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RESEARCH ARTICLE



Stimulation effect of carrageenan on enzymatic defense system of sweet basil against *Cuscuta campestris* infection

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ABSTRACT

Sweet basil is an important medicinal plant used especially for therapeutical potentials. Sweet basil is a common host for *Cuscuta campestris*, which has a negative effect on infected plants. Therefore, natural friendly control of *C. campestris* seems to be useful. It has been shown that carrageenans can act as elicitors of plant defense responses. In this work, the effect of κ -carrageenans on protection against *C. campestris* and suppression of its invasion in basils were studied. Basils were sprayed with a solution of κ -carrageenan (1 g L^{-1}), once a week, three times in total. Infection of basils with *C. campestris* was performed two days after the last carrageenan treatment. *C. campestris* stem and the leaves of basils were collected two weeks after *C. campestris* inoculation for biochemical studies. Treatment with carrageenan significantly increased shoot length and leaf area of basil and decreased *C. campestris* infestation by about 26%. The content of malondialdehyde, other aldehydes, hydrogen peroxide and lipoxygenase (LOX) activity increased significantly in basils parasitized by *C. campestris*. There were significant differences in phenylalanine ammonia lyase (PAL), catalase (CAT), superoxide dismutase (SOD) and peroxidase activity of parasitized basils by *C. campestris* compared with healthy basils. Carrageenan treatment of basils caused a significant increase in H_2O_2 content and the activity of PAL, CAT and SOD, but not of malondialdehyde, other aldehydes content and LOX, polyphenol oxidase (PPO) and peroxidases activity. The activated enzymatic defense system (PAL, PPO, CAT, SOD and peroxidase) in carrageenan-treated basils have a vital role in alleviating oxidative stress damage in infected plants, by removing excess reactive oxygen species and inhibiting LOX activity and lipid peroxidation that was observed in this study. Our results showed that the application of κ -carrageenan-induced beneficial effects in plants, with regard to growth stimulation and the activation of enzymatic defense system. Thus, carrageenan was recommended as a natural biostimulator for protection of plants against *C. campestris*.

ARTICLE HISTORY

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Dodder; enzymatic antioxidant; κ -carrageenan; *Ocimum basilicum*

Introduction

Sweet basil (*Ocimum basilicum* L.) is an important commercial vegetable, widely cultivated in several regions all over the world (Ramasubramania 2012). Basil essential oil is used extensively in the food industry as a flavoring agent, in perfumery and in medical industries (Gülçin et al. 2007).

Cuscuta is classified in the family Convolvulaceae. *Cuscuta campestris* Yunck. (field dodder) is a holostemparasite with leafless, threadlike, orange or yellow stems that twines around the host plants (Toth et al. 2005). *C. campestris* was found on 85 host plant species, including sugar beet, alfalfa, potato, tomato, onion, basil and other vegetable crops (Liao et al. 2005) but not grasses (Dawson et al. 1994). It causes vast damage in agriculture. The interference of *Cuscuta* sp. with crop plants is entirely by parasitism. *Cuscuta* sp. obtains resources entirely from its host plants by withdrawing photosynthates, water and minerals. Thus, it can severely suppress the growth of the host plant (Dawson et al. 1994). In addition, it causes severe damages to ornamental plants and forage crops (Fathoulla and Duhoky 2008). Field dodder can also transmit various viruses and mycoplasma-like organisms (Zhang et al. 2001).

Control of *Cuscuta* sp. is extremely difficult. There are some options to reduce crop infestations by dodders such

as hand removal, spot or field burning, close mowing, later planting time, and crop rotation with cereals and corn (Dawson et al. 1994; Musselman 2001). Control by herbicides is complicated as it could affect the host plant adversely. Therefore, environment-friendly control could be promising to manage this parasitic weed (Toth et al. 2005).

Plants are capable of defending themselves against pests with a variety of preformed structures and inducible reactions. The most effective methods of achieving crop protection would be to introduce the desired resistance into plants by activating their natural protection system with elicitors that are environmentally safe (Ton et al. 2006). Plants can respond in various ways, such as stimulation of the phenylpropanoid pathway, generation of defense-specific signal molecules such as salicylic acid, jasmonates and accumulation of antimicrobial compounds/proteins such as phytoalexins and pathogenesis-related proteins (Halim et al. 2004). The enhanced state of resistance is effective against a broad range of pathogens and parasites, including fungi, bacteria, viruses, nematodes, parasitic plants and even insect herbivores (Vera et al. 2011). The elicitors, which bring about these induced reactions, are diverse and include oligosaccharides, polysaccharides, lipids, glycoproteins, peptides and proteins (Halim et al. 2004).

Seaweeds (marine algae) stimulate the growth and yield and defense responses of plants (Vera et al. 2011; Hashmi et al. 2012), enhance antioxidant properties, and develop tolerance to biotic and abiotic stresses (Jayaraj et al. 2008). For example, macroalgae treatments lessen the impact of heat and low soil moisture; (Zhang et al. 2003), salinity (Nabati et al. 1994), fungal disease (Jayaraj et al. 2008), high UV light intensity (Schmidt and Zhang 2001), herbicides (Zhang et al. 2001) and nematodes (Sun et al. 1997).

The best characterized seaweed elicitors that have potential to activate disease resistance in plants and animals are laminarin (from brown seaweeds *Laminaria digitata*) and carrageenans (Mercier et al. 2001). Carrageenan is a generic name for a family of gel-forming and viscosifying polysaccharides, which are obtained from certain species of red seaweeds of the class Rhodophyceae. Carrageenan is a sulfated polygalactan (Van de Velde et al. 2002). It is formed by alternate units of D-galactose and 3, 6-anhydro-galactose joined by α -1, 3 and β -1, 4-glycosidic union. Carrageenan is divided into various types such as λ , κ , ι all containing 22–35% sulfate groups (Mercier et al. 2001).

κ -carrageenan elicits numerous plant defense responses possibly through the effect of its great sulfate content and induces signaling and defense gene expression in tobacco leaves (Mercier et al. 2001; Shukla et al. 2016). Plants have developed efficient mechanisms to deal with abiotic and biotic environmental stresses. In the latter context, they display a primitive immune system relying mainly on oxidative stress responses occurring at systemic and local levels (Alvarez et al. 1998).

Excessive generation of reactive oxygen species (ROS) is a damaging effect of negative environmental impact (Candan and Tarhan 2003) and biotic stress like *Cuscuta* sp. infestation of host plants (Fürst et al. 2016). To keep the levels of active oxygen species under control, plants have enzymatic antioxidant systems to protect cells from oxidative damage. The enzymes include superoxide dismutase (SOD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT) and polyphenol oxidase (PPO) (Sudhakar et al. 2001). The balance between ROS production and activities of enzymatic antioxidant determines whether oxidative signaling and/or damage will occur (Skyba et al. 2012). The capability of scavenging ROS and reducing their damaging effects may correlate with the tolerance of plants (Gill and Tuteja 2010).

There is no report that shows the effect of carrageenan on plant resistance against dodder infection. In previous work (unpublished), we observed that infection by dodder was reduced and the level of phytoalexins was significantly increased when basil plants were treated with carrageenan before. Therefore, we hypothesized that suppression of dodder infection of carrageenan pretreated plants. This is why we wanted to study the impact of dodder on the enzymatic antioxidant defense system and on other biochemical reactions in sweet basil and evaluate the protective roles of κ -carrageenan as elicitor.

Material and method

Plant material and treatments

Ocimum basilicum L. (sweet basil cv. 'Italian large leaf') seeds were obtained from Kerman Agriculture Research Institute. Seeds were sterilized by immersing them in

0.1% (w/v) sodium hypochlorite solution for 5 min then washed extensively with distilled water and finally rinsed with sterile water. Each pot (14 cm diameter \times 12 cm height) was filled with homogenous mixture of soil and organic manure (4:1). Physicochemical characteristics of the soil were: texture-sandy loam, Electrical conductivity (1:2) (soil: water, v: v) 0.43 ds m^{-1} and pH (1:2) (soil: water, v: v) 7.2 (Hanlon 2015). Basil seeds were planted at 2 cm depth in the center of each pot and thinned to eight plants per pot after seedling emergence. Plants were watered when required. Basil and *Cuscuta campestris* grow best under condition of high light, moisture and temperature; these conditions are provided in natural environment and therefore the pot experiment was conducted in the natural conditions on June, 2015 with an average temperature of 35/18°C day/night and 40% relative humidity. All experiments were repeated twice. The repetition of cultivation and experiments were conducted in the following year (2016).

Pure κ -carrageenan (Sigma-Aldrich, U.S.A.) was used in this study. Carrageenan was dissolved in hot deionized water and a solution of 1 g L^{-1} was prepared for foliar spray treatments with a spray bottle. Basil plants at 2-leaf stage (14 days after sowing) were treated once a week with cool carrageenan solution (15 mL per plant). The treatment was repeated the next two weeks. Control plants were sprayed with deionized water.

Cuscuta campestris is simply transferable to its host plants by vegetative propagation. Significant changes in the amount of metabolites at its apical region indicate that this region is most active during haustorium development for parasitization (Furuhashi et al. 2012). Placement of actively growing *C. campestris* stems on host plants usually results in the formation of haustoria and thus causes a new *C. campestris* plant. *C. campestris* threadlike stems were collected from infested *Alhagi persarum* (Fabaceae) host plants in field of Shahid Bahonar University of Kerman on June, 2015 and 2016. *C. campestris* threadlike stems (120 mm below tip) without visible haustorium were separated and immediately placed on the shoot of 30-day-old (6-leaf stage) basil plants, which had their last carrageenan treatment 48 h before. The pair of basil (44 days after sowing) leaves of the third node and 12 cm of threadlike stems of *C. campestris* (12 days) were collected together two weeks after *C. campestris* inoculation on basil. Carrageenan-treated and untreated basil leaves of the third node (without *C. campestris* inoculation) were harvested at the same time (44 days after sowing).

Infestation percentage of *C. campestris*

For analysis of defense against *C. campestris* invasion and measuring of infestation of basil plants by *C. campestris*, the percentage of *C. campestris* tight coupling (stem attachment and its haustorium penetration) to basil shoot was determined 14 days after the infection.

Shoot length

The height of a plant is the vertical distance from the soil at its base to the shoot tip (apical meristem) in their natural position. The height of the basil plants in its natural position was measured.

Leaf area

The method for leaf area measurement was used by weighing (Chanda and Singh 2002). The shape of the 5th leaf of the test plants was copied on paper. The leaf shape was cut out from the paper and the copies were weighted (W); the leaf area was then gravimetrically evaluated. The leaf area was estimated using following equation: $LA = W / c$

LA is the leaf area (cm^2), W is the weight of the paper (g) and c (0.0082 g/cm^2) is the coefficient of the paper (One cm^2 of the same paper was also cut and weighed).

The following plant materials (basil and dodder) were used for biochemical trials: **B₀**: 5th and 6th leaves of basil sprayed with deionized water (control), **B₁**: 5th and 6th leaves of basil that was treated with carrageenan (1 g L^{-1}), **B₀+D**: 5th and 6th leaves of basil that was parasitized by dodder, **B₁+D**: 5th and 6th leaves of basil that was treated with carrageenan (1 g L^{-1}) and was parasitized by dodder, **D_{B0}**: Threadlike stems of dodder that was attached to basil shoot (control), **D_{B1}**: Threadlike stem of dodder that was attached to basil shoot treated before with carrageenan (1 g L^{-1}).

Lipid peroxidation and hydrogen peroxide (H_2O_2) assay

Lipid peroxidation was estimated from the levels of malondialdehyde and other aldehydes' production using the thio-barbituric acid (TBA) method as described by Heath and Packer (1968) and Meir et al. (1992). Chopped plant material (0.5 g) of each sample (**B₀**, **B₁**, **B₀+D**, **B₁+D**, **D_{B0}** and **D_{B1}**) was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and was centrifuged at $12,000 \times g$ for 5 min. Four mL of 0.5% (w/v) TBA in 20% (w/v) TCA was added to an aliquot of 1 mL of the supernatant of the homogenate. Samples were incubated at 90°C for 30 min. Reaction was stopped immediately by an ice bath. The mixtures were centrifuged at $10,000 \times g$ for 5 min and absorbance of the supernatant was recorded at 532 and 455 nm by spectrophotometry. The values were corrected for non-specific turbidity by subtracting the absorbance at 600 nm.

The H_2O_2 content was determined according to Singh et al. (2007). Chopped plant material (0.5 g) of each sample (**B₀**, **B₁**, **B₀+D**, **B₁+D**, **D_{B0}** and **D_{B1}**) was homogenized with 5 mL of 0.1% (w/v) TCA. The homogenate was centrifuged at $10,000 \times g$ for 15 min, and 0.5 mL of the supernatant was added to 0.5 mL of 100 mM potassium phosphate buffer (pH 7.0) and 1 mL potassium iodide (1 M). The supernatant absorbance was recorded at 390 nm. The content of H_2O_2 was calculated by comparing with a plotted standard calibration curve using different concentrations of H_2O_2 .

Enzymes extraction and assays

Three hundred milligrams plant material of each sample (**B₀**, **B₁**, **B₀+D**, **B₁+D**, **D_{B0}** and **D_{B1}**) was homogenized in an ice-cold mortar using 3 mL of 50 mM potassium phosphate buffer pH 7.0 containing 1 mM Ethylenediaminetetraacetic acid (EDTA), 1% (w/v) soluble polyvinylpyrrolidone (PVP) and 1 mM phenylmethane sulfonyl fluoride (PMSF). After centrifugation at $17,000 \times g$ for 20 min, the supernatant was used for the determination of enzymes' activities and protein content. All spectrophotometric analyses were conducted in a final volume of 3 mL by using UV/visible spectrophotometer

(Biochrom WPA Biowave II, UK). Protein content was determined according to the method of Bradford (1976) using Bovine serum albumin as standard.

Guaiacol peroxidase

(GPX; EC 1.11.1.7) activity was measured in a reaction mixture containing 50 mM potassium phosphate (pH 7.0), 0.3% (v/v) H_2O_2 , 1% (v/v) guaiacol and the enzyme extract by a method described by Plewa et al. (1991). The enhancement in absorbance due to tetraguaiacol formation was recorded at 470 nm and the enzyme activity was determined using $\epsilon = 25.5 \text{ mM}^{-1} \text{ cm}^{-1}$. One unit (U) of GPX activity was defined as the amount of enzyme that produced 1 μmol of tetraguaiacol per minute. The enzyme activity was expressed in U per milligram protein.

Superoxide dismutase

(SOD; EC 1.15.1.1) activity was determined according to Giannopolitis and Ries (1977) by following the photoinhibition of nitroblue tetrazolium (NBT). The reaction mixture contained a 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM Na_2EDTA , 75 μM riboflavin, 13 mM methionine and 50 μL of the enzyme extract. Riboflavin was added as a last component to reaction tubes that were placed for 10 min under 40 W fluorescent lamps. The absorbance of the solution was measured at 560 nm. SOD activity was expressed in U per milligram protein, where one U of SOD activity was defined as the amount of the enzyme that inhibited the NBT photoreduction by 50%.

Catalase

(CAT; EC 1.11.1.6) activity was determined according to the modified method of Dhindsa et al. (1981). The decline in absorbance at 240 nm due to decline of extinction of H_2O_2 was recorded. The assay mixture consisted of a 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 100 μL of the enzyme extract. The activity was calculated using $\epsilon = 40 \text{ mM}^{-1} \text{ cm}^{-1}$. One U of CAT activity was defined as the amount of enzyme that decomposed 1 μmol of H_2O_2 per minute. The enzyme activity was expressed in U per milligram protein (= 1 μM of H_2O_2 reduction $\text{min}^{-1} \text{ mg}^{-1}$ protein).

Ascorbate peroxidase

(APX; EC 1.11.1.11) activity was measured according to Nakano and Asada (1981). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 150 μL of enzyme extract. The absorbance was read as decrease at 290 nm against the blank. The activity of APX was calculated using $\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$. One U of APX activity was defined as the amount of enzyme that decomposed 1 μmol of ascorbate per minute. The enzyme activity was expressed in U per milligram protein.

Lipoxygenase

(LOX; EC 1.13.11.12) activity was measured according to Minguez-Mosquera et al. (1993). One-hundred microlitres of the crude extract and 20 μL of 100 mM linoleic acid containing 0.1% (v/v) Tween-20 were added to 2.88 mL of a

phosphate buffer (200 mM, pH 6.5). The increase in absorbance at 234 nm between 10 and 40 s of the reaction was used and the enzyme U was defined as 1 μ mol of the product formed per minute. The enzyme activity was expressed in U per milligram protein.

Phenylalanine ammonia lyase

(PAL; EC 4.3.1.5) activity was determined according to the method of Şirin et al. (2016). Three hundred milligrams plant material of each sample (B_0 , B_1 , $B_0 + D$, $B_1 + D$, D_{B0} and D_{B1}) was homogenized in an ice-cold mortar with 6.5 mL of 50 mM pH 8.8 Tris-HCl buffer containing 15 mM of β -mercaptoethanol. The mixture was centrifuged at $20,000\times g$ for 20 min and the clear supernatant was desalted in aliquots using an Amicon Ultra-15 Centrifugal Filter Units with Ultracel-50 membrane (Merck Millipore, Germany) and assayed for PAL activity. The enzyme reaction mixture contained 400 μ L of reaction buffer (100 mM Tris-HCl, pH 8.8) and 200 μ L of substrate (40 mM L-phenylalanine, 100 mM Tris-HCl, pH 8.8) and a 200 μ L aliquot of the sample filtrate (or 200 μ L of deionised water used as a blank). The reaction was carried out at 37°C for 30 min and terminated by the addition of 50 μ L of 4 M HCl, and the cinnamic acid concentration was measured spectrophotometrically by the absorbance at 290 nm. One unit of PAL activity is equal to 1 μ mol of cinnamic acid produced per min. The enzyme activity was expressed in U per milligram protein.

Polyphenol oxidase

(PPO; EC 1.10.3.1) activity was measured according to the method of Raymond et al. (1993). The reaction mixture containing 2.7 mL of 200 mM sodium phosphate buffer (pH 6.8), 200 μ L of 20 mM pyrogallol and 100 μ L enzyme extract. The temperature of the reaction mixture was 40°C. The enzyme activity was expressed in U per milligram protein.

Data analysis

All experiments, including plant treatment, growth and the assessment of biochemical parameters, were performed by a factorial arrangement, based on complete randomized design (CRD). All experiments comprised four replication (pots) per treatment and were repeated twice. The repetition experiments were conducted in the following year on June, 2016. Obtained data were averaged over the two repetitions, because there was no interaction. Results were determined using analysis of variance (ANOVA) via Statistical Analysis Software (SAS, Version 9.4, SAS Institute Inc., Cary, NC, U.S.A.). The univariate procedure of SAS was used to test for normality of residuals. Each value indicates mean \pm standard deviation (SD). Means were compared using Fisher's protected least significant differences (LSD) test. Differences at $P < 0.05$ were considered to be significant.

Results

Growth parameters

C. campestris grew and attached itself to basil plants. Already one day after *C. campestris* inoculation of plants, the attached threadlike stems began twining around basil shoots. After

establishing *C. campestris* on host shoot, the twinned stem developed haustoria and penetrated the host tissue to draw water and nourishment. Dodder stem twined itself in counter-clock wise direction tightly around the stem of host plant and grew upward. After two weeks of *C. campestris* infestation, by debilitating and inhibiting the host plants growth, the shoot length and leaf area of basil plants decreased significantly. The carrageenan treatment significantly increased the growth parameters (shoot length and leaf area) in comparison with carrageenan untreated set of basil. Therefore, it seems that the foliar application of carrageenan significantly alleviated the negative effect of parasite (*C. campestris*) on growth of infested host plants (Table 1). In the present study, we observed that infestation percentage of *C. campestris* to basils which were not treated with carrageenan was 60.48%, but in carrageenan-treated basil plants recorded a significantly lower attachment of *C. campestris*, which were 33.93%. These findings showed that infestation of *C. campestris* to basil plant treated with carrageenan decreased by about 26% (Table 1).

Lipid peroxidation, hydrogen peroxide contents and LOX activity

The content of malondialdehyde, other aldehydes and H_2O_2 and the LOX activity increased remarkably in basil plants that were parasitized by *C. campestris* (Figures 1–4). Carrageenan treatment of basil plants caused a significant increase in H_2O_2 content (Figure 3). But, between carrageenan-treated and untreated basil plants, no significant differences could be noticed in the malondialdehyde and other aldehyde contents and LOX activity (Figures 1, 2 and 4). Carrageenan treatment of basil plants parasitized by dodder caused a decrease in contents of malondialdehyde and other aldehydes and LOX activity (Figures 1, 2 and 4).

The malondialdehyde and other aldehydes contents and the LOX activity in *C. campestris* were on the same level as untreated basil plants (Figures 1, 2 and 4). However, the amount of H_2O_2 was about 3.5-fold higher than that of basil control plants (Figure 3).

Antioxidant enzyme activities

Enzyme activity analyses revealed that PAL, CAT and GPX activities in basil were elevated after infestation by dodder (Figures 5, 7 and 9). Significantly enhanced activities of PAL, CAT and SOD enzymes were observed in carrageenan-treated basil plants compared to control plants (Figures 5, 7 and 8). Moreover, carrageenan treatment of basil

Table 1. Effect of *Cuscuta campestris* (dodder) infestation and foliar application of carrageenan (1 g L^{-1}) on the growth parameters (shoot length and leaf area) and infestation percentage of basil plants (*Ocimum basilicum* L.) by *C. campestris*. B_0 : Basil (Control); B_1 : Basil sprayed with carrageenan; B_0+D : Basils that were parasitized by dodder; B_1+D : Basils that were sprayed with carrageenan and parasitized by dodder. Means were compared using Fisher's protected least significant differences (LSD) test.

Treatments	Shoot length (cm)	Leaf area (cm^2)	Infestation of basils by <i>Cuscuta</i> sp. (%)
B_0	22.05 ^b \pm 1.56	5.91 ^b \pm 0.43	
B_1	24.325 ^a \pm 0.99	8.43 ^a \pm 0.95	
B_0+D	13.625 ^d \pm 0.54	4.06 ^c \pm 0.73	60.48 ^a \pm 2.69
B_1+D	16.375 ^c \pm 0.60	6.09 ^b \pm 0.71	33.93 ^b \pm 2.23

Note: Values are mean \pm SD. The different letters in the same column indicated significant difference at $P \leq 0.05$.

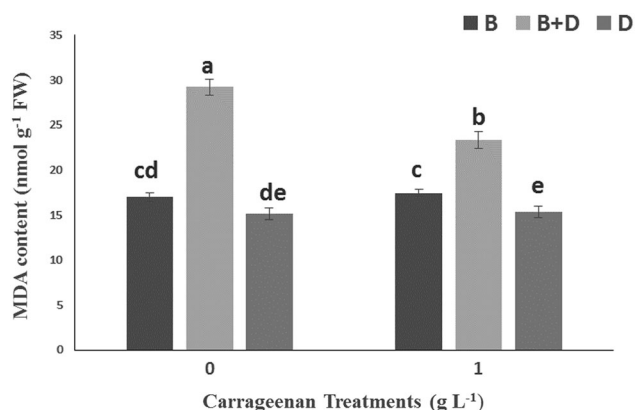


Figure 1. Malondialdehyde (MDA) content ($\text{nmol g}^{-1}\text{FW}$) of basil leaves and *C. campestris* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

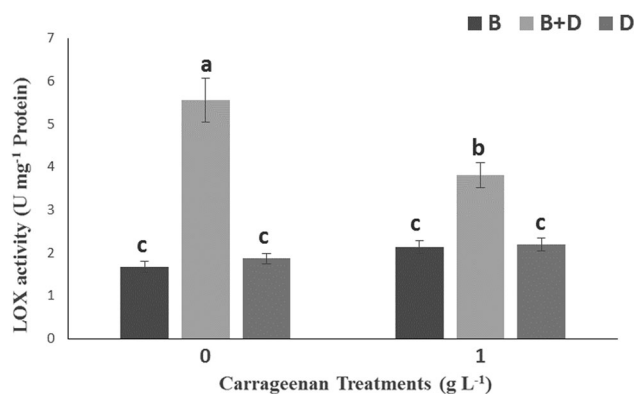


Figure 4. Lipoxygenase (LOX) Activity (U mg^{-1} protein) of basil leaves and *C. campestris* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

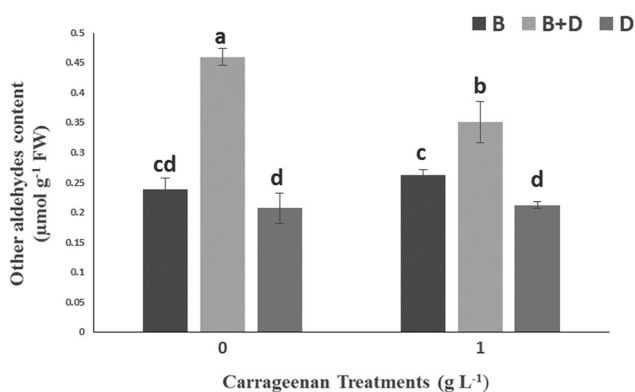


Figure 2. Other Aldehydes a content ($\mu\text{mol g}^{-1}\text{FW}$) of basil leaves and *C. campestris* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

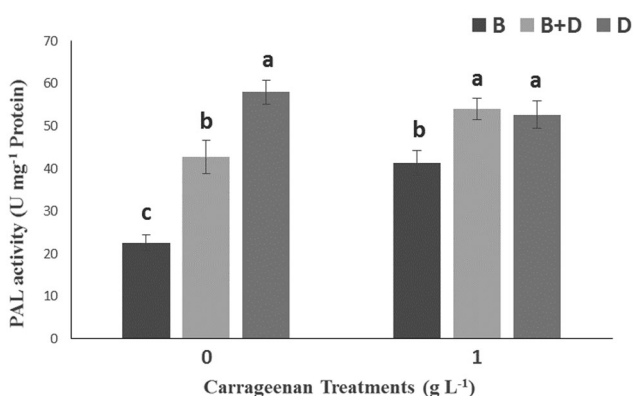


Figure 5. Phenylalanine ammonia lyase (PAL) Activity (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

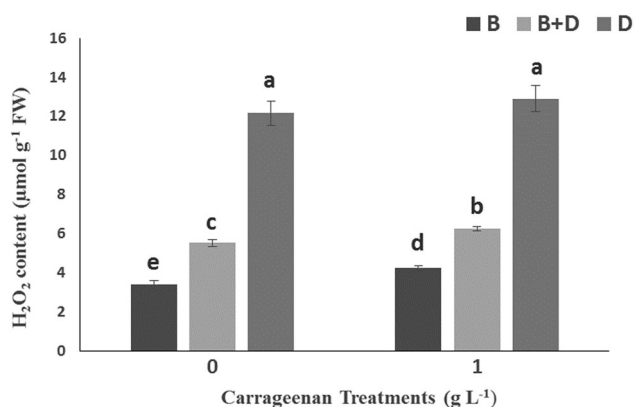


Figure 3. H_2O_2 content ($\mu\text{mol g}^{-1}\text{FW}$) of basil leaves and *C. campestris* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

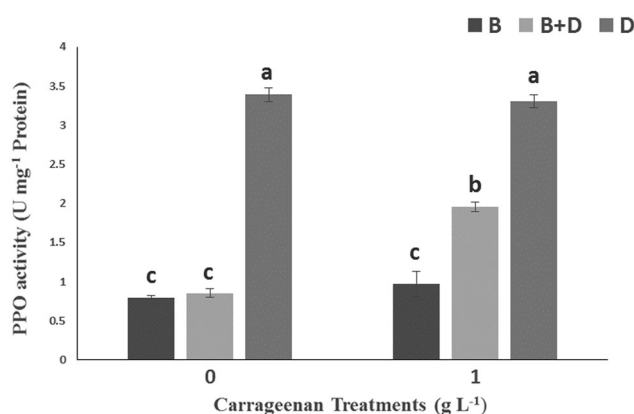


Figure 6. Polyphenol oxidase (PPO) (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basil that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

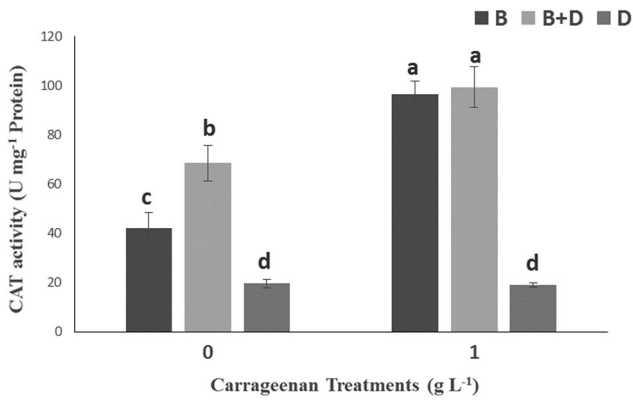


Figure 7. Catalase (CAT) Activity (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

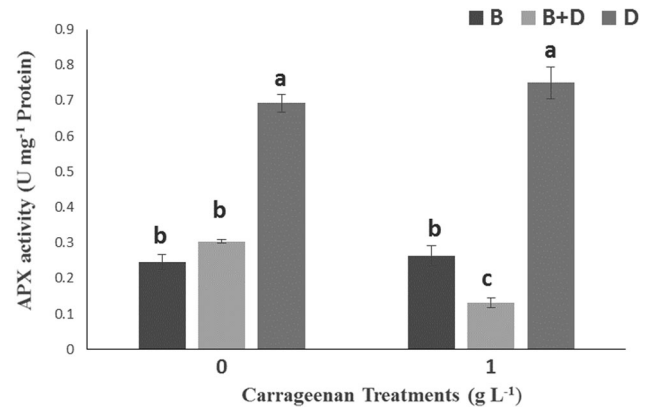


Figure 10. Ascorbate peroxidase (APX) Activity (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

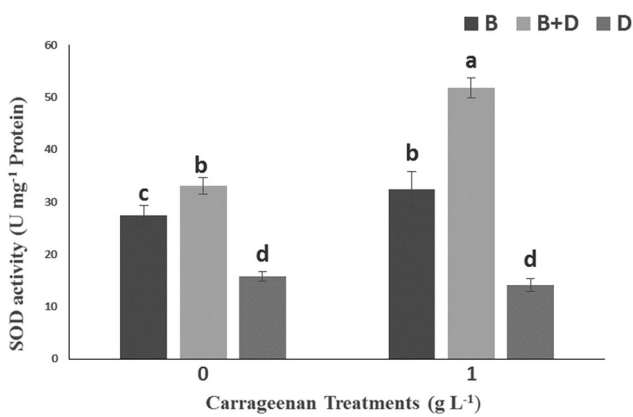


Figure 8. Superoxide dismutase (SOD) Activity (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

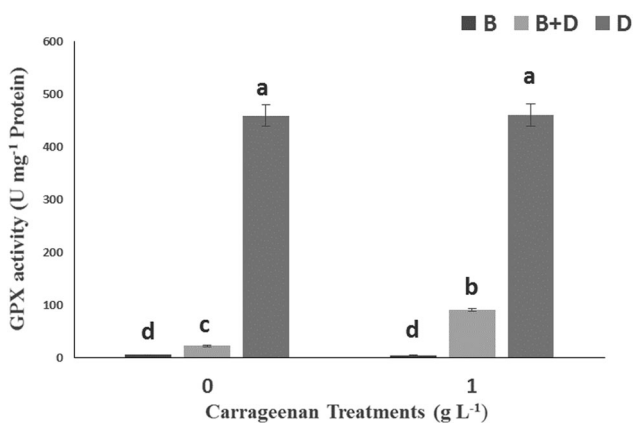


Figure 9. Guaiacol peroxidase (GPX) Activity (U mg^{-1} protein) of basil leaves and *Cuscuta* stems. **B:** Basil; **B+D:** Basils that were parasitized by *C. campestris*; **D:** Dodder (*C. campestris*). Carrageenan treatment: **0:** Control group, foliar spray of water, **1:** Foliar spray of carrageenan 1 g L^{-1} . Columns stand for mean values \pm SD. Means were compared using Fisher's protected least significant differences (LSD) test. Different letters indicate significant differences among treatments ($p \leq 0.05$).

parasitized by dodder caused a significant increase in the activity of PAL, PPO, CAT, SOD and GPX, but not of APX activity (Figures 5–10).

Significant differences were observed in enzyme activity of *C. campestris* compared to the host plant. CAT and SOD activities in *C. campestris* were about 2- and 1.73-fold less than in basil leaves, respectively (Figures 7 and 8). On the other hand, PAL, PPO, GPX and APX activities in *C. campestris* were ca. 2.5-, 4.3-, 85- and 2.82-times higher than in basil leaves, respectively (Figures 5, 6, 9 and 10).

Discussion

In this study, the foliar application of carrageenan enhanced shoot length and leaf area of basil, and alleviated the negative effect of parasite on the growth of infested plants. It is known that κ , λ and ι -carrageenans at a concentration of 1 mg mL^{-1} increased shoot height and leaf biomass in tobacco plants by increasing the net photosynthesis, rubisco activity, glutamate dehydrogenase activity, which is involved in nitrogen assimilation, basal metabolism and cell proliferation (Vera et al. 2011).

In this research, anti-infestation activities of carrageenan were observed, the infestation of *C. campestris* to basil plant treated with carrageenan decreased by about 26%; therefore, spraying of carrageenans significantly increased the resistance of basil plants to *C. campestris* invasion and carrageenan-treated basil plants attracted fewer *C. campestris* as compared to the control.

In the present experiment, dodder invasion induced oxidative stress in basil plants by the generation of ROS such as H_2O_2 , which can directly attack membrane lipids and increase the content of malondialdehyde (MDA). According to Farooq et al. (2009), this is considered as the indicator of oxidative damage. Besides direct effect of H_2O_2 on the pests, it also can stimulate a cascade of reactions that lead to induced defense response, which prevents plants from subsequent attack by pests (Cheeseman 2007). It has been reported that with H_2O_2 application in Arabidopsis, ROS act as secondary messengers to activate many defense-related genes (Desikan et al. 2000). ROS mediate the defensive gene activation and establish additional defenses by regulating the transcription and/or by interacting with other signal

components such as phosphorylation in plant systems in response to different stresses (Cheeseman 2007).

In this research, carrageenan treatment of dodder-parasitized and non-parasitized basil plants caused H₂O₂ accumulation as a consequence of transient production of ROS, which acts as a hardening process for increasing antioxidant capacity of plants (Cheeseman 2007). Low concentration of H₂O₂ can stimulate antioxidant activity; therefore we suppose that carrageenan induced the redox signal (H₂O₂ as a secondary messenger) and lead to increase of enzyme activities.

LOX activity in basil was elevated after infestation with dodder. Polyunsaturated fatty acids are considered to be the most susceptible targets to oxidative stress mediated by ROS. They are converted to oxilipins and further to fatty acid hydroperoxides by lipoxygenase or by auto-oxidation (Porta and Rocha-Sosa 2002). Therefore, we assume that increasing LOX activity contributed to lipid peroxidation in basil plants that were parasitized by dodder. Induction of LOX activity has been reported in several species during plant-pest interactions such as mechanical pressure, wounding or feeding (Creelman and Mullet 1997; Porta and Rocha-Sosa 2002). The function of LOX in defense against pests seems to be related to the synthesis of a number of different compounds with signaling activity, antimicrobial activity or to the development of the hypersensitive response (Porta and Rocha-Sosa 2002).

In the present study, PAL activity was enhanced in infected basil plants. In previous work (unpublished), phenolic and flavonoid contents were increased remarkably in basil plants that were parasitized by dodder. Plant phenolics are secondary metabolites involved in the defense mechanisms of plants and PAL is first and key enzyme of phenylpropanoid pathway (Lattanzio et al. 2006). Albert et al. (2006) showed that the responses of tomato varieties resistant to *C. reflexa* were due to the elongation of hypodermal host cells and a subsequent hypersensitive-like response and accumulation of phenolics and peroxidases at the attachment site that created a mechanical barrier that can block haustorial formation (Albert et al. 2006).

Based on the findings of our experiments, we assume that carrageenan induces resistance pathways. We observed in carrageenan-treated basil plants enhanced activities of different defense enzymes (PAL and PPO). A similar increase in activity of defense enzymes was observed in tobacco plants when sprayed with carrageenan (Mercier et al. 2001). These enzymes are involved in defense reactions and can be stimulated through various elicitors in potato and tomato (Vasuiikova et al. 2001; Jayaraj et al. 2008). Phenolic compounds are believed to mediate resistance to pests in plants, and polyphenol oxidase enzyme has been reported to be responsible for *in vivo* synthesis and accumulation of these compounds (Vaughn and Duke 1984). Therefore, a close correlation found between the enhanced activity of polyphenol oxidase and PAL and the concentration of phenolic is justifiable (Lattanzio et al. 2006). Bi et al. (2008) also reported of induced pathogen resistance compounds in various crop plants such as chickpea, carrots and potatoes caused by k-carrageenan. These results suggest that carrageenan enhance plant resistance against *C. campestris* infestation probably through induction of the defense enzymes and phenolic compounds' accumulation.

Plants possess several defense systems to scavenge ROS to protect themselves from the oxidative stress (Cheeseman 2007). In our experiments, dodder invasion lead to a

significant increase in the activity of the enzymatic antioxidants CAT and GPX of the host plants. One of the most important defense reactions of plants to overcome stress is to synthesize oxidative products (Gill and Tuteja 2010). Apoplastic burst of ROS acts as a first barrier against subsequent attack by the pests (Cheeseman 2007). However, to prevent the self-toxicity of ROS, it has been demonstrated that plant cells have developed free radicals scavenging systems for removing the excess ROS to keep a relatively low and constant ROS concentration (War 2012). An efficient H₂O₂ scavenging system is required to enable rapid removal of ROS in the plant cells (Gill and Tuteja 2010). A number of enzymes regulate H₂O₂ levels. SOD and CAT are enzymes that represent the first line of antioxidant defense in plant cells. SOD catalyzes dismutation of superoxide radicals (O₂^{•-}) into H₂O₂ for decreasing the hydroxyl radical (OH[•]) content. CAT, GPX and APX are contributed in decreasing the content of plant H₂O₂ (Gill and Tuteja 2010). In our study, CAT and GPX were considered the most important.

In the present study, we observed the enhanced activities of enzymatic antioxidants in carrageenan-treated plants (CAT and SOD in healthy basil, and CAT, SOD and GPX in basil parasitized by dodder). These activated antioxidant systems are useful for plant performance and have an essential role in alleviating oxidative stress damage in plants, by removing excess ROS, inhibiting of LOX activity and lipid peroxidation.

Furuhashi et al. (2012) suggest *Cuscuta* for studies on plant-plant interactions. To date, however, no comprehensive study on antioxidative activities between parasitic plant and its host has been performed. In this research, enzyme activity of *C. campestris* was compared to its host. We observed that CAT, SOD activities in *C. campestris* were about 2- and 1.73-fold lower than in basil leaves, respectively. Also PAL, PPO, GPX and APX activities in *C. campestris* were about 2.5, 4.3, 85 and 2.82 times more than in basil leaves, respectively.

It seems that high activity of GPX in *C. campestris* was due to its essential role in the processes of invasion as Lopez-Curto et al. (2006) have determined for the invasion of *Coffea arabica* by *C. jalapensis*. They consider the role of peroxidase in association with decomposition and/or rearrangement of the components of cell walls and other barriers, together with the wall loosening, which are mechanisms necessarily involved in the processes of attachment and invasion. Therefore, they estimate that these enzymes interact with the host, breaking the defense barriers of the host plant and facilitate the invasion and growth of the haustorium.

The present findings support our hypothesis that high efficiency of antioxidants induced after carrageenan application is responsible for increasing of basil tolerance to dodder infection. We assume that this is due to the action of carrageenan, which stimulates the synthesis of secondary metabolites and activates natural plant-resistance mechanisms. Therefore, Carrageenan treatment at a concentration of 1 g L⁻¹ can directly suppress the damaging infestation of basil plant by *C. campestris*.

Conclusion

The degree of *Cuscuta* sp. parasitization of basil plants treated with carrageenan was significantly lower than that of untreated basil. The results of biochemical reaction of basil

plants indicate that foliar spraying of basil plants with k-carrageenan stimulated PAL, catalase and superoxide dismutase activities. The high activities of antioxidant enzymes after carrageenan treatment could be responsible for increasing host plant defense system. It is suggested that carrageenan is able to induce the natural plant-resistance mechanisms and the synthesis of secondary metabolites. The use of carrageenan as an environment-friendly biostimulator is a promising approach to protect basil against dodder.

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Disclosure statement

There is no potential conflict of interest was reported by the authors.

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