

**“Identification of resistance and its confirmation in
lentil germplasm against wilt caused by *Fusarium
oxysporum* f. sp. *lentis*”**

THESIS



**Submitted to the
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya,
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Degree of
MASTER OF SCIENCE
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By
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2022

CERTIFICATE – I

This is to certify that the thesis entitled “**Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis***” submitted in partial fulfilment of the Degree of **MASTER OF SCIENCE IN AGRICULTURE (Plant Pathology)** of **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** is a record of the bonafide research work carried out by **Mr. Lokesh Chandrawanshi (ID No. 20131806)** under my guidance and supervision. The subject of the thesis has been approved by the Student’s Advisory Committee and the Director of Instruction.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of these investigations has been acknowledged by the scholar.

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This is to certify that the thesis entitled “**Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis***” submitted by **Lokesh Chandrawanshi (ID No. 20131806)** to the **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** in partial fulfilment of the requirements for the degree of **M.Sc. (Ag.)** in the **Department of Plant Pathology, RAK College of Agriculture, Sehore**, has after evaluation, been approved by the external examiner and by the Students Advisory Committee after an oral examination of same.

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(Lokesh Chandrawanshi)

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol	Legend
-	<i>In- vitro</i>
-	PSI
;	Semicolon
/	Per
&	And
@	At the rate of
°C	Degree Celsius
C.D.	Critical Difference
Cm	Centimeter
C.C.	Correlation Coefficient
D.F.	Degree of Freedom
<i>et al.</i>	Et alia (and others)
etc.	Etcetera
Fig.	Figure (s)
G	Gram
Ha	Hectare
i.e.	That is
Kg	Kilogram (s)
No.	Number (s)
NS	Non-significant
Sp.	Species (singular)
Spp.	Species (plural)
R.H.	Relative humidity
S.Em.±	Standard error of mean
M.S.S.	Mean sum of square
Viz.	Videlicet (namely)
√	Square root
%	Percent

CHAPTER - I

INTRODUCTION

Lens culinaris Medik is the botanical name of lentil, which is the oldest crops among legumes and belongs to the leguminosae family. It is originated from the Near East and Egypt at the Center and Southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India, Pakistan, China and later spread to Latin America (Cubero 1981, Duke 1981). India is one of the prime pulse producing countries in the world. In India the total area under lentil was 13.00 lakh ha with a total production of 11.00 lakh tonnes and its average productivity was 847 kg/ha. The major lentil producing states in the country are Madhya Pradesh, Uttar Pradesh, Bihar, Uttarakhand and Bengal. In Madhya Pradesh lentil is grown in 3.79 lakh ha with a total production of 2.94 lakh tonnes with 777 kg/ha productivity (Anon., 2020).

Lentil is grown as a *Rabi* crop or winter crop in India and sown in between months of October and November. Lentil is adapted in semi-arid environments, where it is usually grown in rotations. It is usually cultivated in all continents except Antarctica. Lentil is known by various names in different regions and languages like adas (Arabic), mercimek (Turkey), messer (Ethiopia), masoor (India), and heramme (Japanese) (Muehlbauer and McPhee., 2005). It can be cultivated on a wide range of soil from light loam to black cotton soils and it can also stand in less fertile moderately alkaline soils and even in rainfed conditions. The crop matures in arounds 90 to 120 days.

Lentil is among the first crops domesticated globally and it has become a prime food legume crop in the farming and food systems in many countries. It is a rich source of protein, minerals, and vitamins and plays an important role in human nutrition. The straw of lentils are pretty much valued animal feed and its ability in nitrogen fixation and carbon sequestration improves the nutrient status of soil, which in turn gives sustainability in production system (Sarker and Erskine, 2006).

Lentil crop is at risk to a various diseases caused by fungi, viruses, nematodes, insect pests, parasitic plants and abiotic stress. The most significant and serious soil-borne disease among them is Fusarium wilt. In many countries like in India, Algeria, Syria and Iran the pathogen of lentils showed high levels of phenotypic and genotypic diversity. The

disease develops at the temperature of 22-25°C and affects lentil either at seedling and vegetative or at the reproductive stages (Tiwari *et. al.*, 2018).

In India lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis* is the major disease in lentil growing regions. The disease was first reported in Hungary but in India, it was first observed in 1941 in Delhi and Karnal. The annual yield losses are 10-15 per cent due to this disease alone which valued approximately Rs. 2000-2500 crores (Chaudhary and Amarjit, 2002).

If infection takes place in early stage of the plant then it does not produces seeds and if infection occurs at later stage than only few number of seeds are formed. Wilt causes more damage during flowering and pod formation stage of the crop. The *Fusarium* wilt pathogen that primarily infects lentils, *F. oxysporum* f. sp. *lentis* (Vasudeva and Srinavasan) is present in many countries in Asia, Europe, Africa and America. Frequently the fungus spreads through the plant debris and soil contamination and it infects the plant through the root system. There is also an evidence of dissemination of fungus through seeds (Bayaa *et al.*, 1990). Temperature and soil moisture are the most important factors in determining symptoms expression and fungal growth rates (Saxena and Khare, 1988).

Host resistance is one of the most economic methods to manage wilt in lentil. Use of fungicide seed treatment is effective only at initial stage of wilting, while late stage wilting can't be avoided. Therefore, present investigations were conducted to identify sources of wilt resistance and its confirmation through pot screening under artificially infected soil. The response of soil temperature at different depths of soil on wilt incidence in lentil was also studied in the present investigation as it plays an important role in wilt symptoms expression.

With this context, the existing research work entitled **“Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis*”** has been taken up with the following objectives

1. Evaluation of lentil genotypes against wilt under sick plot conditions
2. Confirmation of wilt resistance under pot culture conditions
3. Effect of soil temperature at different depths of soil on incidence of wilt in selected lentil genotypes

CHAPTER - II

Review of literature

The literature pertaining to the thesis problem entitled “**Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis***” is reviewed here on the studies carried out in India and abroad.

2.1 History

Fusarium wilt is the most important seed and soil borne disease of lentil (*Lens culinaris* M.) through out the world and is prevalent in almost all the lentil growing areas.

Prissyajnyuk (1931) described a serious seedling blight of lentil caused by an unknown species of Fusarium in Russia. However, Flieschmann (1937) found that lentils in Hungary, particularly on acid soils, were susceptible to wilting.

Padwick (1941) isolated a *Fusarium* species from wilted lentil plants, *Fusarium orthoceras* App. var. *ciceri*, and found it may cause heavy economic losses to gram crop also.

2.2 Isolation:

Khare (1981) reported that *F. oxysporum* f. sp. *lentis* produces three types of spores microconidia, macroconidia and chlamydospores.

Honnareddy and Dubey (2007) conducted a study to characterize the morphological and cultural variability of 25 isolates of *F. oxysporum* collected from major chickpea growing areas of India.

Chaudhary and Singh (2008) conducted an experiment to find out the growth and pigmentation of 40 *F. oxysporum* f. sp. *lentis* isolates cultured on potato dextrose agar (PDA) or Czapek's-Dox-Agar (CDA) medium.

Naimuddin and Chaudhary (2009) worked on pathogenicity of 102 isolates of fusarium wilt disease of lentil.

Saxena *et al.* (2018) conducted a study to *F. oxysporum* f. sp. *lentis*, isolates were white, dull white, pinkish white, yellowish white and brownish white. The pigmentations on Potato Dextrose Agar medium were yellowish brown, dark brown, creamy and dark tan.

The rate of mycelia growth was grouped into three groups based on the size of macroconidia; size more than 15 µm was considered as large.

2.3 *Fusarium oxysporum* f. sp. *lentis* Identification and pathogenicity test

Tosi *et al.* (2001) took twenty-five samples of lentil roots and stems from each field. Seven to eight tissue sections were taken from the lower stems and roots of each sample, which were surface-treated with 0.1 percent HgCl₂ for 30 seconds, rinsed in sterile deionized water, placed on petri dishes containing potato dextrose agar (PDA, pH 5.5), and incubated at 25 °C for seven days. *F. oxysporum* was found in affected tissues on a regular basis.

Belaibidet *al.* (2004) tested thirty-two strains of *F. oxysporum* f. sp. *lentis* (Fol) isolated from wilted lentil plants taken from several lentil growing sites in north-western Algeria. All isolates were subjected to pathogenicity testing. Using nitrate-intolerant mutants, the isolates were also tested for vegetative compatibility (nit). Isolates that generated mutual heterokaryons were grouped together with terms of vegetative compatibility (VCG). The FOL isolates were all from the same race, but their aggressiveness on susceptible lines varied.

Lindbeck (2008) looked at microconidia from *F. oxysporum* f. sp. *lentis* that were numerous, aseptate, reniform to oval, and generated in false heads on monophialide conidiophores. These ranged in length from 5 to 12 meters, with a maximum depth of 2-3.5 meters. Macroconidia were quite rare. In culture, chlamydospores were abundant and developed singly or in pairs.

Choudhary *et al.* (2009) isolated *F. oxysporum* f. sp. *lentis*, causal agent of wilt disease of lentil, isolated from root samples collected from Banda, Hamirpur, Jalaun, Jhansi, Lalitpur and Mahoba districts in Budelkhand area of U.P. was worked out. Data on percent wilting induced in wilt susceptible genotypes of lentil.

Nikam *et al.* (2011) confirmed Pathogenicity of the *Fusarium oxysporum* f. sp. *ciceris* by sick soil inoculation technique in earthenpots under greenhouse conditions by using susceptible cultivar JG-62.

Mohammadi *et al.* (2012) used the root dip method to investigate the pathogenicity of 47 FOL isolates in the greenhouse on a particularly susceptible lentis line (ILL4605). The pathogenicity test was performed on the highly vulnerable to *Fusarium* wilt lentil

cultivar (ILL 4605). ILL 4605 seeds were surface sterilized for 3 minutes with 1 percent sodium hypochlorite, washed in sterile water, and germinated for ten days in sterilized per lit. pots. The roots of the ten-day-old plants were carefully removed and washed under tap water to remove superfluous soil. To make it easier for the pathogen to get into the roots, the pathogen's root tips were clipped off around 0.7cm long. The roots of the seedlings were then dipped in the inoculums of each isolate (5×10^6 conidium/ml) for 5 min to enable conidia to cohere to the roots. Inoculated seedlings were transplanted in ore-irrigated sterile soil: sand: pit: per lit (1:1:1:1) in pots and incubated at $25 \pm 3^\circ\text{C}$. Control plants were dipped in the sterile water for 5 minutes and then planted in pots.

Dubey and Singh (2018) studied the size of *F. oxysporum* f. sp. *lentis* microconidia varied between 7.30×3.43 (FOL-L4) TO 13.25×2.68 μm (FOL-S10) and macroconidia varied from 24.11×4.67 (FOL-A2) to 40.05×4.71 μm (FOL-V8). In macro-conidia, septa were generally 3-6, while micro-conidia had mostly no septum and some were 0-1. Chlamydospores were at intercalary terminals.

Hiremani *et al.* (2018) analysed 50 isolates of *F. oxysporum* f. sp. *lentis* on differentials in 2013-2014 and 2014-2015, the isolates belonging to different regions of the country showed marked variability with respect to incident ranging from 0 to 100% but the reaction patterns were more or less same in both the years.

Arya *et al.* (2019) showed that the reactions of the host against the pathogen were recorded in the wilt sick plot of lentil by calculating the percent of number of infected plants over total plants observed.

Kumari *et al.* (2021) studied of *F. oxysporum* f. sp. *lentis* (Fol) were isolated from wilted lentil plants that collected from different major lentil growing parts a pathogenicity test was tested in pot house condition by seed and soil inoculation techniques.

2.4 Taxonomic position of *Fusarium oxysporum* f. sp. *lentis*:

Vasudeva and Srinivasan (1952) identified the pathogen as *Fusarium oxysporum* f. sp. *lentis* and described it in detail. Snyder and Hansen (1940) placed the species *Fusarium oxysporum* Schlecht in the section elegance. *Fusarium oxysporum* f. sp. *lentis* belong to the

Kingdom	-	Fungi
Division	-	Eumycota

Subdivision	-	Deuteromycotina
Phylum	-	Ascomycota
Class	-	Hyphomycetes
Order	-	Moniliales
Family	-	Tuberculariaceae
Genus	-	<i>Fusarium</i>
Species	-	<i>oxysporum</i> f. sp. <i>lentis</i> (G.C. Ainsworth)

2.5 Symptoms of wilt

Khare *et al.* (1975) reported that out of 16 fungi, four seed borne, fungi namely *Fusarium* sp., *S. rolfsii* and *Rhizoctonia bataticola* are pathogenic. Seedling diseases were due to *F. moniliforme*, *F. oxysporum*, *Macrophomina phaseolin* and *S. rolfsii*. Ultimate wilting was due to *F. oxysporum* f. sp. *lentis*.

Agrios (2005) stated that *F. oxysporum* usually produces wilting, chlorosis, necrosis, vascular system browning, stunting, and shedding. It occurs with other root rot pathogens as a complex, which leads to serious loss of lentil seedlings.

Stoilova and Chavdarov (2006) reported that the symptoms produced by *F. oxysporum* f. sp. *lentis* stunting, marked reduction of the root system, internal vascular discolouration of the lower stem, and wilting. *F. oxysporum* wilt occurs in the field, which is patchy and occurs in the seedling stage or adult reproductive stage of crops. Seedling wilt is characterized by rapid drooping, followed by drying and death of the seedling. Though roots look healthy, proliferation and nodulation are reduced, and usually, the vascular system does not have external discoloration. Tosi *et al.* (2001) found stunting, wilting, a significant loss in root system size, and internal vascular discolouration of the lower stem in wilted lentil plants.

Sallam and Abdel-Monaim (2012) found that *F. oxysporum* f. sp. *lentis* was the common fungi associated with wilt symptoms and was pathogenic to lentil plants. Isolates of *F. oxysporum* f. sp. *lentis* were varied in their virulence. The influences of some agricultural practices on severity of wilt disease in lentil plants were studied under greenhouse and field condition.

Haji *et al.* (2015) reported the wilt disease appears in the field in patches at both seedling and adult stages. Seedlings 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. In adult plants wilt symptoms appear from flowering to pod formation stage in which leaflet close without premature shedding, foliage become dull green followed by wilting of the whole plant or of individual branches. Seeds from mid-pod-fill to late pod-fill affected plants are often shrivelled.

2.6 Evaluation of lentil genotypes against Fusarium wilt

Khare and Sharma (1969) reported that out of ten varieties from All India coordinated Trial, varieties L 9-12, B-25, NP-11 and T-36 exhibited the lowest percentage of wilt (1.82, 2.75, 3.12, and 3.55 respectively) under field condition. *F. oxysporum* f. sp. *lentis* was isolated from the roots of all the ten varieties. They have also observed minimum wilt incidence in variety S-61-7 (1.01%) followed by S-64-388, pushore-1 and 2, S64-376 and Vidisha-4 in the field screening of 72 other varieties of lentil.

Khare *et al.* (1971) reported that out of 485 germplasm lines collected, 440 were screened for wilt resistance in wilt sick plots (*F. oxysporum* f. sp. *lentis*) out of which 110 lines exhibited promise.

Bhat *et al.* (2006) screened thirteen Indian lentil genotypes. The results of the study classified the genotypes into different groups, immune, highly resistant, moderately resistant and moderately susceptible. No genotypes were found immune to the disease. SKL-16 was found to be highly resistant and exhibited highest yield. SKL-1, SKL-29 and SKL-9 were moderately resistant and SKL-12, SKL-8, SKL-13, SKL-3 and SKL-15 were moderately susceptible to the disease.

Joshi (2006) reported that lentil varieties i.e. Bari Masur-4, Shital, Simal, Khajura-1, Simrik and Maheswor Bharati possessed field resistance to vascular wilt at Khumaltar, Nepal.

Chaudhary *et al.* (2008) reported that Flip 2006- 11L was highly resistant seven lines (flip 2005-5l, flip- 2005-12L, flip 2008-sl, flip- 2008- 8l, Flip- 2007-10L, Flip- 2007-10L, Flip-2007-63L and Flip- 2007-85L) were resistant to Fusarium wilt.

Singh *et al.* (2009) studied the effect of sowing dates on the incidence of wilt in lentil sehore 74-3 caused by *F. oxysporum* f. sp. *lentis*. Late sowing (19 October) resulted in minimum disease incidence & higher yield compared to the early sowing.

Ghartiet *al.* (2011) observed that genotypes ILL 7715 and ILL 9993 were resistant and ILL 7164, ILL 590, PL 406 and F 2003-49L revealed moderate resistance to wilt / root rot complex in Nepalgunj condition.

Fatima *et al.* (2015) screened twenty eight lentil lines in a previously developed wilt sick bed in the research area of Pulses Research institute, Faisalabad using augmented design. Results revealed that sixteen lines were found highly susceptible, seven remained susceptible, and three lines were moderately resistant whereas two demonstrated resistance responses against the disease.

Yadav *et al.* (2017) screened 185 lentil genotypes for wilt and reported resistance in 15 genotypes (Arun, Sagun, M-Bharati, RL- 13, DPL- 62, ILL8191, ILL1672, RL- 85, ILL6811, RL-77, ILL6468, ILL6260, ILL9996, ILL7164, ILL6024 and RL- 21). Twenty-three genotypes (ILL8187, ILL9949, L 39-S-66, ILL8132, ILL7980, LN- 0135, ILL1920, FLIP05-24L(ILL10045), RL-51, LN-0111, FLIP2009, ILL9932, ILL7157, ILL6025, RL-44, ILL602, RL6, ILL3490, LN0137, ILL2526 and ILL6256 expressed moderate resistance.

Hiremani and Dubey (2018) tested the eighty-nine lentil genotypes during 2012–2013 against virulent isolate of *F. oxysporum* f. sp. *lentis* (FLS 75) to constitute a set of differential cultivars for race/pathotype determination. The wilt incidence was highly variable ranging from 6.7 to 92.9% and 7 genotypes were found resistant, 3 moderately resistant and remaining were susceptible. Considering the reactions, genetic background and area of cultivation, 10 genotypes namely, JL 3, L 4149, PL 4, PL 101, DPL 62, K 75, MC 6, L 6183, Sehore 74-3 and Vidhokar local were selected as differential cultivars for race/pathotype profiling. Based on the resistant and susceptible reactions on the differential cultivars, the isolates were grouped into eight races/pathotypes and differential cultivar for each race/pathotype was identified.

Neelkanth *et al.* (2018) screened eighty-nine lentil genotypes during 2012–2013 against virulent isolate of the pathogen (FLS 75) to constitute a set of differential cultivars for race/pathotype determination. The wilt incidence was highly variable ranging from 6.7 to 92.9% and 7 genotypes were found resistant, 3 moderately resistant and remaining were susceptible.

Soomro *et al.* (2018) revealed that from ten varieties none was observed immune to wilt. NIA-Masoor-05, lentil-25 were comparatively resistant against the infection followed by Masoor-93 and Markaz-09, while varieties like DL-2, VM-4 and DL-1 were highly susceptible to disease, as compared to other varieties. Maximum disease incidence, disease severity and mortality percentage were recorded in DL-2, VM-4, DL-3 and DL-1 followed by Lentil-85, Masoor-6 and Markaz-9, whereas, minimum was noted in NIA-Masoor-05, Lentil-25 and Masoor-93.

Arya and Kushwaha (2019) screened ninety-two germplasms along with the local check (Sehore) under field conditions during the two consecutive years 2016-17 and 2017-18. The germplasms were further categorized into Immune (0 germplasm), Highly Resistant (11 germplasm), Resistant (13 germplams), Moderately Resistant (18 germplams), Susceptible (40 germplams) and Highly Susceptible (10 germplams) depending on the disease reaction. Wilt incidence was varied from 0.33 percent to 96.67 percent during the year 2016-17 and 0.75 percent to 93.56 percent during the year 2017-18. None of the screened germplasm was found immune against the disease. Eleven germplasms viz., DL16-5, DL16-7, VL152, IPL339, IPL340, PL237, RL7-3, RVL15-5, IPL227, IPL338 and IPL332 were found highly resistant to the disease under field condition.

Chandra *et al.* (2019) tested 150 germplasm and cultivars for *In-vivo* against wilt of lentil and reported sixty-six genotypes were found resistant and thirty-seven were reported moderately resistant.

Chandra *et al.* (2019) evaluated one hundred forty germplasm and cultivars were evaluated in field condition against wilt of lentil and reported 53 genotypes were found highly resistant and 35 were reported resistant genotypes were noticed susceptible to highly susceptible.

Chandra *et al.* (2019) tested 150 germplasm and cultivars for *In-vivo* against wilt of lentil and reported forty nine genotypes were found highly resistant and thirty four were reported resistant rest genotypes were noticed susceptible to highly susceptible.

2.7 Resistance against Fusarium wilt under pot culture conditions

Voluzneva and Goluber (1982) found varietal differences in resistance of lentil to *Aschochyta lentis* and *F. oxysporum* f. sp. *lentis* under artificial inoculation conditions.

Mishra and Dhar (2005) carried out a study to find out the most efficient method of creating pigeon pea wilt under controlled conditions for inheritance and variability studies and reported that, out of three methods of inoculation soil inoculation and spore suspension and inoculation in pot culture were highly effective and could be efficiently utilized in screening for disease resistant sources / varieties under pot culture.

Stoilova and Chavdarov (2006) evaluated thirty-two lentil genotypes with different geographical origin under greenhouse condition for reaction to *F. oxysporum* f. sp. *lentis*. During 2003-2004. None of the accessions was immune or resistance to this pathogen under glasshouse conditions.

Tivoli *et al.* (2006) found the ectotrophic pathogens of the cool season food legumes (pea), lentil, chickpea, faba bean and lubin cause wide spread disease and severe crop losses throughout the world. Measures to control these diseases have relied on identification of resistant germplasm and development of resistant varieties through screening in the field and controlled environment.

Pouralibaba *et al.* (2015) evaluated 196 lentil landraces under wilt sick plot and in green house under controlled condition. Only twelve accessions were identified as resistant while remaining was found susceptible.

Rafique *et al.* (2016) tested 23 germplasm and cultivars for *In-vitro* for host resistance by root dip method revealed five cultivars viz. Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09 found resistance.

2.8 Effect of soil temperature on wilt incidence

Vasudeva and Srinivasan (1952) studied effect of temperature ranges on wilt incidence and found 7.5, 96.4 and 41.7 % wilt incidence at soil temperature ranges of 13 - 23 °C, 17 - 31 °C and 13 - 38 °C. Saxena and Khare (1988) also observed wilt to be more severe at 20-30 °C soil temperature. In further studies (Saxena and Khare, 1994), sandy loam soils at 30°C were found to be more conducive for development of wilt. Kaushal and Sharma (1998) reported 24-27 °C to be the optimum temperature range for development of lentil wilt at Palampur.

Mina and Dubey (2010) tested pusa 212' and 'BGD 1005' chickpea cultivars for influence of environmental parameters on wilt caused by *F. oxysporum* f. sp. *ciceris*. Lowest mean wilt incidence (22.6–25.5%) and maximum mean grain yield (13.5–14.3 tonnes / ha) were recorded in the crop sown on 10 and 20 November. Maximum and minimum ambient temperature and soil temperature were positively and significantly correlated with wilt incidence in Pusa 212.

Garkoti *et al.* (2013) reported that *F. oxysporum* f. sp. *lentis* is soil borne and can survive in the soil and plant debris for a period of 3-4 years. The disease is favoured by low soil temperature, 30% soil water holding capacity and increasing plant maturity. Yield losses depend on the stage at which the plant wilts - it can be 100% when wilt occurs at prepod stage or 67% when it occurs at the pre harvest stage.

Fatima *et al.* (2015) collected disease severity data along with weekly environmental conditions and were subjected to correlation and stepwise regression analysis. All the environmental factors were positively correlated with disease development and best explained using linear regression. Maximum severity of lentil wilt was recorded at 17-18°C and 7-10°C air temperature (Max. and Min.), 60-78% relative humidity and 2 mm rainfall.

Ghatak *et al.* (2015) studied the effect of temperature and sowing time against lentil wilt caused by *F. oxysporum* f. sp. *lentis*. Increased amount of inoculum resulted higher number of diseased plants. However, increased inoculum proved to be ineffective under cooler conditions. Early sowing associated with high soil temperature (22.6 °C) rendered maximum wilt incidence (97.2%). Singh *et al.* (2019) the test entries showed positive correlation with soil temperature and contributed 54.7% (JG 315), 56.32%, (IGP 187), 54.88% (IGP 29) and 39.42% (JG 62) in development of wilt at three different levels of temperatures, viz. 20, 27 and 34.

Pandit (2020) found mean temperature and relative humidity to be positively correlated; therefore it is evident that disease severity progressed significantly with rise in mean temperature and mean relative humidity. Rainfall and bright sunshine hours were found to influence disease in the significantly.

CHAPTER - III

MATERIAL AND METHODS

The present investigation entitled “**Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis*.**” was carried out in wilt sick plot of the Department of Plant Pathology of Rafi Ahmed Kidwai College of Agriculture, Sehore, (M.P.) during *Rabi* season 2021-22. The details of materials used, experimental procedures followed and techniques adopted are given as under:

3.1 Materials

The following are the materials used during the present research work:

3.1.1. Experimental site

The field experiment was conducted in wilt sick plot of the Department of Plant Pathology of R.A.K. College of Agriculture, Sehore, during *Rabi* season of 2021-2022. The experimental site is geographically situated in sub-tropical zone of Vindhyan Plateau of Madhya Pradesh at an elevation of 498.77 meters from mean sea level and lies between altitude and longitude 23° 18', North and 23° 18', East, respectively.

3.1.2. Collection of lentil Seed

Seeds of lentil varieties/germplasm were collected from the Department of Plant Pathology R.A.K. College of Agriculture, Sehore. (M.P.)

3.1.3 Collection of disease samples

Wilt samples of lentil plants were collected at vegetative and flowering phases from a sick plot at R.A.K College of Agriculture, Sehore (MP). Samples were taken into the lab, and symptoms were recorded in detail. The presence of the causal agents was examined under a microscope in the tissues of affected plants showing indications of wilt.

3.1.4 Culture media

Potato dextrose agar (PDA)

Peeled potato	200g
Dextrose	20g
Agar-agar	20g
Distilled water	1000ml (volume to makeup)

3.1.5 Chemicals

a. Cleaning solution:

Cleaning solution contained 80g potassium dichromate; 300 ml distilled water and concentrated sulphuric acid 400 ml. This was used to clean the glass wares.

b. Mercuric chloride solution:

Solution of 0.1% mercuric chloride solution was prepared and used for surface sterilization of plant, plant roots and seed samples.

C. Formaldehyde

1 part formalin in 49 parts of water (approximately 0.5 liter in 25 liter) is used to sterilize 1 m² to 23 cm depth of soil.

3.1.6 Glass-wares

The common glass-wares (Borosil and J-sil) viz., Petri-dishes, test tubes, conical flasks, volumetric flasks, measuring cylinder, glass rods, beakers, funnel, pipette etc. were received from the Department of Plant Pathology, R.A.K. College of Agriculture, Sehore.

3.1.7 Equipment's

The laboratory equipment's viz., autoclave, hot air oven, laminar airflow cabinet, BOD Incubator, refrigerator, binocular research microscope, electronic balance etc. available at the Department of Plant Pathology, R.A.K. College of Agriculture, Sehore were utilized as and when required.

3.1.8. Miscellaneous

Earthen pots (30 cm dia.), plant protection appliances, inoculation needle, cork borer, forceps, blotter paper, paper bags, polythene bags, spirit lamp, mercuric chloride, labels, tag, scales, sand, soil, FYM, screen house etc. were used during the course of present investigation.

3.2 Methodology

3.2.1 General procedure

3.2.1.1 Cleaning and sterilization of materials

Glassware's were sterilized in a hot air oven at 180°C for 2 hours. The culture media were sterilized in an autoclave at a pressure and temperature of 15 lbs per square inch (PSI) and at 121°C for 20 minutes.

The metallic equipment like inoculating needle and forceps, cork borer and other metallic instruments were sterilized by dipping them in spirit and heating till it gets red hot over the flame of a spirit lamp. All inoculation and isolation studies were conducted under aseptic conditions in laminar air flow chamber.

3.2.1.2 Sterilization of the laminar air flow chamber

Isolation and cultural studies were conducted under aseptic conditions in the laminar air flow cabinet. The working surface of laminar air flow was sterilized by swabbing with 70 per cent ethanol. Any material coming from outside was sterilized with alcohol or ethanol and hands were sterilized with alcohol.

3.2.1.3. Surface sterilization of plant parts:

Plant materials were surface sterilized using 0.1% mercuric chloride solution for 1 minutes and then washing thrice in sterile water.

3.2.1.4. Soil sterilization:

With formalin

Soil is sterilized systematically by drenching the soil with 1 part formalin in 49 parts of water (500 ml in 25 liters) per m² to 23 cm depth and covered with transparent polyethene sheets. After 8-10 days water vapours appear on the underside of the polyethene sheets after that the sheets were removed and soil is left in open to evaporate the formalin.

By autoclaving

Another method used for soil sterilization is by this method. Moist soil and sand were filled in gunny bags of volume 3 kg and were kept for sterilization in autoclave at temperature 121°C for 20 minutes.

3.2.1.5 Preparation of culture media

Potato dextrose agar (PDA)

The method was following as described by **Johnston and both (1983)** was used for present study.

Peeled potato	200.0g
Dextrose	20.0g
Agar	20.0g
Distilled water	1000 ml

The peeled potato was cut into 12 mm cubes. Two hundred grams of potato cubes were rinsed in water and boiled for 20 minutes in 500 ml water potato broth was filtered through cheese cloth. At the same time agar was also melted in 500 ml of water by heating then Potato broth was poured into the melted agar and dextrose was added in it. The final volume was made up to 1000 ml by adding distilled water. The pH was adjusted to 7.0 than it was sterilized at 15 psi for 20 minutes in an autoclave.

3.2.2 Symptomatology

The symptoms of the wilt disease were carefully observed and recorded at seedling and adult plant stage of lentil plant starting from the disease appearance till the maturity of the crop.

3.2.3. Method of isolation:

The diseased part of lentil plant showing distinct characteristic of wilt, were selected for isolation of the pathogen. The selected roots, collarr region and stem were first washed in tap water to remove soil particles and surface contaminant. The washed diseased parts were cut into small bits along with some healthy portions, with the help of sterilized blade. The cut pieces were surface sterilized with 1 per cent NaClO₂ solution under aseptic condition inside the laminar flow and washed thoroughly 3 to 4 times with sterilized water to remove the traces of mercuric chloride. Excess moisture was removed by placing them in the fold of sterilized blotting papers.

Three to four cut pieces of diseased parts of lentil were placed in Petri dish at equal distance from each other. Petri dishes were properly marked with glass marking pencil indicating date of isolation. The Petri dishes were transferred in B.O.D. incubator at 25 ± 20°C for 7 days. These incubated plates were observed for mycelial growth of the fungus after 24 hours of inoculation. The growth of the fungus was regularly observed. The

mycelium growth of the fungus was visible around infected plant parts, the hyphae tips from the advancing mycelium were cut and transferred into the culture tubes containing sampling PDA medium for further purification, identification and maintenance of culture.

3.2.4 Pure culture of the pathogen:

Pure culture of pathogen was prepared by single spore isolation. A dilute spore suspension was poured on plain agar Petri dishes to form a very thin layer on it and spores allowed to settle down on the agar surface. Settled spores were supported out from each other, selected under the microscope and encircled with the help of dummy culture in Petri dishes. They were lifted along with agar block and transferred to Petri dish containing sterilized 2 per cent PDA medium. After proper growth of fungus obtained by single spore culture regular sub-culturing was done to check contamination, till pure culture was obtained. Thus, obtained culture was sub cultured at monthly intervals and maintained on Potato Dextrose Agar slants under refrigeration at 6 to 80°C for further studies.

3.2.5 Pathogenicity tests

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 1 kg sterilized soil and 20 g of inoculums 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. Seeds of variety L 9-12 were sown in each plot.

3.3 Evaluation of lentil genotypes against wilt under sick plot condition

In order to locate the sources of resistance against *F. oxysporum* f. sp. *lentis* causing wilt, the lentil cultivars were screened in wilt sick plot of lentil in R.A.K. College of Agriculture, Sehore.

All the cultivars including three checks collected were sown on 10 November 2021 in rows of 2m length and 30 cm x 15 cm row to row and plant to plant spacing in augmented RBD design to test them against wilt of lentil at well maintained sick plot. The check genotypes L 9-12, Sehore 74-3 and DPL 62 were sown after every 10 genotypes in augmented RBD design.



Plate 3.1: RESEARCH TRIAL

Border								
Border	<i>T1</i>	<i>C3</i>	<i>T141</i>	<i>C3</i>	<i>T281</i>	<i>C3</i>	<i>T421</i>	Border
	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	
	<i>T10</i>	<i>C1</i>	<i>T150</i>	<i>C1</i>	<i>T290</i>	<i>C1</i>	<i>T430</i>	
	<i>C1</i>	<i>T140</i>	<i>C1</i>	<i>T280</i>	<i>C1</i>	<i>T420</i>	<i>C1</i>	
	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	
	<i>C3</i>	<i>T131</i>	<i>C3</i>	<i>T271</i>	<i>C3</i>	<i>T411</i>	<i>C3</i>	
	<i>T21</i>	⋮	<i>T151</i>	⋮	<i>T291</i>	⋮	<i>T431</i>	
	⋮	<i>C3</i>	⋮	<i>C3</i>	⋮	<i>C3</i>	⋮	
	<i>T30</i>	<i>C2</i>	<i>T160</i>	<i>C2</i>	<i>T300</i>	<i>C2</i>	<i>T440</i>	
	<i>C1</i>	<i>C1</i>	<i>C1</i>	<i>C1</i>	<i>C1</i>	<i>C1</i>	<i>C1</i>	
	<i>C2</i>	<i>T90</i>	<i>C2</i>	<i>T230</i>	<i>C2</i>	<i>T370</i>	<i>C2</i>	
	<i>C3</i>	⋮	<i>C3</i>	⋮	<i>C3</i>	⋮	<i>C3</i>	
	⋮	<i>T81</i>	⋮	<i>T221</i>	⋮	<i>T361</i>	⋮	
	<i>T61</i>	<i>C3</i>	<i>T200</i>	<i>C3</i>	<i>T341</i>	<i>C3</i>	<i>T491</i>	
	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	
	<i>T70</i>	<i>C1</i>	<i>T210</i>	<i>C1</i>	<i>T350</i>	<i>C1</i>	<i>T500</i>	
	<i>C1</i>	<i>T80</i>	<i>C1</i>	<i>T220</i>	<i>C1</i>	<i>T360</i>	<i>C1</i>	
	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	⋮	<i>C2</i>	
	<i>C3</i>	<i>T71</i>	<i>C3</i>	<i>T211</i>	<i>C3</i>	<i>T351</i>	<i>C3</i>	
				Border				

Layout of lentil screening Experiment

All five hundred genotypes were (Table 3.1) tested in the *Rabi* season 2021-22. The observations on the incidence of disease were made after every fortnight on the basis of wilt incidence, the genotypes were categorized into resistant (upto 10% wilt), moderately resistant (11-20% wilt), susceptible (21-50 %wilt), highly susceptible (above 51% wilt) (Singh *et. al.*, 2009). Percent wilting was calculated by the following formula.

$$\text{Wilt incidence (\%)} = \frac{\text{Total number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

Table 1: List of lentil genotypes evaluated

S. No.	Genotpes	S. No.	Genotypes
1	IC78552	251	IC392561
2	EC0267580	252	IC241447
3	EC0267563	253	L-4076
4	IC396044	254	EC0042392
5	EC27659	255	EC0267538
6	EC440747	256	EC0267601
7	IC11871	257	IC0014190
8	EC223214	258	IC0078420
9	IC424864	259	IC0078520
10	EC03001191	260	EC0078477-C
11	EC0223205-B	261	EC0223244
12	IC0014215	262	IC0078421
13	IC381127	263	EC0267613-A
14	IC98401	264	IC78406
15	IC0078552	265	EC223243
16	IC0014214	266	IC14158B
17	EC955432	267	EC0078545
18	IC260062	268	IC0078453
19	IC241144	269	IC201748
20	EC0223217	270	EC0078462
21	EC255504	271	EC329161
22	EC0267577	272	EC373712
23	IC53237	273	EC0027158
24	IC199779	274	EC0078526-F
25	IC33920	275	EC78531
26	EC0267569-B	276	EC0078552-B
27	IC0053235-A	277	EC0267613
28	IC361467	278	EC0267614-A
29	EC0267574	279	EC0267676
30	IC59038	280	ICIC0048546
31	IC241533	281	IC145259

32	IC565035	282	EC299650
33	IC0078399	283	IC22666
34	IC0078542	284	EC0078526-E
35	IC385824	285	EC0267584
36	DPL-62	286	IC0060375
37	IC267656	287	IC0060377
38	EC0267619	288	IC0078422
39	IC342718	289	IC0078525
40	EC0267539	290	IC0078556
41	IC145305	291	IC447768
42	IC384445	292	IPL-520
43	IC241531	293	EC0267709
44	IC54563	294	IC0073525
45	EC0267557-B	295	IC0078513
46	IC520809	296	IC0078526
47	EC0267619-A	297	EC0267565
48	IC0078516	298	EC0267693-A
49	IC381285	299	IC0078526-B
50	IC260010	300	EC0223237-C
51	IC384473	301	IC0078430
52	EC0267574-B	302	IC0078507
53	IC0078506	303	IC0078512
54	EC0078517	304	EC0267609
55	EC0255497	305	IC0053326-A
56	EC440870	306	EC0267544-B
57	IC148333	307	EC0267597
58	IC95658	308	EC0267691
59	IC241501	309	IC0062857
60	EC0223242	310	IC0078565
61	EC0267598	311	IC139850
62	EC0267631	312	IC241473
63	EC0267693-B	313	EC0267708
64	IC0020458	314	IC0078419
65	EC0267555	315	IC0060376
66	IC361417	316	IC0078418
67	EC0213199-A	317	IC0078566
68	EC0223236	318	IC0078391
69	EC0267634	319	IC0014158
70	EC0267579-B	320	IC345433
71	IC0014202	321	IC98364
72	IC0078518	322	IC22651
73	EC397812	323	EC267578
74	EC0213197-B	324	L-4594
75	IC421976	325	EC0223233
76	EC267362	326	EC0267573-A
77	IC201710	327	EC0267591

78	IC201562	328	EC0267593
79	EC0223237-A	329	IC0073125
80	EC0225484	330	IC0078506-A
81	EC0225995	331	IC0078533
82	IC0078528	332	IC0078568
83	EC0223197-C	333	IC0095652
84	IC0014159	334	IC201684
85	EC0267659	335	EC0299638
86	IC0027659	336	IC78529
87	IC0014225	337	EC0078553-A
88	IC199461	338	IC201693
89	IC201557	339	IC0078537
90	EC223212A	340	EC0223210
91	IC0014250	341	EC0223211
92	IC0078388	342	IC0078386
93	IC201772	343	IC201697
94	IC201661	344	EC0223207
95	IC73121	345	EC0267622
96	IC78549	346	EC0267641
97	IC201776	347	IC0078527
98	IC280887	348	IC0078536
99	EC0078530	349	IC0078546
100	EC0223229-B	350	IC0095660
101	EC0267571	351	IC267105
102	EC0267595-C	352	EC28514
103	EC0267652	353	IC0078409
104	EC0199459	354	EC0078526-A
105	EC33920	355	IC560153
106	EC0267614-B	356	EC0078442
107	IC201533	357	IC0078425
108	EC0241429	358	IC0078396
109	EC225503	359	IC078412
110	IC0057645	360	EC0078403
111	IC0078530	361	EC267607
112	EC0267526-D	362	EC0267612
113	EC027569-A	363	IC201582
114	EC0267617	364	EC0267549-A
115	IC316127	365	EC0225493
116	IC240885	366	EC0267628-B
117	IC274090	367	EC0267677
118	IC329110	368	IC53238
119	EC0267562	369	EC0223240
120	EC0267588	370	EC0267510
121	EC0267618	371	EC0267620
122	EC0267690	372	IC0060966
123	EC0255491	373	IC0078545

124	EC0267626	374	IC201694
125	EC955431	375	EC0299692
126	EC0267596	376	IC396758
127	EC78387	377	IC405259
128	IC521438	378	EC0078433
129	EC0078541-A	379	EC0078506-D
130	EC0255502-A	380	EC0267569
131	IC0078559	381	EC0267648
132	IC283540	382	EC0267678
133	EC0267579-A	383	EC0267706
134	IC0016458	384	EC0299632
135	IC00784117-A	385	IC0078460
136	IC0078423	386	IC0078555
137	EC0267579-C	387	EC0078518
138	EC0303711	388	EC267615
139	IC258265	389	IC0034704
140	IC201518	390	IC0095655
141	IC614705	391	EC0016371
142	IC208326	392	EC0255492
143	EC2675958	393	EC0267625-B
144	JL-3	394	IC201553
145	EC0078519	395	IC0078541
146	EC0078542-A	396	EC16391
147	EC0267528	397	IC78398
148	IC0078414	398	EC299732
149	IC241507	399	EC0241442
150	EC0267651-C	400	EC0267600
151	IC201788	401	IC201561
152	IC201537	402	EC0225456
153	IC201548	403	EC0078540
154	IC398793	404	IC0078424
155	IC241488	405	IC0078476
156	EC267595-D	406	IC0078510
157	IC0014172	407	EC0267606
158	EC241480	408	EC0299647
159	EC0225486	409	IC321501
160	IC240886	410	EC0223191
161	EC0267614	411	EC0267545-B
162	EC0267647	412	EC0267586
163	L-4717	413	EC0267696
164	EC0267575	414	IC0024089
165	IC616579	415	IC0078524
166	EC223237B	416	EC0267527
167	IC346268	417	IC201555
168	EC0225460	418	EC0267545-A
169	EC0267544-A	419	EC0267567

170	EC0267577-D	420	EC0299644
171	EC0267613-B	421	IC384447
172	IC241532	422	EC0267583
173	EC267554	423	EC0299649
174	NC60969	424	IC0078381
175	LL-931	425	IC0078395
176	IC0078526-A	426	IC0014195
177	IC0078532	427	IC0057170
178	EC0267572	428	EC0241434
179	IC267088	429	EC0299655
180	EC0267688	430	IC345478
181	IC584611	431	IC316132
182	IC201781	432	IC0028514
183	EC0267619-B	433	EC0078496
184	IC0078538	434	IC0078557
185	EC0299697	435	IC241543
186	IC0042393	436	EC0299668
187	IC0078563	437	IC0024088-A
188	EC0267575	438	IC078408
189	EC0267570	439	IC78455
190	IC0007809	440	EC0267692
191	IC201656	441	EC0299587
192	IC98351	442	IC0034679
193	EC0078526-B	443	EC0267572-A
194	EC0267541	444	EC0299673
195	IC0078417	445	EC0300190
196	IC0078544	446	IC241454
197	EC0267651-B	447	IC267668
198	EC0267694	448	EC0078512
199	EC0267710	449	EC0267640
200	EC0225488-B	450	IC0078415
201	IC0078550	451	EC299645
202	IC201564	452	EC0223188
203	IC0078567	453	EC0267604
204	IC98392	454	EC0223199-B
205	IC0078397	455	EC0299676
206	IC201568	456	EC0078547
207	IC267078	457	EC0267611
208	EC0299687	458	IC0014194
209	IC0078416	459	IC0078426
210	EC0223209-B	460	IC0093392
211	EC0267576	461	EC0299643
212	IC0014224	462	IC0062514
213	EC223235	463	IC0034650
214	IC417864	464	EC0078503-A
215	IC277173	465	EC0267576-B

216	IC241222	466	IC0073122
217	IC329109	467	IC0078405
218	IC317520	468	L-4147
219	HUL-57	469	IPL316
220	EC0078446	470	IC78477
221	EC0078463	471	EC0267702
222	EC0223209-C	472	EC0223224
223	EC0267577	473	IC22658
224	EC0267577-C	474	EC0269676
225	EC0267595-E	475	IC560225
226	IC0001843	476	EC0003379
227	IC0024087	477	EC0011371
228	IC0078521	478	EC0078454
229	IC0078534	479	EC0078486
230	IC0078535	480	EC0078524-A
231	IC0078547	481	EC0225487
232	IC0095653	482	EC0267526-B
233	IC201798	483	EC0267535
234	IC541007	484	EC0267587
235	IC553069	485	EC0267592
236	IC0078387	486	EC0267595-B
237	EC0225501	487	EC0267608
238	IC0078509	488	EC0267672
239	IC16453	489	EC0267686
240	EC22369	490	EC0294646
241	IC241475	491	EC0299612
242	EC0223226	492	EC0299646
243	IC95654	493	IC0014167
244	EC0267536	494	IC0014171
245	EC0267540	495	IC0053228
246	EC0267585	496	IC0053233
247	EC0267594	497	IC0059902
248	IC0078511	498	IC0073120
249	IC0078548	499	IC0078523
250	EC0267543	500	IC0095649
1	Check 1	L 9-12	
2	Check 2	Sehore 74-3	
3	Check 3	DPL-62	

3.4 Confirmation of wilt resistance under pot culture conditions.

During the *Rabi* season 2021-22, the pot experiment was conducted under controlled conditions (greenhouse). The soil was collected from the wilt infested field and 1 kg soil was filled into each pot. The pathogen was mass multiplied on soil maize medium. A light irrigation was applied after soil inoculation. Other pots without inoculum served as control. Ten lentil genotypes viz. EC440747, IC11871, IC396044, EC223214, IC424864, EC27659, IC78552, IC02675563, EC0267580 and EC0300191 showing resistance in wilt sick plot were selected for confirmation under pot culture condition by soil inoculation method.

Mass multiplication of isolates *F. oxysporium* f. sp. *lentis* was done using soil maize medium. This was prepared by mixing sterilized soil and coarsely ground maize grains in the ratio of 9:1. It was transferred to 250 ml conical flasks and added 2ml of 2% glucose solution and 15 ml of deionised water before autoclaving for 25 minutes at 121°C and 15 lbs pressure. After cooling to room temperature, these were inoculated separately with 4-6 mycelial discs (5 mm diameter.) taken from 7 days old *F. oxysporium* cultures, inoculated conical flasks were incubated at 30°C temperature, for nearly 14 days. Such mass multiplied inoculum of *F. oxysporium* f. sp. *lentis* were used for inoculating the test pots.

Pots of 25 cm face diameter were used during experiments. The pots were filled with 1 kg 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.

3.5 Effect of soil temperature at different depths of soil on incidence of wilt at weekly intervals.

Influence of soil temperature at different soil depths (5cm, 10cm and 20cm) on wilt incidence was examined in selected susceptible lentil genotypes grown in factorial RBD design.

Percent wilting was calculated by the following formula:

$$\text{Wilt incidence (\%)} = \frac{\text{Total number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

The absolute disease intensity was calculated as the difference between two successive observations of wilt incidence and correlation coefficient ('r') was computed between the absolute disease intensity and meteorological factors like

soil temperature at different depths (5 cm, 10 cm and 20 cm). The significance of correlation coefficient was judged by 't' test as per standard statistical methods.

The data on wilt intensity were plotted against soil temperature at different depths to predict the correlation between wilt and soil temperature. Regressions equations were also computed and scatter diagrams were drawn.

Data on soil temperature at different depths during the period were obtained from Agro-meteorological section, at R.A.K. College of Agriculture, Sehore. (Table-2).

Table 2: Soil temperature at different depths during November to March, 2021 at Sehore

Month	Standard Week	Week	Soil temperature (°C)		
			5 cm	10 cm	20 cm
November	45	5-11	19.157	20.429	23.043
	46	12-18	17.600	20.286	22.643
	47	19-25	21.700	22.414	23.886
	48	26-2 Dec.	16.429	20.171	22.443
December	49	3-9	17.429	19.086	22.000
	50	10-16	16.800	19.257	21.586
	51	17-23	12.714	15.571	18.886
	52	24-31	13.913	17.088	19.388
January	1	1-7	15.243	16.914	19.357
	2	8-14	13.714	15.771	18.486
	3	15-21	12.586	15.114	17.543
	4	22-28	12.200	15.243	17.929
	5	29-4 Feb	13.129	15.600	18.600
February	6	5-11	14.357	16.886	19.414
	7	12-18	15.029	17.729	20.086
	8	19-25	14.829	18.686	21.529
	9	26-4 March	16.457	20.029	22.786
March	10	5-11	17.943	21.229	23.886
	11	12-18	19.886	22.343	25.100
	12	19-25	22.929	25.529	27.214

3.6 Statistical Analysis

The data obtained in all the experiments was subjected to the statistical analysis (Panse and Sukhatme, 1978). The percentage values were transformed into arcsine values. The standard error (SE) and critical difference (C.D.) at level $P = 0.01$ were worked out and results obtained were compared statistically. All the statistical analysis was done using (OP STAT) and R-studio statistical programmer at Computer Laboratory, R.A.K. College of Agriculture, Sehore.

(1) Standard error for treatment means:

$$S. Em. \pm = \frac{\sqrt{Ems}}{r}$$

(2) Critical difference:

$$C.D. = S.Em. \sqrt{2} \times t$$

Where,

Ems ($\pm$)	=	Error means sum of square
R	=	Number of replications
T	=	't' value at 5% probability levels

(3) Correlation coefficient:

Correlation per cent disease index and per cent seed infection were worked out as per method suggested by Chandel (1991).

$$r(x, y) = \frac{COV. (x, y)}{\sqrt{Var. (x) Var. (y)}}$$

Where,

$r(x, y)$	=	correlation coefficient between character x and y.
$COV(x, y)$	=	covariance between character x and y.
$V(x)$	=	variance of x character.
$V(y)$	=	variance of y character.

CHATER – IV

EXPERIMENTAL RESULTS

This investigation entitled “Identification of resistance and its confirmation in lentil germplasm against wilt caused by *Fusarium oxysporum* f. sp. *lentis*.” was carried out with the following heads.

1. Evaluation of lentil genotypes against wilt under sick plot conditions.
2. Confirmation of wilt resistance under pot culture conditions.
3. Effect of soil temperature at different depths of soil on incidence of wilt in selected lentil genotypes.

4.1 Isolation, identification and pathogenicity test of *F. oxysporum* f. sp. *lentis*.

4.1.1 Symptomatology

The first symptom of wilt observed in patches which are almost round in outline and enlarge as the season advances. Curving of the leaves starts from the lower part of the plant and extends to upward which is mainly drooping and drying of leaves, stem branches and finally death of plant. The root system is badly grown and exhibits discolored brown color. The discoloration of roots may be partial or whole. The concerned root vessels were discolored brown and fungal hyphae were observed in some vessels which are running close to the walls. The successive evaluation of disease symptoms were recorded in inoculated wilt sick plot. The occurrence of disease was first recorded after flowering and podding stage. Initially the appearance of disease was slight vein clearing on the outer portion of younger leaves, after that collapse (downward dropping) of older leaves. The seedling was hanged followed by sudden death. The infected main stem, collar region and root showing the definitive symptoms and browning of the vascular tissue is powerful verification of the disease (Plate 4.1a).

4.1.2 Isolation and identification

The fungus was isolated in the laboratory under aseptic conditions from infected roots of lentil plants and cultured on PDA medium in Petri plates by transferring them after surface sterilization. After mycelial growth, the fungus was purified through hyphal tip culture method (Plate 4.1b).



a. Wilted Plant

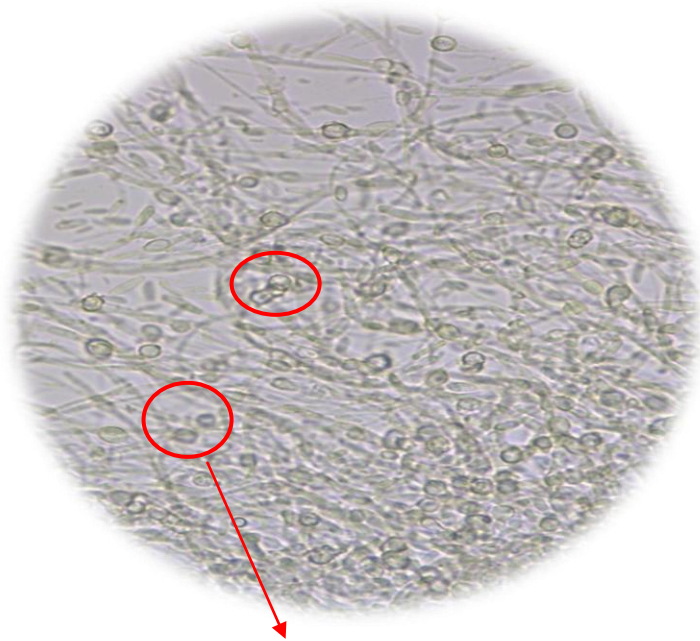


b. Wilted Plant

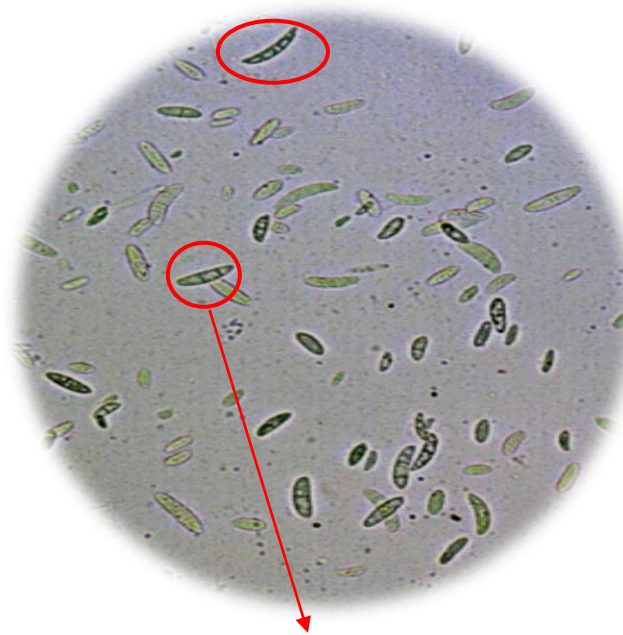


c. Infected root

Plate 4.1a: Symptom of lentil wilt



a. Chlamydospores



b. Macro conidia



c. Micro conidia

Plate 4.1c: Conidia and chlamydospores of *Fusarium oxysporum* f. sp. *lentis*



Plate 4.1b: Mycelium growth of *Fusarium oxysporum* f. sp. *lentis* in petridish



Plate 4.1d: Pathogenicity test

The fungus isolated from wilted lentil plant revealed typical wilt symptoms that developed into a white fluffy colony of the fungus that is identified as *F. oxysporum* f. sp. *lentis*. Mycelium of test fungus was hyaline, septate and branched with cottony, whitish to pinkish growth. Micro-conidia were comma-shaped and more or less curved, with 1-2 septa and measured 2.5-3.5 × 5-11µm, whereas, Macro-conidia has 3-5 septa, was sickle shaped, smooth and hyaline, measuring 3.5-4.5 × 25-65 µm. The chlamydospores were spherical, thick-walled spores that seemed like intercalary on older mycelium. (Plate 4.1c).

4.1.3 Pathogenicity

The pathogenicity test of isolated fungus *F. oxysporum* f. sp. *lentis* was carried out on wilt susceptible cultivar L 9-12 grown in pots under glasshouse condition. Wilting of the seedlings started after 15 days and after 30 days more than 90 percent plants were wilted in infested soil. The plants were normal and no wilt symptoms were observed in uninfested control pots. *F. oxysporum* f. sp. *lentis* was reisolated from the wilted lentil plants, thus proving the Koch's postulates. (Plate 4.1d)

4.2 Evaluation of lentil genotypes against Fusarium wilt under sick plot conditions.

This study was conducted on lentil genotypes in *Rabi* season 2021-22 in wilt sick plot under field condition for evaluation for resistance and susceptibility. Wilt incidence varied from 3.45 to 100% in different genotypes. A total of 500 genotypes were classified in four groups based on disease incidence: resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS). Among these 500 genotypes, 10 genotypes were found resistant showing up to 10% wilt incidence, 35 genotypes were found moderately resistant showing 11-20% wilt incidence, while 187 genotypes were susceptible showing 21-50% wilt incidence. All 268 genotypes were found highly susceptible (more than 50% wilt incidence).

Table 3: Incidence of lentil wilt in different genotypes

Block No.	Genotypes	Wilt incidence (%)
Block 1	L 9-12	30.77 (33.71)
	Sehore 74-3	50.00 (45.00)
	DPL-62	28.57 (32.33)
	EC241480	41.67 (40.22)
	EC267362	28.57 (32.33)
	EC223243	57.14 (49.08)
	EC397812	27.78 (31.86)
	EC223237B	42.86 (40.92)
	EC225503	35.00 (36.27)

	EC223235	50.00 (45.00)
	IC584611	45.00 (42.17)
	IC241507	40.63 (39.58)
	IC145259	58.82 (50.07)
Block 2	L 9-12	25.00 (30.00)
	Sehore 74-3	28.57 (32.33)
	DPL-62	25.00 (30.00)
	IC240886	41.94 (40.34)
	IC201798	51.61 (45.92)
	IC14158B	57.14(49.08)
	IC560225	100.00 (90.00)
	EC440870	23.08 (28.73)
	IC392561	55.17 (47.93)
	IC240885	36.36 (37.11)
	IC614705	40.00 (39.13)
	IC553069	52.63 (46.49)
	IC201568	47.62 (43.62)
Block 3	L 9-12	12.50 (20.70)
	Sehore 74-3	9.09 (17.56)
	DPL-62	15.00 (22.79)
	IC258265	39.29 (38.83)
	IC145305	20.00 (26.56)
	IC381285	21.74 (27.76)
	EC440747	9.09 (17.56)
	IC16453	53.33 (46.89)
	IC201533	34.78(36.15)
	IC98401	11.76 (20.09)
	IC53237	15.00 (22.79)
	IC59038	16.67 (24.12)
	IC381127	11.54 (19.82)
Block 4	L 9-12	50.00 (45.00)
	Sehore 74-3	50.00 (45.00)
	DPL-62	42.86 (20.92)
	IC201564	46.88 (43.22)
	IC384445	20.00 (26.56)
	EC955432	13.04 (21.13)
	EC255504	14.29 (22.22)
	IC384473	22.22 (28.11)
	IC241531	20.00 (26.56)
	EC78387	37.50 (37.76)
	EC22369	53.85 (47.24)
	IC520809	21.43 (27.56)
	IC421976	28.00(21.95)
Block 5	L 9-12	100.00 (99.00)
	Sehore 74-3	96.67 (79.53)
	DPL-62	20.00 (26.56)
	IC417864	50.00 (45.00)
	IC78529	68.00 (55.55)
	IC321501	80.00 (63.44)
	IC361417	26.09 (30.72)

	EC329161	58.33 (49.78)
	IC274090	36.36 (37.11)
	IC521438	37.50 (37.76)
	IC78455	88.00 (69.73)
	IC277173	50.00 (45.00)
	IC54563	20.00 (26.56)
Block 6	L 9-12	33.33 (35.24)
	Sehore 74-3	59.09 (30.24)
	DPL-62	50.00 (45.00)
	IC73121	33.33 (35.24)
	IC95654	54.55 (47.64)
	IC78549	33.33 (35.24)
	IC398793	41.18 (39.93)
	IC201582	72.00 (58.05)
	IC98392	47.37 (43.51)
	IC385824	18.18 (25.25)
	IC201557	30.77 (33.71)
	IC241533	16.67(24.20)
	IC95658	25.00 (30.00)
Block 7	L 9-12	42.31 (40.57)
	Sehore 74-3	20.00 (26.56)
	DPL-62	16.67 (24.20)
	IC199779	15.00 (22.79)
	IC208326	40.00 (39.23)
	IC260062	13.04 (21.13)
	EC33920	34.48 (35.37)
	IC201788	40.74 (39.64)
	IC260010	21.74 (27.83)
	IC33920	15.00 (22.79)
	IC11871	9.38 (17.85)
	IC241222	50.00 (45.00)
	IC396044	7.69 (16.11)
Block 8	L 9-12	29.41 (32.83)
	Sehore 74-3	37.50 (37.76)
	DPL-62	50.00 (45.00)
	IC201661	32.43 (34.70)
	IC201776	33.33 (35.24)
	IC201693	68.18 (55.56)
	IC201656	46.15 (42.82)
	IC98364	65.71 (54.15)
	IC201697	68.75 (56.04)
	IC361467	15.79 (23.42)
	EC16391	77.78 (61.89)
	IC329109	50.00 (45.00)
	IC396758	75.00 (60.00)
Block 9	L 9-12	70.00 (56.79)
	Sehore 74-3	28.57 (32.33)
	DPL-62	100.00 (90.00)
	IC241475	53.85 (27.24)
	IC241501	25.00 (30.00)

	IC22651	66.67 (54.76)
	IC267088	44.44 (40.78)
	IC267656	18.37 (25.40)
	IC139850	62.96 (52.53)
	IC201555	80.77 (64.01)
	EC28514	69.57 (56.54)
	IC22658	96.43 (79.06)
	EC223212A	31.43 (34.08)
Block 10	L 9-12	29.63 (32.96)
	Sehore 74-3	57.14 (49.08)
	DPL-62	50.00 (45.00)
	IC241454	89.66 (71.23)
	IC241144	13.33 (21.30)
	EC267554	43.75 (41.44)
	IC201748	57.58 (49.37)
	IC241543	86.21 (59.19)
	NC60969	43.75 (41.44)
	IC98351	46.15 (42.76)
	IC447768	60.00(50.77)
	IC201781	45.00 (42.13)
	IC345433	65.52 (54.03)
Block 11	L 9-12	18.92 (25.77)
	Sehore 74-3	40.00 (39.23)
	DPL-62	16.67 (24.12)
	IC201710	28.57 (32.33)
	IC316127	36.11 (36.93)
	IC342718	18.92 (25.77)
	IC283540	37.93 (38.00)
	IC201772	32.26 (34.63)
	IC280887	33.33 (35.244)
	IC201553	77.27 (61.55)
	IC241488	41.18 (39.93)
	EC223214	9.38 (17.95)
	IC201684	67.74 (55.37)
Block 12	L 9-12	30.00 (33.21)
	Sehore 74-3	33.33 (35.24)
	DPL-62	42.86 (40.92)
	EC373712	58.33 (49.78)
	IC201518	39.39 (38.88)
	IC329110	36.36 (37.11)
	EC299645	90.48 (72.05)
	EC299650	58.82 (50.07)
	IC345478	84.21 (66.58)
	IC201537	40.91 (39.76)
	IC267668	90.00 (71.56)
	IC384447	81.82 (64.57)
	IC53238	72.73 (58.50)
sBlock 13	L 9-12	50.00 (45.00)
	Sehore 74-3	33.33 (35.24)
	DPL-62	60.00 (50.77)

	IC78398	77.78 (61.89)
	IC424864	9.68 (18.15)
	IC201548	40.91 (39.76)
	IC346268	42.86 (40.92)
	IC267105	69.23 (56.29)
	IC78406	56.67 (45.85)
	EC955431	36.84 (37.35)
	IC616579	42.42 (40.63)
	IC201694	73.91 (59.28)
	IC201562	28.57 (32.33)
Block 14	L 9-12	43.75 (41.44)
	Sehore 74-3	50.00 (45.00)
	DPL-62	40.00 (39.23)
	IC565035	16.67 (24.12)
	EC299732	77.78 (61.89)
	IC241447	55.17 (47.98)
	IC405259	75.00 (60.00)
	IC201561	78.26 (62.24)
	IC78477	94.74 (76.69)
	IC267078	47.62 (43.62)
	IC148333	24.00 (29.33)
	IC541007	52.38 (46.38)
	EC27659	8.57 (17.05)
Block 15	L 9-12	50.00 (45.00)
	Sehore 74-3	60.00 (50.77)
	DPL-62	75.00 (60.00)
	IC316132	84.62 (66.89)
	IC22666	58.82 (50.07)
	IC241532	43.48 (41.27)
	EC267615	76.19 (60.83)
	IC199461	30.00 (33.21)
	EC2675958	40.00 (39.23)
	IC317520	50.00 (45.00)
	IC241473	63.64 (52.89)
	IC78552	3.45 (10.78)
	EC267578	66.67 (54.76)
Block 16	L 9-12	28.57 (32.33)
	Sehore 74-3	50.00 (45.00)
	DPL-62	100.00 (90.00)
	IC560153	70.00 (56.79)
	DPL-62	18.18 (25.25)
	HUL-57	50.00 (45.00)
	IPL316	93.94 (75.00)
	IPL-520	60.00 (50.77)
	JL-3	40.00 (39.23)
	L-4076	55.56 (48.22)
	L-4147	93.55 (75.35)
	L-4717	42.31 (40.57)
	LL-931	43.75 (41.44)
Block 17	L 9-12	16.00 (23.58)

	Sehore 74-3	36.36 (37.11)
	DPL-62	56.25 (48.62)
	L-4594	66.67 (54.76)
	EC0003379	100.00 (90.00)
	EC0011371	100.00 (90.00)
	EC0016371	76.47 (61.00)
	EC0027158	58.33 (49.78)
	EC0042392	55.56 (48.22)
	EC0078403	71.43 (57.67)
	EC0078433	75.00 (60.00)
	EC0078442	70.59 (57.17)
	EC0078446	50.00 (45.00)
Block 18	L 9-12	66.67 (54.76)
	Sehore 74-3	62.50 (52.24)
	DPL-62	42.31 (40.57)
	EC0078454	100.00 (90.00)
	EC0078462	57.89 (40.51)
	EC0078463	50.00 (45.00)
	EC0078477-C	56.25 (48.62)
	EC0078486	100.00 (90.00)
	EC0078496	85.71 (64.78)
	EC0078503-A	93.33 (75.00)
	EC0078506-D	75.00 (60.00)
	EC0078512	90.00 (71.56)
	EC0078517	22.73 (28.45)
Block 19	L 9-12	100.00 (90.00)
	Sehore 74-3	26.32 (38.85)
	DPL-62	13.33 (21.39)
	EC0078518	76.00 (60.67)
	EC0078519	40.00 (39.23)
	EC0078524-A	100.00 (90.00)
	EC0078526-A	69.70 (56.60)
	EC0078526-B	46.15 (42.82)
	EC0078526-E	58.82 (50.07)
	EC0078526-F	58.33 (49.78)
	EC0078530	33.33 (35.24)
	EC78531	58.33 (49.78)
	EC0078540	78.57 (62.44)
Block 20	L 9-12	50.00 (45.00)
	Sehore 74-3	50.00 (45.00)
	DPL-62	40.00 (39.23)
	EC0078541-A	37.50 (37.76)
	EC0078542-A	40.00 (39.23)
	EC0078545	57.14 (49.08)
	EC0078547	91.30 (72.84)
	EC0078552-B	58.33 (49.78)
	EC0078553-A	68.00 (55.55)
	EC0199459	34.38 (35.91)
	EC0213197-B	27.78 (31.82)
	EC0213199-A	26.09 (30.72)

	EC0223188	90.48 (72.05)
Block 21	L 9-12	40.00 (39.23)
	Sehore 74-3	60.00 (50.77)
	DPL-62	40.00 (39.23)
	EC0223191	80.00 (63.44)
	EC0223197-C	29.17 (32.71)
	EC0223199-B	90.63 (72.15)
	EC0223205-B	11.11 (19.46)
	EC0223207	68.75 (56.04)
	EC0223209-B	47.83 (43.74)
	EC0223209-C	50.00 (45.00)
	EC0223210	68.42 (55.80)
	EC0223211	68.42 (55.80)
	EC0223217	13.33 (21.39)
Block 22	L 9-12	54.55 (47.64)
	Sehore 74-3	38.46 (38.35)
	DPL-62	100.00 (90.00)
	EC0223224	96.30 (78.91)
	EC0223226	53.85 (47.24)
	EC0223229-B	33.33 (35.24)
	EC0223233	66.67 (54.70)
	EC0223236	26.32 (30.85)
	EC0223237-A	28.57 (32.33)
	EC0223237-C	61.54 (51.65)
	EC0223240	73.08 (58.76)
	EC0223242	25.00 (30.00)
	EC0223244	56.25 (48.62)
Block 23	L 9-12	41.18 (45.87)
	Sehore 74-3	24.00 (29.33)
	DPL-62	50.00 (45.00)
	EC0225456	78.26 (62.24)
	EC0225460	42.86 (40.92)
	EC0225484	28.57 (32.33)
	EC0225486	41.67 (40.22)
	EC0225487	100.00 (90.00)
	EC0225488-B	46.67 (43.11)
	EC0225493	72.22 (58.18)
	EC0225501	52.94 (46.66)
	EC0225995	28.57 (32.33)
	EC0241429	34.78 (36.15)
Block 24	L 9-12	40.00 (39.23)
	Sehore 74-3	100.00 (90.00)
	DPL-62	42.31 (40.57)
	EC0241434	82.61 (65.35)
	EC0241442	77.78 (61.89)
	EC0255491	36.67 (37.29)
	EC0255492	76.92 (61.27)
	EC0255497	22.73 (28.45)
	EC0255502-A	37.50 (37.76)
	EC0267510	73.33 (58.89)

	EC0267526-B	100.00 (90.00)
	EC0267526-D	35.71 (36.69)
	EC0267527	80.65 (63.94)
Block 25	L 9-12	37.50 (37.76)
	Sehore 74-3	44.44 (41.78)
	DPL-62	39.29 (29.82)
	EC0267528	40.00 (39.23)
	EC0267535	100.00 (90.00)
	EC0267536	54.55 (47.64)
	EC0267538	55.56 (48.22)
	EC0267539	19.05 (25.92)
	EC0267540	54.55 (47.64)
	EC0267541	46.15 (42.76)
	EC0267543	55.00 (47.87)
	EC0267544-A	42.86 (40.92)
	EC0267544-B	62.50 (52.24)
Block 26	L 9-12	60.00 (50.77)
	Sehore 74-3	12.50 (20.70)
	DPL-62	60.00 (50.77)
	EC0267545-A	80.95 (64.16)
	EC0267545-B	80.00 (63.44)
	EC0267549-A	72.00 (58.05)
	EC0267555	25.93 (30.59)
	EC0267557-B	20.83 (27.13)
	EC0267562	36.36 (37.11)
	EC0267563	7.14 (15.45)
	EC0267565	60.87 (51.30)
	EC0267567	80.95 (64.16)
	EC0267569	75.00 (60.00)
Block 27	L 9-12	57.14 (49.08)
	Sehore 74-3	63.33 (52.71)
	DPL-62	54.55 (47.64)
	EC027569-A	35.71 (36.69)
	EC0267569-B	15.00 (22.79)
	EC0267570	45.83 (42.59)
	EC0267571	33.33 (35.24)
	EC0267572	44.00 (41.55)
	EC0267572-A	88.89 (70.54)
	EC0267573-A	66.67 (54.76)
	EC0267574	16.13 (23.66)
	EC0267574-B	22.22 (28.11)
	EC0267575	45.71 (42.53)
Block 28	L 9-12	40.00 (39.23)
	Sehore 74-3	66.67 (54.76)
	DPL-62	45.45 (42.42)
	EC0267575	42.31 (40.57)
	EC0267576	47.83 (43.74)
	EC0267576-B	93.33 (75.00)
	EC0267577	50.00 (45.00)
	EC0267577	14.29 (22.22)

	EC0267577-C	50.00 (45.00)
	EC0267577-D	42.86 (40.92)
	EC0267579-A	37.93 (38.00)
	EC0267579-B	26.67 (31.11)
	EC0267579-C	38.89 (38.59)
Block 29	L 9-12	41.67 (40.22)
	Sehore 74-3	66.67 (54.76)
	DPL-62	75.00 (60.00)
	EC0267580	6.06 (14.30)
	EC0267583	81.82 (64.75)
	EC0267584	58.82 (50.07)
	EC0267585	54.55 (47.64)
	EC0267586	80.00 (63.44)
	EC0267587	100.00 (90.00)
	EC0267588	36.36 (37.11)
	EC0267591	66.67 (54.76)
	EC0267592	100.00 (90.00)
	EC0267593	66.67 (54.76)
Block 30	L 9-12	66.67 (54.76)
	Sehore 74-3	100.00 (90.00)
	DPL-62	77.78 (61.89)
	EC0267594	54.55 (47.64)
	EC0267595-B	100.00 (90.00)
	EC0267595-C	33.33 (35.24)
	EC267595-D	41.18 (39.93)
	EC0267595-E	50.00 (45.00)
	EC0267596	37.04 (37.47)
	EC0267597	62.50 (52.24)
	EC0267598	25.00 (30.00)
	EC0267600	77.78 (61.89)
	EC0267601	55.56 (48.22)
Block 31	L 9-12	40.00 (39.23)
	Sehore 74-3	53.85 (47.24)
	DPL-62	50.00 (45.00)
	EC0267604	90.48 (77.57)
	EC0267606	79.17 (62.87)
	EC267607	71.43 (57.67)
	EC0267608	100.00 (90.00)
	EC0267609	61.90 (51.88)
	EC0267611	91.67 (73.26)
	EC0267612	71.43 (57.67)
	EC0267613	58.33 (49.78)
	EC0267613-A	56.52 (48.73)
	EC0267613-B	42.86 (40.92)
Block 32	L 9-12	50.00 (45.00)
	Sehore 74-3	87.50 (69.30)
	DPL-62	50.00 (45.00)
	EC0267614	42.11 (40.46)
	EC0267614-A	58.33 (49.78)
	EC0267614-B	34.78 (36.15)

	EC0267617	35.71 (36.69)
	EC0267618	36.36 (37.11)
	EC0267619	18.75 (25.70)
	EC0267619-A	21.43 (27.56)
	EC0267619-B	45.00 (42.13)
	EC0267620	73.33 (58.89)
	EC0267622	68.75 (56.04)
Block 33	L 9-12	50.00 (45.00)
	Sehore 74-3	37.04 (37.47)
	DPL-62	38.89 (38.59)
	EC0267625-B	76.92 (61.27)
	EC0267626	36.67 (37.29)
	EC0267628-B	72.22 (58.18)
	EC0267631	25.00 (30.00)
	EC0267634	26.32 (30.85)
	EC0267640	90.00 (71.56)
	EC0267641	68.75 (56.04)
	EC0267647	42.11 (40.46)
	EC0267648	75.00 (60.00)
	EC0267651-B	46.43 (42.94)
Block 34	L 9-12	66.67(54.76)
	Sehore 74-3	43.48 (41.27)
	DPL-62	38.46 (38.35)
	EC0267651-C	40.63 (39.58)
	EC0267652	33.33 (35.24)
	EC0267659	29.41 (32.83)
	EC0267672	100.00 (90.00)
	EC0267676	58.33 (49.78)
	EC0267677	72.41 (58.31)
	EC0267678	75.00 (60.00)
	EC0267686	100.00 (90.00)
	EC0267688	44.44 (41.78)
	EC0267690	36.36 (37.11)
Block 35	L 9-12	35.71 (36.69)
	Sehore 74-3	70.00 (56.79)
	DPL-62	66.67 (54.76)
	EC0267691	62.50 (52.24)
	EC0267692	88.24 (69.91)
	EC0267693-A	61.11 (51.41)
	EC0267693-B	25.00 (30.00)
	EC0267694	46.43 (42.94)
	EC0267696	80.00 (63.44)
	EC0267702	95.24 (77.34)
	EC0267706	75.00 (60.00)
	EC0267708	63.64 (52.89)
	EC0267709	60.00 (50.77)
Block 36	L 9-12	33.33 (35.24)
	Sehore 74-3	27.27 (31.50)
	DPL-62	46.15 (42.82)
	EC0267710	46.43 (42.94)

	EC0269676	97.14 (80.19)
	EC0294646	100.00 (90.00)
	EC0299587	88.24 (69.21)
	EC0299612	100.00 (90.00)
	EC0299632	75.00 (60.00)
	EC0299638	67.74 (55.37)
	EC0299643	92.00 (73.57)
	EC0299644	81.25 (64.38)
	EC0299646	100.00 (90)
Block 37	L 9-12	61.54 (51.65)
	Sehore 74-3	46.67 (43.11)
	DPL-62	100.00 (90.00)
	EC0299647	79.17 (62.87)
	EC0299649	81.82 (64.75)
	EC0299655	84.00 (66.42)
	EC0299668	87.50 (69.30)
	EC0299673	88.89 (70.54)
	EC0299676	90.91 (72.00)
	EC0299687	47.62 (43.62)
	EC0299692	74.07 (59.41)
	EC0299697	45.45 (42.42)
	EC0300190	89.47 (71.09)
Block 38	L 9-12	69.57 (56.54)
	Sehore 74-3	100.00 (90.00)
	DPL-62	60.00 (50.77)
	EC03001191	10.00 (18.44)
	EC0303711	39.13 (38.70)
	IC0001843	50.00 (45.00)
	IC0007809	45.83 (42.59)
	IC0014158	65.38 (53.97)
	IC0014159	29.17 (32.71)
	IC0014167	100.00 (90.00)
	IC0014171	100.00 (90.00)
	IC0014172	41.18 (39.93)
	IC0014190	55.56 (48.22)
Block 39	L 9-12	50.00 (45.00)
	Sehore 74-3	57.14 (49.01)
	DPL-62	50.00 (45.00)
	IC0014194	91.67 (73.26)
	IC0014195	82.35 (65.20)
	IC0014202	27.27 (31.50)
	IC0014214	12.50 (20.70)
	IC0014215	11.11 (19.46)
	IC0014224	48.65 (44.25)
	IC0014225	29.63 (32.96)
	IC0014250	31.82(34.33)
	IC0016458	38.10 (38.12)
	IC0020458	25.00 (30.00)
Block 40	L 9-12	60.00 (50.77)
	Sehore 74-3	50.00 (45.00)

	DPL-62	50.00 (45.00)
	IC0024087	50.00 (45.00)
	IC0024088-A	87.50 (69.30)
	IC0024089	80.00 (63.44)
	IC0027659	29.41 (32.83)
	IC0028514	84.62 (66.89)
	IC0034650	92.86 (74.15)
	IC0034679	88.24 (69.91)
	IC0034704	76.19 (60.80)
	IC0042393	45.45 (42.42)
	ICIC0048546	58.33 (49.78)
Block 41	L 9-12	100.00 (90.00)
	Sehore 74-3	71.43 (57.67)
	DPL-62	12.50 (20.70)
	IC0053228	100.00 (90.00)
	IC0053233	100.00 (90.00)
	IC0053235-A	15.00 (22.79)
	IC0053326-A	61.90 (51.88)
	IC0057170	82.35 (65.20)
	IC0057645	35.00 (35.27)
	IC0059902	100.00 (90.00)
	IC0060375	58.82 (50.07)
	IC0060376	64.29 (53.31)
	IC0060377	58.82 (50.70)
Block 42	L 9-12	35.71 (36.69)
	Sehore 74-3	66.67 (54.76)
	DPL-62	45.00 (42.13)
	IC0060966	73.33 (58.89)
	IC0062514	92.59 (74.21)
	IC0062857	62.50 (52.24)
	IC0073120	100.00 (90.00)
	IC0073122	93.33 (75.00)
	IC0073125	66.67 (54.76)
	IC0073525	60.00 (50.77)
	IC0078381	81.82 (64.30)
	IC0078386	68.42 (55.80)
	IC0078387	52.63 (46.49)
Block 43	L 9-12	50.00 (45.00)
	Sehore 74-3	58.33 (49.78)
	DPL-62	61.54 (51.65)
	IC0078388	32.00 (34.45)
	IC0078391	65.00 (53.75)
	IC0078395	82.14 (64.97)
	IC0078396	70.83 (57.29)
	IC0078397	47.37 (43.51)
	IC0078399	16.67 (25.12)
	IC0078405	93.33 (75.00)
	IC078408	87.50 (69.30)
	IC0078409	69.57 (56.54)
	IC078412	70.83 (57.29)

Block 44	L 9-12	40.00 (39.23)
	Sehore 74-3	33.33 (35.24)
	DPL-62	62.50 (52.24)
	IC0078414	40.00 (39.23)
	IC0078415	90.00 (71.56)
	IC0078416	47.62 (43.62)
	IC0078417	46.15 (42.82)
	IC0078417-A	38.46 (38.35)
	IC0078418	64.29 (53.31)
	IC0078419	64.00 (53.13)
	IC0078420	55.56 (48.22)
	IC0078421	56.25 (48.62)
	IC0078422	58.82 (50.07)
Block 45	L 9-12	12.50 (20.70)
	Sehore 74-3	41.18 (39.93)
	DPL-62	33.33 (35.24)
	IC0078423	38.46 (38.35)
	IC0078424	78.57 (62.44)
	IC0078425	70.59 (57.17)
	IC0078426	91.67 (73.26)
	IC0078430	61.54 (51.65)
	IC0078453	57.14 (49.08)
	IC0078460	75.00 (60.00)
	IC0078476	78.57 (62.44)
	IC0078506	22.22 (28.11)
	IC0078506-A	66.67 (54.76)
Block 46	L 9-12	50.00 (45.00)
	Sehore 74-3	100.00 (90.00)
	DPL-62	64.29 (53.31)
	IC0078507	61.54 (51.65)
	IC0078509	52.94 (46.66)
	IC0078510	78.57 (62.44)
	IC0078511	54.55 (47.64)
	IC0078512	61.54 (51.65)
	IC0078513	60.00 (50.77)
	IC0078516	21.43 (27.56)
	IC0078518	27.27(31.50)
	IC0078520	55.56(48.22)
	IC0078521	50.00 (45.00)
Block 47	L 9-12	100.00 (90.00)
	Sehore 74-3	31.82 (34.33)
	DPL-62	61.11 (51.41)
	IC0078523	100.00 (90.00)
	IC0078524	80.00 (63.44)
	IC0078525	58.82 (50.07)
	IC0078526	60.00 (50.77)
	IC0078526-A	43.75 (41.44)
	IC0078526-B	61.11 (51.41)
	IC0078527	68.75 (56.04)
	IC0078528	28.57 (32.33)

	IC0078530	35.29 (36.45)
	IC0078532	43.75 (41.44)
Block 48	L 9-12	13.33 (21.39)
	Sehore 74-3	77.78 (61.89)
	DPL-62	35.29 (36.45)
	IC0078533	66.67 (54.76)
	IC0078534	50.00 (45.00)
	IC0078535	50.00 (45.00)
	IC0078536	68.75 (56.04)
	IC0078537	68.18 (56.67)
	IC0078538	45.00 (42.13)
	IC0078541	77.27 (61.55)
	IC0078542	16.67 (24.12)
	IC0078544	46.15 (42.76)
	IC0078545	73.68 (59.15)
Block 49	L 9-12	13.33 (21.39)
	Sehore 74-3	77.78 (61.89)
	DPL-62	35.29 (36.45)
	IC0078546	68.75 (56.04)
	IC0078547	50.00 (45.00)
	IC0078548	54.55 (47.64)
	IC0078550	46.67 (43.11)
	IC0078552	11.76 (20.09)
	IC0078555	75.00 (60.00)
	IC0078556	59.09 (50.24)
	IC0078557	85.71 (67.78)
	IC0078559	37.50 (37.76)
	IC0078565	62.50 (52.24)
Block 50	L 9-12	35.71 (36.69)
	Sehore 74-3	66.67 (54.76)
	DPL-62	45.00(42.13)
	IC0078563	45.45(42.42)
	IC0078566	64.52(53.43)
	IC0078567	47.06(43.34)
	IC0078568	66.67(54.76)
	IC0093392	91.67(73.26)
	IC0095649	100.00(90.00)
	IC0095652	66.67(54.76)
	IC0095653	50.00(45.00)
	IC0095655	76.19(60.80)
	IC0095660	68.75(56.04)
Overall.adjusted.mean		54.98
		SE (m)±
A Test Treatment and a Control Treatment		24.42
Control Treatment means		4.19
Two Test Treatments (Different Blocks)		34..2
Two Test Treatment (Same Block)		29.62
		C.D. at 5%
		48.41
		8.3
		67.79
		58.71

Figure in the parentheses are angular transformed values

A total of 500 lentil genotypes were evaluated against Fusarium wilt in sick plot. The observations were recorded at regular interval and final disease incidence was recorded before harvesting. Ten genotypes; IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864 and EC03001191 were classified as resistant against Fusarium wilt (Table 4.2). Thirty five genotypes; EC0223205-B, IC004215, IC381127, IC98401, IC0078552, IC0014214, EC955432, IC260062, IC241144, EC0223217, EC255504, EC0267577, IC53237, IC199779, IC33920, EC0267569-B, IC0053235-A, IC361467, EC0267574, IC59038, IC241533, IC565035, IC0078399, IC0078542, IC385824, DPL-62, IC267656, EC0267619, IC342718, EC0267539, IC145305, IC384445, IC241531, IC54563 and EC0267557-B were found moderately resistant against Fusarium wilt (Table 4.2).

Table 4: Grouping of lentil genotypes in different classes against Fusarium wilt under sick plot conditions

Grading	Wilt incidence (%)	Total No. of Genotypes	Name of Genotypes
Highly resistant	Less than 1%	—	—
Resistant	1- 10%	10	IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864, EC03001191.
Moderately resistant	11-20%	35	EC0223205-B, L 21-22-385, IC381127, IC98401, IC0078552, IC0014214, EC955432, IC260062, IC241144, EC0223217, EC255504, EC0267577, IC53237, IC199779, IC33920, EC0267569-B, IC0053235-A, IC361467, EC0267574, IC59038, IC241533, IC565035, IC0078399, IC0078542, IC385824, DPL-62, IC267656, EC0267619, IC342718, EC0267539, IC145305, IC384445, IC241531, IC54563, EC0267557-B.

Susceptible	21-50%	187	IC520809, EC0267619-A, IC0078516, IC381285, IC260010, IC384473, EC0267574-B, IC0078506, EC0078517, EC0255497, EC440870, IC148333, IC95658, IC241501, EC0223242, EC0267598, EC0267631, EC0267693-B, IC0020458, EC0267555, IC361417, EC0213199-A, EC0223236, EC0267634, EC0267579-B, IC0014202, IC0078518, EC397812, EC0213197-B, IC421976, EC267362, IC201710, IC201562, EC0223237-A, EC0225484, EC0225995, IC0078528, EC0223197-C, IC0014159, EC0267659, IC0027659, IC0014225, IC199461, IC201557, EC223212A, IC0014250, IC0078388, IC201772, IC201661, IC73121, IC78549, IC201776, IC280887, EC0078530, EC0223229-B, EC0267571, EC0267595-C, EC0267652, EC0199459, EC33920, EC0267614-B, IC201533, EC0241429, EC225503, IC0057645, IC0078530, EC0267526-D, EC027569- AEC0267617, IC316127, IC240885, IC274090, IC329110, EC0267562, EC0267588, EC0267618, EC0267690, EC0255491, EC0267626, EC955431, EC0267596, EC78387, IC521438, EC0078541- A, EC0255502-A, IC0078559, IC283540, EC0267579- A, IC0016458, IC0078417-A, IC0078423, EC0267579-C, EC0303711, IC258265, IC201518, IC614705, IC208326, EC2675958, JL-3, EC0078519, EC0078542-A, EC0267528, IC0078414, IC241507, EC0267651-C, IC201788, IC201537, IC201548, IC398793, IC241488, EC267595-D, IC0014172, EC241480, EC0225486, IC240886, EC0267614, EC0267647, L-4717, EC0267575, IC616579, EC223237B, IC346268, EC0225460, EC0267544- AEC0267577-D, EC0267613-B, IC241532, EC267554, NC60969, LL-931, IC0078526-A, IC0078532, EC0267572, IC267088, EC0267688,
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			IC584611, IC201781, EC0267619-B, IC0078538, EC0299697, IC0042393, IC0078563, EC0267575, EC0267570, IC0007809, IC201656, IC98351, EC0078526-B, EC0267541, IC0078417, IC0078544, EC0267651-B, EC0267694, EC0267710, EC0225488-B, IC0078550, IC201564, IC0078567, IC98392, IC0078397, IC201568, IC267078, EC0299687, IC0078416, EC0223209-B, EC0267576, IC0014224, EC223235, IC417864, IC277173, IC241222, IC329109, IC317520, HUL-57, EC0078446, EC0078463, EC0223209-C, EC0267577, EC0267577-C, EC0267595-E, IC0001843, IC0024087, IC0078521, IC0078534, IC0078535, IC0078547, IC0095653.
Highly susceptible	More than 50%	268	IC201798, IC541007, IC553069, IC0078387, EC0225501, IC0078509, IC16453, EC22369, IC241475, EC0223226, IC95654, EC0267536, EC0267540, EC0267585, EC0267594, IC0078511, IC0078548, EC0267543, IC392561, IC241447, L-4076, EC0042392, EC0267538, EC0267601, IC0014190, IC0078420, IC0078520, EC0078477-C, EC0223244, IC0078421, EC0267613-A, IC78406, EC223243, IC14158B, EC0078545, IC0078453, IC201748, EC0078462, EC329161, EC373712, EC0027158, EC0078526-F, EC78531, EC0078552-B, EC0267613, EC0267614-A, EC0267676, IC0048546, IC145259, EC299650, IC22666, EC0078526-E, EC0267584, IC0060375, IC0060377, IC0078422, IC0078525, IC0078556, IC447768, IPL-520, EC0267709, IC0073525, IC0078513, IC0078526, EC0267565, EC0267693-A, IC0078526-B, EC0223237-C, IC0078430, IC0078507, IC0078512, EC0267609, IC0053326-A, EC0267544-

		B, EC0267597, EC0267691, IC0062857, IC0078565, IC139850, IC241473, EC0267708, IC0078419, IC0060376, IC0078418, IC0078566, IC0078391, IC0014158, IC345433, IC98364, IC22651, EC267578, L-4594, EC0223233, EC0267573-A, EC0267591, EC0267593, IC0073125, IC0078506-A, IC0078533, IC0078568, IC0095652, IC201684, EC0299638, IC78529, EC0078553-A, IC201693, IC0078537, EC0223210, EC0223211, IC0078386, IC201697, EC0223207, EC0267622, EC0267641, IC0078527, IC0078536, IC0078546, IC0095660, IC267105, EC28514, IC0078409, EC0078526-A, IC560153, EC0078442, IC0078425, IC0078396, IC078412, EC0078403, EC267607, EC0267612, IC201582, EC0267549-A, EC0225493, EC0267628- B, EC0267677, IC53238, EC0223240, EC0267510, EC0267620, IC0060966, IC0078545, IC201694, EC0299692, IC396758, IC405259, EC0078433, EC0078506-D, EC0267569, EC0267648, EC0267678, EC0267706, EC0299632, IC0078460, IC0078555, EC0078518, EC267615, IC0034704, IC0095655, EC0016371, EC0255492, EC0267625-B, IC201553, IC0078541, EC16391, IC78398, EC299732, EC0241442, EC0267600, IC201561, EC0225456, EC0078540, IC0078424, IC0078476, IC0078510, EC0267606, EC0299647, IC321501, EC0223191, EC0267545-B, EC0267586, EC0267696, IC0024089, IC0078524, EC0267527, IC201555, EC0267545-A, EC0267567, EC0299644 IC384447, EC0267583, EC0299649, IC0078381, IC0078395, IC0014195, IC0057170, EC0241434, EC0299655, IC345478, IC316132, IC0028514, EC0078496, IC0078557, IC241543, EC0299668, IC0024088-A, IC078408, IC78455, EC0267692,
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			EC0299587, IC0034679, EC0267572-A, EC0299673, EC0300190, IC241454, IC267668, EC0078512, EC0267640, IC0078415, EC299645, EC0223188, EC0267604, EC0223199-B, EC0299676, EC0078547, EC0267611, IC0014194, IC0078426, IC0093392, EC0299643, IC0062514, IC0034650, EC0078503-A, EC0267576-B, IC0073122, IC0078405, L-4147, IPL31, IC78477, EC0267702, EC0223224, EC0223224, IC22658, EC0269676, IC560225, EC0003379, EC0011371, EC0078454, EC0078486, EC0078524-A, EC0225487, EC0267526-B, EC0267535, EC0267587, EC0267592, EC0267595-B, EC0267608, EC0267672, EC0267686, EC0294646, EC0299612, EC0299646, IC0014167, IC0014171, IC0053228, IC0053233, IC0059902, IC0073120, IC0078523, IC0095649.
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4.3 Confirmation of wilt resistance under pot culture conditions

Ten lentil genotypes showing wilt resistance under field condition viz. IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864 and EC03001191 were selected for confirmation of wilt resistance under *in-vitro* condition in polyhouse where they were planted in the earthen pots filled with pathogen infested soil. These genotypes did not show any symptoms of wilt and remained healthy at 15 days and 30 days of sowing which confirmed that these genotypes were resistant. Plate

(4.3)



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Plate 4.3: Confirmation of wilt resistance under pot conditions

4.4 Effect of soil temperature at different depths of soil on incidence of wilt in selected susceptible lentil genotypes.

4.4.1. Soil temperature and incidence of wilt in L 9-12 lentil cultivar

Wilt incidence in L 9-12 lentil genotype was studied in relation to soil temperature at different depths. The wilt incidence and temperatures were recorded every week and correlated. Correlation coefficient (r) and t statistic was computed at each depth level i.e. 5 cm, 10 cm and 20 cm for is given in (Table 5.) To quantify the effect of soil temperature depth levels the linear regression equation was also worked out (Table 6).

Table5: Correlation coefficient (r) values between incidence of wilt in L 9-12 and soil temperature at different depth levels

Week Interval	Standard week	Wilt incidence (%) L 9-12	Soil temperature (°C)		
			5 cm	10 cm	20 cm
17 Dec -23 Dec	51	4.392	12.714	15.571	18.886
24 Dec -31 Dec	52	5.166	13.913	17.088	19.388
1 Jan.-7 Jan.	1	7.987	15.243	16.914	19.357
8 Jan.-14 Jan.	2	11.327	13.714	15.771	18.486
15 Jan.-21 Jan.	3	13.892	12.586	15.114	17.543
22 Jan.-28 Jan.	4	23.734	12.200	15.243	17.929
29 Jan.-4 Feb.	5	28.491	13.129	15.600	18.600
5 Feb -11 Feb.	6	35.373	14.357	16.886	19.414
12 Feb -18 Feb	7	47.496	15.029	17.729	20.086
19 Feb -25 Feb	8	49.169	14.829	18.686	21.529
26 Feb-4 March	9	50.025	16.457	20.029	22.786
Coefficient of correlation (r)			0.568	0.700*	0.702*
Test of significance (t)			2.070	2.941	2.957

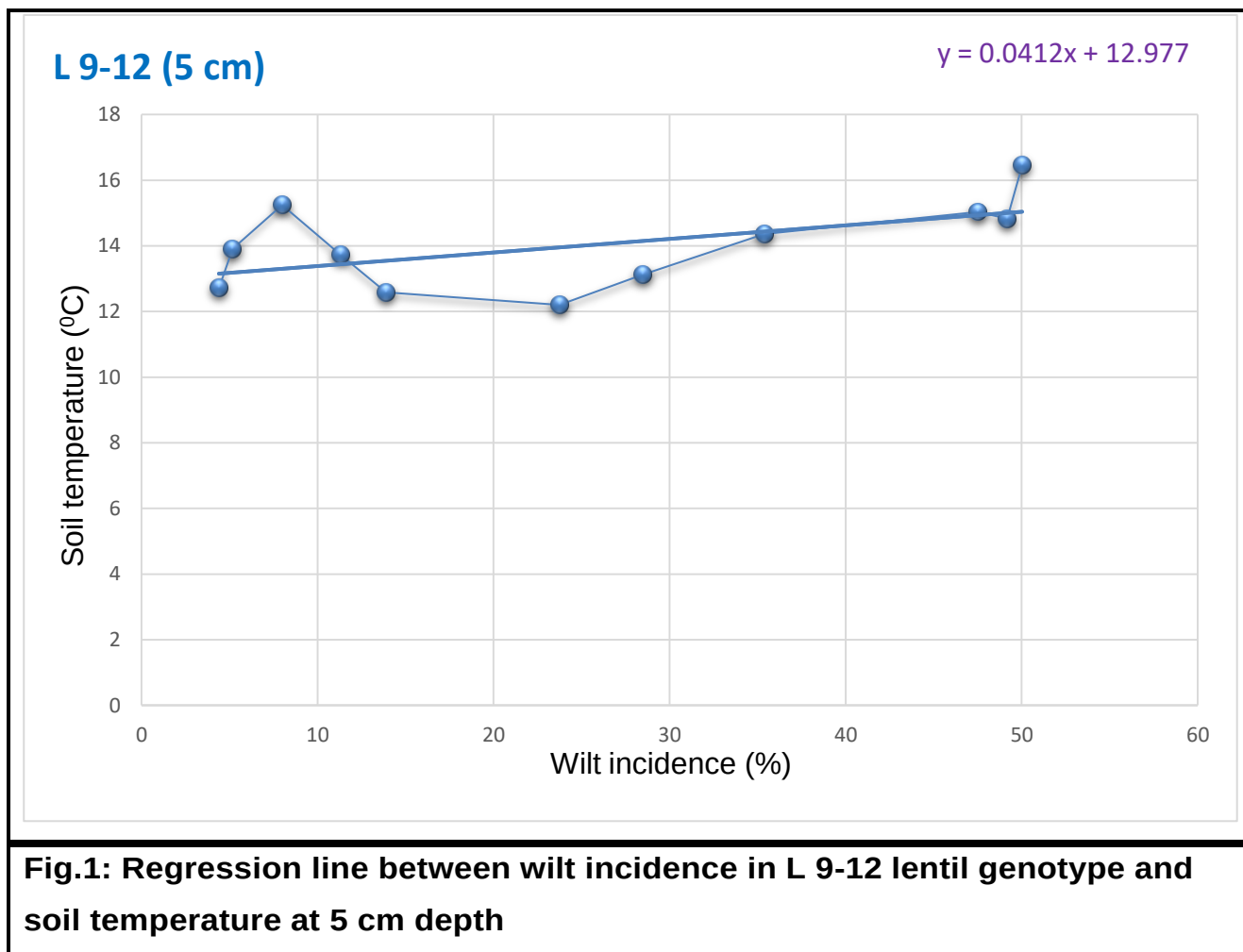
* significant at 5% level of significance, respectively

Table 6: Regression equation of soil temperature at different depth levels with incidence of wilt in L 9-12 lentil genotype

Soil temperature depth levels	Regression equation
5 cm	$y = 0.0412x + 12.977$
10 cm	$y = 0.0603x + 15.265$
20 cm	$y = 0.06x + 17.943$

Wilt susceptible L 9-12 lentil genotype did not show significant correlation at 5 cm soil depth the observed correlation coefficient (r) was 0.568 The regression equation of L 9-12 lentil genotype showed that wilt incidence of lentil increased by 0.0412% with the increase of 1°C of the soil temperature at 5 cm depth. (Fig.1).

Soil temperatures at 10 cm and 20 cm depths of soil were found to have significant positive correlation (at 5% level of significance) with lentil wilt, the observed correlation coefficient (r) 0.700 and 0.702 with wilt incidence in L 9-12 lentil genotype, respectively (Table 5).The regression equation showed that wilt incidence of lentil increased by 0.603% and 0.06% with the increase of 1°C of the soil temperature at 10 cm and 20 cm depth. (Fig. 2 and 3).



L 9-12 (10 cm)

$$y = 0.0603x + 15.265$$

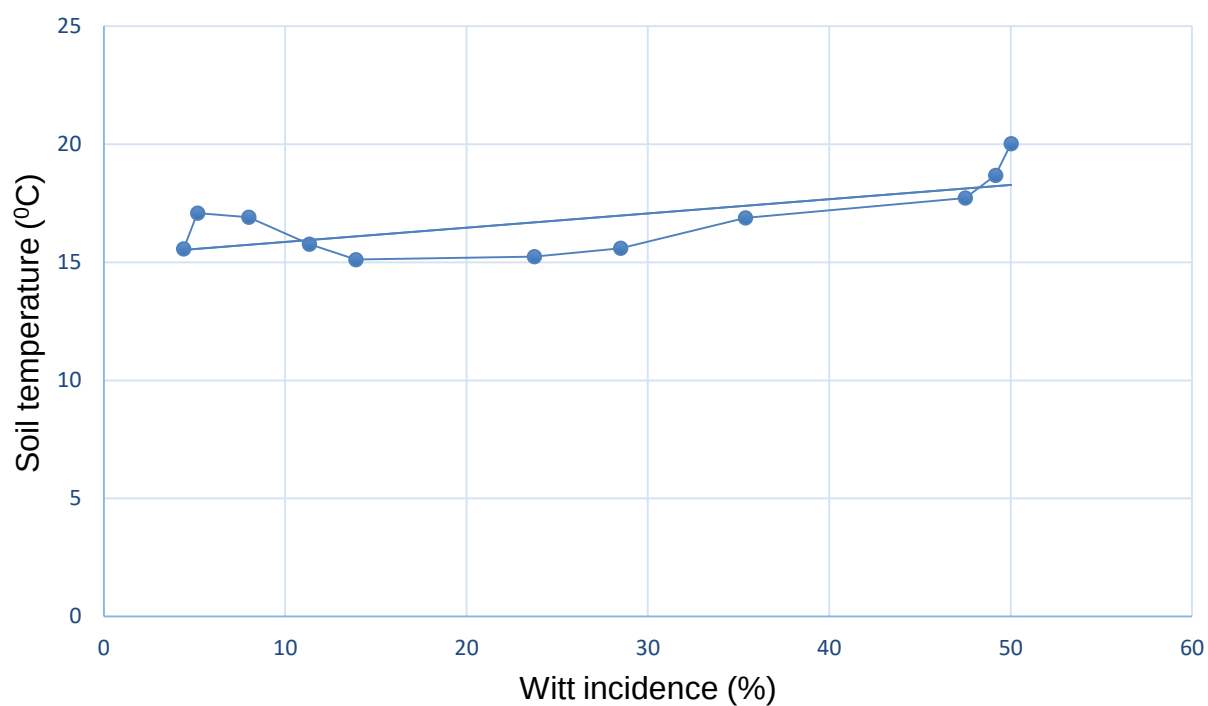


Fig. 2: Regression line between wilt incidence in L 9-12 lentil genotype and soil temperature at 10 cm depth

L 9-12 (20 cm)

$$y = 0.06x + 17.943$$

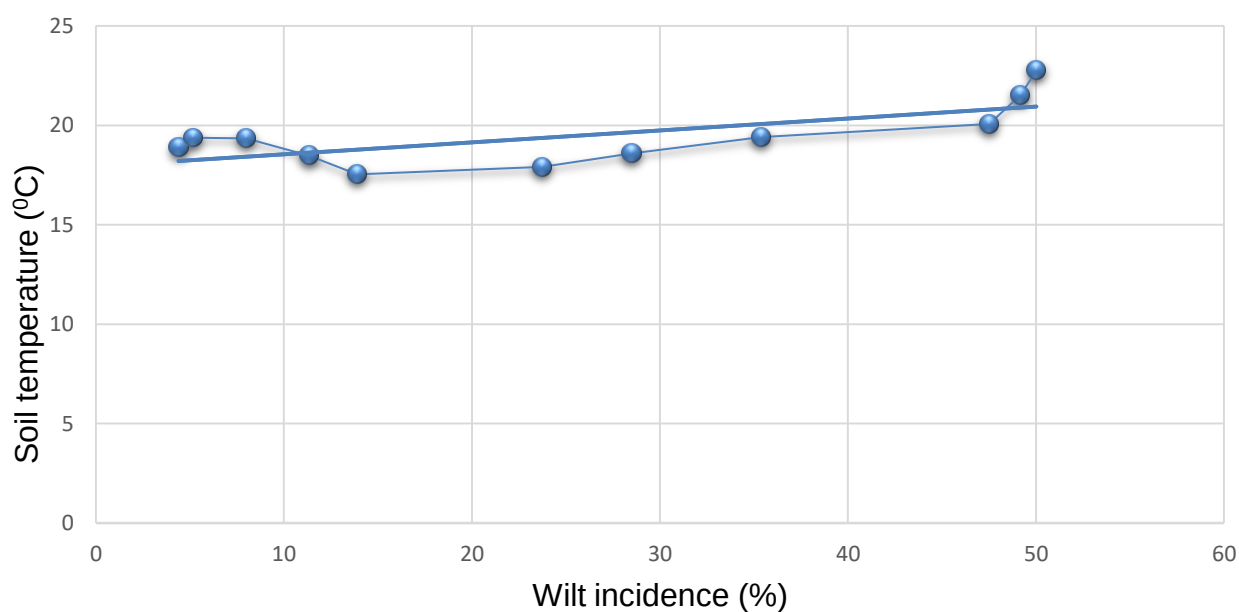


Fig. 3: Regression line between wilt incidence in L 9-12 lentil genotype and soil temperature at 20 cm depth

4.4.2. Soil temperature and incidence of wilt in Sehore 74-3 lentil cultivar

Wilt incidence in Sehore 74-3 lentil genotype was studied in relation to soil temperature at different depths. The wilt incidence and temperatures were recorded every week and correlated. Correlation coefficient (r) and t statistic was computed at each depth level i.e. 5 cm, 10 cm and 20 cm is given in (Table 7). To quantify the effect of soil temperature depth levels the linear regression equation was also worked out (Table 8).

Table 7: Correlation coefficient (r) values between incidence of wilt in Sehore 74-3 lentil genotype and soil temperature at different depth levels

Week Interval	Standard week	Wilt incidence (%) Sehore 74-3	Soil temperature (°C)		
			5 cm	10 cm	20 cm
17 Dec -23 Dec	51	3.72	12.714	15.571	18.886
24 Dec -31 Dec	52	4.91	13.913	17.088	19.388
1 Jan.-7 Jan.	1	6.52	15.243	16.914	19.357
8 Jan.-14 Jan.	2	9.93	13.714	15.771	18.486
15 Jan.-21 Jan.	3	13.72	12.586	15.114	17.543
22 Jan.-28 Jan.	4	19.16	12.200	15.243	17.929
29 Jan.-4 Feb.	5	23.63	13.129	15.600	18.600
5 Feb -11 Feb.	6	30.32	14.357	16.886	19.414
12 Feb -18 Feb	7	36.31	15.029	17.729	20.086
19 Feb -25 Feb	8	38.99	14.829	18.686	21.529
26 Feb-4 March	9	41.08	16.457	20.029	22.786
Coefficient of correlation (r)			0.558	0.691*	0.692*
Test of significance (t)			2.017	2.868	2.876

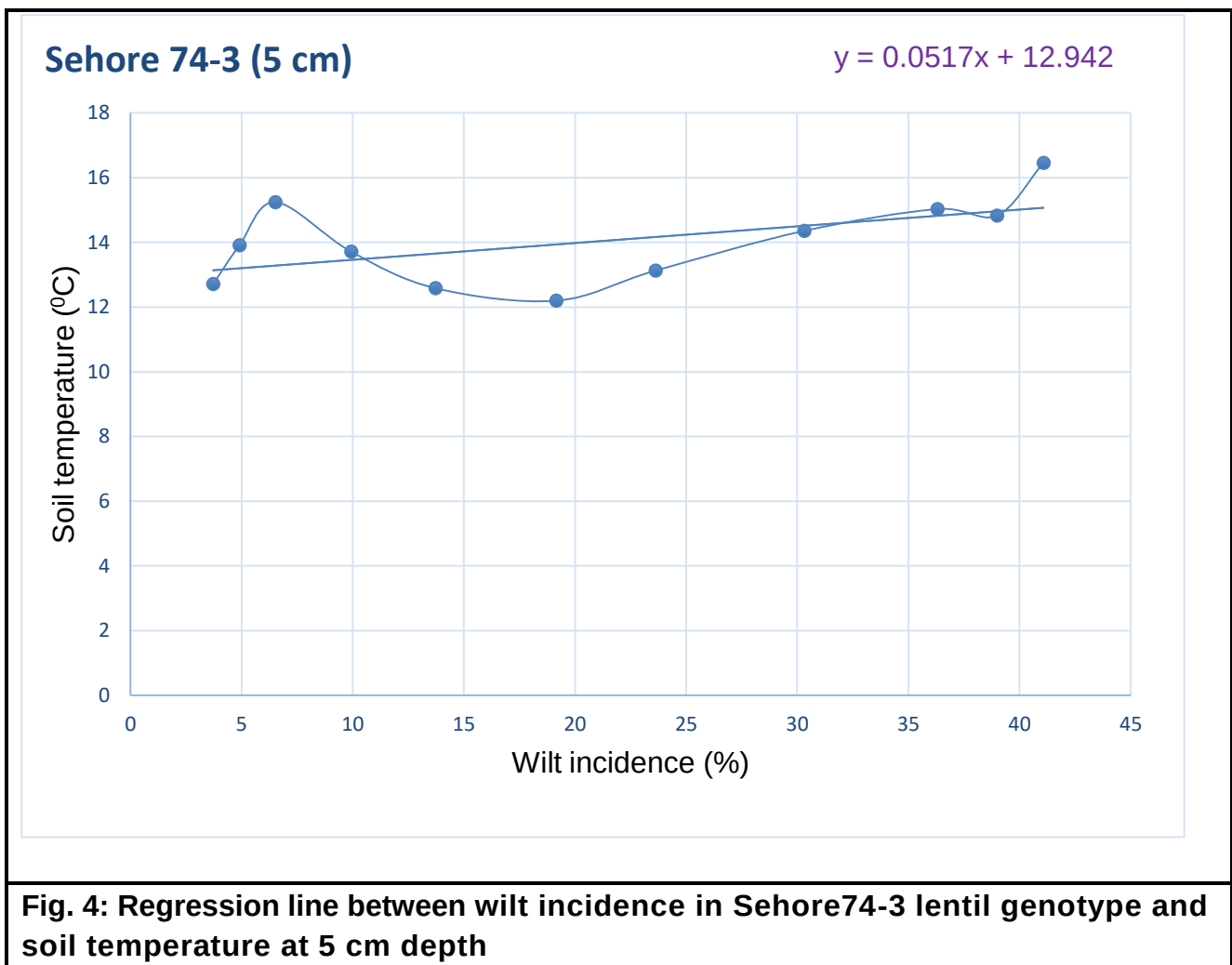
*denotes significant at 5% level of significance, respectively

Table 8: Regression equation of soil temperature different depth levels with incidence of wilt in Sehore 74-3 lentil genotype

Soil temperature depth levels	Regression equation
5 cm	$y = 0.0517x + 12.942$
10 cm	$y = 0.0661x + 15.471$
20 cm	$y = 0.0755x + 17.888$

The Sehore74-3 lentil genotype did not show significant correlation at 5 cm depth of soil, the observed correlation coefficient (r) was 0.558 The regression equation of Sehore 74-3 genotype showed that wilt incidence of lentil increased by 0.0517% with the increase of 1°C of the soil temperature at 5 cm depth. (Fig. 4).

Temperatures at 10 cm and 20 cm depth of soil had significant positive correlation (at 5% level of significance) with lentil wilt, the observed correlation coefficient (r) 0.691 and 0.692. The regression equation of Sehore 74-3 lentil genotype showed that wilt incidence of lentil increased by 0.0661% and 0.0755% with the increase of 1°C of the soil temperature at 10 cm and 20 cm depth. (Fig. 5 and 6).



Sehore 74-3 (10 cm)

$$y = 0.076x + 15.207$$

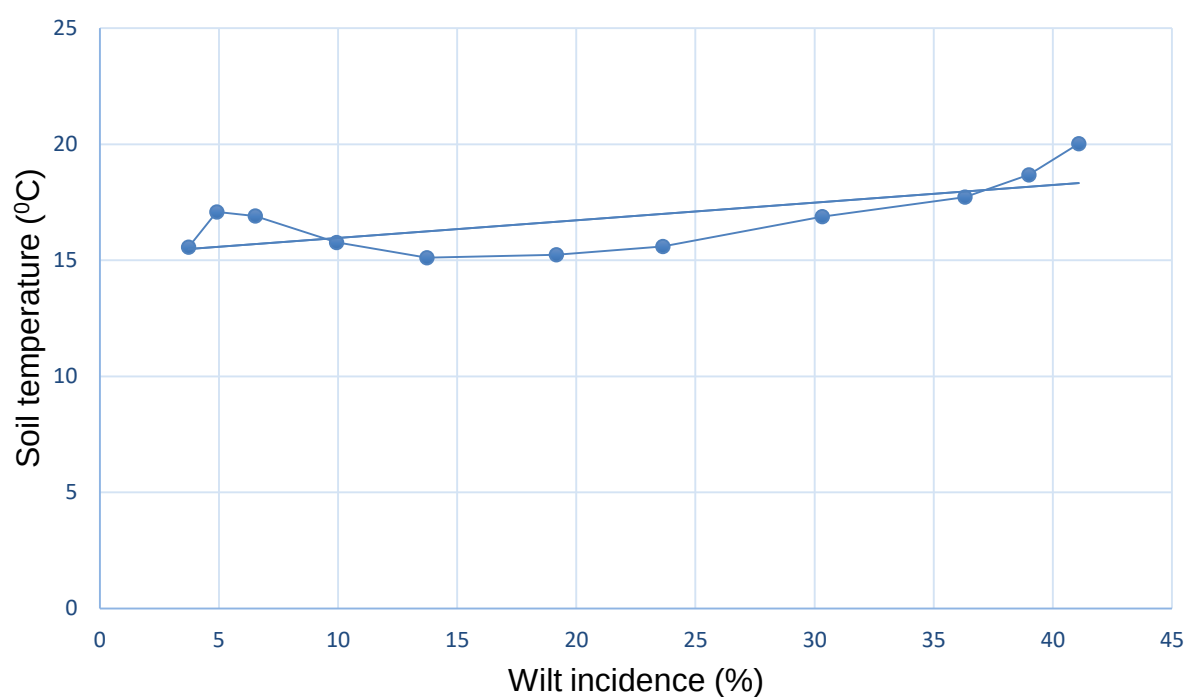


Fig. 5: Regression line between wilt incidence in Sehore74-3 lentil genotype and soil temperature at 10 cm depth

Sehore 74-3 (20 cm)

$$y = 0.0755x + 17.888$$

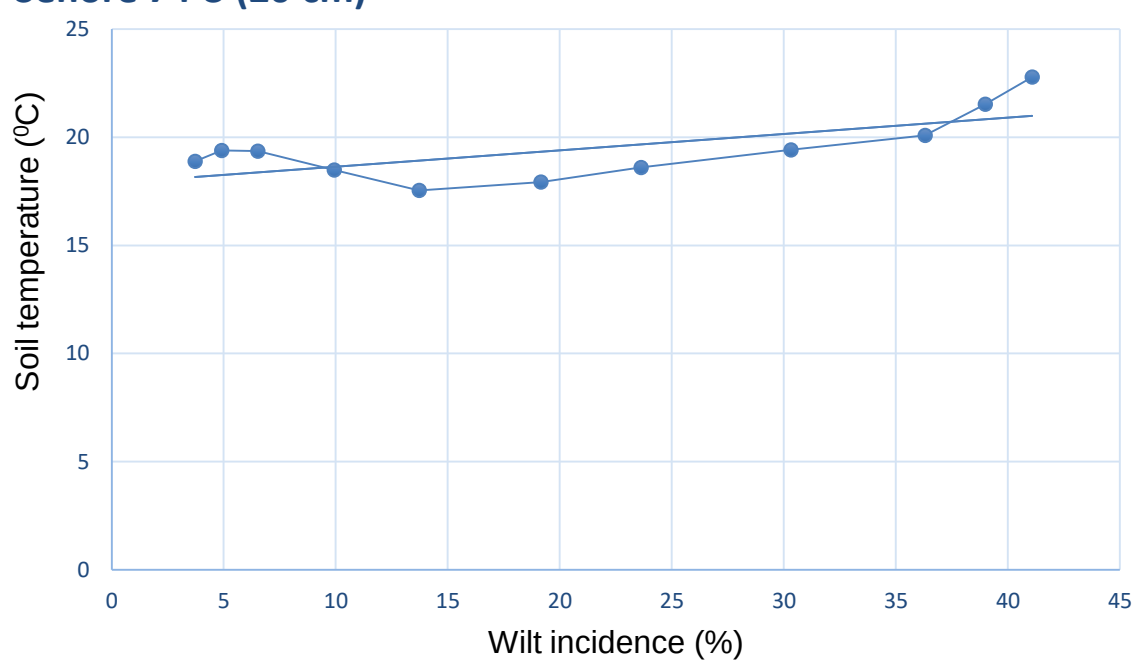


Fig. 6: Regression line between wilt incidence in Sehore74-3 lentil genotype and soil temperature at 20 cm depth

4.4.2. Soil temperature and incidence of wilt in DPL-62 lentil cultivar

Wilt incidence on DPL-62 lentil genotype was studied in relation to soil temperature at different depths. The wilt incidence and temperatures were recorded every week and correlated. Correlation coefficient (r) and t statistic was computed at each depth level i.e. 5 cm, 10 cm and 20 cm (Table 9). To quantify the effect of soil temperature at different depth levels the linear regression equation was also worked out (Table 10).

Table 9: Correlation coefficient (r) values between incidence of wilt in DPL-62 lentil genotype and soil temperature at different depth levels

Week Interval	Standard week	Wilt incidence (%) DPL-62	Soil temperature (°C)		
			5 cm	10 cm	20 cm
17 Dec -23 Dec	51	3.60	12.714	15.571	18.886
24 Dec -31 Dec	52	9.49	13.913	17.088	19.388
1 Jan.-7 Jan.	1	11.88	15.243	16.914	19.357
8 Jan.-14 Jan.	2	13.27	13.714	15.771	18.486
15 Jan.-21 Jan.	3	15.73	12.586	15.114	17.543
22 Jan.-28 Jan.	4	18.14	12.200	15.243	17.929
29 Jan.-4 Feb.	5	21.57	13.129	15.600	18.600
5 Feb -11 Feb.	6	24.22	14.357	16.886	19.414
12 Feb -18 Feb	7	29.72	15.029	17.729	20.086
19 Feb -25 Feb	8	32.01	14.829	18.686	21.529
26 Feb-4 March	9	44.01	16.457	20.029	22.786
Coefficient of correlation (r)			0.672 [*]	0.781 ^{**}	0.770 ^{**}
Test of significance (t)			2.722	3.752	3.620

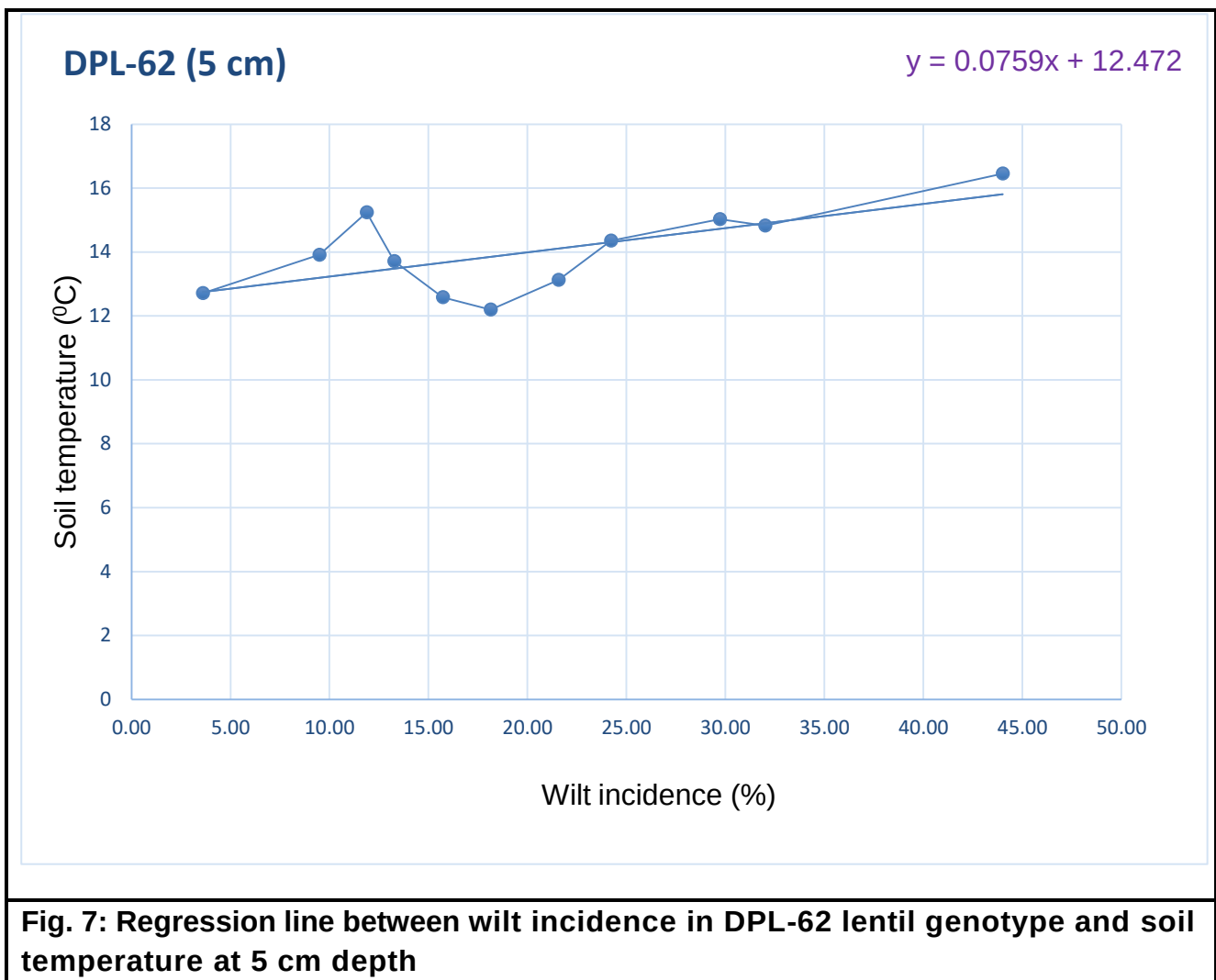
^{**} and ^{*} significant at 1% level and 5% level of significance, respectively

Table 10: Regression equation of soil temperature different depth levels with incidence of wilt in DPL-62 lentil genotype

Soil temperature depth levels	Regression equation
5cm	$y = 0.0759x + 12.472$
10cm	$y = 0.1047x + 14.657$
20cm	$y = 0.1024x + 17.373$

The DPL-62 lentil genotype was found to have a significant positive correlation (at 5% level of significance) at 5 cm depth of soil, the observed correlation coefficient (r) was 0.672). The regression equation of DPL-62 genotype showed that wilt incidence of lentil increased by 0.0759% with the increase of 1°C of the soil temperature at 5 cm depth. (Fig. 7).

Temperatures at 10 cm and 20 cm depth of soil was found to have significant positive correlation (at 1% level of significance) with lentil wilt, the observed correlation coefficient (r) 0.781 and 0.770. The regression equation of DPL-62 genotype showed that wilt incidence of lentil increased by 0.01047% and 0.1024% with the increase of 1°C of soil temperature at 10 cm and 20 cm depth. (Fig. 8 and 9).



DPL-62 (10 cm)

$$y = 0.1047x + 14.657$$

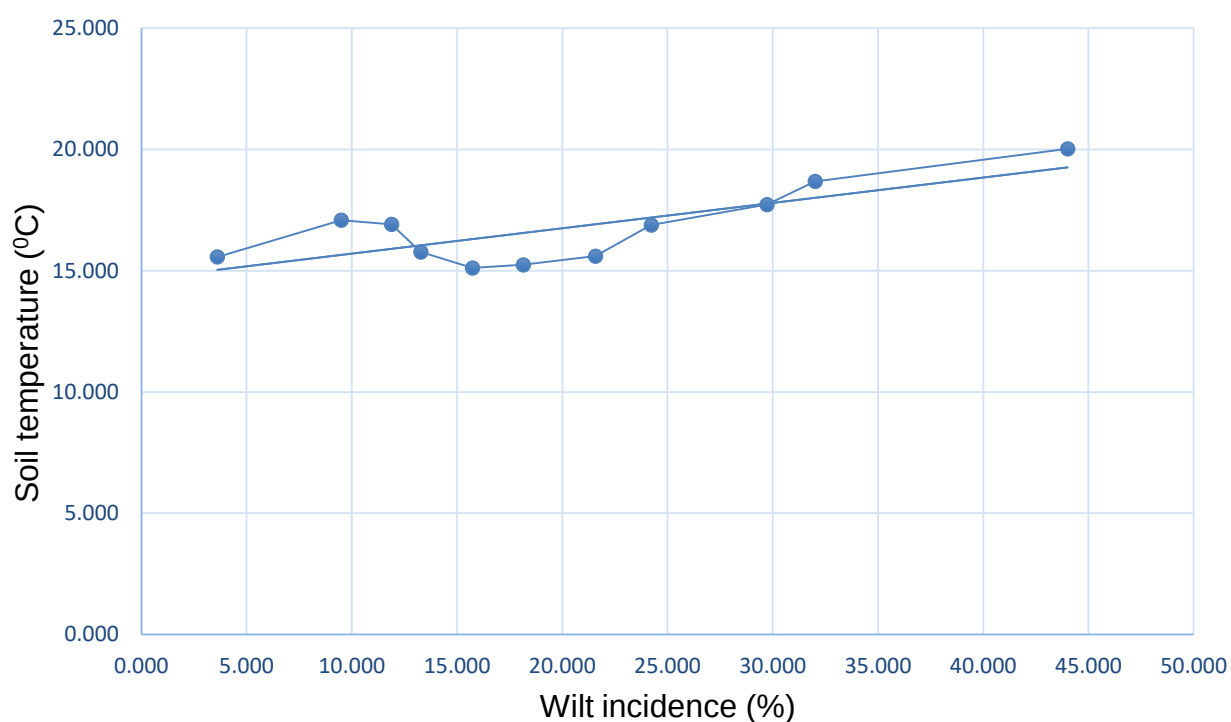


Fig. 8: Regression line between wilt incidence in DPL-62 lentil genotype and soil temperature at 10 cm depth

DPL-62 (20 cm)

$$y = 0.1024x + 17.373$$

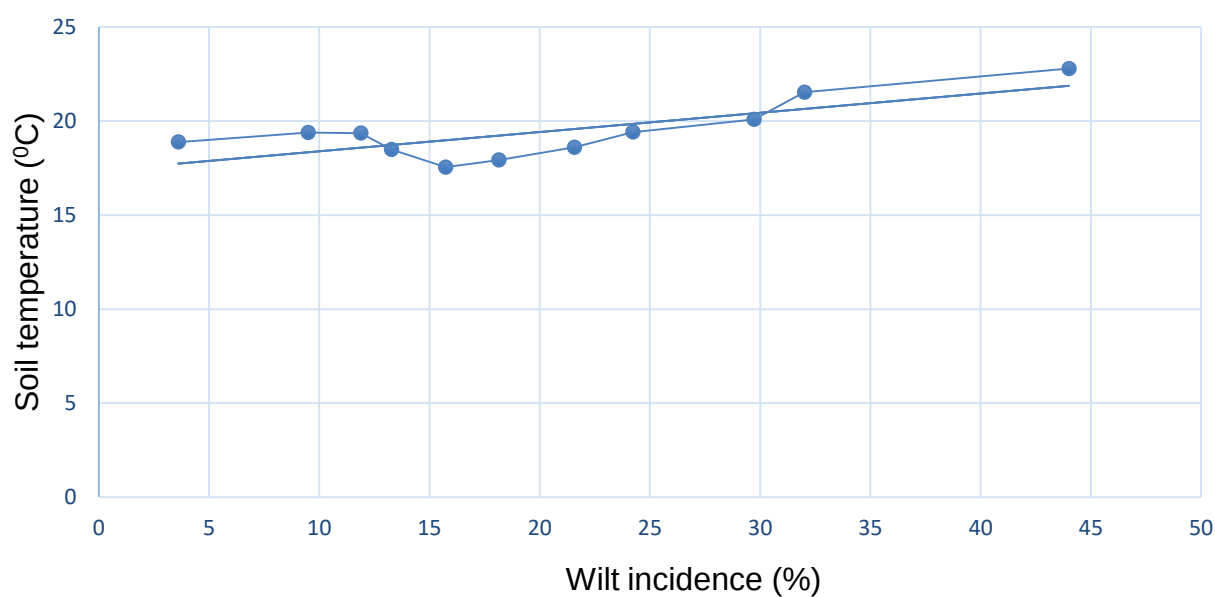


Fig. 9: Regression line between wilt incidence in DPL-62 lentil genotype and soil temperature at 20 cm depth

CHAPTER - V

DISCUSSION

Field symptoms of *Fusarium* wilt first appeared on younger leaves, followed by downward dropping of older leaves. The seedlings were droop down followed by sudden death. The infected root, collar region and main stem showing the typical symptoms and browning of the vascular tissue is strong evidence of the disease. Further on older plant symptoms appear during flowering & pod maturation. These findings are in confirmation of the reports of earlier workers (*Tosi et al.*, 2001; *Stoilova and Chavdarov*, 2006).

The fungus was isolated in the laboratory under aseptic conditions from infected roots of lentil plants and cultured on PDA medium in Petri plates by transferring them after surface sterilization. After mycelial growth, the fungus was purified through hyphal tip culture method. Similar characteristics were also reported by (*Vasudeva and Srinivasan*, 1952; *Chaudhary and Singh*, 2008; *Saxena et al.* 2018).

White fluffy colony of *F. oxysporum* f. sp. *lentis* was seen on PDA and the microscopic studies revealed that the mycelium was septate and hyaline. Micro conidia were slightly curved and comma shaped and had 1-2 septa. Macro conidia were strongly curved or hooked at apex, smooth hyaline and had 3-5 septa. The chlamydospores were rounded, thick walled spore, produced inter terminally or intercalary on older mycelium or on macroconidia. These cultural and morphological characters of *F. oxysporum* f. sp. *lentis* were similar as described by *Khare*, (1981), *Agrios*, (1988) and *Dubey and Singh*, 2018).

The pathogenicity tests of *F.oxysporum* f. sp. *lentis* were carried out on wilt susceptible cultivar L 9-12 grown in pots under glasshouse condition by inoculating *F. oxysporum* f. sp. *lentis* multiplied on sand maize medium. Wilting of seedling was started after 15 and after 30 days more than 90 per cent plants were wilted in the infested soil. The plants were normal and no wilted plants were observed in control pots. *F. oxysporum* f. sp. *lentis* was re-isolated from the wilted plants thus, the Koch's postulates were proved. *Kumari et al.* (2021) also described the pathogenicity test in pot house condition by seed and soil inoculation technique.

Lentil genotypes were evaluated for wilt resistance and susceptibility under wilt sick field condition. Total 500 genotypes were classified in four groups based on wilt reaction. Among these 10 were found resistant having low wilt incidence up to 10%, 35 were moderately resistant having wilt incidence 11-20%, 187 were susceptible having wilt

incidence 21-50% and the remaining ones were highly susceptible more than 50% to wilt. Ten lentil genotypes viz. IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864 and EC03001191 were found resistant against Fusarium wilt. Thirty five genotypes; EC0223205-B, IC004215, IC381127, IC98401, IC0078552, IC0014214, EC955432, IC260062, IC241144, EC0223217, EC255504, EC0267577, IC53237, IC199779, IC33920, EC0267569-B, IC0053235-A, IC361467, EC0267574, IC59038, IC241533, IC565035, IC0078399, IC0078542, IC385824, DPL-62, IC267656, EC0267619, IC342718, EC0267539, IC145305, IC384445, IC241531, IC54563 and EC0267557-B were found moderately resistant to Fusarium wilt. (Singh *et al.*, 2009) also found 10 resistant lentil genotypes among the 100 genotypes. Yadav *et al.* (2017) screened 185 lentil genotypes for wilt and reported resistance in 15 genotypes. Chandra *et al.* (2019) tested 150 germplasm *In-vivo* and reported 66 genotypes resistant and 37 moderately resistant.

Ten lentil genotypes showing wilt resistance under field condition viz. IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864 and EC03001191 were selected for confirmation of wilt resistance under *in-vitro* condition also. These were planted in the earthen pots filled with pathogen infested soil. These genotypes did not show any symptoms of wilt and remained healthy at 15 days and 30 days of sowing, thus confirming their resistance. Rafique *et al.* (2016) also found five cultivars viz. Markaz-09, Masoor-86, Masoor-2006, Punjab Masoor-00518 and Punjab Masoor-09 resistant to wilt under *in-vitro* condition.

Wilt incidence was studied in three genotype (L9-12, Sehore 74-3, DPL-62) in relation to soil temperature at three different soil depths viz. 5 cm, 10 cm and 20 cm, and it was found that there was a significant positive correlation between soil temperature and wilt incidence. Soil temperature at 5 cm soil depth did not show positive correlation with wilt incidence in L 9-12 lentil genotype. While soil temperature at 10 cm and 20 cm depths of soil had significant positive correlation with wilt incidence. Similarly, soil temperature at 5 cm soil depth did not show positive correlation with wilt incidence in Sehore 74-3 lentil genotype. while soil temperature at 10 cm and 20 cm depths of soil had significant positive correlation with wilt incidence. Soil temperature at all the three soil depths showed positive correlation with wilt incidence in DPL-62 lentil genotype. Fatima *et al.*, (2015) reported that environmental factors were positively correlated with disease development. Singh *et al.*, (2019) found that aerial temperature (0.7226^{**}) and inoculum load (0.6438^{**}) showed significant positive correlation wilt incidence.

CHAPTER - VI

SUMMARY, CONCLUSION AND SUGGESTION FOR FUTURE WORK

The present investigations on wilt of lentil caused by *Fusarium oxysporum* f. sp. *lentis* were carried out during *Rabi* 2021-2022, the results obtained are summarized below:

- **Summary**

- The symptoms first appeared on younger leaves, followed by downward dropping of older leaves. The seedlings drooped down followed by sudden death. The infected root, collar region and main stem showing the typical symptoms and browning of the vascular tissue is strong evidence of the disease. Further on older plants symptoms appeared during flowering & pod maturation.
- The fungus was isolated from infected roots of lentil plants and cultured on PDA medium in Petri plates by transferring them after surface sterilization. After mycelial growth, the fungus was purified through hyphal tip culture method.
- The mycelium was septate and hyaline. Micro conidia were slightly curved and comma shaped, and had 1-2 septa. Macro conidia were strongly curved or hooked at apex, smooth hyaline and had 3-5 septa. The chlamydospores were rounded, thick walled spores.
- Pathogenicity of the isolated fungus, *F. oxysporum* f. sp. *lentis* was proven on wilt susceptible lentil variety L 9-12. Wilt symptoms appeared 15 days after-inoculation in the soil inoculated plants, and thus Koch's postulates were validated.
- Out of 500 genotypes evaluated, 10 were found resistant, 35 were moderately resistant, 187 were susceptible, and 268 genotypes were found to be highly susceptible.
- Ten lentil genotypes showing wilt resistance under field condition viz. IC78552, EC0267580, EC0267563, IC396044, EC27659, EC440747, IC11871, EC223214, IC424864 and EC03001191 were selected for confirmation of wilt resistance under *in-vitro* condition. They were planted in the earthen pots filled with pathogen infested. These genotypes did not show any symptoms of wilt.
- Wilt incidence in L 9-12 lentil genotype was studied in relation to soil temperature at different depths. Soil temperature at 5 cm soil depth did not show correlation ($r=0.568$) with wilt incidence while soil temperature at 10 cm and

20cm, had significant positive correlation with wilt incidence ($r=0.700$ and 0.702 , respectively)

- Wilt incidence in Sehore 74-3 lentil genotype was studied in relation to soil temperature at different depths. Soil temperature at 5 cm soil depth did not show correlation ($r=0.558$) with wilt incidence while soil temperature at 10cm and 20cm depths had significant positive correlation with wilt incidence ($r=0.691$ and 0.692 , respectively)
- Wilt incidence in DPL-62 lentil genotype was studied in relation to soil temperature at different depths. Soil temperature at all the three depths viz., 5 cm, 10 cm and 20 cm showed positive correlation with wilt incidence ($r=0.558$, 0.781 and 0.770 , respectively).

CONCLUSION

- Soil inoculation method was found effective in testing pathogenicity.
- Five hundred genotypes of lentil could be grouped in to four classes viz. resistant, moderately resistant, susceptible and highly susceptible.
- Ten lentil genotypes showing wilt resistance were selected for confirmation of wilt resistance under *in-vitro* condition. These were also planted in the earthen pots filled with pathogen infested soil. Their resistance was confirmed.
- L 9-12 lentil genotype was studied in relation to soil temperature at different depths. Soil temperature at 5 cm depth did not show significant correlation with wilt incidence while soil temperature at 10cm and 20 cm depths of soil had significant positive correlation with wilt incidence.
- Sehore 74-3 lentil genotype was studied in relation to soil temperature at different depths. Soil temperature at 5 cm depth did not show significant correlation with wilt incidence while soil temperature at 10 cm and 20 cm depths of soil had significant positive correlation with wilt incidence.
- DPL-62 lentil genotype was studied in relation to soil temperature at different depths. Temperature at all the three soil depths viz. 5 cm 10 cm and 20 cm had significant positive correlation with wilt incidence.

Suggestion

- More genotypes should be screened to find additional source of resistance.
- Genotypes found resistant should be re-evaluated in pathogen favourable environmental control conditions or at hot spot locations for confirming the stability of resistance.
- Effect of soil temperature at different depths (5 cm, 10 cm and 20 cm) on incidence of lentil wilt needs to be studied for five to six years at more locations in order to establish precise disease of prediction modal.

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