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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 05

Res Jr of Agril Sci (2021) 12: 1876–1879

CARAS

Res. Jr. of Agril. Sci. (Sep-Oct 2021) 12(5): 1876–1879
www.rjas.org

Full Length Research Article

Morphological Characters of *Fusarium oxysporum* and *Colletotrichum capsici* Isolates and their Sensitivity using Biopesticide

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Received: 04 Jul 2021 | Revised accepted: 23 Sep 2021 | Published online: 20 Oct 2021

A B S T R A C T

Anthracnose and wilt diseases of chilli samples were observed and collected the infected material from field and storage regions of Maharashtra. *F. oxysporum* f.sp. capsici and *C. capsica* were severe pathogens on chilli. Management of anthracnose and wilt is equally important to increase productivity of chili in Maharashtra. Recently biopesticides was the alternative of fungicide therefore, today uses bio-pesticides are eco-friendly without hazardous to crop and also soil. The management of crop diseases is much more significance applied aspects in the present investigation. Morphological characters and their sensitivity were studied. All isolates of each pathogen were tested their sensitivity against Biopesticide (*Lawsonia inermis* L). MIC values ranged between of 36.29µg/ml to 160 .07µg/ml while 19.31 µg/ml -110.12 µg/ml respectively. The isolate *Fo*-3 was sensitive (MIC-36.29 µg/ml) while *Fo*-4 was resistant (MIC -160.07 µg/ml) while *Cc*-10 was sensitive (MIC-19.31 µg/ml) and *Cc*-7 resistant (MIC- 110.12 µg/ml) *in vitro.* The highest resistance factors went up to 4.41 and 5.70 respectively.

***Key words*:** *Fusarium, Colletotrichum,* Characters, MIC, Biopesticide, Chilli

Chilli (*Capsicum annuum* L.) is an important part of vegetable belongs to family Solanaceae. It is originated in southern American tropics and is presently being cultivated throughout the world including tropical, subtropical and temperate regions [10]. Chilli is used in fresh cooked, pickled, canned in sauces and powdered for hot spices in daily kitchens. So, it is one of the most important commercial and industrial crops of India. It is grown almost all over the country. Different varieties of capsicum i.e., *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. pubescens* are also cultivated in different parts of the world. Chilli play is an important role in Indian food i.e., spice and condiments in all kitchen. It contains numerous biochemical compounds like steam volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, proteins, fibre and mineral elements [1]. Chilli contains pigments like capsaicin and rich source of vitamins especially vitamin C [14-15]. India is the world's largest producer, consumer and exporter country of chillie. In India chilli contributes about 36% to the total world production [13]. Nandurbar is larger producer district in Maharashtra. In India during 2019-20 Andhra Pradesh tops the list in dry chilli production of 6.66 lakh tonnes covered under 1.43 lakh ha with 4657 kg/ha productivity. According to 3rd advance estimates of the government of Andhra Pradesh, chilli production is estimated at 8.36 lakh tonnes grown under the area of 1.8 lakh ha with a productivity of 4644 kg/ha during 2020-21 [16]. The fruit of chilli is a main creature of Indian food and sustainability of chilli productions day to day threatened by various types of biotic and abiotic factors like fungi, bacteria, viruses, aphids, nematodes and temperature, light, rainfall, herbicides, pesticides which cause directly or indirectly significant yield losses in chili production all over the world. *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus niger*, *Phythium*, *Rhizoctonia*, *Penicillium expansum*, *Botrytis cinerea*, *Colletotrichum capsici*, *Rhizopus nigricans*, *R. stolonifer* and *Phytophthora cinnamon* are caused different disease to chilli [12]. Out of these *Colletotrichum capsica* (Syd.) E. J. Butler & Bisby *(Cc)* and *Fusarium oxysporum* f.sp. capsici Schlecht. Emend. Snyder & Hansen *(Fo)* caused anthracnose and wilt diseases to chilli respectively to reduce the crop productivity. Both diseases are the major economic constraints to chilli production worldwide. Total 15 isolates of *C. capsici* and *F. oxysporum* were isolated from collected rotted chilli of each pathogen and their sensitivity tested against *Lawsonia inermis* L. *Fusarium oxysporum* is a pathogenic as well as non-pathogenic fungi which caused a severe disease to plant crop and reduces the productivity of yield. The interaction of between biopesticide and

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inoculated *Fusarium* did not significantly affect the parameters of nitrogen and phosphorus content but it was significantly affected potassium content [3]. Morphological characters of *S. rolfsii* on ground nut was observed [7]. Recently farmer avoid chemical fungicides for the management of chilli diseases and alternative of fungicides use plant extract and maintained fertility of soil and reduce the chemical fertilizers as well as fungicides.

MATERIALS AND METHODS

Collection and isolation of pathogen

Chilli (*Capsicum annuum* L) is an important spices and condiments crop India. Survey was made in field and storage condition of infected chilli frequently. Observing symptoms of anthracnose and wilt of chilli were collected in polythene bags and brought at laboratory. Same infected chillies were kept for 24 hrs. in laboratory at room temperature. Rotted chillies were cut into small pieces 3-4 mm sterilized with 1% $HgCl_2$ and washed 3-4 times with D/W. Three treated small pieces of each disease symptoms were kept on PDA in each petri dish and incubate for two days at room temperature. The hyphal tips of pathogen were emanating from the tissue of infected chilli parts and transferred to new PDA petri plates for purification and further growth of pathogen. Fungal growth was observed and isolated it using single spore isolation technique.

Cultural and morphological characters

Same pathogens were inoculated on sterilized PDA in 90mm petri plates and incubated at 28±2°C at room temperature. Parameters of each isolate like colony, size, pigmentation, conidia and formation pattern was recorded after the incubation of 7-8 days. The mycelial growth rate was observed after every 24-hour interval till the last petri plate was completely colonized. The *F. oxysporum* and *C. capsica* were identified using literature and conidia with mycelium on the basis of their morphological character. *Fusarium* was showed white cottony growth, mycelium septate, circular, micro and macro conidia with 3-4 septa while *Colletotrichum capsici* showed white fluffy, cottony, white, irregular colony, hyaline spore [11].

Sensitivity of F. oxysporum and Colletotrichum capsici

Fifteen isolates of each pathogen were isolated from rotted chillies. Sensitivity tested against *Lawsonia inermis* L. aqueous extract taking the same isolates at different concentration (10-100%). Fresh culture of each pathogen was used for the same and transferred aseptically on *Lawsonia inermis* L. aqueous extract containing PDA petri plates at different concentration. The plates were kept at incubation for further growth. The growth of the pathogens was observed after every 24 hours till control petri plate was not completely colonized. After that the sensitivity result were recorded. Similarly, Sensitivity of all isolates was tested against *Lawsonia inermis* by food poisoning method [8].

RESULTS AND DISCUSSION

Morphological characters of F. oxysporum f. sp. capsici Schlecht. emend. snyder & Hansen and C. capsica (Syd.) E. J. Butler & Bisby

Morphological characters of *Fusarium oxysporum* Link produced red pigments, colourless sporulation and scattered cottony mycelium growth, large quantity of microconidia, one celled, comma shaped, hyaline and macroconidia,3-4 septate, half-moon shaped, pointed ends and resting chlamydospores (Plate 1) and *Colletotrichum capsici* Corda is produced regular margin with cottony fluffy mycelium with small conidia with or without setae (Plate 2). This result is correlate with cultural and morphological characterization of *Colletotrichum capsici* produces colony colour white to grey, yellow pigmentation, with fluffy mycelium [5].

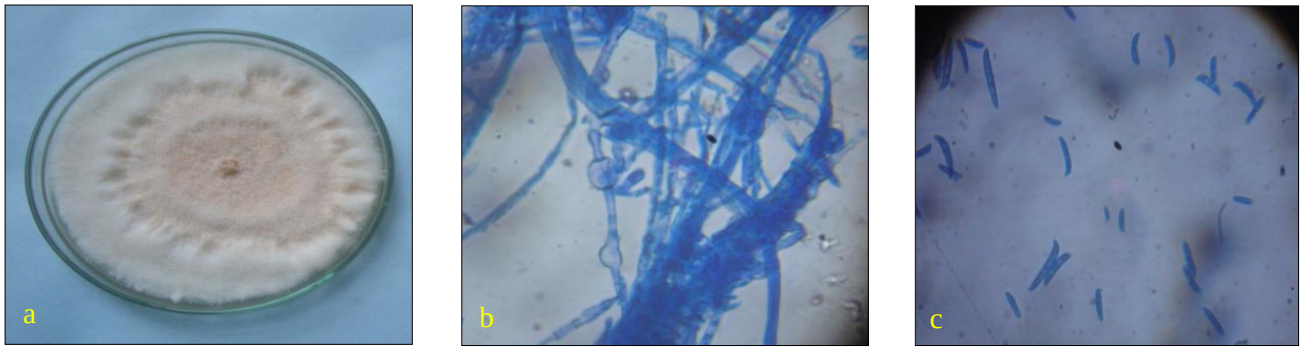

Plate 1 a) Pure culture of *F. oxysporum* L., b) Mycelium with microconidia and c) macroconidia

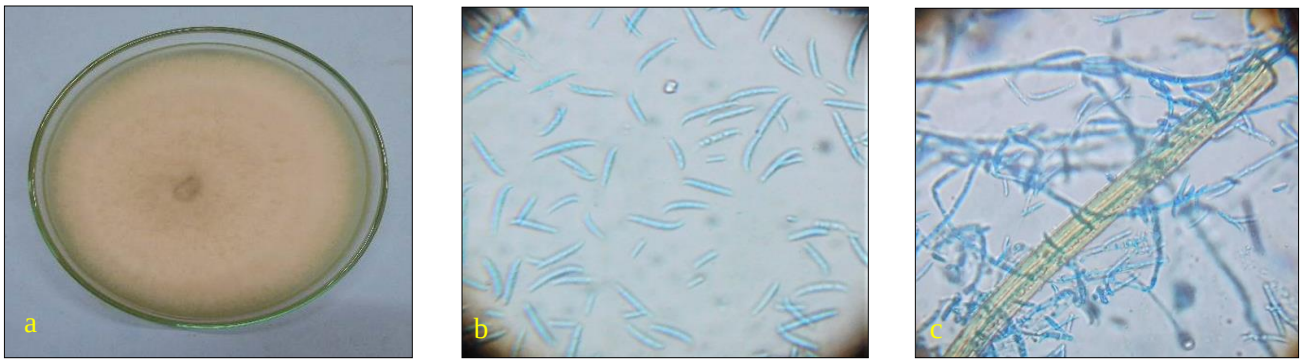

Plate 2 a) Pure culture of *Colletotrichum capsici* Corda, b) Conidia and c) Mycelium with conidia

Sensitivity tested against Lawsonia inermis L. aqueous extract

All these isolates were tested their sensitivity against *Lawsonia inermis* L. Results are depicted in (Table 1-2). The sensitivity of *Fusarium oxysporum* and *Colletotrichum capsica* were tested using *Lawsonia inermis* L. aqueous extract. MIC values were in ranged of 36.29µg/ml to 160 .07µg/ml while 19.31µg/ml -110.12µg/ml respectively. The isolate *Fo*-3 was sensitive MIC-36.29 µg/ml while *Fo*-4 was resistant MIC -160.07 µg/ml while *Cc*-10 was sensitive (19.31 µg/ml) and *Cc*-7 resistant (MIC- 110.12 µg/ml) *in vitro* (Plate 3-4). The highest resistance factors went upto 4.41 and 5.70 respectively. Also, the results of the present study are similar to in vitro evaluation of bio-control agents, biopesticide and botanicals against *Colletotrichum truncatum* causing anthracnose of horse gram [9]. Similar results correlated with the antifungal activity in aqueous extracts, ethyl acetate and ethanol extract at 1mg/100µl of flowers and leaves against some selected phytopathogenic fungi are suitable sources for further screening of bio-pesticides [4]. Fungicide resistance in fungal pathogens of various crops and its integrated management was illustrated [17]. Similar results correlated with the management approach towards disease of anthracnose caused by *Colletotrichum spp.* to chilli [6]. Results of present study is correlated with using plant extract for inhibitory growth of *C. capsici* under in vitro observed highest radial growth (57.78%) in *Polyalthia* methanol and highest inhibition of biomass production was observed in ginger chloroform (32.78%) [2]. Statistical analysis of (Table 1-2) indicated that standard error, critical difference was hypothetically significance.

Table 1 Sensitivity of *Fusarium oxysporum* f. sp. capsici against *Lawsonia inermis* L. using agar medium

Isolate No.	Location	Data characteristic to dose response curve (*In-vitro*)					
		Regression constant	Regression coefficient	Correlation coefficient	ED50 (µg/ml)	MIC (µg/ml)	R/F
Fo - 1	Akole	87.12111	-0.88613	-0.98771	65.92	133.25	3.67
Fo - 2	Alephata	76.68583	-0.94558	-0.96053	28.56	85.33	2.35
Fo - 3	Bhandardara	50.52778	-0.70833	-0.86377	21.71	36.29	1.00
Fo - 4	Ghoti	93.80556	-0.585	-0.98041	37.26	160.07	4.41
Fo - 5	Kalyan	44.41667	-0.253	-0.99306	52.36	106.46	2.93
Fo - 6	Karjat	86.91667	-0.29833	-0.98358	56.47	105.70	2.91
Fo - 7	Kolhapur	84.63389	-0.86383	-0.9254	40.17	84.19	2.31
Fo - 8	Malegaon	42.74056	-0.4089	-0.92952	38.03	80.05	2.20
Fo - 9	Nashik	81.77778	-0.645	-0.99798	51.67	108.37	2.98
Fo - 10	Osmnabad	65.37111	-0.7378	-0.93875	46.73	94.38	2.60
Fo - 11	Pune	80.86111	-0.66167	-0.99872	39.16	104.06	2.86
Fo - 12	Satara	88.09333	-0.8678	-0.98874	50.03	110.81	3.05
Fo - 13	Shahapur	81.37111	-0.87113	-0.9949	25.76	95.13	2.62
Fo - 14	Sinner	74.12083	-0.85502	-0.97054	33.28	98.99	2.72
Fo - 15	Yavatmal	89.47222	-0.57167	-0.98944	22.36	94.64	2.60
			SE	: 3.38			
			SE	: 6.90			
			CD at 0.05	: 2.624			
			0.01	: 1.761			

*Values of three replicate

Whereas *Fo* – *Fusarium oxysporum*, ED_{50} - Ethal Dose, MIC –Minimum Inhibition, Concentration, R-Resistant, F-factor, SE – Standard Error, CD –Critical Difference

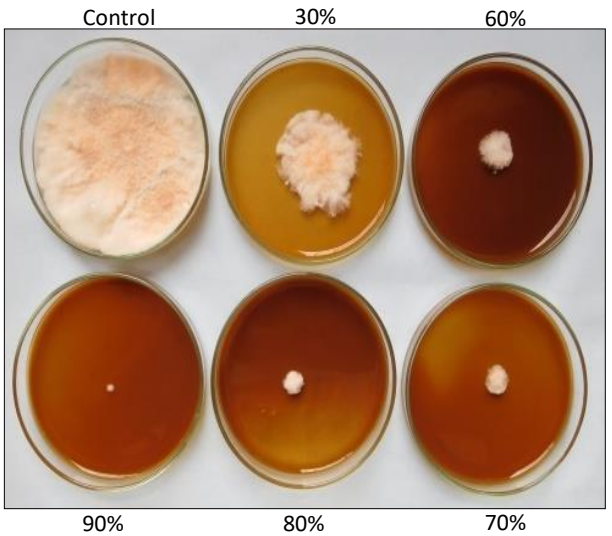

Plate 3 Sensitivity of *F. Oxysporum* Link against *Lawsonia inermis* L. using PDA

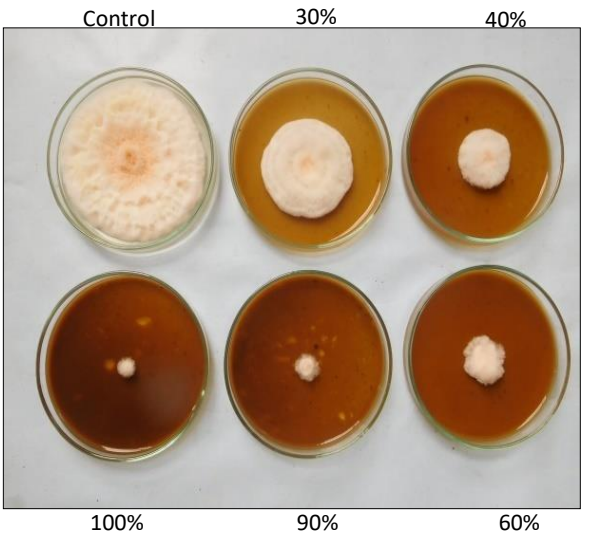

Plate 4 Sensitivity of *Colletotrichum capsici* Corda against *Lawsonia inermis* L. using PDA

Table 2 Sensitivity of *Colletotrichum capsici* against *Lawsonia inermis* L. using agar medium

Isolate No.	Location	Data characteristic to dose response curve (*In-vitro*)					
		Regression Constant	Regression Coefficient	Correlation Coefficient	ED50 (µg/ml)	MIC (µg/ml)	RF
Cc - 1	Akole	75.08333	-0.88833	-0.9822	42.02	90.06	4.66
Cc - 2	Alephata	64.38889	-0.59667	-0.99028	52.69	67.38	3.48
Cc - 3	Bhandardara	67.55556	-0.87333	-0.96691	25.78	52.27	2.70
Cc- 4	Ghoti	26.95333	-0.365	-0.92264	27.23	57.02	2.95
Cc- 5	Kalyan	57.65833	-0.69837	-0.90583	47.00	95.34	4.93
Cc- 6	Karjat	77.45444	-0.8128	-0.98866	51.67	108.29	5.60
Cc- 7	Kolhapur	56.91667	-0.53833	-0.89889	51.32	110.12	5.70
Cc- 8	Malegaon	37.72222	-0.29667	-0.84311	41.46	87.52	4.53
Cc- 9	Nashik	90.69444	-0.805	-0.99565	50.89	108.37	5.61
Cc- 10	Osmnabad	55.38889	-0.79667	-0.78006	09.45	19.31	1.00
Cc- 11	Pune	53.09306	-0.59668	-0.99625	42.36	90.26	4.67
Cc- 12	Satara	73.17639	-0.91168	-0.93745	49.98	102.42	5.30
Cc- 13	Shahapur	67.02778	-0.75167	-0.9985	45.90	95.03	4.92
Cc- 14	Sinner	68.13889	-0.89833	-0.93661	35.48	77.47	4.01
Cc- 15	Yavatmal	57.04667	-0.7128	-0.95678	40.46	83.46	4.32
			SE :	3.12			
			SE :	6.46			
			CD at 0.05 :	2.624			
			0.01 :	1.761			

*Values of three replicate

Whereas, *Cc-Colletotrichum capsici*, ED_{50}-Ethal Dose, MIC –Minimum Inhibition, Concentration, R-Resistant, F-factor, SE – Standard Error, CD –Critical Difference

CONCLUSION

The present paper investigation that altogether 15 isolates of *F. oxysporum* and *Colletotrichum capsci* was isolated from anthracnose and wilt of chilli was studied. For their 15 isolates sensitivity was tested against biopesticides. There was quite large variation in MIC of biopesticide. Fo-3 isolate was sensitive while Fo-4 was resistant where as in case of *Colletotrichum*, Cc-10 isolate was sensitive while Cc-7 was resistant.

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