

Original Research Article

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Isolation of Causal Organism of Wilt and Collar Rot of Lentil and its Pathogenicity Tests

Abhishek Rathod¹, Babli¹, Shivangi Tiwari², Dharmendra Mishra³, Ravi Patel³, Kamlesh Choubey³, Jyoti Kurmi² and Dwarka^{4*}

¹Department of Plant Pathology, Rajmata Vijayaraje Scindhia Krishi Vishva Vidyalaya, Gwalior, Madhya Pradesh, India

²Eklavya University, Damoh, Madhya Pradesh, India

³Swami Vivekanand University, Sagar, Madhya Pradesh, India

⁴Department of Entomology, Jawahar Lal Nehru Krishi Vishva Vidyalaya, Jabalpur, Madhya Pradesh, India

*Corresponding author

ABSTRACT

Keywords

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The disease appears in the field in patches at both seedling and adult stages. Seedling wilt is characterized by sudden drooping, followed by drying of leaves and seedling death. The roots appear healthy, with reduced proliferation and nodulation and usually no internal discoloration of the vascular system. The fungus *Fusarium oxysporum* f. sp. *lentis* is septate, profusely branched growth on potato sucrose / dextrose agar at 25°C, initially white turning light buff or deep brown later and fluffy or submerged. The growth becomes felted or wrinkled in old cultures. Various types of pigmentation (yellow, brown, whitish /cream, crimson dark purple, light orange) may be observed in culture on solid medium. Microconidia were usually borne on simple and short conidiophores, which arise laterally on the hyphae. They are oval to cylindrical, straight or curved and measure 2.5 - 3.5 x 5 - 11µm. Macroconidia are borne on branched conidiophore, thin walled, 1 to 6 septate, fusoid, pointed at both ends and measures 3.5 -4.5 x 25 - 65µm. Chlamydospores are formed in old cultures, which are smooth or rough walled, terminal intercalary and may be formed singly or in pairs or in chains. The pathogen causes a lesion localized at or about the collar between the stem and the root. The lesions develop around the stem eventually forming a "collar" region.

Introduction

Lentil production in India has always been important as it is one of the most important

rabi crops in the country. Lentil (*Lens culinaris* Medik.) is an important pulse crop and the second major source of protein (25%) after soybean in human and animal diet. Lentil

is important source of energy (353 kcal), protein (25%), carbohydrate (63%), sugar (2%), fat (1%), vitamin and antioxidant compounds. It is mainly cultivated in Madhya Pradesh, Uttar Pradesh, Bihar, and West Bengal, which account for 85% of total production of India. Lentil is grown as a *rabi* crop or winter crop in India and sown from October to November. It can be grown on a wide range of soils from light loams to black cotton soils and can stand even less fertile moderately alkaline soils and rainfed conditions. The crop matures in 90 to 120 days. The total area under lentil in India was 12.76 lakh ha with a total production of 9.76 lakh ton and 765 Kg/ha productivity.

In Madhya Pradesh lentil was cultivated in an area about 5.5 lakh ha with production of 2.33 lakh tone and 424 kg/ha productivity (Anonymous 2016-17)⁽¹⁾. Lentil suffers from attack of a number soil born diseases such as vascular wilt (*Fusarium oxysporum* f.sp. *lentis*), collar rot (*Sclerotium rolfsii*), root rot (*Rhizoctonia solani*) etc. Productivity of lentil is reduced by the diseases by infecting to leaves, stems, roots and pods. It also reduces marketability due to discoloration of seeds (Khalequzzaman, 2016)⁽³⁾. *Fusarium* wilt causes yield losses upto 50% in India. The disease appears in either early stage of crop growth (seedling) or during the reproductive stage (adult stage) (Khare 1981; Stoilova and Chavdarov, 2006)⁽⁴⁾⁽⁷⁾.

Materials and Methods

Equipments and apparatus

The equipments and apparatus which have been used in the study are given below:- Laminar air flow, BOD incubator, Refrigerator, Autoclave, Glassware, Microscope, Hot air oven, Electronic balance, Forceps, Inoculation Needle, Cork borer, Blade etc.

Cleaning and sterilization of equipments

Corning make glassware were used during the period of investigation. All the glassware were cleaned with chronic acid, followed by thorough washing with detergent powder and then rinsing tap water before use.

The sterilization of media was done at 15lbs, pressure for 20 min. Petriplates were sterilized in hot air sterilizer at 180°C for 2 hrs. The plastic petriplates used in bio control study, were sterilized by alcohol. The isolation chamber was sterilized by alcohol, followed by ultraviolet exposure for 20 min.

The other equipments used in isolation chamber like forceps, inoculation needle, cork borer, blade, etc. were sterilized by dipping them in alcohol, followed by heating on flame.

Media

Potato Dextrose Agar (PDA) medium

Peeled and sliced potato - 200 g
Dextrose - 20 g

Agar-agar - 20 g

Distilled water - 1000 ml

Trichoderma-selective agar medium

MgSO₄ 7H₂O - 0.2 g

K₂HPO₄ - 0.9 g

KCl - 0.15 g

NH₄NO₃ - 1.0 g

Glucose - 3.0 g

Hloramphenicol - 0.25 g
P-dimethylamino-benzenediazo sodium - 0.3 g
Pentachloronitro-

benzene - 0.2 g Rose-bengal - 0.15 g

Agar - 20 g

Distilled water - 1 lit.

Sterilization procedure Sterilization of glass wares

Glass wares were washed in liquid detergent under running tap water, and rinsed with distilled water 2-3 times. These were air-dried and then kept in oven for sterilization at 180°C for at least 2 hrs. Plastic wares were autoclaved at 121°C, 15 psi for 15 min.

Sterilization of inoculating needles, forceps, cork – borer and working table

Clean inoculating needle was sterilized by dipping the loop of needle in spirit and heating over the flame until red – hot. The process was repeated 2 –3 times. Forceps and cork – borer were also sterilized in the way of needle. The working table of laminar air flow was disinfected by sweeping with cotton soaked in absolute alcohol and exposing it to UV light for 30 minutes.

Sterilization of media and distilled water

Sterilized glassware and plastic wares were used for dispensing media and distilled water. All media were autoclaved at 121°C, 15 psi pressure for 15-30 min.

Sterilization of laminar air flow

Prior to the day of inoculation of fungus sample, the laminar air flow was saturated with alcohol vapors. At the time of inoculation the laminar air flow chamber was wiped with 70% alcohol or general spirit. Then only required instruments were kept in the chamber and exposed to UV rays for 15-20 min.

Sterilization of media

In all cases 100 ml medium was poured in 150 ml Erlenmeyer flask, separately plugged with non-absorbent cotton and sterilized in an autoclave.

Source of seeds and other materials

Lentil seeds and fungicides, talc based formulation of *Trichoderma* strains were obtained from the Department of Plant Pathology R.A.K. College of Agriculture, Sehore (M.P.)

Collection of diseased plant samples

Wilt and Collar rot of lentil plants were collected from sick plot of R.A.K. College of Agriculture, Sehore (M.P.) at vegetative and flowering stages. Samples were brought in laboratory and symptoms were recorded in detail. The tissues of affected plants showing the symptoms of wilt and collar rot were observed under microscope for the presence of the causal agent.

Isolation and purification of the pathogen

Small pieces of infected tissues 1 to 2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Pieces were dipped in 1 per cent sodium hypochloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water.

The bits were then transferred to PDA petri plates or slants with the help of inoculating needle under aseptic condition and incubated at $28 \pm 1^\circ\text{C}$. After 72 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA petri plates or slants. Pure culture was made; following repeated hyphal

tip transfer. Pure culture was maintained on PDA slants by sub culturing it at 30 days intervals. For preservation of cultures the plugged end of the culture tubes were dipped in melted wax and stored in a refrigerator at 5 ± 1 .

Pathogenicity tests Pot inoculation method

The test fungus was multiplied on soil was sand maize medium for 7 days. Sterilized soil was used in pot experiment. The soil was collected from the local field and dried thoroughly sterilized. Pots of 25 cm face diameter were during experiments. The pots were filled with 1kg sterilized soil and 20 g of inoculum was added in each pot. One inch of soil was placed over the inoculum. The contents were then mixed and surface sterilized seeds were sown and covered by half inch layer of soil. In control, no inoculum was added and pots were filled with sterilized soil. Ten seeds of variety RVL-31 were sown in each pot.

Results and Discussion

The collar rot and wilt pathogen was isolated from the diseased lentil plants. The infected tissues with healthy tissues were cut into small pieces of 0.5 to 1.0 cm long bits. The bits were surface sterilized by dipping in 1% sodium hypochlorite (NaHCl) solution for 1 min. The excess water on the surface of the pieces was removed by blotting on sterile blotting paper. The sterilized pieces were placed on potato dextrose agar medium supplemented with 200 ppm streptomycin. The plates were incubated at $28 \pm 1^\circ\text{C}$ and examined daily for the growth of mycelium of *Sclerotium rolfsii*. Hyphal tip transfer was made aseptically to potato dextrose agar (PDA) plates amended with 200 ppm streptomycin. The fungus grew upto 90 mm in 3 days on potato dextrose agar (PDA) medium. It produced extensive and white mycelium in culture medium. The mycelium

was hyaline, much branched and hyphae thin walled, septate, connection and sclerotia were small, mustard shape, white round bodies with clamp in the beginning, later becoming light to dark brown with shine and measuring 1.0 to 1.15 mm in size of *Sclerotium rolfsii* (Plate-1) and The mycelium was oval to cylindrical, straight or curved and measure 2.5 - 3.5 x 5 - 11 μm . Macroconidia are borne on branched conidiophore, thin walled, 1 to 6 septate, fusoid, pointed at both ends and measures 3.5 - 4.5 x 25 - 65 μm . Chlamyospores are formed in old cultures, which are smooth or rough walled, terminal intercalary and may be formed singly or in pairs or in chains of *Fusarium oxysporum* f. sp. *lentis*.

Antifungal activity of *Trichoderma* spp. against *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *lentis*.

The result of interaction between *Trichoderma* strains mycelia and *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *lentis* mycelia in dual culture indicated that there is a statistical difference in the overall growth. More than 60 mm diameter was observed in *T. viride*, *T. harzianum-5*, *T. harzianum-4*, *T. harzianum-1*. The mycelial growth of *T. harzianum-5* and *Sclerotium rolfsii* in dual culture were 66.2 mm and 24.2 mm followed by *T. viride* 65.1mm and 26.3 mm and the mycelial growth of *T. viride* and *Fusarium oxysporum* f. sp. *lentis* in dual culture were 75.3 mm and 15.7 mm followed by *T. harzianum-5* 70.1 mm and 20.6 mm respectively after 168 hrs of incubation.

The findings of the investigation entitled Studies on Biological and Chemical Control of wilt and Collar rot of lentil caused by *Fusarium oxysporum* f. sp. *lentis* and *Sclerotium rolfsii* has been described and explained with support of relevant research work published by earlier workers on the subject as follow.

Table.1 Antifungal activity of *Trichoderma* strains on radial growth of *Sclerotium rolfsii* after seven days of incubation.

| Treatment | Colony diameter of <i>Sclerotium rolfsii</i> (mm)* | Colony diameter of antagonist (mm)* | Inhibition growth of <i>Sclerotium rolfsii</i> |
|-----------------------|--|-------------------------------------|--|
| <i>T. harzianum-1</i> | 30.5 | 60.2 | 51.2 |
| <i>T. harzianum-2</i> | 34.4 | 56.4 | 49.2 |
| <i>T. harzianum-4</i> | 25.6 | 65.1 | 53.8 |
| <i>T. harzianum-5</i> | 24.2 | 66.2 | 55.1 |
| <i>T. viride</i> | 26.3 | 64.3 | 57.6 |
| <i>T. mutant</i> | 46.1 | 44.4 | 39.9 |
| SE(m) | 0.79 | 1.33 | - |
| C.D at 5% | 2.4 | 4.10 | - |

Table.2 Antifungal activity of *Trichoderma* strains on radial growth of *Fusarium oxysporum* f. sp. *lentis* after seven days of incubation.

| Treatment | Colony diameter <i>Fusarium Oxysporum</i> f.sp. <i>lentis</i> (mm)** | Colony diameter of antagonist (mm)* | Inhibition growth of <i>Fusarium Oxysporum</i> f.sp. <i>lentis</i> |
|-----------------------|--|-------------------------------------|--|
| <i>T. harzianum-1</i> | 23.2 | 67.2 | 58.9 |
| <i>T. harzianum-2</i> | 33.4 | 57.5 | 50.2 |
| <i>T. harzianum-4</i> | 28.5 | 62.4 | 57.2 |
| <i>T. harzianum-5</i> | 20.6 | 70.1 | 61.2 |
| <i>T. viride</i> | 15.7 | 75.3 | 65.9 |
| <i>T. mutant</i> | 33.5 | 57.4 | 49.8 |
| SE(m) | 0.83 | 1.67 | - |
| C.D at 5% | 2.56 | 5.16 | - |

*Average of 3 replications.

Fig.1 Colony diameter of *Trichoderma* strains and *Sclerotium rolfsii* after seven days of incubation

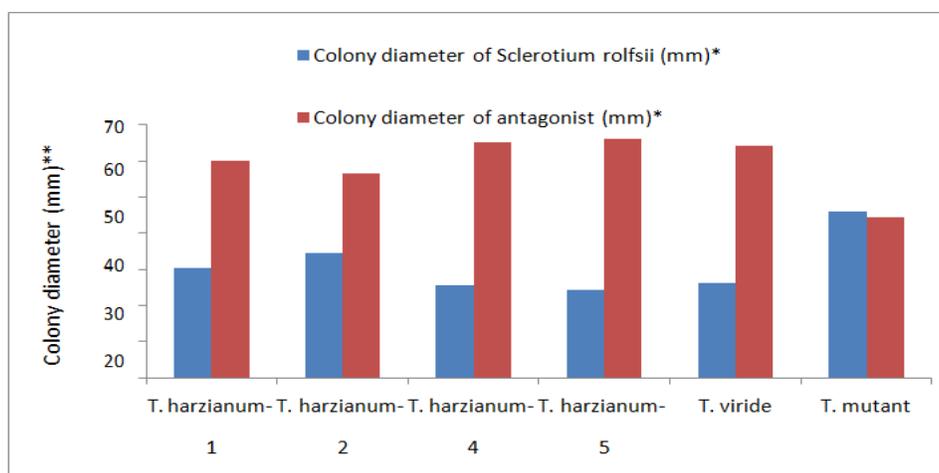
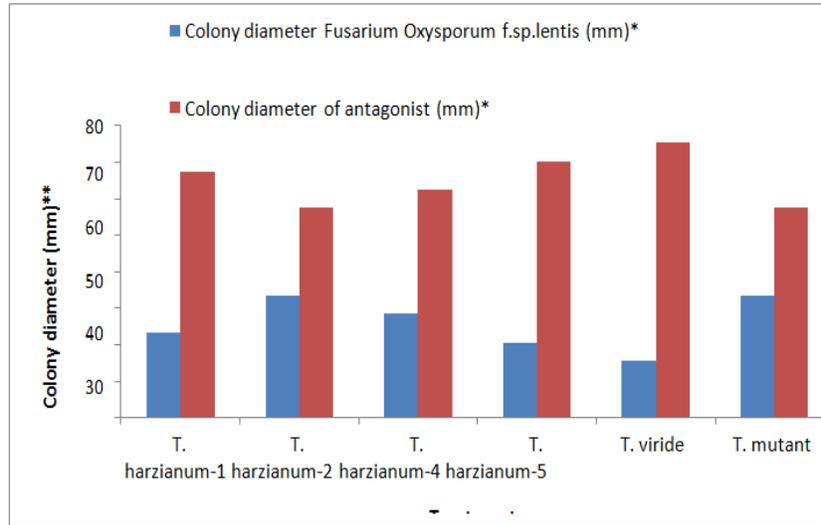


Fig.2 Colony diameter of *Trichoderma* strains and *Fusarium oxysporum* f.sp. *lentis* after seven days of incubation



The wilt disease appeared in the field in patches at both seedling and adult stages. Seedling wilt is characterized by sudden drooping, followed by drying of leaves and seedling death. The roots appeared healthy, with reduced proliferation and nodulation and usually no internal discoloration of the vascular system. Adult wilt symptoms appear from flowering to late pod-filling stage and are characterized by sudden drooping of top leaflets of the affected plant, leaflet closure without premature shedding, dull green foliage followed by wilting of the whole plant or of individual branches. Haji *et al.*, (2015)⁽²⁾ also described such wilt symptoms in lentil.

Pathogenicity of isolated fungi *F. oxysporum* f. sp. *lentis* was confirmed on lentil wilt susceptible variety L 9-12 by root dip and soil inoculation methods. Wilt symptoms were appeared after 15 days of inoculation in both the cases and Koch's postulates were confirmed by obtaining similar pathogen. Both these techniques were also demonstrated by Mohammadi *et al.*, (2011)⁽⁶⁾. Isolation *Sclerotium rolfsii* fungi from infected lentil plant. The mycelium of the fungal culture on

PDA medium was white and fluffy. Small white tufts were formed on mycelium which later turned to dark brown, round, sclerotia and measured 1-2 mm in diameter. On inoculation of *S. rolfsii* the lentil plants exhibited seedling mortality after ten days and cottony white growth of the fungal mycelium was found around the collar region on which several mustard like sclerotia were observed, confirming the *S. rolfsii*. Xie *et al.*, (2014)⁽⁸⁾ also adopted. Similar methodology to prove pathogenicity of *S. rolfsii* in tomato plants. In this present investigation germination percentage in lentil seed treated with *Trichoderma* strains was significantly higher than control.

The germination percent ranged between 56.5 to 74.5 percent. Maximum germination was recorded in *T. viride* (74.5%) followed by *T. harzianum-5* (72.5%) and *T. harzianum-1*(71.5%). Which was significantly superior to rest of treatments including control. Kumar *et al.*, (2014)⁽⁵⁾ reported enhanced seed germination and vigor of chickpea by the seed treatment of *Trichoderma* strains. *Trichoderma* strains for bio-efficacy,

compatibility with fungicides and growth promoting ability (GPA) of lentil, it could be concluded that *Trichoderma* strains viz. *T. viride* and *T. harzianum-5*. Among the *Trichoderma* strains *T. harzianum-5* and *T. harzianum-4* were highly compatible with hexaconazol+zineb, while strains *T. harzianum-2*, *T. harzianum-4* were compatible with carboxin +thiram. *Trichoderma* strains *T. viride* increased growth of lentil plant followed by other *Trichoderma* strains viz. *T.harzianum-5*, *T.harzianum-4*, *T. harzianum-2* and *T.mutant*.

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