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# COLLECTION OF *FUSARIUM OXYSPORUM F.SP. LENTIS* ISOLATES FROM BHIND, MORENA AND GWALIOR DISTRICTS OF MADHYA PRADESH

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**ABSTRACT**
Fifteen isolates of *Fusarium oxysporum f.sp. lentis* were collected from lentil growing (infected root sample of lentil plants) fields of three districts of Madhya Pradesh for finding cultural and morphological variability among them. The color, growth and growth rate of mycelium of collected isolates were found varied and can be distinguishable in different categories. Out of fifteen isolates 6 (40%) isolates were found as white at first, later flesh pink on places, 5 (33.33%) isolates were found as pinkish white and 4 (26.67%) isolates were found as white turning pale color of mycelium. Maximum isolates showed fluffy type of mycelia growth and 33.33 percent isolates showed the slow growth (19.8-30.07 mm), 40 percent showed medium growth (30.08-40.35 mm). Micro-conidia were 0-1 septate, length of micro-conidia was recorded in the range of 5.2 to 9.2 $\mu$m, while its width was in the range of 3.2 to 1.6 $\mu$m. Macro-conidia were 3-5 septate, length of macro-conidia was varied from 26.4 to 48.4 $\mu$m, while its width was in the range of 4.8 - 2.6 $\mu$m.

## INTRODUCTION

Lentil has great impact in world agriculture because of its high protein content (23.7%) in seeds, while the straw serve as high value animal feed (Kashem *et al.,* 2014). It is chief protein source in comparison with high cost animal protein (Mondal *et al.,* 2013). In addition to food value lentil also plays an important role in cropping system because of its ability to fix nitrogen in soil (101Kg/hac/annum) and thereby enrich the soil (BINA, 2012). Various causes are associated with its low yield. One of them is disease causing remarkable yield loss. Diseases are major contraints to lentil production all over world (Bayya and Erskine, 1998). *Fusarium* is one of the devastating phytopathogenic fungi belongs to Division *Ascomycota*, Class: *Sordariomycetes*, Order *Hypocreales*, Family: *Nectriaceae*. This filamentous fungi, can smite any crop since having a broad host range including lentil (Pulses), rice, wheat, horticultural crops, ornamentals and in almost all agricultural commodities (Supyani and Widadi, 2015). *Fusarium* wilt in lentil by *Fusarium oxysporum f.sp. lentis*, Sugarcane wilt by *Fusarium sacchari* (Lin *et al.*, 2014), Bakanae in rice by *Fusarium fujikuroi* (Jain *et al.*, 2014), are some important diseases caused by *Fusarium* spp. Species of *Fusarium* serving as pathogens to many diseases in crops such as vascular wilt, root rot, corm rot, damping-off, yellows, and others. (Sharma *et al.,* 2016). Lentil wilt appears in the field at both seedling and adult stage. In seedling wilt sudden droopping followed by drying of leaves and the whole seedlings and apparently healthy looking roots with reduced proliferation. Adult maturity stage symptoms first appears during floweringto late pod filling stage, sudden drooping of top leaf lets of the affected plant leaflet closure without premature shedding apparently healthy looking root system with a slight reductionin the lateral roots.(Kumar *et al.,*2014).In India mention of the wilt disease is made in a report from Bengal, where one of the imported varieties suffered badly from wilt. To study and to manage the problem of wilt of lentil, investigations were undertaken with a view to know the variability among isolates of *F. oxysporum f.sp. lentis* (cultural, morphological, and pathological variability) collected from survey of 15 locations of Gwalior, Bhind and Morena districts of Madhya Pradesh. The studies were carried out during winter season of 2010-11 at College of Agriculture, Gwalior.

## MATERIALS AND METHODS

Lentil wilted fields of three districts viz., Gwalior, Bhind and Morena districts were surveyed to find out the isolates during Rabi season of 2010-11. Five locations from Gwalior (Research Farm, Badagaon, Utila, Berja, Rayru), five from Bhind (Rampura, Dandraua, Mehgaon, Piproli, Gormi) and Morena (Ambah, Dimni, Khera, Morenagaon, Shanichara) were randomly selected. Thus total of 15 localities (Table-1) were selected and in each locality 5 fields were randomly selected.

### Isolation of *Fusariumoxysporumf.sp.lentis* from the plant samples

The leaves of diseased lentil plant showing the wilt symptoms were washed thoroughly with tap water, small pieces from infected leaves were cut with the help of sterilized blade. These

pieces were surface sterilized with 1:1000 mercuric chlorides ($HgCl_2$) solution for one minute followed by three changes in sterilized distilled water to remove trace of $HgCl_2$. The pieces were then transferred aseptically to petriplates containing PDA 20ml in each. Inoculated petriplates were incubated at 28 ± 1°C for three to five days and examined at frequently intervals to see the growth of the fungus developing from different pieces.

**Purification and identification of isolates**

The culture of *F. oxysporumf.sp.lentis*was purified by sub-culturing the single hyphal tip method and maintained by mass transfer on potato dextrose agar medium at room temperature. After purificationof isolates of *F. oxysporum f.sp. lentis* were identified by observing the colony against light with the naked eyes and later confirmed with the help of microscope.

**Cultural and morphological characteristics of pathogen**

**Cultural variability**

The cultural characters of isolates of *F. oxysporum f.sp. lentis* were recorded from culture grown on PDA. Twenty ml of PDA was poured in each of previously sterilized petri plates. Five mm discs were cut through sterilized cork borer from the margin of seven days old colony of the fungal culture grown in petri plates. One disc was placed in the centre of each plate and incubated at 28 ± 1°C for seven days. Three replications were maintained for each isolate. The differences between observations regarding colony color, Growth rate and type of mycelial growth of each isolate were taken.

**Table 1: Collection of diseased plant / root samples of lentil from different districts of Northern Madhya Pradesh**

| Isolate no. | Isolate Code | Districts | Locations |
|---|---|---|---|
| 1 | $GWL_1$ | Gwalior | Research Farm |
| 2 | $GWL_2$ | Gwalior | Badagaon |
| 3 | $GWL_3$ | Gwalior | Utila |
| 4 | $GWL_4$ | Gwalior | Berja |
| 5 | $GWL_5$ | Gwalior | Rayru |
| 6 | $BND_1$ | Bhind | Rampura |
| 7 | $BND_2$ | Bhind | Dandraua |
| 8 | $BND_3$ | Bhind | Mehgaon |
| 9 | $BND_4$ | Bhind | Piproli |
| 10 | $BND_5$ | Bhind | Gormi |
| 11 | $MRN_1$ | Morena | Ambah |
| 12 | $MRN_2$ | Morena | Dimni |
| 13 | $MRN_3$ | Morena | Khera |
| 14 | $MRN_4$ | Morena | Morenagaon |
| 15 | $MRN_5$ | Morena | Shanichara |

**Morphological variability**

The slides of 15 isolates were prepared in lactophenol from 10 days old culture. For morphological studies, 10 observations for size of macro and micro conidia and number of conidia per microscopic field belonging to each isolates were taken under high power (40x) microscopic field. These isolates were categorized in various groups according to size of macro and micro conidia, number of conidia, and shape of conidia.

## RESULTS AND DISCUSSION

**Cultural characters**

**Color of mycelium**

Color of mycelium was white which later turned pink or pale. Details of color of mycelium for all 15 isolates after 7th day of incubation are given in (Table 2,3,4). Color of myceliium was observed and categorized into three groups as white at first, later flesh pink on places, pinkish white and white turning pale. Out of the 15 isolates 6 (40%) Isolates were found as white at first, later flesh pink on places, 5 (33.33%) isolates were found as pinkish white and 4 (26.67%) isolates were found as white turning pale color of mycelium.

**Morphological characters**

To know the variability in morphological characters of different isolates of *Fusarium oxysporium f.sp.lentis* regarding number of septa and size of micro-conidia and macro-conidia have been studied and summarized in (Table 5). Five microscopic observations under high power (40 X) were carried out for each isolate and the average value is presented in Table-5. Morphological studies about conidial characteristics reveal that the conidia formed freely on mycelium at the end of free conidiophores. Micro-conidia often agglutinated into false heads, 0-1 septate, ellipsoid to ovoid, cylindrical, oblong or

**Table 2: Type of mycelial growth and growth rate of various isolates of *Fusarium oxysporium f.sp. lentis***

| IsolateNo. | Type of mycelial growth | Growth rate (mm / day) * | | | | | Average growth per day |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| 1 | Fluffy | 5 | 13 | 28 | 35 | 45 | 25.2 |
| 2 | Fluffy | 9 | 21 | 37 | 49 | 56 | 34.4 |
| 3 | Fluffy | 0 | 5 | 15 | 30 | 49 | 19.8 |
| 4 | Submerged | 7 | 17 | 31 | 42 | 61 | 31.6 |
| 5 | Fluffy | 11 | 28 | 39 | 54 | 67 | 39.8 |
| 6 | Fluffy | 0 | 12 | 27 | 38 | 51 | 25.6 |
| 7 | Submerged | 17 | 29 | 37 | 52 | 72 | 41.4 |
| 8 | Partial submerged | 11 | 25 | 34 | 43 | 61 | 34.8 |
| 9 | Partial submerged | 13 | 29 | 47 | 60 | 77 | 45.2 |
| 10 | Fluffy | 21 | 34 | 50 | 65 | 83 | 50.6 |
| 11 | Submerged | 0 | 9 | 22 | 40 | 59 | 26.0 |
| 12 | Fluffy | 13 | 37 | 46 | 65 | 72 | 46.6 |
| 13 | Partial submerged | 12 | 23 | 38 | 52 | 71 | 39.2 |
| 14 | Fluffy | 7 | 18 | 31 | 46 | 64 | 33.2 |
| 15 | Submerged | 0 | 8 | 20 | 37 | 58 | 24.6 |

**Table 3: Grouping of isolates of *Fusariumoxysporiumf.sp. lentis* on the basis of mycelium color**

| Characters | Isolates | Total | Per cent |
|---|---|---|---|
| White at first, later flesh pink on places | 1, 5, 6, 7, 13, 14 | 6 | 40.00 |
| Pinkish white | 3, 9, 10, 12, 15 | 5 | 33.33 |
| White turning pale | 2, 4, 8, 11 | 4 | 26.67 |
| | Total | 15 | 100 |

**Table 4: Grouping of isolates of *Fusarium oxysporium f.sp. lentis* on the basis of growth rate**

| Characters | Isolates | Total | Per cent |
|---|---|---|---|
| Slow growth (19.80-30.07 mm) | 1, 3, 6, 11, 15 | 5 | 33.33 |
| Medium growth (30.08-40.35 mm) | 2, 4, 5, 8, 13, 14 | 6 | 40.00 |
| Fast growth (40.36-50.63 mm) | 7, 9, 10, 12 | 4 | 26.67 |
| | Total | 15 | 100 |

**Table 5: Morphological characters of various isolate of *Fusarium oxysporium f.sp. lentis***

| Isolate No | Micro-conidia | | | Macro-conidia | | |
|---|---|---|---|---|---|---|
| | No of Septa | Size ($\mu$m) | | No of Septa | Size ($\mu$m) | |
| | | L | W | | L | W |
| 1 | 0 | 5.2 | 2.2 | 3 | 28.2 | 3.6 |
| 2 | 0 | 5.6 | 3.0 | 3 | 27.2 | 2.6 |
| 3 | 1 | 5.4 | 3.2 | 4 | 39.8 | 4.8 |
| 4 | 1 | 5.8 | 2.6 | 3 | 27.2 | 3.0 |
| 5 | 0 | 6.0 | 3.0 | 3 | 37.2 | 2.6 |
| 6 | 1 | 6.8 | 2.8 | 5 | 41.0 | 3.8 |
| 7 | 0 | 9.2 | 2.0 | 3 | 35.4 | 3.2 |
| 8 | 1 | 8.4 | 2.6 | 4 | 43.0 | 3.2 |
| 9 | 1 | 7.0 | 2.0 | 3 | 48.4 | 2.8 |
| 10 | 1 | 8.2 | 1.6 | 5 | 43.0 | 3.4 |
| 11 | 1 | 7.0 | 2.2 | 4 | 42.2 | 4.8 |
| 12 | 0 | 6.0 | 2.6 | 4 | 27.6 | 3.8 |
| 13 | 0 | 6.2 | 2.2 | 3 | 26.4 | 3.6 |
| 14 | 0 | 6.8 | 2.4 | 5 | 47.2 | 4.2 |
| 15 | 1 | 5.8 | 2.4 | 4 | 46.4 | 3.8 |

*Average of five observations

slightly curved, hyaline. It differed in their size also length was recorded in the range of 5.2 to 9.2 $\mu$m. Maximum of 9.2 $\mu$m length was recorded in Dandraua while minimum of 5.2 $\mu$m length was recorded in Gwalior research farm. In case of width of micro-conidia maximum width of 3.2 $\mu$m was observed in Berja while minimum of 1.6 $\mu$m was observed in Gormi. Macro-conidia were found nearly straight to fusiform-falcate, slender with thin delicate walls and indistinct septa. Number of septa was varied from 3-5, out of 15 isolates maximum (7) isolates showed 3 septa. Length of macro-conidia was varied from 26.4 to 48.4 $\mu$m. Among these maximum of 48.4 $\mu$m length was recorded in Piproliwhile minimum of 26.4 $\mu$m was observed in Khera isolate. In case of width of macro-conidia maximum of 4.8 $\mu$m was observed in Berja and Ambah isolates, while minimum of 2.6 $\mu$m width was recorded in the isolates of Badagaon and Milavali. Present investigations were undertaken with a view to know the variability among isolates of *F. oxysporumf.sp. lentis* (cultural, morphological, and pathological variability) collected from survey of 15 locations of Gwalior, Bhind and Morena districts of Madhya Pradesh. A very little information on variability among isolates of *F. oxysporumf.sp.lentis* is available in the literature particularly in lentil wilt.Vasudeva and Shrinivasan (1952) also observed the growth characteristics of *F. oxysporumf.sp. lentis*and recorded that the aerial mycelium was short and dense. Gupta *et al*. (1988) reported seven different strains of *Fusariumudum* on the basis of colony characters, growth rate, sporulation and dry mass production.Paulkar and Raut (2004) were found 4 isolates of *Fusariumoxysporumf.sp. ciceri* (from Amaravati ( Foc-1), Akola (Foc-2), Buldhana (Foc-3) and Nagpur (Foc-4), Maharashtra, India) morphologically different. Mandhare*et al*. (2007) also detected a total of 53 isolates of *Fusarium sp*., causing chickpea wilt in Maharashtra, India from 66 samples.The isolates were categorized into 6 groups based on variation in the morphological, cultural characters and pathogenicity.Chaudhary*et al*. (2009) reported that lentil (*Lens culinaris*), the second most important rabi pulse crop of India, suffers heavy plant mortality due to wilt, root rot and collar rot at different stages of crop growth.

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