International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 1568-1572

Received: 10-07-2019
Accepted: 12-08-2019

Kalpana Yadav
Ph. D Scholar Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

NL Meena
Assistant Professor, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Kulchander
M.sc. Plant Pathology, Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Nitisha Gahlot
Ph.D. Scholar, Department Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Correspondence
Kalpana Yadav
Ph. D Scholar Department of Plant Pathology, MPUAT, Udaipur, Rajasthan, India

Morphological, cultural and pathogenic variability of the different isolates of *Fusarium oxysporum* f. sp. *radicis cucumerinum* causing root and stem rot of cucumber

Kalpana Yadav, NL Meena, Kulchander and Nitisha Gahlot

Abstract
An experiment was conducted to study the variability using 5 isolates of *Fusarium oxysporum* f.sp. *radicis cucumerinum* causing Root and Stem Rot of cucumber collected from different cucumber growing areas of nearby Udaipur region. Studies were made on cultural, morphological, and pathogenic variation like mycelial colour, mycelial growth, macro and micro conidial size and formation of chlamydospores, and disease incidence of several isolates on seedling stage of cucumber. The isolates produced moderate, profuse fluffy, white colour, thin flat to slight fluffy growth and submerged growth had yellow and light pink pigmentation. The size of macroconidia ranged from 25.0-35.0× 2.7-4.8 µm with 1–4 septa in different isolates. The size of microconidia varied from 3.0-7.4× 2.3-2.6 µm with 0–1 septa in different isolates. All isolates of *Fusarium sp*. were inoculated on cucumber for their pathogenic variability in Cage house by soil inoculation technique. Isolate FORC-5 and FORC-3 were highly virulent and causing more than 60% disease. FORC- 1 exhibited moderate virulence and FORC-2 was least virulent pathogen with 17.50 per cent disease incidence recorded.

Keywords: *Fusarium oxysporum* f. sp. *radicis cucumerinum* (FORC), morphological variability, pathogenic, cultural characters, micro and macro conidia, chlamydospore etc

Introduction
Cucumber (*Cucumis sativus* L.) belongs to family cucurbitaceae which is a major source of human edible products and useful fibers. Cucumber probably originated in the foothills of the Himalayas and have been cultivated for at least 3,000 years (Kroon *et al.,* 1979) [6]. Cucumber popularly known in India as '*khira*' is extensively grown in tropics, subtropics and milder temperate zones of India. It was probably introduced throughout Europe by the Romans, and records of cucumber cultivation appear in France in the 9th century, England in the 14th century, and North America by the mid. 16th century. Among the diseases affecting cucumber, stem and root rot caused by *Fusarium oxysporum*. f. sp. *radicis cucumerinum*. A *Fusariurn* root and stem rot disease on greenhouse cucumber (*Cucumis sativus* L.) has only been reported in Greece, where during the 1989-90 growing season, the disease was limited to a few greenhouses on the island of Crete. Since then, the pathogen has spread to most of the other growing regions of Crete; severe losses occured only 3 years after first being reported. *Fusarium* root and stem rot of cucumber has been reported to be favoured at lower soi1 temperatures (17 °C) (Vakalounakis, 1996, Agrios, 2005) [8, 2]. Root and stem rot is the most destructive disease of glasshouse cucumber crops in Canada in 1994, in France in 1998, in China in 1999, and in Spain in 2000, causing significant losses in the yield (Punja & Parker, 2000) [7]. The present investigation was therefore, undertaken with the Morphological, Cultural and Pathogenic variability of the different isolates of *Fusarium oxysporum* f.sp. *radicis cucumerinum* using *in vitro* techniques.

Materials and Methods
Five isolates of *F. oxysporum* f. sp. *radicis cucumerinum* were collected from different cucumber growing regions of udaipur during Kharif-2017 growing seasons. The samples showing characteristic root and stem rot symptoms were uprooted and brought into laboratory for isolation. The roots of such diseased plants were washed with running tap water to remove all adhering soil particles, and they were subjected to tissue isolation.

The typically infected root and stem portions from the collar region were cut into small pieces with the help of sterilised knife and again washed with sterilised distilled water. These pieces were then disinfected for 5min with 2.5% sodium hypochlorite solution. To remove residue of 2.5% sodium hypochlorite solution, the pieces were washed thrice in sterilised distilled water for one minute each time and pieces were then transferred aseptically under laminar air flow system on sterilised Petri plates containing 20ml potato dextrose agar (PDA) medium. The pathogenicity of the isolated fungus was tested on 10 seeds of cucumber cultivar Cucumber long desi sown in pots (15cm) filled with 2kg of autoclaved soil and inoculated seeling with inoculum multiplied on sorghum grains. The fungi were multiplied on sorghum (Sorghum vulgare Pers.) grains presoaked for 12 h in water and autoclaved at 1.1kg/cm2 for 30 min for two days subsequently for sterilisation. Soaked grains (200g) filled in 500ml flasks were sterilised and inoculated with seven-day-old culture. Inoculated flasks were incubated in BOD incubator at 25±1 °C for 15days. Cultures on PDA slants were stored at 4 °C for use.

Cultural Variability

Five pathogenic isolates were grown on PDA medium (Potato 200g, dextrose 20g, agar 20g and water 1L) and incubated at 28±2 °C for 7 days for cultural variability. After 7 days of incubation period, diameter of the fungal mycelial growth, colony characters and pigmentation were recorded. The colony diameters were measured on PDA medium poured into 90mm Petri dishes (20ml/plate) with four replications.

Morphological Characters

The five isolates were also cultured on potato dextrose agar medium. The autoclaved medium was dispensed in plate and allowed to solidify. Five mm disc of the individual isolate of *F. oxysporum* removed from the periphery of five day old culture was aseptically placed in the center of plate, keeping as four plates as four replications for each isolate. These plates were incubated at 28±2 ˚C for seven days. After incubation, average measurements were taken by the micrometry method. The morphological characters like size (length and width) of macroconidia, microconidia and chlamydospore, were recorded. The observations were recorded in four replications within each isolate. The study was carried out using ocular and stage micrometer and were taken by measuring 50 spores of each isolate and after mounting them. Data were analyzed statistically using complete randomized design.

Pathogenic Variability

The pathogenic variability of five isolates of *Fusarium sp*. was tested on cucumber variety "Cucumber Long Desi" in pots under cage house condition. Ten plants in each pot separately grown as four replications for each isolate and pots were inoculated with different culture of *Fusarium sp*. then variability among different isolates was studied through counting the dead plants in each pot after 15-20 days of inoculation.

Results and Discussion

Cultural Character

The five isolates of *Fusarium spp*. collected from different locations showed variations in colony diameter, colour of colony, shape and size of micro conidia, macro conidia and chlamydospore on PDA medium at 28±2ºC after 7 days of inoculation. Maximum mean colony diameter (87.50mm) was recorded in FORC-5. This was followed by 81.00 mm in FORC-3, 76.50 mm in FORC-4 and FORC 1 with 49.25 mm. Minimum mean colony diameter 46.25 mm was recorded in FORC-2. The margin/shape and colour of the culture of different isolates varied from cottony, plenty growth, aerial to fluffy, irregular submerged growth that varied in colour from white cottony with light yellow pigmentation, (FORC-1 and FORC-4) or clear White growth with pink pigmentation in FORC-2, FORC-3, FORC-5. Ashwathi *et al.* (2017) [1] found similar result that colony colour of Fusarium isolates varied from white, white with pinkish and white and margin of the colony was smooth to irregular. The mycelial topography was flat to fluffy.

Table 1: Mycelial growth and cultural characteristics of five isolates of *F. oxysporum* f.sp. *Radicis cucumerinum* on PDA

S. No.	Name of isolates	Diameter* (mm) at 7th days after incubation	Growth Characters	Pigmentation
1.	FORC 1	49.25	White mycelia colour, fluffy	Light yellow
2.	FORC 2	46.25	Serrated margin with fluffy growth	Light Pink
3.	FORC 3	81.00	Fluffy,aerial growth	Pink
4.	FORC 4	76.50	Fluffy growth	Light Yellow
5.	FORC 5	87.50	Plenty growth	Pink
SEm±		1.06		
CD at 5%		3.19		

*Mean of four replications

Isolate-I (Madar) Isolate-II (RCA Polyhouse) Isolate-III (RCA Field)

Plate 1: Cultural characteristics among the five isolates of *F. oxysporum*. f. sp. *radicis cucumerinum*

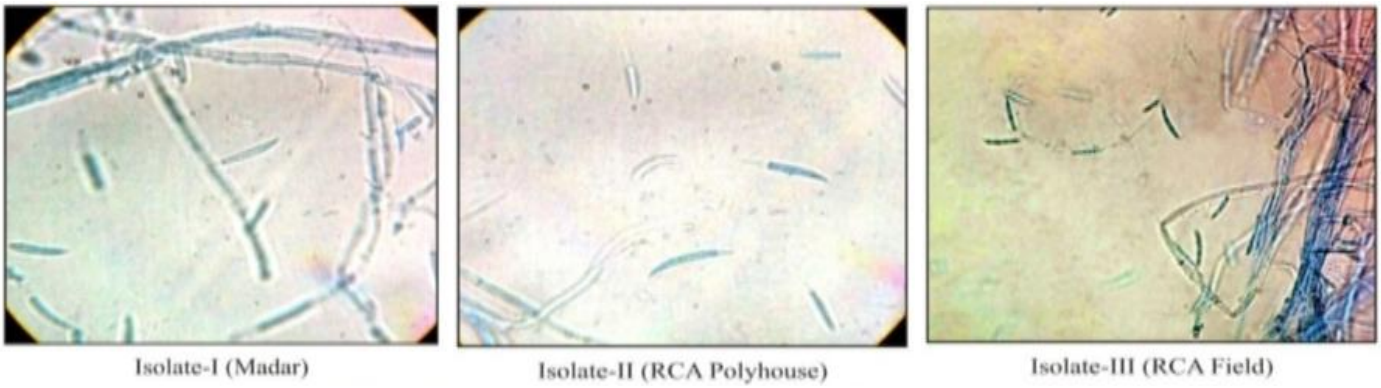
Variation in Conidial Morphology

All the five isolates of *Fusarium spp*. showed significant variation in conidial morphology. Result presented Table 3 shows that length and width of macro conidia ranged from 25.0-35.0× 2.7-4.8 µm and mean length and width of micro conidia in different isolates ranged from 3.0-7.4× 2.3-2.6 µm. The chlamydospore size in different isolates of *Fusarium oxysporum* f.sp. *radicis cucumerinum* ranged from 4.12-7.00 µm. Among *F. oxysporum* f.sp. *radicis cucumerinum* isolates, Maximum length of macroconidia was with the isolate FORC-4 which measured 35 (23-51.1) µm and the width 3.5(3.5-4.2) µm. In FORC-5 the mean size of macroconidia was 30.5 (30-40.3) × 3.6 (3-4.6) µm and isolate FORC-3 was 30 (31.2-40.5) × 4.8 (4-4.5) µm. In case of FORC-2, macroconidia size was 26.5 (16-47) × 4 (2.6-4.8) µm and FORC-1 it was 25 (19-40) × 2.7 (1.74.2) µm. While the maximum length of microconidia FORC-4 was 8.6 (7-8.9) × 2.6 (2.6-2.9) µm. In FORC-2, length and width ranged between 8.5 (7.4-10) × 2.6 (2.6-3.0) µm followed by FORC-1 with 7 (6-11.5) × 2.6 (2.2-3.2) µm. FORC-5 was it measured between 5 (3-5.11) × 2.6 (2.1-2.8) µm followed by FORC-3 with 5 (4.5-5.2) × 2.3 (2.1-2.3) µm. Size of chlamydospore also varied among the isolates and FORC-5 exhibited maximum length of chlamydospore was 7.0 µm followed by FORC-4 with 6.18 µm. In FORC-2, FORC-1 and FORC-3 was measured with 5.70 µm, 5.12 µm and 4.12 µm respectively. Chopada *et al*. (2015) found similar result that macro conidia ranged from 15.46–21.8×4.91–5.45µm in SGFOL-1 isolate to 21.42–44.28×7.35–9.14µm in SGFOL-3 isolate. The microconidia were hyaline, size of microconidia ranged from 3.57–14.28× 2.68–4.46µm in SGFOL-2 and SGFOL-6 isolates to 7.14–14.28×3.57–5.35µm in SGFOL-4 isolate. Chlamydospores were round and size varied from 6.85–7.73×6.67–7.90µm in SGFOL7 isolate to 8.97–13.70×8.78–10.18µm in SGFOL-2 isolate. These results were supported by the research findings of Benaouali *et al*. (2014) [3].

Table 2: Conidial morphology of five different isolates of *F. oxysporum* f.sp. *radicis cucumerinum* on PDA at 15 days after incubation (28±2 °C)

S. No	Isolates	Conidial Morphology									
		Macroconidia (µm) 10X				Microconidia (µm) 10X				Chlamydospore (µm)10X	
		Length		Width		Length		Width		Length	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	Madar	25±8	19-30	2.7±1.02	1.7-4.2	7±1.14	6-7.0	2.6±0.16	2.2-3.2	5.12	4.5-5.1
2.	RCA, Polyhouse	26.5±10.3	16-32.5	4 ± 0.40	2.6-4.0	8.5±0.44	7.4-8.0	2.5±0.15	2.6-3.0	5.70	5.2-5.7
3.	RCA, Horticulture Farm	30±8	29-40.5	3.5 ± 0.44	3.5-4.2	5±0.4	4.5-5.2	2.3±0.35	2.1-2.3	4.12	6.1-6.7
4.	Salumber	35±8.9	32-51.5	4.8 ± 0.25	4-4.9	8.6±0.55	8-9.1	2.6±0. 12	2.6-2.9	6.18	5.1-6.7
5.	Dabok	30.5±7.5	31-40.3	3.6 ± 0.5	3-4.6	5±0.50	3-5.1	2.6±0.32	2.1-2.6	7.00	6.2-8.1
SEM+		0.08		0.01		0.02		0.07		0.01	
CD at 5%		0.23		0.03		0.06		0.02		0.04	

*Mean no. of 50 conidia and ± S.D. of mean valve



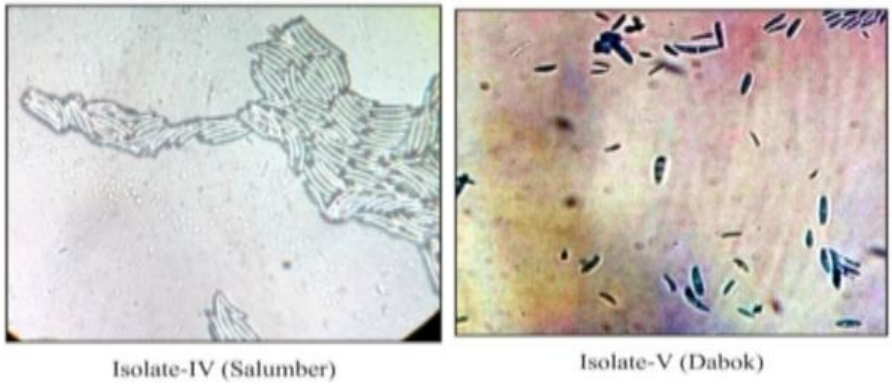
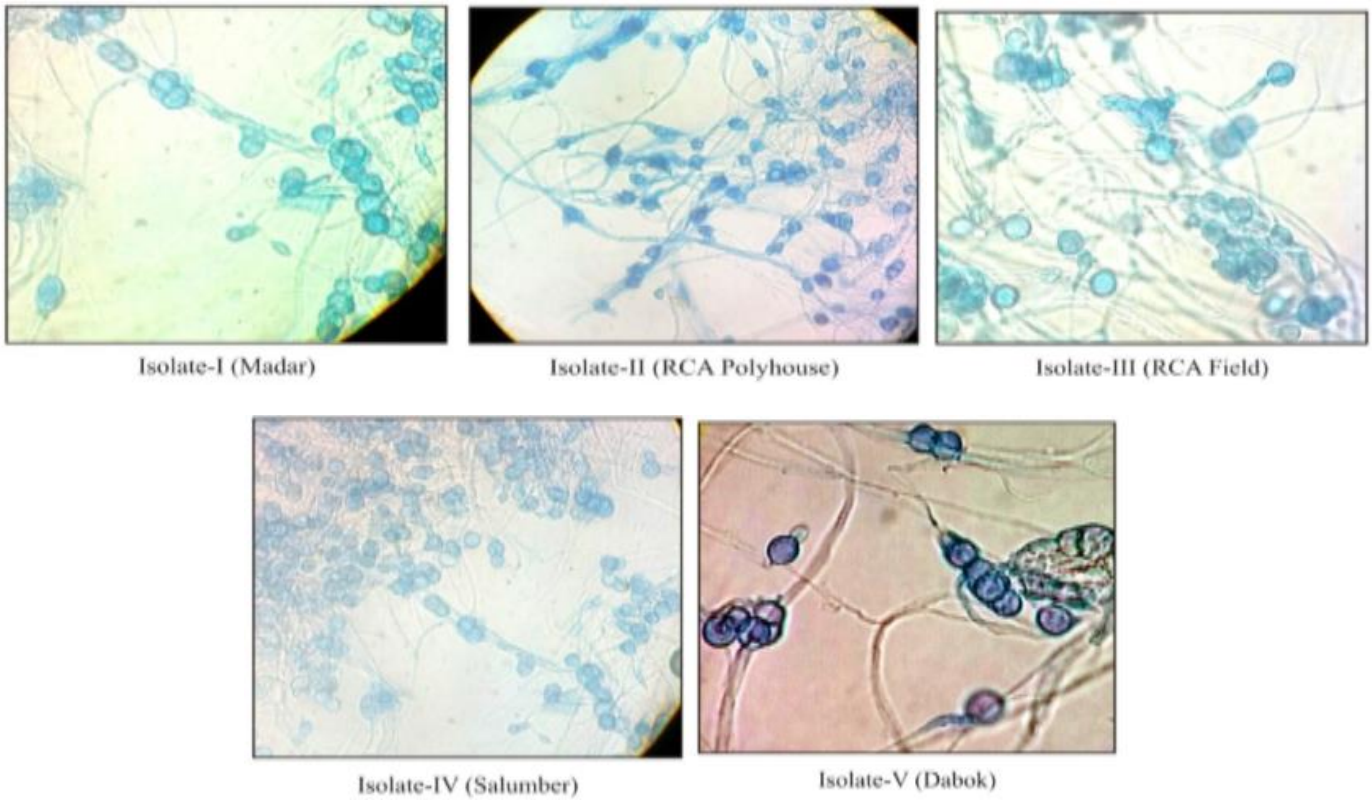


Plate 2: Conidial variability among the five isolates of *F. oxysporum*. f. sp. *radicis cucumerinum*



Plato 5: Chlamydospore variability among the five isolates of *F. oxysporum*. f. sp. *radicis cucumerinum*

Pathogenic Variability

The five isolates of *Fusarium sp*. were evaluated on cucumber variety (Long Desi) for their pathogenic variability in Cage house by soil inoculation technique. The observation of disease severity were recorded after 20 days of inoculation. Isolate FORC-5 and FORC-3 were highly virulent and produced 80.00 and 70.00 per cent disease incidence in pots condition, respectively. FORC- 1 exhibited moderate virulence with 65 per cent disease incidence followed by FORC-4 with 55 per cent disease incidence. FORC-2 was least virulent pathogen with 17.50 per cent disease incidence recorded. Among the pathogenic variability, FORC 5 was more virulent isolate with 80.00% mortality of cucumber plants (Cucumber Long Desi) followed by FORC 3 (70.00%). FORC 2 was least virulent pathogen with 17.50% mortality. These results were supported by the research findings of Joshi *et al.*, 2013 [5].

Table 3: Pathogenic variability among different isolates of *F. oxysporum* f.sp. *radicis cucumerinum* on variety Cucumber Long Desi of cucumber in the pot condition

S. No.	Name of isolates	Place of collection	Mortality Percent
1	FORC 1	Madar, Udaipur	65.00%
2	FORC 2	Polyhouse, RCA	17.50%
3	FORC 3	Horticulture Farm, RCA	70.00%
4	FORC 4	Salumber, Udaipur	55.00%
5	FORC 5	Dabok, Udaipur	80.00%
	SEm±		2.14
	CD at 5%		6.45

Plate 3: Pathogenic variability among the five isolates of *F. oxysporum*. f. sp. *radicis cucumerinum*

Conclusion

The morphological and cultural and pathogenic variation serves as an aid in differentiation of isolates. Present study clearly indicated the variation among 5 isolates of *F. oxysporum* f.sp. *radicis cucumerinum* collected from Udaipur region in terms of cultural and morphological character and pathogenic variability. On the basis of such investigation, here we conclude that there may be chance of presence of new race of this pathogen as far as regional occurrence is concern. Further extensive study is required to identify the variation among *F. oxysporum* f.sp. *radicis cucumerinum* up to races.

References

1. Ashwathi S, Ushamalini C, Parthasarathy S, Nakkeeran S. Morphological and molecular characterization of *Fusarium* spp. associated with Vascular Wilt of Coriander in India. Journal of Pharmacognosy and Phytochemistry. 2017; 6:1055-1059.
2. Agrios GN. Plant Pathology, Academic Press, London, UK, 2005, 922.
3. Benaouali H, Hamini-Kadar N, Bouras A, Benichou SL, Kihal M, Henni JE. Isolation, pathogenicity test and physicochemical studies of *Fusarium oxysporum* f.sp *radicis lycopersici.* Advances in Environmental Biology. 2014; 8(10):36-49.
4. Chopada GB, Singh P, Chandulal K. Cultural and morphological variability among *Fusarium oxysporum* f.sp. *lycopersici* causing wilt of tomato in south Gujarat region. Archives of Phytopathology and Plant Protection. 2015; 48:104-110.
5. Joshi M, Srivastava R, Sharma AK, Prakash A. Isolation and characterization of *Fusarium oxysporum*, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato (*Solanum lycopersicum*). Journal of Applied and Natural Science. 2013; 5:108-117.
6. Kroon GH, Custers JBM, Kho YO, Den niris APM, Varekamp HQ. Intraspecific hybridization in *Cucumis* (L.) need for genetic variation, biosystematic relations and crossability barriers. Euphytica. 1979; 28:723-728.
7. Punja ZK, Parker M. Development of *Fusarium* root and stem rot, a new disease on greenhouse cucumbers in British Columbia, caused by *Fusarium oxysporum* f. sp. *radicis cucumerinum*. Canadian Journal of Plant Pathology. 2000; 36:393-410.
8. Vakalounakis DJ. Root and stem rot of cucumber caused by *Fusarium oxysporum* f.sp. *Radicis cucumerinum* f.sp. nov. Plant Disease. 1996; 80:313-316.