

# Assessing Pathogenicity and Cultural Characteristics of *Fusarium oxysporum* f. sp. *ricini* Isolates from Major Castor Growing Regions of India

## ABSTRACT

The present study was undertaken to investigate the pathogenicity and cultural characteristics of 20 isolates of *Fusarium oxysporum* f. sp. *ricini*, the causal fungus of wilt disease of castor (*Ricinus communis* L.). Pathogenicity tests on the susceptible cultivar JI-35 revealed that all isolates were pathogenic, with symptoms progressing from yellowing and drooping of leaves to marginal necrosis, wilting and root discoloration. The incubation period varied significantly among the isolates, ranging from 10.0 to 22.5 days, while the per cent disease incidence (PDI) ranged from 51.7 % to 100 %. Cultural characterization that includes mycelial colour, colony morphology, pigmentation, growth habit and sporulation highlighted significant variability among the isolates of *F. oxysporum* f. sp. *ricini*. The colour of mycelium among the isolates ranged from white to various shades of pink and yellow, with growth habits observed as fast, moderate, or slow. Sporulation rates varied from very high to sparse among the isolates. The observed diversity in pathogenicity and cultural traits emphasized the presence of genetic variability in *F. oxysporum* f. sp. *ricini* isolates. These results are vital for understanding the pathogen virulence and for developing effective management strategies for wilt disease in castor crop.

*Keywords:* Castor, *F. oxysporum* f. sp. *ricini* isolates, Incubation period, PDI

## 1. INTRODUCTION

Castor (*Ricinus communis* L.), a member of the *Euphorbiaceae* family is a vital non-edible oilseed crop predominantly cultivated in arid and semi-arid regions, holding significant economic and industrial importance worldwide. India is the largest producer of castor seed in the world and meets most of the global demand for castor oil. However, the cultivation of castor crop is significantly threatened by the wilt disease, caused by *Fusarium oxysporum* f. sp. *ricini* (Nanda and Prasad, 1974). This disease poses a serious challenge to castor production resulting in substantial economic losses (Desai *et al.*, 2003). The wilt disease is characterized by progressive symptoms that include yellowing, wilting and eventual plant desiccation, significantly affecting both yield and quality. The fungus thrives in warm and humid conditions making it devastating in regions where castor is predominantly grown. Although the pathogen is primarily soil-borne, the seed-borne nature of *F. oxysporum* has also been documented (Naik, 1994), allowing the pathogen to infect plants at various growth stages. Castor plants are susceptible to wilt disease at all stages of crop growth and the disease appears mostly in patches. The extent of yield loss depends on the stage at which plant wilts with reported losses reaching 77 % at flowering, 63 % at 90 days and 39 % in later stages on secondary branches (Pushpavathi *et al.*, 1997). Additionally, reductions of 10-40 % in yield, 8-14 % in seed weight and 1-2 % in oil content have been reported (Lakshminarayana and Raof, 2005). Cultural and chemical control measures have proven ineffective against wilt disease in castor, primarily due to the soil-borne nature of *F. oxysporum* f. sp. *ricini* and its ability to spread systemically within the vascular tissues of plant (Dange *et al.*, 2006). To effectively manage wilt disease in castor,

breeding and cultivating resistant varieties is considered the most practical and economical strategy. Significant advancements have led to the development of wilt resistant castor hybrids and varieties. However, maintaining genetic resistance is challenging due to the pathogen ability to adapt, which diminishes the effectiveness of resistance traits over time (Niks *et al.*, 1993). For instance, the widely cultivated resistant hybrid GCH-4 became susceptible to wilt disease (Patel *et al.*, 1991). Similarly, Anjani *et al.* (2004) reported that the previously resistant variety DCS-9 exhibited wilt incidence up to 60 %, highlighting the gradual breakdown of resistance. This highlights the need for continuous efforts to identify and develop new resistant sources, as the pathogen adaptability drives the emergence of new, virulent *Fusarium* pathotypes. Understanding the variability within the *F. oxysporum* f. sp. *ricini* population is crucial, as it enables the pathogen to thrive under diverse environmental conditions and overcome host defences. Therefore, the present study aims to collect *F. oxysporum* f. sp. *ricini* isolates from major castor-growing regions of India, isolating the pathogen, assess its pathogenicity on susceptible castor cultivar and evaluating the cultural characters among these isolates.

## 2. MATERIAL AND METHODS

The study was conducted at the ICAR-Indian Institute of Oilseeds Research (IIOR) to evaluate the cultural characteristics of *F. oxysporum* f. sp. *ricini* isolates grown on potato dextrose agar (PDA) medium. A total of 20 *F. oxysporum* f. sp. *ricini* isolates including the most virulent isolates identified in earlier research at ICAR-IIOR and newly collected isolates from major castor growing areas across India were selected for studying the cultural characteristics (Table 1). The pathogen was isolated from the diseased root samples of castor as per methods described by Dhingra and Sinclair (1985). Diseased root samples were collected and 2 mm sections containing both infected and healthy tissues were thoroughly washed with sterilized water. These sections were surface-sterilized with a 1 % sodium hypochlorite solution for 1 minute and rinsed with sterile distilled water to remove any residual disinfectant. The sterilized pieces (4-5 per dish) were aseptically placed in sterilized Petri dishes containing potato dextrose agar (PDA). The dishes were incubated at  $27\pm 1^{\circ}\text{C}$  and fungal growth was observed after 2-3 days. The growing mycelium was subsequently transferred to fresh PDA for maintenance and storage. All the isolated *F. oxysporum* f. sp. *ricini* cultures were designated as *For*-1 to *For*-20 (Table 1).

**2.1 Cultural Characteristics of Isolates of *F. oxysporum* f. sp. *ricini*:** Cultural characteristics of all the isolates of *F. oxysporum* f. sp. *ricini* were recorded by culturing them on potato dextrose agar (PDA) medium for 10 days at  $27\pm 1^{\circ}\text{C}$ . Various cultural characters including colour of mycelium, colony morphology, pigmentation, growth patterns and sporulation were recorded following the 10-days incubation period.

**2.2 Proving the Pathogenicity:** The pathogenicity of 20 isolates of *F. oxysporum* f. sp. *ricini* was assessed using the sick pot method. The evaluation was conducted using the susceptible castor cultivar JI-35, with the experiment organized in a completely randomized design consisting of four replications under shade net conditions. Plants were monitored regularly for wilt symptoms using the Standard Evaluation System (SES) scale (Shaw *et al.* 2016; Bharathi *et al.*, 2024) up to 45 days after sowing (DAS). Additionally, re-isolations of each isolate from the artificially inoculated plants were

performed and the cultures obtained were compared to the original isolates to validate Koch's postulates.

### 3. RESULTS AND DISCUSSION

#### 3.1 Cultural Characterization of Different Isolates of *F. oxysporum* f. sp. *ricini*

The cultural characterization of *F. oxysporum* f. sp. *ricini* isolates revealed significant variability across several phenotypic traits, including mycelial colour, colony morphology, pigmentation, growth patterns and sporulation (Table 2, Plate 1). These findings indicated a presence of substantial diversity among the isolates collected from various castor growing regions of India, supporting previous observations by Piplani *et al.* (1985), Desai *et al.* (2003), Santhalakshmi Prasad *et al.* (2008), Mulekar *et al.* (2017) and Sangava *et al.* (2018).

**3.1.1 Mycelial Colour:** The colour of aerial mycelium of the isolates grown on PDA medium ranged from white to diverse shades of pink and yellow (Table 2). Specifically, the variations recorded included whitish purple (*For-1*), pinkish white (*For-2*), whitish pink (*For-3*, *For-11*, *For-16*), cottony white (*For-7*, *For-8*, *For-13*, *For-15*, *For-17*, *For-19*, *For-20*), whitish yellow (*For-9*), milky white (*For-6*, *For-10*, *For-12*, *For-18*) and pale white (*For-4*, *For-5*, *For-14*). The observed differences in colour of mycelium among the isolates may be attributed to genetic variation, influence of secondary metabolites. Furthermore, the composition of the growth medium including variations in carbon and nitrogen sources as well as nutrient availability, could also affect pigment production, resulting in the wide spectrum of observed mycelial colours. Similarly, Mishra and Dhar (2007) observed wide variation among the isolates in respect of mycelia growth and sporulation of *F. udum* isolates. Kumar and Upadhyay (2014) also observed variability among the cultural characters of *F. udum* isolates.

**3.1.2 Colony Morphology:** The isolates of *F. oxysporum* f. sp. *ricini* exhibited variability in colony morphology, with textures varying from fluffy to dense or sparse cottony forms, accompanied by either smooth or irregular margins (Table 2). Most isolates displayed fluffy colonies characterized by smooth or irregular margins (*For-1*, *For-2*, *For-3*, *For-5*, *For-9*, *For-16*, *For-18*). In contrast, other isolates showed either dense or sparse cottony growth patterns with smooth or irregular margins (*For-7*, *For-8*, *For-12*, *For-13*, *For-15*, *For-17*, *For-19*, *For-20*). Additionally, some isolates exhibited submerged colony growth (*For-4*, *For-6*, *For-10*, *For-11*, *For-14*). Nanda and Prasad (1974) observed white fluffy mycelial growth of *F. oxysporum* f. sp. *ricini* with pinkish pigmentation. Desai *et al.* (1994) suggested that growth variability was useful in distinguishing 4 races of *F. oxysporum* f. sp. *ciceris*. Similarly, Chopada *et al.* (2014) reported that isolates of *F. oxysporum* f. sp. *lycopersici* has moderate, profuse fluffy, thin flat to slight fluffy and submerged growth. Singh (2016) also observed considerable variation in colony morphology among isolates of *F. solani*, with colony texture ranging from smooth to rough and pigmentation appearing 3-5 days after inoculation.

**3.1.3 Pigmentation:** The isolates produced varied pigmentation from dull white and pale yellow to multiple shades of pink (Table 2). Pinkish pigmentation was observed in isolates *For-1*, *For-2*, *For-3*, *For-11* and *For-16*. Pale yellow pigmentation was recorded in isolates *For-4*, *For-6*, *For-9*, *For-10*, *For-12*, *For-13*, *For-14*, *For-15*, *For-17* and *For-20*. Additionally, several isolates displayed dull white pigmentation, including *For-5*, *For-7*, *For-8*, *For-18* and *For-19*. Previous studies by Chauhan (2007) and Santhalakshmi Prasad *et al.* (2008), who observed diverse mycelial growth patterns and

pigmentation among the isolates of *F. oxysporum* f. sp. *ricini*, ranging from light to dark pink, violet and orange. Reddy *et al.* (2010) also observed the wide variations in cultural characters among the isolates of *F. oxysporum* f. sp. *ricini*. Sumangala *et al.* (2013) reported that most of the isolates of *F. oxysporum* f. sp. *lycopersici* showed white cottony to pink mycelium. Chopada *et al.* (2014) also reported that isolates of *F. oxysporum* f. sp. *lycopersici* showed white, yellow, light pink, dark pink, orange and purple-orange pigmentation on PDA medium.

**3.1.4 Growth Habit:** Variability in growth habits among isolates ranged from fast to slow growth rates (Table 2). Isolates identified as fast-growing included *For-1*, *For-2*, *For-3*, *For-5*, *For-8*, *For-12*, *For-16* and *For-20*. Moderate growth rates were observed in isolates *For-6*, *For-7*, *For-9*, *For-10*, *For-13*, *For-14*, *For-15* and *For-18*, while isolates *For-4*, *For-11*, *For-17* and *For-19* displayed slow growth. The observed growth rates may reflect intrinsic factors related to genetic makeup of each isolate and environmental conditions. Fast-growing isolates often exhibit higher metabolic efficiencies and superior nutrient assimilation abilities, which facilitate pronounced colony growth. Okiror and Kimani (1997) reported similar variability in growth habits such as growth rate, growth habit and morphology in isolates of *F. udum* of pigeon pea wilt fungus.

**3.1.5 Sporulation:** Sporulation rates among the isolates ranged from very high (++++), high (+++), moderate (++) to sparse (+) (Table 2). Very high sporulation levels were recorded in *For-1*, *For-2* and *For-16*, while high sporulation was noted in *For-3*, *For-8*, *For-12*, *For-14*, *For-15*, *For-18* and *For-19*. Moderate sporulation was observed in *For-4*, *For-5*, *For-6*, *For-9*, *For-10*, *For-11*, *For-13* and *For-20*, whereas *For-7* and *For-17* demonstrated sparse sporulation. The variability in sporulation levels of the isolates may be influenced by genetic traits that enhance sporulation, which is crucial for pathogen aggressiveness. Light, humidity, and nutrient levels also affect sporulation rates of the pathogen, as observed by Desai *et al.* (2003) and Das and Sengupta (1998).

The present findings are in agreement with work done on different formae specialis of *Fusarium* wilt by several workers. Previously, Piplani *et al.* (1985), Desai *et al.* (2003), Chauhan (2007) and Santhalakshmi Prasad *et al.* (2008) reported cultural and morphological variability among different isolates of *F. oxysporum* f. sp. *ricini*. Presence of genetic variation in different isolates of *F. oxysporum* f. sp. *ricini* isolated from different castor growing regions has been reported by Santhalakshmi Prasad *et al.* (2008). Diverse cultural, morphological and pathogenic characteristics were recorded in different *F. oxysporum* f. sp. *ricini* isolates and it was also observed that highly virulent isolates produce abundant spores as compared to moderately virulent isolates (Nanda and Parasad, 1974; Desai *et al.*, 2003). Mulekar *et al.* (2017) also recorded morphological variability in 24 isolates of *F. oxysporum* f. sp. *ricini* representing various castor growing regions of India in Andhra Pradesh, Gujarat, Rajasthan Tamil Nadu, Telangana states. Sangava *et al.* (2018) also observed significant variation in growth and sporulation of five isolates of *F. oxysporum* f. sp. *ricini* representing various castor growing areas of Gujarat. These results indicated existence of variability in cultural characters among the twenty isolates of *F. oxysporum* f. sp. *ricini* causing wilt disease in castor.

**3.2 Pathogenicity Test:** The results of pathogenicity studies of 20 isolates of *F. oxysporum* f. sp. *ricini* indicated that all the isolates were found pathogenic on the susceptible castor cultivar, JI-35 (Table 3, Plate 2). Initially, infected plants exhibited symptoms such as yellowing and drooping of

leaves, which is progressed to marginal and inter-veinal necrosis, ultimately leading to wilting and desiccation. Examination of the roots revealed brown discoloration in the xylem vessels. Un-inoculated control plants remained healthy and showed no symptoms of wilt disease. These findings highlighted the varying levels of aggressiveness among the isolates.

The pathogenicity studies revealed considerable differences in both the incubation periods and percentage disease incidence (PDI) among the isolates (Table 3 and Figure 1). A broad range of incubation periods varying from 10.0 to 22.5 days was observed across the isolates. Isolates *For-1* and *For-9* had the shortest incubation period at 10.0 days, while *For-6* and *For-16* exhibited the longest at 22.5 days. Disease incidence varied significantly, ranging from 51.7 % to 100 %. The highest disease incidence of 100 % was recorded in isolates *For-1*, *For-2*, *For-3*, *For-4*, *For-6*, *For-9*, *For-12*, *For-13* and *For-17*. In contrast, isolate *For-15* showed the lowest PDI of 51.7 %. On an average, the mean incubation period for all isolates was 16.4 days, with an average PDI of 91.1 %. To verify pathogenicity, roots from the artificially inoculated and infected plants of the susceptible JI-35 cultivar were collected and re-isolated on PDA media. The morphological characteristics of the re-isolated cultures were found consistent with those of the original isolates, thereby confirming Koch's postulates. Similarly, Desai *et al.* (2003) also confirmed the pathogenic potential of 15 isolates of the castor wilt pathogen using the susceptible cultivar VI-9. Similar variations in virulence have been noted by Chauhan (2007) and Reddy *et al.* (2010).

#### 4. CONCLUSION

This study revealed significant variability among the *F. oxysporum* f. sp. *ricini* isolates in pathogenicity, incubation period, disease incidence and cultural characteristics. Pathogenicity tests highlighted differences in aggressiveness with certain isolates exhibiting shorter incubation period and higher disease incidence. Cultural characterization further showed diversity in mycelial colour, colony morphology, pigmentation, growth habit and sporulation. Majority of the isolates which exhibited fast or moderate growth and very high or high sporulation generally demonstrated higher aggressiveness and pathogenic potential. These observations suggested that these cultural characteristics could serve as reliable factors for virulence of the isolates. Moreover, the complex interactions between environmental conditions and the physiological adaptability of the isolates, along with the pathogen virulence factors indicated the intricate nature of pathogenicity in *F. oxysporum* f. sp. *ricini* isolates. The observed variations likely reflected the genetic diversity among isolates and environmental influences, emphasizing the need for effective management strategies against castor wilt disease. These findings provided valuable insights for developing effective disease management approaches and enhancing castor production.

#### Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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**Table 1. Collection of *Fusarium oxysporum* f. sp. *ricini* isolates from various castor growing regions in India**

S. No.	Location	District	State	Isolate Code
1	Palem	Nagarkurnool	Telangana	<i>For-1</i>
2	Rajendranagar, Hyderabad	Rangareddy	Telangana	<i>For-2</i>
3	Bangalore	Bangalore	Karnataka	<i>For-3</i>
4	Yethapur	Salem	Tamilnadu	<i>For-4</i>
5	Bhawanipatna	Kalahandi	Odisha	<i>For-5</i>
6	Sardarkrushinagar	Surat	Gujarat	<i>For-6</i>
7	Islampura, Vadgam	Banaskantha	Gujarat	<i>For-7</i>
8	Prempur, Himatnagar	Sabarkantha	Gujarat	<i>For-8</i>
9	Charada, Mansa	Gandhi nagar	Gujarat	<i>For-9</i>
10	Balad, Kheralu	Mehesana	Gujarat	<i>For-10</i>
11	Dadhial, Visnagar	Mehesana	Gujarat	<i>For-11</i>
12	Junagadh	Junagadh	Gujarat	<i>For-12</i>
13	Jaliya, Rajkot	Junagadh	Gujarat	<i>For-13</i>
14	Bholgamda, Dhoraji	Junagadh	Gujarat	<i>For-14</i>
15	Devarkadra	Mahabubnagar	Telangana	<i>For-15</i>
16	Mandore	Jodhpur	Rajasthan	<i>For-16</i>
17	Mallapura, Hosdurga	Chitradurga	Karnataka	<i>For-17</i>
18	Ganganagar, Mandore	Jodhpur	Rajasthan	<i>For-18</i>
19	Hanumangarh, Mandore	Jodhpur	Rajasthan	<i>For-19</i>
20	Jalore, Mandore	Jodhpur	Rajasthan	<i>For-20</i>

**Table 2. Cultural characteristics of isolates of *F. oxysporum* f. sp. *ricini* collected from various castor growing locations of India**

S. No.	Isolate	Mycelial colour	Colony characters	Pigmentation	Growth Habit	Sporulation
1	For-1	Whitish purple	Fluffy, smooth margin	Pinkish	Fast	++++*
2	For-2	Pinkish white	Fluffy, smooth margin	Pinkish	Fast	++++
3	For-3	Whitish pink	Fluffy, smooth margin	Pinkish	Fast	+++
4	For-4	Pale white	Submerged, irregular margin	Pale yellow	Slow	++
5	For-5	Pale white	Fluffy, smooth margin	Dull white	Fast	++
6	For-6	Milky white	Submerged, smooth margin	Pale yellow	Moderate	++
7	For-7	Cottony white	Dense cottony, smooth margin	Dull white	Moderate	+
8	For-8	Cottony white	Sparse cottony, smooth margin	Dull white	Fast	+++
9	For-9	Whitish yellow	Fluffy, smooth margin	Pale yellow	Moderate	++
10	For-10	Milky white	Submerged, irregular margin	Pale yellow	Moderate	++
11	For-11	Whitish pink	Submerged, smooth margin	Pinkish	Slow	++
12	For-12	Milky white	Sparse cottony, irregular margin	Pale yellow	Fast	+++
13	For-13	Cottony white	Sparse cottony, irregular margin	Pale yellow	Moderate	++
14	For-14	Pale white	Submerged, irregular margin	Pale yellow	Moderate	+++
15	For-15	Cottony white	Sparse cottony, smooth margin	Pale yellow	Moderate	+++
16	For-16	Whitish pink	Sparse fluffy, smooth margin	Pinkish yellow	Fast	++++
17	For-17	Cottony white	Dense cottony, smooth margin	Pale yellow	Slow	+
18	For-18	Milky white	White Fluffy, irregular margin	Dull white	Moderate	+++
19	For-19	Cottony white	Sparse cottony, smooth margin	Dull white	Slow	+++
20	For-20	Cottony white	Dense cottony, irregular margin	Pale yellow	Fast	++

\*+: Sparse      ++: Moderate      +++: High      ++++: Very high

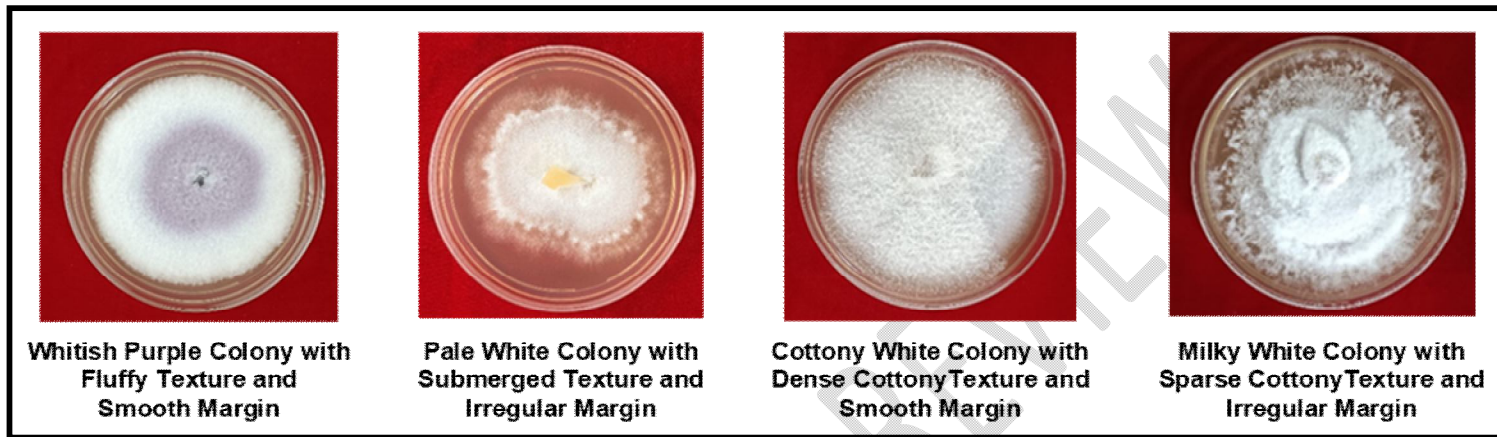


Plate 1. Colony characteristics of *F. oxysporum* f. sp. *ricini* isolates

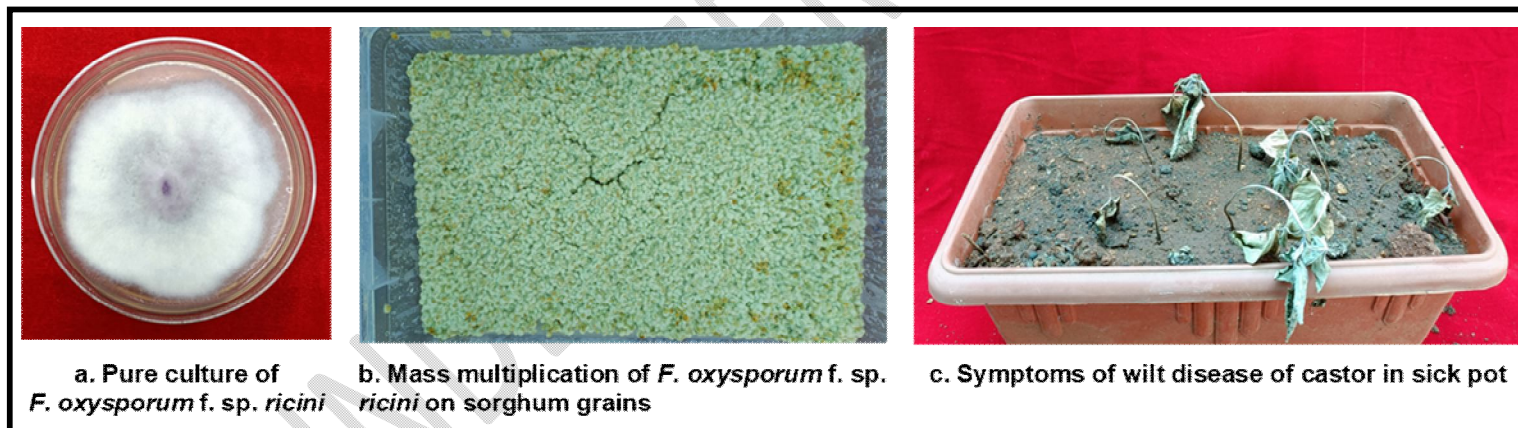
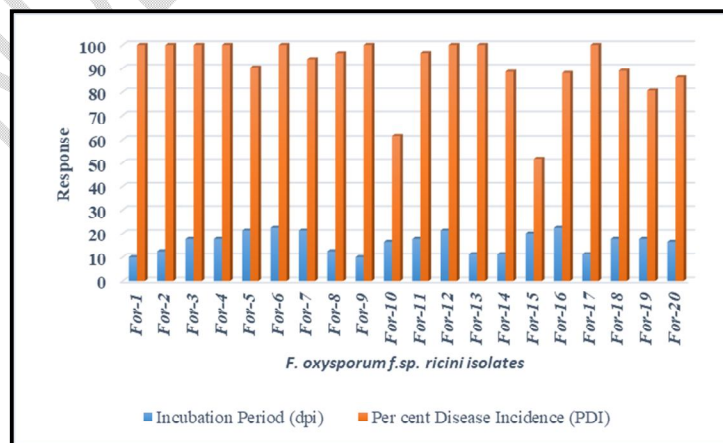


Plate 2. Proving pathogenicity of *F. oxysporum* f. sp. *ricini* on susceptible castor cv. JI-35 under sick pot conditions

**Table 3. Pathogenicity of *F. oxysporum* f. sp. *ricini* isolates on the susceptible castor cultivar JI-35**

S. No.	Isolate	Incubation Period (dpi)	Per cent Disease Incidence (PDI)
1	For-1	10.0	100.0 (90.0)*
2	For-2	12.5	100.0 (90.0)
3	For-3	17.5	100.0 (90.0)
4	For-4	17.5	100.0 (90.0)
5	For-5	21.3	90.3 (71.9)
6	For-6	22.5	100.0 (90.0)
7	For-7	21.3	93.8 (75.5)
8	For-8	12.5	96.3 (78.9)
9	For-9	10.0	100.0 (90.0)
10	For-10	16.3	61.5 (51.7)
11	For-11	17.5	96.4 (79.1)
12	For-12	21.3	100.0 (90.0)
13	For-13	11.3	100.0 (90.0)
14	For-14	11.3	88.5 (70.1)
15	For-15	20.0	51.7 (46.0)
16	For-16	22.5	88.0 (69.7)
17	For-17	11.3	100.0 (90.0)
18	For-18	17.5	88.9 (70.5)
19	For-19	17.5	80.6 (63.9)
20	For-20	16.3	86.2 (68.2)
Mean		16.4	91.11 (72.7)
SE(d)		0.7	0.2
SE(m)		0.5	0.2
CD		1.4	0.4
CV		2.3	2.2

\*Figures in parenthesis are arc sin transformed values; The figures are mean of four replications; dpi-Days of Post Inoculation; PDI-per cent disease incidence



**Figure. 1 Incubation period and wilt incidence of twenty isolates of *F. oxysporum* f. sp. *ricini* on susceptible castor cultivar (JI-35)**