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R Arutselvan
Professor, Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

RD Prasad
ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, Telangana, India

G Uma Devi
Professor, Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

C Sarada
ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
R Arutselvan
Professor, Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

In vitro evaluation of the chemical fungicides against the fungus *Botrytis ricini*

R Arutselvan, RD Prasad, G Uma Devi and C Sarada

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Abstract
Castor (*Ricinus communis* L.) is an important non-edible oilseed crop which experienced serious declines in area under cultivation in Telangana state due to yield losses caused by gray mold pathogen *Botryotinia ricini*. *B. ricini* fungus were isolated from infected castor plants in ICAR-IIOR, Hyderabad and maintained in oat meal gar medium. Use of chemical fungicides is an important tool the management of gray mold disease in castor. Five systemic fungicides, Bavistin (Carbendazim), Score (Difenoconazole), Amistar (Azoxystrobin), Ergon (Kresoxim methy) and three combination formulation fungicides such as Merivon (Fluxapyroxad + Pyraclostrobin), Cabrio Top (Pyraclostrobin + Metiram) and Sectin (Fenamidone + Mancozeb) were evaluated for *in vitro* efficacy at concentrations of 100, 250, 500, 750 and 1000 ppm against gray mold pathogen. The poisoned food technique was used and radial mycelial growths were measured seven day after inoculation. Bavistin was found to be the most effective fungicide followed by Merivon have shown high fungitoxicity against *B. ricini*. Amistar were the least effective. The results of this present investigation will be helpful for future research on botrytis fungicide management and for castor growers in controlling gray mold disease of castor.

Keywords: Castor, gray mold, fungicides

Introduction
Castor, *Ricinus communis* L., is important non-edible oilseed crop belong to the family Euphorbiaceae. It is widely cultivated in the tropics and sub tropics regions for its seed from which 50-55% castor oil is extracted (Weiss, 1973; Purseglove, 1968; Gobin et. al., 2001; Adefris and Nigussie, 1993) [21, 17, 7, 1]. Castor oil is used in industries (Cosmetic base, lubricant, paints) and pharmaceutics (Gobin *et al*., 2001 and Purseglove, 1968) [7, 17]. Among the various diseases in castor, gray mold disease caused by *Botryotinia ricini* Godfrey. emerged as a serious destructive disease especially in Telangana and Andhra Pradesh state when maturity of the racemes coincides with the cyclonic rains. Successful control could not be achieved causing great damage under favourable weather conditions leads to huge yield loss. Anjani *et al*., (2004) [3] reported that botrytis fungus can cause yield loss of 80-100% and this affects the income of castor farmers in terms of foreign exchange.

Very little information is available regarding its management of gray mold disease of castor. Some workers with varied success have used different chemicals and fungicides. *In vitro* studies conducted reveals that some fungicides restrict or prevent the growth of *B. ricini* pathogens (Yamuna, 2015; Gurvinder Kaur, 2015; Suyal *et al*., 2011) [22, 9, 20]. Fungicides like tebuconazole and iprodione are being recommended (Chagas *et al*., 2014) [5] and there is still a need to identify a better fungicide to control *Amphobotrys ricini* infection on castor. In view of the economic importance of the crop and the effect of gray mold diseases on the seed yield, there is a need to recommend specific fungicidal management for disease under field conditions. The aim of the study was to determine *in vitro* effects of chemical fungicides on the mycelial growth of *B. ricini* pathogen isolated from castor raceme.

Materials and Methods
Isolation and maintenance of the pathogen
Isolation of the test fungal pathogen was made from gray mold infected capsules collected from the ICAR-IIOR, Rajendranagar, Hyderadad. The infected capsules were cut into small pieces and were surface sterilized by immersing in 0.1% mercuric chloride for 30 seconds and

placed on oat meal enriched medium containing petri plates after thorough washings in sterile distilled water. The plates were incubated at 23±1ºC temperature for 3 to 4 days. The fungal growth emerging from diseased capsule pieces were picked up and the culture was further purified by single spore isolation method. The pure culture of the pathogen was maintained on oat meal enriched medium by periodical transfers (Prasad and Bhuvaneswari *et al.,* 2014) [16].

Identification of the pathogen

The fungus associated with the disease was brought into pure culture on Oat meal enriched medium and identified based on the descriptions given by Stevens (1918) [19], Godfrey (1923) [8] and Moses and Reddy (1989) [15].

Screening of fungicides against *B. ricini* under *in vitro* conditions

The laboratory experiment was conducted at the ICAR-IIOR, Hyderabad during 2018 on gray mold disease of castor. The study was conducted in a Complete Randomized Design (CRD) with seven treatments and four replications. Seven different fungicides belonging to different groups/ mode of action (Table 1) such as Bavistin (Carbendazim), Score (Difenoconazole), Amistar (Azoxystrobin), Ergon (Kresoxim methy) and three combination formulation fungicides *viz*., Merivon (Fluxapyroxad + Pyraclostrobin), Cabrio Top (Pyraclostrobin +Metiram) and Sectin (Fenamidone + Mancozeb) were screened against the *B. ricini* under laboratory conditions to find out their relative efficacy in inhibiting the mycelial growth of the pathogen in culture media by the "Poisoned food technique" (Schmitz, 1930) [18] at 100, 250, 500, 750, 1000 ppm concentrations respectively. Oat meal agar medium was prepared and amended with different concentrations of the fungicides. About 20 ml of sterilized culture medium was poured in each 9 cm petri dish. After solidification, the plates were inoculated with a 5 mm disk of seven days-old *B. ricini* culture. Four replications were maintained for each concentration and radial growth was recorded. The Petri plates were incubated at 23±1⁰C. One set of control was maintained without adding any fungicide to the medium. The observations were recorded until the control plate was full of growth of the pathogen. The data of radial growth of fungal colony was measured in millimeters. The per cent mycelial inhibition over control was calculated by the following formula given by Bliss (1934) [4].

Per cent inhibition over control= $[(C-T)/T] \times 100$

Where, C = Growth of fungus in control; T = Growth of fungus in treatment

Results and Discussion

The efficacy of seven fungicides were tested under *in vitro* by poisoned food technique and the results are presented in Table 2 and 3. Most of the fungicides were effective in inhibiting the mycelial growth of the *B. ricini* pathogen to varying degrees. Significant difference in inhibiting the mycelial growth of the pathogen among the fungicides was observed.

Out of four systemic fungicides tested, complete mycelial inhibition (100%) of growth of the pathogen over control was observed with Bavistin (Carbendazim) followed by Score (Difenoconazole) at 100 ppm concentration when compared to control. Amistar (Azoxystrobin) was least effective with 16.6 per cent mycelial growth inhibition at 100 ppm concentration (Table 2). Similarly, results of combination fungicide reveals that Merivon (Pyraclostrobin + Metiram) shown complete inhibition (100%) of growth of the pathogen over control followed by sectin (Fenamidone + Mancozeb) with mean per cent inhibition of 87.5 per cent whereas CabrioTop (Pyraclostrobin+ Metiram) was least effective with 78.3 per cent in inhibiting the growth of the pathogen at 100 ppm concentration (Table 3).

The benzimidazole fungicides were the first systemic fungicides that exhibited broad spectrum of activity against *Botrytis* spp. (Delp, 1995) [6]. These fungicides do not prevent conidial germination, but at low concentrations, they inhibit hyphal growth and cause distortion of germ tubes (Leroux *et al*., 1999) [13]. Carbendazim fungicide can inhibits the development of fungi by interacting with β-tubulin and stopping hyphal growth and cell division (Hollomon *et al*., 1998) [10]. Similar *in vitro* results were obtained by Yamuna (2015) [22] that fungicides such as Bavistin (carbendazim), Folicur (tebuconazole), Tilt (propiconazole) and Nativo (trifloxystrobin + tebuconazole) showed 100% mycelial inhibition of the *B. ricini* pathogen at 250 and 500 ppm concentrations by poison food technique. The laboratory data revealed that Quintal and Contaf at 50, 100, 150 and 200 ppm showed complete mycelial growth inhibition of *Botrytis cinerea* under *in vitro* condition followed by Avtar (91.16%) and Score (63.99%), respectively whereas Hilnate was least effective (11.68%) (Gurvinder Kaur, 2015) [9]. Hosen *et al.* (2010) [12] and Hosen (2011) [11] concluded that the iprodione and carbendazim was effective in inhibition of spore germination *B. cinerea.* The reduction in disease severity by iprodione + carbendazim, difenaconazole and hexaconazole are in consonance with the findings of Madhu Meeta *et al*. (1986) [14] and Agarwal and Tripathi (1999) [2]. Suyal *et al.* (2011) [20] tested seven fungicides under *in vitro* for the control of grey mold of chickpea. Fungicides Bavistin (0.1%), Quintal (0.1%) and Derosal Plus (0.3%) showed complete mycelial inhibition of *B. cinerea* in chickpea.

Table 1: Details of fungicides employed in the present investigation

Trade Name	Fungicide name	IUPAC name	Formulation *	Source of Supply
Bavistin	Carbendazim	Methyl (1H-benzimidazol-2-yl)carbamate	50 WP	BASF
Score	Difenoconazole	1-((2-(2-Chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl)-1H-1,2,4-triazole	25 EC	Syngenta
Amistar	Azoxystrobin	Methyl (2E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	25 SC	Syngenta
Ergon	Kresoxim methy	(2E)-(methoxyimino){2-[(2-methylphenoxy) methyl] phenyl}acetic acid	44.3 SC	Rallis India Limited
Merivon	Fluxapyroxad + Pyraclostrobin	1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)- 1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl) + [2-[[[1-(4-chlorophenyl)- 1H-pyrazol-3-yl]oxy] methyl] phenyl] methoxy-, methyl ester	25 SC	BASF
Cabrio Top	Pyraclostrobin + Metiram	Methyl[2-({[1-(4-chlorophenyl)-1H-pyrazol-3yl] oxy} methyl) phenyl] methoxycarbamate Tris [ammine [ethylenebis (dithiocarbamato)]zinc(2+)][tetrahydro -1,2,4,7-dithiadiazocine-3,8 dithione], polymer	20 WG	BASF

Sectin	Fenamidone + mancozeb	5S)-5-Methyl-2-(methylsulfanyl)-5-phenyl-3-(phenylamino)-3,5-dihydro-4H-imidazole-4-one+ Manganese(2+) zinc 1,2-ethanediyldicarbamodithioate	60 WG	Bayer Crop Science

*WP - Wettable Powder; EC- Emulsifiable Concentrate; SC- Suspension Concentrate; WG- Wettable Granules

Table 2: Radial colony growth along with %inhibition of *B. ricini* with systemic fungicides

Concentration (ppm)	**Fungicides***							
	Bavistin		**Score**		**Amistar**		**Ergon**	
	Radial growth (mm)	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**
100	0 (0.0)	100	6.5 (14.5)	92.7	75 (60.3)	16.6	51 (45.5)	43.3
250	0 (0.0)	100	0 (0.0)	100	57 (49.0)	38.1	0 (0.0)	100
500	0 (0.0)	100	0 (0.0)	100	19.5 (26.1)	82.7	0 (0.0)	100
750	0 (0.0)	100	0 (0.0)	100	7.5 (15.7)	98.3	0 (0.0)	100
1000	0 (0.0)	100	0 (0.0)	100	0 (0.0)	100	0 (0.0)	100
Control (Untreated)	90 (100)	0.0	90 (100)	0.0	90 (100)	0.0	90 (100)	0.0
Factors	SE(d)				C.D.			
Fungicides (A)	0.56				1.11			
Concentrations (B)	0.62				1.25			
Factor(A X B)	1.25				2.49			

*Mean of four replications, () Data in parentheses are arcsine transformed values

Table 3: Radial colony growth along with %inhibition of *B. ricini* with combination fungicides

Concentration (ppm)	**Fungicides***					
	Merivon		**Cabrio Top**		**Sectin**	
	Radial growth (mm)	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**
100	0 (0.0)	100	19.5 (26.1)	78.3	11 (19.2)	87.7
250	0 (0.0)	100	4.0 (11.4)	95.5	0 (0.0)	100
500	0 (0.0)	100	0 (0.0)	100	0 (0.0)	100
750	0 (0.0)	100	0 (0.0)	100	0 (0.0)	100
1000	0 (0.0)	100	0 (0.0)	100	0 (0.0)	100
Control (Untreated)	90 (100)	0.0	90 (100)	0.0	90 (100)	0.0
Factors	SE(d)			C.D		
Fungicides (A)	0.25			0.51		
Concentrations (B)	0.33			0.66		
Factor(A X B)	0.57			1.14		

*Mean of four replications, () Data in parentheses are arcsine transformed values

Conclusion
Bavistin (50 WP) systemic fungicide showed the complete mycelial inhibition of *B. ricini* pathogen at 100 ppm using poison food technique. So, Bavistin 50 WP may be suggested to use by the castor grower for the management of castor gray mold disease.

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