Indian Phytopathology
https://doi.org/10.1007/s42360-022-00556-y

RESEARCH ARTICLE

Management of gray mold disease of castor using fungicides

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Received: 25 June 2020 / Revised: 29 June 2022 / Accepted: 16 August 2022

Abstract

Castor (*Ricinus communis* L.) crop experienced serious decline in area under cultivation in Telangana state due to yield losses caused by gray mold pathogen *Botryotinia ricini*. Use of chemical fungicides is an important tool to control the gray mold disease in castor. Investigations have been carried out at ICAR -IIOR, Hyderabad, India during *kharif* 2018 and 2019 cropping season to identify an effective fungicide and manage castor gray mold disease under in vitro and in vivo conditions. Fungicides viz., propiconazole, carbendazim and pyraclostrobin + fluxapyroxad have shown high fungitoxicity against *B. ricini*. In polyhouse and field experiment, propiconazole at 0.1 per cent was most effective in reducing the disease by 89.7 and 91.3 per cent, respectively followed by pyraclostrobin + fluxapyroxad at 0.1 per cent concentration with 81.8 and 84.0 per cent disease control. Propiconazole at 0.1 per cent was found most effective against castor gray mold and significantly increased the seed yield. Hence, the fungicide propiconazole can be recommended for the management of castor gray mold disease.

Keywords Gray mold · Castor · Fungicides · Management

Introduction

Castor bean, which is a native plant of Ethiopia and North Africa, is cultivated as an oil or medicinal plant in tropical Asia and the Indian continent (Sung Kee et al. 2001). In India, castor is an important non-edible oil seed crop. Castor is known to suffer from many fungal and bacterial diseases at different crop growth stages. The crop is most commonly affected by wilt, gray mold, root rot and seedling blight. Among them, gray mold of castor caused by the fungus *Botryotinia ricini* is one of the most destructive diseases of castor (Godfrey 1923; Hennebert 1973; Kolte 1995; Prasad et al. 2016) and has become a major production constraint in several castor growing areas of the world. Initially symptoms appear as small blackish spots on inflorescence and later turn into grayish necrotic patches. Fungal threads which grow from these spots, spread the infection and the entire raceme turns into grayish mass of mycelium and powdery conidia. The disease is problematic when rains occur during capsule formation and during prolonged favourable weather (Soares 2012). Need based chemical control measures mostly systemic fungicides, constitutes the principle means of efficient and reliable crop protection against gray mold. Control of diseases due to *Botrytis* and related species represents roughly 8 per cent of the global fungicide market (Phillips and Mc dougall 2012). Cultivated varieties or hybrids of castor possess no resistance to gray mold, therefore farmers have to rely on fungicides for disease management. By considering the importance and the economic damage caused by the disease, the present investigation was carried out to select effective chemical fungicides for management of gray mold disease of castor under *in-vitro*, glass house, polyhouse and field conditions.

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Materials and methods

Isolation and maintenance of *Botryotinia ricini*

The pathogen causing gray mold of castor was isolated from the naturally infected castor capsules on oat meal enriched medium (Bhuvaneswari et al. 2013). The pure culture of the fungus was obtained and purified by single spore isolation method and maintained on oat meal enriched medium.

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

The laboratory experiment was conducted at the ICAR-IIOR, Hyderabad during *kharif* 2018. The study was conducted in a Complete Randomized Design (CRD) with ten treatments and four replications. Ten different fungicides belonging to different groups/ mode of action viz., carbendazim, hexaconazole, kresoxim - methyl, propiconazole, difenoconazole, tebuconazole, azoxystrobin, fenamidone + mancozeb, pyraclostrobin + fluxapyroxad and metiram + pyraclostrobin were screened against the *B. ricini* under laboratory conditions to find out their relative efficacy in inhibiting the growth of the pathogen in culture by the "Poisoned food technique" (Schmitz 1930) at 20, 50, 100, 250, 500, 750, 1000 ppm concentrations. Oat meal agar medium was prepared and amended with different concentrations of the fungicides. About 20 ml of sterilized culture medium was poured in 9 cm Petri dish. After solidification, the plates were inoculated with a 5 mm disk of seven day-old *B. ricini* culture. The Petri plates were incubated at 23 ± 1^{o}C. One set of control was maintained without adding any fungicide to the medium. Four replications were maintained for each concentration and radial growth was recorded. The observations were recorded until the control plate showed complete growth of the pathogen. The data of radial growth of fungal colony was measured and the per cent inhibition over control was calculated by the following formula given by Bliss (1934).

Per cent inhibition over control= [(C-T)/T] × 100.

Where, C = Growth of fungus in control.

T = Growth of fungus in treatment.

Testing the efficacy of fungicides against *B. ricini* under glasshouse conditions

Testing of the fungicides was carried out using detached racemes (spikes) of susceptible castor cv. DCH-519 in a glass house where temperature was maintained at 25 ± 2 °C with relative humidity above 90% and continuous wetness on racemes (Prasad et al. 2017). Spikes / racemes of 15–20 day along with 10 cm stalk was cut from castor plants, cut end of stalks was immersed in 2% sucrose solution in conical flask and sprayed with spore suspension (10^6 conidia/ml) of *Botryotinia ricini.* Ten different fungicides was sprayed at their respective doses on detached racemes of castor by using an atomizer sprayer. The conidial suspension was spray inoculated on detached racemes of castor by using an atomizer sprayer. Severity of disease was recorded.

Testing the efficacy of fungicides against *B. ricini* under polyhouse conditions

To identify suitable fungicide for the management of gray mold disease, castor cultivar DCH-519 was grown in rows at a spacing of 90 × 60 cm in polyhouse at ICAR-IIOR, Hyderabad with four replications using randomized complete block design during 2018 and 2019 cropping season. Artificial inoculation of the pathogen was done on 10 day old racemes. Humidity and temperature (average humidity > 90% and temperature around 25^{o}C) were maintained by drawing air through cooling pad and fan system. Wetness on racemes was maintained by fogging for 3 min per hour during the day (Prasad et al. 2016). Optimum soil nutrient status were maintained through application of fertilizers, manures, or other sources of plant nutrients. Conidial inoculum (10^6 conidia $ml^{-1)}$ from seven-day-old *Botryotinia* culture and fungicides were sprayed at 0.1 per cent concentration on 10- day-old racemes. Gray mold disease severity was recorded as per disease assessment scale of Chagas et al. (2010) and disease severity recorded every day starting from 3rd day after inoculum spray till complete spread of gray mold on racemes of a susceptible genotype.

Field experiment

The research experiment was conducted at ICAR-IIOR, Rajendranagar during 2019-20 and 2020-21 to evaluate the efficacy of three fungicides viz., propiconazole, pyraclostrobin + fluxapyroxad and carbendazim selected based on glasshouse and polyhouse screening on gray mold disease of castor with one check (unsprayed). Randomized complete block design was laid with four treatment and five replications using cultivar DCH-519 at a spacing of 90 × 60 cm. Minimum temperature of 21^{o}C, maximum temperature of 28^{o}C, a mean RH of 94 per cent and 14 h of continuous wetness for a period of 4–5 days as recorded by the wireless sensor network (WSN) deployed in the field and weather data was collected from the central server during the season which favoured the disease development.

Based on alerts on gray mold occurrence generated by castor gray mold -decision support system (DSS) developed by ICAR-IIOR (Annual Progress Report of Castor 2020), the fungicides were sprayed at 0.1 per cent concentration 3 days before initiation of gray mold infection. Inoculation methods, fungicides, spray timings and assessment methods were the same as those used for the polyhouse experiment.

Table 1 Efficacy of fungicides against *B. ricini* under in vitro conditions using poisoned food technique

Fungicides	Per cent inhibition of mycelial growth*						
	Concentration (ppm)						
	20	50	100	250	500	750	1000
Propiconazole	95.2 (8.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Carbendazim	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Fenamidone + Mancozeb	36.3 (57.2)	46.6 (48.0)	87.5 (13.7)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Pyraclostobin + Fluxapyroxad	84.4 (14.0)	88.8 (10.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Difenoconazole	82.7 (15.5)	92.7 (6.5)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Azoxystrobin	5.5 (82.5)	6.9 (81.2)	16.6 (73.3)	38.1 (45.5)	82.7 (21.5)	98.3 (5.5)	100 (0.0)
Tebuconazole	81.3 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Hexaconazole	76.6 (3.0)	93.4 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Meitarm + Pyraclostobin	28.5 (64.3)	51.6 (43.5)	78.3 (19.5)	96.2 (9.5)	100 (0.0)	100 (0.0)	100 (0.0)
Kresokim-methyl	33 (60.2)	35.5 (58.0)	43.3 (51.0)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
Factors	SE(d)			C.D.			
Fungicides (A)	0.90			1.78			
Concentrations (B)	0.75			1.49			
Factor (A X B)	2.39			4.71			

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

Results and discussion

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

The efficacy of ten systemic fungicides viz., carbendazim, hexaconazole, kresoxim - methyl, propiconazole, difenoconazole, tebuconazole, azoxystrobin, fenamidone + mancozeb, pyraclostrobin + fluxapyroxad and metiram + pyraclostrobin was tested under in vitro by poisoned food technique at 20, 50, 100, 250, 500, 750 and 1000 ppm concentration and the results are presented in Table 1. Most of the fungicides were effective in inhibiting the colony growth of the fungal pathogen to varying degrees. Significant difference in growth inhibition the mycelial growth of the pathogen among the fungicides was observed.

Of all the fungicides tested, complete inhibition (100%) of growth of the pathogen over control was observed with two fungicides viz., carbendazim and propiconazole at 50 ppm concentration when compared to control. Combination fungicide pyraclostrobin + metiram with mean per cent inhibition of 78.3 per cent and fenamidone + mancozeb with 87.5 per cent of inhibition of pathogen at 100 ppm concentration were at par in inhibiting the growth of the pathogen. Azoxystrobin was least effective with 16.6 per cent mycelial growth inhibition at 100 ppm conc.

Similar results were obtained by Koycu et al. (2012) who tested the sensitivity of *Botrytis cinerea* to cyprodinil + fludioxonil, fenhexamid, procymidone, pyrimethanil and tebuconazole and reported the effectiveness of cyprodinil + fludioxonil and tebuconazole against the *B.cinerea* at 100 ppm concentration compared to other fungicides. Yamuna (2015) reported that fungicides such as carbendazim, tebuconazole, propiconazole and trifloxystrobin + tebuconazole showed 100 per cent mycelial inhibition of the *B. ricini* pathogen at 250 and 500 ppm concentrations by poison food technique. Suyal et al. (2011) tested seven fungicides under in vitro for the control of grey mold of chickpea. Fungicides carbendazim (0.1%), iprodione (0.1%) and carbendazim (+) thiram (0.3%) showed complete mycelial inhibition of *B. cinerea*.

The three effective fungicides viz., propiconazole, pyraclostrobin + fluxapyroxad and carbendazim were further used for testing their efficacy under polyhouse and field conditions against *B. ricini*.

Evaluation of fungicides against *B. ricini* under glass house conditions

Ten fungicides at seven concentrations were evaluated against castor gray mold using detached spike/raceme technique. The detached spikes were first inoculated with fungicides and then with pathogen spore suspension (10^6 conidia/ml) 24 h after fungicide application. The disease progress in various treatments was assessed using the disease severity scale, when maximum disease severity was observed on susceptible check (cv. DCH 519) and the data presented in Table 2. Results revealed that all the fungicides have significantly reduced the infection on capsules compared to infection on susceptible check. The mean per cent infected capsules were nil or minimum in the racemes sprayed with propiconazole, pyraclostrobin + fluxapyroxad and carbendazim at 100 ppm concentration, which were significantly at par with each other. Fungicide difenoconazole, hexaconazole and tebuconazole were significantly superior to combination fungicide pyraclostrobin + metiram. The per cent

Table 2 Efficacy of fungicides against *B. ricini* under glasshouse conditions using detached spike technique

Fungicides	Disease severity*						
	Concentration (ppm)						
	20	50	100	250	500	750	1000
Propiconazole	6.5 (25.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Carbendazim	37.5 (52.7)	18.5 (36.2)	5.0 (23.8)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Fenamidone + Mancozeb	86.0 (58.9)	63.0 (50.7)	50.0 (46.9)	37.5 (43.0)	18.5 (43.0)	10.0 (31.0)	4.0 (23.8)
Pyraclostobin + Fluxapyroxad	10.0 (28.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Difenoconazole	37.5 (41.1)	16.3 (27.6)	10.6 (16.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Azoxystrobin	91.3 (66.1)	85.3 (61.1)	76.0 (61.1)	50.0 (43.0)	37.5 (35.2)	33.3 (27.2)	15.0 (19.2|)
Tebuconazole	50.0 (43.0)	33.3 (37.2)	16.3 (30.9)	4.0 (16.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Hexaconazole	50.0 (41.1)	37.5 (33.1)	12.0 (22.5)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Meitarm + Pyraclostobin	82.6 (61.1)	58.6 (52.7)	33.3 (43.0)	20.6 (29.9)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Kresokim-methyl	93.3 (66.1)	70.6 (57.8)	58.6 (48.8)	41.6 (39.1)	25.0 (21.1)	0.0 (0.0)	0.0 (0.0)
Factors	SE(d)			C.D.			
Fungicides (A)	0.87			1.72			
Concentrations (B)	0.73			1.44			
Factor (A X B)	2.31			4.57			

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

Table 3 Efficacy of fungicides against *B. ricini* under polyhouse conditions

Fungicides (0.1%)	Disease severity (%)*	
	2018–2019	2019–2020
Propiconazole	5.3 (13.7)	6.9 (15.0)
Carbendazim	24.2 (30.6)	23.3 (29.8)
Fenamidone + Mancozeb	45.2 (42.2)	43.7 (40.9)
Pyraclostobin + Fluxapyroxad	10.2 (18.5)	11.4 (19.5)
Control	66.6 (54.7)	69.6 (56.9)
C.D	2.6	2.4
SE(d)	1.0	1.1
C.V	4.2	4.7

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

infected capsules were maximum in raceme sprayed with azoxystrobin and kresoxim-methyl which were significantly at par with each other. The efficacy of various treatments in terms of disease control is depicted in Table 3. It was evident from the results that the per cent infected capsules were significantly reduced in all the fungicidal treatments tested at 100 ppm concentration compared with control.

Similar results were obtained by Siva Kumar et al. (2011) who reported carbendazim as the most effective treatment with low incidence of gray mold on detached castor racemes under greenhouse conditions. Yamuna (2015) evaluated seven fungicides against castor gray mold under a closed polythene humid chamber using detached spike technique. The mean per cent infected capsules were minimum in the racemes sprayed with carbendazim (5.0%), trifloxystrobin + tebuconazole (7.3%) and tebuconazole (9.6%) at a concentration of 250 ppm, which were significantly at par with each other. The fungicide mancozeb was least effective in reducing castor gray mold with per cent infected capsules of 85.6% respectively.

Evaluation of fungicides against *B. ricini* under poly house conditions

The results of two seasons revealed that all the fungicides used in the experiment significantly reduced disease severity as compared to control plot (Table 3). The treatment propiconazole (0.1%) was found to be significantly superior over pyraclostrobin + fluxapyroxad, carbendazim and fenamidone + mancozeb fungicides in controlling the per cent disease severity. Highest disease severity was recorded in control (unsprayed) plots.

James and Woo (1984) reported that the fungicides viz., chlorothalonil, iprodione and captan reduced *Botrytis* infection and improved the survival of western larch seedlings in the green house. Moorman and Lease (1992) reported that the fungicide vinclozolin applied singly, provide the best in control of *B. cinerea* in geranium followed by chlorothalonil and mancozeb under greenhouse conditions. Foliar application of Triazole fungicides (Tebuconazole and fenbuconazole) are highly effective against *B. cinerea* under controlled conditions. (Yunis et al. 1990; Elad et al. 1992). According to Maiti et al. (1988), application of carbendazim (0.05%) or thiophanatemethyl (0.05%) twice at 15 day interval after emergence of racemes gave good control of castor gray mold caused by *B. ricini*.

Table 4 Effect of different fungicides against gray mold under field conditions during 2019-20

Fungicides (0.1%)	Disease severity (%)*	Seed yield (kg/ha)	Increase in yield over control (kg/ha)	Increase in yield over control (%)	Cost of increased yield (Rs.) (A)	Plant protection cost* (Rs.) (B)	Net profit (Rs.) A-B	IBCR**
Propiconazole	8.0 (16.3)	1814	1184	65.2	52,096	11,250	40,846	1:3.6
Carbendazim	29.3 (32.7)	1269	639	50.3	28,116	8,750	19,366	1:2.2
Pyraclostobin + Fluxapyroxad	14.1 (22.0)	1544	914	59.1	40,216	11,250	28,966	1:2.6
Control	77.5 (61.6)	630						
C.D	6.6	103.1						
SE(d)	2.6	41.3						
C.V	9.2	7.4						

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

**Market price of castor seed: Rs.44/kg; ICBR = Net profit/Plant protection cost

Table 5 Effect of different fungicides against gray mold under field conditions during 2020-21

Fungicides (0.1%)	Disease severity (%)*	Seed yield (kg/ha)	Increase in yield over control (kg/ha)	Increase in yield over control (%)	Cost of increased yield (Rs.) (A)	Plant protection cost* (Rs.) (B)	Net profit (Rs.) A-B	IBCR**
Propiconazole	7.9 (16.3)	1827	1204	65.9	52,976	11,250	41,726	1:3.7
Carbendazim	29.8 (33.1)	1258	635	50.4	27,940	8,750	19,190	1:2.2
Pyraclostobin + Fluxapyroxad	14.2 (22.0)	1539	916	59.5	40,304	11,250	29,054	1:2.6
Control	78.0 (62.0)	623						
C.D	6.5	102.8						
SE(d)	2.4	40.9						
C.V	9.1	7.3						

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

**Market price of castor seed: Rs.44/kg; ICBR = Net profit/Plant protection cost

Evaluation of fungicides against *B. ricini* under field conditions

Three effective fungicides viz., propiconazole, carbendazim and pyraclostrobin + fluxapyroxad were tested against *B. ricini* under field conditions and the results are indicated in Table 4 & 5. Perusal of the two years field data indicated that the fungicide propiconazole 0.1 per cent significantly reduced the gray mold severity of 89.7 per cent over control under field condition and proved to be superior in managing gray mold disease of castor than other fungicides. Castor seed yield data was also found to be significantly influenced by the application of fungicides during 2019- 20 and 2020-21. Among the fungicides, propiconazole sprayed plots recorded highest seed yield (1814–1827 kg/ha) was found most effective and proved to be most economical with higher benefit- cost ratio of 1:3.7 followed by pyraclostrobin + fluxapyroxad and carbendazim respectively. The lowest seed yield (623–630 kg/ha) was obtained in control (unsprayed) plot (Table 4 & 5).

Propiconazole fungicide have a single site mode of action which inhibits sterol 14a-demethylation in Botrytis and exhibits systemic action *via* the xylem to different plant tissues. High antifungal activity was found towards fungal species such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Alternaria* spp. and *Leptosphaeria* spp. makes these triazoles fungicides very effective at low doses (Pernak et al. 2015). The present study also indicated that propiconazole a systemic fungicide could inhibit the growth of *B. ricini* when compared to other fungicides. Application of tebuconazole, iprodione, and procymidone fungicides are highly effective against gray mold, only if they are applied at the start of disease development and on a weekly basis (Soares 2012; Bhat and Rajasri 2015; Dange et al. 2005) found that two prophylactic sprays of propiconazole or carbendazim (0.05%) can effectively reduce castor gray mold severity. According to Sudhakar et al. (2010), the use of two prophylactic sprays with carbendazim (0.05%) at the flowering stage and immediately after the appearance of the first symptoms decreases the disease spread. Under field conditions, efficacy of propiconazole, difenoconazole, fenamidone + mancozeb, carbendazim, tebuconazole were tested against gray mold of chickpea. Fenamidone + Mancozeb sprayed at the rate of 1 g/L with 7 days interval gave the lowest disease severity with increased yield (Shahiduzzaman 2015).

In summary, investigations carried out under in vitro have shown that the fungicides viz., propiconazole, carbendazim and pyraclostrobin + fluxapyroxad were highly effective in reducing the mycelial growth and disease severity in castor racemes against *B. ricini.* In field trials timely spray of fungicide propiconazole 0.1%- one spray before disease initiation followed by 2nd spray after 7 days was very effective in reducing the disease severity by 89.6 per cent and increasing the seed yield to 1184 kg/ha for the management of gray mold disease of castor and economic benefit to the castor growers.

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