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In vitro evaluation of fungicides against diplocarpon rosae causing black leaf spot in rose

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Abstract
The rose leaves showing typical symptoms of black spot were collected from Horticulture Field, College of Agriculture, Nagpur and used for isolation of pathogen on potato dextrose agar media. The culture of the pathogen was examined for morphological and cultural characteristics. On the basis of hyphae, shape of spore produced, colour and type of colony, the fungus was identified as *Diplocarpon rosae* and it proved the Koch's postulates. The fungicides *viz*., Azoxystrobin (Amistra), Mancozeb+Carbendazim (Bendaco), Thiophenate methyl (Topsin-M), Mycobutanil (Systhane), Tebuconazole (Folicure) and Chlorothalonil (Daconil) were evaluated against *Diplocarpon rosae in-vitro* by poisoned food technique. Results revealed that, all the fungicides significantly inhibited the growth of *Diplocarpon rosae* over untreated control. Chlorothalonil 75 WP @ 2% and Topsin M 70 WP @ 0.05% showed minimum colony diameter of 8.33 mm and 16.45 mm respectively and found significantly superior over all the test fungicides. Inhibition of growth varies from 48.14 to 90.74 per cent. Highest inhibition was observed by Chlorothalonil 75 WP @ 2% (90.74%) and Topsin M 70 WP @ 0.05% (81.72%).

Keywords: *Diplocarpon, rosae*, fungicides, rose, black spot

Introduction
Rose (*Rosa* spp.) is known as "Queen of Flowers" and symbol of love and beauty. Cut roses play an important role in interior decoration. Total area under floriculture in India is 1044 thousand ha with production of 36.51 lakh MT and 3.49 MT ha^{-1} of productivity. The cut flower production in year 2017-18 was 5.70 lakh MT while that of loose flower was 29.1 lakh MT (Anonymous, 2018) [1]. The flower yield is considerably decreasing in recent days due to the invading of pest and diseases. The crop is vulnerable to several diseases *viz*., black spot, powdery mildew, die back, rust, blight, crown gall, downy mildew. Out of these diseases, the black spot caused by *Diplocarpon rosae* is pre-dominant and cause great losses and economically divasting disease especially in hot and humid climate (Horst and Cloyd, 2007) [6]. Looking to the threat of rose black spot and subsequent damage to the rose cultivation, the *in-vitro* studies were undertaken to evolve suitable and effective disease management strategy in the interest of farming community.

Material and Methods
In-vitro evaluation of fungicides against *Diplocarpon rosae* causing black leaf spot in rose was carried out at Plant Pathology section, College of Agriculture (Dr. PDKV), Nagpur during 2019.

The rose leaves showing typical symptoms of black spot were collected from Horticulture Field, College of Agriculture, Nagpur and used for isolation of pathogen on potato dextrose agar media. The culture of the pathogen was examined for morphological and cultural characteristics. On the basis of hyphae, shape of spore produced, colour and type of colony, the fungus was identified as *Diplocarpon rosae* and it proved the Koch's postulates. To find out the most effective fungicide, six fungicides *viz*., Azoxystrobin (Amistra), Mancozeb+Carbendazim (Bendaco), Thiophenate methyl (Topsin-M), Mycobutanil (Systhane), Tebuconazole (Folicure) and Chlorothalonil (Daconil) were evaluated against *Diplocarpon rosae in-vitro* by poisoned food technique (Nene and Thapliyal, 1979). The colony diameter was recorded in mm and per cent mycelia growth inhibition was calculated as per Vincent (1947) [15]. Data obtained was statistically analyzed by using standard statistical methods (Gomez and Gomez, 1984) [7].

Result and Discussion

Symptoms of black spot fungus (Plate 1)

The rose leaves showing typical symptoms of the black spot were collected from the horticulture field, College of Agriculture, Nagpur. The infected specimen was used for isolation of organism.

Initially the black spot disease symptoms appeared as tiny dots like purplish spots with irregular radiating feathery margins ranging from 3 to 5mm in size with an average of 4mm in size. Later the spots turned from purplish to black over the time and coalesced to form bigger flecks. Sporulation was observed at the center of the lesions. These spots were also found in the peduncles, petioles and sepals. In severe infections, leaf spots coalesced to form bigger spots. Later lesions turned chlorotic and attained maximum diameter of 12 to 13mm. The leaf surface gradually turned yellow except for the black spot and the area around the spot that remained green and later resulted into severe defoliation. Infected leaves easily detached from the plant with the slight finger touch of slight wind movements. The symptoms recorded in the present investigation were almost matched with those reported by various earlier workers. Horst (1983) [5] gives the compendium of rose disease and reported the symptoms include lesions on leaves and stems as used as frequent leaf yellowing and defoliation. The disease caused dark brown to black circular lesions of up to 15mm in diameter. Mangadi and Peres (2009) [9] studied black spot of rose and reported that spots can also be seen on peduncles, fruits and sepals. Henn (2010) [4] studied the black spot and reported that infected leaves developed characteristics dark spots. Chlorosis and drop prematurely. When left untreated the disease can lead to reduce plant vigor, fever blossom compromised aesthetics and eventual failure of the plant. Sinha (2017) [13] studied rose diseases their identification, detection and cure and reported that the severity of the disease is at its peak post the warm spring. Blechert and Debener (2005) [2] studied morphological characterization of the interaction between *D. rosae* and various rose species and noticed the symptoms of black spot disease, which are similar to the present investigation.

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.

Morphological characters of pathogen

The morphological characters of the fungus were studied on potato dextrose agar (PDA) medium. Details of various morphological characters observed were as follows. The fungus grew profusely with submerged growth on potato dextrose agar medium at optimum temperature of 25+1°C, fungus grew profusely at this temperature range and attained colony diameter of40mm within 7-8 days. The mycelium under microscope was hyaline, septate and whitish at early stage but later of the colour changed from whitish to dark grey (Plate 2) and pinkish cinnamon colour. The pathogen was characterized with cylindrical, two celled and hyaline conidia. The detail of the morphological character were compared with keys documented by Wolf (1912) [6] and identified as *D. rosae*. Same characteristics for identifying fungus were also described by Wiggers *et al.* (1997) [14] and Kumar *et al*. (2013) [8].

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 (8×10^5 conidial ml^{-1}). 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'postulates and pathogenicity of the isolated fungus which proved that *D. rosae* is causal organism of black spot disease of rose.

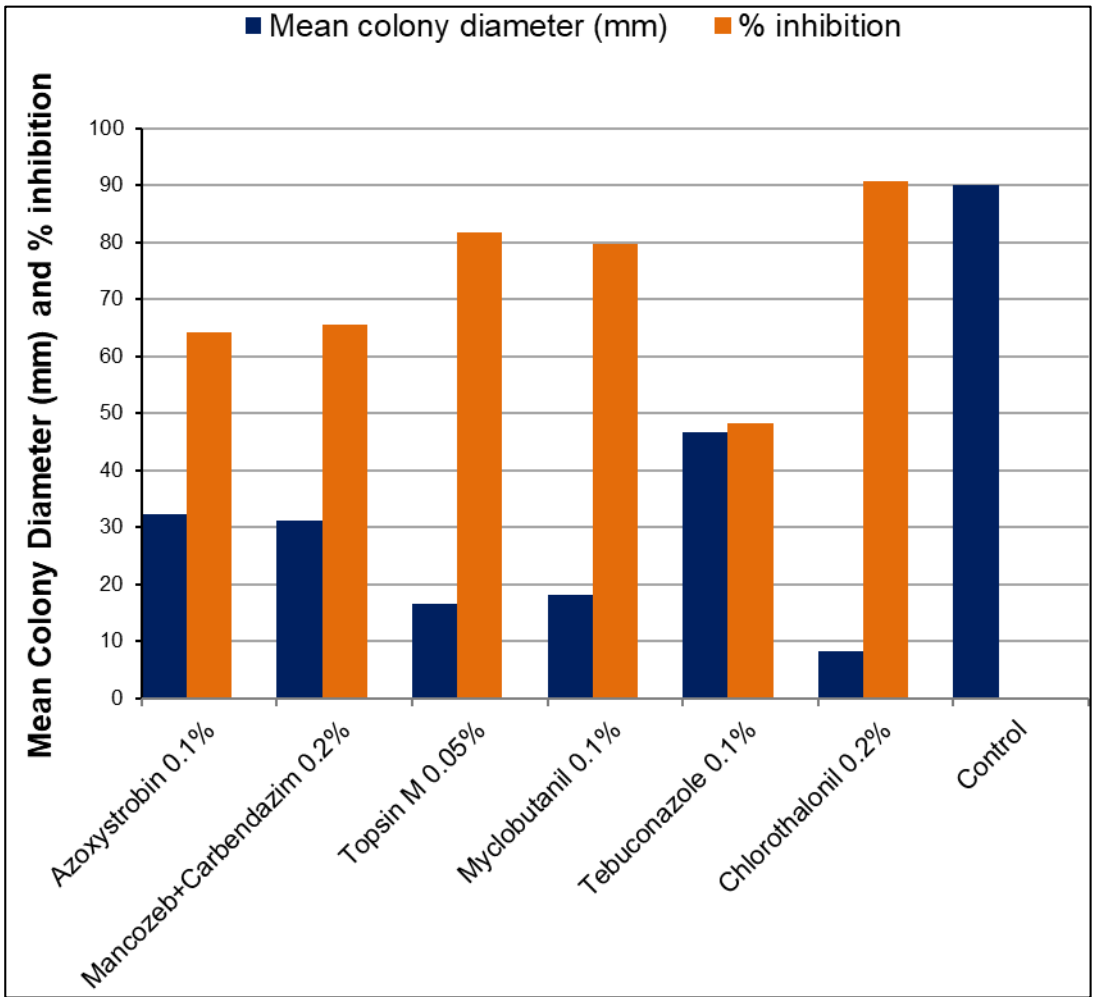
In-vitro evaluation of fungicides

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 1. Result from the table No.1 and Graph 1 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 70WP @ 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 tested 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. 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) [11] 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: *In vitro* evaluation of fungicides against *D. rosae*.

Treatments	Concentration (%)	Mean Colony diameter (mm)*	% inhibition over control
Azoxystrobin 23SC	0.1	32.33	64.07
Mancozeb + Carbendazim 50WP	0.2	31.08	65.46
Topsin M 70WP	0.05	16.45	81.72
Myclobutanil 10WP	0.1	18.19	79.78
Tebuconazole 25EC	0.1	46.67	48.14
Chlorothalonil 75WP	0.2	8.33	90.74
Control	-	90.00	-
'F' test		Sig.	
SE ± (m)		1.10	
CD P=0.01		3.39	

*=Mean of three replications



Graph 1: *In vitro* evaluation of fungicides against *D. rosae*.

Plate 1: Symptoms on leaf and stem of rose plant

Plate 2: Microscopic view of the conidia of *D. rosae*

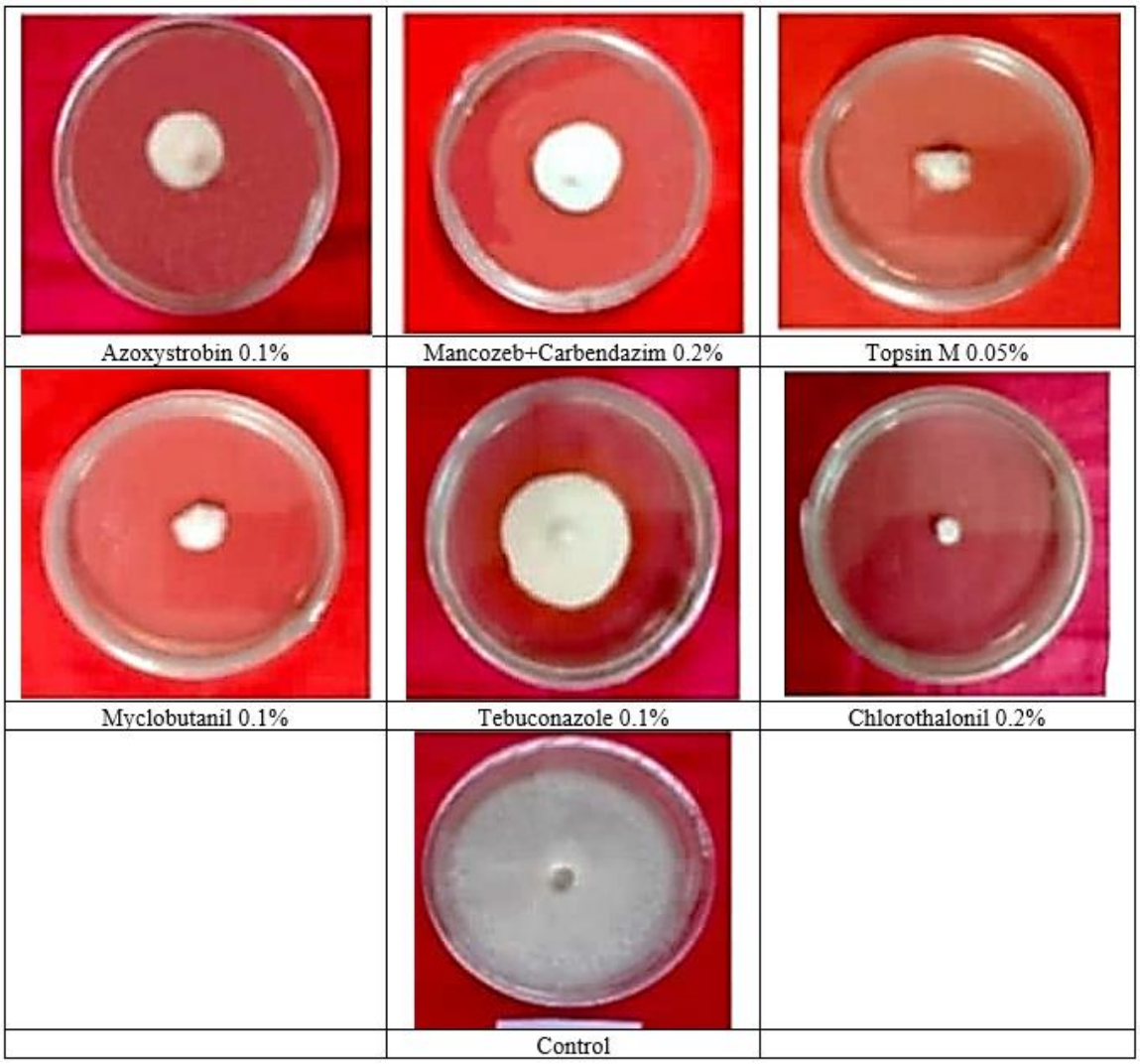


Plate 3: Effect of fungicides against *D. rosae* by Poisoned food method.

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