P-ISSN: 2618-0723
E-ISSN: 2618-0731

Extension Journal

www.extensionjournal.com

International Journal of Agriculture Extension and Social Development

Volume 1; Issue 1; Jan-Jun 2018; Page No. 17-19

Received: 11-04-2018
Accepted: 15-05-2018

Indexed Journal
Peer Reviewed Journal

# Efficacy of plant extracts on detection and location of seed borne infection against *Fusarium oxysporum* F. sp. Lentis wilt in Lentil

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**Abstract**
Lentil wilt a serious disease induced by *Fusarium oxysporum* f. sp. lentis cause heavy losses of the crop. A few compounds of plant origin have been proved to be possible alternatives to fungicides use. Out of 5 species tested plant extract from *Azadirachata india, Parthenium hysterophorus, Lantana camara, Argemone mexicana and Ocimum* sp. Were effective in reducing the redial growth of the pathogen. Fifteen seed samples were found infected by *Fusarium oxysporum* f.sp. Lentis. Presence of the fungus was detected in pericarp, endosperm and embryo by employing component planting and microtone techniques, disease appeared in all samples except two when seeds were sown in pots, confirming the seed borne nature of the inoculum of the pathogen.

**Keywords:** lentil, wilt, seed borne, plant extracts, antifungal activity

## Introduction

## Material and Methods

Fusarium wilt of lentil caused by *Fusarium oxysporum* schlecht f. sp. lentis (Vasudevce and Srinivasan) Godon, recognized as potential destructive disease in India (Chattopadhyay and Maiti, 1990) produce symptom in patches in the field when the crop was one month old. Losses ranging between 5-60 per cent and infection ranging from 70-80 percent in the field. The pathogen basically soil borne but same workers (Khare, 1981 and Verm and Lahori 2004) have suggested possible of seed transmission. Studies were therefore, made to detect and locate the pathogen and evaluate pathogenicity of inoculum in seed.

Hundred randomly seeds were selected from each samples of lentil. Whereas lentil is generally cultivated using traditionally/cultivation practices and store in different structure to 7-8 months after harvest. Sampling was done by the method suggested in ISTA (1999). All seed samples were used separately for the isolation of *Fusarium oxysporum* f.sp. lentis from seeds.

## Result and Discussion

### Detection of *Fusarium oxysporum* f. sp. lentis on seed

Randomly selected seed and discoloured Shriveled seeds were attempted by using Botter and Agar plate's methods (ISTA, 1999). The fungal colonies emanating from seeds were observed from 3 to 7 days after inoculation. The percentage infection in seed samples (based on number of seed showing infection was calculated). The specific identity of the fungus was confirmed under compound microscope on the basis of morphological characters.

### Seeding symptom test

One hundred seeds from each sample showing highest percentages of infections were shown in pots (25 cm dia) @ 10 seeds/pots) test transmission of *Fusarium oxysporum* f. sp. lentis seeds to plant. Thirty days after showing percentage of seedling showing wilt symptoms was recorded.

### Location of *Fusarium oxysporum* f.sp. lentis in lentil seed

For location of fungus in seed the method suggested by Madan *et al* (1975) with slight modification was followed. One hundred seeds selected at random were washed individually three times with tap water and finally with sterilized water and then soaked in distilled water for 24 hours. Each seed was then dissected in to its components i.e. pericarp, endosperm and embryo with a pair of sterilized needles under sterobinocular microscope. Each components were surface strilized with $HgCl_2$ (0.1%) and tested by Blotter method for the presence of fungus. Fifty seeds were boiled in hydrochoric acid (2%) for 2 min and fixed in 70 percent ethanol for ethanol for 48 hrs they were dehydrated through a tertiary butyl alcohol series, infiltrated and embedded in paraffin wax. The embedded material was softened by immersing the paraffin blocks in aqueous solution of sodium lauryl sulphate (1%) for 25 hrs. the blocks were washed in distilled water (1.1 v/v) for seven days. Serial microtome sections were cut (12-20 µm thick) stained with sarfranin and fast green combition and mounted in caedax solution two parts of caedax with one part of xylen). Microtome sections were examined under a compound microscope. The fungus produced white to greyish white colonies on incubation seeds in Blotter and Agar plate methods. The microcondia were 1-celled or

rarely 1- septate ovate or somewhat elongated. The microcondia were muticelur (3-5 sepate) fusiform-s falcate and curved inwards at both ends. In both seed incubation methods out of 15 samples tested. *Fusarium oxysporum* f.sp. lentis was detected in pericarp endosperm and embryo of fourteen seed samples. The seed infection detected by Blotter method ranged between 1 to 12 percent in randomly selected seed category whereas in discoloured and shrivelled seed category it ranged between 5 to 2 percent in agar plate method. It ranged from 0.5 to 7 percent in randomly selected category whereas in discoloured and shivelled seed. Shrivelled and discoloured seed showed higher level of infection as compared to randomly selected seeds.

The results in presented table-1 revealed that the pathogen was present in all components of the infected seeds of all the samples. It was present in the seed coat of all the infected seeds and then enters into the extent in to deep to cause infection in tissue of cotyledons and embryonal axis to the extent of 60.46 to 37.21 percent respectively. The establishment of perfection in cotyledon and empbryonal axis varied with varieties it was maximum in seed coat and minimum in emprynal in so for as establishment of inoculum in the cotyledon was concerned. The experiment indicated that the pathogen was carried with the lentil seeds externally on its surface in the form of mycelial fragments and micro and macro conidia and internally in the tissues of seed coat, cotyledons and embryonal axis.

**Efficacy of plant extracts against *Fusarium oxysporium* f. sp. Lentis wilt in Lentil**

Lentil (*Lens culinaris* M.) is an important spice crop and occupies a significant place among the food grown crop in the country. One of the major threats to lentil cultivation is the onslaught of wilt disease caused by *Fusarium oxysporum* f. sp. lentis. While is lightly destructive and cause losses up to 80 percent. (Erskine *et al*., 1989) [1]. The presence of antifungal compounds in higher plant has long been recognized as an important factor to disease control (Singh *et al*, 2000) [2] such compounds being biodegradable and selective in their toxicity are consider valuable for controlling some plant disease (Singh and Dwivedi, 1987) [3]. Therefore the study was conducted to evaluate extracts of five different plants species for their antifungal properties against *Fusarium oxysporum* f. sp lentis.

Plant extracts of *Azacdirachata india*, Lantana camara, Parthenium hystreophres, Argemone mexicana and Ocimum sp. Were prepared (Singh and Tripathi, 1992) [2]. The extracts were prepared by crushing the plant in mechanical grinder with equal quantity of distilled water and they strained through chese cloth. The extracts were subjected low speed centrifugation (1000 pm for is 15 min) and clear supernatant were diluted with sterile distilled water to arrive at the required concentration. Plant extracts were assayed by poison food technique (Grover and Moore, 1962) [4]. Supernatant (10 ml) of plant extract was mixed separately in 90 ml sterilized potato dextrose agar medium and were poured in to plates. Control plates received sterilized distilled water. Each treatment was replicated four times. The amended and control agar plate from 7 days old culture grown on PDA. The linear growth of colony was measured 7 days after inoculation.

Among 5 plant species the extract from Azadirachata indica and Lantana camara were found equally effective in inhibility the growth and sporaulation of the *Fusarium oxysporum* f. sp. s lentis is followed by Parthenium hyterophorees and Ocimum sp. Was found least effective to inhibit mycelial growth and sporudtion.

**Table 1:** Percent infection of *Fusarium oxysporum* f. sp. lentis in different seed components.

| Seed samples | Seeds showing infection | Seed components | | |
|---|---|---|---|---|
| | | Seed coat | Cotyledon | Embryonal axis |
| Sehore-74-3 | 19.5 | 19.7 | 13.9 | 7.5 |
| K-75 | 18.9 | 18.7 | 11.8 | 6.8 |
| DPL-53 | 17.9 | 17.8 | 10.8 | 6.8 |
| IPL-76 | 14.8 | 14.9 | 7.8 | 4.8 |
| DPL-15 | 13.9 | 14.1 | 6.9 | 4.9 |
| Average | 17 | 17.04 | 10.24 | 6.16 |

**Table 2:** Effect of some plant leaf extracts on colony growth and sporulation of *Fusarium oxysporum* f. sp. lentis.

| Leaf extracts | Colony growth (mm) | Percent Inhibition | Average sporulation | Per cent reduction in sporulation |
|---|---|---|---|---|
| Azadirchata indica | 29.2 | 63.2 | 2.9 | 89.2 |
| Lantana camara | 32.0 | 59.7 | 3.9 | 85.71 |
| Parthenium hysterpohorus | 44.8 | 43.7 | 9.9 | 64.2 |
| Argemone mexicana | 62.0 | 22.7 | 14.9 | 46.4 |
| Ocimum sp. | 68.6 | 14.2 | 20.8 | 24.8 |
| Control | 80.0 | - | 28 | - |
| CD at 5% | 2.44 | - | 3.77 | |

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