

# Symptomless Carriers of the Causal Agent of Tomato Wilt Pathogen

A. Fassihiani<sup>1</sup>

## ABSTRACT

A study was carried out to determine whether naturally-occurring weeds and other cultivated plants in tomato growing regions could act as symptomless reservoirs of infection to *Fusarium oxysporum f. sp. lycopersici*. A number of *F. oxysporum* isolates from weeds and oubergines were used in this investigation. A susceptible tomato cultivar was used for comparison. The plants were artificially inoculated at the five to six leaf stage by root dip method. Only tomato showed wilt symptoms and died three weeks after inoculation. These isolates were identified as *F. o. f. sp. lycopersici*. Weeds including *Amaranthus retroflexus*, *Amaranthus. sp.*, *Chenopodium album*, and aubergines were colonized to various degrees and determined as symptomless carriers. Therefore, in infested areas, aubergines should not be rotated consecutively with tomatoes and proper measures should be adopted to control the weeds.

Keywords: *Lycopersicom esculentum* Mill , Symptomless carrier , *Fusarium oxysporum f. sp. lycopersici* .

## INTRODUCTION

Vascular wilt *Fusaria* can survive in the absence of susceptible host plants by invasion and colonizaion of other plants that show no symptoms of the disease [1, 8, 17, 18]. These plants are often described in the literature as symptomless carriers [1,8], non-susceptible hosts [7,12], or non-hosts [3]. Katan [8] studied symptomless carriers of *Fusarium oxysporum* Schlecht. f. sp. lycopersici (Sacc.) Synd. and Hans (Fol). A number of weeds growing in soil naturally infected with the pathogen did not show wilt symptoms, although they harbored the pathogen. These weeds included the genera of *Oryzopsis*, *Digitaria*, *Amaranthus*, and *Malva*. The plants were also colonized when planted under controlled conditions in

soil naturally infested with race 1 and 2 of the pathogen, or when artificially inoculated with isolates of the pathogen from roots of weeds or tomatoes [*Lycopersicom esculentum* Mill.]- In a more recent study, Benincasa (a wild cucurbit), also a non-host, showed no wilt symptoms following inoculation with *F. o. f. sp. melonis*. The fungus occurred in the root, but never in the stem [3].

In Hormozgan province, where *Fusarium* wilt of tomato can be a serious problem [5], aubergine (*Solarium melongena* L.) is widely used in rotation with tomato. In addition, several species of common weeds including *Amaranthus retroflexus*, *Amaranthus. sp.*, and *Chenopodium album* occur in these crops. The following study was conducted to determine whether naturally occurring weeds and

<sup>1</sup> Plant Pests and Disease Research Department, Fars Agricultural Research Centre, P.O. Box 73415-121, Zargan, Islamic Republic of Iran.

aubergines could act as symptomless carriers of *Fol*

## MATERIALS AND METHODS

### Culture of the Host and Pathogen

Aubergine seeds cv. Long Purple were germinated in compost at 20-25°C in a glasshouse for two weeks. When emergence was complete, seedlings were pricked off into 12 cm pots, usually after the first true leaf had clearly emerged. Seedlings were selected for uniformity and deformed ones discarded. At the two leaf stage, seedlings were transferred to 12 cm pots and kept for a period of 5-6 weeks until the sixth leaf stage when these were suitable for inoculation. From the fourth week onwards, the plants were supplied with 50 ml Hoagland solution on alternate days. Seeds of weeds, *A. retroflexus*, *A. sp.* and *C. album* were also sown in compost.

Isolates of *F. oxysporum*, obtained from aubergines and weeds, were used in the present study. These isolates were identified as *Fol* race 1 using a method similar to that previously described [6]. Conidial suspensions of *Fol* isolates were prepared by adding 10 ml aliquots of sterilized distilled water (SDW) to a one week-old potato dextrose agar (PDA) culture, and agitating with a glassrod to remove spores. From this suspension, 200  $\mu$ l was added to PDA plates and incubated for one week at 28±2°C. Conidia were washed off the agar surface by agitating with 10 ml SDW by a glassrod. The suspension was passed through four layers of muslin and washed twice with SDW by centrifugation.

Plants were inoculated at the five to six leaf stage by the root dip method. Plants were de-potted and the root system was immersed in spore suspension ( $10^6$  spores ml<sup>-1</sup>) for five minutes. After inoculation, plants were re-potted in the same compost and returned to the glasshouse.

### Estimation of Fungal Propagules in Plants

*Fusarium* propagules (spores and mycelial fragments) in the plants' root tissues were determined at weekly intervals for four weeks after inoculation using the comminution dilution technique based on the method of Pegg and Street [16]. Plants were uprooted and the compost removed by a tap water rinse. Roots were further washed by a similar method to that of Banihashemi and De Zeeuw [2]. One gram of root sample was comminuted in 10 ml SDW using a Sorvali Omnimixer at 10000 rpm in an ice bath. Serial dilutions were made for susceptible ( $10^4$ ), and nonhost ( $10^2$ ). 0.5 ml suspension was pipetted into sterilized dishes, and 15 ml PCNB-pepton agar (14) which had been held at 50°C was added. The plates were incubated for 2-4 days at 28°C. Ten plates were used for each dilution. *Fol* colonies were expressed as numbers of colony forming units (CFU) per gram of root fresh weight.

In the second experiment, 1 cm of the tap root (2.5 cm above the point of inoculation) and 1 cm of hypocotyl (2 cm below the cotyledonary node) were severed, the cortex removed aseptically, and the pericycle cut into the smallest pieces possible with a razor blade. The degree of colonization in each section was estimated using the same technique as mentioned above, the comminution time for lateral root, tap root, and hypocotyl was 30, 30, and 60 seconds, respectively.

### Effect of Inoculum Concentration on Root Colonization

Plants were prepared for inoculation as previously described. Four groups of aubergine plants were inoculated with  $5 \times 10^6$ ,  $5 \times 10^4$ ,  $5 \times 10^3$ , and  $5 \times 10^2$  spores ml<sup>-1</sup> of *Fol* race 1 in SDW. The fifth group was treated with SDW. The numbers of CFU g<sup>-1</sup> root fresh weight, as well as the heights of the plants

were determined four weeks following inoculation.

## RESULTS

### Determination of Fungal Growth in Host and Non-Host Plants.

Results in Table 1, show that the roots of different plants are colonized to various degrees. In the first week after inoculation, similar numbers of propagules were recovered from the roots of host and non-host plants indicating that these plants were colonized to the same extent at the earlier stage. In the third and fourth weeks, after inoculation, larger numbers of propagules were obtained from the roots of susceptible tomato plants than from roots of non-susceptible hosts.

Root colonization of solanaceous plants (aubergine, tomato) and *Chenopodium* increased with time, up to the third week. In the case of the two *Amaranthus* weeds, colonization increased, at least, up to the end of the fourth week when the experiment was terminated. Of all the plant species inoculated, only tomato plants showed symptoms of the disease and wilted. Aubergine was slightly stunted, other weeds remained symptomless.

Table 1. Population of *Fusarium oxysporum f. sp. lycopersici* race 1 in the roots of susceptible tomato and non-hosts

plant species	No. of propagules <sup>a</sup> g <sup>-1</sup> fresh rootx10 <sup>3</sup>			
	Week after inoculation			
	1	2	3	4
<i>Lycopersicon esculentum</i>	8.8	10.9	68.8	70.5
<i>Solanum melongena</i>	9.5	11.5	15.6a	8a
<i>Chenopodium album</i>	0.2	4.7	13.6a	13.2a
<i>Amaranthus retroflexus</i>	3	2.6a <sup>b</sup>	5a	15.3a
<i>Amaranthus. sp.</i>	1.8	2.4a	2a	7.4a

a Average of three plant replicates.

b Significantly lower number of propagules compared to tomato at the same sampling date LSD at 0.01 level = 7.633

### Effect of Inoculum Concentration on Aubergine Colonization

The reaction of aubergine to increasing levels of inoculum concentration is presented in Figure 1. Increasing inoculum concentration had no significant effect on the level of colonization except at  $5 \times 10^6$  (Fig. 1b). A similar relationship between the effect of increased inoculum concentration and plant height was observed. Only the highest concentration ( $5 \times 10^6$  spore ml<sup>-1</sup>), significantly decreased the height of the plants in comparison to the control (Fig. 1a).

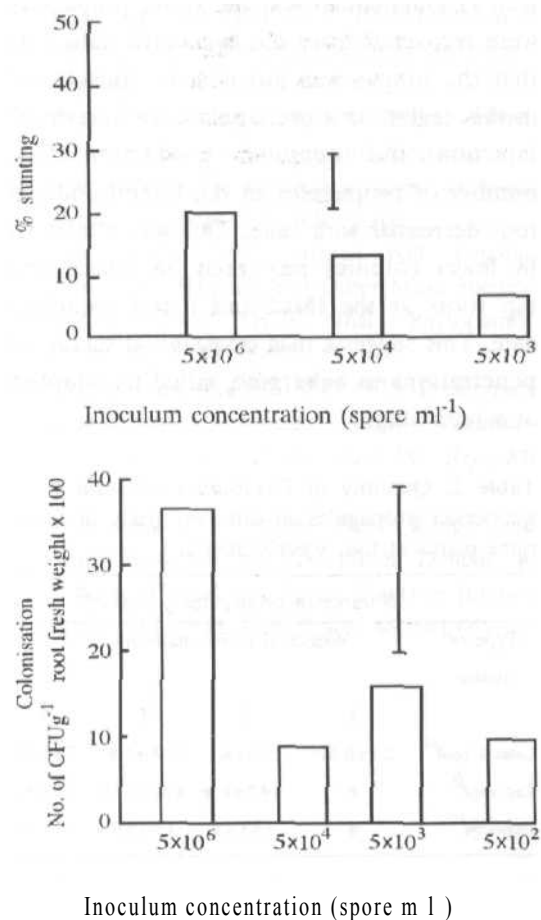


Figure 1. Effect of inoculum concentration of *Fusarium oxysporum f. sp. lycopersici* on (a) stem height and (b) colonization of aubergine cv. Long Purple four weeks after inoculation. Means of five plant replicates. Vertical bars indicate LSD (P=0.05).

### Estimation of *Fol* in Aubergine Roots and Shoots

An attempt was made to determine whether different parts of the plant had a similar pattern of colonization.

Results presented in Table 2 show that the level of colonization at each date during the four week intervals was always greater on the lateral roots than on the tap root and hypocotyl. The quantity of fungal propagules decreased with increasing distance from the site of inoculation. There was slight vascular browning at the base of the tap root when seen in longitudinal section. Fewer propagules were recovered from the hypocotyl, indicating that the fungus was not able to consolidate in this region as a prerequisite for successful infection and symptom production. The number of propagules in the lateral and tap root decreased with time. This was illustrated by fewer colonies recovered on lateral and tap roots at the third and fourth sampling date. This suggests that many initial successful penetrations in aubergine, failed to establish at a later stage.

**Table 2. Quantity of *Fusarium axysporum* f. sp, *lycopersici* propagules in different parts of aubergine plants at four weekly intervals.**

Type of tissue	Number of propagules g <sup>-1</sup> tissue <sup>a</sup>			
	Weeks after inoculation			
	1	2	3	4
Lateral root	2.3±0.34*	17.5±4.1	5.49±1.8	7.1±1.8
Tap root <sup>b</sup>	0	19.2±1.9	4.80±0.73	5.6±0.9
Hypocotyl <sup>c</sup>	0	5.6±0.8	4.6±0.6	7.2±0.9

a Number of propagules g<sup>-1</sup> fresh lateral rootx10 .

b Number of propagules cm<sup>-1</sup> of tap root cut 2.5 cm above the inoculation point.

c Number of propagules cm<sup>-1</sup> of hypocotyl cut 1 cm below the cotyledonary node.

D Each point is the average of five plant replicates.

\* Standard error

### DISCUSSION

This investigation revealed that several nonhost plants, were colonized by *Fusarium* wilt of tomato at various degrees and can act as symptomless carriers. The difference in colonization between hosts and non-hosts, was because the pathogen failed to colonize the main stele system of the non-host while in the tomato host more propagules were recovered from the roots due to a systemic invasion and proliferation in the vascular system. Other workers [4, 8, 9, 10] also found similar results with wilt fungi such as *Fusarium* and *verdcillium* which infecting many non-host plants.

It has been suggested that the invasion of non-hosts by *Fusarium* which results in root infection, but is not accompanied by the production of any symptoms, is due to the inability of the pathogen to produce toxins, or enzymes, in non-host plants [13,15).

The effect of increasing levels of inoculum concentration on root colonization of aubergine by *Fol*, indicated that only the highest concentration, 5x10<sup>6</sup> spore ml<sup>-1</sup>, significantly reduced the height of the plants by 19% . At the same spore concentration, root colonization was significantly higher when compared to the plants inoculated with lower spore concentrations. A similar result was obtained by Katan [8] who found that concentrations below 1x10<sup>6</sup> spores ml<sup>-1</sup> were ineffective in decreasing aubergine stem height. At 1x10<sup>6</sup> spore ml<sup>-1</sup> stem height was reduced by 25% and 18% in two separate experiments.

Woolliam [ 19] also reported similar stunting to various degrees in weeds, inoculated with *V. dahliae*. With *F. O. f. sp. niveum* both squash and watermelon, showed increased susceptibility as the concentration of inoculum increased from 10<sup>3</sup> to 10<sup>6</sup> microconidia ml<sup>-1</sup> . Cultivars with a high level of resistance were unaffected by increasing inoculum concentration [11].

## REFERENCES

1. Armstrong, G.M. and Armstrong, J.K. 1948. Non-susceptible Hosts as Carriers of Wilt Fusaria. *Phytopathology*, 38: 808-826.
2. Banihashemi, Z. and De Zeeuw, D.J. 1969. Two Improved Methods for Selectively Isolating *Fusarium Oxyspomm* from Soil and Plant Roots. *Plants Dis. Rep.*, 53: 589-591.
3. El Mahjoub, M. 1985. Susceptibility Varietale du Melon a la Fusariose Vasculaire: Approche Biochimique et Ultrastructurelle. These Doct. Etale. Univ. Bretagne occidentale., PP. 171.
4. Hvang, G. and Gleeson, A.C. 1973. Observation of Origin and Nature of *Verticillium Dahliae* Colonising Plant Roots. *Aust. J. Agric. Sci.*, 26: 151-161.
5. Fasihiani, A. 1985. Occurrence of *Fusarium* Wilt of Tomato in the Hormozgan Province of Iran. *Iran. J. Plant Path.*, 21: 9-11.
6. Fasihiani, A. 1992. The Physiological Race of *Fusarium Oxyspomm* f. sp. *Lycopersici* in Hormozgan Province of Iran. *Iran. J. Plant pathology*, 78: 19-26.
7. Health, M.C. 1980. Reaction of Nonsusceptibles to Fungal Pathogens. *Ann. Rev. Phytopathol.*, 18: 211-236.
8. Katan, J. 1971. Symptomless Carriers of the Tomato Wilt Pathogen. *Phytopathology*, 61: 1213-1217.
9. Lacy, M.I. and Homer, C.E. 1966. Behavior of *Verticillium Dahliae* in the Rhizosphere and on Roots of Plants Susceptible, Resistant, and Immune to Wilt *Ibid.*, 56: 427-430.
10. Levy, J. and Isaac, I. 1976. Colonisation of Host Tissue of Varying Resistance to *Verticillium Dahliae*. *Trans. Br. Mycol. Soc.*, 67: 91-94.
11. Martyn, R.D. and McLaughlin, R.J. 1983. Effects of Inoculum Concentration on the Apparent Resistance of Watermelon to *Fusarium Oxyspomm* f. sp. *niveum*. *Plant. Dis.*, 67: 493-495.
12. Matta, A. 1971. Microbial Penetration and Immunization of Uncongenial Host Plants. *Ann. Rev. Phytopathol.*, 9: 387-410.
13. Mussell, H.W. and Green, J.R. 1970. Host Colonization and Polygalacturonase Production by Two Trachcomycotic Fungi *Phytopathology*, 60: 192-195.
14. Nash, S.M. and Synder, W.C. 1962. Quantitative Estimations by Plate Counts of Propagules of the Bean Root Rot of *Fusarium* in Field Soils. *Ibid.*, 52: 567-572.
15. Pegg, G.F. and Parry, D.W. 1983. Infection of lucerne (*Medicago sativa*) by *Fusarium species*. *Ann. Appl. Biol.*, 103: 45-55.
16. Pegg, G.F. and Street, P.F.S. 1984. Measurement of *Verticillium Albo-atrum* in High and Low Resistance Hop Cultivars. *Tram. Br. Mycol. Soc.*, 82: 99-106.
17. Stover, R.H. 1962. Fusarial Wilt (Panama Disease) of Bananas and Other *Musa* Species. Commonwealth Mycol. Inst. Phytopathol. Paper, No. 4, PP. 117.
18. Waite, B. and Dunlap, V.C. 1953. Preliminary Host Range Studies With *Fusarium Oxyspomm* f. sp. *cubense*. *plant Dis. Rep.*, 37: 79-84.
19. Woolliams, G.E. 1966. Host Range and Symptomatology of *Verticillium Dahliae* in Economic Weed and Native Plants in Interior British Columbia. *Can. J. Sci.*, 46: 561-669.

## میزبان‌های بدون علائم، عامل بیماری پژمردگی فوزاریومی گوجه‌فرنگی

### چکیده

در این بررسی تعدادی از گیاهان زراعی و علف‌های هرز جمع‌آوری شده از مزارع گوجه‌فرنگی در استان هرمزگان به عنوان میزبان‌های بدون علائم قارچ *Fusarium oxysporum f.sp. Lycopersici* مورد مطالعه قرار گرفته است. برای این منظور قارچ *F. oxysporum* جدا شده از گیاهان بادمجان (*Solanum melongena*)، تاج خروس (*Amaranthus retroflexus*) و سلمه تره (*Chenopodium album*) تحت شرایط گلخانه به گیاهچه گوجه‌فرنگی و علف‌های هرز از طریق ریشه، مایه‌زنی گردید. قارچ‌های مزبور تنها بر روی گیاهچه گوجه‌فرنگی علائم بیماری را ایجاد نمودند و پس از سه هفته منجر به مرگ آن گردیدند. این نتیجه نشان داد که تمام قارچ‌های جدا شده، *F.o.f.sp. lycopersici* می‌باشد. ریشه سایر علف‌های هرز مانند تاج خروس و سلمه تره و بادمجان با درجات متفاوت مورد حمله قرار گرفتند ولیکن علائمی ایجاد نکردند و در نتیجه نسبت به این قارچ، میزبان‌های بدون علائم محسوب می‌شوند. بنابراین در مناطق آلوده بایستی از کشت متناوب گوجه‌فرنگی و بادمجان خودداری و به روش مطلوبی با علف‌های هرز مبارزه کرد.