Plant Pathology Journal

ISSN 1812-5387

Plant Pathology Journal 11 (1): 32-37, 2012
ISSN 1812-5387 / DOI: 10.3923/ppj.2012.32.37

Influence of Some Agricultural Practices on Suppression of Lentil Wilt Disease

[1]Nashwa M.A. Sallam and [2]Montaser Fawzy Abdel-Monaim
[1]Department of Plant Pathology, Faculty of Agriculture, Assiut University, 71526 Assiut, Egypt
[2]Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

Abstract: Isolation and pathogenicity trails revealed clearly that *Fusarium oxysporum* f. sp. *lentis* was the common fungi associated with wilt symptoms and were 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 conditions. In tests of varietals response, all tested Egyptian local lentil cultivars were susceptible to infection in greenhouse and field while the Canadian cultivars (Eston, Laird and Richlea) showed high resistance to the disease. On the other hand, Richlea cv. resulted highly seed yield followed by Sinaa 1. The first of December was more suitable to minimize the infection with wilt disease in greenhouse and field. Sowing lentil at 1st November gave the highest seed yield in both growing seasons. Lentil seeds sowing at depth 2 cm were the most appropriate depth of cultivation where gave the least of wilt severity and recorded the highest seed yield. Increase or a lack of the planting depth led to increase the rate of infection and decreased seed yield.

Key words: Lentil, wilt, *Fusarium oxysporum*, varietals response, sowing dates, sowing depth

INTRODUCTION

Lentil (*Lens esculenta* L.) is a major grain and widely distributed legume crop grown under a broad range of climates in many developing countries (Turk *et al*., 2004; Abd-Allah and Hashem, 2006; Akem *et al*., 2006). This crop has been grown mainly as an inexpensive source of high quality protein in human diets (Sadiq *et al*., 2002; Rahman *et al*., 2010).

Lentil plants are affected by a wide range of pathogens. Fungal diseases cause sever damage to leaves, stems, roots and pods as well as reduced marketability by declaration seeds (Taylor *et al*., 2007). Fusarium wilt disease caused by *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen f. sp. *lentis* Vasudeva and Srinivasan, is the most important disease affecting lentils in Egypt, causing significant economic losses (El-Hersh *et al*., 2011; Hamdi and Hassanein, 1996). The pathogen persists in the soil as chlamydospores that can remain viable for several seasons (Erskine and Bayaa, 1996) and is capable of colonizing crop residue and roots of most crops grown in rotation with lentil. The disease is best controlled through host plant resistance. No physiological races of the pathogen have been reported so far (Bayaa *et al*., 1997) although *Fusarium oxysporum* f. sp. *lentis* isolates exhibit great variability in morphology and aggressiveness (Abbas, 1995).

Several agricultural factors affecting spread of this disease in lentil fields and most important of which are selected of resistance cultivars, sowing depth, sowing dates and soil types. Generally, effects of these factors on lentil wilt disease are complex and often interrelated, because they affect both the host lentil and root pathogens. Some factors may affect the lentil negatively and the fungus positively, leading to a clear-cut increase in lentil wilt, while other may affect both the lentil and the fungus positively, leaving the resultant disease increase or decrease of wilt in a matter of speculation.

Many authors studied the response of local lentil cultivars to infection with *Fusarium* sp., they found that the most local cultivars susceptible to infection with the disease (El-Garhy, 2000; Abdel-Monaim, 2002), it is therefore essential to test the sensitivity of foreign varieties of susceptibility to these pathogen to be used in breeding programs in order to develop a resistance cultivar for cultivation of such crop.

Hwang *et al*. (2000) also reported that, sowing dates were found to play a vital role in the development of lentil wilt disease. In a controlled-environment study of the impact of temperature on infection of lentil seedlings (cv. Eston) by *Fusarium* sp., root rot symptoms, the disease was severe at warm temperatures (20 to 27.5°C) and declined in warmer or cooler soils.

Corresponding Author: Nashwa M.A. Sallam, Department of Plant Pathology, Faculty of Agriculture, Assiut University, Assiut, 71256, Egypt Tel: +20882412726 Fax: +20882331384

This study aimed to isolate and identify the pathogens that cause wilt disease of lentil. Evolution of available local and foreign cultivars to susceptibility of infection and the effect of certain agriculture process i.e., planting depth, sowing dates on lentil wilt disease were also investigated.

MATERIALS AND METHODS

Isolation and Identification of causal organism: Isolation trails were carried out from diseased samples collected from different locations at Minia, Assuit and New Valley governorates. Roots of wilting lentil plants at different stages of plant growth were used for isolation. They were washed carefully under tap water to remove the adhering soil particles. The washed roots were cut into small pieces and surfaces sterilized by immersing the root pieces in 1% sodium hypochlorite solution for 5 min and then washed several times in sterilized distilled water to remove any residues of sodium hypochlorite then transferred to Potato Dextrose Agar (PDA) amended with streptomycin sulfate (0.01%) in Petri dishes and incubated at 25±1°C for 7 days. The growing fungi were individually transferred to PDA medium. Pure cultures of fungi were obtained using single spore or hyphal tip technique. The fungal isolates were then identified according to Nelson *et al*. (1983) and Booth (1985), Pure cultures were kept in a refrigerator at 4°C for further studies.

Greenhouse experiments

Seeds and growth of plants: Lentil (*Lens esculenta* L. cv. Giza 9) used in this study was procured from the Ministry of Agriculture, Egypt. Seeds were planted in plastic pots 25 cm diameter (3.2 kg soil), filled with a pasteurized mixture of soil and sand (4:1 w/w). Seedlings were kept in the greenhouse at temperatures ranging between 15 and 25°C with 46-50% RH and received about 11 h of natural light and 13 h of darkness daily. The plants were watered as required and each pot received about 200 mL of N:P:K fertilizer suspension (10 g L^{-1}) per week.

Pathogenicity tests: Ten isolates obtained from isolation process were tested on lentil plants cv. Giza 9 in a greenhouse. Sterilized Conical flasks, each containing 100 g of corn grains: sand (3:1, w /w), were inoculated with cultures from each isolate using agar plugs from 7-day-old cultures. The inoculated medium was incubated at 25°C for 10 days. Plastic pots (25 cm in diameter) were filled with sterilized soil and infested with each of the fungal isolates at a rate of 3% of soil weight. Pots were irrigated and left for 7 days to ensure the establishment of the tested isolates in the soil. Seeds of lentil Giza 9 were disinfested by dipping in 1% sodium hypochlorite solution for 2 min and then washed several times in sterilized distilled water then sown in infested pots at the rate of 4 seeds/pot. Four pots were used for each isolate. Pots containing sterile soil mixed with corn grains free of any fungi were sown similarly with lentil seeds at the same rate to be used as a control treatment. All pots were irrigated after soil infestation and later when it is needed.

Disease assessment: After 90 days from planting, Disease Severity Index (DSI) was recorded with a disease scale proposed by Sreeramanan *et al*. (2006)

$$DSI = \sum d/(d_{max} X\ n) \times 100$$

where, d is the disease rating of each plant, d_{max} is the maximum disease rating and n is the total number of plants examined in each replicate.

Varietals response: Five local lentil cultivars namely Giza 4, Giza 9, Giza 51, Giza 370, Sinaa 1 and three Canadian namely Eston, Laird and Richlea were tested for their susceptibility to highly pathogenic isolates (isolates 3) *Fusarium oxysporum* f. sp. *lentis*. Data were recorded for wilt after 90 days from planting, as above described.

Effect of sowing depth: In this study, plastic pots (25 cm diameter) were filled with soil infested with isolate 3 of *Fusarium oxysporum* f. sp. *lentis* at rate 3% from inoculation prepared as above then irrigated and left 1 weak. Lentil disinfested seeds cv. Giza 9 Planted in the various depths, 1, 2, 4 and 6 cm. Four pots per treatment as replicates and 4 seed/pot were used. In control treatment disinfested seeds sowed with the same method in sterilized soil. Data were recorded for wilt after 90 days.

Field experiments: In this study, five experiments were conducted during 2009/10 and 2010/11 growing seasons under field conditions. The experiments were carried out in a field naturally infested with the causal organisms of wilt disease of lentil located at the experimental farm of El-Kharga Agriculture Station, New Valley governorate. The experimental design was Randomized Complete Block Design (RCBD) with four replicates. The experiments were conducted in plots 10.5 m^2, 3.5 m in length and 5 rows, between both of them 0.60 m. Two seeds/hill were sown with 5 cm apart between hills on one side from row. Wilt was recorded 90 days after sowing. At the end of the growing season, seed yield was harvested, weighed and calculated as (kg fed^{-1}) (feddan = 2400 m^2). Experiments that have been studied were as follows:

- **Varietal response:** Response of five local lentil cultivars, namely Giza 4, Giza 9, Giza 51, Giza 370 and Sinaa 1 as well as three Canadian cultivars namely Eston, Laird and Richlea were used in this study
- **Effect of sowing dates:** Lentil seeds cv. Giza 9 were planting at three different dates i.e., 1st October, 1st November and 1st December in both seasons
- **Effect of sowing depth:** Lentil seeds cv. Giza 9 were planted in the various depths, 1, 2, 4 and 6 cm

All previous field experiments have been cultivated in the first of November, except to sowing dates.

Statistical analysis: All experiments were performed twice. Analyses of variance were carried out using MSTAT-C (1991). Least Significant Difference (LSD) was employed to test for significant difference between treatments at $p \leq 0.05$.

RESULTS

Isolation, identification and pathogenicity tests of the causal organisms: Ten fungal isolates were obtained from different naturally infected lentil plants showing wilt symptoms collected from different locations at Minia, Assuit and New Valley governorates. These isolates were identified as *F. oxysporum* f. sp. *lentis*. These isolates were showed various ability to cause wilt symptoms on artificial inoculated lentil plants. Isolate 3 caused the highest percentage of wilt symptoms (50.7%), whereas isolates 2 and 4 caused 37.1 and 35.0% wilt, respectively. Isolates 6, 8, 9, 10 were the weak ones due to wilt symptoms (less than 15% wilt) (Fig. 1). Therefore, isolate 3 was selected for further studies.

Greenhouse experimental reaction of certain lentil cultivars to wilt disease: The analysis of variance of the wilt reveled significant differences between the tested cultivars in their reaction to wilt disease caused by *F. oxysporum* f. sp. *lentis* (Fig. 2). The obtained data summarize that all local lentil cultivars were highly susceptible to infection with *F. oxysporum* f. sp. *lentis* isolate 3. Giza 9 recorded the highest infection with wilt disease (48%). Cultivar Sinaa 1 exhibited the lowest infection (25 %) wilt. On the contrary, the Canadian cultivars were highly resistance to infection with *F. oxysporum* f. sp. *lentis*. Laird cv. showed the highest disease resistance cultivars to infection with wilt pathogen, where the infection rate does not exceed 8.6%.

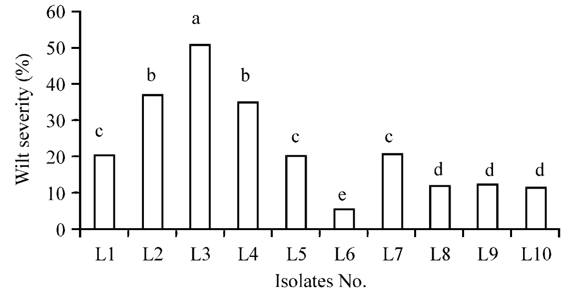

Fig. 1: Pathogenicity tests of ten fungal isolates obtained from naturally diseased lentil roots. Different letters indicate significant differences among treatments within the same column according to least significant difference test (p = 0.05), where as L1-L10: *Fusarium* isolates

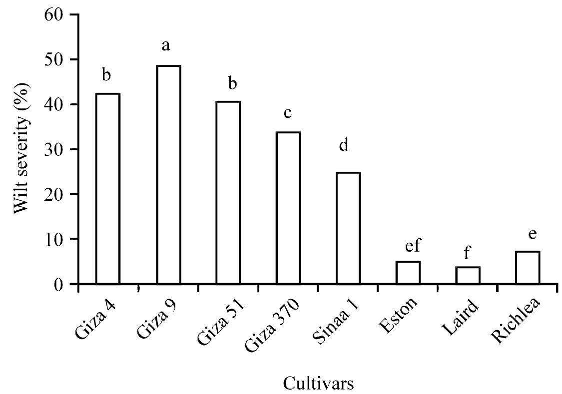

Fig. 2: Varietal response of eight lentil cultivars towards *Fusarium oxysporum* f. sp. *lentis* isolate 3 obtained from diseased lentil plants Different letters indicate significant differences among treatments within the same column according to least significant difference test (p = 0.05)

Effect of sowing depth: Wilt disease of lentil plants caused by *F. oxysporum* f. sp. *lentis* was significantly affected by sowing depth. Data present in Fig. 3 indicate that wilt severity was affected with planting depth but the effecting was not clear especially when planting at depth of 1 to 4 cm.

Field studies

Reaction of certain lentil cultivars to wilt disease: Data present in Table 1 show that all the tested cultivars were susceptible to infection by Fusarium wilt pathogens under field conditions. The Canadian cultivars (Eston, Laird, Richlea) were highly resistant than the Egyptian local tested cultivars. Giza 9 cv. recorded the highest wilt disease (18.4 and 17.5%) in both seasons, respectively.

Table 1: Varietal response of eight lentil cultivars towards wilt disease and seed yield under natural infection in field during seasons 2009/10 and 2010/11

	Season 2009/10		Season 2010/11	
Cultivars	Wilt (%)	Seed yield (kg fed^{-1})	Wilt (%)	Seed yield (kg fed^{-1})
Giza 4	14.3^{bc}	438.3^{e}	12.5^{b}	455.2^{d}
Giza 9	18.4^{a}	446.3^{e}	17.5^{a}	486.3^{d}
Giza 51	12.5^{cd}	512.3^{c}	12.0^{b}	529.3^{bc}
Giza 370	14.7^{b}	483.6^{d}	11.4^{b}	565.2^{b}
Sinaa 1	10.8^{d}	582.5^{b}	10.9^{b}	614.3^{a}
Eston	5.0^{ef}	405.3^{f}	4.5^{c}	418.0^{e}
Laird	3.2^{f}	522.3^{c}	4.2^{c}	550.0^{b}
Richlea	6.0^{e}	622.8^{a}	5.1^{c}	636.2^{a}

Different letters indicate significant differences among treatments within the same column according to least significant difference test (p = 0.05)

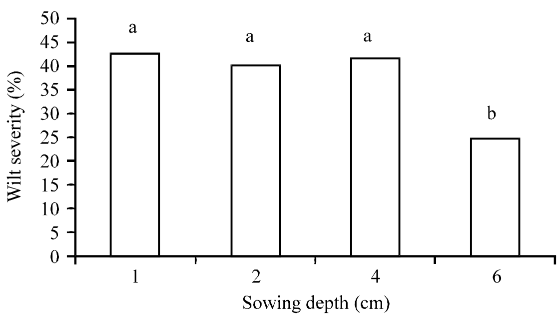

Fig. 3: Effect of sowing depth on the development of wilt disease caused by *Fusarium oxysporum* f. sp. *lentis*. Different letters indicate significant differences among treatments according to least significant difference test (p = 0.05)

While, Laird cv. recorded the lowest wilt (3.2 and 4.2%) in both seasons. On the other hand, Richlea cv. resulted highly seed yield (622.8 and 636.2 kg fed^{-1}) in both seasons, respectively compared with the other tested cultivars followed by Sinaa 1 (582.5 and 614.3 kg $fed.^{-1}$), whereas Eston cv. gave less seed yield (405.3 and 418.0 kg $fed.^{-1}$) followed by Giza 4 (438.3 and 455.3 kg $fed.^{-1}$) in both tested seasons.

Effect of sowing dates: Wilt of Giza 9 was significantly affected by sowing dates (Table 2). The highest average of wilt plants was occurred at the sowing date 1st October (16.4 and 14.5% wilt) in both tested seasons, respectively, while planting at 1st December recorded the lowest wilt (16.4 and 14.5%). On the other hand, also seed yield significantly affected by sowing dates whereas the highest seed yield were recorded when lentil seeds sowing at 1st November in both seasons, where produced 461.2 and 499.3 kg $fed.^{-1}$) followed by sowing date at 1st December (375.2 and 368.1 kg $fed.^{-1}$) while sowing date at 1st October produced the lowest seed yield (352.6 and 344.2 kg $fed.^{-1}$) in both seasons, respectively.

Table 2: Effect of sowing dates on wilt and seed yield of Giza 9 lentil variety under field conditions during seasons 2009/10 and 2010/11

	Season 2009/10		Season 2010/11	
Showing depth	Wilt (%)	Seed yield (kg fed^{-1})	Wilt (%)	Seed yield (kg fed^{-1})
1st October	25.7^{a}	352.6^{b}	29.6^{a}	344.2^{b}
1st November	19.2^{ab}	461.2^{a}	17.9^{b}	499.3^{a}
1st December	16.4^{b}	375.2^{b}	14.5^{b}	368.1^{b}

Different letters indicate significant differences among treatments within the same column according to least significant difference test (p = 0.05)

Table 3: Effect of sowing depth on the development of wilt disease and seed yield under field conditions during 2009/10 and 2010/11

	Season 2009/10		Season 2010/11	
Showing depth (cm)	Wilt (%)	Seed yield (kg fed^{-1})	Wilt (%)	Seed yield (kg fed^{-1})
1	22.4^{ab}	410.1^{c}	19.0^{b}	428.6^{c}
2	19.3^{b}	459.3^{a}	18.7^{b}	499.2^{a}
4	23.1^{ab}	431.9^{b}	24.3^{a}	466.2^{b}
6	26.8^{a}	312.3^{d}	28.4^{a}	328.4^{d}

Different letters indicate significant differences among treatments within the same column according to least significant difference test (p = 0.05)

Effect of sowing depth: Data present in Table 3 show that lentil wilt severity and seed production significantly affected by planting depth in field. Lentil seed planting at depth of 6 cm caused highly wilt and recorded less seed yield compared with the other planting depth, whereas caused 26.8 and 28.4% wilt and produced 312.3, 328.4 kg $fed.^{-1}$ of seed yield in both seasons, respectively. While planting at 2 cm depth recorded the lowest wilt 19.3, 18.7% and produced the highest seed yield (459.3 and 499.2 kg $fed.^{-1}$).

DISCUSSION

Lentil wilt, caused by *Fusarium oxysporum* f. sp. *lentis* is one of the main limiting factors to successful cultivation. Ten fungal isolates were obtained from naturally diseased lentil plants and identified as *F. oxysporum* f. sp. *lentis*. The most isolated fungi were pathogenic towards lentil plants cv. Giza 9, causing wilt disease, *F. oxysporum* f. sp. *lentis* isolate 3, was highly pathogenic isolates caused the highest wilt severity (50.7%). These results are in agreement with those obtained by Hamdi and Hassanein (1996), Bayaa *et al.* (1997), Ahmed *et al.* (2002) and Morsy (2005).

The effect of certain agricultural practices that affect incidence and severity of the disease was investigated. In varietals trials carried out under greenhouse and field conditions showed that all local lentil cultivars (Giza 4, Giza 9, Giza 51, Giza 370 and Sinaa 1) were susceptible towards infection with wilt disease, while the tested Canadian cultivars (Eston, Laired and Richlea) showed highly resistance to the disease. Data are in harmony with those reported by Bayaa *et al.* (1997), El-Garhy (2000), Abdel-Monaim (2002), Morsy (2005) and Sreedharan *et al.* (2010).

Sowing date is considered as one limiting factor for disease incidence and onset and effect seed production in field (Ahmed *et al*., 2002; Akem *et al*., 2004; Landa *et al*., 2004; Tahir *et al*., 2004).The current study revealed that sowing dates have significant effects on lentil wilt disease. Sowing date of 1st December was more suitable to minimize the disease severity but sowing at 1st November produced the highest seed yield therefore preferred of sowing at the first of November and may have to see that the impact of weather. Hwang *et al.* (2000) found that sowing dates of lentil plants play a vital role in the development of lentil root disease.

Lentil seeds sowing at depth 2 cm were the most appropriate the depth of cultivation where gave the least of wilt and produced the highest seed yield under greenhouse and field conditions. While that increase of the planting depth to increase the rate of infection with these fungi. The increased of infection when lentil seeds were planted more than 2 cm may be due to higher moisture levels found deeper in the soil and/or to increase the period of seedling out above the soil surface, exposing it to severe infection with soil borne fungi, (Broscious and Frank, 1986; Sweetingham, 1991). In conclusion, present study demonstrated that sowing depth, sowing date and selected the resistance cultivars can be used for protecting of lentil plants from infection by wilt disease.

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