Print : ISSN 0970-7662
Online : ISSN 2455-7129

Journal of Tree Sciences
online available at www.ists.in

Volume 35 No.2 December, 2016

Survey and Pathological Investigation on Fusarial Wilt of Garmalo (*Cassia fistula* L.)

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DOI: 10.5958/2455-7129.2016.00006.6

ABSTRACT

Occurrence of fusarial wilt of garmalo (*Cassia fistula*) was conducted in three districts of Southern Gujarat. The maximum percent disease incidence (75 per cent) was observed at Karatha Sarvajanik Forest Nursery of Narmada district. Microscopic examination and the tissue isolation from roots of infected seedlings of different forest nurseries yielded culture of *Fusarium oxysporum*. Most of the plants were found infected by showing varying degree of infection indicating their susceptibility to the pathogen at almost all stages of seedling growth. Leaves became yellow with initially brown and eventually black streaks in the vascular system. Plants were showing severe stunting and initiation of wilting symptoms and at later stage found to be completely wilted. On critical examination, the fungal growth was observed on the roots of the wilting plants. Microscopic examination revealed the presence of micro and macro conidia of *F. oxysporum* with dirty white mycelium. The pathogenicity tests were carried out by soil inoculation, seed inoculation, seed cum soil inoculation, and root dip inoculation in plastic pots. All the methods successfully produced typical wilt symptoms similar to those observed under natural condition and described in the literature, confirming pathogenic nature of the fungus. Among the different techniques root dip inoculation method was found to be the quickest and most effective method for proving the pathogenicity followed by seed cum soil inoculation and soil inoculation method. Thus, the causal agent of the Garmalo (*C. fistula*) fusarial wilt was identified and confirmed as *Fusarium oxysporum.*

Key words:

Fusarium oxysporum, Wilt, *C. fistula,* Survey, Pathogenicity

INTRODUCTION

Garmalo (*Cassia fistula* L.) is one of the most important multipurpose tree in the forest. Diseases are major constraint in production of healthy seedlings in nurseries as they inflict heavy losses. Wilt is one of the major threats in *C. fistula* seedling in nursery stage. It caused approximately 25 to 44 percent seedling losses in the forest nurseries of Southern Gujarat districts and it has

been reported to be incited by *Fusarium oxysporum*. The scientific information on this disease is presently lacking. Hence, the investigation on this problem was undertaken for generating more scientific information about the disease.

MATERIALS AND METHODS

Nursery Survey and methodology

Survey of the forest nurseries and other nurseries of the Navsari district was done. The nurseries were frequently visited during October-November 2014. Occurrence of disease or any symptoms or nature of damage caused to seedling were recorded. Other relevant information pertaining to nursery practices, such as total number of bed/container beds, sowing date, quality of seeds per standard bed, soil characteristics, watering schedule, type of shade, beside the date of appearance of disease were recorded. The incidence of a disease was estimated either by counting number of disease patches and the approximate area covered by them or percent seedling affected for a given density of seedling in a seedbed (Table 1). Appropriate specimens of disease seedling and soil samples were collected subjected to isolation of the causal organism. (Sharma et al. 1985).

Table 1. Disease scoring scale for assessing the severity of disease in nursery

Disease severity	Disease scoring scale		
	Percent seedling affected per seedbed	No. of patches of diseased seedling per seedbed	Percent of seed bed area affected
Low(L)	1-25	1-25	1-10
Medium(M)	26-50;10-15%Seedling dead	26-50	11-25
Severe(S)	50-75 or more;20% Seedling dead	>50	>25

Collection of samples

The diseased samples showing yellowing leaves and brownish coloured symptoms collected from the forest nurseries of Navsari district were brought to the laboratory and subjected to microscopic examination.

Survey for the severity of fusarial wilt disease

The survey was carried out at forest nurseries of south Gujarat viz.,Navsari, Narmada and Surat. A roving survey was conducted to know the status of wilt disease in Southern Gujarat districtduring November- December 2014.

Calculation of Per cent Wilt Incidence (PWI):

$$PWI = \frac{\text{No. of Wilted}}{\text{Total no. of plant/bed}} \quad X\ 100$$

Isolation of pathogen

Isolation was done from infected roots of *C. fistula* seedling showing typical wilt symptoms. The infected sample was subjected to tissue isolation. The infected portion of the plant was cut into small bits in such a way that each bit consisted of infected as well as healthy tissues. The bits were surface sterilized with 0.1per cent mercuric chloride ($HgCl_2$) solution for 30 seconds followed by three washing with sterilized distilled water and then transferred aseptically under laminar air flow system on sterilized Petriplates containing 20 ml Potato Dextrose Agar (PDA) medium. These Petriplates were incubated at room temperature (27 ± 2°C). The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA slants. The pure culture thus obtained was microscopically examined for identification and was further purified by using single hyphal tip isolation technique.

Maintenance of cultures

The cultures of obtained pathogens were maintained in refrigerator and sub-cultured periodically at an interval of 30 days during the course of this study.

Raising *Cassia fistula* seedlings for symptomatology.

The diseased samples representing typical wilt disease symptoms were collected from the field and brought to the laboratory for symptomatological studies. *C. fistula* seeds are grown in nursery for detail studies of symptoms development.

Pathogenicity test

Pathogenicity test of Fusarium spp. isolated from C. fistula was carried out in pots by seed inoculation, soil inoculation, seed cum soil inoculation and root dip technique treatment in the Green house. The spores having 11.2 x 104 spores/ml was used as inoculums. The surface of healthy seeds were sterilized by dipping in 0.1 percent mercuric chloride ($Hgcl_2$) solution for a minute followed by 3 subsequent washing of sterile distilled water. Two seeds were sown in the each plastic pot containing autoclave soil. The pots were labeled, watered as and when required and left undisturbed in net house for germination and development of symptoms. Four repetition were kept in each method with control (uninoculated).

Seed inoculation

The healthy and surface sterilize seeds were first dipped in spore suspension (11.2 $x10^4$ spores/ml) for 30 minutes and then were sown in bags.

Soil inoculation

The healthy and surface sterilize seeds were sown in pot and on around the seeds 200ml spore suspension (11.2 $x10^4$ spores/ml) was poured.

Seed cum soil inoculation

The healthy and surface sterilize seeds were first dipped in spore suspension (11.2 $x10^4$ spores/ml) for 30 minutes and then were sown in bags and 200ml spore suspension (11.2 $x10^4$ spores/ml) was poured as mentioned earlier on and around the seeds in each bag.

Root dip technique

The 35-45 days old seedlings carefully uprooted and their roots were washed with sterilized distilled water. These plants were inoculated by dipping roots in to the spore suspension (11.2 $x10^4$ spores/ml) for 30 min and then were replanted in to pots containing sterilized soil.

Identification of pathogens

To identify the pathogens, cultural and morphological characters were recorded in laboratory under microscope and compared with those given in literature.

RESULTS AND DISCUSSION

Survey and collection of samples

During the survey of the Southern Gujarat forest nurseries seven nurseries were showing wilt disease incidence. The nurseries were frequently visited in November and December 2014. The occurrence of disease or any symptoms or nature of damage caused to seedling was recorded. The other relevant information which was also recorded includes total no. of infected seedling, sowing date, soil characteristics, watering schedule, media and severity (Table 2).Among the visited seven forest nurseries of Southern Gujarat, the Karatha forest nursery showed maximum disease incidence 75 per cent followed by Visdaliya forest nursery 36.4 per cent. Rajpipla forest nursery showed minimum disease incidence 4.8 per cent. The obtained results were in conformity with the earlier observations recorded by Chakravarti and Mishra (1986), Malhotra (1989), Harsh (1992), Malhotra (1998), and Kadam (2012) as they proved the various diseases during field survey of *Cassia spp.* caused by *Fusarium spp*. from forest nurseries.

Symptomatology

Under natural and greenhouse condition the symptoms were observed during the visit and infected samples were collected. The symptoms were recorded from college forest nursery. Most of the plants were found infected and showing varying degree of infection indicating their susceptibility to the pathogen at almost all stages of seedling growth. Leaves became yellow with initially brown and eventually black streaks in the vascular system. Plants showed severe stunting and wilting symptoms. Most of the plants were found with varying degree of infection in the different nurseries in south Gujarat condition. The

Table 2: Survey and data collection of different forest nurseries of Southern Gujarat.

Sr. No.	Name of Nursery	Size of bed (m)	Total No. of seedling	Sowing Date/time	Soil (Soil +Sand + F.Y.M)	Watering schedule	Total No. of infected seedling	% of Wilt incidence (%)	Disease severity
1	Vesma Forest Nursery	1×3	300	1st week of July	2:1:1	Daily	76	25.33	L
2	Aamri Forest Nursery	1×1	100	3rd week of July	2:1:1	Daily	20	20	L
3	College of Forestry Nursery	1×1	100	1st week of April	2:1:1	Daily	40	40	M
4	Rajpipla Forest Nursery	1×10	1000	2nd week of June	2:1:1	Daily	48	4.8	L
5	Visdaliya Forest Nursery	1×5	500	2nd week of July	2:1:1	Daily	182	36.4	M
6	Zankhvav Forest Nursery	1×10	1000	15-16 july	2:1:1	Daily	100	10	L
7	Karatha Forest Nursery Rajpipla	1×5	500	3-4 August	2:1:1	1-2 days	375	75	S

Table 3. Pathogenicity of *Fusarium oxysporum* on Garmalo (*Casia fistula* L.) in pot condition.

Sr. No.	Inoculation methods	Total no. of plant sowing/pot	Total no. of germinated plant	Wilting symptoms produced days after inoculation (DAI)	Total no. of wilted plant	Wilt %	Crop phenology
1	Soil inoculation	2	4	47.00	4	100	Leaves became yellow with initially brown, stunting vascular discoloration.
	Control	1	1	00.00	0	00	-
2	Seed inoculation	2	2	45.00	2	100	Leaves became yellow with initially brown, stunting vascular discoloration.
	Control	1	1	00.00	0	00	-
3	Seed cum soil inoculation	2	4	43.00	4	100	Leaves became yellow with initially brown, stunting vascular discoloration.
	Control	1	1	00.00	0		-
4	Root dip technique	2	-	26.00	6	100	Gradually leaves becomes brown and plant wilting up to down, after symptoms develop sudden death of seedling
	Control	1	1	00.00	0	00	-

seedling stage of plants was most attacked. Yellowing and drooping of the leaves as well as complete drying of disease plant were observed at initial stage of seedling growth. On critical examination, the fungus growth was observed on the roots of the wilting plants. Microscopic examination revealed the presence of micro and macro conidia of *Fusarium oxysporum* with white mycelium. The obtained results were in conformity with the observations of Shetty et al.(1974) and Arif et al.(2013) who also reported fusarial wilt symptoms like paling of the leaves, which gradually turned yellow and drooped, followed by premature leaf fall and death of the plant within 4-6 months, dark brown discoloration of diseased roots and gradually decreasing with height and was isolated constantly up to approximately 40 per cent height of the seedling.

Pathogenicity test

The pathogenicity test of the isolates was carried out by four different methods viz., seed inoculation, soil inoculation, seed cum soil inoculation and root dip inoculation. Soil inoculation, seed inoculation and seed cum soil inoculation showed same effect on germination of *C. fistula*. The seedling sprouted after 10-12 days and gradually showed symptoms like necrosis of the lower leaves occurs first, with often the leaves on only one side of the stem turning brownish color then after plant is dead. However, no symptoms of disease was reported in control condition. Root dip inoculation method showed symptoms after 12-15 days of inoculation. The plant leaves and stem gradually turn in to brown color and near the soil surface stem showed whitish color. On the standing plants, wilt symptoms were initiated earlier (26 DAI) in root dip inoculation method. In other methods, the symptoms development was initiated comparatively later. In seed inoculation, soil inoculation and seed cum soil inoculation the first wilt symptoms was observed after 47.00, 45.00 and 40.00 days of inoculation respectively. Cent percent plants were showing typical wilt symptoms in soil inoculation, seed inoculation, seed cum soil inoculation and root dip inoculation methods (Table 3). With the above results root dip inoculation method was found to be the quickest and most effective method for proving the pathogenicity of Garmalo (*C. fistula*) followed by seed cum soil inoculation and soil inoculation method. Various workers have also proved the pathogenicity of Fusarium sp. causing wilt in different crops by this methods confirmed with Chakravarti and Mishra (2006) proved pathogenicity of *F. oxysporum* causing fusarial wilting of *C. tora* by using root inoculation method, Dadwal et al. (2012) proved pathogenicity of *F. oxysporum* infected the seeds of *C. fistula* by using moist blotter technique, Sahu (2006) proved pathogenicity by using agar plate and blotter method and Rajput et al. (2008) proved pathogenicity by using moist blotter technique.

Identification of pathogen

Cultural characters

Colonies of *F. oxysporum* on PDA media showing white color and slow growing 2.5-3 mm growth per day at 28^{0}C incubation. cultural variation like mycelia size, shape, color, mycelial growth, dry mycelium weight, sporulation, conidial size and formation of chlamydospores. The isolates produced moderate, profuse fluffy, thin flat to slight fluffy and submerged growth with white pigmentation. Results were in conformity with the scientists cultural character of isolated *Fusarium oxysporum* showed its close identity with Mandhare (1997), Sharma et al. (2006), Sinha et al. (2007), Araghi et al. (2008), Prasad et al. (2008), Rangaswamy et al. (2012), Lezcano et al.(2012), Chopada et al. (2014) and Poongothai et al. (2014) by fungus mycelium, reproductive structures, macroconidia, microconidia, chlamydospores, mycelial growth, size, shape of *Fusarium oxysporum*.

Morphological character

Morphological variation like Sporulation varied from 5.47×10^{4} spores/ml to 14.68×10^{4} spores/ml. The macro conidia ranged from 15.46-21.8×4.91-5.45 µm to 21.42-44.28×7.35-9.14 µm. The micro conidia varied from 3.57-14.28×2.68-4.46 µm to 7.14-14.28×3.57-5.35 µm. Width of mycelia and chlamydospore dimension also varied in all isolates. Results were

in conformity with the scientists morphological character of isolated *Fusarium oxysporum* showed its close identity with Honnareddy and Dubey (2007) found microconidia ranged from 5.50 to 13.50 X 2.50 to 3.50 μm and that of macroconidia 15.00 to 37.50 X 3.50 to 4.50 μm, Murumkar et al. (2008) proved microconidia ranged from 2.86 to 26.64 µm, macroconidia ranged from 10 to 48 µm, Prasad et al. (2008) proved macroconidia ranged from 23.2 X 4.1 μm to 64.5 X 5.4 μm, Dubey et al. (2010) found and Gupta et al.(2011) microconidia varied from 5.1-12.8 X 2.5-5.0 μm, whereas macroconidia ranged from 16.5-37.9 X 4.0-5.9 μm, Rangaswamy et al. (2012) proved micro- and macro-conidia varied from 7.27 × 2.88 µm to 13.25 × 2.68 µm and 23.37 × 3.17 µm to 40.05 × 4.71 µm, Kumar and Upadhyay (2013) found macro conidia and micro conidia ranged from 15.4-35.0 × 2.0 - 8.2 µm and 4.1 - 16.5 × 2.0- 6.1 µm and Chopada et al. (2014) proved macro conidia ranged from 15.4- 21.8×4.91 - 5.45 µm to 21.42 -44.28 ×7.35 - 9.14 µm and micro conidia varied from 3.57-14.28×2.68-4.46 µm to 7.14-14.28×3.57-5.35 µm.

ACKNOWLEDGMENTS

Corresponding author is thankful to the faculties of College of forestry, ACHF, NAU, Navsari as well as Laboratory, Department of Plant Pathology, ACHF & NMCA, NAU, Navsari provided facilities to conduct research work, their support and guidance.

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