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PATHOGENIC BEHAVIOR OF PATHOTYPES AND EMERGING ISOLATES OF *Colletotrichum falcatum* WENT. ON 20 HOST DIFFERENTIAL IN SUGARCANE

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ABSTRACT

Colletotrichum falcatum Went. is the most destructive pathogen to cause red rot disease in sugarcane worldwide. For the past 10 years, the problem of 'Red Rot' has persisted in North-Central and Western Uttar Pradesh; this has been the primary cause of the breakdown of the popular CV Co0238 variety, leading to a continuous decline in sugarcane production and sugar recovery rates. In this experiment, samples of red rot affected cane were collected from various districts of Uttar Pradesh, and experiment was conducted on 20 hosts to identify new race. By identifying this new strain, cane farmers can be sensitized to preventive measures, thereby safeguarding against future infections by the latest strains of red rot pathogen. In the aforementioned trials wherein 8 standard pathotypes and 10 isolates were inoculated on 20 hosts using the plug method, it was observed that isolate Cf2401 isolated from Co11015 variety exhibited behaviour identical to the standard pathotype CF13, displaying the highest virulence frequency (55%). Furthermore, all isolates derived from the Co0238 variety also demonstrated behavioural characteristics consistent with those of CF13. If an isolates gives a contrast reaction with the North India pathotypes such as CF 07, CF 08, CF09 and CF13, we assume that it is a new strains of the pathogen, which we note in the category of new pathotypes. We can say that this is a new pathotype for this zone.

This isolates *i.e.*; R2406 (Cf11015) and all isolates of Cf0238 may be alarming for other commercial varieties of UP.

Key words : Sugarcane, Red rot, *Colletotrichum falcatum*, Pathogenic behavior, Virulence.

Introduction

Sugarcane (*Saccharum officinairum*) is an important cash crop for the farmer belonging to the *Poaceae*. The genus Saccharum is the richest source of disaccharides sugar accumulated in the stalk internodes. In India, sugarcane covers more than 4.5 million ha land and produce more than 26 million tons of sugarcane (Solomon, 2011). Area under sugarcane in India, is 4.90 million ha with productivity of 70.7 t/ha and sugar production about 27.9 million tones (Anonymous, 2017a). Diseases are a key source of worry for reduced yield, but other biotic and abiotic variables also contribute. In India, fungal diseases re estimated to cause a loss of 18-31%. Sugarcane crops are sensitive to several diseases, including red rot, a deadly fungal disease that poses a significant danger since seeding. Red rot affects practically all sugarcane growing countries worldwide. It reduces cane yield and sugar recovery by 29.07% and 30.8%, respectively (Hussnain and Afghan, 2006). In 1893, Went reported the first case of red rot from Java (today Indonesia). Barber (1901) made the first scientific observation of red rot disease on Red Mauritius in the Godavari delta of Andhra Pradesh, India. Multiple outbreaks were recorded in Jammu and Kashmir in 1922 and Pusa (Bihar) in 1932. The disorder received significant attention between 1938 and 1940. In 1942, an outbreak occurred in Northern Bihar and Western Uttar Pradesh, leading to the collapse of the most popular variety Co 213. In 1946-47, an outbreak of the disease struck various regions in Uttar Pradesh, leading to the failure of the popular variety Co 312 (Mathur, 1946). The disease

outbreak has led to the failure of varieties Co 1148 and CoJ 64 in Punjab and Western U.P (Satyavir, 1984). Red rot is caused by *Colletotrichum falcatum* Went, (Sexual stage *Glomerekka tucumanensis*), a facultative saprophyte. The fungal colonies are filthy and range from white to dark brown to black. The hyphae are thin, septate, branching and brown in colour. The setae range from dark brown to black. Condiophores are short, upright, simple, hyaline and not septate. Conidia are hyaline but pinkish, unicellular, thin walled, falcate to lanceolate, and contain an oil globule in the centre. (Satyavir, 2003) found that disease expression varies depending on the environment. Early detection of red rot disease can be challenging (Nithya *et al*., 2012). Red rot is not only prevalent in the east coat zone and north central & western region of India; it has also spread to the peninsular part like Gujrat (Alexander and Viswanathan, 1996). Resistance variety of sugarcane playing important role to managing red rot (Srinivasan *et al*., 1956; Viswanathan, 2010). Variability in red rot pathogen has been main reason of varietal breakdown of sugarcane varieties, considered resistant at the time of their release. Thus appearance of new strain of the pathogen such as pathotype is an important factor in the red rot epidemics and in the failure of popular varieties. In *C falcatum* pathogenic variation was noticed for the first time (Edgerton and Moreland., 1920). Since then, the existence of pathogen variability has been recognized by various workers (Chhabra *et al*., 2016), in India several workers have studied the variability among *C falcatum* isolates based on morphological, pathological & molecular characterization and host resistant from both tropical and sub-tropical regions. In subtropical India after the break down of the popular variety Co1148 in the previous decades many new pathotypes have emerged during the last three decades especially on the popular cultivers Co7717, CoJ 64, CoJ85 and CoS767 and about 11 pathotypes were designated as CF1 to CF11 (Viswanathan, 2003). The current study examines pathogenic diversity in red rot pathogen *C. falcatum* and compares the virulence potential of recently obtained isolates to the conventional pathotypes of North India's sub-tropical regions.

Materials and Methods

Survey for epidemics observation and samples collection in red rot affected sugarcane growing areas of northern-central and western Uttar Pradesh

Survey were conducting for recording the incidence of red rot in sugarcane varieties commercially growing in farmer field at different sugar factory in various district of central Uttar Pradesh, India. Sugarcane Research Institute, Shahjahanpur is situated at 27.35 N latitude and 79.37 E longitudes. Survey in sugarcane disease was carried out during post monsoon season October and November month in central Uttar Pradesh in the year of 2024-25. The collected samples were covered and bagged with clean paper covers, packed in polythene envelop and transported for pathogen isolation in the laboratory of Plant Pathology, UP Council of Sugarcane Research, Shahjahanpur.

The majority of the isolates were acquired from the popular variety Co0238, which occupied over 80% of the cane producing area in the sub-tropical region. Nine new isolates were isolated from cv., Co0238 variety and one is Co11015 at different district of U.P, India. Assessed level of virulence in new isolates during season 2024-25.

Isolation and maintenance of newly collected isolates of red rot

Affected canes samples were collected from the sugarcane varieties grown commercially by farmers at different district of U.P., the plant showing typical symptoms were taken for further studies. Collected

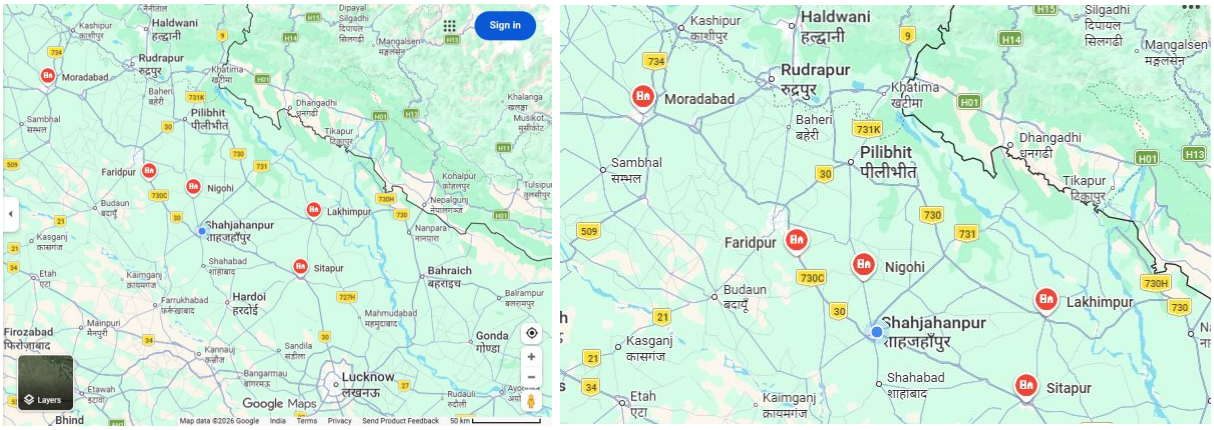


Fig. 1 : Survey work was conducted in various sugar mills areas of the following district and samples were taken from red rot affected area.

isolates of red rot and their place of collection is recorded and listed. Isolation of *Colletotrichum falcatum*, from infected cane samples is first cleaned with fresh water and allowed to dry in the shade in order to be isolated. After that 0.5% concentrated alcohol is used to clean the surface. The infected stem tissue is cut in half under aseptic circumstances, and a cork borer is used to deposit a stem cut in a petri plate. After that, the plate is sealed and left to incubate for 4-5 days at room temperature of 25±2°C with 70% Rh. All the isolates were properly maintained thorough sub-culturing timely 25 days interval on oat meal agar medium and stored for later use in further study. The map of India depicts sugarcane disease surveyed areas and sites where red rot samples were obtained.

Pathogenicity assay

A total of 20 sugarcane differentials, such as Co 419, Co 975, Co 997, Co 1148, Co 7717, Co 62399, CoC 671, CoJ 64, CoS 767, CoS 8436, B0 91, Co 86002, Co 86032, Co 7805, CoV 92102, CoSe 95422, Baragua, Khakai, SES 594 and Co0238 were used to study the pathogenic variation of *C. falcatum*. All the newly collected isolates and eight designated *C.falcatum* pathotypes (CF01, 02, 03, 07, 08, 09, 11 and CF13) were grown on oat meal agar medium under aseptic conditions. For pathogenicity assays, fresh conidial suspensions average containing 1×10^6 conidia/ml were made from 7 days old cultures grown on oatmeal agar in petri plates. The newly collected isolates and pathotypes were inoculated on 20 host differential that display clear differential reactions of compete resistance or susceptible, Co0238, Co1148, Co62399, Co86032, CoC671, CoJ64, CoS8436, CoS767, khakai and SES594 as earlier described (Vishwanathan *et al*., 2022). Two budded sets of sugarcane differential were planted during February month, in 0.9×6 meter rows of each differential, proper agronomical practice follow for better growth. After six months, July-August, 15 healthy canes free of any affection and insect attack were chosen for each isolate/pathotype inoculation. Following the plug inoculation, conidial suspension 0.5ml of each isolate were inoculated on the middle of the third internode above the soil level of the standing canes using piston like red rot inoculating device. A Pasteur pipette was used to transfer the conidial suspension into the borehole. To stop ants and other microbiological pollutants from entering the hole, the cane tissue core was inserted right after inoculation and coated with modeling clay (Mohanraj *et al*., 2012 and Viswanathan, 2012). Sixty days following pathogen inoculation, the canes were cut at ground level below the place of inoculation to examine disease development under field settings. Disease data was collected by splitting the canes lengthwise. The differentials were infected via the plug method (Srinivasan and Bhatt, 1961). Srinivasan and Bhatt,1961) reported observations on a scale of 0 to 9. The results were categorized as resistant (0-4.0), intermediate (4.1-6.0), and susceptible (>6.1). Two years of study (2024-25) were conducted in the plant pathology block at UPCSR-Shahjahanpur. Disease severity was categorized in to 5 categories such as resistant (R), moderate resistant (MR), moderate susceptible (MS), susceptible (S) and highly susceptible (HS) denote with respective scores as well as 0.0-2.0, 2.1-4.0, 4.1-6.0, 6.1-8.0 and above 8.1 to 9.0. The reaction were classified as resistant (R+MR), moderately susceptible (MS), susceptible (S+HS). The pathogenic reactions were categorized as virulent (6.1 to 9.0 scale), moderately virulent (4.1 to 6.0 in 0-9.0 scale) and less virulent (0.0 to 4.0 in 0-9.0 scale) as reported earlier (Viswanathan *at al*., 2022).

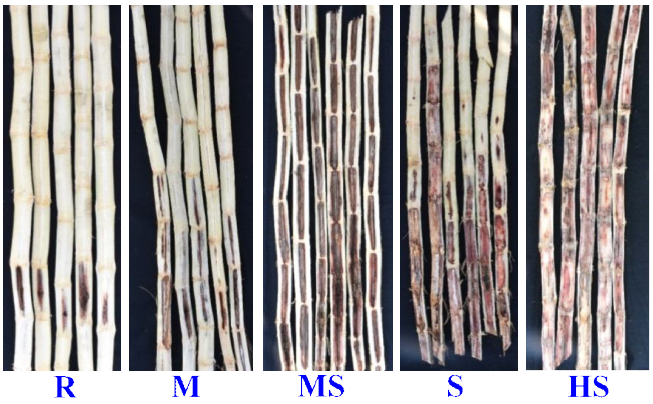


Fig. 2 : Disease reaction graded as R, MR, MS, S and HS.

Results and Discussion

Eight designated Pathotypes and 10 isolates including nine isolates of cv. Co0238 and one isolates of Co11015 were examined for their pathogenic variability on sugarcane 20 host differentials (Co419, Co975, Co997, Co1148, Co7717, Co62399, CoC671, CoJ64, CoS767, CoS8436, B091, Co86002, Co86032, Co7805, CoV92102, CoSe95422, Baragua, Khakai, SES594 and Co0238. Disease intensity was assessed on the basis of resistant (R), intermediate (X) and susceptible (S) reaction. The differential *viz*., CoS8436, CoSe95422, B091 and SES594 exhibited universal resistant behaviour to all the 10 isolates and designated Pathotypes. All the isolates exhibited almost virulent behaviour on host differential such as Co62399, CoC671, Co86032, Co7805 and Co0238. The isolates Cf2401 isolated from Co11015 exhibited almost similar trend to virulent reference Pathotypes CF13 on the host differentials. The designated Pathotypes such as CF07, CF08, CF11 and CF09 showed same virulent pathogenic behaviour with CoJ64 and CoS767, which was

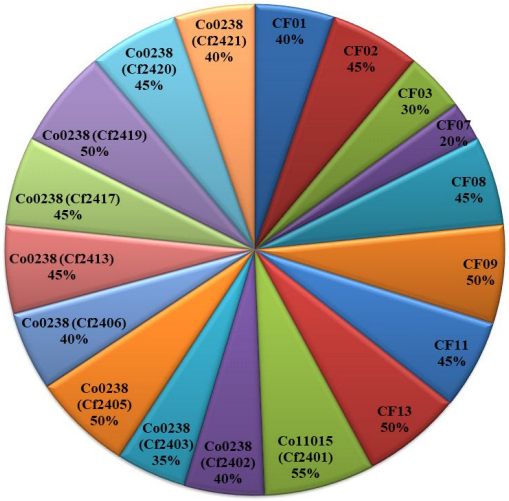


Fig. 3 : Virulence frequency of pathotypes and new isolates of *C. falcatum* Went. on sugarcane 20 host differential.

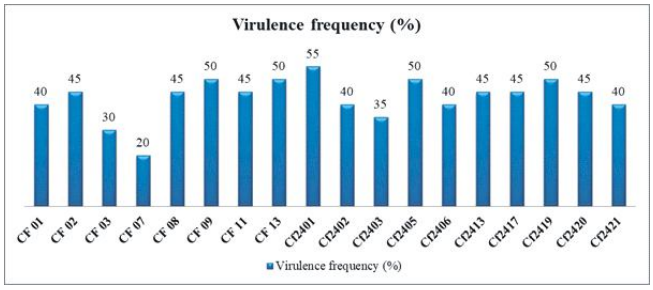


Fig. 4 : Virulence frequency of *Colletotrichum falcatum* on 20 host differentials.

contrast to all the 10 isolates along with CF13. All the 10 isolates exhibited similar pathogenic pattern to CF13, while designated Pathotypes CF07, CF08 and CF09 exhibited the contrast pathogenic behavior on same differentials viz, Co62399, CoC671, Co86032, Co7805 and Co0238. The red rot development on differential hosts indicated that all the new 10 isolates exhibited more or less similar reactions to reference Pathotypes CF13 on all the host differentials. According to Satyanarayana and Satyanarayana (1976) the two isolates of red rot pathogen from coastal Andhra Pradesh's variety Co 419 and Co 997 represented two different races. According to Khirbat *et al*. (1980) there are three races in Haryana in subtropical India based on various responses. Beniwal *et al*. (1989) also reported prevalence of three pathotypes based on differential reaction from varieties Co 7717, Co 1148 and CoJ 64. Detailed studies conducted by Padmanaban *et al*. (1996) revealed existence of three more pathotypes *viz*.; Cf419, Cf997 and Cf671 later designated as CF04, CF05 and CF06 isolated from Co 419, Co 997 and CoC 671, respectively from tropical region. Subsequently, with emergence of new variants, five new pathotypes CF07, CF08, CF09, CF10 and CF11 with distinct pathogenic behaviour on the host differentials were designated (Viswanathan, 2010). Prittesh *et al*. (2016) reported that the different sugarcane cultivar showed strain cfCHA infected maximum cultivar 77.9% virulence frequency. Viswanathan *et al*. (2022) reported that the monoculture generated a selection pressure for pathogens to adapt to the host, leading to resistance breakdown due to waterlogging and flooding. The isolate from Co 0238 has been named *C. falcatum* pathotype CF13 after thorough pathogenicity testing. It is suitable for screening sugarcane cultivars for red rot resistance. New isolates with high virulence emerged as a result of the breakdown of the well-known variety CoJ 64; these isolates were later described and given the names CF03, CF07, CF08, and CF11. Furthermore, CoS767, a common variety in the state of Uttar Pradesh, was used to identify the CF09 pathotype (Viswanathan, 2021b). Behalf of pathogenicity, serological and molecular characterization, two designated pathotypes of *C. falcatum* CF01 and CF02 were clearly distinguished (Mohanraj *et al*. 2002; Viswanathan *et al*., 2003). In sub-tropical India, namely in all five states, this single

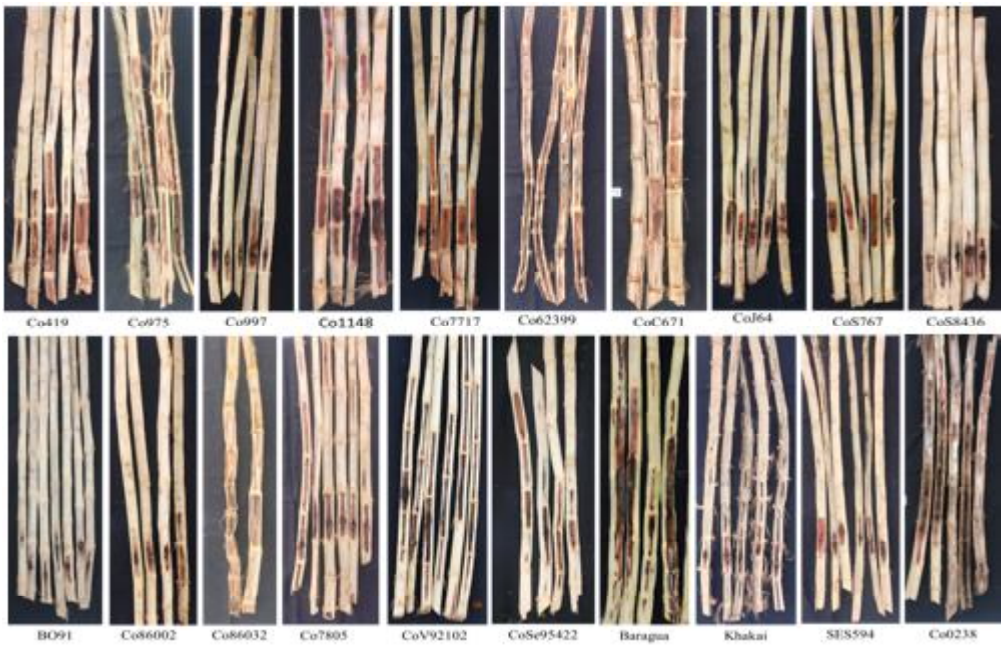


Fig. 5 : Pathogenic behavior of CF13 pathotype on 20 host differential.

Table 1 : Pathogenic behaviour of *C. falcatum* pathotypes/isolates on a set of 20 host differentials during 2024-25 at Shahjahanpur.

S. no.	Pathotype/ Isolate	Source/ Place	914 o C	579 o C	709 o C	9411 o C	3177 o C	9326 o C	1760 o C	46 Jo C	3675 o C	6348 So C	19 0 B	2068 o C	2306 o C	3087 o C	20128 o C	22459 o C	Jakah K	95 S ES	8320 o C
1	**CF 01**	Co1148	X (6.0)	X (2.0)	X (3.3)	X (3.0)	X (3.0)	S (9.8)	X (5.1)	X (7.0)	X (3.5)	R (2.2)	R (3.1)	R (4.4)	R (3.8)	X (3.1)	X (4.3)	X (2.0)	S (4.2)	R (4.0)	R (4.0)
2	**CF 02**	Co7717	X (4.3)	X (4.6)	R (2.6)	R (3.5)	R (3.4)	S (7.3)	X (5.3)	X (5.4)	X (4.5)	R (2.2)	R (3.0)	R (5.2)	R (4.0)	X (5.1)	X (4.3)	X (2.6)	S (9.0)	R (2.0)	R (9.0)
3	**CF 03**	CoJ64	R (2.8)	R (3.0)	X (4.7)	X (4.1)	R (2.0)	R (2.6)	S (6.1)	S (9.0)	R (2.0)	R (2.5)	R (3.1)	S (8.0)	R (3.0)	R (3.2)	R (3.4)	R (2.4)	X (4.3)	R (2.0)	R (2.8)
4	**CF 07**	CoJ64	R (4.0)	R (4.0)	R (2.0)	R (3.8)	R (2.6)	R (3.7)	R (3.9)	S (9.0)	S (6.2)	R (2.0)	R (2.0)	X (5.6)	R (2.8)	R (3.4)	R (3.7)	R (2.0)	S (5.3)	R (2.0)	R (3.0)
5	**CF 08**	CoJ64	R (3.0)	R (2.0)	X (4.5)	X (6.7)	R (3.0)	R (2.5)	R (2.8)	S (9.0)	S (6.1)	R (2.0)	R (3.8)	S (6.5)	X (6.0)	X (4.9)	X (4.1)	R (3.5)	S (6.8)	R (2.0)	R (2.5)
6	**CF 09**	CoS767	R (3.2)	R (4.0)	X (4.5)	X (4.6)	R (3.2)	X (6.0)	X (5.8)	X (5.0)	S (6.6)	R (2.7)	R (3.2)	X (5.5)	R (3.9)	X (5.2)	X (5.0)	R (2.0)	X (5.5)	R (2.0)	R (4.0)
7	**CF 11**	CoJ64	S (6.4)	R (3.0)	R (2.6)	R (3.2)	X (4.8)	R (2.0)	S (6.3)	S (9.0)	X (5.3)	R (2.4)	R (3.0)	R (2.3)	S (6.7)	X (4.8)	X (5.1)	R (2.0)	X (5.5)	R (2.0)	R (2.5)
8	**CF 13**	Co0238	X (5.3)	S (6.2)	R (3.6)	R (3.8)	X (4.8)	S (9.0)	S (9.0)	R (2.6)	R (2.5)	R (2.8)	R (2.8)	R (2.5)	S (6.1)	X (5.4)	R (4.0)	R (2.3)	X (5.3)	R (2.0)	S (9.0)
9	**Cf2401**	Raninagl(Co11015)	X (4.7)	S (6.2)	R (3.3)	S (6.7)	R (3.5)	S (9.0)	S (9.0)	R (2.5)	R (2.2)	R (2.0)	R (3.0)	R (2.0)	S (9.0)	S (6.2)	X (6.0)	R (2.7)	X (5.3)	R (2.0)	S (9.0)
10	**Cf2402**	Faridpur (Co0238)	X (5.5)	S (6.1)	R (2.0)	R (3.0)	R (4.0)	S (9.0)	S (9.0)	R (3.2)	R (2.4)	R (2.2)	R (2.8)	R (2.5)	S (9.0)	S (6.2)	R (2.0)	R (2.0)	X (5.2)	R (2.0)	S (8.5)
11	**Cf2403**	Aira (Co0238)	R (2.0)	X (6.0)	R (3.0)	R (2.8)	R (2.0)	X (5.3)	S (9.0)	R (2.3)	R (3.0)	R (2.0)	R (2.8)	R (3.0)	S (8.0)	S (6.2)	R (2.0)	R (2.2)	X (4.2)	R (2.0)	S (9.0)
12	**Cf2405**	Powayan (Co0238)	S (6.0)	S (6.5)	R (2.6)	R (2.2)	X (4.5)	S (8.2)	S (9.0)	R (3.3)	R (2.5)	R (2.0)	R (2.6)	R (3.2)	S (9.0)	X (6.0)	X (6.0)	R (2.6)	X (4.2)	R (2.0)	S (9.0)
13	**Cf2406**	Hargaon (Co0238)	S (9.0)	S (6.1)	X (4.1)	R (3.5)	R (3.6)	S (9.0)	S (9.0)	R (4.0)	R (2.3)	R (2.0)	R (3.6)	R (2.2)	S (9.0)	S (9.0)	R (2.0)	R (3.0)	R (3.5)	R (2.0)	S (9.0)
14	**Cf2413**	Nigohi (Co0238)	X (6.0)	S (8.5)	R (3.0)	R (2.6)	X (4.2)	S (9.0)	S (9.0)	R (3.2)	R (3.2)	R (2.0)	R (3.0)	R (2.7)	S (9.0)	X (5.6)	R (4.0)	R (2.2)	S (9.0)	R (2.0)	S (9.0)

Table 1 continued...

Table 1 continued...

15	**Cf2417**	Gola (Co0238)	R (3.0)	S (9.0)	X (6.0)	R (3.6)	S (6.8)	S (9.0)	S (9.0)	R (2.7)	R (3.3)	R (3.0)	R (3.1)	R (2.7)	S (6.5)	X (6.0)	R (3.8)	R (4.0)	R (2.0)	S (9.0)
16	**Cf2419**	Nawabganj (Co0238)	X (5.5)	X (4.7)	R (3.4)	R (2.2)	X (4.6)	S (9.0)	S (9.0)	R (2.7)	R (2.6)	R (2.0)	R (3.1)	R (2.6)	S (8.7)	S (7.2)	R (2.4)	S (6.1)	R (2.0)	S (9.0)
17	**Cf2420**	Faridpur (Co0238)	X (4.5)	X (4.8)	R (4.0)	R (3.3)	R (4.0)	S (7.4)	S (9.0)	R (3.1)	R (2.6)	R (2.8)	R (3.5)	R (2.5)	X (6.0)	S (9.0)	R (3.6)	X (6.0)	R (2.0)	S (9.0)
18	**Cf2421**	Rosa (Co0238)	X (4.6)	X (5.6)	R (3.4)	R (2.1)	R (4.0)	S (9.0)	S (9.0)	R (3.5)	R (2.3)	R (2.4)	R (3.0)	R (3.2)	S (9.0)	S (9.0)	R (2.8)	X (6.0)	R (2.0)	S (9.0)

R-resistant, X-moderate susceptible, S-susceptible.

variety Co 0238 covered an area of 25, 88, 129 hectares (79.15%) (Ram and Hemaprabha 2020). It was shown that *C. falcat*um pathotypes CF01 and CF02 may adapt to their respective incompatible host varieties, Co 7717 and Co 1148. According to Malathi *et al*. (2006), the pathotypes developed virulence to make the interactions compatible by repeated inoculations on the incompatible hosts. In Haryana, pathotypes CF02, CF03, and CF09 exhibited higher virulence compared to CF08, CF01 and CF11. The latest isolates from in a study CoS 8436 was shown to be less virulent, causing a sensitive response in three known hosts by Viswanathan *et al*. (2011). Previous findings from Punjab showed that new isolates (CF271 and CF273 from CoJ 88, CF268 from CoJ 83, and CF270 from CoJ 82) were more virulent. Viswanathan *et al*. (2011) identified ancient pathotypes CF01, CF03, and CF09. This suggests that the new isolates on Co. 0238 may have originated from a separate source. The varieties Co 1148, CoJ 83, CoJ 84, CoLk 8102, CoPant 84211, CoPant 84212, CoPk 05191, CoS 767, CoS 8436, CoS 91269, CoS 08279, CoSe 92423, and CoSe 95422 all showed red rot to varied degrees before and after the breakdown of Co 0238 in UP (Viswanathan *et al*., 2011, 2017b, 2018). The introduction of a novel virulent pathotype can cause an outbreak in previously resistant varieties to red rot. Generally, the disease epidemic often develops over several seasons from the first identification of the virulent pathotypes. The severity of the epidemic is determined by the growing area of the variety. In such cases, selection pressure favors the virulent pathotype of the variety (Van der Plank, 1963). According to Agnihotri (1990), hybridization is not the only way for a new race to form in *C. falcatum*, since mutations are also a possibility. Under favourable conditions, the disease spreads rapidly in vulnerable kinds. Previous research support pathogenic behaviour. The study discovered that phytopathogenic organisms are prone to extinction and recolonization, and are seldom detected in equilibrium. Plant and pathogen selection pressure can lead to co-evolution, which can impact virulence diversity (Kumar *et al*., 2013 and Satyavir *et al*., 2001).

Conclusion

All isolates derived from the Co0238 variety exhibit behaviour identical to that of their standard strain; no reduction in their virulence was observed. In fact, the isolate Cf2401 which was isolated from the sugarcane variety Co11015 also behaves similarly to the standard strain CF13, is displaying a virulence level of 55%. Therefore, this strain can be eradicated by replacing the Co0238 variety with new disease resistant sugarcane

varieties, thereby eliminating the host and, consequently, reducing the risk of disease breakdown in other sugarcane varieties. This isolates *i.e.*; Cf2401 (Co11015) and all isolates of Co0238 may be alarming for other commercial varieties of UP.

Authorship contribution statement

Rajneesh Kumar Awasthi: Conceptualization, Methodology, experiment design, Data collection, Formal analysis, Visualization, manuscript drafting, original draft prepration.

Satyendra Nath Singh: Supervision, guidance and suggestion, Investigation, final manuscript review and editing. All authors have read and approved the final version of the manuscript and agree to its submission for publication.

Conflict of interest : Authors have declared that no competing interest exists.

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