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Isolation of *Fusarium* Species from the Infected Lentil Plant

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Abstract: ***Fusarium is a filamentous fungus in the genus Fusarium. It is commonly isolated from soil and plant debris. The fungus has a worldwide distribution, but its frequency as a medically important pathogen is not fully known. Aside from keratitis, it is an infrequent cause of fungal infections but remains the most common disease-causing fungus in its genus. Fusarium species causes major loss in lentil yield, so its disease management practices are necessary to control the diseases caused by Fusarium.***

Keywords: *Fusarium,* lentil plant,Vigour index, Standard blotter paper method

1. Introduction

The lentil (*Lens culinaris*Medik) is an edible pulse which is a bushy annual plant of the legume family, known for its lens-shaped seeds and is about 40 cm in height. lentils have been part of the human diet since aceramic (before pottery) Neolithic times, being one of the first crops domesticated in the Near East. Archeological evidence shows they were eaten 9,500 to 13,000 years ago. As concern the Nutritional value and health benefits lentil have energy content 1477kJ per 100 gm of dry weight of seeds. It is rich source of carbohydrates, proteins and also shows some amount of trace element like calcium, magnesium, phosphorus, iron, potassium, sodium, zinc etc. Lentils also contain dietary fiber, folate, vitamin B_1 and minerals. Red (or pink) lentils contain a lower concentration of fiber than green lentils. The low levels of Readily Digestible Starch (RDS) 5%, and high levels of Slowly Digested Starch (SDS) 30%, make lentils of great interest to people with diabetes. The remaining 65% of the starch is a resistant starch that is classified RS1, being a high quality resistant starch, which is 32% amylose. It has about 30% of calories from protein. As compare to other legume plants the Lentils have third-highest level of protein content by weight. Proteins content that include amino acids like isoleucine and lysine. The lentils are an inexpensive rich source of protein in many parts of the world, especially in West Asia and the Indian subcontinent having large amount of vegetarian populations. Lentils are deficient in two essential amino acids methionine and cysteine.

2. Materials & Method

Collection of sample:
3 seed samples of lentil were collected from local market of Jalna and preserved at laboratory condition.

Detection of seed mycoflora:
The external and internal seed mycoflora was detected by using standard moist blotter paper method as recommended by International Seed Testing Association (ISTA, 1996).

Standard blotter paper method:
For detecting external seed mycoflora 100 healthy seed from each sample were taken .untreated 20 seeds from the samples were taken and placed at equal distance on three layers of moistened blotter paper in pre-sterilized Petriplates.

For detecting internal seed mycoflora 100 healthy seeds from each sample were taken and seeds were treated with 0.1% $HgCl_2$ solution for 5 minutes and then washed thoroughly with sterile distilled water. Treated 20 seeds from the samples were taken and placed at equal distance on three layers of moistened blotter paper in pre-sterilized Petriplates. All the Petriplates were incubated at $25+2^0$ C. (Umesh P. Mogle and Sanjay R. Maske)

Seed Germination:
50 seeds of each treatment were subjected to standard blotter method in which the seeds were incubated according to the standard procedures of ISTA (Anonymous, 1996). On the 8th day of incubation, seeds were evaluated for the incidence of mycoflora.

Vigour Index:
Radical & Plumule lengths were recorded. Vigour index was calculated by using the formula as shown below:

Vigour index (VI) *= (Mean shoot length + mean root length) x Germination (%)*

Recorded data were subjected to statistical analysis for mean values and test of significance. The variations among the respective data were compared following least significant difference (LSD) test.

Isolation and identification of the disease causing fungus
Samples were taken from infected roots of lentil plants. Collected samples were then brought to laboratory for isolation and identification of pathogen causing root rot. The samples were first surface sterilized twice with distilled sterilized water and then were treated with 0.5%NaOCl (Sodium hypochlorite) for 2 minutes. After surface sterilization the samples were dried on sterilized blotter paper placed in Petriplates containing sterilized potato dextrose agar medium. All the petridishes were incubated at 25 ± 1^0C for about seven days. After seven days of inoculation the fungi isolated, were then identified with the help of colony character and microscopic observation. (P.K. Mwaniki , M.M. Abang ,et.al,)

Microscopic characterisation:
Microconidia and macroconidia were observed in 10-day old cultures and described based on colour of conidial masses, shape, septation and basal and apical cell of macroconidia, shape of microconidia, and conidiophores in the aerial mycelium.

3. Result & Discussion

Vigour index for seed sample A:

S. No	Untreated seeds		Treated seeds	
	Root length	Shoot length	Root length	Shoot length
.	4.2	2.5	3.7	1.8
.	4	3.5	2.0	1.2
3.	3.5	3.5	6.0	1.8
4.	4.2	2.3	1.0	2.0
5.	5	2.5	3.0	1.6
6.	4.9	1.0	3.5	0.4
7.	3.5	2.7	3.8	1.0
8.	2.8	1.4	2.5	1.7
9.	2.7	1.7	4.3	1.9
10.	2.5	1.5	3.0	1.3
11.	2.7	1.5	2.1	1.7
12.	2.8	0.5	5.5	3.5
13.	3.7	1.8	4.7	2.6
14.	1.8	1.8	3.0	2.1
15.	4.4	1.9	3.8	2.4
16.	2.5	1.7	3.5	2.7
17.	4.5	1.1	5.0	1.0
18.	4.9	1.7	1.5	1.7
19.	0	0	0	0
20.	0	0	0	0
Total	64.6	34.6	61.9	32.4

Vigour index for Untreated seed A
Mean root length = 64.6/18 = 3.58
Mean shoot length = 34.6/18 =1.92
% germination =90%
Vigour index = (mean root length +mean shoot length) X percent germination
= (3.58+1.92) x 90
= 5.5 x 90 = 495

Vigour index for treated seed A
Mean root length =61.9/18= 3.43
Mean shoot length =34.4/ 18 =1.8
Percent germination =90%
Vigour index = (mean root length +mean shoot length) X percent germination
= (3.43 +1.8) x 90
= 470.7

Vigour index for seed sample B:

S. No	Untreated seeds		Treated seeds	
	Root length	Shoot length	Root length	Shoot length
1.	3.5	2.3	3.0	1.6
2.	5.0	4.3	3.0	0
3.	3.8	3.0	4.2	2.5
4.	4.0	2.8	3.4	2.2
5.	3.0	2.8	4.5	2.2
6.	4.0	3.9	2.2	2.3
7.	3.7	3.0	3.5	2.0
8.	3.5	3.3	3.5	2.4
9.	3.3	2.0	1.8	2.2
10.	3.3	3.5	4.0	3.0
11.	4.4	2.5	3.4	1.7
12.	3.4	2.0	3.5	1.2
13.	3.0	2.5	3.3	1.5
14.	3.0	1.5	2.8	2.2
15.	4.5	1.9	2.0	2.0
16.	3.2	2.0	0	1.5
17.	3.0	3.0	0	0
18.	3.7	2.0	0	0
19.	3.0	1.5	0	0
20.	3.3	1.5	0	0
Total	71.6	5.5	48.1	30.3

Vigour index for untreated seed sample B
Mean root length = 71.6/20 = 3.58
Mean shoot length = 51.5/20 =2.58
% germination =100%
Vigour index = (mean root length +mean shoot length) X percent germination
= (3.58+2.58) x 100
= 6.15 x 100
= 615

Vigour index for treated seed sample B
Mean root length =48.1/16= 3
Mean shoot length =30.3/16 =1.89
Percent germination =80%
Vigour index = (mean root length +mean shoot length) X percent germination
= (3+1.89) x80
= 4.89 x80
= 391.2

Vigour index for seed sample C

S. No	Untreated seeds		Treated seeds	
	Root length	Shoot length	Root length	Shoot length
1.	2.1	6.2	2.8	9.0
2.	0	4.1	2.6	5.6
3.	0	2.9	1.5	3.7
4.	2.3	6.9	1.7	5.3
5.	1.7	4.8	2.1	2.4
6.	1.5	5.2	2	4.4
7.	2	4.6	2.6	3.1
8.	1.5	4.2	1.3	4.7
9.	1.4	4.5	3.0	3.3
10.	1.2	1.8	1.6	2.2
11.	1.6	7.2	2.3	5.6
12.	1.8	8.6	1.7	8.1
13.	2.2	7.5	2.1	4.5
14.	2.1	8.9	2.5	3.9
15.	1.9	7.2	0.8	2.6
16.	1.7	8.8	1.7	4.5
17.	0.7	4.7	1.1	5.0
18.	2.1	1.4	2.0	6.3
19.	2.2	4.9	2.5	5.0
20.	0.9	1.1	0	0
Total	30.9	104.9	37.9	89.2

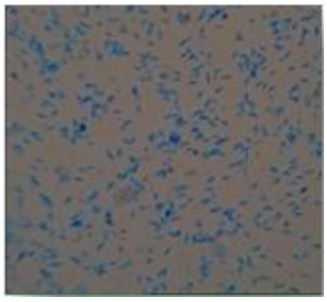
Vigour index for untreated seed sample C
Mean root length = 104.9/20 = 5.245
Mean shoot length = 30.9/20 = 1.545
% germination =100%
Vigour index = (mean root length +mean shoot length) X percent germination
= (1.545+5.245) x 100
= 6.679 x 100

= 679

Vigour index for treated seed sample C

Mean root length =89.2/19= 4.694
Mean shoot length =37.9/19=0.997
Percent germination =95%
Vigour index = (mean root length +mean shoot length) X percent germination
= (4.694+0.0997) x 95
= 4.793 x 95
=455.40

*Fusarium*was successfully isolated using protocol and colony characters are as follows:



Fusarium under microscope

***Fusarium* on PDA**

Shape	Filamentous
Surface	Rough
Colour	White
Elevation	Flat
Opacity	Opaque
Morphology	Conidia

Colony character of *Fusarium*

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