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In vitro and *In vivo* Evaluation of Fungicides against Black Spot of Rose

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A B S T R A C T

An experiment on efficacy of 12 fungicides *in vitro* and 7 fungicides in field against black spot of rose was conducted at University of Horticultural Sciences, Bagalkot. At 500 ppm concentration, carbendazim50 % WP, difenconazole25 % EC, thiophanate methyl70 % WP, fluzilazole40% EC and propiconazole 25 ECgave cent per cent inhibition of mycelia under *in vitro*. In field experiment difenconazole @ 0.1% recorded least (2.86) per cent disease index which was on par with carbendazim @ 0.1% (3.05). These treatments were followed by hexaconazole @ 0.1% (4.95), thiophanate methyl @ 0.1% (6.86) and propiconazole @ 0.1% (7.24).The highest yield of 57754 flowers /ha with highest net income of Rs. 88,106 was recorded in difenconazole @ 0.1%. Carbendazim @ 0.1% was next best with yield 53,013 flowers /ha net returns of Rs. 79325. Hexaconazole @ 0.1% was third best which yielded 52,582 flowers /ha with net return of Rs 79,064.

Introduction

Rose is one of the oldest flowers under cultivation and most popular among all garden flowers throughout the world. It is grown for various purposes, such as garden flower, for aesthetic value, as cut flower for decoration and for making various products such as rose oil, rose water, garland, gulkhand, rose attar *etc.* These spectacular eye catching flowers are complement to any kind of flower arrangement.

In India according to a recent survey about 4826 ha area, with a production of 21618 M tons of rose and 4.48 M. tons/ha of yield. Major rose producing districts in Karnataka are Bangalore urban, Bangalore rural, Chikkaballapur, Kalaburagi, Kolar, Mandya, Mysuru, Raichur, Ramanagar, Tumakuru (Anon, 2019).

The flower yield is considerably decreasing in recent days due to the invading pest and disease. This crop is known to get affected by many diseases. Among all, black spot caused by *Diplocarpon rosae* is one of the major diseases especially in important rose growing areas of Karnataka.

The black spot disease of rose caused by *D. rosae*is economically devastating disease in ornamental rose especially in hot and humid climates. (Horst and Cloyd, 2007). The black

spot of rose is a foliar disease characterized by black spots with an irregular margin on the upper side of the leaf. Spots close to each other merge to form bigger spots. The black spot infections leads to defoliation and in severe cases the plant dies. (Drewes-Alvarez, 2003). Since the pathogen is known to cause huge economical losses in term of yield and quality for flower and flower products, there is a need to conduct studies on developing management strategies using effective fungicide. Therefore the present research work is focused on *in vitro* and field evaluation of fungicides for the management of black spot of rose.

Rehman *et al.,* (2012) found thiophanate methyl as effective fungicide against black spot of rose at a concentration of 150 and 250 ppm. Sunilkumar *et al.,* (2013) reported that hexaconazole was found to inhibit the mycelial growth of *D. rosae* significantly *in vitro*.

Materials and Methods

In vitro evaluation of fungicides against black spot of rose

Fungicides were tested *in-vitro* to evaluate their efficacy on colony growth of *D. rosae* by using poison food technique (Dhigra and Sinclair 1985) laid out using completely randomized design (CRD). Totally 12 fungicides comprising of both contact and systemic fungicides were evaluated at various concentrations *viz.*, 100, 250, 500 and 1000 ppm. Per cent reduction in radial growth of the pathogen over control was calculated by using the formula given by Vincent, 1947.

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

Where,

I = Per cent inhibition
C= growth in control plate
T= growth in fungicide treated plate

In vivo (Field) evaluation of fungicides against black spot of rose

A field experiment was laid out in Randomised Block Design separately for blackspot and powdery mildew disease by selecting seven fungicides which were found effective *in vitro.* Susceptible variety 'Sofia' was used in the present investigation. The variety planted at spacing of 90×60 cm having 8 treatments and 3 replications. The variety was grown as per packages of practices of UHS, Bagalkot.

The fungicides evaluated for black spot are carbendazim (0.1 %), difenconazole (0.1%), fluzilazole (0.1%), hexaconazole (0.1 %), propiconazole (0.1 %), thiophanate methyl (0.1 %) and tebuconazole + trifloxystrobin (0.1 %).Observations on intensity of disease were recorded using five randomly selected plants from each treatment plot and graded as per 0 to 5 scale given by Sharma and Singh (2002). Further percent disease index was calculated as described earlier. Since the observations were taken in percentage, angular transformation was made and data was analysed statistically.

Results and Discussion

In vitro evaluation of fungicides

The results of *in vitro* evaluation of the different fungicides against *D. rosae* at various concentrations are presented in Table 1. Table 1 revealed that there was significant difference found among the different fungicides. At 250 ppm, the fungicides carbendazim, difenconazole thiophanate

methyl and fluzilazolewere found highly effective in inhibiting the growth of the pathogen, as they recorded cent per cent inhibition. The fungicides, propiconazole (93.03 %) and hexaconazole (91.87 %), chlorothalonil (59.69 %), pyraclostrobin (59.50 %) were next best.

At 500 ppm, same trend continued, where in propiconazole also recorded complete inhibition of mycelial growth in addition to previous one. With respect to 1000 ppm, the trend was as that of 500 ppm where in carbendazim, difenconazole, thiophanate methyl, fluzilazole and propiconazole gave cent per cent inhibition of mycelia. These were followed by hexaconazole (96.75 %), tebuconazole + trifloxystrobin (85.87 %), chlorothalonil (74.49 %) and azoxystrobin (73.11 %). Even at higher concentration also mancozeb (43.88 %) found less effective.

Table.1 *In vitro* evaluation of fungicides against *D. rosae*

Sl. No.	Fungicide	Per cent inhibition of radial growth		
		250 ppm	500 ppm	1000 ppm
1	Azoxystrobin 23 % EC	57.83 (49.48)	63.46 (52.79)	73.11 (58.86)
2	Carbendazim 50 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Chlorothalonil 75 % WP	59.69 (50.57)	62.55 (52.25)	74.49 (59.66)
4	Difenconozole 25 % EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
5	Dimethomorph 50 % WP	28.63 (32.33)	48.45 (44.10)	68.28 (55.80)
6	Fluzilazole 40 % EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	Hexaconazole 5 % EC	91.87 (73.47)	95.12 (77.27)	96.75 (79.62)
8	Mancozeb 75 % WP	21.59 (27.66)	23.15 (28.73)	43.88 (41.46)
9	Propiconozole 25 % EC	93.03 (74.68)	100.00 (90.00)	100.00 (90.00)
10	Pyraclostrobin20 % WG	59.50 (50.46)	65.57 (54.05)	65.97 (54.30)
11	Thiophanate methyl 70 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
12	Tebuconazole 50% + Trifloxytrobin25 %WG	56.79 (48.89)	72.53 (58.39)	85.87 (67.92)
	SEm±	0.56	0.63	1.17
	CD (0.01)	1.64	1.86	3.44

*Figures in parenthesis are in angular transformed values

Table.2 Field evaluation of fungicides against black spot of rose

Sl. No.	Fungicide	Percent Disease Index at			Yield (Number of flowers/ha)
		Before spray	15 days After 1st spray	15 days After 2nd spray	
1	Carbendazim 50 % WP@ 0.1%	16.57 (24.06)*	12.00 (20.25)	3.05 (10.04)	53013
2	Difenconazole 23 % EC @ 0.1%	17.91 (25.02)	7.05 (15.39)	2.86 (9.73)	57754
3	Fluzilazole25 % EC @ 0.1%	17.33 (24.59)	13.71 (21.72)	12.19 (20.43)	43531
4	Hexaconazole 5 % EC @ 0.1%	17.33 (24.59)	14.48 (22.34)	4.95 (12.80)	52582
5	Propiconazole 25 % EC @ 0.1%	16.09 (23.63)	11.43 (19.75)	7.24 (15.60)	49996
6	Thiophanate methyl 70 % WP @ 0.1%	18.29 (25.31)	15.62 (23.26)	6.86 (15.15)	45255
7	Tebuconazole 50% + Trifloxytrobin25 %WG @ 0.1%	18.29 (25.31)	12.19 (20.42)	12.38 (20.59)	47410
8	Control	17.52 (24.73)	21.91 (27.89)	23.50 (28.81)	34911
SEm ±		0.41	0.50	0.39	145
CD (0.05)		NS	1.53	1.18	446.64

*Figures in parenthesis are in angular transformed values

Table.3 Economic analysis of the experiment on fungicidal evaluation for the control of black spot of rose

Sl. No.	Fungicides	Yield (No. of flowers/ ha)	Total income/ha (Rs)	Total cost/ ha (Rs)*	Income over control (Rs)	Cost of treatment/ ha (Rs)]	ICBR	Net returns (Rs)
1	Carbendazim 50 % WP @ 0.1%	53013	159039	79714	54306	3013	18.02	79325
2	Difenconazole 23 % EC @ 0.1%	57754	173262	85156	68529	8454	8.10	88106
3	Fluzilazole 25 % EC @ 0.1%	43531	130593	87453	25860	10751	2.40	43140
4	Hexaconazole 5 % EC @ 0.1%	52582	157746	78682	46548	1980	23.50	79064
5	Propiconazole 25 % EC @ 0.1%	49996	149988	80347	45255	3646	12.41	69641
6	Thiophanate methyl 70 % WP @ 0.1%	45255	135765	80409	31032	3708	8.36	55356
7	Tebuconazole 50% + Trifloxytrobin25 %WG @ 0.1%	47410	142230	87354	37497	10653	3.51	54876
8	Control	34911	104733	76701	0.00	0.00	0.00	28032

In vivo (Field) evaluation of fungicides

The fungicides which were found effective *in vitro,* were evaluated for the management of black spot of rose under field condition. Data are presented in table 2. Table 2 revealed that there was significant difference found among the different fungicides with respect to per cent disease index of black spot of rose. Fifteen days after first spray difenconazole recorded (7.05) least per cent disease index than all other treatments followed by propiconazole @ 0.1% (11.43) which was on par with carbendazim @ 0.1% (12.00) and tebuconazole + trifloxystrobin @ 0.1% (12.19) and superior over control. Among fungicides, highest per cent disease index recorded in thiophanate methyl (15.62). Control recorded with PDI of 21.91 per cent.

Fifteen days after second spray difenconazole @ 0.1% recorded 2.86 per cent disease index which was on par with carbendazim @ 0.1% (3.05) and these are significantly superior over all the treatments. These treatments were followed by hexaconazole @ 0.1% (4.95), thiophanate methyl @ 0.1% (6.86) and propiconazole @ 0.1% (7.24). The maximum per cent disease index was recorded in both tebuconazole + trifloxystrobin @ 0.1% (12.38) and fluzilazole @ 0.1% (12.19) which were on par to each other. Control recorded PDI of 23.50 was noticed.

With respect to yield there was significant difference between the treatments. The highest yield of 57754 flowers /ha was recorded in difenconazole@ 0.1% which was superior compared to the rest of the treatments and followed by carbendazim @ 0.1% with yield 53013 flowers /ha. Hexaconazole @ 0.1% yielded 52582 flowers /ha and propiconazole @ 0.1% has given 49996 flowers /ha while least yield of 34911 flowers /ha recorded in control.

Economics of chemical control of blackspot of rose

The economics of different fungicides tested under field condition against black spot of rose are depicted in Table 3. Among the different fungicides tested under field condition, difenconazole was found effective than rest of the treatments with highest net returns of Rs. 88106 which was followed by carbendazim with net returns of Rs. 79325 and hexconazole with net returns of Rs. 79064 while least net returns of Rs. 43140 was noticed in fluzilazole.

Successful control of plant diseases by chemicals depend upon the correct usage of fungicide. The results obtained from the current laboratory study of *D. rosae* revealed that the fungicides, carbendazim, difenconazole, hexaconazole, thiophanate methyl fluzilazole and propiconazole were found highly effective in inhibiting the growth of the pathogen. These results confirmed the earlier findings of Jadhav and Fugro (2015) who reported that carbendazim (0.1%), thiophanatemethyl (0.1%), propiconazole (0.05%), chlorothalonil (0.1%) and myclobutanil (0.05%) completely inhibited the growth of the test fungus *D. rosae*. Rehman *et al.,* 2012 reported that thiophanate methyl was found most effective in reducing the mycelial growth of the *D. rosae*at concentration of 150 and 250 ppm. Reetika, (2017) stated that 300 ppm tebuconazole+ trifloxystrobin found effective in inhibiting growth of *D. rosae*. Sunilkumar *et al.,* (2013) revealed that hexaconazole was found to inhibit the mycelial growth of *D. rosae* significantly at a concentration of 200 ppm and 250 ppm, which is confirmed in the current study.

Field experiment revealed after second spray difenconazole @ 0.1% recorded 2.86 per cent per cent disease index with highest yield of

38502 flowers per hectare, which was on par with carbendazim @ 0.1% (3.05) with yield of 35342 flowers per hectare and these were significantly superior over all the treatments followed by hexaconazole @ 0.1% (4.95), thiophanate methyl @ 0.1% (6.86) and propiconazole @ 0.1% (7.24). This was supported by earlier findings of Sindhan and Roy (1985) who reported the best control of black spot disease was obtained by Carbendazim (0.1%). Maljaja (1997) reported that carbendazim (0.1%) was the most effective against *D. rosae* under field conditions. In present study propiconazole @ 0.1% found effective and recorded 7.24 per cent disease index. This result was in conformity with the results of Devappa *et al.*, (2006) where propiconzaole (0.1%) was found very effective in reducing per cent disease index 8.76 of *D. rosae*.

Carbendazim induced the nuclear instability by disturbing the mitosis and meiosis, also attributed inhibition of biosynthesis process and synthesis of DNA of fungi and being bendamidazole fungicide interferes with energy production and cell wall synthesis of fungi (Nene and Thapliyal, 1979). In present study thiophanate methyl @ 0.1% found effective and recorded (6.86) per cent disease index. This result was supported by Evans (1974) who reported that thiophanate methyl gave good control against *D. rosae*. In this experiment triazole fungicides found more effective than other groups. This was because triazoles are more systemic (Bartlett *et al.*, 2001) during the initial phase of infection. Fungi contain enough ergosterol in the germinating spores to produce germ tubes and infection structures and to penetrate into the host tissue. Triazole inhibits the production of ergosterol and fungus is killed by the depletion of the sterol building blocks necessary for the cell membrane (Noegel, 1998).

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