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Abstract: Effects of dried garlic, peppermint, cabbage, lentil, alfalfa, onion, radish, and garden cress plant materials on *Phytophthora* blight (*Phytophthora capsici* Leon.) of pepper were determined, in both in vitro and in vivo conditions. Extracts of the plant materials were used in vitro. The plant materials were extracted in ethanol and were added to corn meal agar (CMA) at 5 and 10 µg ml^{-1}. The extracts of alfalfa, garlic, cabbage, and peppermint reduced colony diameter of *P. capsici* on corn meal agar between 3.46% and 13.73%, whereas mycelial growth of *P. capsici* was increased by onion, radish, garden cress, and lentil extracts. The plant materials inhibitory to mycelial growth of *P. capsici* were incorporated into soil inoculated with *P. capsici*, in pots and also in the field, in order to determine their effects on *Phytophthora* blight severity. The severity of *Phytophthora* blight of pepper was markedly reduced by cabbage, garlic, and alfalfa materials by 15.3%, 39.8% and 46.9%, respectively, in pot trials. No significant effect of peppermint on disease severity was found. In the field infested with *P. capsici*, disease severity decreased with cabbage, garlic, and alfalfa by 89.5%, 40%, and 10.7%, respectively. Peppermint slightly increased the disease severity (3.4%). In this study, dry cabbage, garlic, and alfalfa materials were effective in reducing the severity of disease caused by *P. capsici*, in both in vitro and in vivo conditions.

Key Words: *Phytophthora capsici*, *Phytophthora* blight, pepper, plant materials

Bazı Bitkisel Materyallerin Biberde *Phytophthora* Yanıklığı (*Phytophthora capsici* Leon.)'na Etkileri

Özet: Kurutulmuş sarımsak, nane, lahana, mercimek, yonca, soğan, turp ve tere bitki artıklarının biberde *Phytophthora* yanıklığı (*Phytophthora capsici* Leon.)'na etkileri in vitro ve in vivo koşullarda belirlenmiştir. In vitro koşullardaki çalışmalarda bitki materyallerinin ekstraktları kullanılmıştır. Bitki materyalleri etil alkolde ekstrakte edilmiş ve mısır unu agara 5 ve 10 µg ml^{-1} dozlarında ilave edilmiştir. Yonca, sarımsak, lahana ve nane, *P. capsici* misel gelişimini % 3.46 ila % 13.73 oranında azaltırken, soğan, turp, tere ve mercimek ekstraktları, misel gelişimini artırmıştır. *P. capsici*' nin misel gelişimine engelleyici etkisi olan bitki materyalleri, biberde *Phytophthora* yanıklığı hastalığının şiddetine etkilerinin belirlenmesi amacıyla, içinde *P. capsici* ile inokule edilmiş toprak bulunan saksılara ve tarla toprağına ilave edilmiştir. Saksı denemelerinde lahana, sarımsak ve yonca artıkları biberde *Phytophthora* yanıklığı hastalığının şiddetini sırasıyla %15.3, %39.8 ve %46.9 oranında azaltmıştır. Nanenin ise hastalık şiddetine önemli bir etkisi olmamıştır. Tarla koşullarında lahana, sarımsak ve yonca *P. capsici*' nin hastalık şiddetini sırasıyla %89.5, % 40 ve %10.7 oranında azaltmış, nane ise %3.4 oranında artırmıştır. Bu çalışmada kuru lahana, sarımsak ve yonca materyalleri in vitro ve in vivo koşullarda *P. capsici*' ye karşı etkili bulunmuştur

Anahtar Sözcükler: *Phytophthora capsici*, *Phytophthora* yanıklığı, biber, bitkisel materyaller

Introduction

Pepper (*Capsicum annum* L.) is an important and very profitable vegetable for growers in Turkey. *Phytophthora* blight of pepper (*Phytophthora capsici* Leon.) is a major disease that limits pepper production in many countries including Turkey. It may cause losses of up to 40% of pepper yield (Yıldız and Delen, 1980). The disease can affect all parts of the pepper plant. It kills seedlings and causes root rot, stem canker, leaf blight, and fruit rot in older plants. Stem infection at the soil line is common. Affected plants exhibit sudden wilting and death (Sherf and MacNab, 1986). The fungus becomes a severe problem when the soil is excessively wet due to over-irrigation, heavy rainfall, or both, especially in heavy soils

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or in low spots in the field (Shannon and Cotter, 1992). The fungus survives in soil and on infested seeds, and is difficult to control, partly because of its wide host range and its ability to survive in the soil. No pepper cultivar with resistance to *P. capsici* is available to help control this disease (Bosland and Lindsey, 1991). Recommended control procedures include avoiding fields with a history of the disease, planting in well-drained soils, following crop rotation schedules that avoid other susceptible crops (peppers, tomatoes, eggplant, and all cucurbit crops), irrigation of alternate rows, and the application of fungicides. However, none of these measures is an effective, practical, or economical control method for *Phytophthora* blight. The use of crop materials with high levels of biologically active substances may be effective in controlling plant diseases.

Organic amendments to soil can affect soil-borne diseases of plants, sometimes enhancing, but frequently decreasing them (Linderman, 1989). Some plant materials decreased the disease incidence of *Rhizoctonia solani*, *R. bataticola*, *Fusarium oxysporum* f.sp. *conglutinans*, *F. oxysporum* f.sp. *ciceris*, *F. sambucinum, F. solani*, *F. equiseti*, *F. culmorum, F. graminearum, Bipolaris sorokiniana, Phytophthora cinnamoni*, *Aphanomyces euteiches*, *Sclerotium cepivorum, Thielaviopsis basicola*, and *Verticillium dahliae* (Synder et al., 1959; Davey and Papavizas, 1960; Gilpatrick, 1969a, 1969b; Singh and Nema, 1987; Ramirez-Villapudua and Munnecke, 1988; Singh et al., 1989; Zavaleta-Mejia and Rojas, 1990; Dolar and Demirci, 1996; Mayton et al., 1996; Williams-Woodward et al., 1997; Demirci and Dolar, 2003; Goicoechea et al., 2004). Control can be achieved through the reduction of the soil-borne pathogen inoculum or an increase of the microbial activity in the soil.

While decomposing in soil, plant materials release compounds, some of which have inhibiting effects on micro-organisms, (Papavizas, 1966; Williams-Woodward, 1997). Furthermore, some plant materials incorporated into soil enhance soil micro-organisms that have inhibiting effects on plant pathogens and have good effects on yield (Smolinska, 2000; Ingemarsson, 2004).

The objective of this study was to determine the effects of soil amendment with some plant materials, such as cabbage, garlic, peppermint, alfalfa, onion, radish, garden cress, and lentil, on *P. capsici* in the laboratory and in the field. The results of this research may help to develop alternative control methods for the reduction of the incidence of *Phytophthora* blight of pepper.

Materials and Methods

Fungal isolate

Phytophthora capsici isolate was obtained from Prof. Dr. Seher Benlioğlu (Department of Plant Protection, Agricultural Faculty of Adnan Menderes University, Aydın, Turkey) and used for inoculations. The culture was maintained on corn meal agar (CMA, Acumedia).

Plant materials

Cabbage (*Brassica oleracea*), garlic (*Allium sativum* L), onion (*Allium cepa* L.), radish (*Raphanus sativus*), garden cress (*Lepidium sativum*), and peppermint (*Mentha piperita* L.) were obtained from commercial sources, and alfalfa (*Medicago sativa* L.) and lentil (*Lens culinaris* Medik.) from the field as fresh material.

Pathogenicity test

Pathogenicity of *P. capsici* isolate was tested on susceptible pepper cv. Ince Sivri-35 by using the inoculation method developed by Pochard et al. (1976). The pepper seeds were surface-sterilised with sodium hypochlorite (0.5%) for 2 min and then rinsed 3 times with sterile distilled water (SDW). Five seeds were sown in each of the 20 cm pots containing sterilised soil, river-bed sand, and peat moss (1:1:1 v/v/v). After germination, seedlings were thinned to 3 seedlings per pot. Plants were maintained in a growth chamber at 25 ± 2 °C and 30%-50% relative humidity, with 12 h/day white fluorescent light (192 µmol s^{-1} m^{-2}). The stem of the 20-day-old pepper plants were cut at the 6th-7th leaf level. Mycelial discs of *P. capsici* grown in V-8 juice (Eden Foods Inc., Michigan, USA) agar for 10 days were placed on the wounded surface of the stem and covered with sterile aluminium foil. Inoculated plants were incubated in the same conditions. Control plants were treated with uninoculated V-8 juice agar plugs in a similar manner. Disease evaluations were conducted 5 days after inoculation.

Inoculum of *P. capsici*

*Z*oospore suspension of *P. capsici* was prepared according to the method developed by Bosland and Lindsey (1991). *P. capsici* isolate was grown at 25 ± 1 °C for 10 days on CMA and then 10-mm CMA plugs were

cut from the colonised agar with a No. 5 cork borer. A maximum of 15 plugs were placed in 150 ml of sterile distilled water for 48-72 h to induce sporangia production. The water dishes containing the agar plugs were kept at 10 °C for 60 min to induce zoospore release. After the cold treatment, the dishes were returned to 25 °C for 45 min and were then checked for zoospore release. When the number of zoospores released was sufficiently abundant, the agar plugs and water were washed through a double layer of cheesecloth with distilled water. The inoculum was adjusted to 10,000 zoospores per millilitre with a haemacytometer.

Extraction of plant materials and the effect of plant extracts on mycelial growth of *P. capsici.*

Above-ground plant parts of cabbage, garlic, peppermint, alfalfa, onion, radish, garden cress, and lentil plant were completely dried at 60 °C for 2-4 days until they reached their constant weight, and then were finely ground in a mill. A 5 g sample of each plant material was placed in 100 ml of 99.5% ethanol (Merck), was left in the dark overnight at room temperature, and then put in a 40 °C water bath for 4 h. These mixtures were filtered through Whatman-1 filter paper and sterilised through a Leitz filter. The ethanol solution was flash evaporated to dryness at 40 °C and the residue was dissolved in 2.5 ml of 99.5% ethanol. The extract was stored at 4 °C until tested. The effects of different concentrations (5 and 10 µl ml^{-1}) of garlic, alfalfa, cabbage, peppermint, onion, radish, garden cress, and lentil extracts on radial growth of *P. capsici* were determined on CMA. The inhibitory effects of these extracts were compared with control petri dishes, which included the same amount of 99.5% ethanol in CMA. Five petri dishes (9 cm diameter) with each extract and each concentration were centrally inoculated with a 5 mm mycelial plug from 12-day-old CMA culture of *P. capsici*. The petri dishes were incubated in the dark at 24 ± 2 °C for 15 days. Colony diameters on all dishes were measured weekly.

Effects of plant materials on *Phytophthora* blight severity in pot trials

Forty-day-old pepper plants were inoculated with *P. capsici* using the inoculation method developed by Pochard et al. (1976). When necrotic lesions developed in stems, the infected pepper stems, in segments 0.5-1 cm long, were incorporated into non-autoclaved field soil at the rate of 1% by weight of dry soil, and then watered. In addition, 50 ml of zoospore suspension (10^4 spores ml^{-1}) was added to each pot. After 3 days, peppermint, garlic, cabbage, and alfalfa materials were added separately in each pot at the rate of 1% by the weight of the soil infested with *P. capsici*, and watered. Plants were closed in polyethylene bags and kept at room temperature during the decomposition period. Three pepper seedlings, in 4 or 5 leaf stages, were planted in each pot after 2 months.

The pots containing *P. capsici* infested soil without plant material amendment were used as controls. Other control pots contained only non-autoclaved field soil. The pots were incubated in the same conditions as mentioned above. Ten pots were used for each treatment and weekly evaluations were conducted over the course of 30 days.

Field experiment

The study was also conducted in micro plots of 1 m^2 in the field. The plants pieces infected with *P. capsici* (150 g m^{-2}) were incorporated into each micro plot to the depth of about 10 cm and 1 l of zoospore suspension (10^4 spores ml^{-1}) per m^2 was added. Every plot was separately flood irrigated. After 1 week, peppermint, garlic, cabbage, and alfalfa materials were incorporated in each plot to the depth of 10 cm, at the rate of 150 g m^{-2}. Plots inoculated with the pathogen and uninoculated plots without any plant material amendment served as controls. Fifteen healthy seedlings with 4 or 5 leaves were planted in each plot after the plant materials decomposed for 2 months. Plots were watered regularly. Plants were observed weekly for 2 months. The plants showing *Phytophthora* blight symptoms were recorded, re-isolation was performed from each of these plants to prove the symptoms were caused by *P. capsici.* The experimental design was a randomised complete block design with 4 replications.

Disease assessment and data analysis

Plants were evaluated on a scale (Bosland and Lindsey, 1991) of 0 to 9: 0 = no response, vigorous, healthy (as in uninoculated control); 1 = slight root darkening, vigorous, healthy; 3 = brown roots, slight stunting, very small lesions on stems; 5 = brown roots, small lesions on stems, lower leaves wilted, stunted plants; 7 = brown roots, large lesions on stems, girdling, whole plants wilted, and stunted; 9 = dead.

Each experiment was performed twice. Statistical analyses were performed with MSTAT statistical software. The data were evaluated by analysis of variance (ANOVA) using Fisher's least significant difference test ($P < 0.05$).

Results

Effects of plant extracts on mycelial growth of *P. capsici*

The effects of garlic, peppermint, cabbage, lentil, alfalfa, onion, radish, and garden cress extracts on the mycelial growth of *P. capsici* are given in Table 1.

Garlic, peppermint, and cabbage extracts at concentrations of 5 and 10 µl ml^{-1} inhibited mycelial growth of *P. capsici*. Although 5 µl ml^{-1} of alfalfa extract did not have any effect, the concentration of 10 µl ml^{-1} had an inhibitory effect on mycelial growth.

The extracts of lentil, onion, radish, and garden cress had stimulatory effects at both concentrations. Increasing the concentration of these extracts, except garden cress, stimulated the mycelial growth of *P. capsici*.

Table 1. The effects of some plant extracts on mycelial growth of *Phytophthora capsici.*

Plant Materials	Mycelial growth (%)	
	Concentration (µl ml^{-1})	
	5	10
Garlic	-7.55 (22.65)[1]	- 13.73 (18.40)
Peppermint	-6.73 (22.85)	- 12.33 (18.70)
Cabbage	-3.46 (23.65)	- 9.51 (19.30)
Lentil	+16.73 (28.60)	+ 24.23 (26.50)
Alfalfa	0.00 (24.5)	- 12.79 (18.60)
Onion	+18.36 (29.00)	+ 22.36 (26.10)
Radish	+ 7.75 (26.40)	+ 39.24 (29.70)
Garden cress	+13.67 (27.85)	+ 5.95 (22.60)
Control	0.00 (24.5)	0.00 (21.33)
LSD (P = 0.05)	2.073	2.240

[1]: (-) percent inhibition, (+) percent stimulation. Figures in parentheses are actual mean values in mm.

Effects of plant materials on disease severity

The effects of garlic, cabbage, peppermint, and alfalfa on *P. capsici* were investigated in pots in a growth chamber and field conditions. The results of the growth chamber experiments are given in Table 2.

Table 2. The effects of some plant materials on the severity of *Phytophthora* blight of pepper in a growth chamber.

Plant materials	Disease severity (%)	Effect (%)[1]
Not inoculated control	0	0
Inoculated control	76.3	-
Alfalfa	40.5	- 46.9
Garlic	45.9	- 39.8
Cabbage	64.6	- 15.3
Peppermint	80.5	+ 5.5
LSD (P = 0.05)	3.7	

[1](-) inhibition (+) stimulation

Alfalfa, garlic, and cabbage significantly reduced ($P < 0.05$) the disease severity from 76.3% to 40.5%, 45.9%, and 64.6%, respectively. Peppermint slightly increased the severity by 5.5% (Table 2).

In the field experiment, the disease severity of *Phytophthora* blight decreased from 74.4% to 7.8% with cabbage amendment, followed by garlic and alfalfa, which reduced disease severity to 44.7% and 66.5%, respectively. The 3 plant materials significantly inhibited *Phytophthora* blight between 10.7% and 89.5%. Peppermint increased disease severity by 3.4%, but the increase was not significant (Table 3). Peppermint was ineffective in both the growth chamber and field experiments, whereas, garlic, cabbage, and alfalfa were highly effective against *P. capsici*.

Table 3. The effects of plant materials on *Phytophthora* blight of pepper in the field.

Plant Materials	Disease Severity (%)	Effect (%)[1]
Control (Not inoculated)	0	-
Control (Inoculated)	74.4	-
Alfalfa	66.5	- 10.7
Garlic	44.7	- 40
Cabbage	7.8	- 89.5
Peppermint	77.0	+ 3.4
LSD (P = 0.05)	3.242	

[1] (-) inhibition (+) stimulation

Discussion

The results of in vitro studies suggest that garlic, peppermint, cabbage, and alfalfa extracts may contain some antifungal substances, whereas the stimulatory effects other the plants' extracts (not including garden cress) increased along with an increase in concentration. Radish, garden cress, lentil, and onion extracts in ethanol stimulated the mycelial growth of *P. capsici*. It was stated that some plant pathogens use some organic materials as a source of energy, which increases their inoculum potential (Linderman, 1989). In this experiment, the fact that 3 plant materials stimulated the mycelial growth of *P. capsici* suggested that *P. capsici* might have used the organic materials of the 4 plant materials as an energy supply, thus promoting mycelial growth.

Lentil extract increased the mycelial growth of *P. capsici* significantly. Peppermint extract inhibited mycelial growth of *P. capsici*. This result is in agreement with the results of Çakır and Yeğen (1991). Peppermint includes menthol, menthone, and methyl ester (Heath and Reineccius, 1986), compounds that may have some inhibitory effect on mycelial growth of *P. capsici*.

Dried plant materials of garlic, alfalfa, and cabbage inhibited mycelial growth and disease severity. Garlic contains diallyl disulphide, diallyl sulphide, diallyl trisulphide, allyl propyl disulphide, allyl alcohol, dimethyl trisulphide, allyl methyl trisulphide, and allycin (diallyl trisulphonate) in its aromatic oils, and these compounds have antimicrobial effects (Akgül, 1993; Ashurst, 1995; Schwartz and Mohan, 1995).

Alfalfa extracts have some inhibitory effects on some plant diseases due to their ammoniac and saponin content. Alfalfa extracts decreased mycelial growth and disease severity of avocado root rot disease caused by *Phytophthora cinnamoni* (Zentmyer and Thompson, 1967; Gilpatrick, 1969a; Gilpatrick, 1969b). Alfalfa plant extracts also restricted the formation of microsclerotia of *Verticillium dahliae* (Bora, 1975). In the present study, alfalfa plant material was effective in reducing mycelial growth and disease severity of *P. capsici*, in both in vitro and in vivo conditions. Cabbage was the most effective plant material in the field condition. Ramirez-Villapudua and Munnecke (1988) found that dried cabbage material released some fungitoxic gases during the decomposition process and that this decreased the severity of chlorosis disease of cabbage caused by *Fusarium oxysporum* f.sp. *conglutinans*.

Sulphide, disulphide, and trisulphide, which are derived from glucosinolates of Brassica tissues during decomposition, are the active compounds (Papavizas, 1966; Williams-Woodward 1997). Cruciferous plants release biocidal compounds, mainly isothiocyanates, produced during the enzymatic degradation of the glucosinolates present in the plant cells. *Brassica juncea* has the highest concentration of isothiocyanates and also the highest fungicidal activity among cruciferous plants (Smolinska and Horbowicz, 1999).

The use of cruciferous plant materials reduced the amount of sclerotia of *Sclerotium cepivorum* and chlamydospores of *Fusarium oxysporum* f.sp. *lycopersici* in the soil. Air-dried and crushed mustard (*Brassica juncea*) added to the soil effectively reduced the viability of fungal propagules and increased the total number of bacteria, spore forming of bacteria, fluorescent *Pseudomonas*, and actinomycetes in the soil (Smolinska, 2000). Cruciferous plants grown as a pre-crop had an inhibiting effect on soil-borne plant pathogens in field experiments with spinach (Ingemarsson, 2004).

In the present study, cabbage and garlic plant materials were effective in reducing mycelial growth and disease severity of *P. capsici*. This shows that the use of cabbage and garlic plant materials, along with other control methods, can be a useful, beneficial, and effective control approach for *Phytophthora* blight of pepper. Although it may not be practical to add cabbage and garlic plant materials to the soil, the rotation of garlic and cabbage crops with pepper may be a more practical and applicable method.

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