635.64: 632.9

INDUCING ANATOMICAL RESISTANCE AGAINST INFECTION WITH TOMATO FUSARIUM WILT BY USING GARLIC AND BLACK PEPPER EXTRACTS

Sagitov, A.O.[1], El-Habbaa, G.M. [2], Ismaiel, F. H.[3], and I.A. El-Fiki[4]

[1-]Professor, Academician of the Kazakh National Academy, Kazakh Scientific Research Institute for Plant Protection and Quarantine – Kazakhstan; [2-] Professor of Plant Pathology, Fac. Agric., Benha Univ. – Egypt, [3-]Associate professor of botany, Faculty of Agriculture, Benha University - Egypt; [4A-] Assistant lecturer of Plant Pathology, Fac. Agric., Benha Univ. – Egypt,

A, The corresponding author, E. mail:ibrahimelfiki@gmail.com

ABSTRACT

In this study, seedlings 4 weeks old of tomato cultivar Carolina Gold were treated before transplanting by immersing (IR) their roots, spraying (SS) their shoots or IR+SS with 4.0% conc. garlic or black pepper extracts. Each treated and untreated (check) seedling was inoculated 20 ml of conidial suspension of *Fusarium oxysporum* f.sp. *lycopersici* (10^6 conidia/ml). Comparing with check treatments, the wilt disease severity was significantly reduced by 52-94-100.0% after 2 months from treatment. The degree of reduction was depending upon kind of extract and application method. Away from site of inoculation, the anatomical studies for petioles of the fifth leaves showed induced positive changes in the water conductive elements particularly xylem vessels and width of the vascular bundles in treated compared with untreated (check) plants. These positive changes might involve in the induced systemic resistance which lead to resist or delay development of the *Fusarium* wilt disease in tomato plants.

INTRODUCTION

Fusarium will of tomato (*Lycopersicon esculentum* Mill), caused by the vascular wilt pathogen *Fusarium oxysporum* Schlechtend. Fr. f. sp. *lycopersici* (Sacc.) W. Q Snyder & H. N. Hans., is a devastating disease that occurs in major tomato-growing regions of the world (27). Most investigations of tomato Fusarium wilt have considered mainly to disease distribution (10, 6, 20), symptoms expression (22, 8), and disease control by using resistant cultivars (15, 23, 22, 5, 26), fungicides (3), bio-agents (25, 13), natural plant products and safe chemicals (17, 1). However, the literatures abut histological response of the host plant to these natural or safe chemical products were not available.

This study was initiated mainly to examine whether there was any anatomical changes in tomato plants (cultivar Carolina Gold) after two months from treating plants with garlic and black pepper extracts under stress of inoculation with a severe isolate of *Fusarium oxysporum f.sp. lycopersici*.

MATERIALS AND METHODS

The garlic (*Allium sativum*) and black pepper (*Piper nigrum*) extracts at 4.0% concentration were used separately form immersing (IR) roots for 10 min., spraying (SS) shoots or combination of IR+SS of 4 weeks old tomato seedlings (Carolina Gold cv.) before transplanting (2 seedlings/pot) in plastic pots 30 cm in diameter containing natural soil. The control plants were treated by plain water. One week after transplanting, spore suspension (10^6 spores/ml) of a virulent isolate of the tomato wilt fungus, *Fusarium oxysporum* f.sp. *lycopersici*, was poured over stem base at rate of 20 ml/seedling. Pots were irrigated and maintained in a glasshouse at 25-30ºC and 70% relative humidity. Three pots (replicates) were used for each particular treatment.

The wilt disease severity (DS) for each treatment was determined 2 months after inoculation using a visual scale of 0-4 as following: 0= No wilting symptoms (healthy plant); 1= Plant slightly wilted, vascular discoloration found in main root region; 2= Plant moderately wilted, yellowing of old leaves, spreading vascular browning; 3= Plant severely wilted, dying of all leaves except end leaves; and 4= Dead plant, seedling entirely wilted (14). Also, disease incidence was determined (24).

Two months after treatments, samples represented petioles of fifth leaves were taken from the main stem basically of each treatment. Specimens were killed and fixed for 48 hr. in FAA solution composed of formalin, glacial acetic acid and ethyl alcohol 70 % at rate of 10:5:85 (by volume), respectively. The selected materials were removed from the FAA solution, washed in 50 % ethyl alcohol, dehydrated in a normal ethyl alcohol series, embedded in paraffin wax (melting point 56ºC.), sectioned to a thickness of 15-25 microns, double stained with safranine-fast green, cleared in xylene and mounted in canada balsam (28). Sections were examined microscopically and read to detect anatomical manifestations of noticeable responses resulted from investigated treatments.

Light photomicrographs were taken with a digital camera (Panasonic, DMC-FX100, Osaka, Japan) fitted to the microscope. The following anatomical characters were determined for each particular treatment:

{1}- Thickness of cuticle (µm), {2}- Thickness of epidermal layer (µm), {3}- Number of collenchyma layers, {4}- Thickness of collenchyma layers (µm), {5}- Number of parenchyma layers, {6}- Thickness of parenchyma layers (µm), {7}- Thickness of cortex (µm), {8}- Thickness of outer phloem in the bi-collateral vascular bundle [VB] (µm), {9}- Thickness of cambium in VB (µm), {10}- Thickness of xylem in VB (µm), {11}- Number of xylem vessels in VB, {12}- Thickness of largest vessels in VB (µm), {13}- Thickness of inner phloem in VB (µm), {14}- Length of Vascular Bundle (µm), {15}- Widest of VB (µm), {16}- Number of pith layers, {17}- Pith layers thickness (µm), and {18}- Whole section thickness (µm).

RESULTS AND DISCUSSION

Wilt disease severity:

The data in **Table** (**1**) revealed that, the garlic extracts reduced the wilt disease by 52.94-100.0% (average 84.33%) while the black pepper extracts reduced it by 88.23-100.0% (average 94.11%) depending upon the application method comparing with the untreated check. The efficiency of garlic (*Allium sativum*) and black pepper (*Piper nigrum*) extracts in controlling tomato wilt and other plant diseases was reported by several authors (4, 1, 2, 14, 16, 7). It was reported that spraying with the aqueous garlic extracts have antibiotic and antifungal properties and suppresses a number of plant diseases, including powdery mildew on cucumbers and, to some extent, black spot on roses (19). Activity may be due to sulfur-containing compounds such as ajoene or allicin. Garlic releases fungicidal chemicals into the soil. Rotation of garlic with tomatoes, for instance, can reduce the likelihood of soilborne tomato diseases. It was reported that the activity of 7 watery extracts including *Allium sativum* and *Piper nigrum* were commensurable to that of marketed botanical fungicides (7).

Table (1): The tomato wilt disease development, in terms of disease severity and % reduction in it, after 2 months from inoculation with the tomato wilt pathogen (*Fusarium oxysporum* F.sp, *lycopersici*) as affected by application methods/ plant extract interaction

Plant extract	Methods of application *			Mean **
	IR	SS	IR + SS	
Garlic	0.00 (100.0%)	0.00 (100.0%)	11.11 (52.94%)	3.70 (84.33%)
Black pepper	1.39 (94.11%)	0.00 (100.0%)	2.78 (88.23%)	1.39 (94.11%)
Check (untreated)	23.61 (0.00%)	23.61(0.00%)	23.61(0.00%)	23.61(0.00%)
Mean	8.33	7.87	12.50	

L.S.D. at 5% for
Methods 1.31
Treatments 1.31
Interaction 3.93

* I = Immersing roots, S = Spraying shoots
** Number between bracts represents the % reduction comparing to the check treatment

Anatomical structure of leaf petiole:

Out of 18 anatomical characters investigated in tomato leaf petiole, 10, 5 and 7 characters in case garlic extracts, 14, 7 and 12 characters in case of black pepper extracts were positively changed in the IR, SS and IR+SS application methods, respectively comparing to the untreated control **(Table, 2** and **Fig., 1)**. Number of xylem vessels (NXV) in the vascular bundle as well as the width of the vascular bundle (WVB) seemed to be correlated with the resistance against the *Fusarium* wilt disease more than any other investigated anatomical structures of the tomato leaf petiole. NXV recorded 80, 46 and 36 (in garlic extracts) and 65, 40 and 39 (in black pepper extracts) for the IR, SS, and IR+SS application methods recorded, respectively comparing with NXV 32 in the untreated control. However, the WVB recorded 1496.7, 968.4 and 1244.3 µm (in garlic extracts) and 1188.9, 1656, 1620.0 µm (in black pepper extracts) for the three application methods, respectively comparing with 893.7 µm in case of the untreated control.

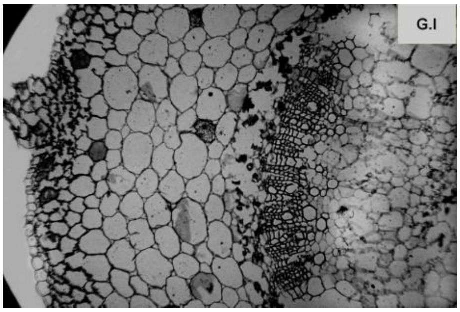
Table (2): Effect of garlic and black pepper extracts used for immersing (I) roots, spraying (S) shoots and I+S of tomato seedlings on the anatomical structure of leaf petiole after two months treatment and inoculation with the tomato *Fusarium* wilt pathogen

Anatomical character	Application method of of garlic extract			Application method of black pepper extract			Control
	IR	SS	IR+SS	IR	SS	IR+SS	
Cuticle thick. (µm)	11.7	13.5	11.7	10.8	13.5	11.7	**13.5**
Epidermal layer thick. (µm)	35.1*	36.9*	37.8*	35.1*	22.7	36.9*	**28.8**
Number of chollenchyma layers	3.5	4.0	3.5	5.5*	5.0	4.5	**5.0**
Chollenchma layers thick. (µm)	180.9	180.0	207.9	211.1	191.7	202.5	**216.5**
Number of parenchyma layers	6.0*	4.0	4.0	5.0*	5.5*	5.5*	**4.0**
Parenchyma layers thick. (µm)	367.2*	331.2*	336.6*	392.4*	286.2	331.2*	**304.2**
Cortex thick. (µm)	548.1*	511.2	544.5*	603.5*	477.9	533.7*	**520.7**
Outer phloem thick. in V.B.(1) (µm)	71.1	111.6*	61.7	99.9*	81.0*	52.2	**74.7**
Cambium thick. in V.B. (µm)	72.0*	39.6	45.9	59.4*	56.7	60.3*	**59.0**
Xylem thick. in V.B. (µm)	435.6	307.8	395.6	501.3*	482.4*	429.3	**446.4**
Number of xylem vessels in V.B.	80.0*	46.0*	36.0*	65.0*	40.0*	39.0*	**32.0**
Largest vessels thick. in V.B. (µm)	75.4*	65.7	78.3*	66.6	54.9	93.6*	**68.0**
Inner phloem thick. in V.B. (µm)	101.7*	52.2	86.0*	117.0*	72.0*	79.7*	**64.8**
Length of Vascular Bundle (µm)	680.4*	511.2	589.1	777.6*	692.1*	621.5	**644.9**
Widest of V.B. (µm)	1496.7*	968.4*	1244.3*	1188.9*	1656.0*	1620.0*	**893.7**
Number of pith layers	14.0	17.0	13.0	22.0	16.0	24.0*	**22.0**
Pith layers thick. (µm)	1136.7	1248.3	1350.0	1710.0*	1287.0	2016.0*	**1531.8**
Whole section thick. (µm)	3687.3	3393.9	3716.1	4563.9*	3699.4	4423.6*	**3947.4**

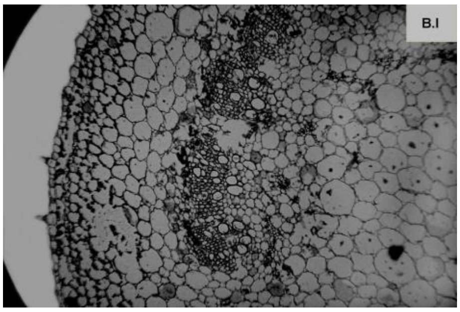
(1) V.B. = Vascular bundle

* Positive changed character comparing to the control

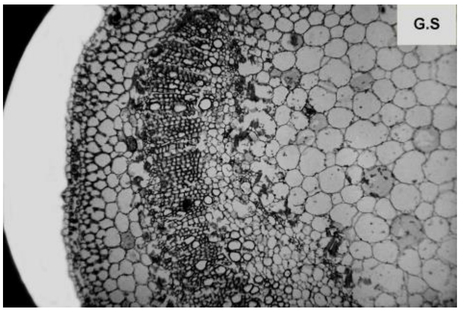
Thus, applying the garlic or black pepper extracts for treating tomato seedlings before transplanting induced positive changes in their water conductive elements, reasonably they resist the wilt disease development by facilitating absorbing more water as the plants are need. In fact, the functional water-conducting system, the tracheary elements of the xylem, is required to sustain plant growth and development. (9). The enlarged number of xylem vessels and width of the vascular bundles caused by the garlic and black pepper extracts might be considered as a probable induced defense mechanism against the tomato fusarium wilt. It is interest to state that neither conidia nor mycelia of the tomato fusarium wilt pathogen were detected in leaf petioles of treated and untreated tomato plants. Such findings agree with other authors (18) who stated that, no conidia were observed in advance of the mycelium in xylem vessel elements of carnation infected with *Fusarium oxysporum* f.sp. *dianthi*. They added that, the absence of conidia in advance of mycelium in the xylem vessel elements is probably the primary reason for the success of culture indexing as a control measure for fusarium wilt of carnation. In fact, xylem plays an important role in strengthening plant bodies as well as in transporting water and minerals. It is a complex tissue composed of vessels, tracheids, fibres and parenchyma. In arabidopsis, secondary xylem does not develop in immature fluorescence stems shorter than 10 cm, although primary xylem does exist in them (12).



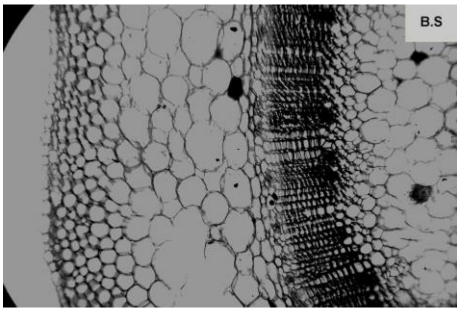
Garlic extract, immersing (IR) roots



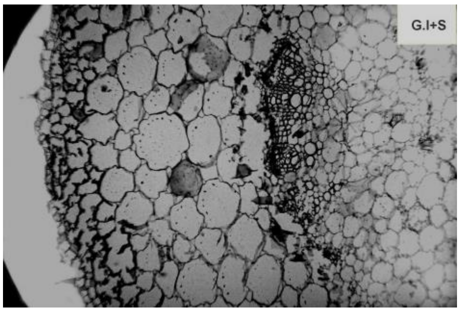
Black pepper extract, immersing (IR) roots



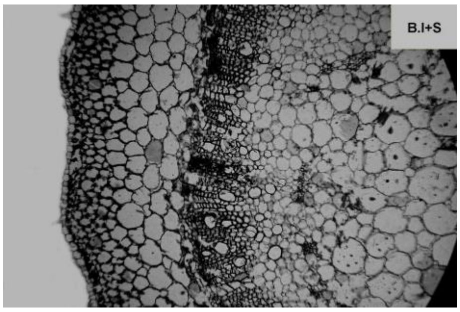
Garlic extract, spraying (SS) shoots



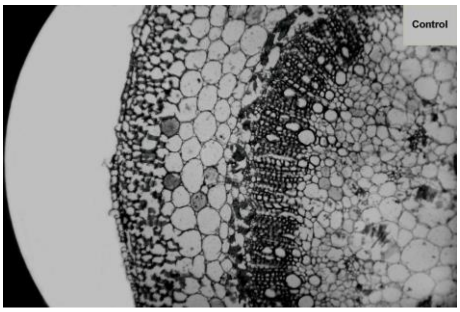
Black pepper extract, spraying (SS) shoots



Garlic extract, IR+SS



Black pepper extract, IR+SS



Untreated control

Fig., (1): Anatomical structure of tomato leaf petiole after two months from immersing (IR) roots, spraying (SS) shoots, and IR+SS of tomato transplants with extracts of garlic (left) and black pepper (right) and inoculation with FOL. Control (untreated).

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