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Full Length Research Paper

Allelopathic potential of para-hydroxybenzoic acid and coumarin on canola: Talaieh cultivar

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Phenolic compounds are secondary metabolites that can affect plant growth and development. In the present study biochemical and physiological responses of canola, Talaieh cultivar, against two allelochemical substance (para-hydroxybenzoic acid and coumarin) was investigated. In this research, chlorophyll, carotenoid and anthocyanin content were determined spectrophotometrically, while guaicol peroxidase and catalase activities were measured. However, lipid peroxidation was measured by thiobarbituric acid procedure. Our results showed that germination was slightly affected by these phenolic compounds, but seedling growth was reduced significantly. Root and shoot length, fresh and dry weight were affected by these compounds. Chlorophyll content was not affected at low concentrations, but decreased with increase in phenolic concentration. Low molecular weight antioxidants such as carotenoids and anthocyanin, increased significantly. Antioxidant enzyme guaicol peroxidase activity increased with increase in the concentration of phenolic compounds, but catalase activity did not change significantly. Despite increase in antioxidant capacity of canola seedlings, lipid peroxidation increased in the presence of these phenolic compounds. Results show that allopathic potential of some plants is related to their phenolic compounds and investigation on allelochemical could result in the development of herbicides with less adverse effect on environment and ecosystem. The presence of these phenolic acids can reduce canola yield, therefore, elimination of weeds, containing these compounds is very important, from the allelopathic point of view for canola production.

Key words: Phenolic compounds, canola, antioxidant enzymes.

INTRODUCTION

Plants secondary metabolites released into the environment can affect plant growth and development (Rice, 1984). Phenolic compounds, alkaloids and terpenoids are compounds that can act as allelochemicals (Inderjit et al., 1995; Reigosa et al., 1999). Many studies have shown that phenolic compounds affect the biochemical and physiological processes such as cell growth, membrane permeability, nutrient uptake from soil, chlorophyll synthesis, photosynthesis, protein synthesis and enzyme activity (Einhellig, 1986; Reigosa et al., 1999). Leather and Einhellig, 1988). Possibility of increasing concentration of reactive oxygen species (ROS), in the presence of phenolic compounds has attracted researchers. Gmerek and Politycka (2010) have shown that in the presence of ferulic and para coumaric acids, hydrogen peroxide, such as superoxide anion and hydroxyl radicals, ROS increase in maize and pea roots. Therefore, in the presence of phenolic compounds increase in the plant oxidants, cause reduction in plant growth and development. To reduce oxidative stresses, plant increase synthesizing low molecular weight antioxidants and also antioxidant enzymes (Lee et al., 2007). Since the presence of these phenolic compounds can reduce canola yield, weed control for canola production seems to be also important from allelopathic point of view. In addition, investigation on allelochemicals may result in the development of herbicides with less adverse effect on environment and ecosystems. Oxidative stress caused by phenolic compounds in canola and its responses to oxidative stress has been less studied, so in the present study, physiological and biochemical responses of canola, Talaieh cultivar, to the stress of phenolic compounds

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Table 1. Effect of different phenolic compounds (mM) on canola germination.

Treatment	Concentration (mM)				
	Control	0.05	0.5	5	10
Coumarin	20(100)	20(100)	19(95)	17(85)	17(85)
Para-hydroxybenzoic acid	20(100)	20(100)	20(100)	18(90)	19(95)

Table 2. Effect of different phenolic compounds (mM) on canola root length (mm).

Treatment	Concentration (mM)				
	Control	0.05	0.5	5	10
Coumarin	68.74±9.94(100)	66.93±7.30(97)	58.96±11.26(87)	23.90±5.70(34)*	19.36±4.56(28)*
Para-hydroxybenzoic acid	68.74±9.94(100)	69.10±5.85(100)	64.86±10.36(94)	39.36±13.10(57)*	43.76±10.15(63)*

Table 3. Effect of different phenolic compounds (mM) on canola shoot fresh weight (mg).

Treatment	Concentration (mM)				
	Control	0.05	0.5	5	10
Coumarin	133.86±9.46(100)	136.66±13.17(102)	111.8±12.78(83)*	93.53±9.09(70)*	85.93±7.05(64)*
Para- hydroxybenzoic acid	133.86±9.46(100)	124.3±14.87(93)	139.6±13.41(104)	126.03±10.92(94)	112.96±12.98(84)

were investigated.

MATERIALS AND METHODS

Canola seeds, Talaieh cultivar, were obtained from oil seed companies of Shiraz, Iran. The canola seeds were sterilized with 10% sodium hypochloride, washed with distilled water several times and placed in Petri dishes containing proper concentration of phenolic compounds (para-hydroxybenzoic acid and coumarin). All materials were purchased from Sigma Chemical Company. After three days, germinated seeds were counted and expressed as percent germination relative to control. For seedling growth determination, the seven days old seedlings were transfered to Hogland solution containing different concentration (0.5, 0.05, 5 and 10 mM) of para-hydroxybenzoic acid and coumarin.

The plants were kept in growth room at 25°C with 16 h light and 8 h dark period. The Hogland solution was aerated for 15 min every hour. After 14 days the seedlings were washed with distilled water and then the root lengths, fresh weights and dry weights were determined.

Each experiment had three replication. Data represent the means ± SE. Statistical analyses were performed using SPSS (version 13). The significant of the differences in the values were performed by one-way ANOVA test and Duncan multiple range test. P-values<0.05 were considered significant.

Pigments extraction and measurement

Chlorophyll and carotenoids were extracted from 200 mg of leaf tissue with 80% acetone and anthocyanins were extracted with methanol and hydrochlorideric acid. Chlorophyll, carotenoid and anthocyanins content were determined spectrophotometrically by Arnon (1956), Eijckelhoff (1997) and Stanciu et al. (2010), respectively.

Enzymes extraction and activity measurements

1 g of leaf tissue was hemogenized in 0.2 molar phosphate buffer, pH = 7. The hemogenized was centrifuged at 5000 rpm were centrifuged for 5 min. The supernatant was used as crude enzyme extract to determine enzyme activities. Catalase activities were measured by decrease in absorbance at 240 nm with (Aebi, 1983) and guaicol peroxidase activity was determined at 436 nm (Mac-adam, 1992).

Determination of lipid peroxidation

0.5 g of canola leaf tissue was hemogenized in 0.1% trichloroacetic acid (TCA). After addition of 0.5 g thiobartituric acid (TBA), the hemogenate was heated, to create manoldialdehyde complex. Degree of lipid peroxidation was measured using thiobarbituric acid (Zhang and kirkham, 1996).

RESULTS AND DISCUSSION

Low concentrations (0.05 and 0.5 mM) of para hydroxy benzoic acid and coumarin had no effect on seed germination, but at high concentrations (5 and 10 mM) of these phenolic compounds, their germination reduced to about 10 to 15% (Table 1). Reduction significantly occured both in root and shoot length (Table 2), and also fresh and dry weight (Tables 3 and 4) of canola seedlings,

Table 4. Effect of different phenolic compounds (mM) on canola shoot dry weight (mg).

Treatment	Concentration (mM)				
	Control	**0.05**	**0.5**	**5**	**10**
Coumarin	12.9±1.11(100)	13.66±1.28(105)	10.76±1.05(83)	9.4±1.45(73)*	7.56±1.45(58)*
Para-hydroxybenzoic acid	12.9±1.11(100)	12.3±1.55(95)	13.56±1.05(105)	10.43±1.06(80)*	10.26±0.45(79)*

Table 5. Effect of different phenolic compounds (mM) on chlorophyll content (mg/g.FW).

Treatment	Concentration (mM)				
	Control	**0.05**	**0.5**	**5**	**10**
Coumarin	1.84±0.19(100)	1.79±0.26(97)	1.80±0.33(98)	1.27±0.18(69)*	1.09±0.16(59)*
Para- hydroxybenzoic acid	1.78±0.12(100)	1.83±0.16(103)	1.66±0.20(93)	1.18±0.12(66)*	1.29±0.15(72)*

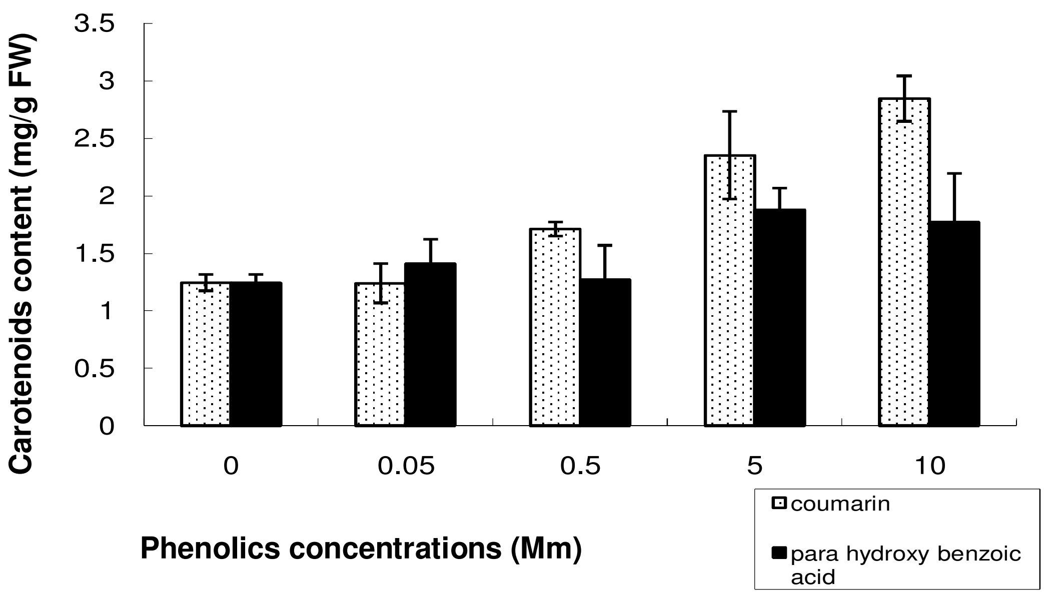

Figure 1. Effect of different phenolic compounds (mM) on carotenoids content (mg/g.FW).

Talaieh cultivar. Decrease in length and weight of canola seedling growth, in some cases occured more than 50%. Different studies have shown that allelochemicals, including phenolic compounds, are affected many bio-chemical and physiological processes such as membrane permeability, respiration and growth in plants (Leather and Einhellig, 1966). Reductions in plant growth in the presence of both phenolic compounds have been reported by researchers (Blum and Dalton, 2008; Reigosa et al., 1999).

Effects of phenolic compounds on pigments content

Low concentrations (0.05 and 0.5 mM) of phenolic compounds did not exert any considerable effect on chlorophyll content. But high concentrations (5 and 10 mM) of these phenolic compounds reduced chlorophyll content significantly (Table 5). About the low molecular weight antioxidants, phenolic compounds have applied significantly increase in carotenoids content (Figure 1).

So, at 10 mM concentration of coumarin, carotenoids content is reduced to 229% different from the control plants. In the presence of para hydroxy benzoic acid and coumarin, another low molecular weight antioxidants, anthocyanins, also increased significantly (Figure 2). Effects of phenolic compounds on break and synthesis of plant pigments (chlorophyll and carotenoids) have been studied by researchers. Usually, low concentrations of some phenolic compounds increase the chlorophyll content, the reverse effect of high concentrations of

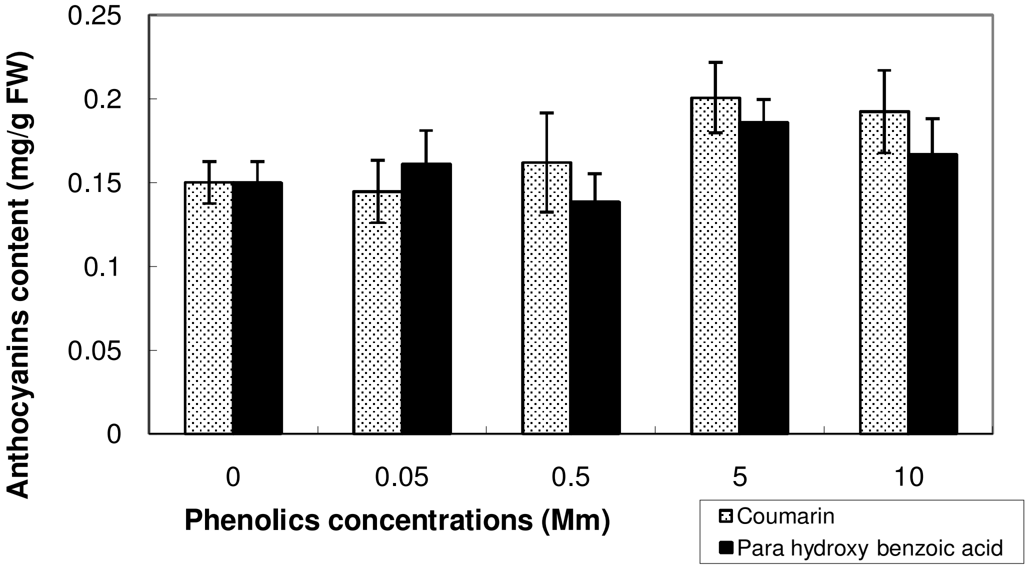

Figure 2. Effect of different phenolic compounds (mM) on anthocyanins content (mg/g.FW).

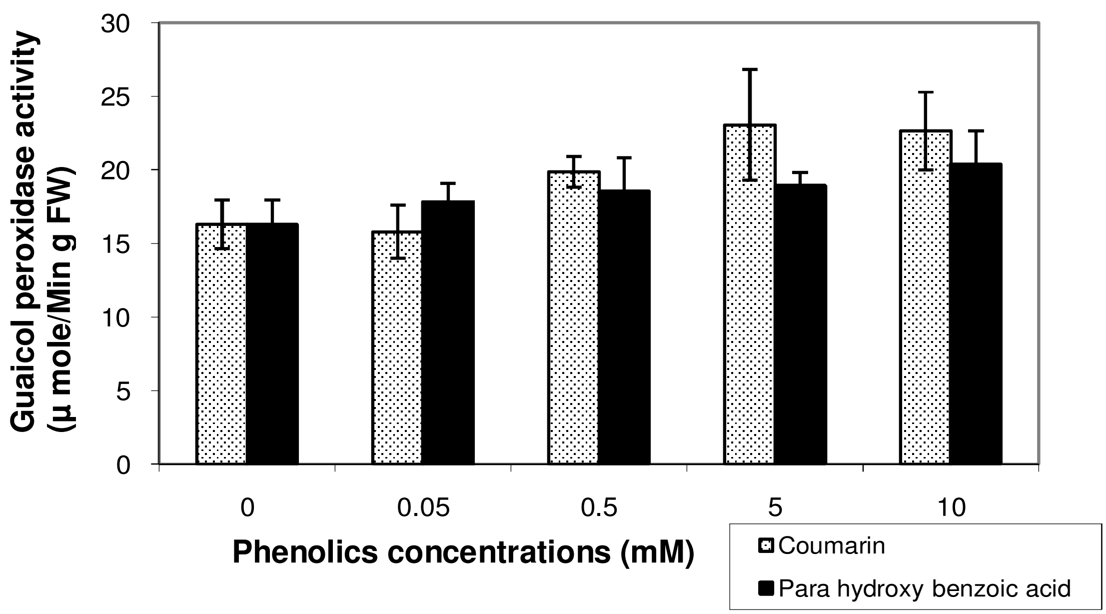

Figure 3. Effect of phenolic compounds (mM) on guaicol peroxidase activity (μ mole/Min g FW).

chlorophyll does not. There is different research in this field, one of them show that some phenolic compounds decrease chlorophyll content in soybean leaves (Einhellig and Eckrich, 1984), but another research show that plant phenolic compounds has no effect on chlorophyll content of sorghum (Baziramakenga et al., 1994).

Also, some studies show that environmental stresses on plant such as salinity, drought stress, heavy metal and secondary metabolites, cause increase in the low molecular weight antioxidants, carotenoids and anthocyanins, in plants and algae (An et al., 1998).

Effects of phenolic compounds on the antioxidant enzymes activity

Guaicol peroxidase, an antioxidant enzyme, showed increase in activity in the presence of para hydroxy benzoic acid and coumarin (Figure 3). At 10 mM concentration of both phenolic compounds increasing in

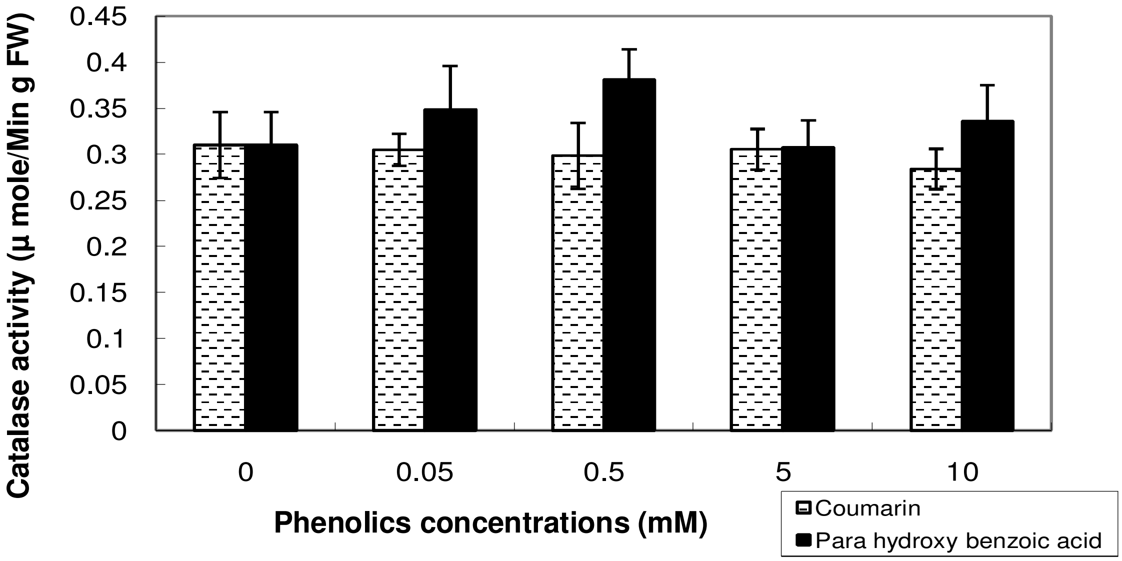

Figure 4. Effect of phenolic ompounds (mM) on catalase activity (μ mole/Min g FW).

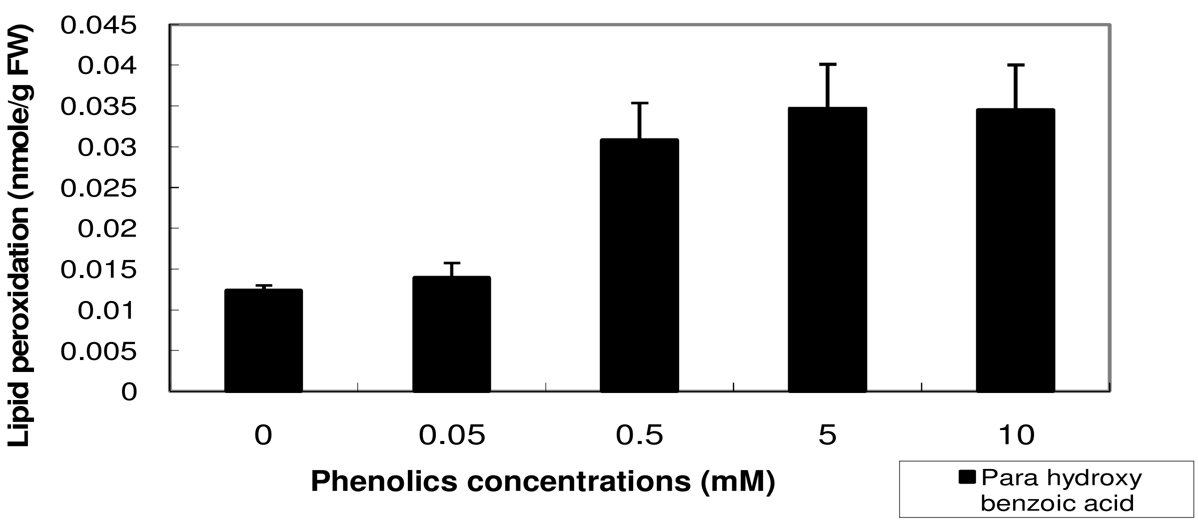

Figure 5. Effect of para-hydroxybenzoic acid (mM) on lipid peroxidation (nmole/g FW).

the enzyme activity of guaicol peroxidase was significant. A slight change occurred in the catalase activity, but this change was not statistically significant (Figure 4). Environment stresses have an effect on antioxidant enzymes activity such as catalase, guaicol peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase. There are some similar results like those of the present study which show that heavy metal stresses increase most of the antioxidant enzymes (Ruley et al., 2004). However, one research showed that benzoic acid can reduce catalase and ascorbat peroxidase activity in sun flower plants (Zhang and Kirkham, 1996).

Effect of para hydroxy benzoic acid on lipid peroxidation

Lipid peroxidation was increased by different concentration (0.05, 0.5, 5 and 10 mM) of para hydroxy benzoic acid (Figure 5). In the presence of para hydroxy benzoic acid, malondialdehyde complexes were significantly seen as 113, 207, 282 and 280% respectively from the control plants. Despite the increase in antioxidants capacity faced with environment stresses, oxidative stress can increase lipid peroxidation and then reduce in the plants growth (Ruley et al., 2004). Either cinnamic and benzoic acid can increase lipid peroxidation in soybean root and reduce plant growth (Baziramakenga et al., 1995).

Conclusion

Results indicate that allelopathic potential of plants could be related to their phenolic compounds. It shows that canola is sensitive to phenolic compounds. We conclude that the presence of some phenolic acids can reduce canola yield, therefore, elimination of weeds, containing these compounds is very important from the allelopathic point of view for canola production. Investigation of allelochemicals could result in the development of herbicides with less adverse effect on the environment and ecosystem production of canola plants with high antioxidant potential by genetic engineering, which can partly confer resistant to adverse effects of abiotic stresses.

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