

Changes in Mineral Profile and Morphological Traits of Pea (*Pisum sativum* L.) as Influenced by *Fusarium oxysporum* F. Sp. *Pisi* under Natural Conditions

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Abstract

Eight pea varieties including three commercial and five advance lines were cultivated under natural conditions for the infection of Pea wilt disease. Only four varieties were found susceptible to *Fusarium* wilt. Infected and healthy samples were collected from each variety. After pathogenicity of *Fusarium oxysporum* F. sp. *pisi*, the mineral and growth parameters from each sample were recorded. The parameters of healthy and infected samples were compared within each variety. Nitrogen was slightly increased (21.09%) after the onset of infection. While infected samples showed a decline in Phosphorus (44.38%), Potassium (53.64%), Calcium (23.60%), Magnesium (57.24%), and Zinc (32.22%). Growth parameters were also influenced negatively after the infection. They included fresh plant weight (68.79%), dry plant weight (57.29%), shoot length (50.43%) and No. of pods/plant (100%) while root length remained un-affected as compared to non-infected plants. These studies explained the role of vascular infection on the physiology and morphology of Pea.

Keywords: Pea wilt; Soil born; Vascular infection

Introduction

Pea is (*Pisum sativum* L.) fourth most important legume crop in the world [1]. Its importance can be estimated from the fact that, 40% of total pulse trade is based over pea [2] and it is important dietary source of proteins that is up to 15.5 to 39.7% of total biomass [3]. Apart from other constraints of pea production, the diseases play a significant role. Pea is infected by several pathogenic fungi in which *Fusarium oxysporum* F. sp. *pisi* is significant causing wilt disease, that may cause partial to complete loss of crop [4,5].

Pea wilt disease is prevailing almost in all pea growing regions of the world. It was firstly reported by Linford in Wisconsin (U.S.A) in 1928 [6]. When the infection takes place it invades the root system and move upward through water conducting xylem tissues. In the result the plant becomes pale. Infection starts from lower leaves and gradually moves to the upper portion. Leaves and stipules show downward curling and plant growth is stunted. In severe cases, the plant wilts and dies due to vessel clogging. Generally pods are not formed, if pod formation occurs the seed is not well developed. Sometimes infected seed is formed which act as a secondary source of disease spread. Root system is not destroyed at all, sometimes remains unaffected in mild cases. Cross-section of root and basal stem shows brick red, orange or yellow the vascular discoloration depending upon the severity of infection and race type [7]. Optimum temperature for the disease development is 21-26°C [8].

When a pathogen attacks the plant, it exhibits some reflective changes in its physiology which ultimately affect the normal metabolic functions. *Fusarium* spp. is also known to cause various biochemical changes in the pea. A little work has been published on such changes and response of Plant to such infections e.g. Morkunas et al. [9] stated that, high carbohydrates level prevented the peas from the invasion of *F. oxysporum* F. sp. *pisi* and the activity of free radicals such as Mn⁺² were extensively raised. Wit-Elshove and Fuchs [10] discovered that, Pisatin breakdown in pea is also increased with the infection of *Fusarium oxysporum*. In the same research it was explored that, malate

dehydrogenase, Superoxide dismutase and Catalase were significantly raised after the infection was occurred.

Fusarium oxysporum also causes morphological changes in the host as described by Benedict [11] who conducted a research over multiple pea cultivars and found the correlation between soil temperature and disease development caused by various soil pathogens including *Fusarium* sp., *Rhizoctonia* sp. and *Pythium* sp. Data was recorded from growth parameters of seed weight, plant weight, plant height, pre-emergence and post emergence killing of seedlings. Infection generally influenced the all growth parameters and growth was retarded. Keeping in view the above mentioned facts, the research was designed to find out the further mineral and morphological changes which are associated with host-pathogen interaction.

Materials and Methods

Pathogenicity test

Eight pea varieties were sown in Faisalabad district of Pakistan. Only four varieties were found susceptible. After the onset of disease the highly infected plants were collected. Healthy samples were collected from the same treatment for the comparison.

Isolation of *Fusarium oxysporum* was done over PDA: Mycelia

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were abundant and ranged in color from white to pale violet. Macroconidia were medium length and slightly curved, relatively slender and thin walled generally contained 3 septations. While microconidia were Oval or kidney shaped without septation and Koch's postulates were confirmed by inoculating the same varieties sown in the sterilized soil in the pots. Wilts symptoms were pronounced on these plants. Later on these inoculated samples were used to re-isolate the same pathogen.

Biochemical studies

Oven drying: All samples were oven dried at 60°C overnight.

Grinding: Dry samples were ground to fine powder for their biochemical studies.

Total nitrogen contents

Total nitrogen in each sample was estimated according to the Kjeldahl's method [12] method No. 46-10. Two grams of each sample were put into the digestion tube. Twenty milliliters of conc. H₂SO₄ (98%) and 2 capsules of digestion mixture (as catalyst) were added into the digestion tube. The digestion was done for 3-4 hours (till the contents attained transparent color). The digested material was cooled at room temperature and diluted to a final volume of 50 ml. The ammonia trapped in H₂SO₄ was liberated by adding 40% NaOH solution by distillation. Then it was collected in a flask containing 4% boric acid solution, possessing methyl indicator. It was titrated against standard 0.1N H₂SO₄ solution and quantity of Nitrogen contents was calculated in %age.

Mineral analysis

All mineral parameters including P, K, Mg, Ca and Zn were determined through Atomic Absorption Spectrophotometer and protocols were followed as described in AOAC [13]. One gram of sample was digested within 10 ml of nitric acid and perchloric acid (7:3) at 180-200°C till the transparent. These contents were further diluted to a volume of 100 ml with double distilled water. Concentration of mineral contents was determined by running the diluted samples on Atomic Absorption Spectrophotometer (Model: Varian, AA-240, Victoria, Australia) using air acetylene flame.

Morphological parameters

Shoot length (cm), Root length (cm), Dry and Fresh plant weight (grams) and No. of pods/plant were recorded from healthy and infected plants after uprooting from soil.

Statistical Analysis

The experiment was laid out according to Randomized Complete Block Design (RCBD). The data of each parameter was subjected to statistical analysis and means from healthy and diseased samples was compared by using LSD test [14].

Results and Discussions

Disease incidence

Although Infection occurred in the late season (Figure 1) and the incidence was calculated as below;

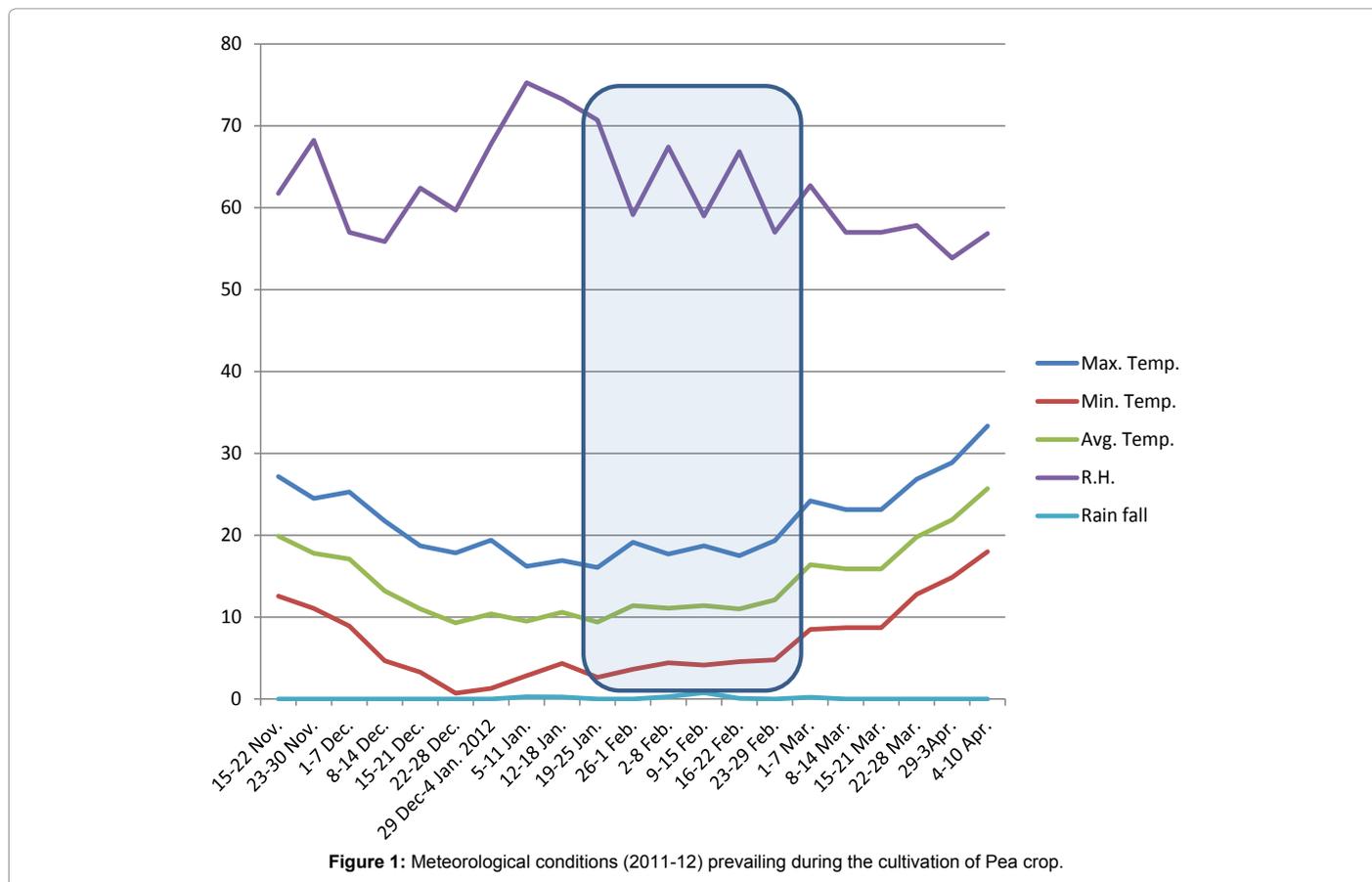


Figure 1: Meteorological conditions (2011-12) prevailing during the cultivation of Pea crop.

$$\text{Disease incidence} = \frac{\text{No. infected plants}}{\text{Total no. of plants}} \times 100 = 35 / 642 \times 100 = 5.45\%$$

Screening test showed that only four varieties Euro, Early green, Advance line A (018337) and Advance line B (019309) were susceptible to *Fusarium* wilt, hence they were selected for biochemical and morphological comparisons.

Changes in pea minerals after infection

Mineral contents were compared among the varieties. Results have indicated that nitrogen was raised in all infected varieties particularly Adv. Line A (21.09%). While there was significant reduction in Phosphorus (Adv. Line B; 44.38%), Potassium (Early green; 53.64%), Magnesium (Adv. line A; 57.24%), Calcium (Euro; 23.60%), and Zinc (Early green; 32.22%) as compared to the healthy plants (Table 1 and Figure 2). That change might indicate the role of fungal mycelium over the translocation of the mineral elements after the vessel clogging.

Morphological parameters

As the results indicated (Table 2 and Figure 3) that *Fusarium* wilt caused significant reduction in the fresh weight (Euro; 68.79%), Dry plant weight (Advance line B; 57.29%), Shoot length (Adv. Line B; 50.43%) and no. of pods/plant (Euro; 100%) as compared to the healthy varieties. But the root length did not showed any significant change after the infection (Figure 3). Each variety has demonstrated varying response for each parameter, as shown in Table 2.

Discussions

Above mentioned facts are somewhere in accordance with previously documented facts of Pea wilt disease. Nitrogen contents were high within the infected samples. These findings agree with the previous

work of Smiley et al. [15] studied the correlation of nitrogen application on the incidence of wilt pathogens *F. roseum* F. sp. *Cerealis*, *F. solani* F. sp. *pisi* and some saprophytic *Fusarium* species. Excess amount of nitrogen favored the infection. Similarly, in case of Nitrogen Burgt et al. [16] evaluated the effect of five organic sources of Nitrogen fertilizer on the growth of Fusarial infection of Head Blight of Wheat. These experiments also showed a positive correlation between the nitrogen and pathogen infestation. In case of Phosphorus, it was recorded in fewer amounts within the infected samples. Our studies are matching with the work of Yergeau et al. [17] who conducted a research on the ecological relationship of *Fusarium* sp. to the environmental factors. This experiment was conducted on Asparagus and Phosphorus was applied in different concentrations. After fertilizer application plants were inoculated with *Fusarium* sp. *Fusarium* infection was recorded more in the Phosphorus deficient plants and vice versa. Potassium was also studied in the healthy and infected plants. As our results indicated that low levels of Potash were recorded in infected samples as compared to the healthy ones, showing the influence of Fusarial infection which causing minimal availability to plant. Spiegel and Netzer [18] discussed the influence of different concentrations of Nitrogen and Potassium on *Fusarium* sp. and Root knot nematode (*Meloidogyne javanica*) singly or in combinations over the muskmelon plants. Parameters of infected plants were compared with healthy ones. These studies also stated that Potassium was depleted in the shoots after the onset of infection.

Calcium level was also reduced in the infected samples. Our results agreed with Blancand and Tramier [19]. They had sown the cuttings of carnation mother plants and six levels of Calcium were added to them. Experiments revealed that lower amount of calcium was favorable to the infection.

In case of Zinc contents, these findings are similar with the work

Mineral parameters	SI. No.	Variety	Healthy	Infected	% Decrease after infection
Nitrogen	1	Euro	3.967 a	4.576 a	-15.37
	2	Early Green	4.266 a	5.100 a	-19.57
	3	Advance line A (No. 018337)	4.033 a	4.883 a	-21.09**
	4	Advance line B (No. 019309)	4.333 a	4.940 a	-14.01
Phosphorus	1	Euro	4.96 a	2.90 a	41.53
	2	Early Green	3.97 b	2.70 ab	31.81
	3	Advance line A (No. 018337)	4.80 a	2.96 a	38.33
	4	Advance line B (No. 019309)	3.83 b	2.13 b	44.38**
Potassium	1	Euro	19.53 a	12.73 a	34.82
	2	Early Green	19.33 a	8.96 a	53.64**
	3	Advance line A (No. 018337)	17.80 b	11.60 a	34.83
	4	Advance line B (No. 019309)	19.16 a	12.46 a	34.97
Magnesium	1	Euro	2.03 c	0.793 c	52.21
	2	Early Green	2.33 b	1.126 b	51.93
	3	Advance line A (No. 018337)	2.76 a	1.186 b	57.24**
	4	Advance line B (No. 019309)	2.43 b	1.660 a	31.68
Calcium	1	Euro	2.966 b	2.266 b	23.60**
	2	Early Green	3.466 a	3.000 a	13.44
	3	Advance line A (No. 018337)	3.333 a	3.000 a	10.0
	4	Advance line B (No. 019309)	3.600 a	3.200 a	11.11
Zinc	1	Euro	0.027 ab	0.0197 ab	27.03
	2	Early Green	0.027 ab	0.0183 ab	32.22**
	3	Advance line A (No. 018337)	0.029 a	0.0240 a	17.24
	4	Advance line B (No. 019309)	0.024 b	0.0177 b	26.25

Level of significance=α=0.05, Critical T Value=2.447

Means followed by same letters are not significantly different from one another according to Fisher's LSD test at 5% level of Significance.

Table 1: All pairwise LSD comparison of mineral profile of healthy and infected pea plants.

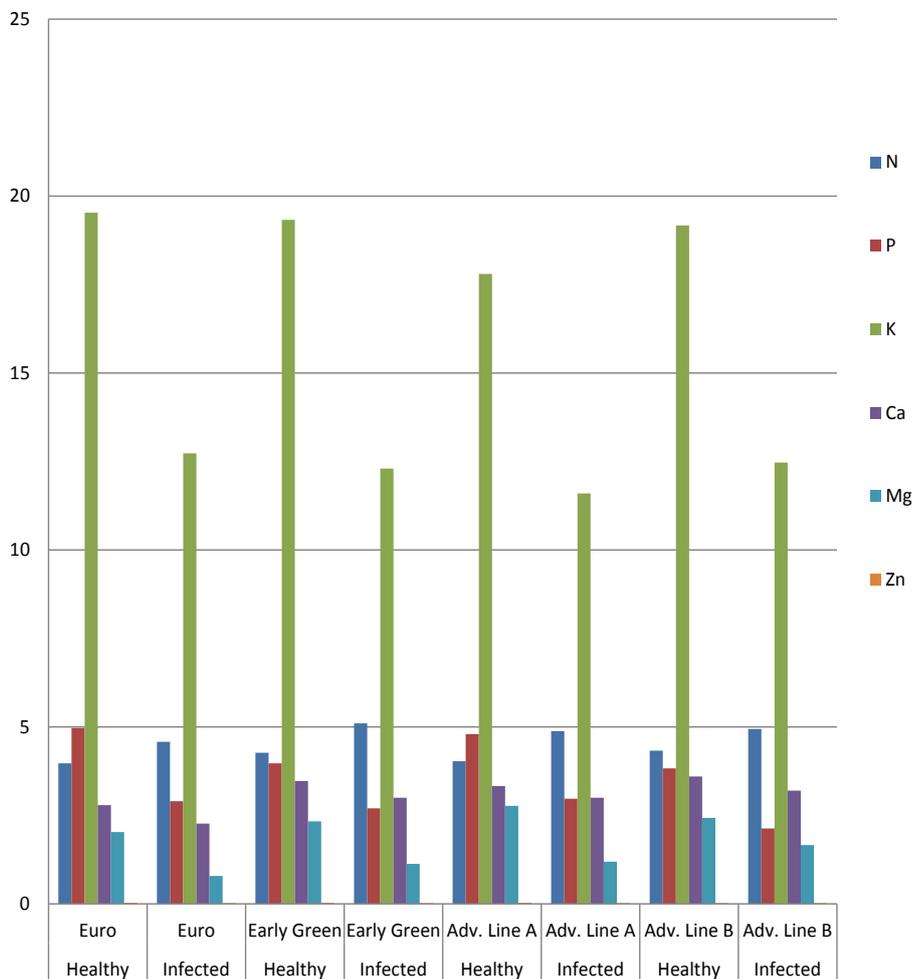


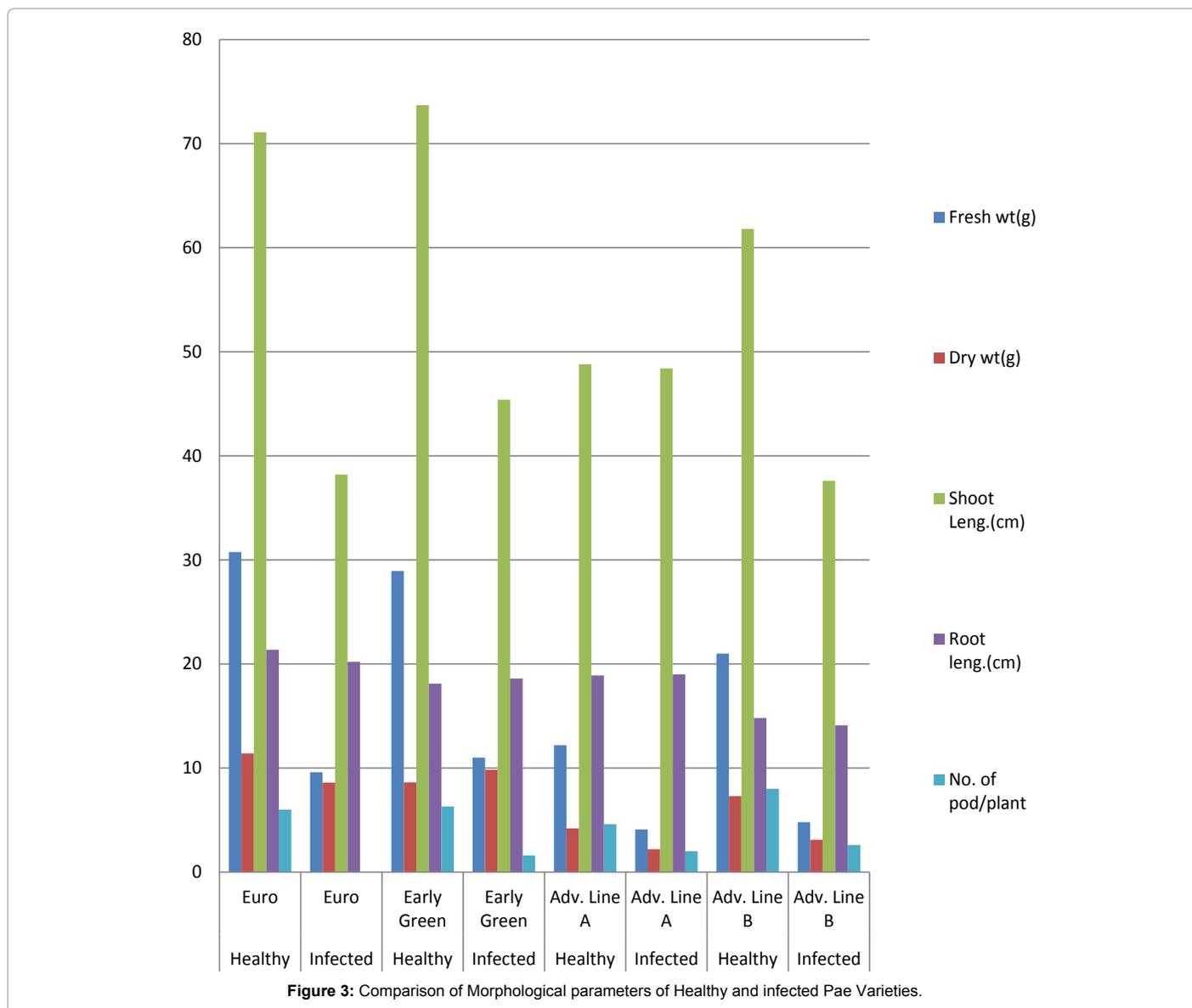
Figure 2: Comparison of Mineral profile of Healthy and infected Pae Varieties (% of total Dry matter).

Morphological Parameters	Sl. No.	Variety	Healthy	Infected	% Decrease after infection
Fresh Plant Weight	1	Euro	30.76 a	9.60 a	68.79
	2	Early Green	28.93 a	11.06 a	61.78
	3	Advance line A (No. 018337)	21.06 a	4.16 a	80.24**
	4	Advance line B (No. 019309)	12.20 a	4.86 a	60.16
Dry Plant Weight	1	Euro	11.40 a	8.68 a	23.85
	2	Early Green	8.63 ab	9.83 a	-13.90
	3	Advance line A (No. 018337)	4.23 b	2.26 a	46.57
	4	Advance line B (No. 019309)	7.33 ab	3.13 a	57.29**
Shoot Length	1	Euro	71.10 a	38.20 a	46.27
	2	Early Green	73.73 a	45.43 a	38.38
	3	Advance line A (No. 018337)	48.80 a	48.40 a	0.4
	4	Advance line B (No. 019309)	37.63 a	37.63 a	50.43**
Root Length	1	Euro	21.36 a	20.20 a	5.43**
	2	Early Green	18.13 ab	18.56 a	-2.37
	3	Advance line A (No. 018337)	18.96 ab	19.03 a	-0.37
	4	Advance line B (No. 019309)	14.83 b	14.10 b	4.92
No. of Pods/Plant	1	Euro	6.00 a	0.0 a	100**
	2	Early Green	6.33a	1.66 a	73.77
	3	Advance line A (No. 018337)	4.66 a	2.00 a	57.08
	4	Advance line B (No. 019309)	8.00 a	2.66 a	66.75

Level of significance=α=0.05, Critical T Value=2.447

Means followed by same letters are not significantly different from one another according to Fisher's LSD test at 5% level of Significance.

Table 2: All pairwise LSD comparison of morphological parameters of healthy and infected pea plants.



of Soshi et al. [20]. They found the influence of Non-pathogenic strains of *Fusarium* sp. on the uptake of different soil elements in tomato. In this research Zn uptake was slightly reduced in few treatment as compared to healthy control. Morphological parameters of shoot length, shoot weight, root weight and No. of pods/plant were reduced after the infection. Al-Askar and Rashad [21] conducted a research find out the role of Arbuscular Mycorrhizal (AM) fungi to check the growth of *Fusarium* sp. in the common beans under natural conditions. These fungi also enhanced the growth parameters as compared to untreated control plants. Shah et al. [22] discussed the pathogenic influence of certain fungi and Bacteria on the growth of the Fenugreek (*Trigonella foenum-graecum* L.). This impact was relevant to infection and plant growth parameters were reduced after the infection. Sartaj et al. [23] conducted an experiment for the biological control of tomato wilt disease caused by *F. oxysporum*. *Penicillium* sp. and Plant growth promoting fungi was taken as biocontrol agents. Untreated infected plants significantly declined their shoot and root weight. While Healthy plants remained unaffected.

Conclusion

Above mentioned research has given the major conclusion that *Fusarium oxysporum* causes significant changes in the physiology and morphology of plant. Even the disease was occurred late in the season but mineral uptake was strongly influenced by infection. Morphological parameters were also minimized. These changes showed the role of vascular infection on the mineral translocation of plant.

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