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Investigation on different systemic fungicides against *Leveillula taurica* causing powdery mildew of cluster bean *in vitro*

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Abstract
Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) is one of the most important legume crops grown in India. Powdery mildew caused by *Leveillula taurica* is one of the major constraints in the production of cluster bean. Different chemicals were evaluated *in vitro* to choose the promising chemicals and their performance was judged in the field conditions against powdery mildew of cluster bean caused by *Leveillula taurica*. Among the systemic fungicides, 78.39 percent spore germination inhibition was observed in the treatment of propiconazole followed by hexaconazole (70.25%). The results of present study indicate that the systemic fungicides from triazole group effectively inhibited spore germination.

Keywords: *Leveillula taurica*, *in vivo*, powdery mildew, fungicides and cluster bean

Introduction
Cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) is an important drought resistant leguminous crop that has been grown in India since ancient times. In India, Rajasthan, Gujarat, Haryana, Uttar Pradesh and Punjab are known to be the leading states for cultivation of cluster bean.

The cluster bean crop is affected by various biotic and abiotic stresses causing considerable yield losses. Among biotic stresses, the damage caused by plant diseases is one of the major constraints. It is affected by a number of diseases caused by fungi, nematodes, virus and phytoplasma. Fungal diseases like anthracnose, angular leaf spot, root rot, rust and charcoal rot *etc*. Among these, powdery mildew of cluster bean caused by *Leveillula taurica* is an important disease. The weather conditions during December and January are most congenial for outbreak of powdery mildew, which has become a limiting factor for successful cultivation of cluster bean. It has also been observed in moderate to severe forms in fields of Junagadh district of Gujarat state. It is a routine practice for farmers to spray fungicides onward from one month crop age to maturity, particularly for powdery mildew control. Fungicidal applications are also mandatory for powdery mildew management after its initiation. Hencelooking to importance of this disease and need of presentera, efficacy of various fungicides was tested against powdery mildew pathogens.

Materials and Methods
Spore germination inhibition activities of different agrochemicals were tested against *L. taurica in vitro* by poisoned food technique using Potato Dextrose Agar (PDA) as a germinating medium (Bagchi and Das, 1968) [1]. The procedure followed is given herein. With micropipette appropriate quantity of each agrochemical required was incorporated into autoclaved PDA medium before solidification and then medium was poured into sterilized Petri dishes in equal quantity (5 ml per Petri dish) to form a thin layer. Conidia of *L. taurica* from young colonies, by using a soft, sterilized, camel hair brush were collected and dusted under aseptic conditions over the solidified PDA medium and then Petri dishes were incubated at room temperature (23° + 1 °C) for 48 hours. Thereafter, observations were recorded at an interval of 12 hours. Inoculated Petri dishes containing PDA medium without agrochemicals served as control. The conidium having germ tube length of more than its width was considered as germinated conidium. Effect of toxicity on conidia and on their germination was observed with the help of compound microscope.

Percent inhibition of spore germination in each treatmet was calculated using following formla (Bliss, 1934) [3].

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

Where,
I = Percent inhibition
C = Number of germinated spore in control
T = Number of germinated spore in treatment

The systemic fungicides mentioned in Table-1 were tested using poisoned food technique. The detail of systemic fungicides and different levels is given in Table-2.

Results and Discussion

Effect of fungicides on powdery mildew of cluster bean

To check the efficacy of systemic fungicides, seven fungicides were tested using poisoned food technique on *L. taurica*. Data on spore germination inhibition are given in Table-3.

It is clear from the data presented in Table-3 that all the fungicides were effective for spore germination inhibition of *L. taurica*. Propiconazole (0.030%), which inhibited 85.71 percent spore germination, was found the most effective systemic fungicide among other systemic fungicides followed by propiconazole 0.025 percent with 78.57 percent spore germination inhibition. Next effective treatment was hexaconazole 0.0075 percent with 75.08 percent spore germination inhibition. Hexaconazole 0.0050 percent, propiconazole 0.020 percent, difenoconazole 0.030 percent and carbendazim 0.075 percent also gave more than 70.0 percent growth inhibition.

Table 1: List of different systemic fungicides tested *in vitro*

Common name	Chemical name	Trade name
Hexaconazole	(RS)-2-(2,4dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl) hexan-2-ol	Contaf 5% EC
Propiconazole	1-[[2-(2,4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2yl] methyl]-1H-1, 2,4-triazole.	Tilt 25% EC
Difenoconazole	1-[2-[2-chloro-4- (4-chlorophenoxy) phenyl]-4-methyl-1, 3-dioxolan-2-yl methyl]-1*H*-1, 2,4-triazole	Score 25% EC
Picoxystrobin	Methyl (*E*)-3-methoxy-2-[2-(6-trifluoromethyl-2-pyridyloxymethyl)-phenyl]acrylate	Acanto 25% EC
Tebuconazole	α-[2-(4chlorophenyl)ethyl]-α-(1,1 dimethylethyl) 1H-1, 2,4-triazole-1-ethanol	Folicur 25% EC
Carbendazim	Methyl-1-H-benzimidazol-2-yl carbamate	Bavistin 50% WP
Thiophanate methyl	1,2-bis (3-methoxycarbonyl-2-thioureido) benzene	Topsin M 70% WP

Table 2: Systemic fungicides tested for their efficacy against *L. taurica in vitro*

Sr. No.	Technical/active Ingredient	Concentration in ppm		
		1	2	3
1.	Carbendazim 50% WP	250	500	750
2.	Propiconazole 25% EC	200	250	300
3.	Picoxystrobilurin 25% EC	200	250	300
4.	Difenoconazole 25% EC	200	250	300
5.	Thiophanate methyl 70% WP	600	700	800
6.	Hexaconazole 5% EC	25	50	75
7.	Tebuconazole 25.9% EC	200	250	300
8.	Control	-	-	-

Table 3: Effect of systemic fungicides on spore germination inhibition of *L. taurica in vitro*

S. No.	Fungicide	Concentration (%)	Percent spore germination	Percent inhibition over control	Mean percent inhibition
1	Hexaconazole 5% EC	0.0025	16.70	64.27 (53.29)*	70.25
		0.0050	13.36	71.42 (57.68)	
		0.0075	11.69	75.08 (60.05)	
2	Propiconazole 25% EC	0.020	13.97	70.71 (57.24)	78.39
		0.025	10.02	78.57 (62.42)	
		0.030	6.68	85.71 (67.78)	
3	Difenoconazole 25% EC	0.020	20.04	57.14 (49.10)	64.23
		0.025	16.70	64.27 (53.29)	
		0.030	13.23	71.51 (57.68)	
4	Picoxystrobin 25% EC	0.020	33.40	28.57 (32.31)	32.12
		0.025	31.73	32.08 (34.50)	
		0.030	30.06	35.71 (36.69)	
5	Tebuconazole 25% EC	0.020	26.72	42.85 (40.88)	50.98
		0.025	23.38	50.00(45.00)	
		0.030	20.04	57.14 (49.10)	
6	Carbendazim 50% WP	0.025	16.70	64.27 (53.29)	67.91
		0.050	15.03	67.90 (55.49)	
		0.075	13.25	71.56 (57.77)	
7	Thiophanate methyl 70% WP	0.060	23.30	50.09 (45.07)	57.13
		0.070	20.04	57.14 (49.10)	
		0.080	16.70	64.27 (53.29)	

8	Control	-	46.76	-	
		Fungicide (F)	Concentration (C)	F x C	
	S. Em. ±	0.46	0.28	0.80	
	CD at 5%	1.35	0.82	2.34	
	CV %	2.54			

*Figures in parentheses show angular transformed values

The above chemicals except carbendazim belong to trizoles group of fungicides. These fungicides interfere with biosynthesis of sterols i.e. inhibits the biosynthesis of ergosterol which is essential for structure of cellwall and its absence causes irreversible damage to cellwall and ultimetaly fungus dies. In additon, they are known to impede condial and haustorial formation. They change the sterol content and saturation of the polar fatty acids leading to alterations in membrane permibiality and behaviour of membrane bound enzymes (Nane and Thapliyal, 1993) [8].

Carbendazim fungicide is known to impact mitosis and cell division in powdery mildew fungi. Previous research revealed the inhibitory effect of this fungicide on the polymerization of tubulin into microtubules. This fungicide binds on β-tubulin in microtubules inhibiting their proliferation and suppressing their dynamic instability. Microtubules are the cytoskeletal polymers in fungal cells and, thus, play a vital role in many cellular functions. The application of carbendazim fungicide suppresses the assembly of spindle microtubules, disturbs the chromosomal alignment at the metaphase plate and microtubule-kinetochore interaction causing chromatid loss, chromosome loss or nondisjunction in target cell (Yang *et al*., 2011) [15].

The effectiveness of propiconazole against *E. polygoni* has been reported earlier by Biju (2000) [2]. Triazole fungicides namely propiconazole, hexaconazole and difenoconazole were highly effective in spore germination inhibition of *L. taurica* in present investigation. The effectiveness of trizole group of fungicides against powdery mildew (*E. polygoni*) of green gram (Venkatrao, 1997, Khunti *et al*. 2002) [14, 6], powdery mildew (*Sphaerotheca pannosa*) of rose (Ravikumar, 1998) [10] and powdery mildew (*E. cichoracearum*) of okra (Shivanna *et al*., 2006) [13] and fenugreek (Chovatiya, 2010) [4] has been reported by various workers. The potentiality of carbendazim in spore germination inhibition of *E. polygoni* (Nawaz and Narayanasamy, 1983; Kunkalikar, 1989), *Oidium erysiphoides* f. sp. *ziziph* (Sataraddi, 1994) [11] and *E. cichoracearum* (Hiremath, 1996) [5] has been reported.

Thiophanate methyl 70 WP and tebuconazole 25 EC were found moderataly effective with 57.13 and 50.98 percent mean spore germination inhibition, respectively. On contrary, Sharma and Gupta (1994) [12] achieved good spore germination inhibited of *Podosphaera leucotricha* causing powdery mildew of apple and reduced gern tube length using thiophanate methyl 70 WP.

The least effective fungicide was picoxystrobin 25 EC with 32.12 percent mean spore germination inhibition.

Conclusion

Different chemicals were evaluated *in vitro* to choose the promising chemicals and their performance was judged in the field conditions against powdery mildew of cluster bean caused by *Leveillula taurica*. Among the systemic fungicides, 78.39 percent spore germination inhibition was observed in the treatment of propiconazole followed by hexaconazole (70.25%). The results of present study indicate that the systemic fungicides from triazole group effectively inhibited spore germination.

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