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Horticulture

Screening of Rose Varieties Against Black Spot Disease and its Management in East Siang District of Arunachal Pradesh

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Abstract

Rose varieties were evaluated in field against *Diplocarpon rosae*, which causes of black spot disease of rose. Black spot resistance was visually evaluated for thirty seven rose varieties against *D. rosae*. Out of thirty seven varieties evaluated, none of the varieties were found immune, very highly resistant, highly resistant, resistant and moderately resistant. However, three varieties namely Paradise, Shabnam and Pixie showed moderately susceptible in reaction. Whereas, eleven varieties *viz*., Angelica Renae, Atago, Folklore, Granada, Hot Cocoa, Mardigrass, Midas Touch, Mrinalini, Revival, Tipus Flame and Victor Hugo showed susceptible reaction. Twelve varieties *viz.* Baccardi, Claudia Ribond, Charles Mallerin, Crimson Lace, Dr. Pal, Impatient, Madam Dulbourde, Marcopolo, Melody, Rainbow End, Sonia and Sugandha were highly susceptible reaction at 75 per cent disease severity. Whereas, eleven varieties namely Angelique, Christian Dior, Gemini, Gladiator, Golden Jubilee, Priyadarsini, Sand. Centenary, R. R. M. Roy, Sweet Promise, Unforgotten and Vale of Cloyd were highly susceptible reaction at 95 per cent disease severity. Further, five fungicides [three systemic fungicides namely Carbendazim, Hexaconazole (Contaf) and Ridomil MZ 72 WP and two contact fungicides *viz*. Blitox-50 and Mancozeb] were evaluated *in vitro* for the management of *D. rosae.* Hexaconazole (Contaf) was found to inhibit the mycelial growth of *D. rosae* significantly at a concentration of 200 and 250 ppm followed by Ridomil MZ 72 WP at same concentration.

Highlights:

- Paradise, Shabnam and Pixie varieties showed moderately susceptible reaction towards black spot disease of rose.
- Hexaconazole and Ridomil MZ72WP can be recommended for managing the disease.

Keywords: Rose, variety, screening, black spot, *in vitro*, fungicides

Rose (*Rosa* x *hybrida* L.) is one of the most economically important ornamental crop used as landscape and cut flower plant in the world. Among cut flowers, rose ranks first in terms of trade and popularity. Rose plays a vital role in manufacturing of various products of medicinal and nutritional importance (Panwar *et al*., 2012). Black spot (*Diplocarpon rosae* Wolf.) disease is economically the most important and devastating disease in ornamental roses, especially in hot and humid climates (Horst and Cloyd, 2007). Disease outbreaks at the beginning of the growing season are initiated by rain-splashed pathogen spores overwintered on fallen leaves. Infected leaves develop

characteristic dark spots, chlorosis and drop prematurely. When left untreated, the disease can lead to reduced plant vigor, fewer blossoms, compromised aesthetics and eventual failure of the plant (Henn, 2010). Previous reports (Lily and Barnett, 1951, Palmer *et al*., 1966a and Svejda and Bolton, 1980) firmly documented differential pathogenicity of *Marssonina rosae* (Lib.) Lind (Imperfect stage of *Diplocarpon rosae* Wolf) isolates to various species and cultivars of rose. Other workers (Jenkins, 1955., Palmer & Semeniuk, 1961, and Palmer *et al*., 1966b) reported different plant response to a single isolate. Arunachal Pradesh is considered as potential area for commercial rose production. However, black spot disease is the major production constraint faced by the growers mainly due to erratic climatic conditions during the growing period. Therefore, the management of *D. rosae* with fungicides intervention becomes an important aspect by testing its effectiveness of active ingredients (*a.i.*) in the pathogen. In order to manage this disease, effect of five different fungicides were evaluated by using different concentrations (ppm). Hence, the present studies were undertaken with objectives *viz*. screening of rose varieties against *D. rosae,* isolation, purification, identification of *D. rosae* from disease specimen and *in vitro* fungicidal management.

Materials and Methods

The experiments were carried out in order to study the screening of rose varieties against *D. rosae* and its management practices. Commercial rose varieties were grown in the Instructional farm, Department of Floriculture, College of Horticulture & Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh. Pasighat, headquarter of East Siang district of Arunachal Pradesh is situated at 28^0 4' North latitudes, 95^0 22' East longitudes and 152 m MSL altitude. Screening of thirty seven rose varieties against black spot disease caused by *Diplocarpon rosae* was performed in natural epiphytotic conditions under open field and data were recorded from April, 2011 to March, 2012 at weekly intervals (Table 1). It has warm and humid climate with distinct rainy season spread over 5 months from May to September. Average weekly temperature, relative humidity and evaporation ranges from 10-36 ^{0}C, 57.0 - 93.3 % and 2.2 - 6.7 mm with annual rainfall ranging between 4000 - 6000 mm respectively. Screening was undertaken in open field conditions using Mc Kinney's Index to estimate the disease severity (Table 2). The experiment was laid out in completely randomized block design (CRBD) with three replications. Diseased leaves of rose plant with symptoms of black spots were collected from the Instructional farm and isolation of *D. rosae* was performed as suggested by Ricker and Ricker (1936).

Isolation of test pathogen (s) was done by simple isolation technique (Ricker and Ricker, 1936). The disease specimens were cut into small pieces (0.5 cm diameter) and surface disinfected by immersing in 70 per cent ethyl alcohol solution for half to one minutes and then rinsed thrice in distilled water under Laminar Flow (aseptic condition). Potato dextrose agar (PDA) was prepared, autoclaved and poured in Petri plates (20 ml PDA for each Petri plates). The sterilized leaf pieces were placed on PDA in Petri plates and incubated at 25°C. After 5 days, the mycelium of fungus *D. rosae* appeared on the diseased leaf pieces were identified and transferred to PDA slants. Later purification was made by single spore culture technique (Douglas and Pavek, 1971).

Pathogenicity Test

The pathogenicity test of the isolated fungus was carried out under field conditions by using spray inoculation method. Highly Susceptible variety Priyadarshini was selected for the pathogenicity test.

Preparation of spore suspension

Potato dextrose broth media was prepared and autoclaved at 121°C at 15 psi (pound per square inch) for 20 minutes. A loop full actively growing inoculum of *D. rosae* was obtained with a sterilized needle and inoculated the Potato dextrose broth media and incubated at 25 ± 2°C for seven days. After this, the broth media was shaken on a rotary shaker for about an hour for preparation of spore suspension. The concentration of spore suspension was adjusted to $1x10^5$ by the use of haemocytometer and inoculum (100 ml / plant) was sprayed over test varieties.

Evaluation of different fungicides against *Diplocarpon rosae*

Five fungicides [three systemic fungicides namely Carbendazim, Hexaconazole (Contaf), Ridomil MZ 72 WP and two contact fungicides *viz*. Blitox-50 and Mancozeb] were tested *in-vitro* to evaluate their effect on colony growth of *D. rosae*, by using poisoned food techniques (Nene and Thapliyal, 1979). One gram of test fungicide on the basis of its active ingredient percentage was dissolved in 100 ml of water for preparation of stock solution. This stock solution was used for preparation of required

Table 1: Weekly meteorological data: April 2011 to March 2012

SMW	Date	Max. Temp (° C)	Min. temp (° C)	RH (%)@ 1400hrs	Rainfall (mm)	No. of rainy days	Evaporation (mm)
13	26 March - 01 April, 2011	25.7	17.1	81.2	45.3	2	3.5
14	02- 08 April, 2011	25.8	17.3	73.6	2.9	0	3.9
15	09 - 15 April, 2011	31.4	19.4	62.8	29.7	3	6.7
16	16 - 22 April, 2011	28.6	19.5	79.8	72.9	4	4.4
17	23- 29 April, 2011	29.3	19.6	66.5	9.3	1	5.5
18	30 April - 06 May, 2011	29.0	19.9	76.2	98.2	4	4.1
19	7-13 May, 2011	32.9	22.4	68.0	8.8	1	5.2
20	14-20 May, 2011	33.5	22.0	72.0	85.6	3	4.0
21	21-27 May, 2011	29.8	22.7	74.0	113.5	4	4.1
22	28 May-03 June, 2011	32.0	23.2	71.0	79.1	5	3.7
23	04-10 June, 2011	30.6	23.1	NA	120.0	3	4.0
24	11-17 June, 2011	33.7	25.0	NA	69.5	2	4.3
25	18-24 June, 2011	32.8	25.2	NA	34.0	3	4.2
26	25June-01 July, 2011	32.8	24.3	NA	222.5	5	5.4
27	02-08 July, 2011	28.5	23.8	93.3	301.9	7	3.3
28	09-15 July, 2011	27.3	23.2	90.7	109.5	5	3.6
29	16-22 July, 2011	31.8	24.6	80.4	219.9	4	3.1
30	23-29 July, 2011	31.4	24.4	81.8	144.9	4	2.3
31	30 July-05 Aug, 2011	32.8	24.8	77.2	48.0	1	4.3
32	06-12 Aug, 2011	32.5	24.5	72.2	3.6	1	4.1
33	13-19 Aug, 2011	28.6	22.4	84.5	309.2	6	3.6
34	20-26 Aug, 2011	32.5	23.7	73.8	39.0	2	4.2
35	27 Aug- 02 Sep, 2011	36.0	24.9	63.8	11.5	1	6.2
36	03-09 Sep, 2011	34.1	24.1	78.3	6.0	1	5.4
37	10-16 Sep, 2011	33.8	24.2	72.4	38.1	2	4.2
38	17-23 Sep, 2011	32.2	24.3	72.8	186.8	2	3.4
39	24-30 Sep, 2011	30.4	23.2	80.8	122.3	3	4.0
40	01-07 Oct, 2011	33.6	23.4	69.2	0.0	0	4.9
41	08-14 Oct, 2011	34.2	22.2	61.4	0.0	0	NA
42	15-21 Oct, 2011	31.9	21.8	65.8	2.0	0	NA
43	22-28 Oct, 2011	31.1	19.1	69.0	13.0	1	NA
44	29 Oct-04 Nov, 2011	29.7	51.9	60.2	0.0	0	5.9
45	05-11 Nov, 2011	29.6	16.5	62.5	0.0	0	4.7
46	12-18 Nov, 2011	24.5	15.9	79.0	2.1	0	3.2
47	19-25 Nov, 2011	28.8	16.1	57.0	0.0	0	5.2
48	26 Nov-02 Dec, 2011	28.6	16.1	58.5	0.0	0	4.5
49	03-09 Dec, 2011	29.4	16.7	64.2	0.2	0	5.1
50	10-16 Dec, 2011	22.4	14.4	71.0	57.5	2	2.9
51	17-23 Dec, 2011	24.9	12.8	63.3	0.0	0	4.4
52	24-31 Dec, 2011	26.8	13.8	NA	0.0	0	4.3
1	1-7 Jan, 2012	20.6	12.5	83.5	20.5	3	2.4
2	8-14 Jan, 2012	22.3	12.5	73.6	2.5	1	3.0
3	15-21 Jan, 2012	20.6	10.8	75.0	13.0	2	2.6
4	22- 28 Jan, 2012	21.7	10.0	73.3	18.0	2	2.2
5	29 Jan- 4 Feb, 2012	24.0	12.2	62.3	0.0	0	3.3
6	5- 11 Feb, 2012	24.8	14.5	70.0	8.2	1	2.9
7	12- 18 Feb, 2012	23.9	16.2	NA	0.0	0	4.6
8	19 - 25 Feb, 2012	26.9	16.0	69.2	22.0	2	4.0
9	26 Feb- 4 Mar, 2012	23.0	14.1	75.7	17.1	4	3.0
10	5- 11 Mar, 2012	23.5	14.6	74.4	43.3	2	3.6
11	12 - 18 Mar,2012	27.9	15.9	65.4	19.5	2	5.4
12	19 - 25 Mar, 2012	29.6	18.7	62.7	0.7	0	5.1
13	26 March -01 April, 2012	26.1	17.5	75.7	54.6	4	3.9

concentration of 50, 100, 150, 200 and 250 ppm. Prior to pouring PDA medium, 0.5 ml of each concentration of fungicides was added in Petri plates. Then about 15 ml of autoclaved medium was poured in sterilized Petri plates. After solidification, the Petri plates were inoculated by placing 5 mm discs of 7 days old PDA culture of *Diplocarpon rosae*. The inoculated Petri plates were incubated at 25^0C in biological oxygen demand (BOD) incubator and data on radial colony growth was recorded after 4-5 days of incubation. The per cent inhibition of fungal growth was estimated by using the formula given by Vincent (1927).

Per cent inhibition over control $I = \frac{C-T}{C} \times 100$

Where,

C = growth of the fungus in control

T = growth of the fungus in treatment

Results and Discussion

Percent Disease Severity Assessment

Varietal reaction on thirty seven rose varieties against black spot disease caused by *D. rosae* were observed and recorded in Table 3. Only three varieties namely Paradise, Shabnam and Pixie showed moderately susceptible reaction. Varieties which showed susceptible reaction were Angelica Renae, Atago, Folklore, Granada, Hot Cocoa, Mardigrass, Midas Touch, Mrinalini, Revival, Tipus Flame and Victor Hugo. Twelve varieties namely Baccardi, Claudia Ribond, Charles Mallerin, Crimson Lace, Dr. Pal, Impatient, Madam Dulbourde, Marcopolo, Melody, Rainbow End, Sonia and Sugandha were responded highly susceptible reaction at 75 per cent disease severity. Whereas, eleven varieties namely Angelique, Christian Dior, Gemini, Gladiator, Golden Jubilee, Priyadarshini, Sand. Centenary, R. R. M. Roy, Sweet Promise, Unforgotten and Vale of Cloyd were highly susceptible in reaction at 89.11 per cent disease severity. In present investigation, out of thirty seven varieties none of the varieties were found to be immune, very highly resistant, highly resistant, resistant and moderately resistant against black spot disease of rose in the open field condition of Pasighat. This might be due to hot and humid weather which favored the disease. These results are in close conformity with Horst and Cloyd (2007), Holcomb (2003) and Colbaugh *et al.* (2001) who evaluated 107 rose cultivars against reaction to naturally happening rose black spot disease. Among them, 40 per cent moderately susceptible and 50 per cent highly susceptible, while 10 per cent were considered to be highly tolerant or resistant to the black spot disease. Disease severity and various level of resistance or susceptibility of thirty seven rose varieties against black spot disease occurred in open field condition at Pasighat during the study period were also recorder (Table 4). It revealed that the black spot disease severity (%) varied widely among the varieties. It is as low as 15% in Paradise while as high as 89.11% in Vale of Cloyd.

Table 2: Disease estimation scale for *Diplocarpon rosae* (Mc Kineys index, 1889)

Grade	% of Disease	Nature of infection level of resistance / susceptibility
0	0.00	No disease (Immune)
1	0.10	1-2 spots per plant (very highly resistant)
2	1.00	5-10 spots per plant (highly resistant)
3	5.00	11-25 spots per plant (resistant)
4	10.00	26-50 spots per plant (moderately resistant)
5	25.00	Every leaf infected (moderately susceptible)
6	50.00	Every plant affected 5% leaf area destroyed (susceptible)
7	75.00	5% leaf area destroyed, field brown nor green (highly susceptible)
8	95.00	5% leaf area destroyed but stem green (highly susceptible)
9	100.00	All leaves dead stem dead (very highly susceptible)

Pathogen isolated from diseased specimens and identified as *D. rosae* on the basis of morphological and physiological characters. The fungus *D. rosae* was observed causing the black spot on leaves with mostly dark to black colour and irregular patches. The pathogen was characterized with cylindrical and hyaline conidia. The mycelium was whitish at early stage but later on the colour changed from whitish to dark grey. Pathogenicity test to confirm the pathogen was carried out in the field by using the spray inoculation method. For the pathogenicity test, highly susceptible variety Priyadarshini was selected to confirm the pathogen. After 12 days of inoculation, the symptoms appeared which were similar to the black spots caused by *D. rosae*. After re-isolation, confirmation was made according to Koch's Postulate (1882) which proved that *D. rosae* is the causal organism of black spot disease of rose.

Table 3: Varietal reaction of thirty seven rose varieties against black spot

Disease Severity	Reaction	No. of Varieties	Name of varieties
0.0	Immune	-	-
0.0-0.1	Very highly resistant	-	-
0.1- 1.0	Highly resistant	-	-
1.0-5.0	Resistant	-	-
5.0- 10.0	Moderately resistant	-	-
10.0- 25.0	Moderately susceptible	3	Paradise, Shabnam, Pixie
25.0- 50.0	Susceptible	11	Angelica Renae, Atago, Folklore, Granada, Hot Cocoa, Mardigrass, Midas Touch, Mrinalini, Revival, Tipus Flame, Victor Hugo
50.0- 75.0	Highly susceptible	12	Baccardi, Claudia Ribond, Charles Mallerin, Crimson Lace, Dr. Pal, Impatient, Madam Dulbourde, Marcopolo, Melody, Rainbow End, Sonia, Sugandha
75.0- 95.0	Highly susceptible	11	Angelique , Christian Dior, Gemini, Gladiator, Golden Jubilee, Priyadarsini, R.R.M.Roy, Sand. Centenary, Sweet Promise, Unforgotten, Vale of Cloyd
95.0- 100.0	Very highly susceptible	-	-

Table 4: Disease severity and level of resistance of thirty seven rose varieties against black spot in open field conditions

Name of variety	Disease severity (%)	Disease rating	Level of resistance
Angelica Renae	28.10	6	susceptible
Angelique	82.00	8	highly susceptible
Atago	31.27	6	susceptible
Baccardii	63.67	7	highly susceptible
Charles Mallerin	60.01	7	highly susceptible
Christian Dior	79.00	8	highly susceptible
Claudia Ribond	62.33	7	highly susceptible
Crimson Lace	65.78	7	highly susceptible
Dr. Pal	66.33	7	highly susceptible
Folklore	38.10	6	susceptible
Gemini	87.33	8	highly susceptible
Gladiator	79.00	8	highly susceptible
Golden Jubilee	87.33	8	highly susceptible
Granada	31.30	6	susceptible
Hot Cocoa	46.10	6	susceptible
Impatient	61.67	7	highly susceptible
Madam Delbourde	59.00	7	highly susceptible
Marcopolo	65.67	7	highly susceptible
Mardigrass	43.10	6	susceptible
Melody	70.00	7	highly susceptible
Midas Touch	42.67	6	susceptible
Mrinalini	31.00	6	susceptible
Paradise	15.00	5	Moderately susceptible
Pixie	20.33	5	Moderately susceptible
Priyadarsini	87.33	8	highly susceptible
R.R.M.Roy	89.00	8	highly susceptible
Rainbow End	64.78	7	highly susceptible
Revival	42.33	6	susceptible
Sand. Centenary	89.00	8	highly susceptible
Shabnam	21.67	5	Moderately susceptible
Sonia	65.33	7	highly susceptible
Sugandha	60.00	7	highly susceptible
Sweet Promise	85.00	8	highly susceptible
Tipus Flame	43.67	6	susceptible
Unforgotten	87.00	8	highly susceptible
Vale of Cloyd	89.11	8	highly susceptible
Victor Hugo	34.67	6	susceptible

Table 5: Efficacy of fungicides on inhibition of *in vitro* mycelial growth (cm)

Fungicides	Concentrations (ppm)				
	50	100	150	200	250
Carbendazim (Bavistin)	3.75	2.99	2.50	2.45	2.10
Hexaconazole (Contaf)	2.17	1.50	1.35	1.25	1.10
Ridomil MZ 72 WP	2.75	2.60	3.45	2.20	1.65
Blitox-50	3.80	2.99	2.75	2.55	2.55
Mancozeb	4.50	2.80	2.60	2.45	2.35
Control	8.75	8.75	8.75	8.75	8.75
SE	0.11	0.07	0.06	0.05	0.04
CD at (1%)	0.35	0.22	0.19	0.16	0.13
CD at (5%)	0.24	0.16	0.13	0.12	0.09
CV	3.17	2.52	1.89	1.82	1.27

In vitro evaluation of fungicides

To investigate the use of fungicides for the management of *D. rosae*, Potato Dextrose Agar (PDA) was amended with the test fungicides. The sensitivity of fungal mycelium varied significantly to five fungicides evaluated (Table 5). None of the fungicides was found to give 100 per cent control at all the concentrations. There was a considerable decrease in mycelial growth with increase in fungicidal concentration of each fungicide. Hexaconazole (Contaf) was found to be the most effective systemic fungicide in reducing the mycelial growth of *D. rosae* at a concentration of 200 ppm and 250 ppm followed by Ridomil MZ 72 WP at the same concentration. There was no inhibition in mycelial growth of *D. rosae* in control. Systemic fungicide Hexaconazole (Contaf) was found to be the best against *D. rosae* in inhibiting the growth of fungus at all concentration. Hang *et al*., 1991 reported good control of disease by application of Tebuconazole and Myclobutanil @ 100 ppm. The present results are also in conformity with earlier work of Kira *et al.* (1996) where Chlorothalonil (Daconil) was found to be effective in managing the black spot disease. Gold *et al.*, (1996) reported that the application of fungicide like Strobilurins restrained the mycelial development and growth of fungus on the leaf margins.

Conclusion

In the present investigation, three varieties of rose namely Paradise, Shabnam and Pixie showed moderately susceptible reaction towards black spot disease in agro-climatic conditions of Pasighat. These varieties can be incorporated in breeding programmes for developing resistant varieties for North Eastern Hill region of India. Furthermore, systemic fungicide namely, Hexaconazole (Contaf) followed by Ridomil MZ 72 WP @ 200 ppm and 250 ppm respectively, can be recommended for managing the black spot disease of rose.

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