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MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *COLLETOTRICHUM FALCATUM* ISOLATES

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ABSTRACT

A survey was conducted to find out the prevalence of red rot incidence and found that maximum red rot incidence was observed in the sugarcane cultivar Co 0238 (45%) dominating over all the varieties growing in both the districts viz. Samastipur and Gopalganj covering more than 70 per cent sugarcane area. Cultivars BO 91 and Rajendra Ganna- 1 were found free from the infection of red rot. Seven isolates were obtained from the collected diseased specimen where the colour of the mycelium of all the isolates varied as whitish grey, greyish white, and pinkish white. The texture of the mycelium was fluffy, abundant, aerial, scanty raised mycelium. The shape of the conidia was observed as falcate. The average length of the conidia ranged from 21.32µm to 27.37 µm and the average width varied from 4.10 µm to 4.39 µm. Spores were counted with the help of haemocytometer. Number of spores ranged from 3.2 x 10^4 spores/ml to 4.6 x 10^4 spores/ml. This study showed about the morphological characterization of isolates of *Colletotrichum falcatum*.

Key words : Sugarcane, Morphological, Cultural, *Colletotrichum falcatum*, Isolates.

Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most significant cash crops cultivated worldwide for its numerous advantages and industrial marketing, showing a high bioenergy potential due to its C_4 nature and high biomass yield. It is a major source of sugar in South Asia and in the rest of the world and it is also an important bioenergy feedstock exemplified by bioethanol and bioelectricity (Hoang *et al*., 2018). Sugarcane is frequently grown for its ability to produce sugar to meet demand, but it also produces a wide range of other products, such as bagasse, molasses, jaggery, khandsari, paper, syrup, and vinegar. It is also used as animal feed. The economically important crop sugarcane dominates the second-largest agro-based sector in India next only to cotton. It is an important source of income and jobs for farming communities in India.

Red rot caused by *Colletotrichum falcatum* Went is considered of great importance as it greatly affects sugarcane production. The disease significantly reduced the quality as well as yield of sugarcane crops, leading to substantial economic losses to farmers. It was found that due to this disease cane weight was reduced up to 29%, reduction of sugar recovery up to 31 % along with sucrose content of 75%. The losses caused by red rot disease has become major constraint in the profitable cultivation of sugarcane and caused a great loss to cane growers and had a great adverse impact on cane industries (Mohan and Sangeeta, 2009).

Seed setts from diseased canes are the main source of primary inoculum and survival of *C. falcatum*. When such sets are planted, they invariably produce affected shoots. If such shoots survive and remain in the field, the secondary infection is caused by conidia produced by

acervuli on such shoots and transmitted by insects, wind, rain splashes, and irrigation water. When these conidia fall on wounds caused by cultivation tools, insects or fall on young unfold leaves, where they travel down to the nodal buds, infection is caused. Singh and Singh (1981) demonstrated the presence of septate mycelium, conidia and appressoria in infected buds. Mid rib of leaves is also affected by the fungus. Red patches with ash coloured centre developed on the mid rib. High humidity, water logged conditions, lack of cultural operations resulting in growth of weeds, continuous cultivation of the same (susceptible) variety in particular locality were some of the factors leading to red rot infection in moderate to severe form. Based on the nature of infection and presence of favourable environment, pathogen starts taking toll by killing the bud. The disease affects the germination and initial establishment of the crop (Duttamajumder, 2008). It is very important to control disease to reduce the quantity and quality losses in the sugarcane production.

Materials and Methods

A survey was conducted in the sugarcane growing areas of Samastipur and Gopalaganj districts of Bihar during the month of July, August and September, 2022 to study the red rot incidence in sugarcane in respective villages of the districts and to collect diseased samples infected with *Colletotrichum falcatum* for morphological and cultural studies. Surveys were conducted in two districts namely Samastipur and Gopalganj. During the course of survey, six villages *viz.* Satmalpur, Paridah, Shasan, Bharpura, Udaipur, Govindpur were taken from Samastipur district, whereas, Bansghat Mansuriya, Fajjullah, Salempur, Bankat,Barahiya were taken from Gopalganj district. During the survey, it was observed that the 7 varieties namely Co 0118, Co 0238, CoP 9301, BO 91, Co 0233, CoP 2061 and Rajendra Ganna-1 were mainly under cultivation in those villages as ratoon crop and as a plant. Among the 7 varieties Co 0238 was found to be dominating over all the varieties in almost every village of the two districts. Forty-two (42) samples were collected and brought to laboratory for confirming the disease and for further studies.

Collection of diseased specimens for isolation and characterization of the isolates

The diseased samples were collected from two sugarcane growing districts of Bihar *i.e.* Samastipur and Gopalganj. Diseased samples from cultivars CoSe 95422, CoSe 92433 and BO 138 were also collected from Experimental Farm, Kalyanpur, Samastipur of Department of Plant Pathology, Sugarcane Research Institute, Dr. Rajendra Prasad Central Agricultural University, Pusa for the study. Pathogen from different cultivars of different locations were isolated and named as CFS1, CFS2, CFS3, CFS4, CFS5 (Samastipur) CFG1, CFG2 (Gopalganj) and presented in Table 1. These isolates were cultured on oat meal agar medium and maintained for further studies. The coding of the isolates was given as CFXi, where CF represents *Colletotrichum falcatum*, X represents the location, i represent the sequence of which they were isolated and presented in Table 1.

Fig. 1 : Map of Bihar indicating Samastipur and Gopalganj districts where survey conducted.

Table 1 : Collection of diseased specimens of *Colletotrichum falcatum* and their sources.

S. no.	District	Number of isolates	Isolate code	Source (cultivar)	Diseased portion taken
1.	**Samastipur**	5	CFS1	Co 0238	Stalk
			CFS2	CoSe 95422	Stalk
			CFS3	CoSe 92433	Stalk
			CFS4	BO 138	Stalk
			CFS5	Co 0118	Stalk
2	**Gopalganj**	2	CFG1	Co 0233	Stalk
			CFG2	CoP 2061	Stalk

Results

The data presented in Table 2 showed that the mean red rot incidence of different cultivars grown in villages, and the overall mean of the two districts. Out of all the villages Paridah village showed maximum disease

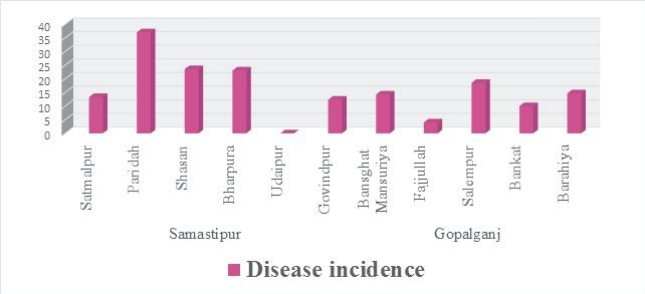

Fig. 2 : Survey on red rot incidence of sugarcane caused by *Colletotrichum falcatum.*

incidence (37%) followed by Shasan (23.5%), Bharpura (23%), Salempur (18.5) Barahiya (14.6%), Bansghat Mansuriya (14.2%), Satmalpur (13.3), Govindpur (12.3%), Bankat (9.95%). Out of all the cultivars Co 0238 showed maximum red rot incidence (45%) in Bharpura village of Samastipur district followed by Shasan (43%), Barahiya (42%), Bansghat Mansuriya (39.6%), Paridah (37%), Salempur (37%), Satmalpur (36.9%). Cultivars like BO 91, Rajendra Ganna-1 were free from red rot infection. Other cultivars like Co 0118, Co 0233 and CoP 2061 showed red rot incidence less than the cultivar Co 0238. Very little or no infection was found in cultivar CoP 9301. Between the two districts Samastipur district recorded more mean disease incidence (18.18%) compared to Gopalganj district (12.26%).

Morphological and cultural characteristics of *Colletotrichum falcatum* isolates

The seven isolates of the red rot pathogen were observed for their colour on the front side of the cultured plate, topography, conidial characteristics and sporulation of the pathogen. The data presented in the table 3 revealed that whitish grey colony colour was shown by four isolates i.e. CFS2, CFS3, CFS4 and CFS5, greyish white colony colour by two isolates i.e. CFS1, CFG2 and pinkish white colony colour by one isolate i.e.CFG1. Out of seven isolates four isolates exhibited fluffy, abundant and aerial

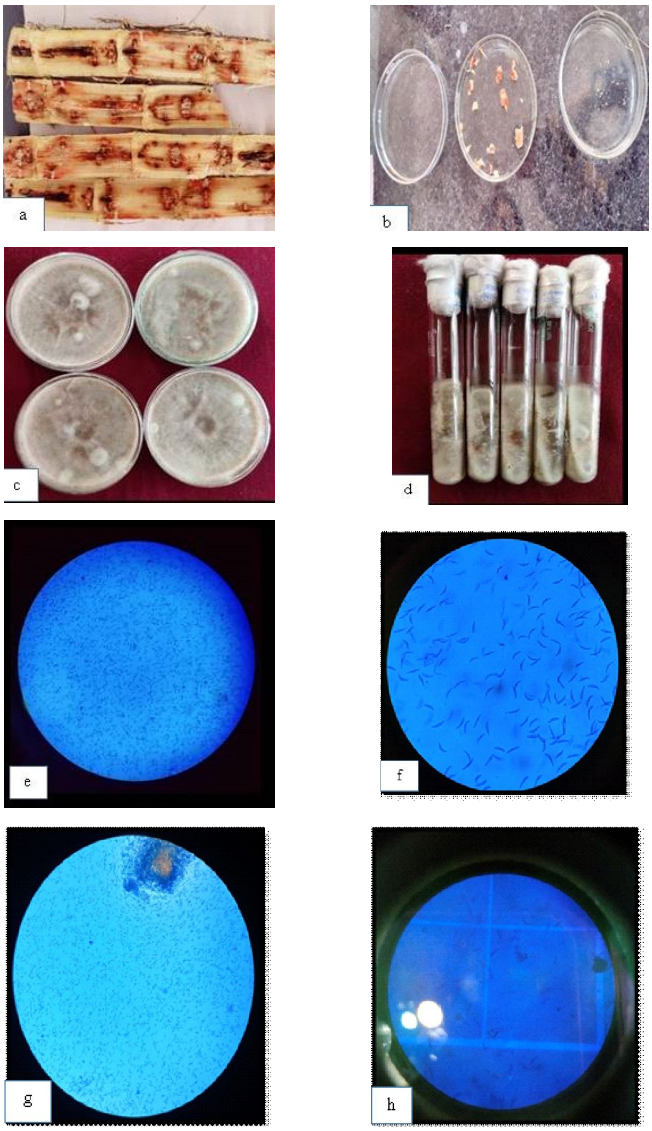

Plate 1 : Isolation and identification of pathogen. **a.** Red rot infected sample **b.** Disinfection **c-d.** Pure culture of Pathogen **e.**10X microscopic view **f.** 40X microscopic view **g.** Acervuli **h.** Counting of spores with haemocytometer.

Table 2 : Survey on red rot incidence of sugarcane caused by *Colletotrichum falcatum*.

District	Village	Cultivar	Disease incidence (%)
Samastipur	Satmalpur	Co 0118	14.0
		Co 0238	36.9
		CoP 9301	2.3
		Rajendra ganna 1	0.0
		Mean	**13.3**
	Paridah	Co 0238	37.0
	Shasan	Co 0118	4.0
		Co 0238	43.0
		Mean	**23.5**
	Bharpura	CoP 9301	1.0
		Co 0238	45.0
		Mean	**23.0**
	Udaipur	BO 91	0.0
	Govindpur	Co 0233	12.3
		Overall mean	**18.18**
Gopalganj	Bansghat Mansuriya	Co 0238	39.6
		Co 0118	3.0
		CoP 9301	0.0
		Mean	**14.2**
	Fajjullah	Co 0118	4.0
	Salempur	Co 0238	37.0
		BO 91	0.0
		Mean	**18.5**
	Bankat	CoP 9301	1.0
		Co 0233	18.9
		Mean	**9.9**
	Barahiya	Rajendra ganna 1	0.0
		Co 0238	42.0
		CoP 2061	2.0
		Mean	**14.6**
		Overall mean	**12.26**
	Overall mean of both the districts		**15.22**

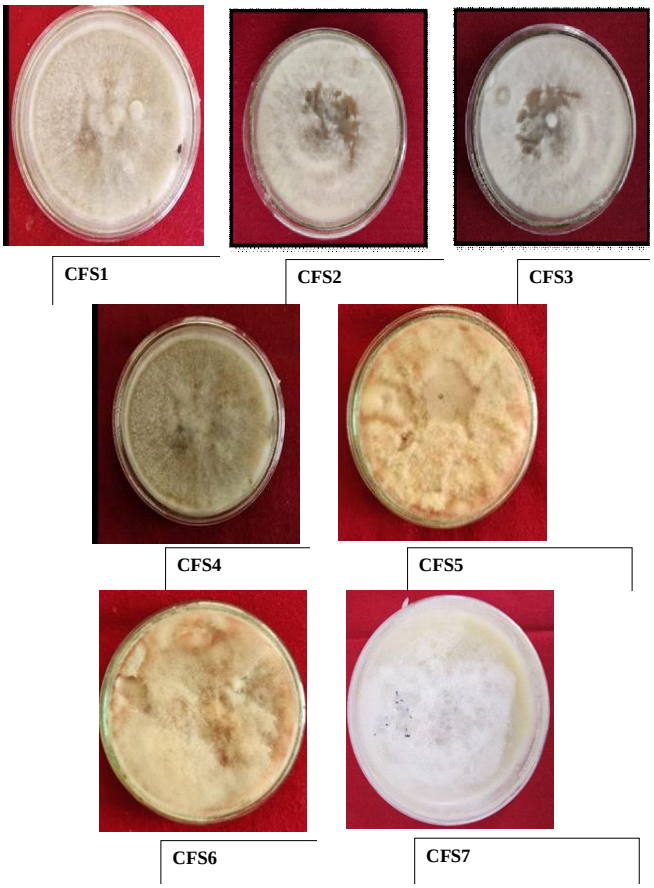

Plate 2 : Mycelial growth of 7 isolates of *Colletotrichum falcatum* on OMA medium.

mycelium i.e.CFS3, CFS5, CFG1 and CFG2, two isolates exhibited slightly raised, abundant aerial mycelium i.e.CFS1 and CFS4 and flat, scanty, sub aerial mycelium was exhibited by one isolate i.e. CFS2. All the isolates showed falcate shaped conidia, which were single celled.

Morphological studies of spores showed that average length of the conidia ranged from 21.32µm to 27.37 µm and the average width varied from 4.10 µm to 4.39 µm. The isolate which showed longest conidia was CFG1 (27.37 µm), followed by CFS3 (26.43 µm), CFS5 (26.32), CFS1 (26.23), CFS2 (24.95 µm), CFG2 (22.17 µm), CFS4 (21.32 µm) which was the least. The highest width of the conidia was shown by the isolate CFG2 (4.72 µm), followed by CFS2 (4.39), CFS4 (4.27 µm), CFS5 (4.19), CFS1 (4.13 µm) and the least was shown by two isolates *i.e.*; CFS3, CFG1 (4.10 µm).

Spores were counted with the help of Haemocytometer. Number of spores ranged from 3.2 x 10^4 spores/ml to 4.6 x 10^4 spores/ml. Maximum number of spores (4.6 x 10^4 spores/ml) was found in isolate CFS1 followed by CFS3 and CFG2 (4.1 x 10^4 spores/ml). Minimum number of spores was found in isolate CFS2 (3.2 x 10^4 spores/ml).

Table 3 : Morphological and cultural characteristics of *Colletotrichum falcatum* isolates.

S. no.	Isolates code	Colony colour	Texture of mycelium	Shape of conidia	Sporulation (x 10^4 spores/ml)	Length (µm)	Width (µm)
1.	CFS1	Greyish white	Slightly, raised abundant aerial mycelium	Falcate	4.6	26.23	4.13
2.	CFS2	Whitish grey	Flat, scanty sub aerial mycelium	Falcate	3.2	24.95	4.39
3.	CFS3	Whitish grey	Fluffy, abundant aerial mycelium	Falcate	4.1	26.43	4.10
4.	CFS4	Whitish grey	Slightly, raised abundant aerial mycelium	Falcate	3.8	21.32	4.27
5.	CFS5	Whitish grey	Fluffy, abundant aerial mycelium	Falcate	3.9	26.32	4.19
6.	CFG1	Pinkish white	Fluffy, abundant aerial mycelium	Falcate	3.3	27.37	4.10
7.	CFG2	Greyish white	Fluffy, abundant aerial mycelium	Falcate	4.1	22.17	4.72

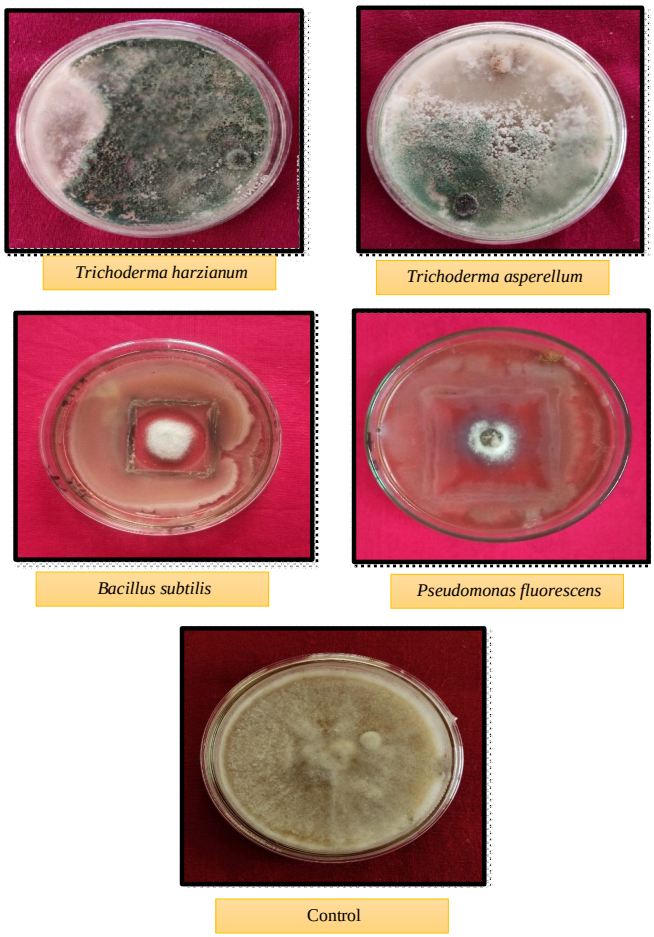

Plate 3 : *In vitro* inhibition of mycelial growth of *Colletotrichum falcatum* by different bio control agents.

Discussion

The present investigation was conducted to know the prevalence of disease incidence in two sugarcane growing districts of Bihar namely Samastipur and Gopalganj. Pertaining to the data, it can be depicted from the survey that out of all the cultivars Co 0238 exhibited maximum red rot incidence (45%) from the village of Samastipur and these cultivars namely CoP 9301, BO 91, Rajendra Ganna 1(0%) were free from the infection of red rot. Other cultivars like Co 0233, Co 0118 showed less incidence of red rot disease. Out of the two districts Samastipur district was more affected with red rot disease with mean disease incidence of 18.18%. Based on an extensive survey conducted by Singh *et al*. (2021) in sugarcane growing areas of Uttar Pradesh during the years 2015-2016 and 2020-2021, it was observed that severe red rot incidence (70-100%) occurred in the variety Co 0238 and devastated the crop in northern states of India.

Results on cultural and morphological characterization of 7 isolates of *Colletotrichum falcatum* isolated from the collected diseased samples depicted that the colour of the mycelium of all the isolates varied as whitish grey, greyish white, and pinkish white. The texture of the mycelium was fluffy, abundant, aerial, scanty raised mycelium. The shape of the conidia was observed as falcate. The average length of the conidia ranged from 21.32µm to 27.37 µm and the average width varied from 4.10 µm to 4.39 µm. The culture was confirmed and specifically identified according to the characteristics given by Abbott (1938), Carvajal and Edgerton (1944). Pandey and Shukla (2017) observed the colour of mycelium of isolates as greyish white, white and greyish. This study gave information about the red rot incidence in two districts and the morphological characterization of the isolates.

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