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Antifungal activity of fungicides and micronutrients against *Diplocarpon rosae* causing black spot of rose

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Abstract
Roses are one of the most important and commercial ornamental flowers, grown worldwide. Black spot disease severely infects the rose crop grown worldwide. In this study, six fungicides at 0.05, 0.10, 0.15, 0.20 and 0.25 per cent concentration were evaluated by poisoned food method and it was observed that hexaconazole 5 per cent EC (Contaf) and tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo) at 0.05 per cent concentration showed highest inhibition percentage. Mancozeb 75 per cent WP showed least inhibition percentage. Three micronutrients and their combinations were evaluated and among them CuSO4+FeSO4+ZnSO4 showed 55.56 per cent mycelial inhibition at 0.1 per cent concentration and completely inhibited the mycelial growth at 0.2 per cent concentration.

Keywords: Rose, black spot, fungicides, micronutrients

Introduction
Roses are one of the most popular and economically important ornamental flowers, grown worldwide. Form, colour, texture and fragrance of flowers are the various positive attributes for the versatile use of roses in landscaping. The flower quality gets affected due to their susceptibility to diseases. Black spot disease of rose caused by *Diplocarpon rosae* Wolf (*Marssonina rosae*, asexual stage) is the most destructive and widespread disease of rose worldwide (Bhaskaran and Ranganathan, 1974 [2]; Nelson, 2012 [7]; Bowen and Roark, 2001 [3]; Wenefrida and Spencer, 1993 [9]). Black coloured circular spots with feathery margins are produced on the upper surface of leaf. The spots are surrounded with yellow halo. The black lesions gradually increase in size and the whole leaf becomes yellow and defoliates. Due to its aesthetic value, the rose plants are used for landscaping but due to the black lesions, yellowing and defoliation of leaves, the plants become unattractive (Debener *et al.*, 1998) [4]. Except the driest regions, this disease is found worldwide in other rose growing regions. The infection of *D. rosae* leads to defoliation and debilitation of the plants (Gachomo *et al.*, 2010) [5]. The present study aimed at evaluating the antifungal activity of chemical fungicides and micronutrients against *Diplocarpon rosae*.

Materials and Methods
Evaluation of various fungicides against the pathogen *in vitro*
To study the efficacy of fungicides against *D. rosae*, carbendazim 50 per cent WP (Bavistin), hexaconazole 5 per cent EC (Contaf), tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo), azoxystrobin 23 per cent EC (Amistar), mancozeb 75per cent WP (Indofil M-45) and tricyclazole 75 per cent WP (Baan) were tested by using poisoned food technique. PDA medium was freshly prepared and autoclaved for 20 minutes at 15 lb psi pressure and 121°C temperature. Using double distilled water, solutions of fungicides at different concentrations (0.05, 0.10, 0.15, 0.20 and 0.25 per cent) were prepared and mixed with 100 ml of autoclaved PDA medium. Then twenty ml mixture was poured into sterilized Petri plates and allowed to solidify. Using sterile cork borer nine mm diameter fungal disc of *D. rosae* was cut from seven-day old culture and placed at the centre of the Petri plate containing solidified medium. Each treatment was replicated thrice. PDA medium without any chemical was used as control. The inoculated plates were incubated at 25±1°C for seven days. The diameter of the mycelial growth was noted and the per cent inhibition was calculated by the formula given by (Vincent, 1947) [8]

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

Where,
I = Per cent inhibition
C = Fungal growth in control plate (cm)
T = Fungal growth in treatment plate (cm)

Evaluation of micronutrients against the pathogen *in vitro*

Copper sulphate, zinc sulphate and iron sulphate are the micronutrients used for the nourishment of rose plants and increasing the yield and quality of flower. These micronutrients individually and in combinations were tested against *D. rosae* by poisoned food technique at 0.1, 0.2 and 0.3 per cent concentrations. PDA medium was freshly prepared and autoclaved for 20 minutes at 15 lb psi pressure and 121°C temperature. Using double distilled water, solutions of micronutrients at different concentrations were prepared and mixed with 100 ml of autoclaved PDA medium. Then twenty ml mixture was poured into sterilized Petri plates and allowed to solidify. Using sterile cork borer, nine mm diameter fungal disc of *D. rosae* was cut from old culture and placed at the centre of the Petri plate containing solidified medium. Each treatment was replicated thrice. PDA medium without any chemical was used as control. The inoculated plates were incubated at 25±1 °C for seven days. The diameter of the mycelial growth was noted and the per cent inhibition was calculated.

Results

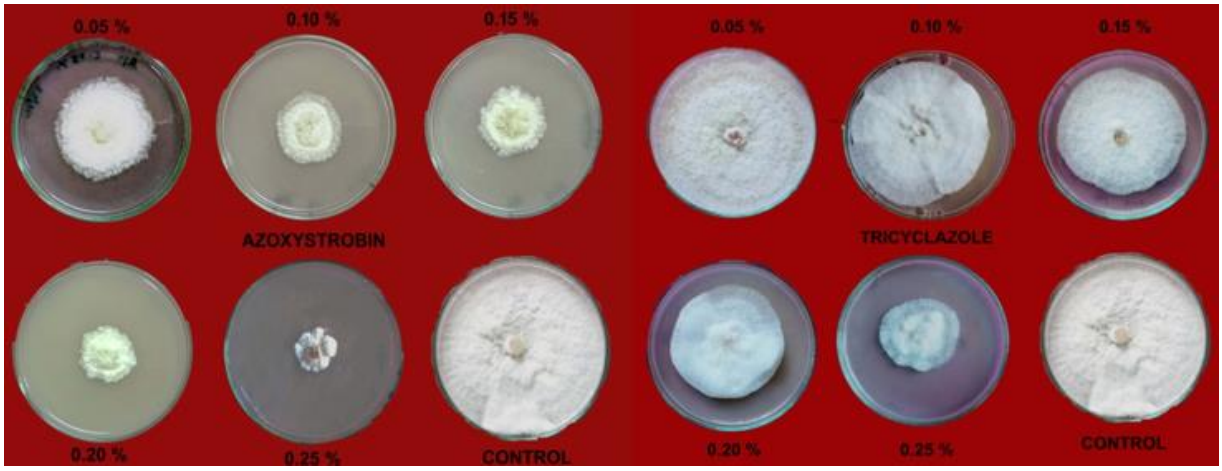
Evaluation of various fungicides against the pathogen *in vitro*

At five different concentrations *viz.*, 0.05, 0.10, 0.15, 0.20 and 0.25 per cent, six different fungicides were examined against *D. rosae* under *in vitro* condition. Two fungicides *viz.*, hexaconazole 5 per cent EC (Contaf) and tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo) completely inhibited the mycelial growth at all the concentrations followed by carbendazim 50 per cent WP (Bavistin), which completely inhibited the mycelial growth at 0.25 per cent concentration. The fungicide tricyclazole 75 per cent WP (Baan) showed least inhibition percentage whereas mancozeb 75 per cent WP (Indofil M-45) did not control the mycelial growth at all. The effect of hexaconazole 5 per cent EC (Contaf) and tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo) at 0.025 and 0.001 per cent concentration were also observed and the result showed that these fungicides effectively inhibited the mycelial growth at lower concentration (Table 1; Fig 1).

Table 1: Effect of fungicides against *Diplocarpon rosae* at different concentrations

Treatment	Fungicide	*Mycelial growth (cm)					*Per cent inhibition over control (%)					Mean
		0.05	0.10	0.15	0.20	0.25	0.05	0.10	0.15	0.20	0.25	
T_1	Carbendazim	4.36	3.29	2.23	1.38	0.00	51.56 (46.36)^b	63.44 (53.51)^b	75.22 (60.38)^b	84.67 (67.63)^b	100.00 (90.00)^a	74.98 (63.15)b
T_2	Azoxystrobin	6.72	5.24	5.00	4.65	2.36	25.33 (30.41)^c	41.78 (40.88)^c	44.44 (41.63)^c	48.33 (44.21)^c	73.78 (59.50)^b	46.73 (43.20)c
T_3	Hexaconazole	0.00	0.00	0.00	0.00	0.00	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)a
T_4	Tricyclazole	9.00	7.78	7.20	6.31	4.23	0.00 (0.00)^d	13.56 (22.37)^d	20.00 (27.17)^d	29.89 (33.59)^d	53.00 (46.89)^c	23.29 (25.60)d
T_5	Mancozeb	9.00	9.00	9.00	9.00	9.00	0.00 (0.00)^d	0.00 (0.00)^e	0.00 (0.00)^e	0.00 (0.00)^e	0.00 (0.00)^d	0.00 (0.00)e
T_6	Trifloxystrobin +Tebucnazole	0.00	0.00	0.00	0.00	0.00	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)^a	100.00 (90.00)a
T_7	Control	9.00	9.00	9.00	9.00	9.00	0.00 (0.00)^d	0.00 (0.00)^e	0.00 (0.00)^e	0.00 (0.00)^e	0.00 (0.00)^d	0.00 (0.00)e
Mean		-	-	-	-	-	39.56 (36.59)e	45.54 (42.09)d	48.52 (44.08)c	51.84 (46.30)b	60.97 (53.69)a	

CD (P=0.05) Treatment = 0.03 Conc. = 0.02 Treatment x Conc. = 0.07
*Mean of three replications. The treatment means are compared using Duncan multiple range test (DMRT).
In a column, mean followed by a common letter (s) are not significantly different (p=0.05). Figures in parentheses are arc sine transformed values.



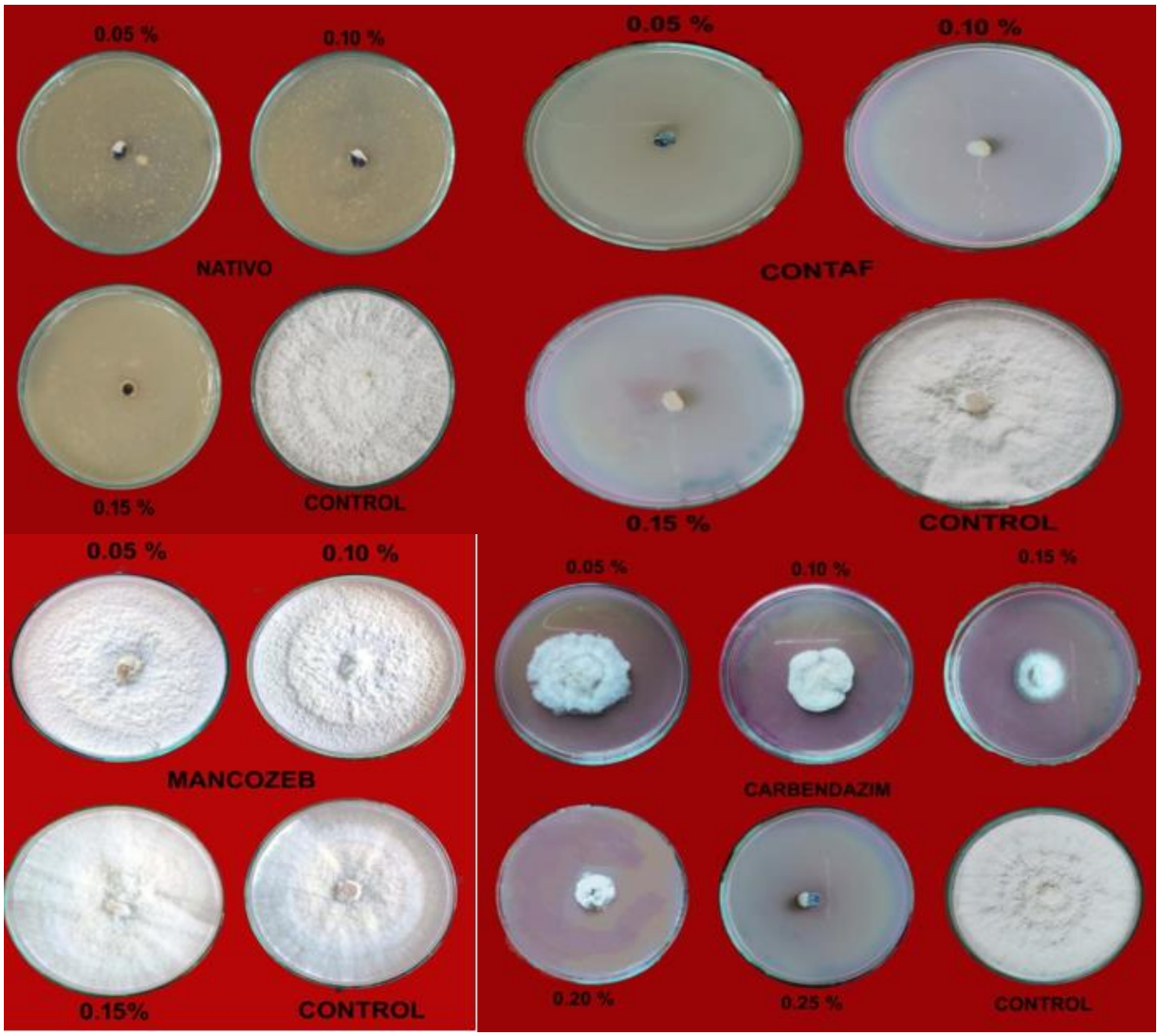


Fig 1: Effect of various fungicides against *D. rosae* under *in vitro* condition

Evaluation of micronutrients against the pathogen *in vitro*

Copper sulphate, zinc sulphate and iron sulphate at three different concentrations *viz.*, 0.10, 0.20 and 0.30 per cent were examined against *D. rosae* under *in vitro* condition. Copper sulphate completely inhibited the mycelial growth at 0.20 per cent followed by zinc sulphate at 0.30 per cent. Copper sulphate effectively inhibited the mycelial growth of the pathogen.

These micronutrients were tested in combinations *viz.*, $CuSO_4$+$FeSO_4$, $CuSO_4$+$ZnSO_4$, $FeSO_4$+$ZnSO_4$ and $CuSO_4$+$FeSO_4$+$ZnSO_4$ each at 0.10, 0.20 and 0.30 per cent concentrations. The result obtained showed that at 0.10 per cent concentration, the micronutrient combination $CuSO_4$+$FeSO_4$+$ZnSO_4$ showed highest per cent inhibition over control (55.56) followed by $CuSO_4$+$ZnSO_4$ (33.67). At 0.20 per cent concentration, $CuSO_4$+$ZnSO_4$ and $CuSO_4$+$FeSO_4$+$ZnSO_4$ showed 100 per cent inhibition of the mycelial growth followed by $FeSO_4$+$ZnSO_4$ which showed 55.56 per cent inhibition. At 0.30 per cent concentration, all the combinations showed 100 per cent inhibition of mycelial growth of pathogen (Table 2; Fig 2a, b).

Table 2: Effect of micronutrients against *Diplocarpon rosae* at different concentrations

Treatment	Chemical	*Mycelial growth (cm)			*Per cent inhibition over control (%)			Mean
		0.10	0.20	0.30	0.10	0.20	0.30	
T_1	$CuSO_4$	2.68	0.00	0.00	70.22 (57.47)^a	100 (90.00)^a	100 (90.00)^a	90.07 (78.98)a
T_2	$FeSO_4$	8.35	4.33	1.30	7.22 (16.21)^e	51.89 (46.29)^d	85.56 (67.68)^b	48.22 (43.11)g
T_3	$ZnSO_4$	6.41	2.52	0.00	28.78 (34.36)^d	72.00 (58.21)^b	100 (90.00)^a	66.93 (60.16)d
T_4	$CuSO_4$+$FeSO_4$	9.00	4.63	0.00	0.00 (0.00)^f	48.56 (44.36)^e	100.00 (90.00)^a	49.52 (44.72)f
T_5	$CuSO_4$+ $ZnSO_4$	5.97	0.00	0.00	33.67 (35.96)^c	100.00 (90.00)^a	100.00 (90.00)^a	77.89 (71.82)c
T_6	$FeSO_4$+ $ZnSO_4$	9.00	4.00	0.00	0.00 (0.00)^f	55.56 (48.25)^c	100.00 (90.00)^a	51.85 (46.06)e
T_7	$CuSO_4$+$FeSO_4$+ $ZnSO_4$	4.00	0.00	0.00	55.56	100.00	100.00	85.19

					(48.20)[b]	(90.00)[a]	(90.00)[a]	(76.06)b
T_8	Control	9.00	9.00	9.00	0.00 (0.00)[f]	0.00 (0.00)[f]	0.00 (0.00)[c]	0.00 (0.00)h
Mean					24.43 (23.58)c	66.00 (58.31)b	85.70 (75.96)a	

CD (P=0.05) Treatment = 0.01 Conc. = 0.01 Treatment x Conc. = 0.02
*Mean of three replications
The treatment means are compared using Duncan multiple range test (DMRT).
Figures in parentheses are arc sine transformed values
In a column, mean followed by a common letter (s) are not significantly different (p=0.05).

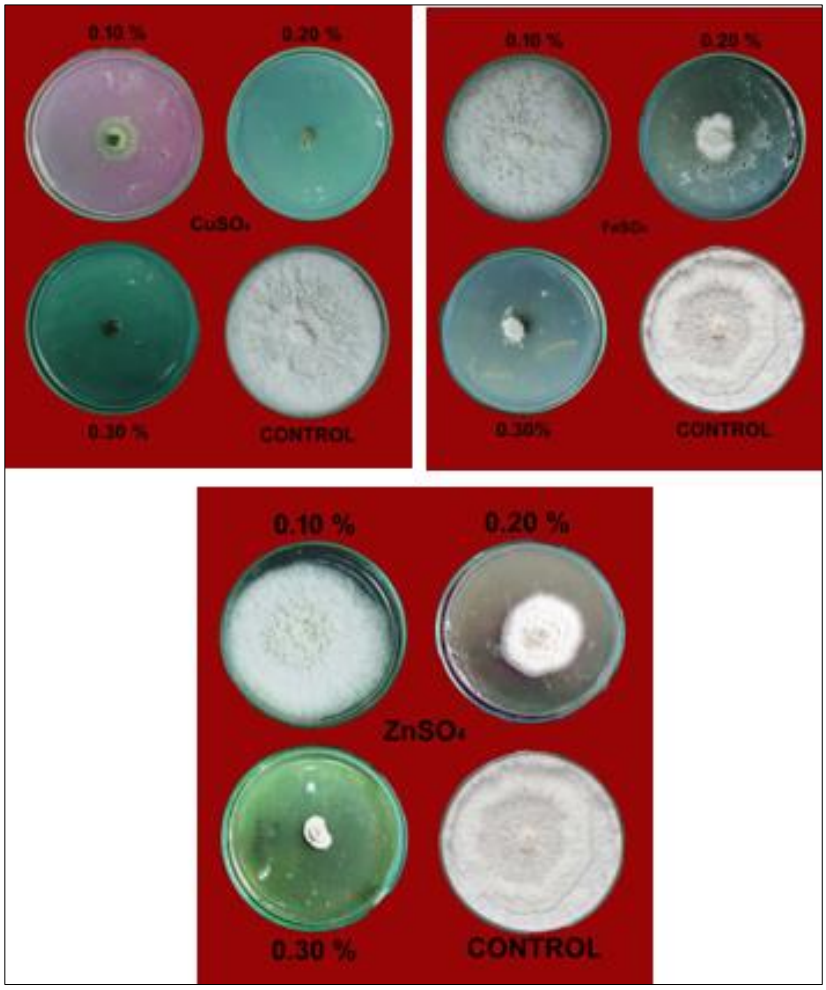


Fig 2(a): Effect of micronutrients against *D. rosae* at different concentrations

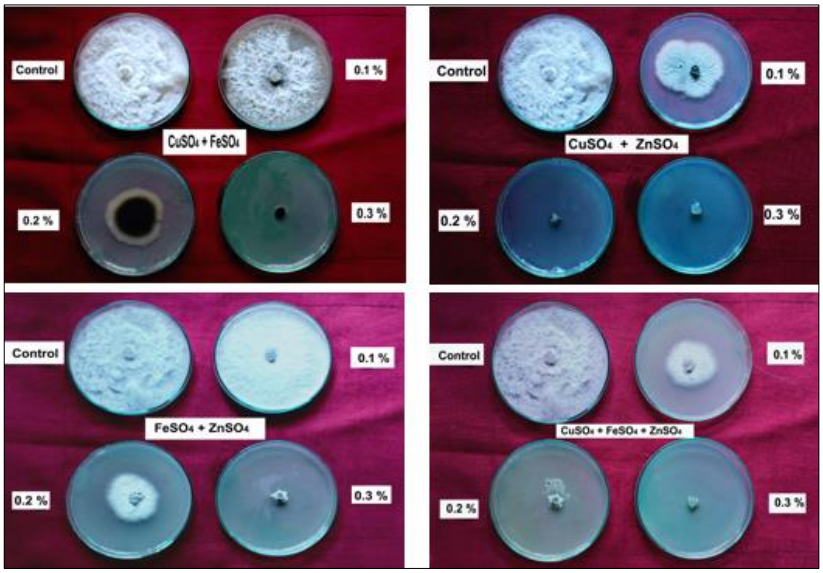


Fig 2(b): Effect of micronutrients combinations against *D. rosae* at different concentrations

Discussion

Among the six different fungicides examined against *D. rosae* under *in vitro* condition, two fungicides *viz.,* hexaconazole 5 per cent EC (Contaf) and tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo) completely inhibited the mycelial growth at all the concentrations. Tricyclazole 75 per cent WP (Baan) showed least inhibition percentage whereas mancozeb 75 per cent WP (Indofil M-45) did not control the mycelial growth at all. Similarly, Kumar *et al.* (2013) [6] evaluated two contact fungicides *viz.,* Blitox, Mancozeb and three systemic fungicides *viz.,* Ridomil, Carbendazim and Hexaconazole under *in vitro* condition and reported that at 200 and 250 ppm concentrations Hexaconazole was effective against *D. rosae.* Previous studies showed that strobuilurin fungicides restrain the growth and development of fungus on the leaf surface. ATP synthesis is blocked by the fungicide which in turn interferes with the mitochondrial energy production (Ammermann *et al.*, 1992) [1]. The germination of spore is dependent on mitochondrial respiration, therefore, application of strobilurin fungicide inhibits the spore germination (Zheng and Koller, 1997) [10].

Copper sulphate, zinc sulphate and iron sulphate and their combination at three different concentrations *viz.,* 0.10, 0.20 and 0.30 per cent were examined against *D. rosae* under *in vitro* condition. Copper sulphate completely inhibited the mycelial growth at 0.20 per cent concentration. And the micronutrient combination $CuSO_4$+$FeSO_4$+$ZnSO_4$ showed highest per cent inhibition over control at 0.10 per cent concentration. This finding reveals that micronutrients can also be used for disease control.

Conclusion

The finding of this study suggests that triazole and strobilurin group of fungicides are highly effective in controlling black spot disease in rose. Hexaconazole 5 per cent EC (Contaf) and tebuconazole 50 per cent + trifloxystrobin 25 per cent WG (Nativo) at lower concentration can be used to control black spot disease at field condition. Among the micronutrients, copper sulphate was highly effective in inhibiting the growth of pathogen both individually as well is in combination with other micronutrients. It showed higher inhibition percentage when applied in combination with zinc sulphate and iron sulphate than when applied individually. It can be concluded that apart from providing nutrition to the plant, micronutrients also act as plant protectants against pathogenic microbes.

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