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Infection, disease and managementof Fusarium wilt of lentil (Fusarium oxysporum f. Sp. Lentis)

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Abstract

Lentil crop suffers from a number of diseases. Among fungal diseases, wilt of lentil soil borne and endemic in nature causes heavy losses if susceptible varieties with infected seeds without proper treatment is grown year after year . The leaf extract of *Aadirachhta indica* and Lantana camara are found effective in inhibiting the growth of the pathogen and spore formation in vitro. The fungicides evaluated in vitro against the pathogen Bavistin and Vitavax completely inhibited the growth of the pathogen. Among the bio-agents *Trichoderma viride* was most effective in reducing the pathogen growth.The leaf extract of *Aadirachhta indica* and Lantana camara are found effective in inhibiting the growth of the pathogen and spore formation in vitro. Out of six fungicides evaluated in vitro against the pathogen Bavistin and Vitavax completely inhibited the growth of the pathogen. Among the bio-agents *Trichoderma viride* was most effective in reducing the pathogen growth.

Keyword- Economics, taxonomy, Lentil Symptoms, inoculation , transmission and Management

Introduction

Lentil (*Lens culinaris*Medik, Leguminosae)is an annual food crop that have been grown as an important food source for ancient time (**Dhupparet *al*.,2012**). Lentil wilt caused by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *lentis* Vasudeva and Srinivasan (Fol), is the most important disease affecting lentils, causing substantialeconomic losses (**Setti and Bouznad, 1998; Belabid*et al*., 2000**). The pathogen persists in the soil as chlamydospores that can remain viable for many seasons (**Erskine and Bayaa, 1996**), and it is capable of colonizing crop residue and roots of most follow crops. It can enter the plant through tips of roots in root elongation region that is assisted by wounding (**Bhalla*et al*., 1992**)

It is largest food crop being produced and consumed globally .it belong to family Leguminosae. In India lentil is mostly growing in Northern plains, Central and Eastern parts of India. It is grown in about 1.46 million hectares with total production of 1.08 million tonnes of grain. The major lentil-growing countries of the world are India, Canada, Turkey, Australia, Nepal, China, and Ethiopia. The total lentil cultivated area in the world is estimated around 4.34 million hectares with annual production and productivity of 4.95 million tons and 1260 Kg/ha respectively. It is cultivated as a rain fed crop in all India about 1.34 million ha area with 1.02mt production and 759 kg/ha productivity (**Abraham, 2015**).

In Uttar Pradesh, it is grown in 438.000 lakh/ha area with 235.000lakhtonnes/ha production and 537.0 kg/ha productivity (**Anonymous, 2015**).In India Lentil is produced mostly in Uttar Pradesh, Bihar, Madhya Pradesh, and Punjab. 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, *etal*., 2014**). It is chief protein source in comparison with high cost animal protein (**Mondal, *etal*.,2013**). A number of biotic and a biotic stresses can limit lentil's yield (**Chen , *etal*.,2009**). Among biotic agents, vascular wilt caused by (Fol) Vasudeva and Srinivasan is an important soil-borne disease of lentil causing important yield losses in dry and warm conditions. Under favourable conditions this disease can cause the complete loss of the crop (**Chen , *etal*.,2011**).

However, adoption of high yielding lentil varieties along with assured irrigation and judicious use of fertilizes results in a serious problem of wilt disease in the lentil growing areas of the country accounting for a enormous loss. Among disease the wilt of lentil caused by *Fol* is the main seed borne diseases of lentil, which is endemic in nature.

Economic importance

The productivity of wilted plants is very low. Infected plants have very low number of seeds and these seedsgerminate poorly and often produce very weak seedlings. Wilting causes more injury towards flowering and pod formation stage of the crop (**Grewal, 1988**)

Moderately high soil temperature (20 to 25° C) which favour fungal growth and sunlight, which enhances transpiration, seem to be the key factors determining symptom expression. This disease causes severe yield losses in the countries where the pathogen is found. Yield losses of up to 60% may occur under favourable conditions (**Singh *et al.* 2007**)

Taxonomic position

Lentil belongs to the genus Lens of the *Viceae* tribe in *the Legumnosae (Fabaceae*) family, commonly known as the legume family **(Fikru, *etal*.,2007**).

Lentil in taxonomy is as follows: **KingdomPlantae**-Plants, **Subkingdom Tracheobionta**-Vascular plants, **SuperdivisionSpermatophyta**-Seed plants, **Division Magnoliophyta**-Flowering plants, **Class Magnoliopsida**-Dicotyledons, **Subclass Rosidae**, **Order Fabales**, **Family Fabaceae**-Pea family, Genus Lens Mill.-lentil, Species Lens culinarisMedik.- lentil (**Anonymous, 2012**).

SYMPTOMATOLOGY

Fusarium wilt of lentil Symptoms can occur at both the seedling and adults stages of plant development. Disease effects both seedlings and adult stages and appear as patches in the field. The disease at the seedling stage originate from India where, Fusarium infection is characterised by a sudden drooping of the leaves (more like wilting and damping off), followed by the leaves drying and the eventual death of the seedling. The root system appears healthy, but with a compact proliferation and rate of nodulation. In most of the cases, there is no discolouration is found of the vascular system. Other symptoms at the seedling stage include seed rot and root rot, drooping and wilting of the uppermost leaflets, which resembles water deficiency, stunting of plants, shrinking and curling of leaves that starts from the lower part of the plants and progressively moves up the stem. The remainder of the plants foliage or individual branches turns a dull green colour. The leaflets undergo closure and premature detaching from the plants. Plants lastly become entirely yellow and perish. When plants are affected during the mid- to late-pod filling stages, seeds are often shrunken. Root symptoms include low growth with clear brown discolouration, tap root tips that are damaged and proliferation of secondary roots above the area of taproot injury. Very often discolouration of vascular tissue may not be visible in all cases.

Spread potential

The entry potential of *F. oxysporum f. sp. lentis* is medium for the following reasons: *F. oxysporum f. sp. lentis* is a seed-borne pest of lentil and it can also survive within infected plant material in the field. This indicates that it is well adapted to survive many adverse abiotic conditions. *F. oxysporum f. sp. lentis* can also be hosted by *Vicia spp.*

The spread potential of *Fol* in India is high for the following reasons: Spores can be splash disseminated. Rain splash and moving water can carry chlamydospores and conidia short distances to surrounding plants and adjoining enclosures. The pathogen can transport over large distances in infected and infested planting material and harvesting equipment into new territories. As a long-term strategy the lentil production in regions where the pest is identified should be postponed for better eradication of the disease. In the year of the outbreak most of the loss in lentil production occurs.This pest causes serious yield losses in those countries where the pathogen is known to occur and causes the complete crop losses under favourable conditions of a warm, dry spring.In addition, screening of lentil breeding lines for resistance to Fusarium wilt found that yield losses can range from 25-95% depending on the variety (Baraimer and Izquierdo 1977).

Diagnosis

Field diagnosis should be done in connection with lentil cropping history. Suspect stunted and wilted plants should be carefully uprooted from the soil so that the roots can be inspected for lower growth without externally showing fungal growth. Lower stems can be fragmented to check for vascular discolouration. Although vascular discolouration is not always symptomatic of Fusarium wilt the presence of discolouration confirms the presence of disease. Culturing of infected plant tissue in the laboratory should be done with molecular characterization because of the possible presence of other saprophytic Fusarium spp. that appears similar to *F. oxysporum* f. sp. *lentis.* A pathogenicity test on lentil is necessary to confirm *F. oxysporum f. sp. lentis.*

Disease cycle

The life cycles of the *Fusarium* wilts of lentil are basically the same, varying only in the hosts. After infecting of host roots, the fungus crosses the cortex and enters the xylem of the plant. The fungus then spreads quickly upward through the plant vascular system and may directly infect the seeds, thus becoming systemic in crop.The root tips of healthy plants growing in contaminated soil are penetrated by the germ tube of spores or fungal mycelium. The pathogen enters in plants is either direct, through wounds, or opportunistic at the point of formation of lateral roots. The mycelium spreads intracellularly through the cortex, and enters xylem vessels through the pits.The pathogen is primarily limited to the xylem vessels in which the mycelium branches and produces microconidia.The microconidia separate and are carried upward in the vascular system until movement is stopped, at this point they germinate and the mycelium pierces the wall of the adjacent vessel. Adjacent movement between vessels is through the pits. The water status of infected plants is severely affected by blockage of xylem vessels, resulting in closure of stomata, wilting and death of leaves, and followed by death of the whole plant.*F. oxysporum* can survive as mycelium and chlamydospores in seed and soil, and also on infected crop residues, roots and stem tissue buried in the soil for more than 6 years (**Singh , *etal.*, 2007**). Chlamydospores can survive in soil either in dormant form or saprophytically for without a suitable host. The disease is favoured by warm and dry soil conditions with an optimal temperature of 22-25°C.Spores may then be dispersed by wind and water or movement of soil or plant debris.

Inoculation technique

The production of artificially inoculated seed and varietal screening under artificialconditions, techniques are applied the pathogen inoculums: A known most highly virulent isolate FWL12 of F. oxysporum f. sp. lentis from lentil growing area of district Layyah (23°54'S; 21°55'E), Punjab, Pakistan was used for the study (**Rafique, *etal.*,2015**). For the inoculation of plants, the inoculum of the pathogen was prepared according to method of **Taheri , *etal.*,(2010**).

Seed transmission

*Fusarium oxysporum*f. sp. *lentis* survives mainly in soil oninfected plant remainders. The competitive saprophytic ability of *F.o. lentis*isfound to be very high in agar plate and straw colonization tests(**Mehrotra and Claudius, 1973a, 1974**). Seed transmission is tremendously important in dissemination of lentil wilt. The fungus can be isolated from all parts of the plants. Systemic infection occurs from root to seed through stems branches pedicel and placenta (**khare, 1981 J 1991**). Transmission of the fungus occurs primarily through plant residues and soil contamination, where it infects the plant through the roots. There is also evidence of transmission through seeds (**Erskine , *etal.*,1990**).

Management

Culture control

Sowing date affects wilt incidence because it determines the growth stage of the crop that is at an optimum or near-optimum temperature for fungal growth. In India, delayed sowing reduces disease incidence, but late sowing dramatically reduces yield potential and its effect on disease development differs over locations and seasons (**Kannaiyan and Nene, 1975a; Mittal, 1997**). In Syria, early sown crops are usually less affected. This is mainly due to differences in temperature at sowing, in the two countries (See Biology and Ecology; Epidemiology). A crop rotation of 4-5 years reduces inoculum density in the field, but does not completely eradicate the disease. In India, cultivation of paddy or sorghum in the rainy season reduced lentil wilt incidence the following winter (**Kannaiyan and Nene,** 1979). Soil amendment with organic matter (wheat or barley straw) enhances antagonism by other soil microorganisms

Certification standard

The certification standard are based on percent plant infection .the central seed certification board has permitted a maximum of 0.10 and 0.20% infected plants in foundation and certified seed plots, respectively. 10 meters for foundation seed production and 5 meters for certified seed production, isolation of the seed crop from other fields of lentil, is sufficient.(**Harpal Singh Tomar, 2011**). Ground to be used for seed production of lentil can be free of volunteer plants. A minimum of two inspections shall be made, the first before flowering and the second at flowering and fruit stage. (**R.K Trivedi 2013).**

Biological management

Singh et al., (2004) have earlierevaluated for antagonists VIZ., *T. Viride*, *T. Harzianum*, *G. virens* and *Aspergillus nidulans* as seed, soil and combined seed and soil treatments for the control of tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopersic*i in green houseexperiments. Application of the *T. harzianum*, *T. viride* and *G. virens*as seed treatment l0gm/kg seed are effective in controlling the seedling mortality up to 80-85% and areequal with carbendazim. Soil treatment with antagonist is less effective than seed treatment.

Chaudhary and Prajapati (2004) evaluated six bioagents VIZ., *T. harzianum* (Pantnagar). *T. viride* (Kanpur), *Penicilliumcitrinum* (Lucknow) against *Fusarium udum* causing pigeon pea wilt in dual culture technique and found maximum colony growthinhibition of *F. udum* by *Gliocladiumvirens* (Pantnagar) and *T. viride* (coimbatore). Mycoparasitism such as coiling, entwining and lysis of *F. udum*was seen with*T. harzianum*, *T. viride* and *G. virens* isolates but not with *A. niger* and *Penicilliumcitnnum*. The culture filtrates of six bio-agents inhibited *F. udum* colony growth by 18.1-53.6% at different concentrations highest being in *A. niger* which showed 36.4% mean growth inhibition.

Biological control can be done either by the introduction or augmentation in numbers of one or more species of controlling organisms, by a change in environmental conditions designed to favour the multiplication and activity of such organisms or by a combination of both. Biological control relies largely upon an interruption of host parasite relationship through biological means (**Snyder,1960)**

Choudhary , *etal.*,(2012) studied the antagonistic potential of three *Trichoderma spp*. viz., *Trichoderma harizanum, Trichoderma viride and Trichoderma koningii* against *Fusarium oxysporum*f.sp.*lentis*causing wilt of lentil under in vitro condition through dual culture technique. All the three bio-agentsare found effective in inhibiting the growth of pathogen**.**

Hassan , *etal.*,(2013) evaluated *Trichoderma hamatum* for its antagonistic potential against *Fusarium oxysporum* f.sp. *lentis*, the causal agent of vascular wilt disease of lentil and found effective.

Patel , *etal.*,(2014) tested seven different strains of *Trichoderma*, isolated from wilt infected leguminous crops of Madhya Pradesh and tested for their antagonistic activity against Fusarium (soil borne pathogen), which is expressed as a zone of inhibition in the culture plates. The seven strains were identified as *Trichoderma viride*, *T. harzianum, T. asperellum, T. koningii, T. atroviride, T. longibrachiatums* and *T. virens*. In this study the *Trichoderma viride*was prepared with a simple bioformulation that is cheap, easy to apply and readily accessible to the farmers.

Raut, *etal.*,(2014) have earlierevaluated two strains of *Trichoderma aspellerum* isolated from soil for their efficacy against some common soil borne plant pathogens by dual culture technique. Trichoderma strains is grown in potato dextrose broth and collected metabolites were amended to PDA medium to obtain 5, 10, 25 and 50% concentration in Petri plates. The solidified agar plates were inoculated with pathogen and incubated at 25 ± 2ºC for 7 days. The colony diameter is measured and percentage inhibition of radial growth was calculated. Both antagonists strains produced nonvolatile metabolites and inhibit the mycelia growth of *Fusarium graminearum, Rhizoctoniasolani* and *Pythium umtimum*.

Ommati, *etal.*,(2012) studied about the biocontrol efficacy of some native Trichoderma isolates against Fusarium oxysporum, an important causal agent of potato wilt disease under laboratory and greenhouse conditions in Shahrood Agricultural Research Center, Shahrood, Iran during 2006-2007. Fourteen isolates were collected among which eight showed promising ability in inhibiting growth of the pathogen through dual culture and production of volatile and non-volatile metabolites but *T. asperellum* and *T. atroviride* were almost more efficient than other isolates in inhibiting the mycelial growth of the pathogen in comparison to control. Eight isolates were evaluated against the disease under green house condition. Potted plants treated with Trichoderma isolates + F. oxysporum showed lower disease incidence in comparison to Fusarium infested control. Best disease control was observed in potted plants treated with *F. oxysporum + T. asperellum* showing 2.5% disease incidence in contrast to Fusarium infested control, in which disease incidence was72- 73%.

CHEMICAL CONTROL

khare, *etal.*,(1974) reported that Capton, Thiram and Rhizoctol were better than other fungicides tested in vivo in reducing pre and post emergence mortality of lentil due to *Sclerotiumrolfsii*. **Kannaiyan et al., (1975)** observed that seed treatment with Benomyl @ 0.30% reduced the wilt of lentil caused by *Fusarium oxysporumf.sp.lentis* under field conditions.

Agrawal and Khare (1977) reported the action of eight systemic and non systemic fungicides. They found that the organomercurials and arsenic fungicides were fungicidal, while Thiram, Captan, Folpet, Difolatan, Oxycarboxin and Benomyl were fungistatic against *Fusarium oxysporum*f.sp.*lentis.*

Maheshwari, *etal.*,(2008) studies the seven fungi toxicants against Fusarium oxysporum f. sp. lentis in vitro. Thesesuggestively checked the growth of the pathogen as compared to control. Carbendazim proved most effective fungi toxicant for checking the fungal growth, monitored by Captan, Hexaconazole and Diniconazole. The efficacy of six fungicides against *Fusarium oxysporum* f.sp.*lentis* revealed that Carbendazim and Carboxin completely inhibited the growth of *Fusarium oxysporum* f.sp.*lentis* .Whereas, Thiram and Captafol inhibited 87.5 and 83.1% of mycelial growth, respectively. Seed treatment with Carbendazim and Carboxin also improved seed germination (90.0& 89.0%), root length (10.1 cm & 10.0 cm), shoot length (4.8 cm & 4.8 cm), and vigour index (1342.0 & 1317.0) as compared to rest of the fungicides, respectively. Similarly, foliar spray with two fungicides separately gave the best results in reducing the wilt incidence from 37.5 to 5.0% in both the cases. (**Singh et al., 2010).**

Hoque, *etal.*,(2014) tested the efficacy of four fungicides in controlling foot and root rot of lentil under field condition. The test fungicides were Rovral (0.2%), Secure 600WP (0.2%), Bavistin 70WP (0.2%) and Captan 50WP (0.2%). Among the fungicides, best performance was found with Secure 600WP (0.2%) in controlling the incidence of foot and root rot.

Singh , *etal.*,(2014) studied the efficacy of different treatment measures viz., Carbendazim, Benomyl, Vitavax against seed borne fungi viz., *Aspergillus spp, Penicilliumspp, Alternariaalternata, Rhizopus and Fusarium spp.* All the three fungicides Benomyl, Carbendazim and Vitavax showed minimum occurrences against all the seed borne fungi.

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Verma, *etal.*,(2002) reported that the growth of *Fusarium oxysporum*f. sp. *pisi* was completely inhibited (1000/0) by Bavistin (200 ppm) followed by Dithane in 45 (Mancozeb) at 2000 ppm in vitro using the poisoned food technique.

De and Dwivedi (2003) evaluated 8 fungicides against *Fusarium oxysporum* f.sp.*lentis*in vitro and found that carbendazim and captan @ 10 ~g/ml were most effective by completely inhibiting (1000/0) the growth *Fusarium oxysporum* f.sp.*lentis*. There was 76, 75, 52, 49, 19 and 12% growth inhibition at 10 g/m1 concentration of pendimethalin. thiram, mancozeb) HgCb, metalaxyl, carboxin and CuS04, respectively.

De,, *etal.*,(2003) observed that in pot experiment presowing seed treatment (ST) with*Pseudomonas fluorescens* + Carboxin resulted in 60.3% \vilt control. The rhizosphere population of *Fusarium oxysporum* f.sp.*lentis*in 10-20 em soil depth at 17 ,30-60 days after sov./lng was 32.7-135.7 cfu x 10 m ST with *Gliocladiumvirens* and it was lower than that in soil drenching with spore suspension of *G. virens or Trichodennaharzianum* ST with Carbendazi+ Thiram and *G. virens* + *P. fluorescens* + carboxin was effective in the field in controlling 48.8 and 44.20/0 lentil wilt, respectively.

Khan and Mehnaz (2003) reported that the seed treatment with Bavistin @, 2 gm/kg controlled *Fusarium oxysporum* f.sp.*lentis*in field**.**

Sharma , *etal.*,(2003) reported that growth of *Fusarium oxysporum* f.sp.*lini*was completely inhibited by Bavistin (CarberdzimLDithane M-45 (Mancozeb) and Kavach (Cholorothalonil) 1500 ppm in vitro.

Sinha and Sinha (2004) reported efficacy of the seed treatment with Bavistin (0.1 %}) and drenching of soil with Blitox 50(0.3%) against the wilt complex caused by R. *solani, F. oxysporнm, Sclerotiumrolfsii and Mcrophonlinaphaseolina*In lentil. Both treatments significantly reduced wilt incidence and increased yield as compared with the control

Conclusion

Lentil crop suffers from a number of diseases. Among fungal diseases, wilt of lentil soil borne and endemic in nature causes heavy losses if susceptible varieties with infected seeds without proper treatment is grown year after year .it occur all the lentil areas of the world but is more severe in moist than dry regions. Consequently upon the infection ,yield and yield attributing factors are adversely affected. Three phases of wilting of lentil plants are distinguished on the basis of various symptoms which are pre-emergence, post-emergence and adult stages. In the pre-emergence stage, newly emerged radicle and plumule died, before complete emergence from surface of the soil. In seedlings wilt, young seedlings collapsed and dried up within 6-8 days of emergence form the soil. The most noticeable symptoms is the adult plant wilting which could be evident- even from a distance as dry plants in circular patch is in broad cast crop and in rows in line sown crops depending upon disease severity. The disease is present in all the locations of Uttar Pradesh being surveyed. The pathogen is isolated on potato dextrose agar medium from diseased plants parts and Koch's postulates are proved. On the basis of morphological and cultural characters the pathogen is identified as *Fusarium oxysporum* .sp. *lentis*. The pathogen survived on/in seeds as macro and micro conidia as mycelium fragments and chlamydospores whereas in soil, pathogen survived in diseased plant residues and as chlamydospores and these also served as primary source of inoculums. When crop is sown late in season the disease incidence is found least and yield of the crop also increased. The leaf extract of *Aadirachhta indica* and Lantana camara are found effective in inhibiting the growth of the pathogen and spore formation in vitro. Out of six fungicides evaluated in vitro against the pathogen Bavistin and Vitavax completely inhibited the growth of the pathogen. Among the bio-agents *Trichoderma viride* was most effective in reducing the pathogen growth. Among the fungicides tested as seed dresser in paper towel method and in pot experiment under glass house conditions Bavistin and Vitavax were found the best in improving seed germination, seedling vigour and in reducing wilt incidence whereas in bio-agents, *Trichoderma viride* was found to be most effective treatment. When the bio-agents are tested in seed treatment either alone or in various combinations with fungicides in the fields, the best results were observed with *T. viride*+ Vitavax and *T- viride*+ Bavisitin. A total of 100 cultivars germplasm of lentil were screened to locate the source of resistance against wilt.

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