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Screening of Tuberose Cultivars and Effect of Fungicides against Tuberose Leaf Spot Caused by *Alternaria polyanthi*

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ABSTRACT

Six systemic fungicides viz., azoxystrobin, pyraclostrobin, difenconazole, hexaconazole, propiconazole, tebuconazole and three non-systemic fungicides viz., chlorothalonil, copper oxy chloride and mancozeb were tested against Alternaria polyanthi at three different concentrations using poison food technique. Among the systemic fungicides hexaconazole (Contaf 5 EC), propiconazole (Tilt 25 EC), tebuconazole (Folicur 25 EC) and pyraclostrobin (Cabriotop 60 WG) (0.1%) showed 100 percent inhibition of the fungus at 1000 ppm, while among the non-systemic fungicides, chlorothalonil at 2000 ppm, was found the best in inhibiting the growth of A.polyanthi with 52. percent inhibition. Eleven varieties of tuberose were screened against Alternaria leaf spot (Alternaria polyanthi) under field conditions. Out of eleven cultivars, none of them was found resistance or immune, while three cultivars viz., Arka Nirantara (13.14%), GKTC-4 (16.48%) and Hyderabad Double (19.91%) were found to be moderately resistant (category II) and seven cultivars viz., Sikkim (22.86%), Phule Rajini (24.01%), Calcutta single (30.75%), Prajwal (32.50%), Rajith Rekha (32.57%), Sringar (34.14%) and Vaibhav (34.73%) were found moderately susceptible (category III). The local susceptible check recorded highest with per cent leaf area infection 43.5% (category IV).

Key words: *Tuberose, Screening, Leaf spot, Alternaria polyanthi, Fungicides.*

INTRODUCTION

Tuberose (*Polianthes tuberosa*) is one of the most important flower crop of tropical and sub-tropical areas . In India, tuberose is commercially cultivated in West Bengal, Karnataka, Maharastra, Tamil Nadu, Haryana, Punjab, Gujarat, Rajasthan, Andhra Pradesh[2]. It is mainly cultivated for cut and loose flower purpose and also for the extraction of its highly valued natural flower oil. This crop is affected by various biotic and abiotic factors which affect growth and cause loss in flower yield. Among the biotic factors, leaf spot caused by *Alternaria polianthi* is an important fungal disease[3] in tuberose. In India, leaf spot in tuberose incited by *A. polyanthi* was first reported from Coimbatore[3]. During our survey it was found that the incidence of tuberose leaf spot is increasing and causing economic loss in Andhra Pradesh.

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Hence, an attempt was made to investigate the efficacy of different systemic and non systemic fungicides and screening for resistance sources for the management of leaf spot of tuberose.

MATERIAL AND METHODS

The experiment on *in vitro* evaluation of fungicides was carried out at Horticultural Research Station, Anantharajupet, Dr. YSR. Horicultural University. In the present study, *Alternaria polianthi* was isolated from the leaves of tuberose leaves showing the typical symptoms of leaf spot disease (Fig. 1) using tissue isolation and purified using single spore isolation method. Further, the pure culture of fungus (Fig. 2) obtained from these two methods was used in various laboratory studies. The efficacy of six systemic fungicides *viz*., azoxystrobin , pyraclostrobin, difenconazole , hexaconazole , propiconazole, tebuconazole were tested at five different concentrations viz., 250,500,1000 ppm and each treatment replicated thrice. Three non-systemic fungicides *viz*., chlorothalonil, Copper oxychloride and mancozeb were tested at 1000, 2000, 2500 ppm and each treatment replicated five times using poison food technique of Sharvelle[8]. The plate without any fungicide served as control. Required quantity of individual fungicides was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later 20 ml of the poisoned medium was poured into sterile petri plates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium.

Then such plates were incubated at 28°C for twelve days and radial colony growth was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by using the formula suggested by Vincent[9].

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

where,

I : Percent inhibition, C: radial growth of fungus in control and T: radial growth of fungus in treatment.

Screening of tuberose cultivars under field conditions

The experiment for screening the resistant genotypes was conducted at Horticultural Research Station, Anantharajupeta, Dr. YSR. Horticultural University during 2015-16 and 2016-17 years. A total of eleven tuberose cultivars were grown in randomized block design with three replications. The net plot size of 4 x 1 m and spacing of 30x30 cm was followed. The observations on disease intensity were recorded using 0-5 scale of [7] at harvest when the disease was developed to its maximum extent and percent disease index was calculated by the following formula given by Wheeler[11].

$$\text{Per cent disease index (PDI)} = \frac{\text{al numerical rating}}{\text{Total No. of units examined X maximum rating (i.e. 5)}} \times 100$$

Further, the varieties were placed in different categories of resistance and susceptibility on the basis of method given by[5] (Table 1).

RESULTS AND DISCUSSION

***In vitro* evaluation of fungicides**

Data for systemic and non-systemic fungicides for inhibition of mycelial growth are presented in Table 2 & 3. There was significant difference among the systemic fungicides tested in inhibiting the growth of *Alternaria polianthi*. All fungicides were effective and gave more than 65% inhibition of test fungus at 250 ppm compared to control. Among the systemic fungicides evaluated hexaconazole (Contaf 5 EC), propiconazole (Tilt 25 EC), tebuconazole (Folicur 25 EC) and pyraclostrobin were fount most effective and showed 100 percent inhibition growth of the *A. polianthi* at 1000 ppm followed by azoxystrobin (1000 ppm) and pyraclostrobin (1000 ppm) with 86.81 and 86.2 percent inhibition respectively. The results are in agreement with Amaresh *et al.*[1] who reported that the systemic fungicides *viz*., propiconzole, hexaconazole and difenoconazole at 500 ppm

found effective against *A. alternata* causing blight of sesame. Sastrahidayat[8] reported that difenconazole (0.08%) inhibited the growth of *Alternaria porri* under *in vitro* condition.

Similarly, among the non-systemic fungicides tested chlorothalonil (0.25%) was showed effective in inhibiting the fungal growth by recording 52.44 percent inhibition followed by mancozeb and copper oxy chloride with percent inhibition of 42.96 % and 35.02% respectively at 2500 ppm concentration. Patel *et al*.[4] reported that mancozeb, zineb, carbendazim, chlorothalonil and thiophanate methyl were effective against *A. alternata* of onion leaf blight. Wen Jiawei *et al*.[10] also found that Daconil (chlorothalonil) was the most effective in the inhibition of spore germination, followed by mancozeb.

The highest toxicity index of (277.12) was found in hexaconazole, followed by propiconazole (271.0) on maximum toxicity index of 300. The least toxicity index was in the treatment of Copper oxy chloride (95.75).

Screening of tuberose cultivars under field conditions

Eleven tuberose cultivars were screened against Alternaria leaf spot (*Alternaria polyanthi*) under field conditions during 2015-16 and 2016-17. The data on disease severity showing the relative reaction of different genotypes are presented in Table 4 & Fig. 3. None of the eleven tuberose cultivars screened against leaf spot disease, was free from the disease. Therefore no variety could be included in the Immune or resistant (category 0 & I). However, three cultivars *viz.,* Arka Nirantar (13.14%), GKTC-4 (16.48%) and Hyderabad Double (19.91%) were found to be moderately resistant (category II) and seven cultivars *viz.,* Sikkim (22.86%), Phule Rajini (24.01%), Calcutta single (30.75%), Prajwal (32.50%), Rajith Rekha (32.57%), Sringar (34.14%) and Vaibhav (34.73%) were found moderately susceptible (category III). The local susceptible check recorded maximum per cent leaf area infection 43.5% (category IV).

Table 1: Scale to indicate degree of resistance against leaf spot of tuberose

Disease severity	Category	Reaction
<5	0	Immune
5-10	I	Resistant
11-20	II	Moderately resistant
21-40	III	Moderately susceptible
41-60	IV	Susceptible
>61	V	Highly susceptible

Table 2: Growth inhibition of *Alternaria polyanthi* at different concentrations of various systemic fungicides after seven days of incubation at 28 ± 2º C

Fungicide	Concentration (ppm)/Per cent inhibition*			Mean Per cent inhibition*	Toxicity Index#
	250 ppm	500 ppm	1000 ppm		
Azoxystrobin (Amistar 25 S.C)	65.49	73.42	86.81	75.24	225.72
Pyraclostrobin (Cabriotop 60 WG)	85.94	73.23	75.68	78.28	234.85
Difenconazole (Score 25 EC)	82.80	85.06	100.00	89.29	267.86
Hexaconazole (Contaf 5 EC)	83.18	93.93	100.00	92.37	277.12
Propiconazole (Tilt 25 EC)	84.82	86.18	100.00	90.33	271.00
Tebuconazole (Folicur 25 EC)	65.125	83.968	86.27	78.46	235.37
Control	0.00	0.00	0.00	0	0
	Fungicide (F)		Concentration(C)	(F X C)	
S.Em.+	0.64		1.30	1.107	
C.D. at 5%	1.84		1.60	3.188	

* Mean of four replications # Maximum toxicity index = 300.00

Table 3: Growth inhibition of *Alternaria polianthi* at different concentrations of various Non-systemic fungicides after seven days of incubation at 28 ± 2º C

Fungicide	Concentration (ppm)/Per cent inhibition*			Mean	Toxicity Index#
	1000 ppm	2000 ppm	2500 ppm		
Mancozeb (Dithane M-45)	26.74	26.83	42.96	32.18	**96.53**
Chlorothalonil (Kavach 75%WP)	44.48	48.77	52.44	48.56	**145.69**
Copper oxychloride (Blitax 50% WP)	26.05	34.69	35.02	31.92	**95.75**
Control	0.0	0.0	0.0	0.0	**0.0**
	Fungicide (F)		Concentration(C)	(F X C)	
S.Em.+	0.364		0.316	0.631	
C.D. at 5%	1.039		0.900	1.800	

* Mean of four replications # Maximum toxicity index = 300.00

Table 4: Reaction of tuberose varieties against *Alternaria polianthi* under field conditions during 2015-16 and 20116-17

Variety	Percent disease index (PDI) %		Mean PDI	Reaction
	2015-16	2016-17		
Arka nirantar	15.78	13.14	14.46	Moderately resistant
Calcutta single	32.51	30.73	31.62	Moderately susceptible
GKTC-4	18.61	16.48	17.55	Moderately resistant
Hyderabad double	17.21	19.91	18.56	Moderately resistant
Phule Rajini	27.86	24.01	25.94	Moderately susceptible
Prajwal	35.81	32.5	34.155	Moderately susceptible
Rajith Rekha	36.54	32.57	34.56	Moderately susceptible
Sikkim	24.56	22.86	23.71	Moderately susceptible
Sringar	37.82	34.14	35.98	Moderately susceptible
Vaibhav	39.13	34.73	36.93	Moderately susceptible
Local variety	59.32	43.5	51.41	Susceptible

Fig. 1: Symptoms of Leaf spot of Tuberose (*Alternaria polyanthi)*

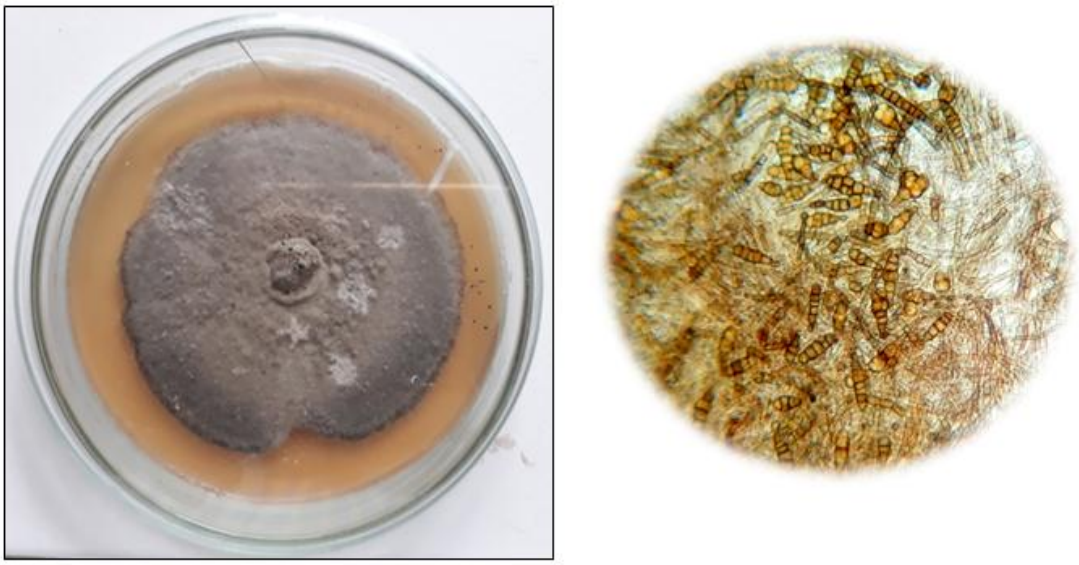

Fig. 2: Pure culture and sporulation of *Alternaria polianthi*

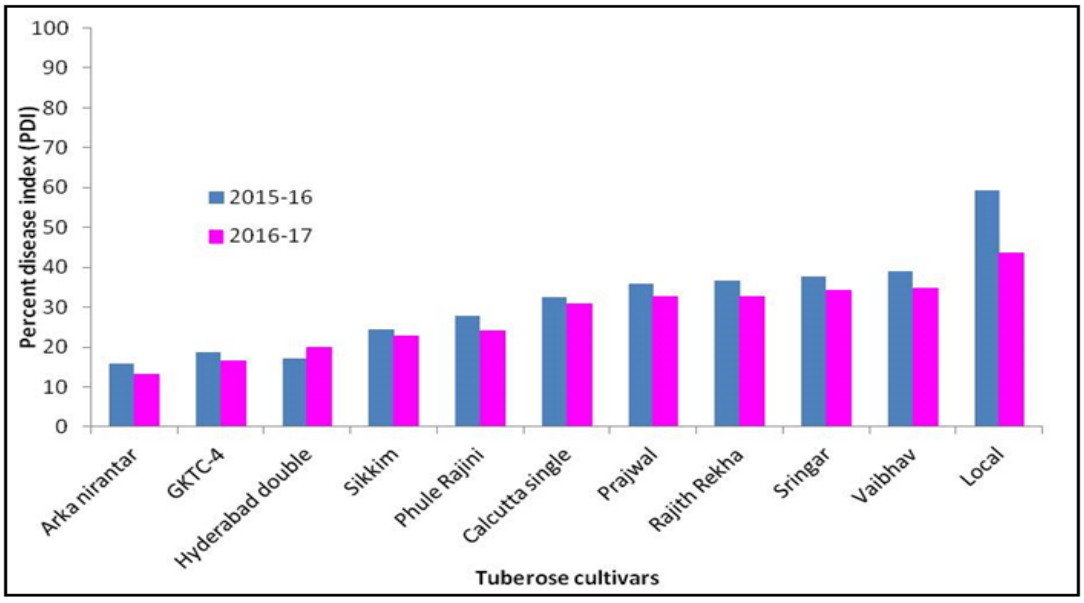

Fig. 3: Screening of tuberose cultivars against leaf spot of tuberose caused by *Alternaria polianthi* during 2015 and 2016

CONCLUSION

Systemic fungicides *viz.*, hexaconazole, propiconazole, tebuconazole at 0.1% found effective *in vitro* condition. The tuberose cultivars *viz.*, Arka Nirantara (13.14%), GKTC-4 (16.48%) and Hyderabad double (19.91%) exhibited moderately resistant reaction against tuberose leaf spot under field conditions.

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