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AD Matte
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

MJ Patil
Assistant Professor, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

SV Gawarkar
Student, College of Agriculture, Nagpur, Maharashtra, India

SC Khawshi
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

Corresponding Author:
AD Matte
Student, Department of Plant Pathology, College of Agriculture, Nagpur, Maharashtra, India

In vitro management of black spot of rose caused by *Diplocarpon rosae* wolf

AD Matte, MJ Patil, SV Gawarkar and SC Khawshi

Abstract
Background: Among the world's flowers, the rose has captured the heart and soul of all. It is the symbol of love. Rose suffers from many foliar diseases and among them black spot caused by *Diplocarpon rosae* Wolf is one of the most important disease of rose, this disease is economically devastating disease in ornamental rose especially in hot and humid climates. Considering severity and its destructive nature, the study was under taken to find out the most effective and economic fungicides against the black spot of rose with objective to test the efficacy of different fungicides against black spot of rose.
Methods: *D. rosae* causing black spot of rose was isolated from infected rose plants collected from Department of horticulture, College of Agriculture, Nagpur on potato dextrose agar medium (PDA) and later its pure culture accomplished. The culture of the pathogen was examined microscopically for morphological and culture characteristics. In the present study of investigation efficacy of various fungicides *viz*., azoxystrobin 0.1%, mancozeb + carbendazim 0.2%, topsin 0.05%, myclobutanil 0.1%, tebuconazole 0.1%, chlorothalonil 0.2% were evaluated *In vitro* and their economics was computed.
Result: The fungicidal along with untreated control were evaluated against *Diplocarpon rosae* by poison food technique and per cent inhibition in mycelia growth. Results indicated that, all the rest fungicides significantly inhibited the growth of *Diplocarpon rosae* over untreated control. Highest inhibition was observed by chlorothalonil 75 WP @ 0.2% (90.74 per cent) and topsin M 70 WP @ 0.05% (81.72 per cent) found effective to control black spot disease of rose over all the test fungicides followed by myclobutanil 10 WP @ 0.1% (79.78 per cent).

Keywords: *In vitro*, black spot, disease management, % growth inhibition, yield

Introduction
The rose is widely studied plant and much has been written about it. Without delving much in to the history sufficient it to say that the wild roses are believed to have their origins in Europe, America, the orient and china, and in the Middle East (Beales, 1997; Joyaux, 2003) [1, 6]. Rose cultivation dates way back to around 3000 B.C. in the ancient Chinese gardens (Beales, 1997) [1]. Rose (*Rosa* spp.) belongs to family Rosaceae and its genus *Rosa*, contains 200 species and more than 18,000 cultivars, most of which are woody perennial shrubs with a basic chromosome no. of seven and ploidy levels ranging from 2x to 8x (Wissmen, 2006; Debener and Linde, 2009) [13, 2]. Rose cultivars are commercially important. Every year 8 billion flowering stems, 80 million potted plants and 220 million garden rose plants are sold world-wide (Roberts *et al*., 2003) [9]. It is also known as "Queen of flowers". Roses are symbols of love and beauty. The fragrance and multiple uses of roses as cut flower or landscape plants have made roses an appreciated crop since ancient times. From as Economical standpoint, roses are the most important plant in ornamental horticulture (Hummer and Jenick, 2009) [5].

Black spot of rose: There are various diseases occurred in the rose, among these black spot is one of the important disease of rose. It is also considered as one on the most serious disease that are caused by the fungus *Diplocarpon rosae* and asexual stage *Marsonnina rosae* (Sinha, 2017) [10]. The fungus belongs to the Kingdom-Fungi, Division-Ascomycota, Class-Leotiomycetes, Order-Helotiales, Family-Dermatacae, Genus-*Diplocarpon* and species-*rosae*. The incidence of black spot disease (*Diplocarpon rosae*) was highest in various rose cultivars *viz*., Arjun, Golden Times, Super star and in Gladiator (Thammaiah *et al*., 1997) [11]. The black spot of rose is a foliar disease characterized by black spot with an irregular margin on the upper side of the leaf. The first visible symptoms are black spots of approximately 1 mm on the upper side of the leaf, which can increase to an average of 15 mm. Spots close to each other merge to form bigger spots.

On some susceptible varieties, infected leaves may turn yellow, but the immediate area around the black spots remains green forming 'green islands'.

Methods and Materials

The present investigation deals with recording intensity of black spot disease of rose and evaluation of efficacy of different fungicides for controlling the black spot *in vitro*. The materials and methods used in present investigation are mentioned below. Infected leaves from rose plants showing black spot symptoms were collected and isolation was done by following standard isolation method under asceptic conditions. The diseased 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 minute and then rinsed thrice in distilled water under laminar air flow. Potato dextrose agar (PDA) was prepared, autoclaved and poured in petri plates (20 ml PDA per 9 cm petri plates). To avoid the bacterial contamination Streptocyclin (30 mg/ml) was added in the medium. The sterilized leaf pieces were placed on PDA in petri plates and incubate at 25±1 °C. After every 24 hrs, the observation on mycelial growth was noticed and 5 days later, the mycelium of fungus *Diplocarpon rosae* which appeared on PDA petri plates were identified and transferred to PDA slants.

Pathogenicity test: Koch's postulates were established to prove the pathogenicity of caused fungus. Pathogenicity of fungus was conducted under field condition by inoculating the healthy plants of rose with spore suspension of the fungus. Potato dextrose broth medium was prepared and autoclaved at 121 °C at lb 15 psi pressure for 20 minutes. A loop full of actively growing inoculum of *D. rosae* was obtained with a sterilized needle and inoculate into potato dextrose broth medium and incubate at 25±1 °C in BOD incubator. After this the broth medium was shaken on a rotary shaker for about an hour for preparation of spore suspension. The concentration of spore suspension was adjusted to $8x10^5$ by the use of hemocytometer and inoculum (100ml/ plant) was sprayed over potted rose plants using atomizer. After inoculation, the plants were immediately covered with perforated polythene bags individually for 48 hrs. Observation were recorded on disease appearance based on symptomatology and days of disease appearance after inoculation.

Evaluation of fungicides against *Diplocarpon rosae* under *In vitro* condition

To find out the most effective fungicides against the test pathogen (*D. rosae*) fungicides were evaluated under *In vitro* conditions by Poison food technique (Nene and Thapliyal, 1979) [7]. The fungicides evaluated during the course of present investigation are as under.

Poison food technique: Six different fungicides (Table:1) were evaluated under *In vitro* against *D. rosae* by adopting poison food technique. Potato dextrose agar (PDA) medium was prepared, equally distributed measuring 100 ml in 250 ml conical flask and sterilized in autoclave. Requisite quantity of each of the fungicides (as per concentration) was added in sterilized melted (45 °C) PDA separately so as to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20 ml of melted poisoned PDA was poured in each sterilized petri plate and allow to solidify. These petri plates were inoculated by test fungus separately. Five mm disc of one week old fungus culture was cut with sterilized cork borer, lifted and transferred aseptically in the center of petri plate containing the medium poisoned with test fungicide. The control plates were kept the culture disc grown in same condition on PDA without fungicides. Treated plates were incubated at room temperature (28±2 °C) for a period of seven days. Colony diameter was recorded in mm and per cent mycelial growth inhibition was calculated as per Vincent's formula based on the average colony diameter. The data was subjected to statistical analysis wherever necessary.

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

Where

I = Per cent inhibition
C = Fungal growth in control plate (mm)
T = Fungal growth in treatments (mm)

(Vincent, 1947) [12]

Result and Discussion

Black spot caused by *Diplocarpon rosae* is a serious disease and a major constrains with regarding the production. Rose is commercial cut flower crop fetching yield of 2-4 tonnes per hector. The flower yield is considerably decreasing in recent days due to the invading of disease. Therefore, the present studies were under taken to check the efficacy of chemical fungicides *In vitro*. The results of the present investigation were discussed here as under.

Isolation and identification of *D. rosae:* *D. rosae* causing black spot of rose was isolated from infected rose plants collected from Department of horticulture, College of Agriculture, Nagpur on potato dextrose agar medium (PDA) and later its pure culture accomplished. The culture of the pathogen was examined microscopically for morphological and culture characteristics. The fungus was identified as *D. rosae* on the basis of their morphological character *viz*. hyphae, shape of spore produced and cultural characteristics *viz*. colour and type of colony.

Pathogenicity: Pathogenicity test to confirm the pathogen i.e. behaviour of *D. rosae* was carried out under pot culture by using the spray inoculation method with conidia suspension (8×105 conidial/ml). It was observed that the symptoms appeared after 15 days of inoculation, which were similar to the black spot caused by *D. rosae*. The pathogen was reisolated from the infected leaf tissues on PDA medium. The microscopic characters of reisolated fungus were same as recorded in the parent culture of the test fungus and colony characteristics of both the culture were same. This proves the koch's postulates and pathogenicity of the isolated fungus which proved that *D. rosae* is causal organism of black spot disease of rose.

Effect of different fungicides on mycelial growth of *D. rosae* causing black spot of rose under *In-vitro* condition

The fungicidal *viz*., azoxystrobin (amistra), mancozeb + carbendazim (bendaco), topsin M (thiophanate methyl), myclobutanil (systhane), tebuconazole (folicure) and chlorothalonil (daconil) along with untreated control were evaluated against *Diplocarpon rosae* by poison food technique and per cent inhibition in mycelia growth is

presented in Table 2. Result from the table No.2 and Fig.2 indicated that, all the rest fungicides significantly inhibited the growth of *Diplocarpon rosae* over untreated control. The chlorothalonil 75 WP @ 0.2% and topsin M 70 WP @ 0.05% shown minimum growth colony diameter 8.33 mm and 16.45 mm respectively and were found significantly superior over all the test fungicides. It was followed by myclobutanil 10 WP @ 0.1% (18.19 mm). The mancozeb + carbendazim 50 WP @ 0.2% shown growth colony diameter 31.08 mm which was at par with azoxystrobin 23 SC @ 0.1% (32.33 mm). The tebuconazole 25 EC @ 0.1% shown maximum growth colony diameter 46.67 mm and found to be inferior over all the test fungicides.

All the test fungicides significantly reduced growth of *Diplocarpon rosae* than control. Inhibition of growth varied from 48.14 to 90.74 per cent in different test fungicides. Highest inhibition was observed by chlorothalonil 75 WP @ 0.2% (90.74 per cent) and topsin M 70 WP @ 0.05% (81.72 per cent) found effective to control black spot disease of rose over all the test fungicides followed by myclobutanil 10 WP @ 0.1% (79.78 per cent). Rest of the fungicides ranged between 48.14 to 65.46 per cent inhibition. The tebuconazole 25 EC @ 0.1% was found least effective to control black spot disease of rose (Plate 6 and Fig. 2). Similar findings were reported by many workers *viz*., Rao and Rajagopalan (1982) who reported that chlorothalonil (0.1%) and thiophanate methyl (0.1%) cause moderate inhibition of *A. alternata*. Hagan *et al*. (1991) [3] studied application rates and spray schedules of ergosterol-biosynthesis inhibitor fungicides and reported that chlorothalonil inhibited maximum growth of mycelium than other fungicides. They also found that myclobutanil and tebuconazole (100 ppm) both of this fungicide gave good controlling disease. Rahman *et al*. (2012) [8] studied *in vitro* effectiveness of fungicides against *D. rosae* and found that topsin M inhibited the mycelial growth of the fungus significantly at concentration of 150 ppm and 250 ppm followed by chlorothalonil. Findings of the present investigation were almost similar with the studies reported by various reporters.

Table 1: Chemical fungicides used *In vitro* concentration.

Sr. No.	Treatments	Conc. (%)
T1	Azoxystrobin 23 SC	0.1
T2	Mancozeb + Carbendazim 50 WP	0.2
T3	Topsin M 70 WP	0.05
T4	Myclobutanil 10 WP	0.1
T5	Tebuconazole 25 EC	0.1
T6	Chlorothalonil 75 WP	0.2
T7	Control	-

Table 2: *In vitro* evaluation of fungicides against *D. rosae*.

Sr. No.	Treatments	Conc. (%)	Mean Colony diameter (mm)*	% Inhibition over control
T_1	Azoxystrobin 23 SC	0.1	32.33	64.07
T_2	Mancozeb + Carbendazim 50 WP	0.2	31.08	65.46
T_3	Topsin M 70 WP	0.05	16.45	81.72
T_4	Myclobutanil 10 WP	0.1	18.19	79.78
T_5	Tebuconazole 25 EC	0.1	46.67	48.14
T_6	Chlorothalonil 75 WP	0.2	8.33	90.74
T_7	Control	-	90.00	-
	'F' test		Sig.	
	SE ± (m)		1.10	
	CD P = 0.01		3.39	

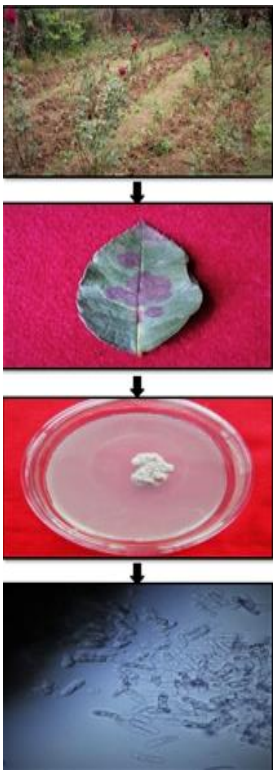

Plate 1: Microscopic view of the fungus

Plate 2: Pathogenicity test

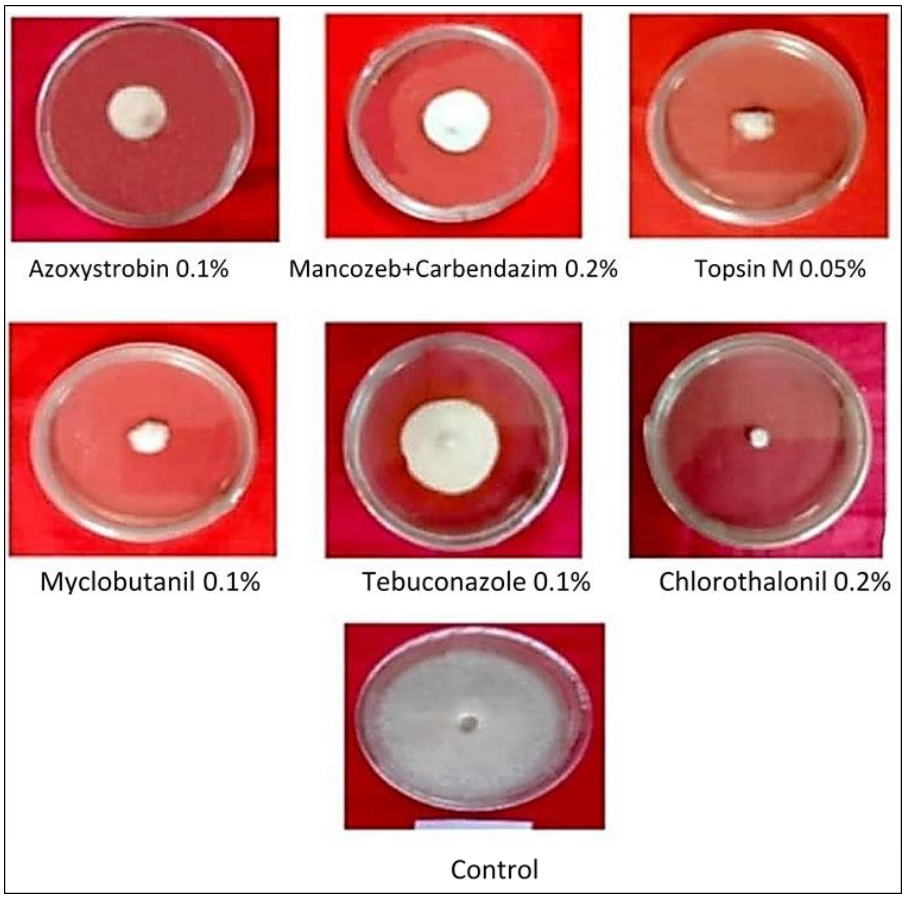

Plate 3: Effect of fungicides against *D. rosae* by Poison food method.

Conclusion

Among the flower crops rose is the most important flower crop grown throughout the world. In terms of area cultivated and production, it is perennial high in put crop with more economical in cultivation and gives maximum income to the farmer. Rose suffers from many foliar diseases. Among them black spot caused by *Diplocarpon rosae* is major destructive disease. It appears in epidemic form almost every year in varying intensity leading to greater yield losses. Most of the rose varieties were reported susceptible to this disease. Therefore, it was necessary to obtain the information on disease initiation, symptoms and management by using different fungicides and estimation of their economics. The experiment results revealed that chlorothalonil found maximum 90.74% inhibition of spore germination which was followed by topsin M, myclobutanil, mancozeb + carbendazim, azoxystrobin and tebuconazole recording 81.72%, 79.78%, 65.46%, 64.07% and 48.14% respectively.

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