifloxystrobin + tebuconazole (35.47%). Carbendazim reduced the conidial formation in powdery mildew of cucumber as reported by Sen and Kapoor (1974) [16] and efficacy of karathane in protecting adult plant from powdery mildew has been reported by Gutierrez *et al.* (1991) [17], however in present study carbendazim and karathane does not gave the promising results as compared to other fungicides. The efficacy of the carbendazim was found by the Gupta and Gupta (1991) [18], Sharma and Gupta (1994) [18] and Singh (2004) [19] in apple powdery mildew but in present study we found the complete opposite results as in our study carbendazim had least conidial reduction compared to other new fungicides molecules though found superior over control. Scanning through the literature revealed that there is not much information available on effects of the all investigated fungicides on anti-sporulant activities against powdery mildew.

**Table 1:** Post symptom anti-sporulant activity of fungicides on formation of conidia of *Podosphaera pannosa* on rose leaves (*in vivo*)

Fungicide (s)	Conc. (%)	Total number of Conidia ( <i>in vitro</i> )				Mean	Per cent reduction of conidia				Mean
		Number of Days (After 1 <sup>st</sup> spray)					Number of Days (After 1 <sup>st</sup> spray)				
		2	4	6	8		2	4	6	8	
Carbendazim	0.10	70.67	74.00	76.67	78.33	74.92	19.99(26.53)	17.16 (24.45)	15.14 (22.88)	13.91 (21.87)	16.55 (23.94)
Tebuconazole	0.05	58.67	67.33	70.67	72.27	67.23	33.58(35.40)	24.62 (29.73)	21.77 (27.80)	20.58 (26.96)	25.14 (29.97)
Hexaconazole	0.05	63.00	64.67	69.00	70.67	66.83	28.68(32.36)	27.59 (31.66)	23.61 (29.05)	22.34 (28.20)	25.56 (30.32)
Dinocap	0.05	66.33	72.00	72.33	76.00	71.67	24.90(29.92)	19.39 (26.11)	19.93 (26.50)	16.48 (23.93)	20.17 (26.61)
Trifloxystrobin+ Tebuconazole	0.04	57.00	68.67	72.00	72.67	67.58	35.47(36.53)	23.12 (28.72)	20.30 (26.76)	20.14 (26.65)	24.76 (29.67)
Difenoconazole	0.04	51.00	62.67	68.67	69.33	62.92	42.26(40.53)	29.85 (33.10)	23.99 (29.31)	23.80 (29.19)	29.98 (33.03)
Pyraclostrobin	0.05	68.67	72.33	73.67	77.47	73.03	22.26(28.13)	19.02 (25.84)	18.45 (25.42)	14.86 (22.65)	18.65 (25.51)
Propiconazole	0.05	64.67	70.67	72.33	73.67	70.33	26.79(31.16)	20.89 (27.18)	19.93 (26.50)	19.04 (25.86)	21.66 (27.68)
Control	-	88.33	89.33	90.33	91.00	89.75	-	-	-	-	
Mean		65.37	71.30	73.96	75.71		25.99 (28.95)	20.18 (25.20)	18.12 (23.80)	16.80 (22.81)	
CD 0.05	Fungicide(F)=0.43 Days (D)= 0.29 F x D= 0.98						Fungicide(F)=0.55 Days (D)= 0.08 F x D= 1.01				

\*Figures in parentheses are arc sine transformed values

#### Post symptom anti-sporulant activity of bio-control agents

The anti-sporulant activities of seven bio-control agents were tested and the data pertaining to this upto 8<sup>th</sup> day is presented in the Table 2. It was revealed that the minimum no. of conidia were recorded in *Ampelomyces quisqualis* (63.33%) after first spray followed by the *Bacillus subtilis* (65.08%), *Verticillium lecanii* (68.45%) while reverse trend was

followed in *Pseudomonas fluorescense* and yeast which recorded maximum no. of conidia 78.18 and 76.54 per cent, respectively compared to other treatments. By 4<sup>th</sup>, 6<sup>th</sup> and 8<sup>th</sup> day all bio-control agents continued to reduce the conidial number, but the reduction level advances with increase in time period.

The overall mean showed the maximum reduction in conidia was found in *Ampelomyces quisqualis* (26.50%) followed by the *Bacillus subtilis* (24.44%) and *Verticillium lecanii* (20.54%) within 8 days of disease observation. However, least reduction in conidia were recorded in *Pseudomonas fluorescens* (9.20%) and yeast (11.10%). There was gradual reduction in conidia after 2<sup>nd</sup> day upto 8<sup>th</sup> day of its observation in all the treatments.

All the bio-control agents possessed some extent of anti-sporulant activities against rose powdery mildew. The highest

anti-sporulant activity among the seven bio-control agents was found in *Ampelomyces quisqualis* followed by the *Bacillus subtilis* and *Verticillium lecanii*. Anti-sporulant activities of bio-control agents might be due the mechanisms of hyper parasitism (*Ampelomyces quisqualis*) and antibiosis in case of the bacterial antagonists. Scanning through the literature revealed that there is no information available on effects of these bio-control agents on anti-sporulant activities against powdery mildew hence the results could not be compared.

**Table 2:** Post symptom anti-sporulant activity of bio-control agents on formation of conidia of *Podosphaera pannosa* on rose leaves (*in vivo*)

BCA (s)	Conc. (%)	Total number of Conidia ( <i>in vitro</i> )				Mean	Per cent reduction of conidia				Mean
		Number of Days (After 1 <sup>st</sup> spray)					Number of Days (After 1 <sup>st</sup> spray)				
		2	4	6	8		2	4	6	8	
<i>Bacillus subtilis</i>	0.8	60.67	64.67	66.67	68.33	65.08	28.03 (31.95)	24.12 (29.40)	23.05 (28.67)	22.58 (28.36)	24.44 (29.60)
<i>Pseudomonas fluorescens</i>	0.8	76.13	78.33	78.63	79.60	78.18	9.69 (18.12)	8.07 (16.49)	9.23 (17.67)	9.82 (18.25)	9.20 (17.63)
<i>Verticillium lecanii</i>	0.8	64.67	67.33	69.53	72.27	68.45	23.29 (28.84)	20.98 (27.25)	19.74 (26.37)	18.13 (25.19)	20.54 (26.19)
<i>Trichoderma viride</i>	0.8	72.27	74.60	76.53	78.53	75.48	14.27 (22.18)	12.45 (20.65)	11.66 (19.95)	11.03 (19.39)	12.35 (20.54)
<i>Ampelomyces quisqualis</i>	0.8	58.67	61.67	64.47	68.53	63.33	30.41 (33.45)	27.63 (31.70)	25.59 (30.37)	22.36 (28.21)	26.50 (30.93)
Yeast	0.8	74.67	76.27	76.67	78.57	76.54	11.42 (19.71)	10.50 (18.89)	11.50 (19.81)	10.99 (19.35)	11.10 (19.44)
<i>Trichoderma harzianum</i>	0.8	69.67	70.93	72.67	74.13	71.85	17.36 (24.60)	16.76 (24.15)	16.12 (23.65)	16.01 (23.58)	16.56 (23.99)
Control	-	84.30	85.21	86.63	88.27	86.10	-	-	-	-	-
Mean		70.13	72.38	73.98	76.03		16.81 (22.36)	15.07 (21.07)	14.61 (20.81)	13.87 (20.29)	
CD <sub>0.05</sub>		Antagonist(A)=0.39 Days (D)= 0.58 A x D= 0.89					Antagonist(A)=0.49 Days (D)= 0.69 A x D= 1.39				

Figures in parentheses are arc sine transformed values

### Post symptom anti-sporulant activity of bio-products

It is evident from the data (Table 3) that the lowest conidial production (62.67) was recorded in neem oil after its spray on second day. While the maximum no. of conidia were recorded in bio-formulation-1 (78.33) and vermiwash (76.33). Overall mean reveals the same trend that highest conidia (80.03) were registered in bio-formulation-1 and the lowest (65.80) in neem oil. The rate of conidial production increased from 73.70 to 78.47 after the day of first spray upto 8<sup>th</sup> day of observation. The neem oil obtained significant reduction (26.50%) in number of conidia two days after first spray followed by the bio-formulation-3 (21.43%), milk (17.51%) and anhydrous milk fat (15.16%) while the least effective bio-product was bio-formulation-1 (8.14%) followed by vermiwash (10.52%), chicken manure (11.90%), FYM (13.17%) and bio-formulation-2 (13.44%). All bio-products continued to reduce the conidial number, in comparison to control (87.22%).

The neem oil, bio-formulation-3, milk and anhydrous milk fat were reported to be statistically superior when overall mean

was compared which ranged within 13.65 to 24.58 per cent in relation to reducing conidial number on rose leaves. While the bio-formulation-1 (8.24%) and vermiwash (9.69%) were less effective in lowering the conidial count.

Thus from the present study it was concluded that all the bio-products show some extent of anti-sporulant activities against rose powdery mildew. However, the highest anti-sporulant activity was observed upto the extent of 16.31 to 24.58 per cent by reducing conidial formation in rose leaves after 8<sup>th</sup> day of disease recording in the neem oil, bio-formulation-3 and milk. The anti-sporulant activity of neem oil might be due to the presence of triterpenoid which has antifungal properties. The anti-sporulant activity of bio-formulation-3 and milk might be due to the presence the microorganism especially the lactic and other bacteria which inhibit the growth of conidial increase. Scanning through the literature indicated that there is no information available on effects of these bio-products on anti-sporulant activities against powdery mildew.

**Table 3:** Post symptom anti-sporulant activity of bio-products on formation of conidia of *Podosphaera pannosa* on rose leaves (*in vivo*)

Bio-product (s)	Conc. (%)	Total number of Conidia ( <i>in vitro</i> )				Mean	Per cent reduction of conidia				Mean
		Number of Days (After 1 <sup>st</sup> spray)					Number of Days (After 1 <sup>st</sup> spray)				
		2	4	6	8		2	4	6	8	
Vermiwash	20	76.33	78.27	78.80	81.67	78.77	10.52(18.92)	10.47(18.86)	9.27 (17.71)	8.51 (16.94)	9.69 (18.11)
FYM	20	74.53	74.67	76.53	78.33	76.02	13.17(21.26)	13.10(21.21)	12.59(20.77)	12.25(20.47)	12.84(20.99)
Milk	20	70.33	72.33	73.67	75.67	73.00	17.51(24.72)	16.35(23.84)	16.15(23.68)	15.23(22.96)	16.31(23.80)
Anhydrous Milk Fat	20	72.33	74.30	76.00	78.67	75.33	15.16(22.90)	13.87(21.85)	13.70(21.72)	11.87(20.15)	13.65(21.65)

Bio-formulation-3	20	67.00	70.47	72.67	73.67	70.95	21.43(27.55)	18.31(25.32)	17.49(24.71)	17.47(24.70)	18.68(25.57)
Neem Oil	5	62.67	64.27	66.33	69.93	65.80	26.50(30.97)	25.50(30.32)	24.68(29.77)	21.66(27.72)	24.58(29.69)
Bio-formulation-1	20	78.33	78.80	80.60	82.40	80.03	8.14 (16.57)	8.65(17.10)	8.48 (16.92)	7.69 (16.09)	8.24 (16.67)
Bio-formulation-2	20	74.20	75.80	76.47	77.87	76.08	13.44(21.50)	12.98(21.11)	12.13 (20.37)	12.77 (20.92)	12.76 (20.91)
Chicken Manure	20	75.33	76.00	78.53	80.53	77.60	11.90(20.17)	11.65(19.95)	10.82(19.20)	9.78 (18.21)	11.04(19.38)
Control	-	85.27	86.27	88.07	89.27	87.22	-	-	-	-	-
Mean	-	73.63	75.12	76.77	78.80		13.64(20.34)	12.92(19.80)	12.83 (19.76)	11.72 (18.82)	
CD <sub>0.05</sub>		Bio-product (BP)=0.34 Days (D)= 0.48 BP x D= 0.96					Bio-product (BP)=0.38 Days (D)= 0.59 BP x D= 1.19				

\*Figures in parentheses are arc sine transformed values

\*Bioformulation-1 (Turmeric + chilli + onion) \*Bioformulation-2 (Garlic paste + turmeric) \*Bioformulation-3 (Reetha + cow urine + neem oil)

### Post symptom anti-sporulant activity of botanicals

The anti-sporulant activities of eight botanicals were tested and the data presented in Table 4 against *Podosphaera pannosa* indicated that maximum reduction in conidia after 2 days of spray was recorded in *Allium sativus* with 25.59 per cent followed by the *Sapindus mukorossi* (21.00%) and *Melia azedarach* (20.53%), however latter two were statistically at par with each other. The least conidial reduction was found in *Glycine max* (6.73%) followed by the *Robinia pseudoacacia* (10.04%). The results were in consonance with number of conidia formed in all above treatments, minimum being formed in *Allium sativus* and maximum in *Glycine max*.

In consequent days (2<sup>nd</sup> to 8<sup>th</sup> day) the best results with regard to conidia reduction was also obtained with *Allium sativus* (21.01%) followed by the *Sapindus mukorossi* (18.95%) and *Melia azedarach* (18.01%) both statistically at par in their effectiveness. While least conidial reduction (6.42%) was recorded in *Glycine max*.

The three botanicals viz. *Allium sativus*, *Sapindus mukorossi* and *Melia azedarach* gave highest reduction in conidial number. The anti-sporulant activity of *Allium sativus* might be due to the presence of sulfur compounds like aliin, allicin, ajoene, allylpropl, diallyl, trisulfide, sallylcysteine, vinylthiines, S-allylmercaptocystein, and others compounds. Moreover, the efficacy of ajoene, a compound derived from garlic (*Allium sativus*) in inhibiting the conidial germination of *Erysiphe pisi* has also been reported by Singh *et al.* (1995)<sup>[13]</sup>. While the anti-sporulant activity of *Sapindus mukorossi* might have been due to the presence high content of saponins which should have checked the conidial formation rapidly and *Melia azedarach* might have reduced the conidial level due the presence of ethanolic extract in fruits of *M. azedarach* possessing both fungistatic and fungicidal activities due to presence of various organic molecules reported as vanillin, hydroxyl -3- methoxycinnamaldehyde and pinosresinol (Carpinella *et al.*, 1999; Mishra *et al.*, 2013)<sup>[14, 15]</sup>.

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**Table 4:** Post symptom anti-sporulant activity of botanicals on formation of conidia of *Podosphaera pannosa* on rose leaves (*in vivo*)

Botanical (s)	Conc. (%)	Total number of Conidia ( <i>in vitro</i> )				Mean	Per cent reduction of conidia				Mean
		Number of Days (After 1 <sup>st</sup> spray)					Number of Days (After 1 <sup>st</sup> spray)				
		2	4	6	8		2	4	6	8	
Drake ( <i>Melia azedarach</i> L.)	20	68.67	71.67	73.27	76.00	72.40	20.53 (26.93)	18.01 (25.10)	17.55 (24.76)	15.94 (23.52)	18.01 (25.08)
Black Locust ( <i>Robinia pseudoacacia</i> L.)	20	78.47	79.53	80.67	81.33	80.00	10.04 (18.46)	9.19 (17.64)	9.01 (17.46)	9.22 (17.62)	9.37 (17.80)
Bougainvillea ( <i>Bougainvillea glabra</i> C.)	20	76.43	78.07	78.67	79.63	78.20	11.92 (20.19)	11.55 (19.85)	11.48 (19.79)	10.69 (19.08)	11.41 (19.73)
Pine ( <i>Pinus roxburghii</i> S.)	20	70.00	72.60	74.27	76.33	73.30	18.99 (25.83)	16.95 (24.29)	16.43 (23.90)	15.57 (23.23)	16.99 (24.31)
Garlic ( <i>Allium sativus</i> L.)	10	64.30	68.27	72.33	74.20	69.78	25.59 (30.38)	21.91 (27.90)	18.60 (25.53)	17.93 (25.04)	21.01 (27.21)
Euphorbia ( <i>Euphorbia resinifera</i> A.)	20	72.20	74.27	74.67	77.97	74.78	16.45 (23.92)	15.98 (23.55)	15.04 (22.81)	13.77 (21.76)	15.31 (23.01)
Reetha ( <i>Sapindus mukorossi</i> )	20	68.27	71.00	72.47	74.53	71.57	21.00	18.78	18.45	17.56	18.95 (25.78)

L.)							(27.26)	(25.66)	(25.43)	(24.77)	
Soybean ( <i>Glycine Max</i> L.)	20	80.60	81.80	83.27	83.77	82.36	6.73 (15.02)	6.42 (14.67)	6.30 (14.52)	6.00 (13.86)	6.42 (14.67)
Control	-	86.41	87.41	88.87	90.41	88.28	-	-	-	-	-
Mean		73.93	76.07	77.61	79.35		14.45 (20.76)	12.98 (19.66)	12.67 (19.46)	12.23 (19.19)	
CD <sub>0.05</sub>		Botanical (B)=0.29 Days (D)= 0.44 B x D= 0.88					Botanical (B)=0.31 Days (D)= 0.46 B x D= 0.92				

\*Figures in parentheses are arc sine transformed values

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