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THE EFFECTS OF *ALLIUM TUNCELIANUM* EXTRACT ON SOME IMPORTANT PATHOGENS AND TOTAL PHENOLIC COMPOUNDS IN TOMATO AND PEPPER

HULYA OZGONEN OZKAYA[1*] AND TUGCE ERGUN[2]

[1]Suleyman Demirel University, Agricultural Faculty, Department of Plant Protection, Isparta, Turkey
[2]Aksu Directorate of District Food Agriculture and Livestock, Antalya, Turkey
[*]Corresponding author's e-mail: hozgonen@yahoo.com

Abstract

The aim of this study was to determine the effects of *Allium tuncelianum* ethanol extract on nine important plant pathogens and the amount of total phenolic compounds in tomato and pepper. Three extract doses (0.5%, 1%, and 1.5%) were applied in the form of irrigation water against soil-borne pathogens while the spraying method was applied for the bacterial speck disease in tomato and disease severity was determined. According to the pot trial results, higher doses of extract reduced the disease severity of *Botrytis cinerea*, *Fusarium oxysporum* f.sp. *lycopersici*, *Pythium deliense*, *Rhizoctonia solani* and *Sclerotium rolfsii* and the 1.5% dose reduced disease severity by 76.8%, 56.6%, 47.4%, 85.8%, and 53.1%, respectively. Total phenolic compounds were determined spectrophotometrically. The extract applications have increased the amount of phenolic compounds in a certain level compared to the control in tomato. However, it was also observed that this level was much higher when the different doses of extract and pathogen combinations were applied. Conclusively, ethanol extract of the garlic used in our study reduced the development of some important pathogens, and promising results were obtained.

Key word: *Allium tuncelianum*, Tomato, Pepper, Soil-borne pathogens, Disease severity, Phenolic compounds.

Introduction

Vegetable production has an economical importance all over the world and it's increased in order to supply the needs of the world population. Modern agricultural techniques have been utilized in order to increase yield from unit area (Özalp, 2010; Yildiz, 2013). Despite the use of modern agricultural techniques, yield losses due to the plant diseases can not be prevented effectively. Among the important agricultural products, tomatoes and peppers are exposed to many fungal, fungal-like, and bacterial diseases throughout the production season in many parts of the world. The most important of these diseases are soil-borne, and they cause different symptoms in plants and lead to product losses. The main important fungal and fungal-like disease in tomatoes are *Botrytis cinerea* (grey mold), *Fusarium oxysporum* f.sp. *lycopersici* (wilt), *Pythium deliense* (seedling rot), *Rhizoctonia solani* (root and stem rot), *Sclerotinia sclerotiorum* (stem rot), *Sclerotium rolfsii* (white mold). Likewise, important bacterial agents are *Clavibacter michiganensis* subsp. *michiganensis* (bacterial wilting) and *Pseudomonas syringae* pv. *tomato* (bacterial speck) (Blancard, 2005; Jones *et al*., 2014).

Botrytis blight caused by *Botrytis cinerea*, a polyphagous pathogen, is a common disease in tomatoes grown in greenhouses and causes flower blights and fruit rot in the plant (Altınok, 2011). One of the most important soil-borne diseases in tomato is *Fusarium oxysporum* f.sp. *lycopersici*, which causes wilting in the seedling period, dwarfing, yellowing and wilting of the leaves and branches, and weak root formation and necrosis around the leaves in the mature plants. Browning is evident in the vascular tissues of the plant (Çolak & Biçici, 2011). The seedling root rot disease *Pythium deliense*, causes damping-off and rots in the stem and the roots of many plants in addition to tomato. *Rhizoctonia solani* infects the seeds, hypocotyls, roots and causes seed and seedling rots. It also causes root collar and stem rots. *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* are among the cosmopolitan pathogens that cause white mold, white rot, and stem and fruit rots (Onaran & Yanar, 2009).

Among bacterial diseases of tomato, bacterial wilting and canker caused by *Clavibacter michiganensis* subsp. *michiganensis*, is an important disease which decreases the quality and quantity by causing leaf necrosis and birdseye in the fruits (Yıldız & Aysan, 2008). *Pseudomonas syringae* pv. *tomato* causes bacterial spot disease on the leaves and the fruits of tomato (Çalış & Çelik, 2011).

Phytophthora capsici which causes root and root-crown rots is another important pathogen for pepper. *Phytophthora capsici*, which is also known as pepper blight, limits pepper production, causes browning in the roots, root collars, and fruit rots and leads to yield loss as high as 40% (Black *et al*., 1991; Demirci & Dolar, 2006).

Control of all the above-mentioned diseases are difficult because of their ability to live in plant debris in the soil and to survive for many years by producing resistant structures, including oospores, sclerotia or chlamydospores. Physical control methods such as soil disinfection, solarization, crop rotation, and removal of harvest residues from the greenhouse; biological control; and intensive chemical control are used against soil-borne diseases. One of the best management tools in disease management is using resistant varieties however, resistant varieties are usually resistant to one or a limited number of diseases. An active control program cannot be established because of the high costs of fungicides used in the control and the pathogens also develop resistance (Yücel *et al*., 1999; Aksoy, 2006).

Current agricultural production is in search of alternative control methods against plant diseases in such commonly produced and consumed vegetables. Additionally, due to the air, water, and soil pollution caused by intensive chemical control, alternative chemical control methods are sought against diseases. Considering the negative effects of synthetic chemicals on human health, studies on the determination of the effects of plant extracts on the diseases have become popular (Gurjar *et al*., 2012).

Allium tuncelianum, belonging to the family Liliaceae, is endemic to Eastern Turkey (Ipek *et al*., 2008). It is also known as Tunceli garlic or Ovacık garlic. This endemic plant is found widely in Ovacık and Pülümür counties located in the foothills of the Munzur Mountains (Ekim *et al*., 2000).Agbaş *et al*. (2013) determined that garlic which contains allicin is a natural and effective antimicrobial.

There are many studies on the effects of different plant extracts and volatile compounds against plant pathogens (Yanar *et al*., 2001; Yildiz & Erkiliç, 2004; Agbenin & Marley, 2006; Soylu *et al*., 2007; Tapwal *et al*., 2011; Nashwa & Abo-Elyousr, 2012.). Some plant-based extracts promote biochemical and morphological resistance factors and provide protection against plant diseases. One of the most important defense reactions in plants is the synthesis of phenolic compounds (Koç & Üstün, 2008). Therefore, phenolic compounds found in the plants have antimicrobial effects and inhibit the growth of bacteria and fungi (Altındag & Aslım, 2005).

The aim of this study was to investigate the effects of *Allium tuncelianum* ethanol extract on some fungal, fungal-like, and bacterial plant pathogens. Accordingly, the effects of three different doses of the garlic extracts on disease severity were examined with pot trials, and the amount of phenolic compounds in plants was determined. This is the first study that focuses on the possible uses of *Allium tuncelianum* extract against plant pathogens.

Materials and Methods

Plant materials and cultures: The standard H2274 variety of tomato (*Solanum esculentum* L.) and standard Demre 8 variety of pepper (*Capsicum annuum* L.) were used in the trials. The seedlings were provided by Asgen Inc. The pathogens tested for biological activity of the extract were *Botrytis cinerea, Fusarium oxysporum* f.sp. *lycopersici, Pythium deliense, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum* in tomato and *Phytophthora capsici* in pepper, bacterial pathogens *Clavibacter michiganensis* subsp. *michiganensis,* and *Pseudomonas syringae* pv. *tomato.Pseudomonas syringae* pv. *tomato* isolate, encoded OY-9-2b, which was isolated and identified in 2011 by Prof. Dr. Yeşim AYSAN from tomato plants in Mersin District Erdemli Province, were used in the study. The remaining cultures were provided by the Süleyman Demirel University Department of Plant Protection Mycology Laboratories culture collection. Experiments were conducted in laboratories and controlled climate room of University of Süleyman Demirel, Agricultural Faculty, Department of Plant Ptotection.

Garlic extract: Garlic (*Allium tuncelianum*) used in the study were collected from the Munzur Mountains located in Ovacık Province of Tunceli on July. Ethanol extract of garlic was used for biological activity studies. Extraction was performed using the head portions after peeling and slicig the garlic skin (Özkan *et al*., 2013). Then 96% ethyl alcohol was added as 50 mL alcohol/g garlic and kept at 25°C overnight. Then the liquid was filtered using Whatman No. 1 filter paper. Ethyl alcohol in the filtrate was evaporated using a rotary evaporator at 35°C. The extract was filtered through 0.45 µm sterile filter and kept at -20°C.

Pot trials: Mixture of soil:turf:pumice (1:1:1, v:v:v) was used in the study as a growing medium after autoclaving two times at 121°C, 1k Pa for 1 hour. For the pot trial, tomato and pepper seedlings were planted in 10-cm diameter pots containing growth medium. Following the plantation, 0.5%, 1%, and 1.5% solutions of garlic extracts (10 ml per plant) were applied, using a hand sprayer, to seedling at two or three leaves stage period for determining the effects on *Pseudomonas syringae* pv. *tomato*. For determining the effect on *Botrytis cinerea, Fusarium oxysporum, Pythium deliense, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, Clavibacter michiganensis* and *Phytophthora capsici in* pepper, volume of 10 mL of solutions containing garlic extract were applied. Distilled water was applied either spraying or to the soil in the control pots. The applications were determined as follows: Control with no application (Control -), positive control with pathogen inoculation (Control +), Garlic extract with0.5%, 1.0%, 1.5% plus pathogens. Pathogen inoculation were performed two days after the garlic extract applications. Fungal and fungal-like pathogens were cultured in PDA medium for 7 days at 25°C for the preparation of inoculum. For the inoculation of *B. cinerea*, conidial suspension was prepared at 10^6 spores mL $^{-1}$, and 10 mL solutions were applied around the roots of the plants. *F. oxysporum* f.sp. *lycopercisi* inoculum was prepared using sterilized wheat. Accordingly, the wheat was boiled and autoclaved at 121°C 1 atm twice for 30 minutes. The wheat was inoculated with 1-cm diameter mycelial disk from the developed culture and incubated for 2 weeks at 25°C. For the inoculation, 2 g of wheat culture was applied to the root collars of the plants. For the *R. solani, S. sclerotiorum*, and *S. rolfsii* inoculations, 10-mm diameter mycelial discs were taken from the freshly developed culture and applied to the root collar parts of the plants. Sand-cornmeal culture was prepared for the inoculation of *P. deliense*. Sand-corn meal which was previously sterilized in the autoclave was inoculated with 6-mm diameter agarose mycelial discs taken from the freshly developed culture and incubated for 2 weeks at 25°C and applied to the root collar parts of the plants. For the inoculation of *P. capsici*, as recommended by Sunwoo *et al.* (1996), zoospore suspension was prepared at $2x10^6$ zoospore/mL concentration from the culture developed in PDA, and 10 mL were applied to the roots of the plants. For the inoculation of the bacterial pathogens, *P. syringae* pv. *tomato* and *C. michiganensis* subsp. *michiganensis* were cultured in King B medium and incubated for 48

hours at 25°C. A bacterial suspension was prepared with sterile water and adjusted to 10^9 cells/mL. The suspension was sprayed onto the upper parts of the plants using a hand sprayer. *C. michiganensis* subsp. *michiganensis* was injected with 2 mL into the stem part of the plants. The plants were placed in humidified polyethylene bags for 24 hours for maintaining the injection conditions, and then the bags were removed. Accordingly, inoculum for each pathogen was prepared separately. The trial was conducted in a climate room with 16 hours of light and 8 hours of dark conditions at 25±2°C. Trial-randomized block design were established with 3 repetitions according to the experimental design having 4 plants in each repetition. Each pathogen was evaluated using disease scales 3 weeks after the trial pathogen inoculation. Symptoms of diseases were evaluated using following scales. 0-4 scale used for *Botrytis cinerea* was 0: Healthy plant, 1: 25% blight and/or defoliation, 2: %50 blight and/or defoliation, 3: %75 blight and/or defoliation, 4: %100 blight or dead plant (Ziogas *et al*., 2005). 1-5 scale for *Fusarium* wilt; 1: Healthy plant, 2: Chlorosis of lower leaves of plant, 3: Chlorosis of 1/3 part of plant or wilting 4: Wilting of the top leaves of plant, 5: Dead plant (Prados-Ligero *et al*., 2007). 0-4 scale for *Pythium deliense*; 0: Healthy plant, 1: Light browning in capilary roots and slight wilting of leaves, 2: Severe browning in root and severe wilting of seedling, 3: Severe root rots of seedling 4: Dead plant (Chen *et al*., 1988). 1-5 scale for *Rhizoctonia solani*; 1: Healthy plant 2: 25% of stem covered with lesion, 3: 26-50% of stem covered with lesion, 4: 51 -75% of stem covered with lesion, 5: Stem covered with lesion completely or dead plant (Cartwright, 1995). 0-4 scale for *Sclerotium rolfsi* was; 0: Healthy plant, 1: 1-25% infection of plant, 2: 26-50% infection in plant, 3: 51-75% infection of plant, 4: Dead plant (Fery & Dukes, 2002). 0-4 scale for *Sclerotinia sclerotiorum;* 0: Healthy plant, 1: Slight infection, small lesions on the stem and branches, 2: Moderate infection, 1-3 cm lesion on the stem and branches and wilted plant, 3: Severe infection, lesions throughout stem and severe wilting, 4: Dead plant (Bora & Karaca 1970). 0-5 scale for *P. capsici*; 0 : no visible disease symptoms; 1: leaves slightly wilted with brownish lesions beginning to appear on stems; 2: 30–50% of entire plant diseased; 3: 50–70% of entire plant diseased; 4: 70–90% of entire plant diseased and 5: plant dead (Sunwoo *et al*., 1996). 0-4 scale for *Clavibacter michiganensis* on tomato ; 0: Healthy plant, 1: 1/4 part of the plant withered or dried, 2: 1/2 part of the plant withered or dried, 3: 3/4 part of the plant withered or dried, 4: Dead plant (Akat &Özaktan, 2011). *Pseudomonas syringae* pv. *tomato* was evaluated according to 0-3 scale (Yunis *et al*., 1980). According to this scale, six leaves next to two leaves, sprayed with the bacterial suspension on each plant were evaluated. Scale used was 0: Healthy plant, 1: 1-5 speck on leaves 2: 6-10 1-5 speck on leaves, 3: over 11 speck.Disease index and severity (%) was calculated using scale values.

Determination of the total phenolic compounds in plants: Phenolic compounds were measured three weeks after the pathogen inoculations when the trial was ended. Total phenolic contents in the control, different garlic extract doses in pot trials, and pathogen applications were determined according to Escarpa & Gonzalez (1998). The measurements were conducted with the plants removed after each application in triplicates. A 1-g sample was weighed, and 0.5 g BHT (2,6-di-tert-butyl-4-methylphenol) and 15 mL extraction solution were added (80% methanol, 20 mL water, and 1 mL HCl). The samples were kept in an ultrasonic bath for 45 minutes. Then the solution was filtered through Whatman No. 4 paper, and a 15 mL extraction solution was added and kept in an ultrasonic bath for 45 minutes and filtered again through Whatman No. 4 paper. Total phenolic contents were determined according to the spectrophotometric method suggested by Kaur & Kapoor (2002). Plant extracts were placed in 0.5 mL falcon tubes. Water (7 mL) and 0.5 mL Folin-Ciocalteu were added and kept for 3 minutes. Then, 2 mL 20% Na_2CO_3 solution was added to the solution and kept in a water bath at 25°C for 1 hour. Absorbance values of samples were measured at 720 nm wavelength in a UV-VIS spectrophotometer. Standard solutions were prepared using catechin at 250, 500, and 1000 ppm concentrations.

Statistical analysis: In pot trials, different extract doses were evaluated for nine pathogens separately, and the Kruskal Wallis test was used to determine the differences between the average ranks of these doses. The Bonferroni–Dunn test was used to determine the differences between the median values of the rank of the doses. Phenolic contents was evaluated with analysis of variance and differences were determined with Tukey ($p<0,05$) test.

Results and Discussion

The effects of garlic extracts on the disease severity of pathogens: The effects of three different concentrations of garlic extracts (0.5%, 1%, and 1.5%) on disease severity were determined (Table 1). In Table 1, different doses of the extract either had no effect or reduced disease severity of pathogens. Therefore, the effects on the diseases varied compared with the control. It was found that the 1.5% dose of extract application was effective on *Botrytis cinerea*. Disease severity in positive control pots was 89.6%, while the disease severity was reduced by 76.8% in plants having 1.5% extract dose. The disease severity of *Fusarium oxysporum* was reduced with the increased doses of extract. It was found that the 1.5% dose of extract was reduced the disease severity by 56.6%. Garlic extracts at 1% and 1.5% doses were successful in reducing the disease severity in *Pythium deliense* by 37.5% and 47.4%, respectively. However, on pepper, the applied doses had no effect on the disease severity of *Phytophthora capsici* while the disease severity of *Pythium deliense* was reduced by 47.4% at the highest dose. Garlic extract showed the highest effect (85.5%) on reducing the disease severity of *Rhizoctonia solani*. The increased doses of extract were more effective in reducing the disease severity in *Sclerotinia sclerotiorum*. The reducing effect of 1.5% dose on *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* was 34.3% and 53.1%, respectively.

Table 1. The effects of different concentrations of garlic extract on disease severity (%) of the pathogens.

Pathogen	Extract doses (%)	Disease index	Disease severity (%)	% Effect
B. cinerea (BC)	0.5	3,00 b*	75,0	16,3
	1.0	2,58 b	64,6	27,9
	1.5	0,83 c	20,8	76,8
	BC	3,58 a	89,6	-
Fusarium oxysporum fsp *lycopersici* (FOL)	0.5	1,92 b	38,3	23,4
	1.0	1,33 c	26,7	46,6
	1.5	1,08 c	21,7	56,6
	FO	2,50 a	50,0	-
P. capsici (PC)	0.5	3,67 a	73,3	4,4
	1.0	3,50 a	70,0	8,7
	1.5	3,50 a	70,0	8,7
	PC	3,83 a	76,7	-
Pythium deliense (PD)	0.5	2,25 b	56,3	32,4
	1.0	2,08 b	52,1	37,5
	1.5	1,75 b	43,8	47,4
	PD	3,33 a	83,3	-
R. solani (RS)	0.5	1,83 b	36,7	37,0
	1.0	1,42 c	28,3	51,5
	1.5	0,42 c	8,3	85,8
	RS	2,92 a	58,3	-
S. sclerotiorum (SS)	0.5	2,67 a	66,7	0,0
	1.0	2,42 a	60,4	9,4
	1.5	1,75 b	43,8	34,3
	SS	2,67 a	66,7	-
S. rolfsii (SR)	0.5	2,50 ab	62,5	6,3
	1.0	1,67 bc	41,7	37,5
	1.5	1,25 c	31,3	53,1
	SR	2,67 a	66,7	-
P. syringae pv. *tomato* (PST)	0.5	2,17 a	72,2	3,7
	1.0	1,67 b	55,6	25,9
	1.5	0,75 c	25,0	66,7
	PST	2,25 a	75,0	-
C. michiganensis subsp. *michiganensis* (CMM)	0.5	3,08 a	77,1	2,7
	1.0	3,08 a	77,1	2,7
	1.5	2,92 a	72,9	8,0
	CMM	3,17 a	79,2	-

* Averages in the same row with different letters for each pathogen is different according to the Bonferroni- Dunn ($p<0,05$)

The increased doses of the extract were effective on *P. tomato* pv. *tomato*, while it was not effective on *C. michiganensis* subsp *michiganensis*. Different doses of the extract reduced the disease severity of *Pseudomonas tomato* between 3.7% and 66.7%. However, the 1.5% dose had only 8.0% effect on *C. michiganensis* subsp *michiganensis*. The increased concentrations of garlic extracts were effective against all pathogens except *Phytophthora capsici* and *Clavibacter michiganensis* and in the pot trial part of our study. The 1.5% garlic extract was 76.8% effective on *Botrytis cinerea*. It has been reported that some plant extracts and oils as natural compounds had positive effects on bacterial plant diseases. Mirik & Aysan (2005) reported that application of plant extracts as seed treatments on bacterial spot disease of tomato and pepper had also positive effect on diseases. In a similar study, Tedeschi *et al*. (2007) investigated the effects of garlic preparations applied as lyophilized and dry sprayed. Both garlic preparations successfully inhibited the fungal growth. Both preparations were highly effective against *B. cinerea* and *F. avenaceum*, while they were effective at moderate or low levels against *S. rolfsii* and *R. solani*. In our study, the effects on *S. rolfsii* and *R. solani* at 1.5% were 53.1% and 85.8%, respectively, and the garlic variety we used in our study was more effective against these two pathogens than in the studies conducted with normal garlic. In another study, the effect of the extract on *S. sclerotiorum* increased with the increased concentrations of the extract, and the highest effect compared to the control was found to be 34.3%. In another study on the effects of garlic extract and iprodion, the effect of garlic extract was 49.34% effective compared with control parcels. It has been reported that garlic extract is used against *S. sclerotiorum* (Onaran & Yanar, 2009). The effects of garlic extracts on plant diseases were also detected in studies conducted under greenhouse conditions. Indeed, Tohamy *et al*. (2002), in their *in vivo* study conducted in a greenhouse, planted cucumber seeds that had been previously infected with disease and kept in 2.5%, 5%, and 10% garlic extracts for 60 minutes. Garlic extracts were also applied to the plants with repeated spraying and powder spraying, and their effects were investigated. It has been reported that both methods of applying garlic preparations successfully reduced the severity of the disease in all concentrations in 4-week-old cucumber seedlings. Garlic extract (10%) reduced the damping-off disease rate 43.3% when applied to the seed and 63.3% when applied as powder. Alhussaen *et al.* (2011) used garlic extract against *Pythium* sp. of tomato successfully. In another study by Islam & Faruq (2012) on the effects of some plant extracts on damping-off diseases in vegetables, plant extracts including garlic extract were used as seed applications. Garlic extract, in addition to enhancing the development parameters in tomato and pepper, reduced the intensity of damping-off disease.

Total phenolic compounds levels in the applications: At the end of the experiment, total phenolic contents ($\mu g.ml^{-1}$) in plants treated with different garlic extracts doses and/or pathogen combinations were determined and the results are shown in Table 2. Total phenolic amount were increased with the doses compared to the control. In control plants, total phenolic amount was determined as 84.83 $\mu g.ml^{-1}$. The amount of phenolic compounds in tomato seedlings inoculated with *B. cinerea* was 171.92 $\mu g.ml^{-1}$, while phenolic contents were increased with the higher doses garlic extracts plus pathogen. The phenolic content reached 261.92 $\mu g.ml^{-1}$ in the 1.5% garlic extract application. Similarly, the amount of total phenolics in the *F. oxysporum* f.sp. *lycopersici* inoculated plant was 134.83 $\mu g.ml^{-1}$. This value increased as the garlic extract dose increased and it reached 226.50 $\mu g.ml^{-1}$in 1.5% of garlic extract. Total phenolic contents was increased by *P. deliense* and *R. solani* compared to control. On the other hand, combining different extract doses with pathogens increased the synthesis of phenolic compounds however all the doses remained in the same statistical group. In *S. sclerotiorum* and *S. rolfsii* inoculated plants, total phenolic contents were 153.17 and 179.00 $\mu g.ml^{-1}$, respectively. These values were increased to 241.92 and 274.00 $\mu g.ml^{-1}$, respectively, in the 1.5% garlic extract application. In control and*P. capsici* applied pepper plants, total phenolic contents were 61.50 and 134.00 $\mu g.ml^{-1}$, respectively. The amounts were increasedto 174.00 and 190.67 $\mu g.ml^{-1}$, respectively in 1% and 1.5% doses of extract. Total phenolic contents ($\mu g.ml^{-1}$) of garlic extracts in the control, bacterial pathogen applications, and different extract doses x pathogen combinations are shown in Table 3. The exclusive applications of both of the bacterial pathogens to the plants increased the synthesis of phenolic compounds as a reaction to the pathogens. After the *C. michiganensis* subsp. *michiganensis* inoculation, the amount of phenolic compound was 139.00 $\mu g.ml^{-1}$ while the phenolic compound synthesis increased with the higher doses of garlic extracts x pathogen combinations. Phenolic content was 184.42 $\mu g.ml^{-1}$ in the 1.5% garlic extract application. In *P. syringae* pv. *tomato* inoculated plants, the amount of phenolic compound was 149.00 $\mu g.ml^{-1}$, while it increased to 231.08 $\mu g.ml^{-1}$ in the 1.5% dose. It is known that phenolic compounds in plants have direct antioxidant activities. In studies on the determination of the effects of components in garlic on the diseases, it was found that they had antibacterial, antifungal, antiviral, and antiprotozoal effects (Kozan, 2012). In our study, pathogen applications increased the phenolic compound synthesis in tomato and pepper compared to the control. However, different extract doses and pathogen combinations promoted the phenolic compounds synthesis with the increasing dose. Latha *et al*. (2009) reported that zimmu leaf extract (*Allium cepa* L. x *Allium sativum* L.) led to an increase in the amount of phenolic compounds against *Alternaria solani* (early blight) which was another fungal factor as a reaction to the disease in tests conducted under pot trial conditions. In some other studies, it has been reported that different plant extracts synthesized phenolic compounds in some plants. Moushib *et al.* (2013) sprayed beet extract on potatoes against *Phytophthora infenstans* and determined an increase in cinnamic acid and flavone compounds in the plants. Additionally, simple phenolics p-hydroxybenzoic acid and vanillic acid were also determined in the study.

Table 2. The total phenolic contents in different applications in tomato and pepper against fungal pathogens.

Pathogen	Extract doses (%)*	Total phenolic substance amount (µg ml-1)
	Control (-)	84,83 ± 6,82 C**
	Control (+)	171,92 ± 13,4 B
B. cinerea	0.5	209,83 ± 5,32A B
	1.0	215,25 ± 4,02A B
	1.5	261,92 ± 19,0A
	Control (-)	84,83 ± 6,82 D
	Control (+)	134,83 ± 6,82 C
F. oxysporum f.sp. *lycopersici*	0.5	148,58 ± 5,83 BC
	1.0	188,58 ± 11,4 AB
	1.5	226,50 ± 17,6 A
	Control (-)	84,83 ± 6,82 C
	Control (+)	135,67 ± 0,417 B
P. deliense	0.5	201,50 ± 22,1 A
	1.0	217,75 ± 10,1 A
	1.5	245,25 ± 11,9 A
	Control (-)	84,83 ± 6,82 B
	Control (+)	174,83 ± 19,2 A
R. solani	0.5	199,00 ± 4,51 A
	1.0	205,25 ± 1,25 A
	1.5	211,08 ± 8,36 A
	Control (-)	84,83 ± 6,82 D
	Control (+)	153,17 ± 3,33 C
S. sclerotiorum	0.5	190,67 ± 16,0 BC
	1.0	203,17 ± 14,0 AB
	1.5	241,92 ± 6,71 A
	Control (-)	84,83 ± 6,82 C
	Control (+)	179,00 ± 27,8 B
S. rolfsii	0.5	206,50 ± 26,2A B
	1.0	223,17 ± 16,2A B
	1.5	274,00 ± 0,72 A
	Control (-)	61,50 ± 4,51 C
	Control (+)	134,00 ± 6,17 B
P. capsici	0.5	139,42 ± 1,10 B
	1.0	174,00 ± 3,61 A
	1.5	190,67 ± 8,97 A

*Control (-): control with any application, Control (+): pathogen application, extract doses conbined with pathogen inoculation
**Averages in the same row with different letters for each pathogen is different according to the Tukey ($p<0,05$) statistical test

Table 3. The total phenolic contents in different applications in tomato against bacterial pathogens.

Pathogen	Extract doses (%)	Total phenolic substance amount (µg ml-1)
	Control (-)	84,83 ± 6,82 D*
	Control (+)	139,00 ± 2,50 C
C. michiganensis subsp. *michiganensis*	0.5	145,25 ± 2,17 BC
	1.0	175,67 ± 9,02 AB
	1.5	184,42 ± 9,61 A
	Control (-)	84,83 ± 6,82 D
	Control (+)	149,00 ± 0,72 C
P. syringae pv. *tomato*	0.5	158,58 ± 1,10 BC
	1.0	191,50 ± 8,20 B
	1.5	231,08 ± 14,3 A

*Control (-): control with any application, Control (+): pathogen application, extract doses conbined with pathogen inoculation
**Averages in the same row with different letters for each pathogen is different according to the Tukey ($p<0,05$) statistical test

Conclusions

In this study, the effects of *Allium tuncelianum* ethanol extract on the disease severity of nine important fungal, fungal-like, and bacterial pathogens in tomato and pepper under pot trial conditions and the amount of total phenolic compounds were determined. According to the pot trial results, extracts at higher doses reduced the severity of disease against *Botrytis cinerea*, *F. oxysporum* f.sp. *lycopersici*, *P. deliense*, *R. solani*, *S. rolfsii*; however, higher doses of extract had no effect on other pathogens. It was determined that the amount of phenolic compounds were increased to a certain level compared to the control in tomato and pepper when the pathogen was applied exclusively. However, it was also reported that this level was much higher when pathogens were applied at different doses and in different combinations. Conclusively, ethanol extract of the garlic used in our *In vivo* study reduced the development of some important pathogens in tomato and pepper, and promising results were obtained on the effects on common pathogens in agricultural production. In addition to the *In vitro* effects of Tunceli garlic on some pathogens, its activity at certain levels under pot trial conditions was also an important factor in the study. In previous studies, it was determined that *Allium porrum* and *A. sativum* were used against some pathogens and they showed *In vitro* activity at different levels. The extracts and essential oils of these garlic species have very high *In vitro* effects on some pathogens. Also, in previous studies it was seen that these herbal extracts and essential oils have limited activities on different pathogens under pot trial conditions. There are a limited number of studies on the effects of extracts on the diseases under greenhouse and field conditions. The biggest disadvantage for garlic and other extracts and essential oils is the low *In vivo* activity in spite of their high *In vitro* activity. *A. tuncelianum* was tested for the first time against plant diseases was found to have moderate and high levels of activity against some important pathogens, and this could be regarded as a feature differentiating this variety from other garlic varieties. Also, the increased synthesis of phenolic compounds induced by this garlic species was of importance in terms of promoting the resistance mechanism against plant diseases. In further studies, other resistance mechanisms against diseases can be investigated. In light of our results, greenhouse and field studies can be conducted to determine the activity against diseases and phenolic compounds, and detailed studies can be conducted on the production of extract preparations. Therefore, we believe that the results obtained in this study are important and needs further investigations.

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