# Effect of Temperature and Some Media and Biotic Factors on the Growth of *Fusarium oxysporum* f.sp. *lentis*, and its Mode of Seed Transmission

**Erskine, W.[1], Bayaa, B.[2] and Dholli,M.[2]**

(1) International Center for Agricultural Research in the Dry Areas (ICARDA), P.O.Box 5466, Aleppo, Syria. (2) Faculty of Agriculture, Aleppo University, Aleppo, Syria.

## Abstract

**Erskine, W.; Bayaa, B. and Dholli, M. 1990. Effect of temperature and some media and biotic factors on the growth of *Fusarium oxysporum* f.sp. *lentis* and its mode of seed transmission. Arab J.Pl. Prot. 8(1): 37 - 34.**

This study was conducted to determine the effects of some factors, namely temperature (10, 15, 20, 25 and 30°C) and media (potato dextrose agar, lentil dextrose agar and (Czapek), on the growth *in vitro* of *Fusarium oxysporum* f.sp. *lentis* in order to produce sufficient inoculum for the development of a screening technique, as well as to clarify such aspects of disease epidemiology as mode of seed transmission and association with nematodes and antagonistic bacteria.

The optimum temperature for fungal growth was 22°C. Maximum mycelial growth and sporulation were obtained on lentil dextrose agar. *In vitro* studies revealed an antagonistic effect between the fungus and a *Pseudomonas* sp. isolated from infected soil. The following nematode genera were associated with wilt in the field: *Ditylenchus dipsaci, Aphelenchoides* spp. *Aphelenchus* spp., *Helicotylenchus* spp., *Heterodera* spp., *Meloidogyne* spp., *Pratylenchus* spp. and *Tylenchorhynchus* spp. with the former being the most prevalent. The fungus was not present either in the endosperm or under the seed coat of seed from a crop showing wilt symptoms.

**Key words:** wilt, lentil, Syria.

## Introduction

Lentil (*Lens culinaris* Medic) is the most important food legume in Syria. Its productivity is adversely affected by vascular wilt caused by *Fusarium oxysporum* f.sp. *lentis* (Vasudeva and Srinivasan) Gordon, the major pathogen of lentil in Syria (2,6).

Since chemicals are ineffective in vascular wilt control, the use of resistant varieties is the most efficient way to reduce disease losses. Another suggested approach to disease control is through the manipulation of sowing date, which has been found to affect wilt incidence (11,12). Changes in sowing date radically alter the growing environment for the crop, of which temperature is a primary component.

Some of the Fusarium wilts of field, vegetable and ornamental crops are transmitted via seeds internally (1,16); but the information concerning lentil wilt is scanty (7, 13). Lentil wilt was first reported as seed-borne by Fleischmann in 1937, but it is not known whether seed transmission occurs internally or externally.

This study was conducted to determine the effects of some factors (temperature, media and antagonistic bacteria) on fungal growth *in vitro*, in order to produce sufficient inoculum for the development of a screening technique, as well as to clarify such aspects of disease epidemiology as mode of seed transmission and association with nematodes.

## Materials and Methods

**Isolation of the fungus.** Stem samples (4cm above the crown region) were collected from lentil plants of the wilt-susceptible cultivar ILL4605 showing wilt symptoms at Tel Hadya, ICARDA farm, N. Syria in May 1987. Samples were surface-sterilized with chlorox (sodium hypochlorite 5.25%) for 5 minutes and then washed many times with sterilized water. The end 0.5 cm of the stem samples were cut and discarded with the remaining parts cut into 0.5 cm fragments, which were plated with one end inserted into the surface of potato dextrose agar in petri dishes and incubated at 20°C.

**Mycelial growth on various media at different temperatures.** A core (0.5cm diameter) covered with mycelial growth was taken from the margin of an actively growing colony of the fungus and plated in the center of a petri dish (10 cm diameter) containing the following media:

1. Potato Dextrose Agar (PDA).
   PDA (Difco) 39g and Dextrose 20g were dissolved in distilled water and the volume made up to one litre. The pH was adjusted to 7.0 by NaOH 0.1N or HCL 0.1N as a compromise between pH 5 - 6 generally used for fungal growth and pH 8 typical of soil used for lentil in Syria. The solution was sterilized at 120°C for 20 minutes and distributed into sterile plastic petri dishes.
2. Lentil Dextrose Agar (LDA):
   Lentil seeds (65g) were boiled in water for 30 minutes, pressed through cheese cloth to obtain the extract, to which 20g dextrose and 20g agar were added. The volume was made up to one litre, the pH adjusted to 7.0 and the medium sterilized and distributed as above.

3. Czapek medium:

The following were dissolved in distilled water and the volume made up to one litre: 1g $KHPO_4$, 2.9g $NaNO_3$, 0.5g $MgSO_4.7H_2O$, 0.5g KCL, 0.1g $Fe_2(SO_4)_3$, 30g sucrose and 20g agar (Difco). The pH was adjusted to 7.0 and the medium was sterilized and distributed as above.

Three dishes of each medium were incubated at each of the temperature 10, 15, 20, 25 and 30°C with the exception of PDA at 15 and 20°C. After 3,6 and 9 days of incubation colony diameter was measured and sporulation (production of micro-conidia) was assessed microscopically.

The responses to temperature on LDA and Czapek media were investigated with the second degree polynomial equation $y=a+bx+cx^2$(17). The goodness of fit of the mean of the observed values over replicates to the curve was tested by the coefficient of determination $R^2$. The optimum temperature for fungal growth was first obtained by equating the first partial derivative of the equation to zero.

**Detection of fungus within seeds by microscopic examination and planting on growth medium.** Seeds were randomly selected from the produce of a field sown with the susceptible cultivar ILL4605, in which wilt symptoms had been observed at Tel Hadya in 1987. It was not possible to collect seed from plants exhibiting wilt symptoms because they were all barren.

For microscopic examination, seeds (400) were soaked in 5% alkaline solution (50g NaOH, 1g Trypan blue, and 11 distilled water) for 36 hours (9), transfered onto a screen, rinsed with running tap water and scraped into a beaker (150 ml). The seeds were covered with Trypan Blue stain (250 ml lactic acid, 1.625 g Trypan Blue and 500 ml distilled water) and the beaker was heated slowly to allow the contents to boil for 15 minutes. The seeds were emptied onto a screen, rinsed with running tap water, scraped into a bottle, covered with water and then refrigerated until microscopic examination. Two seeds were placed on a slide, a drop of water added, and with a cover slip they were gently crushed by fingertip. The slides were examined under the microscope for the presence of fungal structure in the seed coat and within the endosperm.

To detect the fungus within seeds, 400 seeds were surface-sterilized with chlorox (sodium hypochlorite 5%) for 5 minutes, rinsed in sterilized distilled water, plated on LDA medium with 10 seeds per petri dish and incubated at 20°C for a month.

**Antagonistic effect.** The identification of the antagonistic bacterium was based on its shape, gram reaction, motility using hanging drop, gelatine liquification, pigment production and the nature of its growth (3). The antagonistic effect of the bacterium on *F. oxysporum* was estimated from the inhibition zone of the Fusarium colony.

**Collection and separation of nematodes from field soil.** Soil samples were collected from plots of lentil differing in wilt symptom incidence at Tel Hadya in May 1987.

From each plot a total of two kg soil from 25 cm depth was collected, sampling from three or more sites/plot. The nematodes were separated from the soil using Baerman funnel and sugar centrifugal flotation techniques (10).

Nematode identification was based on their morphology and anatomy (stylet, stylet knobs, number of ovaries, vulva position, tail shape and cephalic structure) (4, 8, 17).

## Results

**Effect of different media and temperature on fungal growth.** The mean diameters of the fungal colonies after nine days on LDA and Czapek incubated at 10, 15, 20, 25 and 30°C and PDA incubated at 10,25 and 30°C are shown in Figure 1. It is clear that growth on LDA was greater than on the other two media at temperature of 20 - 30°C. Microscopic observation revealed that the sporulation on LDA was higher than on the other two media, possibly due to the presence of a growth stimulating substance(s) in the lentil seed.

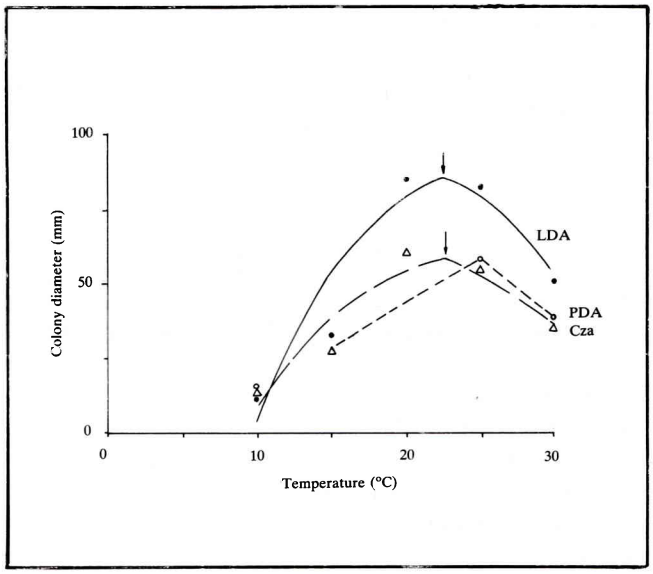


**Figure 1.** The effect of temperature on fungal growth measured by colony diameter (mm) nine days after incubation on three growth media (LDA •, PDA ○, Czapek △). The response curves of the quadratic equations for LDA and Czapek are drawn and temperature optima shown by arrows.

The response of fungal growth to temperature on LDA medium was well described by the quadratic equation $y= -169.8 + 22.31x - 0.49x^2$ as attested by the high coefficient of determination $R^2=0.86$. The optimum temperature for fungal growth was calculated as 22.6°C by the first derivative of the quadratic equation. The maximum were also calculated for earlier growth after 3 and 6 days and the best temperature for fungal growth were both 22.3°C.

The response of fungal growth on Czapek to temperature was also well described by a quadratic equation $y= -96.8 + 13.55x - 0.303x^2$ giving a coefficient of determination $R^2=0.87$. The optimum temperature for growth on this medium was calculated as 22.4°C. In view of the low number of temperature levels tested, it was not appropriate to describe the response on PDA medium to temperature quadratically.

**Detection of fungus within seeds.** Microscopic examination of processed seed failed to detect fungal stucture in either the seed coat or the endosperm of any of the 400 seeds ex-

amined. Surface-sterilized seeds plated onto LDA for a month also did not produce any growth of *Fusarium,* although the percentage of germination of the seeds was about 60%.

**Antagonistic effect.** The antagonistic bacterium was identified as *Pseudomonas sp.* on the basis of its rod shape with round ends, motile nature, gram negative reaction, negative reaction to gelatine liquification and optimum temperature around 25°C. On agar medium the colony was circular and raised with an entire margin and a peripheral, fluorescent zone; whereas on PDA the colony was thin, gray to brown in color and slimy. The cultures emitted a disagreable odour.

The antagonistic effect of the Pseudomonas bacteria on *F. oxysporum* was shown by a ratio of maximum to minimum radii of the colony of 3.

**Associated nematodes.** Both separation techniques yielded the following nematode genera: *Ditylenchus dipsaci, Pratylenchus* spp., *Aphelenchus* spp. *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Aphelenchoides* spp. *Meloidogyne* spp. and *Heterodera* spp. Although nematode counts were not done, visual observation showed clearly that the most frequently observed species was *D. dipsaci* and that the nematode population increased with wilt incidence.

## Discussion

In the vascular wilts of plants caused by *Fusarium* spp. the optimum temperature for the pathogen is often close to that favouring disease development (5). Thus, knowledge of the effect of temperature on the growth of *F. oxysporum* contributes to an understanding of disease epidemiology. Previous research indicated that temperatures between 17 and 30°C favour the disease on lentil (13). Our results are in general agreement with this and show that the growth of *F. oxysporum* f.sp. *lentis* is at its maximum at 22°C. The optimum temperature was confirmed by the results on both LDA and Czapek.

In Syria, symptoms of lentil wilt first appear in the field during the reproductive stage of crop growth in April. The late manifestation of disease symptoms in the field may be because fungal growth is restricted prior to April by the prevailing sub-optimal temperatures. The long-term average temperatures at ICARDA, Tel Hadya, N. Syria are 7.8, 6.9, 8.2 and 10.8°C for December, January, February and March, respectively, rising to 15.5°C for April and reaching 20.5°C in May. However, it is not certain that the relation is as simple as it appears, and in some wilt diseases temperature affects the host and, probably, other soil organisms, which influence the disease (5).

Sowing date is known to affect wilt incidence in many cultivated plants including lentil (12,16). In Syria, late sowing in February compared with normal sowing time (December) increased disease incidence from an average of 0.6% wilted plants to 9.3% (11). The delayed sowing resulted in a greater proportion of the growth cycle of the crop being at optimum or near-optimum temperature for fungal growth than the normal sowing. In India the converse was noted with delayed sowing reducing disease incidence (12); this was associated with low soil temperature in the period following the late sowing dates of November 30 and December 15, which may have saved the lentil from wilt.

This study found no evidence of the presence of *F. oxysporum* inside seeds from an infected field. Thus, the seed-borne nature of the disease is either via an external contamination of the seed, possibly following threshing of pods infected saprophytically by the fungus while in post-harvest heaps in the field, or via trash such as infected stem, soil, etc. which may carry high levels of the pathogen.

The use of microorganisms as biological control agents of *F. oxysporum* on lentil has been studied (15). This research showed an antagonistic effect of a *Pseudomonas* sp. isolated from infected soil on *F. oxysporum* of lentil, a subject worthy of further study.

The study revealed an association between wilt incidence and the occurrence of nematodes, which needs further quantitative investigation. Plants previously invaded by nematodes may predispose a host to infection by a secondary pathogen either directly through increased pathogen penetration following wounding by the primary parasite or through increased metabolic leakage that might affect the ratio of rhizosphere/rhizoplane microorganisms (14).

## Acknowledgments

The authors are grateful to Messrs. M. Dababo and N. Chanaa students at the Agricultural Faculty, University of Aleppo for their technical assistance.

## الملخص

**ارسكين، ويلي، بياعة، بسام وماجد ضلي. 1990. أثر درجات الحرارة وبعض المستنبتات والعوامل غير الحيوية في نمو الفطر *Fusarium oxysporum* f.sp. *lentis* وبحث امكانية انتقاله بالبذور. مجلة وقاية النبات العربية 8(1): 37 - 34.**

استهدفت الدراسة تحديد أثر بعض العوامل، وبخاصة درجات الحرارة (10، 15، 20، 25، 30°م) والمستنبتات (بطاطا - دكستروز - آجار، مستخلص العدس، آجار، وتشابك) في نمو فطر *Fusarium oxysporum* f.sp. *lentis* تحت ظروف المختبر لانتاج لقاح كاف من الفطر بغية تطوير تقنية تقويم لسلالات العدس. وإلى توضيح بعض نواحي وبائية المرض كإمكانية انتقاله بوساطة البذور، وأثر الديدان الثعبانية وبعض الجراثيم المضادة. كانت درجة الحرارة المثلى لنمو الفطر 22°م. وتم الحصول على أكبر كمية من الغزل الفطري والتبوغ على مستنبت مستخلص العدس آجار. وقد أظهرت الدراسات المختبرية علاقة تضاد بين الفطر وجرثوم يتبع جنس *Pseudomonas* تمّ عزله من التربة الموبوءة. وقد ترافق المرض

حقلياً بالديدان الثعبانية التالية، *Ditylenchus dipsaci*, *Aphelenchoides* spp. *Aphelenchus* spp., *Helicotylenchus* spp., *Heterodera* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Tylenchorhynchus* spp., وكان النوع الثاني هو الأكثر تردداً.

وأخفق فحص بذور مجموعة من نباتات تبدي أعراض الذبول في كشف الفطر سواء في سويداء البذور أو تحت غلافها.

**كلمات مفتاحية:** ذبول، عدس، سوريا.

## References


1. Baker, K.F., 1981. Biological control. Pages 523 - 561 in **Fungal wilt diseases of plants.** Eds: Mace M.E., Bell, A.A. and Beckmann, C.H., Academic Press, New York. 640 P.
2. Bayaa, B., Erskine, W. and Khoury, L., 1986. Survey of wilt damage on lentils in Northern Syria. J. Pl. Prot. 4(2):119 - 118.
3. Buchanan, R.E. and Gibbons N.E. (Eds). 1974. **Bergey's manual of determinative bacteriology.** 8th Edition. William and Wilkins. Baltimore. 1246 p.
4. CIH. **Description of Plant Parasitic Nematodes** (sets 1 - - 7), Commonwealth Institute of Helminthology.
5. Cochrane, V.W., 1958. **Physiology of Fungi.** John Wiley and Sons, Inc. New York. 524 P.
6. El Ahmed, M. and Mousalli, N., 1986. Lentil wilt in South Syria. Arab J.Pl. Prot. 4(1):30 Abstract.
7. Fleischmann, R., 1937. Observations on lentil wilt. Pflanzenbau 14(2) 49 - 56.
8. Franklin, M.T., 1971. Taxonomy of Heteroderidae. Pages 139 - 162 in **Plant Parasitic Nematodes** Vol. 1. Morphology, Anatomy, Taxonomy and Ecology. Eds. Zuckerman, B.M., Mai W.F., and Rhode, R.A., Academic Press, New York.
9. Halisky, P.M., Saha, D.C., Bayaa, B.O., Funk, C.R., 1986. Fungal endophytes in *Festuca* and *Lolium.* Can.J. of Plant Pathology 8:350 - 351.
10. Hooper, D.J., 1986. Extraction of free-living stages from soil. Pages 5 - 30 in **Laboratory Methods for Work With Plant and Soil Nematodes.** Ed. Southey, J. F. Reference Book 402 Ministry of Agriculture, Fisheries and Foos, YMSO, London.
11. ICARDA, 1987. Annual Report of Food Legume Improvement Program, International Center for Agricultural Research in the Dry Areas, Aleppo, Syria.
12. Kannaiyan, J. and Nene, Y.L., 1975. Note on the effect of sowing date on the reaction of 12 lentil varieties to wilt disease. Madras Agri. J. 2:77 - 79.
13. Khare, M.N., 1981. Diseases of lentils. Pages 163 - 172 in **Lentils.** Eds. Webb, C. and Hawtin, G.C., CAB and ICARDA.
14. MacDonald, D., 1979. Some interactions of plant parasitic nematodes and higher plants. Pages 157 - 179 in **Ecology of Root Pathogens.** Eds. Krupa, S.V., and Dommergues, Y.R. Elsevier Sci. Pub. Comp. 281 p.
15. Mehrotra, R.S. and Claudius, G.R., 1972. Biological control of the root rot disease of lentils *Lens culinaris* Med. Plant and Soil 36:657 - 664.
16. Nelson, P.E., 1981. Life cycle and epidemiology of *Fusarium oxysporum.* Pages 51 - 80 in **Fungal Wilt Diseases of Plants.** Eds. Mace, M.E., Bell, A.A., and Beckman, C.H. Academic Press. 640 p.
17. Sher, S.A., 1966. Revision of the Hoplolaiminae (Nematoda) VI *Helticotylenchus* Steiner. Nematologica 12:1 - 56.
18. Snedecor, G.W. and Cochran, W.G., 1978. **Statistical Methods.** 6th edition. Iowa State University Press, Iowa. 193 p.