

THE COMBINED EFFECT OF *FUSARIUM OXYSPORUM* F. SP. *CEPAE* AND *TRICHODERMA HARZIANUM* ON THE HISTOLOGY OF ONION ROOTS

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Abstract

Effect of *Fusarium oxysporum* f. sp. *cepae*, *Trichoderma harzianum* and their combined effect on the anatomical structure of onion roots was followed up after 12, 18, 24 hrs and 3, 5, 7, 10 days from inoculation with *T. harzianum*. It revealed that the mycelial growth of *T. harzianum* surrounded the epidermal tissues of the root without penetration. The epidermis, cortex layers and the vascular bundles, were normal in their structure.

No anatomical changes were noticed in the roots inoculated with *Fusarium* up to 24 hrs following inoculation. Three days after inoculation the fungus penetrated the epidermal layer towards the ground tissue. Five days after inoculation it destroyed the cortex tissue with a noticeable invasion of the fungal mycelium through the cortex cells either intercellularly and/or intracellularly. The mycelial mats aggregated in the pericycle layer and progressed towards the vascular bundles. After ten days it completely destroyed the tissues which was occupied with the fungal chlamydospores.

Root tissues inoculated with *F. oxysporum* f. sp. *cepae* and *T. harzianum* after 12, 18, 24 hrs and 3, 5, 7, 10 days looked as the control. The epidermis, cortex layers and vascular bundles were normal in their structure and seemed slightly hypertrophic.

INTRODUCTION

Structural changes of onion roots during parasitism of the biocontrol agent of *Tri-*

Trichoderma harzianum and / or *Fusarium f. sp. cepae* infection were reported by some investigators. Abawi and Lorbeer (1971) found that *F. oxysporum f. sp. cepae* invaded roots by both direct penetration and /or through wounds. Dabash (1976) added that after penetration, the hyphae of the pathogen grow both intercellularly and intracellularly in the cortical parenchyma cells.

Kodama (1983) reported that the mycelial mass of *F. oxysporum f. sp. cepae* was found in the dead tissue of cortex and parenchyma of stem plate and chlamydospores were formed in the collapsed root. Rod (1984) indicated that *Trichoderma* spp. isolated from onion infected by *Sclerotium cepivorum* and *F. oxysporum* had an antagonistic effect on the pathogens *in vivo*.

The present work was carried out to study the effect of infection with *F. oxysporum f. sp. cepae* and /or *T. harzianum* as well as their combination on the tissues of onion seedling roots. The antagonistic effect between both fungi on the root tissues was also studied.

MATERIALS AND METHODS

Seeds of onion "improved Giza 6 cultivar" were surface sterilized in 4% sodium hypochlorite solution for two minutes, then washed several times with sterile distilled water. Seeds were then germinated on sterilized wet filter paper in Petri dishes at room temperature. Seedlings of five days old were placed in contact with the fungal hyphae of *Fusarium oxysporum f. sp. cepae* and /or *Trichoderma harzianum* grown on potato Dextrose agar (PDA) medium. The combined effect of *T. harzianum* and *F. oxysporum f. sp. cepae* was also studied in Petri dishes. Onion seedlings of 5 days old were placed adjacent to fungal discs of both *F. oxysporum f. sp. cepae* and *T. harzianum*. Roots were taken individually at intervals of 12, 18, 24 hours and 3, 5, 7, 10 days after inoculation with the aforementioned fungi. Roots were killed and fixed in formalin acetic acid alcohol (FAA) solution (1:1:18) for at least 36 hrs. Dehydration, infiltration, embedding and mounting procedures were followed according to Johansen (1958). Sections were stained with a combination of safranin and light green according to the method described by Sass (1958). All sections were mounted in canada balsam and examined microscopically.

RESULTS AND DISCUSSION

The histological effect of *F. oxysporum* f. sp. *cepaе* or *T. harzianum* and the combined effect of both on onion roots showed that the root tissue seemed to be normal in feature and size after 12, 18 and 24 h. (Fig. 1).

T. harzianum showed no effect on onion root seedlings except a little hypertrophic effect that caused some elongation in the cortical cells after 24 hrs. Misaghi (1982) reported that attraction and penetration processes of plants by pathogens during the first few hours as well as the infection were influenced by a number of environmental conditions, which might affect the pathogen, the host, or host-pathogen interaction.

Three days after inoculation (Fig. 2), *F. oxysporum* f. sp. *cepaе* showed a progressive advance in the onion seedling tissues where photographs showed little evidence of distortion in the cell wall of the epidermal layer. In general, the fungus appeared to facilitate its penetration through enzymes, toxins and mechanical forces or combination, as was claimed by Misaghi (1982) with respect to *F. oxysporum* causing vascular diseases. *T. harzianum* on the other hand, remained surrounding the epidermal tissues. The cortex and vascular bundles appeared to be as the check.

After five days from inoculation (Fig. 3), there were very wide variations in the subsequent pattern of development and colonization of the host tissues by the fungus. *F. oxysporum* might be first restricted to the epidermal layer of the root, then the pathogen colonized the parenchyma of ground tissues and cortex, and it made further growth into the vascular bundles and pith. However, plants treated with *T. harzianum* seemed to be with much conidiospores surrounding the epidermal tissue. The plants that have been inoculated with the two fungi *F. oxysporum* f. sp. *cepaе* and *T. harzianum* appeared as those of the control. Thus, the presence of *T. harzianum* counteracts the effect of *F. oxysporum* f. sp. *cepaе* and is considered of great value in biological control. Davis (1966) indicated that mycelia were found in root vessels after 3-5 days from inoculation with *Fusarium* wilt fungi. Windham *et al.* (1986) concluded that *Trichoderma* spp. produced growth regulating factors that increased tomato plant growth over that of the control.

The results after seven days of inoculation showed that *F. oxysporum* f. sp. *cepaе* caused a marked deterioration of the root and many cavities were evident in cortex layers surrounding bundles (Fig. 4). Toxins and enzymes that are produced by

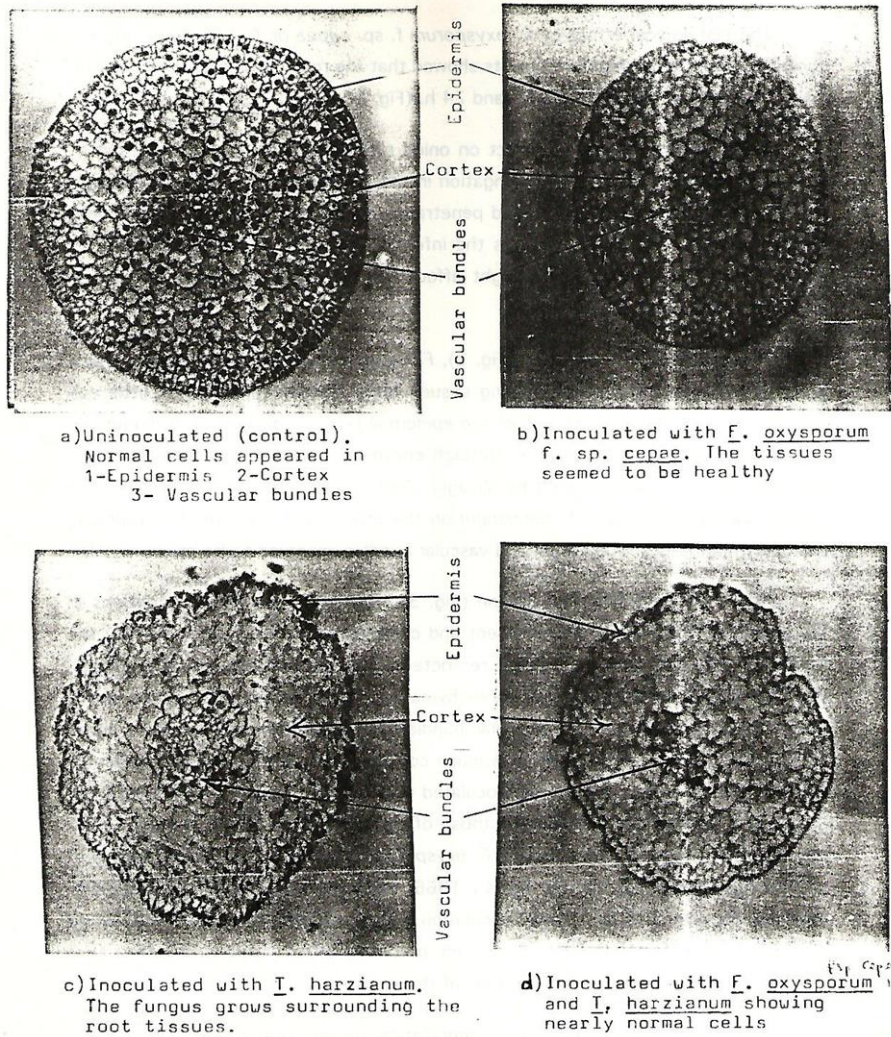


Fig. (1) : Transverse sections of onion root after 24 hrs from inoculation (x 100)

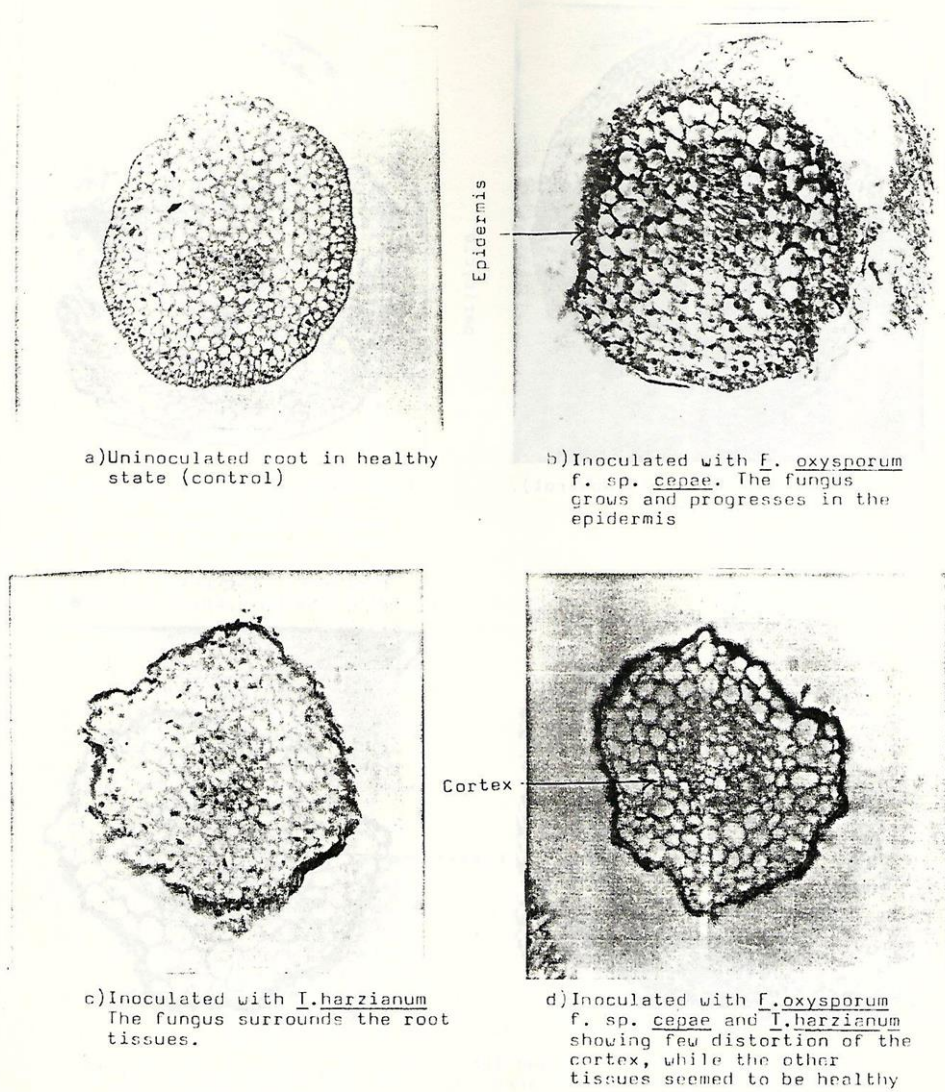
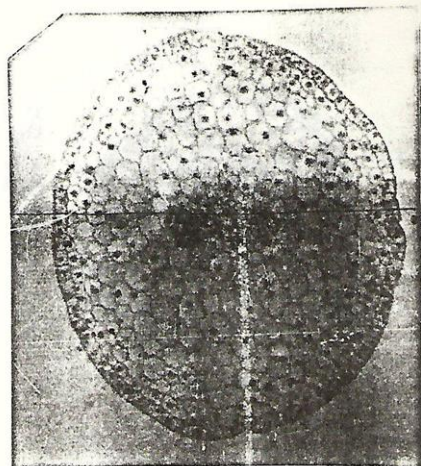
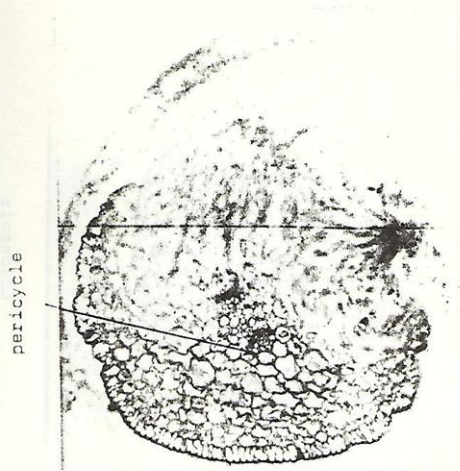


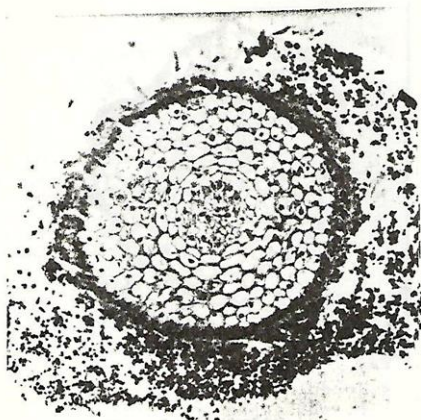
Fig. (2) : Transection of onion root after 3 days from inoculation (x 100)



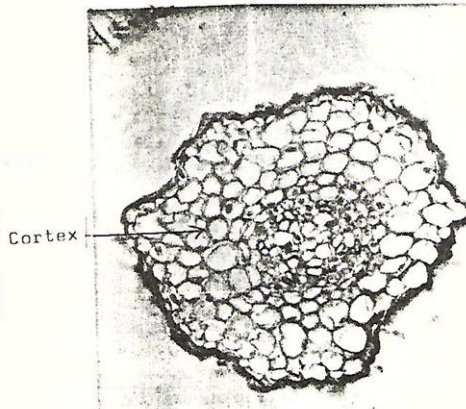
a) Uninoculated root (control).
Normal tissues.



b) Inoculation with *F. oxysporum*
f. sp. *cepae* showing the direct
penetration of the root cells
and the inter- and intracellular
hyphae spreading in various di-
rection in the pericycle layer
progressing towards the stele

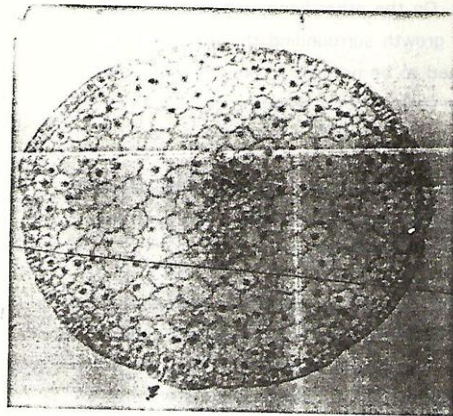


c) *T. harzianum* spores surround the
tissues and cortex cells are
normal

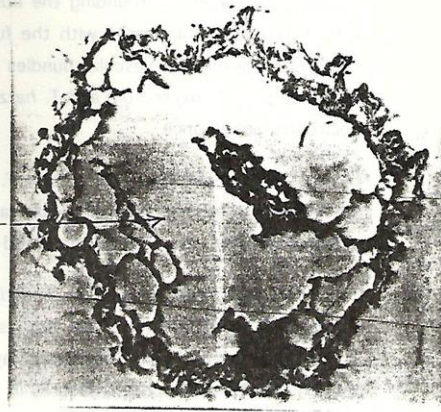


d) *F. oxysporum* f. sp. *cepae* and
T. harzianum showing nearly
normal cells with a little
distortion of the cortex layer

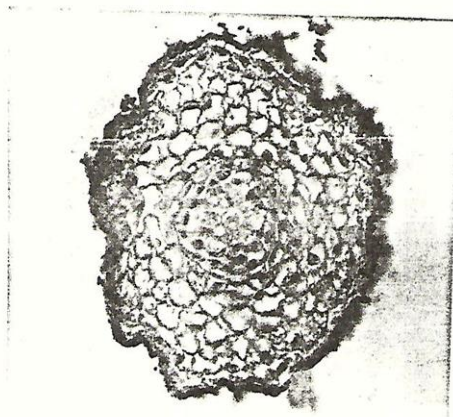
Fig. (3) : Transection in onion root after 5 days from inoculation (x 100)



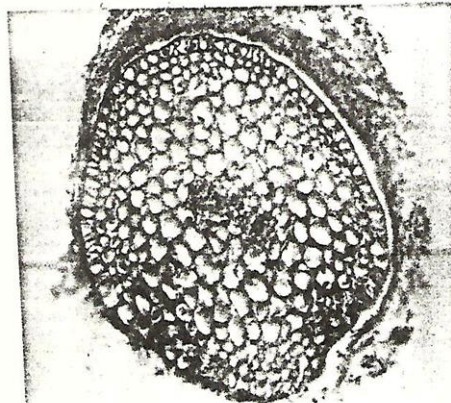
a) Uninoculated root in healthy state (control).



b) Inoculation with *F. oxysporum* f. sp. *cepae* showing an advanced stage of complete disintegration of the root tissues, most of the cortex layers are completely deteriorated.



c) Inoculated with *T. harzianum*. The fungus grows surrounding the root tissue and cells are normal.



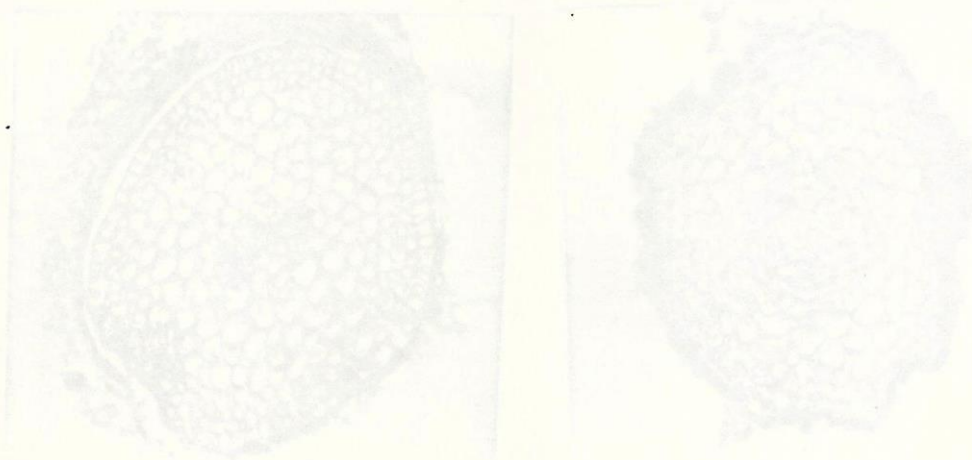
d) *F. oxysporum* f. sp. *cepae* and *T. harzianum* are outside the root tissues without direct penetration of cells, as a result of antagonistic effect.

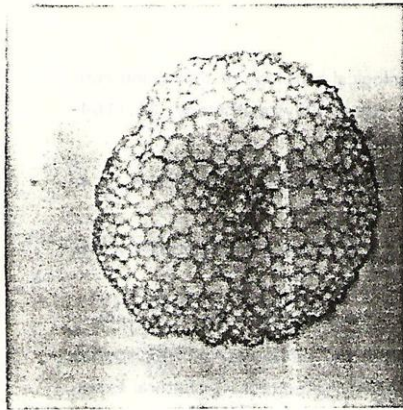
Fig. (4) : Transsection in onion root after 7 days from inoculation (x 100).

the pathogen may cause cell wall breakdown resulting in halo-like zones and cavities in the cortex layers surrounding the bundles. On the other hand, seedlings treated with *T.harzianum*, appeared with the fungal growth surrounding the epidermis but the ground tissue and vascular bundles seemed to be normal. Tissues of roots subjected to both *F. oxysporum* and *T. harzianum* seemed to be as in the check with hypertrophic appearance .

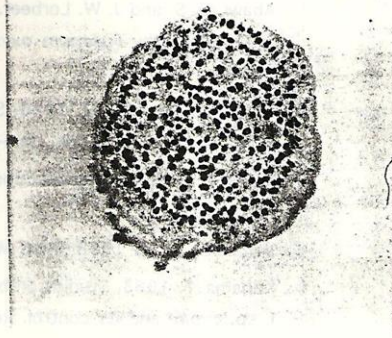
Mahmoud (1985) reported that *F. oxysporum* progressed through all tissues of soybean roots of the susceptible cultivar within 8 days showing fungal spores in the vascular bundles, which were not noted in the resistant cultivar.

Ten days after inoculation, *Fusarium oxysporum* f. sp. *cepae* succeeded in invading and penetrating the host tissues intercellularly and /or intracellularly causing a complete collapse of the epidermal layer, cortex as well as vessels. Then, the pathogen produced the chlamydosores in the tissues (Fig. 5). Similar results were obtained by Abawi and Lorber (1971) and Mehrotra (1983).

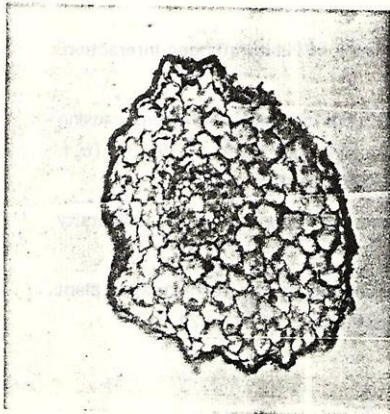




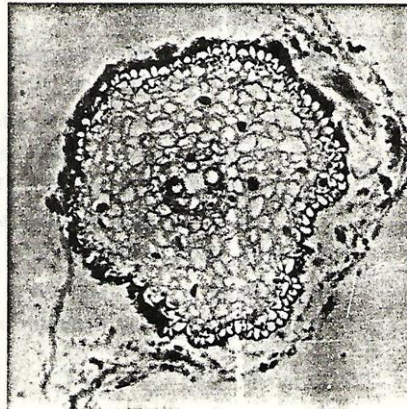
a) Uninoculated (control).
Normal cells—Epidermis, cortex
and vascular bundles.



b) *F. oxysporum* f. sp. *cepae* showing
the distribution of chlamydo-
spores in the root cortex, in the
vascular bundles while the tissues
are completely destroyed.



c) *T. harzianum* surround the tissues
and the cells are normal.



d) *F. oxysporum* and *T. harzianum*
the tissues are irregular but
seemed to be healthy.

Fig. (5) : Transverse section in onion roots after 10 days from inoculation (x 100)

REFERENCES

- 1 . Abawi, G. S. and J. W. Lorbeer. 1971. Pathological histology of four onion cutivars infected by *Fusarium oxysporum* f. sp. *cepae*. *Phytopathology*, 61:1164-1169.
- 2 . Dabash, Thanaa S. M. 1976. Studies on basal rot disease of onion in A. R. E. M.Sc. Thesis, Fac. Agric., Ain-Shams University.
- 3 . Davis, D. 1966. Cross infection in *Fusarium* wilt disease. *Phytopathology*, 56: 825 - 829.
- 4 . Johansen, D. A. 1958. *Plant Microtechnique*, McGraw-Hill Book Co., New York.
- 5 . Kodama, F. 1983. Studies on basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae* and its control. Report of Hokkaido Prefectural Agricultural Exper. Sta. 39,65pp.
- 6 . Mahmoud, Fatma A. F. 1985. Studies on root rot and wilt disease in soybeans plants . Ph. D. Dissertation Fac. Agric., Cairo Univ.
- 7 . Mehrotra, R. S. 1983. *Plant pathology*. Tata, McGraw-Hill Publishing Company Limited, New Delhi.
- 8 . Misaghi, I. J. 1982. *Physiology and Biochemistry of Plant Pathogen Interaction*. Plenum Press, New York.
- 9 . Rod, J. 1984. Antagonistic effect of some fungi on fungal pathogens causing storage rot of onion (*Allium cepa* L.). *Ceska Mykologia*, 38(4) : 235-239. (c. f. *Rev. of Plant Pathology*, 64 (11):527. No. 5269, 1985).
- 10 . Sass, J. E. 1985. *Botanical Microtechnique*. 3rd ed., Iowa State University Press, Ames, Iowa 228 pp.
11. Windham, M. T., Y. Elad and R. Baker. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. *Phytopathology*, 76:518-521.

التأثير المشترك لفطري الفيوزاريوم اوكسيسبورم وترايكودرما هارزيانم على التركيب التشريحي لجذور نباتات البصل

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تم دراسة تأثير الفطر فيوزاريوم اوكسيسبورم سيبا وفطر الترايكودرما هارزيانم وكذلك التأثير المشترك علي التركيب التشريحي لجذور البصل، وقد اوضحت الدراسة أنه بعد ١٨، ١٢، ١٤ ساعة و ٣، ٥، ٧، ١٠ ايام من الحقن بفطر الترايكودرما هارزيانم بمفرده أن الفطر ظل محيطا من الخارج بطبقة البشرة الخارجية للجذور وذلك بدون اختراق للأنسجة وقد ظهرت البشرة والحزم الوعائية في حالة طبيعية

كما ظهرت أنسجة الجذر سليمة بعد ١٢، ١٨، ٢٤ ساعة من حقنها بفطر الفيوزاريوم اوكسيسبورم سيبا ولكن بعد ثلاثة ايام من الحقن لوحظ تقدم الفطر في أنسجة البشرة أما بعد خمسه ايام من الحقن فقد حدث تلف أنسجة القشرة وتقدم الميسليوم بين الخلايا وداخلها ثم بعد ذلك الي الحزم الوعائية وبعد عشرة ايام اتلف الفطر أنسجة الجذر تماما وامتلاءت الأنسجة بجراثيم الفطر الكلاميدية. ولم تتأثر أنسجة الجذور وظهرت كما هي في معاملة المقارنه بعد ١٢، ١٨، ٢٤ ساعة ٣، ٥، ٧، ١٠ ايام من الحقن كما لم تتأثر أنسجة الجذور التي حقنت بفطري الفيوزاريوم اوكسيسبورم سيبا والترايكودرما هارزيانم معا وقد كانت خلايا البشرة والقشرة والحزم الوعائية طبيعية في تركيبها وكبيره نوعا في الحجم.