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Varietal screening of rose (*Rosa* x *hybrida*) cultivars and *in vitro* efficacy of fungicides against black spot disease (*Diplocarpon rosae* Wolf.*)* in Arunachal Pradesh condition

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ABSTRACT

Rose varieties were evaluated in field against *Diplocarpon rosae* cause 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 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 responded 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) & 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 ppm and 250 ppm followed by Ridomil MZ 72 WP at same concentration.

1) INTRODUCTION

Rose (*Rosa* x *hybrida* L.) is one of the most economically important ornamental species 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 [1]. Pasighat, headquarter of East Siang district of Arunachal Pradesh is situated at an altitude of 152 m above MSL and is lying between 27^0 43' and 29^0 20' North latitudes and 94^0 42' and 95^0 35' East latitudes. It has warm and humid climate with distinct rainy season spread over 5 months from April to September. Average annual temperature, relative humidity and rainfall ranges from 16-38 ^{0}C, 60-100 per cent and 4000 mm, respectively. Black spot (*Diplocarpon rosae* Wolf.) disease is economically the most important and devastating disease in ornamental roses, especially in hot and humid climates [2]. 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 [3]. Previous reports Lily and Barnett [4], Palmer *et al*. [5,6], and Svejda and Bolton [7] 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 [8], Palmer & Semeniuk [9] and Palmer *et al*. [5, 6] 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 ingredient in the pathogen. In order to

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manage this disease, five different fungicides were evaluated by using different concentrations (ppm). Hence, the present studies were undertaken with objectives, screening of rose varieties against *D. rosae,* isolation, purification, identification of *D. rosae* from disease specimen and *in vitro* fungicidal management.

2) MATERIALS AND METHODS

The experiments were carried out in order to study the screening of rose varieties against *D. rosae* and its management practices.

2.1 Varietal Screening

Screening of thirty seven rose varieties against black spot disease caused by *Diplocarpon rosae* were undertaken in open field conditions using Mc Kinney's Index to estimate the disease severity **(Table 1)**. The screening was performed in natural epiphytotic conditions under open field and data were recorded from April, 2011 to March, 2012 at weekly intervals.

2.2 Selection of Plant Material

Commercial rose varieties were grown in the Instructional farm, Department of Floriculture, College of Horticulture & Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh. The experiment was laid out in randomized completely block design (RCBD) with three replications.

2.3 Collection of Diseased Samples

Diseased leaves of rose plant with symptoms of black spots were collected from the Instructional farm, Department of Floriculture, College of Horticulture & Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh. The leaves specimens were brought to the Plant Pathology Laboratory, Department of Plant Protection, College of Horticulture & Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh and isolation of *D. rosae* were performed as suggested by Ricker and Ricker [10].

2.4 Isolation and purification of test pathogen (s)

Isolation of test pathogen (s) was done by simple isolation technique [10]. 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. Potato dextrose agar (PDA) was prepared, autoclaved and poured in Petri plates (20 ml PDA per 9 cm 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 [11].

2.5 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.

2.6 Preparation of spore suspension

Potato dextrose broth media was prepared and autoclaved at 121°C at 15 psi pressure 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.

2.7 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 [12]. 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 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 (9 cm). 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) 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 [13].

Table 1. Disease estimation scale for *Diplocarpon rosae* (Mc Kineys index).

Grade	% of Disease	Nature of infection level of resistance / susceptibility
0	0.00	No disease (Immune)
1	0.10	A few spots 1-2 on the 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)

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

Where

C = growth of the fungus in control

T = growth of the fungus in treatment

3) RESULTS AND DISCUSSION

3.1 Percent Disease Severity Assessment

The data were recorded for disease severity of thirty seven rose varieties against black spot disease caused by *D. rosae* **(Table 2)** and various level of resistance/susceptibility of rose varieties showed accordingly **(Table 3)**. However, only three varieties namely Paradise, Shabnam and Pixie showed moderately susceptible reaction.

The varieties which showed susceptible reaction were Angelica Renae, Atago, Folklore, Granada, Hot Cocoa, Mardigrass, Midas Touch, Mrinalini, Revival, Tipus Flame and Victor Hugo, respectively. Twelve varieties *viz.* 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 reaction at 95 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. These results are in close conformity with Holcomb [14] and Colbaugh *et al.,* [15] who evaluated 107 rose cultivars against reaction to naturally happening rose black spot disease. Among 90 per cent varieties, 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.

3.2 Isolation and Identification of Pathogen

Fungus 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.

Table 2. Varietal reaction of thirty seven rose varieties against black spot.

Disease Severity	Reaction	No. of Varieties	Name of varieties
0.00	Immune	-	-
0.10	Very highly resistant	-	-
1.00	Highly resistant	-	-
5.00	Resistant	-	-
10.00	Moderately resistant	-	-
25.00	Moderately susceptible	3	Paradise, Shabnam, Pixie
50.00	Susceptible	11	Angelica Renae, Atago, Folklore, Granada, Hot Cocoa, Mardigrass, Midas Touch, Mrinalini, Revival, Tipus Flame, Victor Hugo
75.00	Highly susceptible	12	Baccardi, Claudia Ribond, Charles Mallerin, Crimson Lace, Dr. Pal, Impatient, Madam Dulbourde, Marcopolo, Melody, Rainbow End, Sonia, Sugandha
95.00	Highly susceptible	11	Angelique, Sand. Centenary, Christian Dior, Gemini, Gladiator, Golden Jubilee, Priyadarsini, R.R.M.Roy, Sweet Promise, Unforgotten, Vale of Cloyd
100.00	Very highly susceptible	-	-

3.3 Morphological Characters

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.

3.4 Pathogenicity Test

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 [16] which proved that *D. rosae* is the causal organism of black spot disease of rose.

3.5 In vitro evaluation of various 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 4)**.

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 and 250 ppm followed by Ridomil MZ 72 WP at a same concentration. There was no inhibition in mycelial growth of *D. rosae* in

Table 3: Disease severity and level of resistance of thirty seven rose varieties against black spot in open field conditions.

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 4. Efficacy of fungicides on inhibition of mycelial growth *in vitro* condition.

Sl. No.	Fungicides	Concentrations (ppm) / mycelial growth (cm)				
		50	100	150	200	250
1	Carbendazim (Bavistin)	3.75	2.99	2.50	2.45	2.10
2	Hexaconazole (Contaf)	2.17	1.50	1.35	1.25	1.10
3	Ridomil MZ 72 WP	2.75	2.60	3.45	2.20	1.65
4	Blitox-50	3.80	2.99	2.75	2.55	2.55
5	Mancozeb	4.50	2.80	2.60	2.45	2.35
6	Control	8.75	8.75	8.75	8.75	8.75

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*., [17] 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.,* [18] where Chlorothalonil (Daconil) was found to be effective in managing the black spot disease. Gold *et al.,* [19] reported that the application of fungicide like Strobilurins restrained the mycelial development and growth of fungus on the leaf margins.

4) CONCLUSION

In the present investigation three varieties of rose namely Paradise, Shabnam and Pixie showed moderately susceptible reaction towards black spot disease. 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) and Ridomil MZ 72 WP @ 200 and 250 ppm can be recommended for inhibition of *Diplocarpon rosae* causing black spot disease of rose.

REFERENCES

1. Panwar, S., Singh, K. P., Prasad, K. V., Satyavathi, C. T. and Namita. 2012. Character association and path coefficient analysis in rose (Rosa x hybrida). Indian Journal of Horticulture, 69(2), 231-238.
2. Horst, R. K. and Cloyd, R. A. 2007. Compendium of Rose Diseases. APS Press, St. Paul, MN. 96 p.
3. Henn, A. 2010. The Plant Doctor - Black spot and powdery mildew of rose. Am. Rose Ann., 155-156.
4. Lily, V. G. and Barnett, H. L. 1951. Physiology of the Fungi. McGraw Hill Book Company, New York, pp. 464.
5. Palmer, J. G., Semeniuk, P. and Stewart, R. N. 1966. Roses and Black spot. I. Pathogenicity to excised leaflets of Diplocarpon rosae from seven geographic locations. Phytopathology, 56, 1277-1282.
6. Palmer, J. G., Semeniuk, P. and Stewart, R. N. 1966. Roses and Black spot. II. Seasonal variation in host susceptibility and decline of virulence in culture of conidia from Diplocarpon rosae. Phytopathology, 56, 1283-1286.
7. Svejda, F. J. and Bolton, A. J. 1980. Resistance of rose hybrids to three races of Diplocarpon rosae. Can. J. Plant Pathol., 2, 23-25.
8. Jenkins, W. R. 1955. Variability of pathogenicity and physiology of Diplocarpon rosae Wolf, the rose black spot fungus. Amer. Rose Ann., 40, 92-97.
9. Palmer, J. G. and Semeniuk, P. 1961. Comparable susceptibilities of fifty species and hybrid roses inoculated with black spot fungus from plants field grown in Maryland in 1959. Amer. Rose Ann., 46, 125-133.
10. Ricker, A. J. and Ricker, R. S. 1936. Introduction to research and plant diseases. John Swift Co., New York, 10, 177-183.
11. Douglas, D. R. and Pavek, J. J. 1971. An efficient method of inducing sporulation of fungus in pure culture. Phytopathology, 61, 239-247.
12. Nene, Y. L. and Thapliyal, P. N. 1979. Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi, pp. 163.
13. Vincent, J. M.1927. Distortion of fungal hyphae in the presence of certain inhibitors. Nature, 59, 850.
14. Holcomb, G. E. 2003. Reaction of rose cultivars to black spot disease. Acta Hort., 424, 209-213.
15. Colbaugh, P. F., Crow, W. T., Mackay, W. A. and George, S. W. 2001. Black spot; *Diplocarpon rosae* Texas A&M Research and Extension Center at Dallas, Dallas, Texas, 75252.
16. Koch, R. 1882. Die aetiologie der tuberculose. Berliner KlinischeWochenschrift, 19, 221-230.
17. Hang, A. K., Gilliam, C. H., Fare, D. C. and Bowen, K. 1991. Application rates and spray schedules of ergosterol-biosynthesis inhibitor fungicides for control of black spot of rose. Plant Dis., 75, 1143-1146.
18. Kira, L. B., Bridget, K. B. and Elizabeth, A. G. 1996.Management key to controlling black spot disease in roses. J. Plant Pathol., 43(2), 141-147.
19. Gold, R. E., Ammerman, E., Koehle, H., Leinhos, G. M. and Sauter, H. 1996.The synthetic strobilurin BAS 490F: profile on the modern fungicide. In Modern Fungicides and Antifungal Compounds, 79-92.