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Influence of *Phytophthora capsici* L. inoculation on disease severity, necrosis length, peroxidase and catalase activity, and phenolic content of resistant and susceptible pepper (*Capsicum annuum* L.) plants

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Abstract: This study explored the level of infection caused by different inoculum concentrations (10^2 , 10^3 , and 10^4 zoospores mL^{-1}) of *Phytophthora capsici* in 3 pepper cultivars at days 2, 4, and 6. The effect that the infection induced on the peroxidase (POX), catalase (CAT), and phenolics of resistant and sensitive seedlings, as well as the defense mechanism against the pathogen, were also investigated. The resistance of PM-702 against the isolate used was high, whereas KM-Hot and DEM-8 displayed sensitive reactions. As a result of analyses of the leaves and stems from the 3 pepper cultivars, important changes in biochemical parameters were observed between resistant and sensitive cultivars after inoculation with the root rot pathogen *P. capsici*. The maximum increase of POX and CAT was observed in the resistant PM-702 cultivar. However, with a high inoculum concentration (10^4 zoospores mL^{-1}), a loss of CAT activity was determined, especially in susceptible cultivars. The maximum increase in phenolics was detected in leaves of susceptible DEM-8 and stems of PM-702 on day 6 following infection. The results suggest that during *Phytophthora* root rot development there is a relation between the disease induced by *P. capsici* and the antioxidant system.

Key words: Antioxidants, defense responses, plant disease, plant-pathogen interaction

Phytophthora capsici L. inokulasyonunun dirençli ve duyarlı biber (*Capsicum annuum* L.) bitkilerinin hastalık şiddeti, nekroz uzunluğu, peroksidaz ve katalaz aktivitesi, fenolik içeriği üzerine etkisi

Özet: Bu araştırmada üç biber çeşidinde *P. capsici*'nin farklı inokulum konsantrasyonlarının (10^2 , 10^3 ve 10^4 zoospor mL^{-1}) belirli zamanlarda (2., 4. ve 6. günlerde) oluşturacağı enfeksiyon şiddeti, meydana gelecek olan enfeksiyonun dayanıklı ve duyarlı biber fidelerinde peroksidaz (POX), katalaz (CAT) ve fenolikler üzerindeki etkisi ile patojene karşı dayanıklılığı sağlayan savunma mekanizması açıklanmaya çalışılmıştır. Çalışmada PM-702'nin kullandığımız izolata karşı dayanıklılığı yüksek bulunurken, KM-Hot ve DEM-8 çeşitlerinin izolata karşı duyarlı reaksiyon verdiği belirlenmiştir. Üç biber çeşidinin yaprak ve gövdelerinde yapılan analizler sonucunda kök çürüklüğü patojeni *P. capsici* ile inokulasyona karşı dirençli ve duyarlı biber çeşitleri arasında biyokimyasal parametrelerde önemli değişimler gözlenmiştir. POX ve CAT'in maksimum artışı dirençli PM-702 çeşidinde gözlenmiştir. Buna rağmen yüksek inokulum konsantrasyonunda (10^4 zoospor mL^{-1}), özellikle duyarlı çeşitlerde CAT'da bir aktivite kaybı belirlenmiştir. Fenoliklerde maksimum artış enfeksiyonu takiben 6. günde PM-702'nin gövdesinde ve DEM-8'in yapraklarında görülmüştür. Sonuçlar *Phytophthora* kök çürüklüğü gelişimi süresince, *P. capsici* ile antioksidan sistem arasında bir bağlantı olduğunu göstermiştir.

Anahtar sözcükler: Antioksidanlar, savunma cevapları, bitki hastalığı, bitki-patojen etkileşimi

Introduction

Pepper (*Capsicum annuum* L.) is cultivated in many areas of the world, including Turkey, for its edible fruits. It is commercially important because of its pungency and color. The crop is attacked by several pathogens, causing serious losses in production. Throughout the world, *Phytophthora* root rot, caused by *P. capsici*, is one of the most economically destructive soil-borne diseases of pepper. The pathogen attacks the roots, stems, leaves, and fruits of the plant. *P. capsici* is also pathogenic in tomato, eggplant, cucumber, watermelon, pumpkin, squash, and cocoa (1). The disease can occur on the plant at any stage, causing damping-off, seedling blight, foliar blight, and plant death preceded by wilting (2).

P. capsici was first detected in Florida in 1931. Extensive losses in pepper, squash, and watermelon were recorded again in 1993, and it caused severe damage in several different fruits and vegetables in 1998 (3).

The pathogen has both a sexual and asexual life cycle; therefore, it is very difficult to control the disease it causes. Since *P. capsici* is a soil-borne pathogen, it is possible to take some cultural precautions such as good soil drainage practices, use of disease-free seeds and seedlings, and balanced fertilization. Moreover, crop alternation can be applied to reduce the disease. There are also several fungicides effective against the disease. However, the effectiveness of these fungicides changes with respect to experimental conditions. The fact that *P. capsici* is a soil pathogen makes natural and chemical control very difficult (4).

Studies aimed at improving pepper cultivars have not succeeded in producing a pepper culture totally resistant against *P. capsici* isolates. It is suggested that the reception property present in the plant before inoculation and the mechanism preventing development of the factor in the plant, or in other words, the elements orchestrating stimulation and sustenance of resistance, materialize in the plant after the disease factor is introduced. In that case, resistance might have a polygenic characteristic (5).

Accordingly, resistibility is related to a chemical mechanism against a disease factor, but not an immunity form, as resistibility can lessen or disappear depending on the age of the plant and its physiology, health, and physical conditions.

Developing cultivars that are resistant to disease and using these for cultivation is the highest value-added disease control method in the long term with regard to health and the environment. Moreover, prevention of product loss due to the disease could provide an increase in economic return. Hence, acknowledgement and utilization of natural defense mechanisms has become important in the control of this pathogen.

Plants develop local and systemic resistance as a result of various agents such as virulent and avirulent pathogens, nonpathogens, and cell wall fragments. Resistance is a long-term and broad-spectrum outcome induced by these resistance stimulants. It has been reported that only 20%-85% of diseases were controlled by applying several methods. The pathogen is increasingly resistant against the fungicides used to control it, and using fungicides leads to the environmental pollution and concerns about human health that constitute a separate dimension of the problem (6). Therefore, it has become very important to determine plant-pathogen interactions and discover which type of resistance is evolved from cultivating resistant species. In addition, it is necessary to develop new strategies to control diseases and increase resistance against disease in economically important plant species.

Today, interest in natural defense mechanisms for the purpose of providing broad-spectrum resistance using a plant's own resistance mechanisms is gradually increasing. The research on fundamental defense mechanisms of plants against disease-inducing factors has increased considerably in recent years (7).

Plants generate some biochemical and physiological reactions when they are faced with biotic or abiotic stress factors, and several chemical compounds are synthesized as a consequence. Defense reactions may develop several hours or a few days after stimulation (8).

Plants have developed appropriate defense mechanisms to recognize and resist inevitable pathogen attacks. Plants use inherent physical and chemical barriers to effectively stop a pathogen invasion, and their inducible defense reactions are activated by pathogen attacks (9).

Studies to obtain pepper species resistant against root-crown rot disease have shown the necessity of analyzing and comparing the metabolisms of pepper species that are susceptible to *P. capsici* and also have different levels of resistance against it. Many current studies aim to analyze the roles of the substances inside the plant that take part in the physiological and biochemical mechanisms controlling this disease.

This study aimed to determine changes in the activities of some antioxidant enzymes [peroxidase (POX) and catalase (CAT)] and the amount of phenolic compounds in the leaves and stems of pepper seedlings (resistant: PM-702; susceptible: KM-Hot and DEM-8) active against the oxidative damage occurring from infection by the disease factor *P. capsici* at different zoospore concentrations (10^2 , 10^3 , and 10^4 zoospores mL⁻¹). Differences in antioxidant activity between diseased and healthy pepper plants were analyzed, and the stage of the disease during which the natural defense mechanisms POX, CAT, and phenolics become effective was detected.

Materials and methods

Plant material and growth conditions

Seeds of 3 *Capsicum annuum* cultivars were used in this study: Kahramanmaraş-Hot (KM-Hot) and Demre-8 (DEM-8) cultivars, which are susceptible to *P. capsici*, and Criollo de Morelos = CM 334 (PM-702) cultivar, which is resistant to *P. capsici*.

Pepper seeds were sterilized by soaking in 0.75% sodium hypochlorite for 2 min and then thoroughly washed with sterile distilled water. After germination, pepper seeds were sown in a plastic pot containing a steam-sterilized soil, fertilizer, and sand mix (1:1:1, v/v/v). The plants were maintained in a growth room under controlled environmental conditions (25 ± 2 °C and a 16-h light, 8-h dark photoperiod).

P. capsici -22 and seedling inoculation

P. capsici-22 (obtained from the Ankara University's Agricultural Faculty Collection, Ankara, Turkey) was grown in the dark on V₈ agar plates at 25 °C. For zoospore production, mycelial plugs were transferred to a flask containing 25 mL of V₈ vegetable juice and incubated in the dark at 25 ± 2 °C. After 1-2 weeks, mycelial plugs were removed from the medium and macerated with sterile distilled water in a sterile

blender for 30 s. Drops of mycelial suspension were placed onto the surface of water-agar plates using a sterile syringe. Sporangial formation in *P. capsici*-22 was induced by removing uncolonized agar from around the mycelium and incubating the culture for an additional 3 days at 25 °C; plates were placed under fluorescent lights (40 W, daylight) at a distance of 12 cm. Zoospores were induced to release by incubating the culture plates in sterile water at 4 °C at room temperature for 1 h. The zoospores were collected and filtered through Whatman No. 54 to remove sporangial cases and mycelial. The concentration was then adjusted to 10^2 , 10^3 , and 10^4 zoospores per milliliter using a hemocytometer (10).

The roots of 2-month-old, uprooted seedlings (5 to 6 true leaves) were washed with tap water and disinfected by sodium hypochlorite (0.75%) for 1-2 min before a final rinse in 1 L of sterile distilled water containing 1-2 drops of Tween 20. Five washed seedlings were bunched together and wrapped with aluminum foil 3-4 cm above the root so that the root necks and plant crowns were level.

A total of 30 seedlings, divided into 6 groups of plants, were put into sterile glass bottles containing 400 mL of Hoagland solution. The plants were incubated for 3 days at 22 ± 3 °C, 60% humidity, and a 14-h light period so that they could acclimate. For each pepper cultivar, 3 glass bottles were prepared. Three days later, samples were taken out of the seedling bunches from the plant breeding room; 100-mL zoospore suspensions, prepared at different concentrations (10^2 , 10^3 , and 10^4), were put into 250-mL beakers; and 100 mL of sterile water was added. Seedling bunches were then dipped into the solutions for 1 h and put into glass bottles again. Bottles were kept under the same conditions for 2, 4, and 6 days. When collecting samples, both control and infected plants were put into nylon bags, labeled, and stored at -70 °C until analysis.

Peroxidase (POX: EC 1.11.1.7) activity

POX was extracted from the leaves and stems (11). The POX activity was calculated by using an absorption coefficient ($26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ at 470 nm) for the tetraguaiacol. The amount of enzyme required for formation of $1 \mu\text{mol}$ tetraguaiacol min^{-1} at room temperature was defined as 1 unit (U) of POX activity (11).

Catalase (CAT: EC 1.11.1.6) activity

CAT was extracted from the leaves and stems (12). The CAT activity was calculated by using an absorption coefficient ($39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm for H_2O_2 (13).

Determination of phenolics

Phenolic content was determined using Folin-Ciocalteu reagent (14). Phenolic compounds were extracted with 80% aqueous methanol in a boiling water bath (80 °C) for 15 min, and extracts were ultracentrifuged for 10 min at $500 \times g$; the pellet was reextracted by the same procedure. With 100 μL of extracts, 750 μL of Folin-Ciocalteu phenol reagent was added to the mixture, and the mixture was shaken. After 5 min, 750 μL of Na_2CO_3 (6%) solution was added. After incubation for 90 min at room temperature, the absorbance against a prepared reagent blank was determined at 765 nm with a UV-visible spectrophotometer (CECIL 5000, Cecil Instruments, Milton, UK). Phenolic content of leaves and stems was expressed in milligrams. Gallic acid was used as a standard.

Disease severity index value

The disease severity index value was rated after inoculation based on a 0-5 scale (15) as a measure of disease severity brought about by the isolate as hand-prepared from 10^2 , 10^3 , and 10^4 zoospores mL^{-1} with inoculated root necks in pepper cultivar seedlings with 5-6 leaves. After inoculation, the average disease severity was observed at days 2, 4, and 6 for 15 seedlings (for each pepper cultivar and zoospore concentration). Scale values from 0 to 3 were accepted as resistant, and 3-5 was accepted as sensitive. The disease severity index value was calculated by Σ (number of seedlings \times scale value) / total number of seedlings.

Statistical analysis

Observation data obtained in terms of disease severity at all concentrations were analyzed by repeated measures analysis of variance (ANOVA). Repeated measures were taken at different levels of the day factor. At the end of variance analysis, Duncan's multiple comparison test was used where necessary. Variance analyses were conducted using SPSS 18, whereas Duncan's test was performed with MSTAT statistical software packages.

The observations obtained in terms of the length of necrosis were analyzed in factorial order by the repeated measures variance analysis technique. At the end of variance analysis, Duncan's multiple comparison test was used where necessary.

The trials were organized to create an experimental design with 3 repetitions in randomized blocks. Samples taken from the leaves and stems of control and infected pepper seedlings with 5-6 leaves were also analyzed by applying factorial variance analysis techniques to the data obtained as a result of the analyses (in terms of POX, CAT, and phenolic characteristics), with 3 repetitions ($n = 3$). Data presented are mean values \pm standard error of measurement (SEM) for 3 replicates. After data were determined by 3-way ANOVA, the significance of differences was determined by Duncan's multiple comparison technique. Variance analysis was conducted using the Minitab 15.1 statistical software package, and Duncan's test was performed using the MSTAT-C statistics software package.

Results

Disease severity

Reaction experiments demonstrated by PM-702, DEM-8, and KM-Hot pepper cultivars against *P. capsici* were implemented under controlled conditions. After inoculation, disease severity index values and necrosis length were observed on days 2, 4, and 6 for 15 seedlings (for each pepper cultivar and zoospore concentration) (Tables 1-4). In the 10^2 zoospores mL^{-1} treatment of *P. capsici*-22 isolate, all 3 pepper cultivars were compared in terms of severity of infection; on day 6 following infection, the highest level of disease among all 3 pepper cultivars was observed in the DEM-8 cultivar ($P < 0.01$) (Table 1).

In the 10^3 zoospores mL^{-1} treatment of *P. capsici*-22 isolate, all 3 pepper cultivars were compared in terms of severity of infection; the highest level of infection was detected in the DEM-8 cultivar on day 4 following infection, and a significant difference was detected among the pepper cultivars ($P < 0.01$). On day 6 after infection, the highest level of disease was observed in the DEM-8 cultivar, and the difference among cultivars was significant ($P < 0.01$) (Table 2).

Table 1. Disease severity index values of pepper seedlings infected with *P. capsici*-22 (10^2 zoospores mL⁻¹) ($P < 0.01$). Statistical significance is indicated by appropriate letters within the table: capital letters represent differences in cultivars on the same day; lowercase letters represent differences among days in the same cultivar.

Cultivars	Day 2	Day 4	Day 6
PM-702	0.0667 ± 0.0667 Aa	0.0667 ± 0.0667 Aa	0.1333 ± 0.0667 Ca
DEM-8	0.1333 ± 0.0667 Ab	0.3333 ± 0.0667 Ab	1.1330 ± 0.1330 Aa
KM-Hot	0.2000 ± 0.0000 Aa	0.2667 ± 0.0667 Aa	0.4670 ± 0.1330 Ba

Table 2. Disease severity index values of pepper seedlings infected with *P. capsici*-22 (10^3 zoospores mL⁻¹) ($P < 0.01$). Statistical significance is indicated by appropriate letters within the table: capital letters represent differences in cultivars on the same day; lowercase letters represent differences among days in the same cultivar.

Cultivars	Day 2	Day 4	Day 6
PM-702	0.1333 ± 0.067 Aa	0.2000 ± 0.000 Ba	0.4670 ± 0.133 Ca
DEM-8	0.1333 ± 0.067 Ac	1.4670 ± 0.240 Ab	3.6000 ± 0.529 Aa
KM-Hot	0.4000 ± 0.115 Ab	0.800 ± 0.200 ABab	1.5330 ± 0.333 Ba

Table 3. Disease severity index values of pepper seedlings infected with *P. capsici*-22 (10^4 zoospores mL⁻¹) ($P < 0.01$). Statistical significance is indicated by appropriate letters within the table: capital letters represent differences in cultivars on the same day; lowercase letters represent differences among days in the same cultivar.

Cultivars	Day 2	Day 4	Day 6
PM-702	0.200 ± 0.000 Ab	0.467 ± 0.1330 Cab	0.867 ± 0.176 Ca
DEM-8	0.6667 ± 0.0667 Ac	3.467 ± 0.1330 Ab	4.533 ± 0.133 Aa
KM-Hot	0.6667 ± 0.0667 Ac	1.667 ± 0.0667 Bb	3.600 ± 0.115 Ba

Table 4. Necrosis length of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). Statistical significance is indicated by appropriate letters within the table: \bar{X} , average; $S_{\bar{X}}$, standard error of average; capital letters represent different days in the same cultivar and same concentration; numbers represent differences in cultivars for the same day and same concentration; lowercase letters represent differences in concentrations for the same cultivar and same day.

Cultivars	<i>P. capsici</i> (zoospores mL ⁻¹)	Necrosis length (mm) [$\bar{X} \pm S_{\bar{X}}$]		
		Day 2	Day 4	Day 6
PM-702	10 ²	1.530 ± 0.0105 B3c	1.660 ± 0.0105 B2c	2.940 ± 0.0235 A3c
	10 ³	2.860 ± 0.0100 B2b	4.420 ± 0.0158 A3b	4.480 ± 0.0152 A3b
	10 ⁴	6.660 ± 0.0108 C3a	13.750 ± 0.0184 B3a	16.730 ± 0.0141 A3a
DEM-8	10 ²	2.530 ± 0.0187 C2c	6.0400 ± 0.0472 B1c	17.400 ± 0.0083 A1c
	10 ³	3.328 ± 0.0351 C2b	30.400 ± 0.0891 B1b	74.700 ± 0.0518 A1b
	10 ⁴	18.31 ± 0.044 C1a	62.270 ± 0.1570 B1a	101.330 ± 0.169 A1a
KM-Hot	10 ²	3.730 ± 0.0173 C1c	6.130 ± 0.0651 B1c	9.600 ± 0.0921 A2c
	10 ³	9.660 ± 0.0219 C1b	27.718 ± 0.0880 B2b	62.804 ± 0.492 A2b
	10 ⁴	15.46 ± 0.155 C2a	42.060 ± 0.618 B2a	72.816 ± 0.434 A2a

In the 10^4 zoospores mL^{-1} treatment of *P. capsici*-22 isolate, all 3 pepper cultivars were compared in terms of severity of infection, and the severity of disease on day 2 after infection was not significant. On days 4 and 6 after infection, the highest level of disease among the 3 pepper cultivars was detected in the DEM-8 cultivar ($P < 0.01$) (Table 3).

Necrosis length

When all 3 pepper cultivars were compared in terms of necrosis length, on day 2 following infection with the 10^2 zoospores mL^{-1} treatment, the difference in necrosis length was significant for all 3 cultivars ($P < 0.05$). At 10^3 zoospores mL^{-1} , the highest necrosis length among the 3 pepper cultivars was observed for the KM-Hot cultivar ($P < 0.05$). At 10^4 zoospores mL^{-1} , the highest necrosis length was determined in the DEM-8 cultivar, and the difference among necrosis lengths was significant for all 3 cultivars (Table 4).

On day 4 following infection at 10^2 zoospores mL^{-1} , the highest necrosis length was detected in the DEM-8 and KM-Hot cultivars, and the difference between them and the PM-702 cultivar was significant ($P < 0.05$). At 10^3 and 10^4 zoospores mL^{-1} , the differences in necrosis lengths among all 3 cultivars were significant ($P < 0.05$) (Table 4).

On day 6 after infection, the greatest necrosis length was observed in the DEM-8 cultivar at all 3 concentrations, and the differences among the necrosis lengths of cultivars were significant ($P < 0.05$) (Table 4).

POX activity in leaves of peppers cultivars

An early induction of POX was observed in the leaves of peppers treated with *P. capsici* within 2 days of all treatments. When the 3 pepper cultivars were compared in terms of POX activity in leaves, it was observed that the amounts of POX in the leaves of the PM-702 cultivar control seedlings on days 2, 4, and 6 were significantly higher than those of the KM-Hot and DEM-8 cultivars ($P < 0.01$) (Figure 1).

POX activity in *P. capsici*-22-infected leaves increased throughout the experimental period (Figure 1). When compared to control leaves, the maximum increase of POX was observed in the leaves of PM-702 seedlings infected with 10^2 , 10^3 , and 10^4 zoospores mL^{-1} on day 6 following infection (P

< 0.01); compared to control leaves, the increases in their values were 132.751%, 259.88%, and 220.104%, respectively. When compared to control leaves, the greatest POX activity in PM-702 inoculated with 10^3 and 10^4 zoospores mL^{-1} of *P. capsici* was detected on day 6 after inoculation ($P < 0.01$) (Figure 1). In the present study, KM-Hot plants showed an increase in POX activity 2, 4, and 6 days after inoculation (Figure 1). POX activity on day 2 after inoculation with 10^3 and 10^4 zoospores mL^{-1} of *P. capsici* was significantly higher ($P < 0.01$) in KM-Hot leaves. When compared to control leaves, the maximum increase of POX was observed in the leaves of KM-Hot seedlings that were infected with 10^2 , 10^3 , and 10^4 zoospores mL^{-1} on day 6 following infection ($P < 0.01$) (Figure 1). POX content in *P. capsici*-22-infected DEM-8 seedlings increased at all times. When compared to the control, the maximum increase of POX activity was observed in the leaves of DEM-8 seedlings that were infected with 10^4 zoospores mL^{-1} on days 4 and 6 following infection ($P < 0.01$) (Figure 1); compared to control leaves, the increases in their values were approximately 132.183% and 102.682%, respectively.

POX activity in stems of pepper cultivars

POX activity in *P. capsici*-22-infected stems increased throughout the experimental period ($P < 0.01$). The highest POX content ($125.79 \pm 1.890 \text{ U g}^{-1} \text{ FW}$) in infected stems was observed in the PM-702 cultivar at 10^4 zoospores mL^{-1} on day 4 following infection; when compared to the control, the increase in value was 57.394% ($P < 0.01$). When compared to control stems, the maximum increase in POX activity was observed in the stems of KM-Hot seedlings infected with 10^2 , 10^3 , and 10^4 zoospores mL^{-1} on day 6 following infection and DEM-8 seedlings infected with 10^4 zoospores mL^{-1} on day 4 following infection ($P < 0.01$); compared to control stems, the increases in value were 265.815%, 226.004%, 225.172%, and 246%, respectively (Figure 2).

CAT activity in leaves of pepper cultivars

When all 3 pepper cultivars were compared in terms of CAT activity in the leaves, the amounts of CAT in the leaves of PM-702 and DEM-8 control seedlings on days 2, 4, and 6 were significantly higher than those of the KM-Hot cultivar ($P < 0.01$) (Figure 3). CAT content in *P. capsici*-22-infected PM-702 seedlings increased at all times. When CAT activity

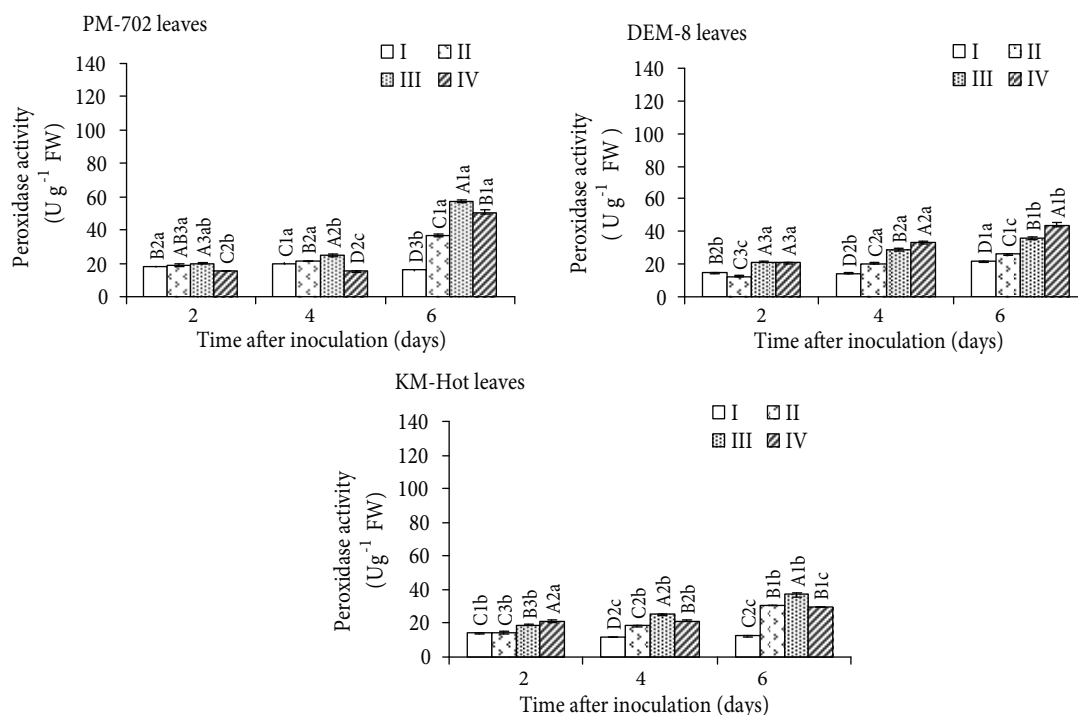


Figure 1. Peroxidase activity in leaves of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: 10^4 zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

in the leaves of all 3 pepper cultivars were compared on day 6 after infection at 10^2 zoospores mL^{-1} , the highest CAT content was detected in the PM-702 cultivar ($P < 0.01$). When compared to control leaves, the maximum increase in CAT activity was observed in the leaves of KM-Hot seedlings infected with 10^2 zoospores mL^{-1} on day 4 following infection ($P < 0.01$). When compared to control leaves, the maximum increase in CAT activity was observed in the leaves of PM-702 seedlings infected with 10^3 zoospores mL^{-1} on day 6 following infection ($P < 0.01$); compared to control leaves, the increase in value was 108.404%. When compared to control leaves, the maximum increase in CAT activity was observed in the leaves of PM-702 seedlings infected with 10^4 zoospores mL^{-1} on days 4 and 6 following infection ($P < 0.01$) (Figure 3).

CAT activity in stems of pepper cultivars

When all 3 pepper cultivars were compared in terms of stem CAT activity, the amounts of CAT in the

stems of PM-702 cultivar control seedlings on days 2, 4, and 6 were significantly higher than those of the DEM-8 and KM-Hot cultivars ($P < 0.01$) (Figure 4). Generally, the CAT content in *P. capsici*-22-infected PM-702 seedlings increased at all times. When compared to control stems, the maximum increase in CAT activity was observed in the stems of DEM-8 seedlings infected with 10^2 zoospores mL^{-1} on day 4 following infection ($P < 0.01$). The increase in its value was approximately 168.88% compared to control stems. When compared to control stems, the maximum increase of CAT activity was observed in the stems of KM-Hot seedlings infected with 10^3 zoospores mL^{-1} on days 2, 4, and 6 following infection ($P < 0.01$); compared to control stems, the increases in these values were approximately 224%, 328%, and 641%, respectively. When CAT activity in the stems of all 3 pepper cultivars was compared on days 2 and 4 after infection at 10^4 zoospores mL^{-1} , the highest CAT content was detected in the PM-702 cultivar ($P < 0.01$) (Figure 4).

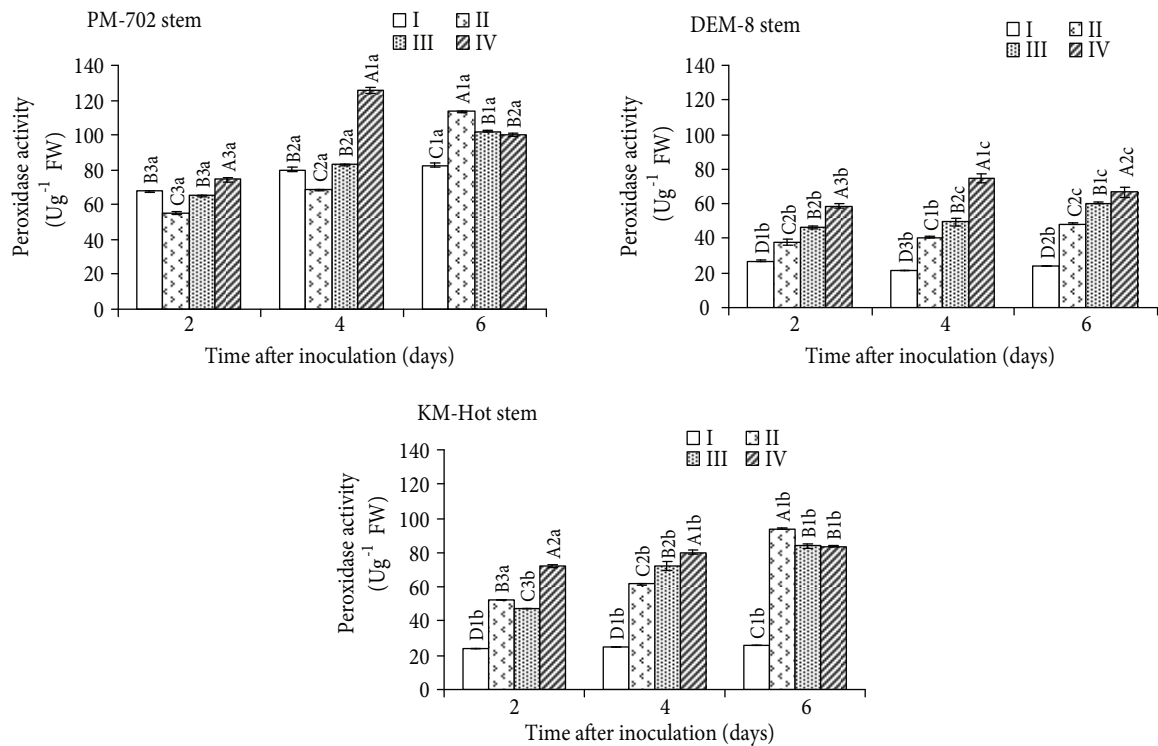


Figure 2. Peroxidase activity in stems of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: 10^4 zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

Phenolic content in leaves of pepper cultivars

When all 3 pepper cultivars were compared in terms of total phenolic properties in the leaves, the amounts of phenolics in the leaves of DEM-8 cultivar control seedlings on days 2, 4, and 6 were significantly lower than those of the PM-702 cultivar ($P < 0.01$) (Figure 5). When the amounts of phenolics in the leaves of all 3 pepper cultivars were compared on day 2 after infection at 10^2 zoospores mL^{-1} , the highest amount of phenolic content was detected in the PM-702 and KM-Hot cultivars ($P < 0.01$). When the amounts of phenolics in the leaves of all 3 pepper cultivars were compared at 10^3 zoospores mL^{-1} , the highest level of phenolics was detected in the KM-Hot cultivar. No significant difference was found between PM-702 and DEM-8 ($P < 0.01$). Among all 3 cultivars at 10^4 zoospores mL^{-1} , the highest amount of phenolics was detected in the PM-702 cultivar. The differences in the amount of phenolics in the 3 cultivars were

significant ($P < 0.01$). On day 4 following infection, the amounts of phenolics in the leaves of all 3 pepper cultivars at 10^2 zoospores mL^{-1} were compared, and the highest amount of phenolics was detected in the DEM-8 cultivar ($P < 0.01$). At 10^3 zoospores mL^{-1} , the highest amount of phenolics was detected in the KM-Hot cultivar. The difference in the level of phenolics was significant for all 3 cultivars ($P < 0.01$). However, at 10^4 zoospores mL^{-1} , the highest level of phenolics was detected in the PM-702 cultivar ($P < 0.01$). On day 6 following infection at 10^2 zoospores mL^{-1} , the difference in phenolics among the cultivars was found to be significant ($P < 0.01$). At 10^3 zoospores mL^{-1} , the difference in phenolics among DEM-8, PM-702, and KM-Hot was significant ($P < 0.01$), and the highest amount of phenolics was detected in PM-702 and KM-Hot. At 10^4 zoospores mL^{-1} , the highest level of phenolics was measured in the PM-702 cultivar, and the difference among all 3 cultivars was statistically significant ($P < 0.01$) (Figure 5).

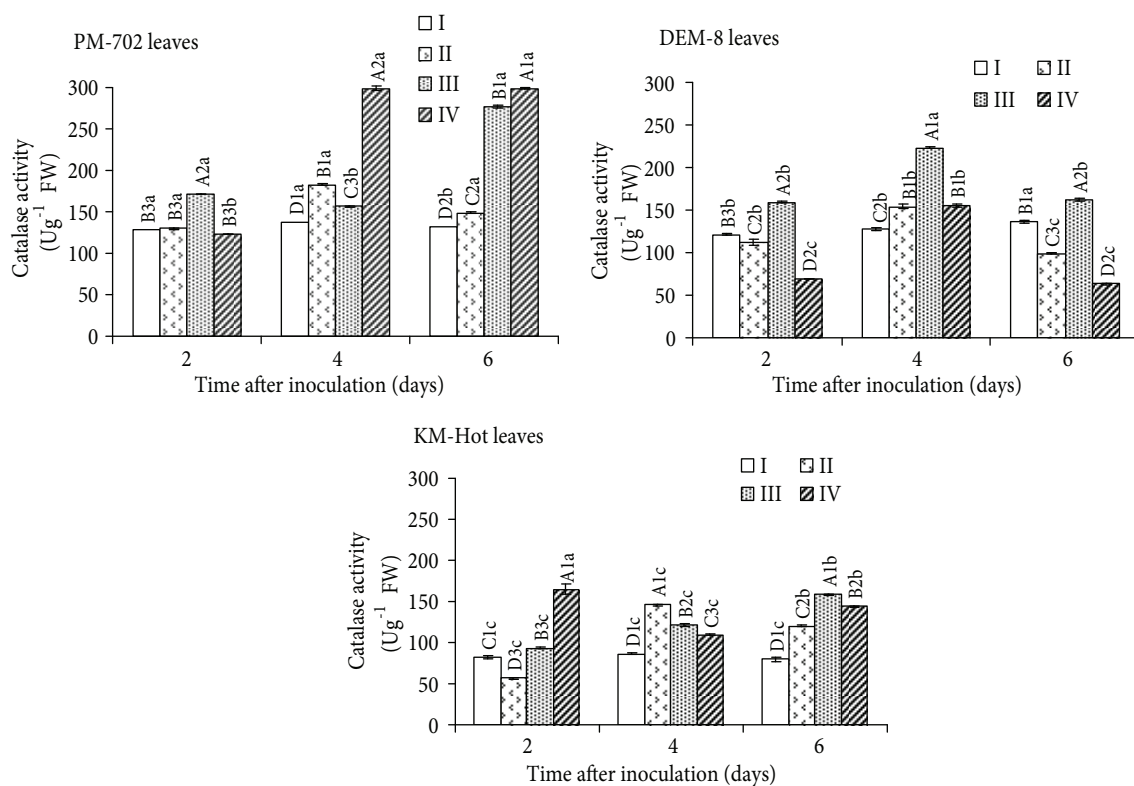


Figure 3. CAT activity in leaves of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: 10^4 zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

Phenolic content in stems of pepper cultivars

When the amounts of phenolics in the stems of all 3 pepper genotypes were compared at 10^2 zoospores mL^{-1} , the highest level of phenolics was detected in the DEM-8 cultivar at day 6 following infection ($P < 0.01$) (Figure 6); compared to control stems, the increase in value was approximately 18%. When the amounts of phenolics in the stems of all 3 pepper cultivars were compared at 10^3 zoospores mL^{-1} , the highest level of phenolics was detected in the KM-Hot cultivar on day 6 following infection ($P < 0.01$); when compared to control stems, the increase in value was approximately 32% (Figure 6). Among all 3 cultivars at 10^4 zoospores mL^{-1} , the highest amount of phenolics was detected in the PM-702 cultivar on day 6 following infection ($P < 0.01$); the increase in value was approximately 42% compared to control stems.

Discussion

One of the parameters that could be used to explain the resistance mechanism is the severity of disease in the stem and leaves of the plant. For this purpose, the severity of disease in leaves and stems of pepper cultivars and necrosis length were determined.

We observed that PM-702 resistance to the isolate used was high, whereas the KM-Hot and DEM-8 cultivars were susceptible to the disease factor and reacted against it. The disease factor progressed far more quickly in KM-Hot and DEM-8, and most of the seedlings, especially those of the DEM-8 cultivar, were severely damaged on day 6. Differences in terms of resistance increased among the cultivars over time. Resistance in KM-Hot and DEM-8 increased from day 2 onward, whereas the level of disease was much lower in the PM-702 cultivar. Despite regions

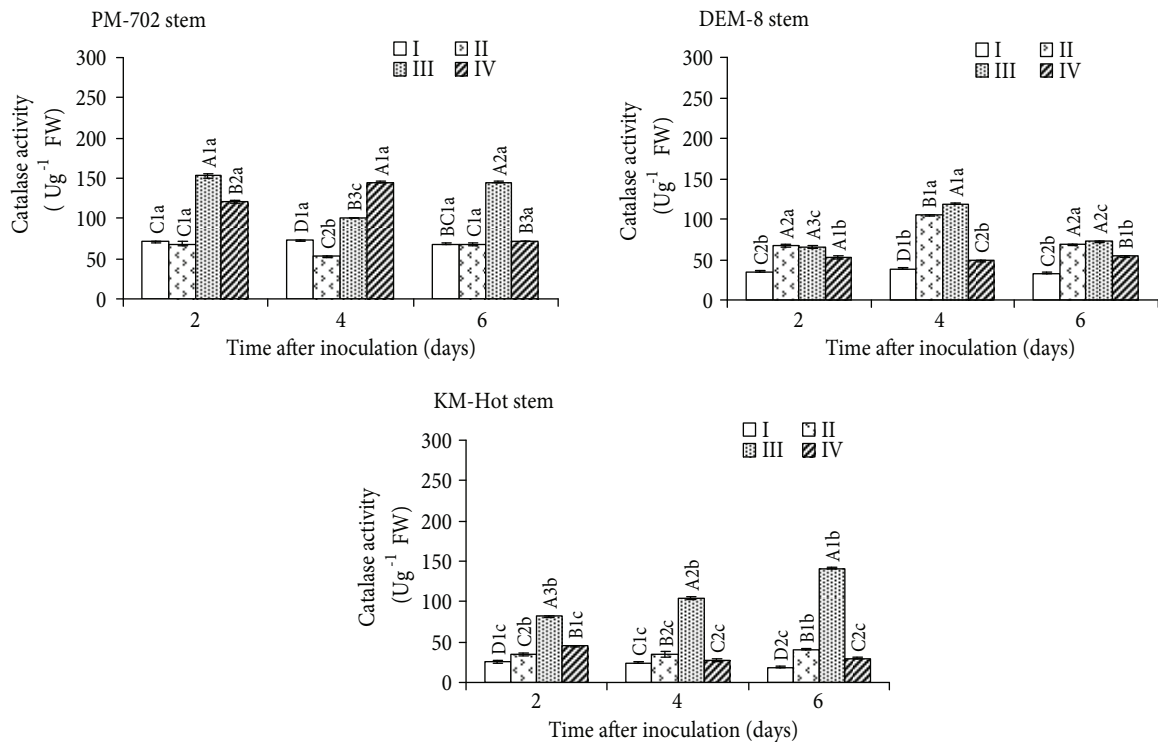


Figure 4. CAT activity in stems of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

of brown lesions progressing along the stem in small amounts in the PM-702 cultivar, the change in green parts was much smaller.

Plants with induced resistance respond to infection by some type of host response that restricts the development of pathogens (16). POX activity is related to the resistance against disease in plant-pathogen interactions. The increase in POX activity indicates that it has a key role in local and systemic resistance (17). POX accumulates both in the extracellular environment and in the cell wall in cell suspension studies. This result might be related to lignin biosynthesis, which prevents fungi from entering the cell (18).

The severity of infection increased over time after inoculation in the leaves of cucumber seedlings that were infected with fungi. Depending on the increase in the severity of the disease (19), the highest POX activity was observed on day 9 after inoculation.

In other words, increases in enzyme activity were detected on days when severity of the disease also increased. This mechanism is also supported by the findings of our study. The earliest response in the leaves and stems was generated after 48 h in our study, whereas the highest level of POX activity was observed on days 4 and 6 for all 3 cultivars at all 3 zoospore concentrations (10^2 , 10^3 , and 10^4). The increase in POX activity in pepper seedlings infected with different inoculum concentrations just 2 days after inoculation indicates that this is among the earliest responses, and the increase in enzyme activity is considered a general reaction. POX enzyme expedites cell necrosis in the infection region and blocks progression of the infection. It generates a toxic environment and inhibits the pathogen from growing toward inner cells (20). This enzyme is required in phenylpropanoid pathways. Stimulation of phenylpropanoid pathways, depending on the

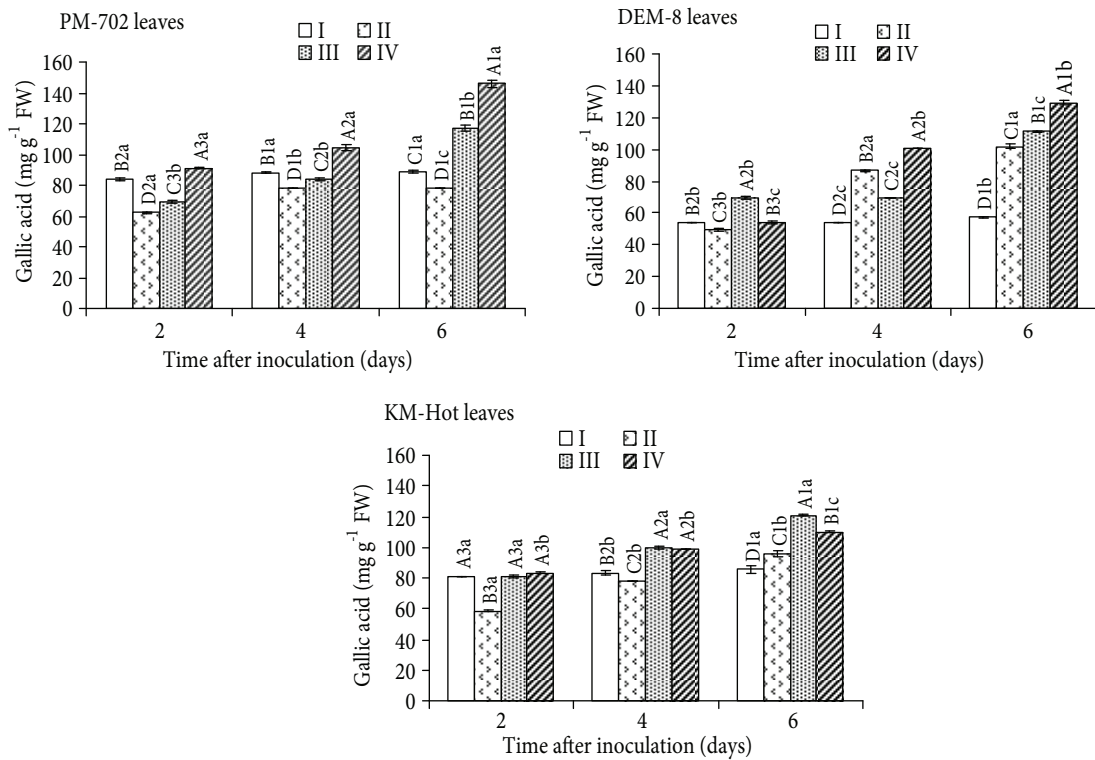


Figure 5. Phenolics in leaves of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: 10^4 zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

pathogen infection and hence the synthesis of antioxidant compounds and phenolics, is known to be one of the most characteristic properties of this hypersensitive response (21).

CAT is also considered a general stress response to reactive oxygen species that are produced in excess amounts. However, the antioxidant potential of the CAT in the tissues of *Capsicum annuum* is not sufficient to block the oxidative damage in some cases. When CAT is inactivated under severe stress conditions, toxic properties of H_2O_2 are inhibited by another antioxidant enzyme such as POX. CAT incurs activity losses, and other antioxidant enzymes are stimulated under severe stress conditions (22). The fact that free radicals such as H_2O_2 are removed by other antioxidant enzymes such as POX, if CAT is inactivated under severe stress conditions, indicates that POX has vital importance in plant defense mechanisms. H_2O_2 accumulation under severe stress conditions is considered to be one of the factors

causing the inactivation of the CAT enzyme. CAT is not a stable enzyme, and it is inhibited under the high concentrations of H_2O_2 produced in plants under stress (23,24).

In our study, a general increase in CAT activity was detected in the leaves and stems of inoculated seedlings. However, at high inoculum concentrations (10^4 zoospores mL^{-1}), a loss of activity was determined, especially in susceptible cultivars. CAT activity continued to increase in the leaves and stems despite the increasing inoculum concentration only in PM-702, which is a resistant cultivar. H_2O_2 accumulation occurs in different tissues as a consequence of plant and pathogen interaction (25). The increase in CAT activity may contribute to the reduction of H_2O_2 content. CAT activity is important for the elimination of H_2O_2 (26). However, it may not be sufficient for CAT to separately block decomposition pathways generated by H_2O_2 .

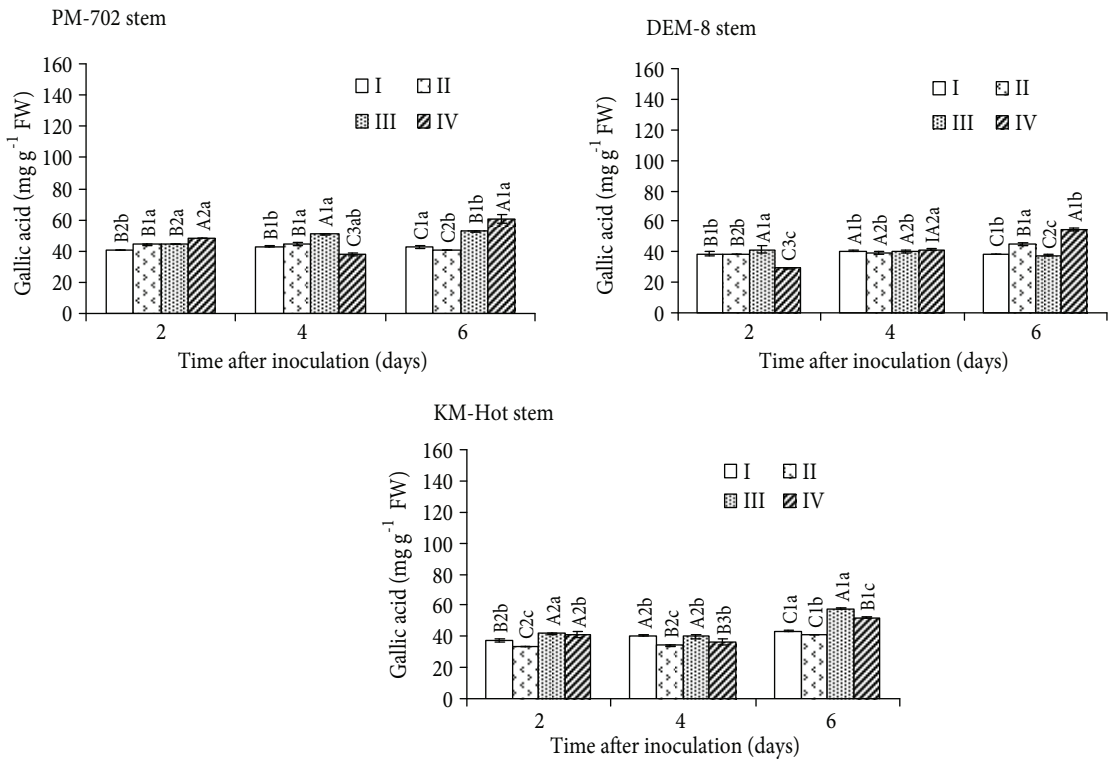


Figure 6. Phenolics in stems of pepper seedlings infected with different zoospore concentrations ($P < 0.01$). I: control, II: 10^2 zoospores mL^{-1} , III: 10^3 zoospores mL^{-1} , IV: 10^4 zoospores mL^{-1} . Statistical significance is indicated by appropriate letters: capital letters represent concentration differences in the same cultivar on the same day; numbers represent differences in days for the same cultivar and same concentration; lowercase letters represent differences in cultivars for the same concentration and same day.

Previous studies demonstrated that the duration of stress treatment causes different reactions in both POX and CAT enzymes in plants. In cotton leaves exposed to aphid (*Aphis gossypii*), POX activity increased with respect to the control group after 6 days following treatment. On day 9 after treatment, it was still higher than in the control group; however, it decreased with respect to day 6. The same reaction was observed for the CAT enzyme (27). In other words, changes were detected in activity levels depending on the time after treatment. In our study, POX activity in the leaves and stems of all 3 pepper cultivars was observed to generally increase in comparison to the control groups as the inoculation period increased (days 2, 4, and 6). This result contradicts the findings of Gomez et al. (27). On the other hand, CAT activity in DEM-8, which is a susceptible cultivar, decreased on day 6 following infection, especially at 10^4 zoospores mL^{-1} . This result indicates that a concentration of 10^4 zoospores mL^{-1} generates severe

stress conditions for cultivars that are susceptible to the pathogen.

Another defense mechanism developed against pathogens is phenolic compounds. As far as responses to pathogen infections are concerned, one of the most significant responses is an increase in phenylpropanoid metabolism, which causes regional synthesis of phenolic compounds. The functional importance of this response is not yet completely understood. However, it is suggested that accumulation of phenolic polymers and lignin in the infection region to inhibit the invasion of the pathogen might be a result of increased synthesis of phenolics (28).

Research to date has demonstrated that phenolic compounds assist in strengthening the cell walls against pathogens and inhibiting fungal growth. Changes in the amount of phenolic compounds in plants are indicators of susceptibility to disease (29).

Enzymes secreted by fungal pathogens during the infection are involved in the decomposition of the cell wall. It is known that various cell wall compounds inhibit the activation of cell wall-decomposing fungal enzymes. For example, phenolics and proteins bound to the cell wall inhibit fungal enzymes. In fact, decomposition and strengthening of the cell wall are considered to be the key events in fungal pathogen infection (30).

Phenolic content in the resistant cultivar and susceptible cultivar KM-Hot decreased after infection with low inoculum concentrations (at 10^2 and 10^3 zoospores mL^{-1} on days 2 and 4) in comparison to the control groups. In other words, phenolic materials are depleted when the plant is infected. It is estimated that this depletion is related to the secretion of metabolites that destroy and decompose the structure of pathogens (31).

With fungi, the deposition of structural barriers commonly occurs in response to infection (16). The POX enzyme stimulates phenolic formation in the cell wall of the plant to defend against pathogenic agents (32). In our study, all 3 pepper cultivars were observed to have the highest POX activity on day 6 in comparison to the control groups. The highest increases in the amount of phenolics were detected on days 4 and 6. Therefore, interaction between *Capsicum annuum* and *P. capsici* causes a significant increase in oxidative stress, and the increases in enzyme activity and phenolic compounds are also linked to this interaction. The fact that POX stimulates synthesis of lignin, which is a phenolic, also indicates that there is a correlation between POX and phenolics. The elevated activity of antioxidant enzymes could reduce membrane damage by eliminating reactive oxygen species (33). The reason for synthesizing and increasing phenolic materials against a pathogen may be to decrease the effects of these reactive oxygen species.

Studies of *P. capsici* (34) and 3 pepper cultivars revealed that the susceptibility level of the pathogen is also different in our study. Moreover, genetic factors of both the host and the microorganism determine the specificity of the local responses, which are very effective for limiting the invasion of the infection.

Since the pathogens, which are capable of recognizing the host plant and possess properties to

suppress plant defense mechanisms, use the plant's nutrition materials to sustain their development and reproduction, they do not kill the plant cell in the early stages of the infection. Since tissue damage is low in the early stages, plant defense mechanisms are also at low levels. Moreover, the reactions occurring during defense can take place a few hours after stimulation or a couple of days later (8). In our study, despite the fact that the earliest responses to the pathogen infection occurred on day 2, the greatest changes in defense activities were detected on days 4 and 6.

Previous research has also reported differences among cultivars of the same species. As indicated earlier, fungal stimulants interact with pathways at different points (35), and, therefore, they induce different defense reactions in their hosts. These different defense reactions are linked to the possession of different genotypes. This is supported by studies conducted on different cultivars of the same species and species susceptible to or resistant against biotic stress.

In our research, enzyme activity and phenolic accumulation in the 3 pepper cultivars occurred at different levels. When the control groups of these 3 pepper cultivars are compared, the PM-702 cultivar resistant to *P. capsici* generally has the highest level of enzymes and the greatest amount of phenolic compounds. Increases in enzyme activity and phenolic amounts were detected with respect to time after infection and inoculum concentration in the treatments; differences were observed among all 3 pepper cultivars, and the highest enzyme and phenolic compound contents were determined in the PM-702 cultivar, which has the greatest resistance to the pathogen. Nonetheless, days and inoculum concentrations were also recorded at which susceptible cultivars showed increases in enzyme activity with respect to their control groups that were higher than those of resistant cultivars.

In fact, the relationship between the product of the avirulence gene of the pathogen and the resistance gene of the plant determines the emergence of the disease (36). If the product of the resistance gene of the plant can perceive the product of the avirulence gene of the pathogen, the general defense mechanism of the plant is activated and the infection does not succeed. In other words, resistance is not related to

the type of immunity but rather the activation of a chemical mechanism against the disease factor. Resistance can decrease or disappear depending on the level of aggression of the factor (4).

This study presents data that indicate that plant defense mechanisms are activated to control the root-crown rot disease induced by *P. capsici* in pepper plants treated with different inoculum concentrations. After treatment, changes in POX and CAT activities and accumulation of phenolic compounds, depending on the number of days, are viewed as defense mechanisms developed against the disease. In other words, the results suggest that there is a relation between the

disease induced by *P. capsici* and the antioxidant system. POX and CAT activities and accumulation of phenolics have different effects on the tolerance of the 3 pepper cultivars to the pathogen.

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