STUDIES ON TOMATO WILT CAUSED BY *FUSARIUM OXYSPORUM* F .SP. *LYCOPERSICI* IN KAZACHESTAN. 1B: EFFECT OF EXOGENOUS APPLICATION OF GARLIC AND BLACK PEPPER EXTRACTS AS RESISTANCE INDUCER TREATMENTS ON THE WILT DISEASE INCIDENCE AND SOME PLANT GROWTH PARAMETERS

Sagitov, A.O.1, G.M. El-Habbaa2, and I.A. El-Fiki3A

[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; [3A-] Assistant lecturer of Plant Pathology, Fac. Agric., Benha Univ. – Egypt, A; The corresponding author, E. mail:ibrahimelfiki@gmail.com

ABSTRACT

In this study, seedlings of tomato cultivar Carolina Gold were treated by garlic (G) and black pepper (BP) extracts using different application methods and inoculated with *F. oxysporum* f.sp. *lycopersici*. Immerging roots (IR) in G@4.0%, spraying shoots (SS) with G@4.0% or BP@4.0% caused 100% reduction in the incidence of tomato fusarium wilt. IR+SS/BP@4.0% induced the highest increase in plant height (40.8%) followed by IR/G@0.5% (38.7%) and IR+SS/G@0.5% (33.2%) whereas IR/G@0.5% caused the highest increase in the root length (64.5%) followed by IR/BP@0.5% (57.0%) comparing to the untreated control**.**

INTRODUCTION

Tomato fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*) become one of a limiting factor in the production of tomato and accounts for yield losses annually. It has become one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen persists indefinitely in infested soils. The use of resistant varieties is the best strategy for disease control **(Silva and Bettiol, 2005)** and **(Sheu and Wang, 2006)**. Because of hazards of pesticides in general, and fungicides in specific, on public health and environmental balance, a relatively recent direction of pest control management was introduced. The so called "induced resistance" is a promising modern approach with a broad spectrum in plant disease control **(Reglinski *et al.*, 2001)**. For this reason, alternative methods with emphasis on biological control using the resistance inducers for controlling the disease have been studied by several researchers to reduce fungicide application and decrease cost of plant production. Recently, there have been many reports stated that some plant extracts and safe chemicals become a necessary to make a fungicide to control the soil borne diseases including tomato *Fusarium* wilt **(Dong and Beer, 2000; Aba AlKhail, 2005; Deepak, *et al.*, 2005; Abogharsa1, *et al.*, 2006; Taheri and Hofte, 2006; Mandal *et al.*, 2009)**. This work was conducted to evaluate effect of applying garlic (G) and black pepper (BP) extracts @ 0.5% and 4.0% concentrations on suppressing development of the tomato fusrium wilt disease under glasshouse condition in Kazakhstan. Effects of tested treatments on % wilted plants, wilt disease severity, plant height and root length were investigated.

MATERIALS AND METHODS

In this study, garlic extract (G) and black pepper extract (BP) each at 0.5% and 4.0% concentrations were used as natural resistance inducer treatments for treating 4 weeks-old tomato (*Solanum lycopersicum*) seedlings (Carolina Gold cv.) immediately before transplanting into plastic pots (30cm. in diameter) each containing 11 Kg of natural soil mixture consisted of clay and sand at rate of 2:1 (by weight). Each inducer treatments was performed by immersing roots (IR) for 10 min., spraying shoots (SS) until dropping or combination between IR and SS application methods (IR+SS). The plain water was used instead of inducer treatments for treating tomato seedling in the control treatment. Spore suspension of an aggressive isolate of *Fusarium oxysporum* f.sp. *lycopersici*, which was isolated from wilted tomato plants grown under glasshouse conditions in

Kazakhstan, was prepared and adjusted according to **Beshir, 1991 and Amini, 2009** and immediately used for inoculating 4-weeks old tomato seedlings by pouring 20 ml of spore suspension (10^6 spores/ml) over stem base of each seedling one week after transplanting. All pots were irrigated and maintained at 25-30ºC and 70% relative humidity under glasshouse conditions. After tow months from inoculation, the wilt disease severity (DS) for each treatment was determined 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 (**Vakalounakis and Fragkiadakis, 1999**). The wilt disease severity was determined according to **Song *et al.*, (2004)** meanwhile; percentage of disease reduction (efficiency) was calculated according to **Elhenawy, *et al.*, 2007**. At the same time, plant height and root length were measured in all tested treatments. All data were subjected to analysis of variance according to **Snedecor and Cochran, 1982**. The least significant difference at 0.05 was calculated.

RESULTS

Percentage of wilted plants and wilt disease severity:

The data in **Table (1)** stated that the percentage of wilted tomato plants was significantly affected only by tested treatments but not by methods or method/treatment interactions. The SS method recorded the lowest % wilted plants (33.3%) followed by IR (40.0%) and IR+SS (43.3%) without significant difference between them. As for treatments, BP@4.0% and G@4.0% were the most effective, decreasing % wilted plants by 86.7 and 80.0% whereas, G@0.5% was the least effective, decreased % wilted plants by 46.7% comparing to the untreated control. The investigated interactions proved that using IR/G and SS/G@4.0% in addition to SS/BP@4.0% were the most effective which completely suppressed disease infection (100.0% reduction) followed

Table (1): Effect of garlic (G) and black pepper (BP) extracts at 0.5% and 4.0% using different application methods on % wilted tomato plants under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
G @0.5%	33.3	50.0	50.0	**44.4**	60.0	40.0	40.0	**46.7**
G @4.0%	0.0	0.0	50.0	**16.7**	100.0	100.0	40.0	**80.0**
BP@0.5%	66.7	33.3	16.7	**38.9**	20.0	60.0	80.0	**53.3**
BP@4%	16.7	0.0	16.7	**11.1**	80.0	100.0	80.0	**86.7**
Control (untreated)	83.3	83.3	83.3	**83.3**	0.0	0.0	0.0	**0.0**
Mean	**40.0**	**33.3**	**43.3**		**52.0**	**60.0**	**48.0**	

L.S.D. at 5% for:

Methods	NS
Treatments	11.69
Interaction	NS

* IR = immersing roots, SS = spraying shoots

Table (2): Effect of garlic (G) and black pepper (BP) extracts at 0.5% and 4.0% using different application methods on wilt disease severity % (DS) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
G @0.5%	11.1	11.1	4.2	**8.8**	52.9	52.9	82.3	**62.7**
G @4.0%	0.0	0.0	11.1	**3.7**	100.0	100.0	52.9	**84.3**
BP@0.5%	19.4	6.9	1.4	**9.3**	17.6	70.6	94.1	**60.8**
BP@4%	1.4	0.0	2.8	**1.4**	94.1	100.0	88.2	**94.1**
Control (untreated)	23.6	23.6	23.6	**23.6**	0.0	0.0	0.0	**0.0**
Mean	**11.1**	**8.3**	**8.6**		**52.9**	**64.7**	**63.5**	

L.S.D. at 5% for:

Methods	NS
Treatments	2.96
Interaction	8.89

* IR = immersing roots, SS = spraying shoots

by IR/BP@4.0%, IR+SS/BP@4.0% and IR+SS/BP@0.5% (80.0% reduction) while, IR/BP@0.5% was the least effective as it decreased wilt infection only by 20.0% comparing to the untreated control.

The data in **Table (2)** showed that the tested application methods were not significantly varied concerning % wilt disease severity (DS). The recorded DS for IR, SS and IR+SS methods were 11.1, 8.3 and 8.6%, respectively. As for treatments, the BP@4.0% was the most effective treatments as it decreased the DS by 94.1% followed by G@4.0% (84.3%), G@0.5% (62.7%), and BP@0.5% (60.8%), respectively comparing to the untreated control (0.0% reduction). Concerning interactions, IR/G@4.0%, SS/G@4.0% and SS/BP@4.0% completely suppressed disease development (100.0% reduction in DS) followed by IR/BP@4.0% and IR+SS/BP@0.5% (94.1% reduction), SS/BP@0.5% (70.6% reduction) whereas IR/BP@0.5% was the least effective in this respect, as it decreased the DS by 17.6% comparing with the control treatment.

Plant height and root length:

The data in **Tables (3)** stated that, the plant height was significantly affected by tested application methods, inducer treatments as well as by method/treatment interactions. As for application methods, the IR+SS recorded the tallest plant height (153.7cm) followed by IR (138.8 cm) and SS (135.1 cm), respectively. All treatments significantly increased plant height comparing to the untreated control. In this respect, G@0.5% was the best treatment for recording the highest average of plant height (163.9 cm) followed by BP@4.0% (150.5 cm), G@4.0% (137.6 cm) and BP@0.5% (136.6 cm) without significant difference between the latter two treatments. These four treatments increased plant height by 32.0, 21.2, 10.8 and 10.0%, respectively comparing to the untreated control. Regarding interactions, IR+SS/BP@4.0 was best of all and increasing plant height by 40.8% (174.8 cm) followed by IR/G@0.5% which increased plant height by 38.7% (172.2 cm) while, the

Table (3): Effect of garlic (G) and black pepper (BP) extracts at 0.5% and 4.0% using different application methods on plant height (cm) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
G @0.5%	172.2	154.2	165.3	**163.9**	38.7	24.2	33.2	**32.0**
G @4.0%	137.5	125.0	150.3	**137.6**	10.7	0.7	21.1	**10.8**
BP@0.5%	127.0	128.8	154.0	**136.6**	2.3	3.8	24.0	**10.0**
BP@4%	133.3	143.3	174.8	**150.5**	7.4	15.4	40.8	**21.2**
Control (untreated)	124.2	124.2	124.2	**124.2**	0.0	0.0	0.0	**0.0**
Mean	**138.8**	**135.1**	**153.7**		**11.8**	**8.8**	**23.8**	

L.S.D. at 5% for:

Methods	1.31
Treatments	2.18
Interaction	6.54

* IR = immersing roots, SS = spraying shoots

Table (4): Effect of garlic (G) and black pepper (BP) extracts at 0.5% and 4.0% using different application methods on root length (cm) under stress of infection with *F. oxysporum lycopersici*

Treatments	* Application methods			Mean	Efficiency %			Mean
	IR	SS	IR+SS		IR	SS	IR+SS	
G @0.5%	25.5	16.5	16.3	**19.4**	64.5	6.5	5.4	**25.4**
G @4.0%	20.0	23.5	18.2	**20.6**	29.0	51.6	17.2	**32.6**
BP@0.5%	24.3	20.0	21.5	**21.9**	57.0	29.0	38.7	**41.6**
BP@4%	23.0	23.8	18.5	**21.8**	48.4	53.8	19.4	**40.5**
Control (untreated)	15.5	15.5	15.5	**15.5**	0.0	0.0	0.0	**0.0**
Mean	**21.7**	**19.9**	**18.0**		**39.8**	**28.2**	**16.1**	

L.S.D. at 5% for:

Methods	0.48
Treatments	0.80
Interaction	2.40

* IR = immersing roots, SS = spraying shoots

lowest significant increase was recorded by IR/BP@0.5% which increased the plant height by 7.4% (133.3 cm). However, IR/BP@0.5%, SS/G@4.0% and SS/BP@0.5% showed no significant effects on the plant height when compared with the untreated control.

The data in **Tables (4)** indicated that, the root length (cm)/plant (RL) was significantly affected by tested application methods, inducer treatments as well as by the interaction in between. The IR method recorded the highest significant increase in the RL (21.7 cm) followed by SS method (19.9 cm) and IR+SS method (18 cm), respectively. All tested inducer treatments induced significant increases in the RL comparing to the untreated control. The highest significant increase was produced by BP@0.5% (41.6%) and BP@4.0% (40.5%) followed by G@4.0% (32.6%) and G@0.5% (25.4%), respectively in relation to the untreated control. Concerning method/treatment interactions, SS/G@0.5% induced the highest significant increase in the RL (64.5%) followed by IR/BP@0.5% (57.0%), SS/BP@4.0% (53.8%) and IR/BP@4.0% (48.4%) while, the lowest significant increase was produced by IR+SS/G@4.0% (17.2%) comparing to the untreated control. On the other hand, the RL was not significantly affected by SS/G@0.5% and IR+SS/G@0.5% comparing to the untreated control.

DISCUSSION

As for plant extracts, the highest concentration (4.0%) of G and BP was significantly better than the lowest concentration (0.5%) for reducing % wilted plants and wilt disease severity (DS) comparing to the untreated inoculated control. G and BP@4% decreased % wilted plants by 80.0 and 86.7% and DS by 84.3 and 94.1%, respectively comparing with 46.7 and 53.3% (for % wilted plants) and 62.7 and 60.8% (for DS) at 0.5%, respectively. However, G@0.5 and 4.0%, BP@0.5 and 4.0% increased plant height by 32.0, 10.8. 10.0 and 21.2%%, root length by 25.4, 32.6, 41.6 and 40.5 %, respectively comparing to the untreated inoculated control. These results declared that the tomato plants treated with G or BP@0.5 and 4.0% concentrations rendered plants healthier then their fruit yield production was increased (unpublished data). Actually, the natural plant extracts may provide an alternative to fungicides. *Allium* genus revered to possess anti-bacterial and anti-fungal activities and include the powerful antioxidants, sulfur and other numerous phenolic compounds which arouse significant interests **[Yin and Cheng, 1998; Phay et al., 1999; Harris et al., 2001; Kyung and Lee, 2001; Rivlin, 2001; Griffiths et al., 2002; Benkeblia, 2004; Haciseferogullari et al., 2005]**. The inhibitory activity of garlic [*Allium sativum* L.], onion [*Allium cepa* L.] and leek [*Allium porrum* L.] extracts [aqueous, acetone and ethyl alcohol] against mould has been reported by numerous authors. It has also been observed that alliicin, thiosulfonates and other compounds show fungistatic activities against several fungi **[Topal, 1989; Hafez and Said, 1997; Ankri and Mirelman, 1999; Harris et al., 2001]**. Similarly, ajoene compound which is a derivative of alliicin and obtained from garlic with ethyl alcohol extraction is very inhibitory against *A. niger*, *Candida albicans* and *Paracoccidioides brasiliensis* **[Naganawa et al., 1996]**. Ajoene compound from garlic have stronger antifungal activity than alliicin. Ajoene damages the cell walls of fungi **[Yoshida et al., 1987]**. Activity of the garlic extract may be due to sulfur-containing compounds such as ajoene or allicin. Sprays with the aqueous garlic extracts have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and, to some extent, black spot on roses. Garlic extracts controlled diseases such as mildew, rusts, fruit rots, blights, and black spot **[Quarles, 2000]**. Activity may be due to sulfur-containing compounds such as ajoene or allicin. Garlic releases fungicidal chemicals into the soil. Garlic extract shows high inhibitory activity against *Aspergillus niger*, *Penicillium cyclopium* and *Fusarium oxysporum* for all tested concentrations i.e. 50, 100, 200, 300 and 500 ml/l **[Benkeblia, 2004]**. Also, several biologically important phytochemicals have been extracted from *Piper nigrum*

plants **(Miyakado et al., 1980; Kiuchi et al., 1988; Bandyopadhyay et al., 1990)**. Alkaloids in fruits of *P. nigrum* ranges from 4 to 5% [**Dev and Koul, 1997**]. The combinations of extracts of pepper and mustard, the cassia extract alone and the essential oil of clove suppress the development of *Fusarium oxysporum* in melon **[Bowers and Locke, 2000]**. Plant extracts of six plant species, cloves [*Dianthus caryophyllus*], cinnamon [*Cinnamum zeylamicum*], thyme [*Thymus vulgaris L.*] fenugreek [*Trigonella fonicum*], amme [*Ammi visnagal*], black pepper [*Piper nigrum*] and three essential oils, geranium [*Pelargonium gravedens*], black cumin seeds [*Nigella sativa L.*] and blue gum [*Eucalyptus globulus*] were evaluated for their antifungal effect on the mycelial growth, incidence and disease severity of onion neck rot disease [*Botrytis allii*]. The antifungal properties of clove extract were more effective than black pepper on inhibiting mycelial growth and disease incidence **[Abo-Elnaga** and **Ahmed, 2006]**. Aqueous extracts of 15 plant species were tested against onion white rot fungus *Sclerotium cepivorum* that was grown in potato dextrose agar culture. Eeach extract presented a fungicidal effect, at a concentration of 5%, when applied on allspice [*Pimenta dioica*] and clove [*Syzygium aromaticum*]. Only clove extract retained its effect at a concentration of 1%, while allspice lost it at 3%. Cinnamon [*Cinnamomum zeylanicum*] and yam bean [*Pachy erosus*] extracts produced total inhibition of sclerotial production besides a poor mycelial growth. Different types of interactions were present when the extracts were mixed: all combinations presented a lost of fungicidal effect [antagonistic effect], including allspice extract; a retained fungicidal effect [single fungicidal effect] occurred in most clove mixtures and in the combination of clove and black pepper [*Piper nigrum*] the retained fungicidal effect was even below the minimal lethal dose [synergistic effect]. The combination of extracts showed that the effect of each plant extract could be modified by the reactions of the complex mixture of plant compounds **[Montes-Belmont** and **Prados-Ligero, 2006]**. *Piper nigrum,* commonly known as ``Black-pepper``, has gained a global consideration because of its volume in the spice industry. This plant has shown great potential for the discovery of novel biologically active compounds and need for techniques to enhance the production of high quality consistent plant material for feasible accumulation of metabolites **[Abbasi *et al.,* 2010]**. Infection with *FOL* significantly reduced the crop yield and quality. Several plant extracts were found to be highly effective on different isolates of Fusarium wilt in the laboratory, and were tested with other control methods on two tomato varieties artificially inoculated with the fusarium wilt fungus. Results showed that these extracts reduced wilt infection rate 49 days after planting on both tested varieties. The most effective treatment after the fungicide Tachigaren was garlic extract **[Silva and Bettiol, 2005; Abogharsa1, *et al.,* 2006]**. The fresh weight of plant stem, number and weight of tomato fruits were significantly lower in tomato plants inoculated than those non-inoculated with the wilt pathogen "*Fusarium oxysporum* f.sp. *lycopersici*" **[Sibounnavong *et al.,* 2010]**. **Abo-Elnaga and Ahmed (2006)** found that, the antifungal properties of cloves extract was more effective than black pepper on inhibiting mycelial growth and disease incidence of onion neck rot disease caused by *Botrytis allii*. **Abo-Elnaga** and **Ahmed [2006]** evaluated plant extracts of six plant species including cloves [*Dianthus caryophyllus*] and black pepper [*Piper nigrum*] for their antifungal effect on the mycelial growth, incidence and disease severity of onion neck rot disease [*Botrytis allii*]. The antifungal properties of clove extract were more effective than black pepper on inhibiting mycelial growth and disease incidence. **Montes-Belmont** and **Prados-Ligero [2006]** tested the aqueous extracts of 15 plant species including black pepper [*Piper nigrum*] against onion white rot fungus *Sclerotium cepivorum* that was grown in potato dextrose agar culture. Each extract presented a fungicidal effect, at a concentration of 5%. **Abbasi *et al.,* (2010)** recorded that, *Piper nigrum* (Black-pepper), shown great potential for the discovery of novel biologically active compounds and need for techniques to enhance the production of high quality consistent plant material for feasible accumulation of metabolites.

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