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# Screening of Resistant Varieties and Antagonistic *Fusarium oxysporum* for Biocontrol of *Fusarium Wilt* of Chilli

**Mamta Joshi[1,2]*, Rashmi Srivastava[1], Anil Kumar Sharma[1] and Anil Prakash[2]**

[1]*Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar – 263145, Uttarakhand, India*
[2]*Department of Biotechnology and Bioinformatics, Barkatullah University, Bhopal, MP, India*

## Abstract

Chilli is an important vegetable/spice, and its socio-cultural role is remarkable worldwide. The enormous popularity and demand for chilli is providing a boost to the chilli industry, but its production is increasingly constrained by diseases. Chilli producers have reported *Fusarium wilt*, as the frequently encountered disease. The present investigation focuses on biological control, which is found effective to manage this disease.

A survey was conducted at Uttarakhand and Uttar Pradesh states in India, for collection of soil and plant samples. A total of eighty isolates of *Fusarium* were isolated from these samples. Among these, forty eight isolates of *Fusarium oxysporum* were identified on the basis of morphological and molecular characteristics, using species-specific primers. Pathogenicity test on chilli was conducted. One isolate of *F. oxysporum* was found most virulent pathogen, while eleven isolates were non- pathogenic isolates. Isolate no. 65 was found most antagonistic towards *F. oxysporum*, under *in-vitro* dual culture assay. Thirty chilli varieties were screened for evaluation of resistance. Among these, two varieties were found resistant against the *Fusarium wilt*. The present investigation focused on recovery of antagonistic Fusarium and resistant varieties of chilli, for controlling and resisting wilt and improving the soil health.

**Keywords:** Biological control; Chilli; Resistant variety; Antagonistic *F. oxysporum*

## Introduction

Chilli (*Capsicum annuum*) has been an important commodity used as a vegetable and spice crop worldwide, that is produced and consumed as fresh or processed. Today, *Capsicum* is found throughout the world, and plays a significant socio-economic role. Major producers of chilli include Asia, Latin America, Africa, Europe, and North America [1].

Chilli grows all over India. In 2003, it was grown on an area of 945.5 thousand hectares, with an annual production of 4.5 million tonnes in fresh weight. India is one of the largest producers of chilli in the world, accounting for over forty six percent of its total area and production. India exported 349 thousand tonnes of fresh weight equivalent chilli, worth US$ 62 million [2]. Chilli is a major source of income for poor farmers in India, but it suffers from many diseases, making it difficult to grow in hot humid conditions [3]. Chilli growers have, for many years, experienced considerable economic loss due to *Fusarium oxysporum*.

In 1919, Leonian [4] described a wilt disease of chilli caused by *Fusarium*. The symptoms of *Fusarium wilt* included leaf chlorosis, vascular discoloration, and wilting of chilli plants. High temperature and high moisture were conducive to symptom development of wilt [5].

If economically justified, soil fumigants and solarization can be used to reduce pathogen populations in soil; but increasing use of pesticides in the past two decades has led to several problems, such as environmental degradation health hazards for human, pest resistance and decrease in the population of beneficial insects, which has direct impact on disease resistance [6]. There is a worldwide need to adopt the practice of sustainable agriculture, using strategies that are environment-friendly, less dependent on agricultural chemicals, and less damaging to soil and water resources. One of the key elements of such sustainable agriculture is the application of biological controlling strategies, for plant protection.

Chilli growers need new highly productive varieties with superior horticultural characteristics, that are also resistant to *Fusarium wilt*. Selection of chilli variety is a top criterion for higher production of chilli, followed by composite measure of all good appearance characteristics, disease and insect resistance [2].

Another natural biological control strategy is soil's suppressiveness towards soil borne plant diseases, in which disease development is minimal even in the presence of virulent pathogen and susceptible plant host. The phenomena of disease suppressive soils have been documented for numerous plant pathogen systems, around the world for over hundred years. Suppressive soils have been characterized for the fungus, *F. oxysporum*. [7,8]. The role of nonpathogenic *F. oxysporum* in the natural suppressiveness of some soils to *Fusarium wilt* has been established [9], and has led to the screening of effective strains as biocontrol agents [10].

Though, all biological methods have given some control, but no single control method has provided better and sustainable control from the disease. Hence, integrating more than one strategy to manage the disease is a must.

In the present investigation, we had found out safer alternative of chemical control for effective biological control of pathogenic *Fusarium* of chilli, using antagonistic *F. oxysporum* and disease resistance variety,

***Corresponding author:** Mamta Joshi, Department of Biological Sciences, CBSH, G.B. Pant University of Agriculture & Technology, Pantnagar – 263145, Uttarakhand, India, Tel: +5944-233309; Fax: +5944-233473; E-mail: mamta.biotech@gmail.com

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which were possibly best lines of defence of the *Fusarium wilt* for the farmers. The present investigation was conducted to explore the possibility of screening and identifying potential antagonist, which could be capable of suppressing pathogenic *F. oxysporum* of chilli, the causal organism of *Fusarium wilt* disease, and assess the efficacy of resistant varieties using *in-vivo* studies, for its incorporation as biological control agents.

## Material and Methods

### Sample collection

A total of one hundred twenty four fields were selected for this study. These fields were located in different agricultural farms at Uttarakhand and Uttar Pradesh states in India. Out of one hundred twenty four fields, fifty nine fields were of chilli (Table 1) and sixty five were of tomato (Table 2). Five subsamples of soil with root from every field, were taken, pooled, well-mixed into a single composite and sample were kept within a plastic bag. Moist soil samples were immediately stored, in sealed plastic bags at 4°C. Fungus isolation was conducted within one week of sampling.

### Recovery and identification of *F. oxysporum* from soil and plant samples

All rhizosphere samples were cultured on potato dextrose agar, amended with streptomycin (Hi-Media, Mumbai). One gram soil was used for serial dilutions, as described by Ofunne [11] and $10^{-4}$ dilution was used for pour plating into potato dextrose agar (PDA) plates. Incubation was done at 27°C and plates were regularly monitored for visible fungal growth. Fungal isolation was performed from plates containing five to eight arbitrarily chosen colonies, and each single colony was transferred to fresh PDA petri plates.

Fungal isolation from plant sample, was done by washing the roots under tap water, split in half, the surface was sterilized in with 0.5% NaOCl for two minutes, rinsed twice with triple distilled water and placed in petri-dishes containing PDA. After five days of incubation at 27°C, colonies of fungus were transferred to fresh potato dextrose agar (PDA) containing petri-plates. All the isolates of fungus were examined morphologically by microscopic (Motic image plus 2.0 software at 400X magnification.) observation, for the identification of *Fusarium* spp. according to the criteria of Booth [12]. Out of eighty *Fusarium* isolates, forty eight isolates were of *F. oxysporum*, which were used in the present study.

### Identification of *F. oxysporum* using molecular tools

DNA extraction, of all eighty isolates of *Fusarium* along with positive control (*F. oxysporum*, MTCC No. 4353) was done, according to the method of Reader and Broda [13] for the molecular identification of *F. oxysporum.*

DNA was visualized on a 1% agarose gel, after staining with 1.6 µg $ml^{-1}$ ethidium bromide. Species specific primers, FOF1 (5'-ACA TAC CAC TTG TTG CCT CG-3') and FOR1 (5'-CGC CAA TCA ATT TGA GGA ACG-3') were used for the identification of *F. oxysporum* [14]. PCR reactions were carried out in a 20 µl reaction volume containing PCR buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl; pH 8.3) (Fermentas), 0.2 mM each dNTP (Fermentas), 0.3 µM of each primer FOF1 and FOR1, nuclease free water was used to achieve the final volume of 20 µl. DNA amplification was performed on a gradient

| Sample No. | Isolated from | Location | Isolate no. | Sample No. | Isolated from | Location | Isolate no. |
|---|---|---|---|---|---|---|---|
| 1 | Endorhizo sphere | Barabanki 1 | 1 | 30 | Endorhizosphere | Barabanki 18 | 38 & 39 |
| 2 | Rhizosphere | Barabanki 2 | - | 31 | Rhizosphere | Barabanki 19 | - |
| 3 | Endorhizosphere | Barabanki 3 | 2 &3 | 32 | Rhizosphere | Barabanki 20 | - |
| 4 | Endorhizosphere | Barabanki 4 | 4 | 33 | Rhizosphere | Barabanki 21 | - |
| 5 | Rhizosphere | Barabanki 5 | - | 34 | Rhizosphere | Barabanki 23 | 41 |
| 6 | Rhizosphere | Barabanki 6 | 5 | 35 | Rhizosphere | Barabanki 24 | - |
| 7 | Endorhizosphere | Barabanki 7 | 6 & 7 | 36 | Endorhizosphere | Barabanki 25 | 42 |
| 8 | Rhizosphere | Barabanki 8 | - | 37 | Rhizosphere | Barabanki 26 | 43 |
| 9 | Rhizosphere | Barabanki 9 | - | 38 | Rhizosphere | Barabanki 27 | - |
| 10 | Rhizosphere | Barabanki 10 | - | 39 | Rhizosphere | Barabanki 28 | 44 & 45 |
| 11 | Endorhizosphere | Barabanki 11 | 8 | 40 | Rhizosphere | Barabanki 29 | 46 |
| 12 | Rhizosphere | Kanpur 4 | 12 | 41 | Rhizosphere | Pilibheet 2 | - |
| 13 | Rhizosphere | Baheri 1 | 26 | 42 | Rhizosphere | Bareilly 4 | 48 |
| 14 | Endorhizosphere | Hardoi 2 | - | 43 | Rhizosphere | Bareilly 5 | 49 |
| 15 | Endorhizosphere | Hardoi 3 | - | 44 | Rhizosphere | Bareilly 6 | - |
| 16 | Endorhizosphere | Barabanki 14 | 29 | 45 | Rhizosphere | Bareilly 7 | - |
| 17 | Endorhizosphere | Furrukhabad 1 | 30 | 46 | Rhizosphere | Bareilly 8 | 50 |
| 18 | Rhizosphere | Furrukhabad 2 | - | 47 | Rhizosphere | Bareilly 9 | 51 |
| 19 | Rhizosphere | Furrukhabad 3 | - | 48 | Rhizosphere | Bareilly 10 | - |
| 20 | Rhizosphere | Lakhimpur 1 | 31 | 49 | Rhizosphere | Bareilly 11 | - |
| 21 | Endorhizosphere | Bareilly 1 | 32 | 50 | Rhizosphere | Baheri 4 | 52 |
| 22 | Rhizosphere | Bareilly 2 | - | 51 | Rhizosphere | Bareilly 12 | 53 |
| 23 | Rhizosphere | Bareilly 3 | - | 52 | Rhizosphere | Pilibheet 4 | - |
| 24 | Rhizosphere | Lakhimpur 2 | 33 | 53 | Endorhizosphere | Pilibheet 5 | - |
| 25 | Rhizosphere | Kanpur 24 | - | 54 | Rhizosphere | Bareilly 13 | 55 |
| 26 | Rhizosphere | Barabanki 15 | 35 & 36 | 55 | Rhizosphere | Pilibheet 6 | 56 |
| 27 | Endorhizosphere | Barabanki 16 | - | 56 | Endorhizosphere | Barabanki 30 | 57 |
| 28 | Endorhizosphere | Barabanki 17 | - | 57 | Endorhizosphere | Barabanki 31 | - |
| 29 | Rhizosphere | Lucknow | 37 | 58 | Rhizosphere | Barabanki 32 | - |

**Table 1:** Soil survey and sample collection of plant and soil, for isolation of *Fusarium* spp. from chilli crop.

| Sample No. | Isolated from | Location | Isolate no. | Sample No. | Isolated from | Location | Isolate no. |
|---|---|---|---|---|---|---|---|
| 1 | Rhizosphere | Barabanki 12 | - | 34 | Rhizosphere | Teenpani 1 | 61 |
| 2 | Rhizosphere | Barabanki 13 | - | 35 | Rhizosphere | Teenpani 2 | - |
| 3 | Endorhizosphere | Kanpur 1 | 9 | 36 | Rhizosphere | Haldwani | 62 |
| 4 | Rhizosphere | Kanpur2 | 10 | 37 | Rhizosphere | Golapar 1 | 63 |
| 5 | Endorhizosphere | Kanpur 3 | 11 | 38 | Rhizosphere | Golapar 2 | - |
| 6 | Rhizosphere | Kanpur 5 | 13 | 39 | Rhizosphere | Golapar 3 | 64 |
| 7 | Rhizosphere | Kanpur 6 | - | 40 | Rhizosphere | Golapar 4 | - |
| 8 | Rhizosphere | Kanpur 7 | - | 41 | Rhizosphere | Chorgalia 1 | 65 |
| 9 | Endorhizosphere | Kanpur 8 | 14 | 42 | Rhizosphere | Chorgalia 2 | - |
| 10 | Rhizosphere | Kanpur 9 | 15 | 43 | Endorhizosphere | Chorgalia 3 | 66 & 67 |
| 11 | Rhizosphere | Kanpur 10 | - | 44 | Rhizosphere | Talla pachonia 1 | 68 |
| 12 | Rhizosphere | Kanpur 11 | - | 45 | Rhizosphere | Talla pachonia 2 | 69 |
| 13 | Rhizosphere | Kanpur 12 | 16 | 46 | Endorhizosphere | Talla pachonia 3 | 70 |
| 14 | Rhizosphere | Kanpur 13 | 17 | 47 | Rhizosphere | Talla pachonia 4 | 71 |
| 15 | Rhizosphere | Kanpur 14 | 18 | 48 | Rhizosphere | Talla pachonia 5 | - |
| 16 | Rhizosphere | Kanpur 15 | 19 | 49 | Rhizosphere | Talla pachonia 6 | 72 |
| 17 | Rhizosphere | Kanpur 16 | 20 | 50 | Rhizosphere | Talla pachonia 7 | - |
| 18 | Rhizosphere | Kanpur 17 | 21 | 51 | Rhizosphere | Talla pachonia 8 | 73 |
| 19 | Rhizosphere | Kanpur 18 | 22 | 52 | Rhizosphere | Buwanipur 1 | 74 |
| 20 | Rhizosphere | Kanpur 19 | 23 | 53 | Rhizosphere | Buwanipur 2 | - |
| 21 | Rhizosphere | Kanpur 20 | 24 | 54 | Endorhizosphere | Buwanipur 3 | 75 |
| 22 | Rhizosphere | Kanpur 21 | - | 55 | Rhizosphere | Buwanipur 4 | - |
| 23 | Rhizosphere | Kanpur 22 | 25 | 56 | Rhizosphere | Buwanipur 5 | 76 |
| 24 | Rhizosphere | Baheri 2 | 27 | 57 | Rhizosphere | Buwanipur 6 | 77 |
| 25 | Rhizosphere | Baheri 3 | - | 58 | Rhizosphere | Buwanipur 7 | - |
| 26 | Rhizosphere | Hardoi 1 | 28 | 59 | Rhizosphere | Buwanipur 8 | - |
| 27 | Endorhizosphere | Kanpur 23 | 34 | 60 | Rhizosphere | Buwanipur 9 | - |
| 28 | Rhizosphere | Barabanki 22 | 40 | 61 | Rhizosphere | Buwanipur 10 | 78 |
| 29 | Rhizosphere | Pilibheet 1 | 47 | 62 | Rhizosphere | Buwanipur 11 | 79 |
| 30 | Endorhizosphere | Pilibheet 3 | 54 | 63 | Rhizosphere | Buwanipur 12 | 80 |
| 31 | Endorhizoshere | Kanpur 26 | 59 | 64 | Rhizosphere | Buwanipur 13 | - |
| 32 | Rhizoshere | Kanpur 27 | - | 65 | Rhizosphere | Buwanipur 14 | - |
| 33 | Endorhizosphere | Kanpur 28 | 60 | | | | |

**Table 2:** Soil survey and sample collection of plant and soil for isolation of *Fusarium* spp. from tomato crop.

PCR machine (Biometra), using an initial denaturation temperature of 94°C for 60s, followed by 25 cycles of template denaturation for 60s at 94°C,primer annealing for 30s at 58°C and chain elongation for 60s at 72°C, with a final extension of 7 min at 72°C. Amplification conditions were similar to those described by Mishra, et al. [15]. The amplified products were verified, using 2% agarose in gel electrophoresis.

## Pathogenicity tests

Nursery preparation for the pot experiment was performed by surface sterilized seeds of chilli (*var*. Nun 2066), sterilized with 2% sodium hypochlorite for 2 min, washed with distilled water and sown into plastic trays filled with steam sterilized soil. The seeds were germinated at 27°C and 70% relative humidity for 14 hours light period (500 lux). All forty eight isolates identified as *F. oxysporum*, were evaluated for their ability to cause disease, and non pathogenic characteristic on three leaf stage chilli plantlets. The mycelia of these *F. oxysporum* cultures were inoculated into Armstrong *Fusarium* sporulation media [12], in 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth. The flasks were placed on a rotary shaker, operating at 120 rpm at 27°C for five days.

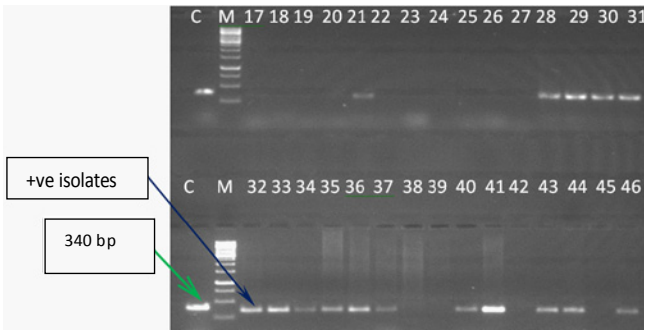
Pathogenicity tests were carried out using a root-dip inoculation method [16]. Seedlings of chilli were uprooted gently from the nursery. The roots of seedling were dipped in a spore suspension of *F. oxysporum* broth containing $10^6$ spores $ml^{-1}$ for 5 min, dried briefly on a paper towel and then, two seedlings were transplanted into root trainer containing 80g of sand.

Four replications were maintained for each isolate. The experiment was conducted in a glass house at 27°C and 14 hours light period (500 lux). Plants were irrigated regularly and supplied with Hoagland's solution weekly. The disease incidence was calculated after twenty days of transplantation.

## *In-vitro* screening of *F. oxysporum* isolates for antagonism

Selected non pathogenic *F. oxysporum* isolates (Isolate no. 27, 32, 49, 62, 65, 66, 73, 75, 77, 79), which did not gave any disease symptom on pathogenicity test, were screened for their activity toward most virulent strains of *F. oxysporum* (Isolate no. 35) by *in-vitro* dual-culture assay.

*In-vitro* screening of antagonistic property of nonpathogenic isolates, which were cut from the edges of full grown lawn of non pathogenic and pathogenic *F. oxysporum* strain, was performed by co-inoculation of 5 mm diameter discs. Discs were placed 5 cm apart of assay onto petri plate containing PDA medium plates, and incubated at 27°C in a BOD incubator. After five days of incubation, the diameter of the colonies of pathogenic *F. oxysporum* was measured. The antagonistic activity of different isolates of *F. oxysporum* was observed, by measuring the percent inhibition of the growth of the pathogenic *F. oxysporum.*




Lane M: 1Kb DNA ladder
Lane C: *Fusarium oxysporum* (Control)
Lanes 1-30: Amplicon of *Fusarium* isolates

**Figure 1:** PCR amplification products of the internal transcribed spacer region of the ribosomal DNA of *Fusarium* isolates using species-specific primers.

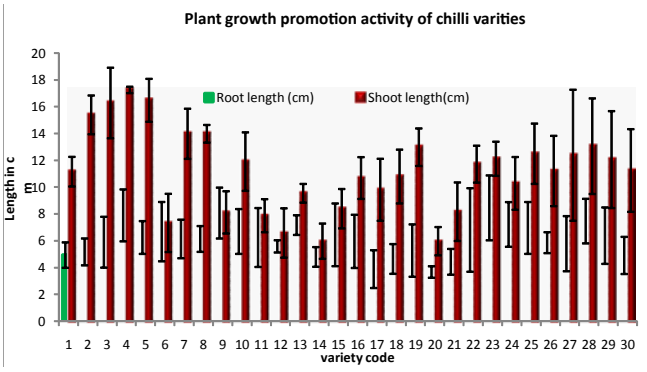



**Figure 2:** Shoot and root length (in centimeter) 30 days of transplantation.

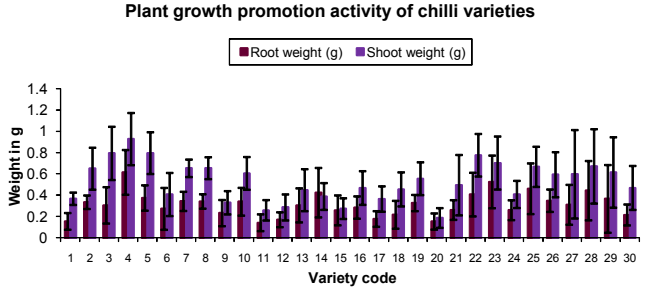



**Figure 3:** Fresh weight (in grams) of shoot and root 30 days of transplantation.

The percent inhibition of growth of pathogenic *Fusarium* by nonpathogenic *Fusarium* was calculated, according to the following formula

Percent inhibition= (C-T) X 100/C

Where C, indicated as the radial growth of the test pathogen in control plate

T= Radial growth of the test pathogen in treatment

### Evaluation of resistance varieties against *Fusarium wilts*

A total of thirty varieties of chilli were collected from the Seed Research Center, Pant nagar. The chilli variety Nun-2066 was taken as control, to be used in pathogenicity test. All thirty varieties of chilli including control, were taken for screening of resistant varieties against *Fusarium wilt* induced by pathogenic *F. oxysporum*. The screening was performed at the three leaf stage plantlets. Pathogenicity test was carried out using root-dip inoculation method (described above as in pathogenicity test), using most virulent pathogen of *F. oxysporum* (Isolate no. 35). The disease incidence in all the varieties was calculated after twenty days of transplantation, and plants were harvested to record plant growth responses by measuring root weight, shoot weight, root length and shoot length.

### Statistical analysis

Statistical analysis of pathogenicity test was done by using one way ANOVA, after angular transformation. Results were compared using least significant difference (LSD) test at p≤5%.

## Results

### Soil survey and sample collection for fungal isolation

Nine different geographical locations [Bareilly (13 fields), Barabanki (32 fields), Kanpur (28 fields), Baheri (4 fields), Hardoi (3 fields), Pilibheet (6 fields), Furrukhabad (3 fields), Lakhimpur (2 fields) and Lucknow(1 field)] at Uttar Pradesh, and 6 locations at Uttarakhand [Teenpani(2 fields), Haldwani (1 field), Golapar (4 fields), Talla pachonia (8 fields), chorgalia (3 fields) and Buwanipur (14 fields)] were surveyed (Table 1 and 2).

Each field represented area of five hundred square meters. Five samples of soil and root were taken from every field (samples were collected from each corner and the center of the field), pooled, well-mixed into a single composite sample and kept within a plastic bag. Moist soil samples were immediately stored in sealed plastic bags at 4°C. Fungus isolation was done within one week of sampling

### Identification of *Fusarium*

Eighty isolates were identified as *Fusarium*. Among eighty *Fusarium* isolates, forty eight isolates were identified as *F. Oxysporum* on the basis of colony morphology and characteristics of macro - and micro conidia. The macro conidia were identified on the basis of characteristics, like thin walled generally 3-5 septate, fused, falcate, and the micro conidia were identified as somewhat hooked apex and particulate base. Chlamydospores were both rough and smooth walled, generally intercalary and sometimes terminal.

### Identification of *F. Oxysporum* using molecular tools

Forty eight isolates (Isolate nos. 2, 5, 10, 12, 13, 14, 15, 16, 18, 19, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 37, 38, 39, 40, 47, 48, 49, 50, 53, 54, 56, 57, 58, 59, 60, 62, 65, 66, 67, 72, 73, 75, 77and 79) of *F. oxysporum* were confirmed on the molecular basis, using species specific primer set FOF1and FOR1, amplifying the DNA fragment of 340 bp of all *F. oxysporum* isolates (Figure 1).

### Percent disease incidence by *F. oxysporum* isolates

The dead plants were counted after twenty days of transplantation. Percent disease incidence was recorded for different isolates of *F. oxysporum* in chilli, and all eight dead plants were considered as 100% disease incidence. Disease incidence was found within the range of 0 to 78.75%. The isolate having disease incidence 78.75 % were recorded as most pathogenic, while the 0.0 % disease incidence isolates were recorded as nonpathogenic. *F. oxysporum* Isolate no. 35 was found to be most pathogenic, while Isolate no. 27, 32, 49, 56, 62, 65, 66, 73, 75,

77 and 79 were found nonpathogenic, on the basis of percent disease incidence (Table 3).

### *In-vitro* screening of isolates for antagonism

Pathogenic *F. oxysporum* was inhibited by various nonpathogenic isolates of *F. oxysporum* to different extent, which was in the range of 24.59% to 37.66 %. The percent inhibition by antagonistic *F. oxysporum* Isolate no. 65 was maximum, while Isolate no. 77 showed minimum inhibition (Table 4).

### Evaluation of resistant varieties against *Fusarium wilt*

Evaluation of resistance in varieties was measured on the basis of total number of live plants of each variety, after induction of pathogen. A total of thirty varieties of chilli were screened for evaluation of resistant varieties. Among thirty varieties screened, only two varieties CO-4 (variety code No. 5) and DLC-352 variety code No. 6) were found 100% resistant, while five varieties, *viz*., Ajeet-3 DLC-524, F-112-5-83, KCS-2013, Hot pepper Nun-2060 (variety code numbers, are given in Table 5) were moderately resistant (83.34%), and Sel 11 was most susceptible variety against wilt of chilli induced by pathogenic *F. oxysporum* (Isolate no. 35) (Table 5).

Among thirty varieties of chilli, highest plant growth was observed in BC-40, on the basis of shoot length, shoot weight and root weight. Maximum shoot length and shoot weight were observed as 17.263 cm and 0.926 g respectively in the variety BC-40, followed by 16.488 cm and 0.794 g respectively in variety CO-4, and 16.288 cm and 0.791 g respectively in variety BC- 25. Maximum root length was observed in the variety G-4 Hot pepper (8.461 cm) followed by DSC-2 (8.075 cm) and BC-40 (7.9cm) (Figure 2 and 3). Maximum root weight was observed in the variety BC-40 (0.61 g), followed by G-4 Hot pepper (0.52 g) and Chilli-Nun-2070 (0.46 g)

| Isolate no | %Disease | Isolate no | %Disease | Isolate no | %Disease | Isolate no | %Disease |
|---|---|---|---|---|---|---|---|
| 2 | 22.50$^{ab}$ | 24 | 11.25$^{ab}$ | 37 | 22.5$^{ab}$ | 58 | 11.25$^{ab}$ |
| 5 | 11.25$^{ab}$ | 25 | 33.75$^{b}$ | 38 | 22.5$^{ab}$ | 59 | 11.25$^{ab}$ |
| 10 | 11.25$^{ab}$ | 26 | 22.50$^{ab}$ | 39 | 33.75$^{b}$ | 60 | 11.25$^{ab}$ |
| 12 | 33.75$^{b}$ | 27 | 0$^{a}$ | 40 | 67.50$^{c}$ | 62 | 0$^{a}$ |
| 13 | 56.25$^{bc}$ | 28 | 11.25$^{ab}$ | 47 | 22.50$^{ab}$ | 65 | 0$^{a}$ |
| 14 | 45.0$^{bc}$ | 29 | 11.25$^{ab}$ | 48 | 11.25$^{ab}$ | 66 | 0$^{a}$ |
| 15 | 11.25$^{ab}$ | 30 | 33.75$^{b}$ | 49 | 0$^{a}$ | 67 | 22.5$^{ab}$ |
| 16 | 33.75$^{b}$ | 31 | 11.25$^{ab}$ | 50 | 22.5$^{ab}$ | 72 | 22.5$^{ab}$ |
| 18 | 22.5a$^{b}$ | 32 | 0$^{a}$ | 53 | 33.75$^{b}$ | 73 | 0$^{a}$ |
| 19 | 45.0b$^{c}$ | 33 | 11.25$^{ab}$ | 54 | 11.25$^{ab}$ | 75 | 0$^{a}$ |
| 21 | 56.25$^{bc}$ | 34 | 22.5$^{ab}$ | 56 | 0$^{a}$ | 77 | 0$^{a}$ |
| 22 | 22.5$^{ab}$ | 35 | 78.75$^{d}$ | 57 | 56.25$^{bc}$ | 79 | 0$^{a}$ |

**Table 3:** Percent disease incidence of *Fusarium oxysporum* isolates (chilli var. Nun 2066).

| Non pathogen | % Inhibition | Non pathogen | % Inhibition |
|---|---|---|---|
| 27 | 31.07663$^{bc}$ | 65 | 37.65706$^{d}$ |
| 32 | 30.64638$^{bc}$ | 66 | 26.03842$^{ab}$ |
| 49 | 34.42258$^{c}$ | 75 | 25.5694$^{ab}$ |
| 56 | 31.51777$^{c}$ | 77 | 24.59543$^{a}$ |
| 62 | 28.35935$^{b}$ | 79 | 31.93872$^{c}$ |

Each figure represents the mean of three replicates. In every column, any two values without common latter in their superscript are significantly different. Values were compared using least significant difference at P≤5%

**Table 4:** *In-vitro* percent inhibition of pathogenic *F. oxysporum* by different antagonistic *F. oxysporum*.

| Code No. | Variety | % Live plants | Code No. | Variety | % Live plants |
|---|---|---|---|---|---|
| 1 | Ajeet-3 | 83.34$^{b}$ | 16 | KCS-2013 | 83.34$^{b}$ |
| 2 | ACS-2002 | 50$^{ab}$ | 17 | LCA-206 | 50$^{ab}$ |
| 3 | BC-25 | 66.66$^{ab}$ | 18 | LCA-353 | 50$^{ab}$ |
| 4 | BC-40 | 50$^{ab}$ | 19 | Sel 11 | 33.33$^{a}$ |
| 5 | CO-4 | 100$^{b}$ | 20 | SKAV101 | 50$^{ab}$ |
| 6 | DLC-352 | 100$^{b}$ | 21 | Utkal yellow | 50$^{ab}$ |
| 7 | DLC-524 | 83.34$^{b}$ | 22 | Ashok Teja | 50$^{ab}$ |
| 8 | DL-1 | 50$^{ab}$ | 23 | G-4 Hot pepper | 66.66$^{ab}$ |
| 9 | DSC-2 | 50$^{ab}$ | 24 | Soldiar MH-1 | 50$^{ab}$ |
| 10 | F-112-5-83 | 83.34$^{b}$ | 25 | Chilli-Nun-2070 | 50$^{ab}$ |
| 11 | HS HS III | 66.66$^{ab}$ | 26 | Hot pepper Nun-2060 | 83.34$^{b}$ |
| 12 | HDC-25 | 50$^{ab}$ | 27 | Hotpepper Nun-2066 | 50$^{ab}$ |
| 13 | HS HS 154 | 66.66$^{ab}$ | 28 | F1 AK-47 Golden seed | 50$^{ab}$ |
| 14 | Indra-Chilli | 50$^{ab}$ | 29 | Advanced seeds | 50$^{ab}$ |
| 15 | JCA-283 | 50$^{ab}$ | 30 | Hot pepperNun-2067 | 50$^{ab}$ |

Any two values without common latter in their superscript are significantly different at LSD p≤5%

**Table 5:** Chilli varieties with their code numbers and evaluation of resistance varieties against *Fusarium wilt*.

## Discussion

The objectives of the present investigation were to evaluate resistant varieties of chilli, isolation and identification of nonpathogenic and pathogenic *F. oxysporum* strains from the rhizosphere and endorhizosphere of chilli, for controlling vascular wilt disease of chilli.

Considerable diversity was observed within the *F. oxysporum* isolates, recovered from soil and the root samples which were collected from the different location at Uttarakhand and Uttar Pradesh states in India. Differences were seen on the basis of cultural and conidial characteristics, among *F. oxysporum* isolates. The mycelium was delicate white, peach, pink, orange and usually with a purple tint. Further confirmation of *F. oxysporum* was done, based on microscopic study. Generally, micro conidia were thin walled, one to six septate and fusoid. In previous report of Groenewald [6], morphological characterization of *F. oxysporum* was based on the shape of macro conidia, the structure of micro conidiophores and the formation and disposition of chlamydospores.

The accurate morphological identification of *Fusarium* species had always been problematic, even for expert mycologists. This was because of the contradictory classification systems proposed by various researchers, primarily based on cultural and morphological characters [15]. Several mycologists had found use of morphological features, often complicated and confusing [17]. But the fungal identification could still be achieved through traditional phenotypic typing that requires an expert taxonomist, takes a longer time, and had often led to misidentification of species due to paucity, plasticity of the characters used, media, cultural conditions, loss of cultural viability, degeneration of the cultures [18]; production of mutants may further add to the problems in fungal identification and diagnosis [19].

Due to shortcomings of morphological characters for delineating species and sub generic groupings of *Fusarium,* the research focus had shifted to molecular tools for identification and determination of evolutionary relationships among species. These molecular tools included Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and the most robust and informative techniques used in fungal diagnosis was used to design species-specific primers and/or probes [6,20], where DNA sequence

variations had been investigated. This study was used to evaluate the efficacy of PCR primers designed from the sequences of the rDNA of ITS region, for identification of F. oxysporum. The PCR primers used in this study exhibited species specific identification. Hence in recent years, the increased use of molecular methods in fungal diagnostics had emerged as a possible answer to the problems, associated with the existing phenotypic identification systems [15,21]. In the present investigation, two primers FOF1 and FOR1 were used for identification of F. oxysporum, which were specific to the ITS region of the rDNA operon of *F. oxysporum* [14,15]. All forty eight isolates of *F. oxysporum* were found positive for these primers.

Determining formae special in and non pathogenic F. oxysporum, unfortunately, still relied on the time consuming procedure of testing the fungus for pathogenicity to plant species [22]. In this study of pathogenicity test, results indicated that though many *F. oxysporum* were pathogenic, but Isolate no. 35 was found to be most virulent pathogen that caused 78.75% disease incidence, and eleven isolates were found nonpathogenic on the basis of percent disease incidence. On inoculation of these non pathogenic isolates on the chilli plant, wilting was not observed. There were reports of non-pathogenic *F. oxysporum* demonstrating competition for infection sites and for nutrients to the pathogenic fusaria, and by induction of resistance [23-25].

We had compared the antagonistic activity of non pathogenic isolates, and the potential antagonists were ranked according to their *in-vitro* ability to inhibit the growth of the pathogen, which was evident through the formation of an inhibition zone on the agar. Effect of antagonistic *F. oxysporum* on *in-vitro* growth of *F. oxysporum* showed much more influence over the colony growth of pathogenic F. oxysporum. Amongst antagonistic F. oxysporum, results showed that the best performance of antagonistic F. oxysporum, Isolate no. 65 was recorded to suppress the colony growth of pathogen by 37.65%, while Isolate no.77 showed minimum inhibition against most virulent pathogen Isolate no. 35.

The practical method of control of the *Fusarium wilt* was the use of resistant varieties. Many resistant varieties had been screened for bacterial wilt [26] and yellow mite [27]. Present investigation was designed to find out resistant variety of chilli for *Fusarium wilt*, which would also be acceptable from a commercial standpoint. Thirty varieties of chilli were tested in pot experiment, to determine their wilt resistance or susceptibility for *Fusarium wilt* of chilli. CO- 4 and DLC- 352 were found to be most resistant varieties. Use of these two strategies, antagonistic *F. oxysporum* and resistance cultivar, was an ecologically sound approach to biocontrol *Fusarium wilt* of chilli.

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