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IDENTIFICATION OF RESISTANT SOURCE IN LENTIL GERMPLASM AGAINST FUSARIUM WILT IN RELATION TO ENVIRONMENTAL FACTORS

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Lentil is a valuable human food and one of the oldest known protein rich food legumes which is also known as poor men's meat. Owing to biotic and abiotic stresses, the crop yield is below attainable levels which are mainly attributed to pathological factors especially lentil wilt caused by *Fusarium oxysporum* f.sp*. lentis*. It is a potential threat to lentil production not only in Pakistan but worldwide as well that can cause complete crop failure. Current studies were designed to screen the available lentil germplasm against the lentil wilt disease in relation to environmental factors. Twenty eight lentil lines were sown in a previously developed wilt sick bed in the research area of Pulses Research institute, Faisalabad using augmented design. Natural inoculum was also relied upon. Disease severity data were recorded and area under disease progress curve was calculated. 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. Furthermore, disease severity data along with weekly environmental conditions were subjected to correlation and stepwise regression analysis to screen environmental variables not having a significant influence on lentil wilt development. All the environmental factors were positively correlated with disease development and best explained using linear regression. Maximum disease severity was recorded at 17-18^{o}C and 7-10^{o}C air temperature (Max. and Min.), 60-78% relative humidity and 2 mm rainfall respectively. Stepwise regression employed to predict lentil wilt incidence, the model developed significantly fit to the data and their predicted values of lentil wilt incidence were in close conformity with the observed values.

Key words: Lentil, Lentil wilt, *Fusarium oxysporum* f.sp*. lentis*, Correlation, Stepwise regression and linear regression.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) commonly known as "masoor" is an important pulse crop of Pakistan. On account of its high protein content it is regarded as quality pulse and used as meat substitute in many countries. In

Pakistan it is largely sown in arid areas of Punjab where it is grown over more than 60,000 hectares producing 30,000 tons (GoP, 2013). However, its production seems adequate but current status of lentil is prone to a number of pathological threats including lentil wilt, rust, *Ascochyta* blight, collar rot and root rot in Pakistan (Ahemad and Khan, 2010). Among these diseases, lentil wilt caused by *Fusarium oxysporum* f.sp*. lentis* (*Fol*) is believed to be the most important constraint in lentil production worldwide (Taylor *et al.*, 2007) and in Pakistan (Altaf *et al.*, 2014) that is characterized by a sudden drooping of the leaves (more like wilting and damping off), followed by the leaves drying and the eventual death of the seedling. Apparently, the root system seems healthy but with a reduced proliferation and nodulation rate. Other symptoms at the seedling stage include seed rot (Kraft *et al.*, 1981).

Being the soil born disease of lentil worldwide, it may cause 5-10% yield losses but sometimes severe damage may result complete crop failure under favorable conditions for disease development (Chaudhary and Kaur, 2002), especially in a warm spring and dry and hot summer. Temperature and soil moisture are the main factors in determining fungal growth rates and symptom expression (Falahati Rastegar *et al.*, 2010). *Fol* is considered as warm-weather pathogen that is generally found in sandy and acidic soil. It can remain in the soil for up to 10 years. The optimal soil and air temperature for the pathogen is about 28^{o}C (Eagling, 2009)

The management of pathogenic *Fol* is difficult because of its wide host range and ability to grow saprophytically or survive for extended periods in the form of thick walled chlamydospores in the absence of a susceptible crop. Use of resistant varieties is the only practical measure for controlling the disease in the field (Tamietti and Valentino, 2006). The search for sources of resistance to diseases is a primary and most eminent research for most of the work carried out in the past and also is continuing presently (Shankar *et al.*, 2013).

Stoilova and Chavdarov (2006) screened thirty two lentil cultivars from the Institute of Plant Genetic Resourses, Sadovo under greenhouse conditions with different geographical origin for reaction to *Fol* during 2003-2004. Three of the studied genotypes (91-001, 91-028 and 98-001) with 45 and 50 % of total wilted plant were susceptible. Similarly, Singh *et al.* (2013) evaluated 44 lines for resistance against *Fusarium* wilt and reported 14 lines as totally free from infection.

Most of the resistance genes in the cultivated crops have been searched through screening of the breeding material/lines, germplasms, landraces etc. for getting the resistance against several dreaded pathogens of crop plants. Therefore, current study was planned to screen the available local adapted advance lines/germplasm against lentil wilt disease under the local environmental conditions.

MATERIALS AND METHODS

Collection of Germplasm and establishment of screening nursery:

Lentil germplasm comprising twenty eight test varieties/ lines was collected from grain market of Faisalabad and Ayub Agricultural Research Institute (AARI) Faisalabad. Prepared mass culture of wilt fungus was used to develop wilt sick bed in an augmented design in the research area of Pulses Research institute, Faisalabad. M-85 (a highly susceptible line) was repeatedly planted after every two test entries to increase the inoculum pressure of *Fol* under natural conditions. The lentil crop was raised by following recommended agronomic practice.

Isolation, Purification and Identification of Pathogen:

Diseased leaves and stems of lentil plants were collected form naturally infected plants showing typical symptoms of lentil wilt. These samples were brought to Pulses Laboratory, AARI and stored in refrigerator for isolation of causal fungus. Potato dextrose agar (PDA), a general purpose medium was used for isolation of fungus associated with diseased samples.

The infected samples were cut into small pieces (4-5 mm length) and surface sterilized using 0.1 % $HgCl_2$ for 1-2 minutes following twice rinsing with distilled water and drying on sterilized filter paper in petri plates. Sterilized pieces were plated on petri plates containing PDA which has been found suitable for the development of this fungus, growth of the mycelium and the development of chalaymadospores (Lee *et al.*, 2007). All Petri plates were incubated at 27^{o}C±2^{o}C for 5-7 days.

After four days the conidia formed in the Petri plates were picked off using single spore method and identified (Ellis and Waller, 1974; Choi *et al.*, 1999). The pure culture was maintained in the test tubes slants and stored in a refrigerator for using in pathogenicity test under study. Colony growth on PDA was rapid with white aerial mycelium that might become slightly tinted with orange. Mycelium was floccose; sparse or abundant; and range in colour from white to pale and violet. Microconidia (ranging from 5-12 µm ×2.-3.5 µm) were abundant, aseptate, Reni form to oval, produced in false heads on short monophialide conidiophores. Few number of orange or voilet macroconidia was found in the centre of the plate chlamydospore were profuse in culture and are formed singly or in pairs (Onyike and Nelson, 1993). Small pale brown, blue to blue-black or violet sclerotia were produced abundantly in some isolates.

Data collection

Response of lentil germplasm exhibiting various levels of

Table 1. Iccarda scale used to record lentil wilt (*Fusarium oxysporum* f. sp. *lentis*) severity during study.

Rating	Infection %	Reaction
0	0	I
1	1-10	HR
2	11-20	R
3	21-30	MS
4	31-50	S
5	**>50**	HS

I: Immune HR: Highly resistant R: Resistant MR: Moderately resistant S: Susceptible HS: Highly susceptible

resistance/ susceptibility was assessed using modified Icarda scale (ICARDA, 1994) at seven day interval on the appearance of *Fusarium* wilt lesions on the stem, leaves, inflorescence and pods was recorded (Table 1). The final data was recorded when the disease status on spreader/check variety was 80-100% disease severity.

Environmental data comprising maximum and minimum air temperature (^{o}C), relative humidity (%), rain fall (mm), were collected from Agro metrology situated at AARI, Faisalabad 150 meter away from the experimental areas of both Pulses research institute (PRI) Faisalabad. These variables were recorded on daily basis from February to first week of March and the weekly average was calculated.

Area under disease progress curve (AUDPC):

The AUDPC was calculated by the trapezoidal integration of the disease severity over time, considering the whole period evaluated, as follows:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{xi + xi + 1}{2}\right)(ti + 1 - ti)$$

Where n is number of assessments; X, disease severity and (*ti*+1 – *ti*), time interval between two consecutive assessments. In order to allow comparison between different treatments that were assessed during different periods of time, the AUDPC integral variable was divided by its respective observation period (*ti*+1 – *ti*), Thus AUDPC was the standard area under the lentil wilt severity progress curve and interpreted as the mean severity of disease. The AUDPC standardized by tn-t_1 of the epidemic (Shaner and Finney, 1977).

Statistical analysis:

Disease severity and environmental data were subjected to pair wise correlation (Ezekiel, 1930). The germplasm revealing significant disease correlation coefficient with meteorological parameters were graphically plotted. Then, all the data were subjected to stepwise regression to screen the environmental variables having non-significant influence on disease development. All possible regressions were determined and the most conducive environmental conditions for lentil wilt disease development were characterized.

RESULTS

Screening of Lentil Germplasm:

Disease severity of twenty eight lentil lines was recorded and AUDPC was determined to study the resistance status of these lines against the disease (Table 2). AUDPC revealed two lines including 11502 and 11504 as resistant having AUDPC 175 and 187 respectively. Three lines encompassing 11501, 11503, and 11514 were moderately resistant with AUDPC 230, 272 and 222 respectively. Five lines comprising 10514 (336), 10508 (363), 10506 (369), 10507 (354), 10511 (389), 10512

Table 2. Disease incidence, level of resistance /susceptibility and total AUDPC on lentil lines

Rating	Infection (%)	Reaction	AUDPC	Genotypes
0	0	I	0	0
1	1-20	HR	1-100	0
2	21-30	R	101-200	11502, 11504
3	31-40	MR	201-300	11501, 11503, 11514
4	41-50	S	300-400	10506, 10507, 10508, 10511, 10512, 10414, 11513
5	>50	HS	401-500	10501, 10502, 10503, 10504, 10505, 10509, 10510, 10513, 11505, 11506, 11507, 11508, 11509, 11510, 11511, 11512

(377) and 11513 (370) remained susceptible. Remaining lines including 10501 (426), 10502 (484), 10503 (457), 10504 (468), 10505 (419), 10509 (461), 10510 (464), 10513 (476), 11505 (461), 11506 (438), 11507 (401), 11508 (476), 11509 (465), 11510 (454), 11511 (414) and 11512 (475) were found highly susceptible.

Correlation of Environmental factors with lentil wilt disease on different lines/cultivars:

Disease severity data were correlated with environmental variables (Table 3). Weekly environmental conditions were significantly different. Maximum disease severity was recorded at air temperature (Max. and Min.) 17-18^{o}C and 7-10^{o}C respectively. At this temperature range disease kept rising continuously. This relationship was best explained by linear regression (Figure 1 and 2). Similarly, relative humidity was positively correlated with disease development and it was best explained by linear regression (Figure 3). Maximum disease was recorded at 60-78% relative humidity. Moreover, rainfall also demonstrated positive correlation with disease development and was best explained by linear regression (Figure 4). Maximum disease was recorded at 2 mm rainfall.

Summary of stepwise regression

Since the environmental conditions during disease-rating period were significantly different, the data were not lumped to avoid interactive effects. The data were subjected to stepwise regression to screen environmental variables not having a significant influence on lentil wilt development. Due to varying environmental conditions, different models consisting of different environmental parameters gave different disease predictions (Table 4). The models consisting of those environmental variables exerted a significant influence on the lentil wilt development. Stepwise regression employed to predict lentil wilt incidence, the model developed significantly fit to the data and their predicted values of lentil wilt incidence were in close conformity with the observed values. Minimum temperature and rainfall explained 98% variability in lentil wilt development on advance line 10501. While these two predictors explained 99% disease variation on line 10503. Maximum temperature and relative humidity explained 81% variability in lentil wilt development in line 10511. On the other hand, on line 10514 and 11506 rainfall explained 92 and 94% variability in lentil wilt development respectively (Table 4). Moreover, observed and predicted values were found in most of the conformity (Table 5).

DISSCUSION

Only two lines including 11502 and 11504 remained resistant while three lines encompassing 11501, 11503, and 11514 were found moderately resistant. All other

Table 3: Correlation of Environmental factors with lentil wilt disease on different lines/cultivars

Sr. No	Genotypes	Temperature (°C)		Relative Humidity (%)	Rainfall (mm)
		Max.	Min.		
1	10501	0.9535 0.1950*	0.6321 0.5644	0.9997 0.0161**	0.9441 0.0691 *
2	10502	0.9483 0.2056	0.6449 0.5538	0.9991 0.0267**	0.9922 0.0797*
3	10503	0.9316 0.2369	0.6817 0.5225	0.9958 0.0580**	0.9848 0.1110*
4	10504	0.9080 0.2752	0.7245 0.4842	0.9886 0.0963*	0.9726 0.1493*
5	10505	0.7562 0.4541	0.8872 0.3052	0.9080 0.2753	0.8700 0.3283
6	10506	0.9054 0.2792	0.7288 0.4802	0.9876 0.1003*	0.9711 0.1533*
7	10507	0.8417 0.3631	0.8124 0.3963	0.9584 0.1842*	0.9314 0.2372
8	10508	0.7294 0.4796	0.9050 0.2798	0.8905 0.3007	0.8496 0.3537
9	10509	0.8766 0.3197	0.7708 0.4397	0.9756 0.1408*	0.9540 0.1938*
10	10510	0.8675 0.3314	0.7825 0.4279	0.9714 0.1526*	0.9483 0.2056
11	10511	0.9233 0.2510	0.6978 0.5083	0.9936 0.0722*	0.9807 0.1252*
12	10512	0.8981 0.2899	0.7402 0.4695	0.9848 0.1110*	0.9670 0.1640*
13	10513	0.9075 0.2761	0.7254 0.4833	0.9884 0.0972*	0.9723 0.1502*
14	10514	0.9137 0.2664	0.7149 0.4929	0.9905 0.0876*	0.9757 0.1406*
15	11501	0.9656 0.1674*	0.5979 0.5920	0.9998 0.0115**	0.9979 0.0415**
16	11502	0.9712 0.1533*	0.5800 0.6061	0.9992 0.0256**	0.9991 0.0274**
17	11503	0.9818 0.1216*	0.5388 0.6377	0.9960 0.0572**	1.0000 0.0042**
18	11504	0.9926 0.0776*	0.4793 0.6818	0.9874 0.1012*	0.9971 0.0482**
19	11505	0.9926 0.0776*	0.4793 0.6818	0.9874 0.1012*	0.9971 0.0042**
20	11506	0.8981 0.2899	0.7402 0.4695	0.9848 0.1110*	0.9670 0.1640*
21	11507	0.8357 0.3701	0.8189 0.3892	0.9552 0.1913*	0.9273 0.2443
22	11508	0.8695 0.3289	0.7800 0.4305	0.9724 0.1500*	0.9496 0.2030
23	11509	0.8456 0.3585	0.8083 0.4008	0.9604 0.1797*	0.9339 0.2327

Table 4: Continuation

24	11510	0.8846 0.3089	0.7599 0.4505	0.9792 0.1301*	0.9589 0.1831*
25	11511	0.8766 0.3197	0.7708 0.4397	0.9756 0.1408*	0.9589 0.1831*
26	11512	0.8827 0.3115	0.7626 0.4478	0.9756 0.1408*	0.9578 0.1857*
27	11513	0.8725 0.3250	0.7761 0.4344	0.9738 0.1461*	0.9515 0. 1991 *
28	11514	0.9806 0.1254*	0.5439 0.6339	0.9965 0.0534**	1.0000 0.0004**

** = Significant at 0.05% P * = Significant at 0.1% P

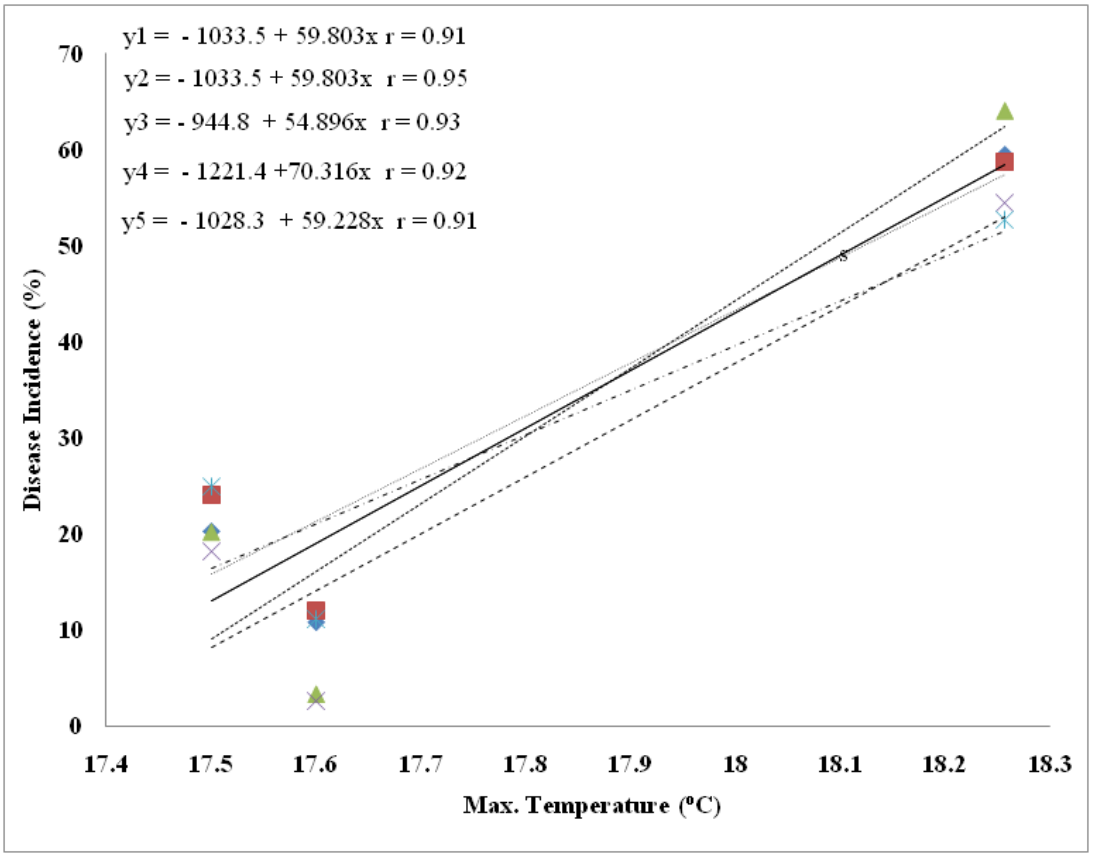

Figure 1. Relationship of weekly maximum air temperature to *Fusarium* wilt development on line 10501 (y_1), 10503 (y_2), 10511 (y_3), 10514 (y_4) and 11506(y_5) during disease rating period.

lines were susceptible against pathogen showing the aggressiveness and dominance of pathogen. Kaiser (1997) experienced 100% mortality of plants in all the ridges sown with the susceptible cv. JG-62 indicates that the *Fusarium* wilt pathogen was uniformly spread throughout the plot. Belabid *et al.* (2004) study the prevalence of different *Fol* isolates in lentil growing regions makes it essential to identify region specific pathogen to devise strategies for conferring resistance against them in the respective agro-climatic regions. Thus, our results were consistent with those of previous studies.

Maximum disease severity was recorded at 17-18^{o}C and 7-10^{o}C air temperature (Max. and Min.), 60-78%

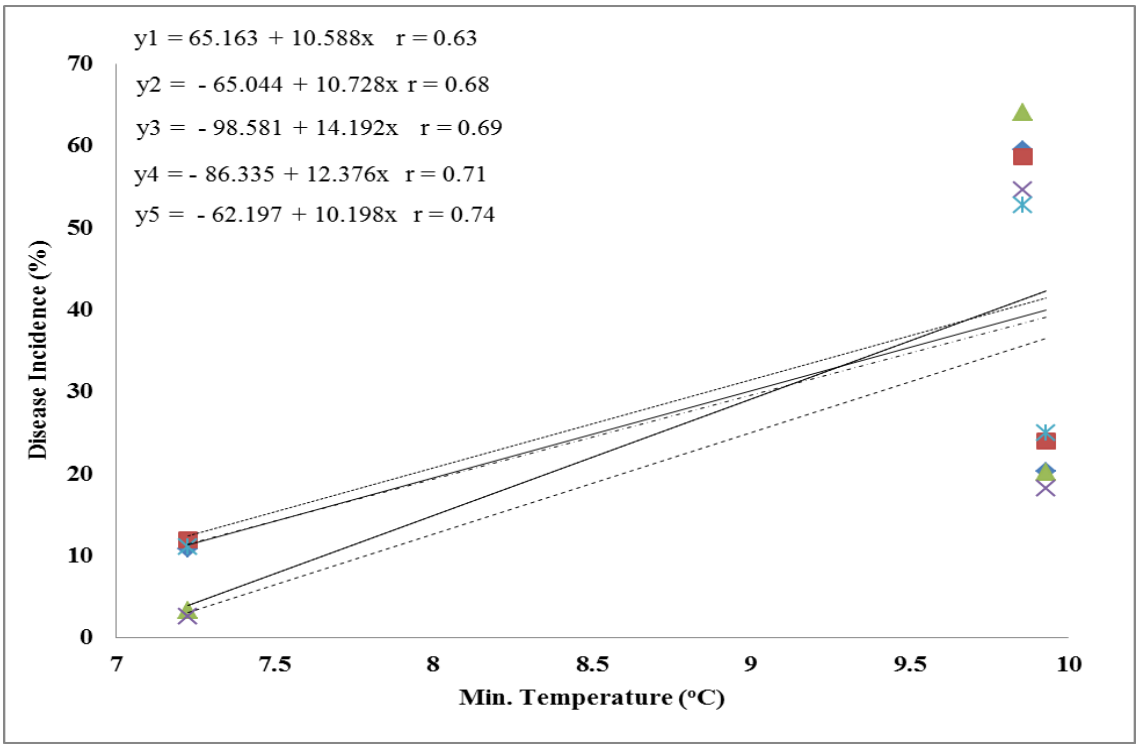

Figure 2. Relationship of weekly minimum temperature to *Fusarium* wilt development on line 10501 (y_1), 10503 (y_2),

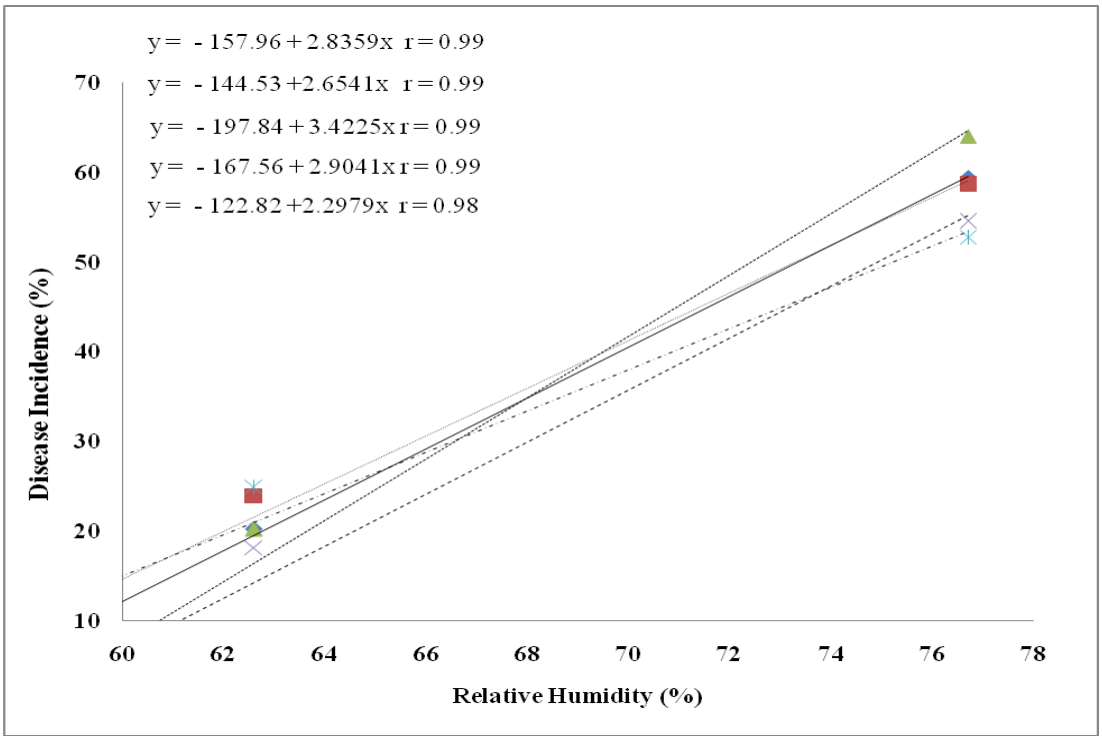

Figure 3: Relationship of weekly relative humidity to *Fusarium* wilt development on line 10501 (y_1), 10503 (y_2), 10511 (y_3), 10514 (y_4) and 11506(y_5) during disease rating period.

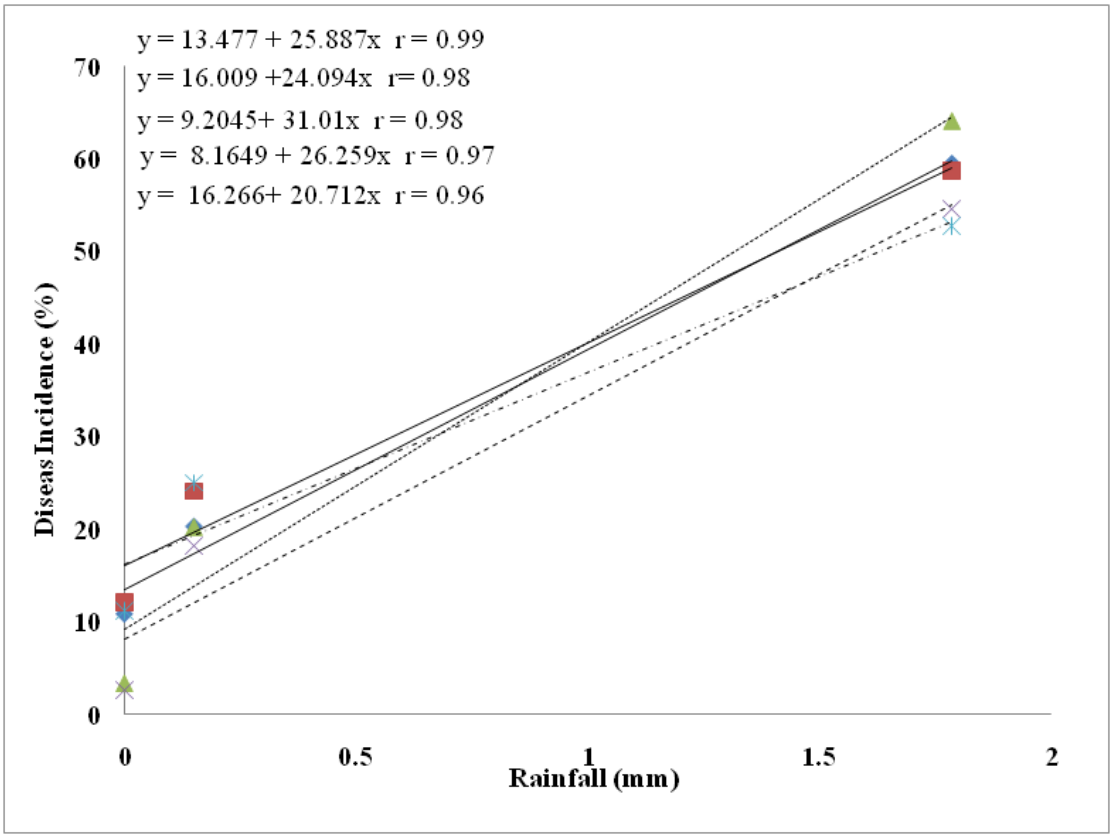

Figure 4: Relationship of weekly rainfall to *Fusarium* wilt development on line 10501 (y_1), 10503 (y_2), 10511 (y_3), 10514 (y_4) and 11506(y_5) during disease rating period.

Table 5: Multiple regression equations (y = Full Model, y1 = 10501, y2 = 10503, y3 = 10511, y4 = 10514, y5 = 11506) based on weekly environmental conditions and predicted *Fusarium* wilt severity.

Regression Equations	**Lentil Wilt Incidence**		
($y = b_o + b_1x + b_2x…$)	**Observed**	**Predicted**	**R^2**
$y = -19.28675+ 22.39952x1+ 3.77263x2$	59.45	57.89	0.98
(where x_1 indicates rainfall, and x2 minimum respectively)	58.66	57.89	
	64.04	57.89	

Table 6: Continuation

y1 = **-4.88619 + 2.17098x1 + 23.98901x2 -** (where x_1 indicates minimum temperature, x_2 rainfall respectively)	59.46 10.81 64.04	59.46 10.81 57.89	0.99
y2 = **142.83599+ -19.29841x1+ 3.49658x2** (where x_1 indicates maximum temperature and x2 indicate relative humidity)	58.67 12.00 24.00	58.67 12.00 24.00	0.81
y3 = **9.20821 +30.93060** x1 (where x_1 indicates rainfall)	64.04 03.37 20.22	64.57 09.20 13.84	0.92
y4 = **8.17063+26.19491x1** (where x_1 indicates Rainfall)	54.55 18.18 10.00	55.0595 16.17 12.0999	0.94
y5 = **-19.20860 +4.19344x1+17.11801x2** (where x_1 indicates minimum temperature, x_2 rainfall)	52.78 11.11 25.00	52.78 11.11 25.00	0.99

Table 7: Summary of Stepwise Regression developed to predict *Fusarium* wilt on Lentil germplasm where V_1= 10501, V_2 = 10503, V_3 = 10511, V_4 = 10514, V_5 = 11506.

Environmental Parameters	R^2	Adj. R_2	Cp	MSE	Prob> F
Overall summary of Model Minimum Temperature (^oC) Rainfall (mm)	0.98	0.98	2	21.51	<0.0001*
V_1 Minimum Temperature (^oC) Rainfall (mm)	0.99	0.99	2	1.08	<0.0001*
V_2 Maximum Temperature (^oC) Relative Humidity (%)	0.99	0.98	2	2.39	<0.0001*
V_3 Rainfall (mm)	0.94	0.92	1.96	115.22	<0.0001*
V_4 Rainfall (mm)	0.94	0.91	1.00	95.33	<0.0001*
V_5 Minimum Temperature (^oC) Rainfall (mm)	0.99	0.98	2.00	16.74	<0.0001*

relative and 2 mm rainfall respectively. The fungus *Fol* can cause severe disease under hot and dry conditions (Eujayl *et al.*, 1998) and optimum temperature for the disease is 22–25°C; for this reason, the fungus causes high yield losses in Moghan, Ardebil and Korasan (Kari Dolatabadi *et al.*, 2011).

Fusarium wilt disease still remains a problem in modern agriculture. Excessive use of chemical fungicides may apparently lower the density of pathogens in soil for short time duration but might give rise to mutant strains of pathogens with altered pathogenicity; making the previously resistant varieties of crops susceptible. Selection of durable resistant germplasm and effective breeding programs are needed to employ. Use of bio-control methods, crop rotations and genetically modified crops are some of the methods of disease management to curb down extreme use of chemicals on field to control pathogens. The efficient use of bio-chemicals or bio-

products requires knowledge of epidemiology of diseases because it provides the basis for forecasting disease outbreaks. The importance of quantitative informations and the relationship between initial inoculum and the rate of disease development represent key elements for the most useful models employed for prediction (Nutter, 2007).

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